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THE LIPIDS

Volume I

CHEMISTRY

Volume II

BIOCHEMISTRY

Digestion, Absorption, Transport and Storage

Volume III

BIOCHEMISTRY

Biosynthesis, Oxidation, Metabolism and Nutritional Value

THE LIPIDS

Their Chemistry and Biochemistry

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Volume II: BIOCHEMISTRY

Digestion, Absorption, Transport and Storage

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PREFACE

The present volume encompasses the available information on the digestion, absorption, transport (in the blood and lymph), and storage of fats and other lipids in the animal body. Although it was the original plan to include all the biochemical data in a single volume, the recent great increase of new findings reported in these fields has forced a revision of these plans. Rather than limit the topics to be discussed or curtail the extent of discussion of such topics, it was decided to expand the scope of the biochemical section of *The Lipids* to make up two volumes. Volume III will include Biosynthesis, Metabolism, Oxidation, and the Nutritional Value of the lipids.

Although Volumes II and III are concerned chiefly with the biochemical phases of lipids, it was necessary to include some topics of a more chemical nature. The description of the properties, composition, and behavior of the lipases and lipoxidases belongs essentially in this category. The description of the chemistry, structure, and composition of the bile acids is included in Volume II; these products are not lipids, but their chemistry must be understood to ensure the proper comprehension of fat absorption.

An attempt has been made to list all the investigators who have contributed to any study, irrespective of whether they were the first to report these findings or among the numerous workers who have confirmed and extended the data. The author realizes that he has been only partially successful in recognizing all workers; he will be most grateful for any suggestions calling to his attention mistakes of omission or commission.

Acknowledgment should again be made to Mrs. Margaret Ritter, who has continued with redoubled energy as an editorial assistant throughout the preparation of the volume. Recognition should also be given Mrs. Lilla Aftergood, who abstracted much of the recent information on lipids, and so made it possible to render this volume fairly current. Above all, the author is truly grateful to his wife, who willingly accepted the verdict that the manuscript on *Lipids* should be the most important task during the past eight years. Again, mention should be made of the skill and accuracy of Mrs. Marie Visser in furnishing a satisfactory typescript for the publisher.

HARRY J. DEUEL, JR.

Pasadena, California
November, 1954

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THE LIPIDS

Volume II: BIOCHEMISTRY

Digestion, Absorption, Transport and Storage

CHAPTER I

INTRODUCTION

The chemical nature of most of the components of the lipids has become fairly well understood within recent years. Methods of synthesis have been developed for many of the substances, and precise data on many of the physical constants have been obtained.

At the present time, it is fairly safe to say that the fatty acids are the best known group of the lipids. Although the chemical information concerning most of these acids is quite complete, the biochemical data are fragmentary. This is especially the case with the unsaturated acids. For example, although oleic acid was described as early as 1815 by Chevreul, and its structure has been understood since 1894 when Baruch[1] established the position of the double bond, little is definitely known of its degradation products and of the compounds involved in its *in vivo* synthesis. Information as to the precursors and decomposition products of the doubly unsaturated acids is even more limited than is knowledge concerning oleic acid.

The neutral fats comprise the type of lipids most widely distributed in animal and plant tissues. The physical and chemical properties of various simple mixed triglycerides have been carefully recorded; considerable data are likewise available on the different mono- and diglycerides. A wide variety of methods for the synthesis of these products has been published. However, little is known of the biochemical factors which control the type of triglycerides which are synthesized in the tissues. Our knowledge of the mechanism of formation of the neutral fats from carbohydrate and from protein is largely hypothetical.

Biochemical information concerning the other classes of lipids is likewise much less complete than are the strictly chemical data regarding them. Probably most is known about the biochemical changes of lecithin and related products, since such reactions are closely tied in with metabolic changes of neutral fats and fatty acids. Cholesterol is still an enigma,

[1] J. Baruch, *Ber.*, *27*, 172–176 (1894).

although a large amount of work is in progress on the mechanism of synthesis and on its metabolism in the animal body.

The chemical approach to the subject of lipids has been made in the first volume of this work. In the current volumes, this aspect will be touched upon only insofar as the information contributes to the problem under discussion.

The biochemistry of the lipids will be considered from the classical viewpoints: First, in Volume II, the subject of lipids will be discussed from the standpoint of digestion and absorption in the gastrointestinal tract. This will be followed by a review of their transport in the blood, with sections on the variations to be found in health and disease. The discussion will next center on the storage of lipids in the tissues in general, with a consideration of the factors which alter fat deposition in such tissues. The nature of the fat deposited in specific tissues and organs will be the subject of another chapter. In each chapter, the changes of the fats, phospholipids, sterols, carotenoids, and fat-soluble vitamins will be considered in separate sections. Data on the deposition of carotenoids and fat-soluble vitamins are not included in the discussion of storage, since they can more appropriately be discussed in the section on nutritional aspects of these compounds.

In Volume III, the intermediary metabolism of the lipids will be described from the viewpoint of their synthesis, oxidation, and metabolism under biological conditions. The role of the several types of lipids in nutrition will be covered in another section of Volume III.

CHAPTER II

THE DIGESTION AND ABSORPTION OF FATS IN THE GASTROINTESTINAL TRACT

1. Introduction

The media in which biochemical transformations take place in the animal organism are almost exclusively aqueous. Moreover, the two most important fluids concerned in the transport of nutrients and waste products, namely the blood and urine, dissolve only water-soluble substances. It is therefore apparent that, in contrast to carbohydrates and proteins, which are readily soluble in water as such or as their digestion products, it is only with difficulty that lipids can undergo metabolic changes or be transported in solution by such a medium as the blood. However, the blood does contain small microscopic particles, the *chylomicrons*, which are discrete lipid droplets. It is believed that these elements may offer an explanation for one mechanism of transfer of lipid materials which are not capable of transformation into water-soluble compounds. Another possible avenue of transport of such hydrophobic lipids as vitamin A alcohol and ester, the carotenoids,[1] and also the sterols, is in the form of protein complexes. These proteins should probably all be classed as lipoproteins; there is evidence that a variety of such proteins exist which present marked differences as regards solubility in ammonium sulfate solutions. It seems that a considerable degree of specificity exists between the lipid component involved and the protein with which it is combined.

2. Enzymes Concerned with the Digestion of Lipids

The predominant enzymes involved in the several phases of lipid metabolism are classed as ester-hydrolyzing enzymes. Table 1 lists the most common representatives, together with the sites of occurrence, the substrates on which they act, and the reaction products which result.

[1] J. Ganguly, N. Krinsky, J. W. Mehl, and H. J. Deuel, Jr., *Arch. Biochem. Biophys.*, *38*, 275–282 (1952).

TABLE 1
THE CLASSIFICATION, OCCURRENCE, SUBSTRATE, AND END-PRODUCTS OF SOME ENZYMES CONCERNED WITH THE METABOLISM OF LIPIDS

Name	Principal sites of occurrence	Substrate	Reaction products
Lipase	Gastric juice (?); pancreatic juice; waxy plants	Neutral fats	Diglycerides, monoglycerides, fatty acids, glycerol
Esterases (*a*-group)	Liver; plants	Esters of monohydroxyalcohols	Alcohol + acid
Cholesterase	Blood; other tissues	Cholesterol esters	Cholesterol + fatty acid
Lecithinase A	Kidney, brain, intestinal mucosa	Lecithin	Diglycerides, choline, phosphate
Phytase	Plants, especially cereals	Phytic acid	Inositol + phosphate
Cholinesterase	Animal tissues	Acetylcholine	Choline + acetate
Choline acetylase	Nervous tissue; muscle	Acetic acid + choline	Acetylcholine
Chlorophyllase	Green plants	Chlorophyll	Phytol + chlorophyllide-a
Sulfatase	Animal and plant tissues	Sulfuric acid esters	Alcohol + sulfate

The lipases, esterases, and other enzymes concerned with the metabolism of lipids are water-soluble substances. The action of such molecules on fats or esters which results in their hydrolysis can occur only if the enzyme molecule comes into proximity with that of the lipid or ester. One site at which proximity can be produced between these components is the water: lipid interface. Since it is known that emulsification of fat precedes the action of the enzyme, the area between the water and oil phases is greatly increased. The large surface produced by this emulsification might be sufficiently great to allow lipolysis to proceed at the speed that is known to occur in the gut, in spite of the fact that the substrate is in a different phase than is the enzyme. Schulman[2] has shown that the digestion of esters by pancreatin is related to the nature of the surface film. The reaction rate is related to the length of the alcohol and acid radical chain. Apparently the acid radical plays the more important role; complete inhibition usually obtains when the acid contains 5 or 6 carbons or possesses the C_6H_5-ring. Activity is noted when the acid is increased to a chain length of 8 carbon atoms. On the other hand, although the length of the alcohol chain does not influence the results in compounds otherwise indigestible, it was found that the ethyl esters are somewhat more easily digested by pancreatin. These variations in hydrolysis are interpreted by Schulman[2] as being related to difference in orientation of the esters in the surface films. In 1951,

[2] J. H. Schulman, *Trans. Faraday Soc.*, *37*, 134–139 (1941).

Desnuelle[3] and Favager[4] discussed, in a comprehensive manner, the enzymatic hydrolysis of triglycerides.

(1) *Lipases*

a. Gastric Lipase. There would appear to be little doubt that fat may, under some conditions, undergo hydrolysis in the stomach. However, it is still a moot question whether this hydrolysis of fat is to be traced to a fat-splitting enzyme produced by the stomach mucosa, or whether the lipolytic action observed in this organ is due to pancreatic lipase which has been regurgitated.

Although Magendie,[5] as early as 1825, reported changes of fat in the pyloric region of the stomach, most of the work of later investigators gave opposite results. However, Hull and Keeton[6] found that gastric juice, obtained from dogs with Pavlov accessory stomachs, did possess appreciable lipolytic activity when the secretion was neutral or when it was neutralized as soon as produced. The failure of earlier workers to obtain positive results was thus explained as due to the hyperacidity of the gastric juice. The later work of Willstätter and Memmen[7] offers considerable evidence that gastric lipase does actually exist. According to these workers, gastric lipase is secreted in active form and not as a zymogen; it occurs in greater concentration in the cardiac than in the fundic end of the stomach, but the extracts are from 40 to 600 times weaker than those obtained by similar means from the pancreas. The optimum *p*H is on the alkaline side of neutrality, as in the case of pancreatic lipase; in fact, Willstätter and Memmen[7] could not demonstrate any qualitative differences between the above two lipolytic enzymes.

In any event, the *p*H usually existing in the stomach is unfavorable, not only for the emulsification of fat but also for the action of gastric lipase. Such highly emulsified fats as those in egg yolk and milk may undergo some hydrolysis; the extent of this reaction is somewhat increased in the infant stomach, where the prevailing acidity more nearly approximates the optimum *p*H of both gastric and pancreatic lipases. Everett[8] states that, even in the infant, less than 5% of fat is hydrolyzed in the stomach.

[3] P. Desnuelle, *Bull. soc. chim. biol.*, *33*, 909–923 (1951).

[4] P. Favager, *Bull. soc. chim. biol.*, *33*, 924–960 (1951).

[5] F. Magendie, *Précis elementaire de physiologie*, Vol. II, 2nd ed., Mequignon-Marvis, Paris, 1825, p. 142; cited from W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 112.

[6] M. Hull and R. W. Keeton, *J. Biol. Chem.*, *32*, 127–140 (1917).

[7] R. Willstätter and F. Memmen, *Z. physiol. Chem.*, *133*, 247–259 (1924).

[8] M. R. Everett, *Medical Biochemistry*, 2nd ed., P. B. Hoeber, New York and London, 1946, p. 211.

The regurgitation of intestinal contents may aid in lipolysis, both by furnishing additional lipase, and by producing a reaction of the gastric contents near to the optimum *p*H of gastric lipase. While the gastric digestion of fats is of only minor importance in itself, it does provide some free fatty acids which accelerate the rate of emulsification of the lipids, once the chyme is passed into the small intestine.

A suggestion as to another possible source of gastric lipase in certain species has been offered by Farnham.[8a] He stated that a lipase is secreted in the calf, kid, and lamb by a gland located at the base of the tongue and extending downward into the gullet area. This enzymatic secretion would naturally pass into the stomach. The gland is now being employed commercially as a source of enzymes which are utilized in the cheese industry for the development of flavor.[8b]

b. Pancreatic Lipase (Steapsin). The action of pancreatic juice in causing emulsification and hydrolysis of fats was first recognized in 1856 by Claude Bernard.[9] This enzyme was further studied by Herter (1880),[10] by Glaessner (1904),[11] by Bradley (1909),[12] and by Wohlgemuth (1912),[13] while Pavlov[14] reported at length on the lipase present in the pancreatic juice of the dog. The collection of pancreatic juice was greatly aided by the discovery that the principal stimulus to secretion is controlled by a humoral mechanism.[15] Ivy[16] reviewed the mechanism of secretion of pancreatic juice, as well as that of other digestive secretions.

Steapsin is the most important enzyme involved in the digestion of neutral fat. It is secreted by the acinar cells of the pancreas, along with the accompanying enzymes, trypsin, chymotrypsin, and amylopsin.

(*a*) *Activation of Pancreatic Lipase by Bile Salts.* Steapsin, as secreted, is partially inactive. It was formerly believed that this lipase was produced as an inert zymogen called "steapsinogen," which was converted to the active form in the small intestine by contact with bile salts. However, bile salts have been shown to activate both gastric and pancreatic lipase

8a M. G. Farnham, *personal communication* to the author (March, 1954).

8b M. G. Farnham, *U. S. Patent* No. 2,531,329 (Nov. 21, 1950).

9 C. Bernard, *Mémoire sur le pancreas et sur la rôle du suc pancréatique dans les phénomènes digestifs, particulièrement dans la digestion des matières grasses neutres,* Ballière, Paris, 1856.

10 E. Herter, *Z. physiol. Chem., 4,* 160–164 (1880).

11 K. Glaessner, *Z. physiol. Chem., 40,* 465–479 (1904).

12 H. C. Bradley, *J. Biol. Chem., 6,* 133–172 (1909).

13 J. Wohlgemuth, *Biochem. Z., 39,* 302–323 (1912).

14 I. P. Pavlov, *The Work of the Digestive Glands,* translated by W. H. Thompson, 2nd English ed., Griffin, London, 1910.

15 W. M. Bayliss and E. H. Starling, *J. Physiol., 30,* 61–83 (1904).

16 A. C. Ivy, *Physiol. Revs., 10,* 282–335 (1930).

only after the enzyme is purified[17] while, at high concentrations, the effectiveness of the bile salts is decreased.[18] Sodium glycocholate has been found to be a better activator than is sodium taurocholate.[19,20] Bile salts can likewise accelerate the hydrolysis of soluble esters by lipase,[21] although these same compounds inhibit the action of esterases.[22]

(*b*) *Activators and Inhibitors of Pancreatic Lipase.* A number of substances other than bile and bile salts, such as egg albumen, calcium oleate and other soaps, blood serum, saponin, and alcohols are now known to bring about this activation of the enzyme. Yamamoto[23] reported that dicarboxylic amino acids and diamino acids, including ornithine, have a notable effect in stimulating the action of pancreatic lipase on triacetin and on olive oil. However, arginine was without effect in increasing lipolysis of triacetin by pancreatic lipase. Histidine increases the rate of splitting of olive oil by more than 100%. L-Histidine was shown to increase the speed of hydrolysis of triacetin at a *p*H of 8.6 to 56%, and that of tributyrin to 70%; it was without influence on the breakdown of butylbutyrate ($CH_3CH_2CH_2COOCH_2CH_2CH_2CH_3$).[24] L- and DL-Histidine were found to be equally potent in augmenting the action of steapsin in the range of 0.005 to 0.04 *M*. Histamine, likewise, was shown to be an activator (30%) with a potency identical with that of tyrosine.[24] The action of lysine in stimulating lipolysis is believed to be related to the presence of two free amino groups,[23] although the free amino groups of the dicarboxylic acids have little effect. While succinic and citric acid were found to be lipase stimulants, fumaric acid (*trans*-COOHCH:CHCOOH) was inactive, although the corresponding *cis* form, maleic acid, was quite active.[23] Willstätter and Memmen[18] have reported that proteins inhibit the hydrolysis of tributyrin, although certain amino acids and peptides were found to favor the hydrolysis of this simple triglyceride.

Rosenheim[25] separated a coenzyme from lipase, in the absence of which the enzyme was inactive. However, according to the more recent work of Willstätter and co-workers,[19,20] such non-specific activating agents exert their effect by affording an especially efficient adsorption environment

[17] R. Willstätter and E. Bamann, *Z. physiol. Chem.*, *173*, 17–31 (1928).
[18] R. Willstätter and F. Memmen, *Z. physiol. Chem.*, *129*, 1–25 (1923).
[19] R. Willstätter, E. Waldschmidt-Leitz, and F. Memmen, *Z. physiol. Chem.*, *125*, 93–131 (1923).
[20] R. Willstätter and F. Memmen, *Z. physiol. Chem.*, *133*, 229–246 (1924).
[21] E. F. Terroine, *Biochem. Z.*, *23*, 429–462 (1910).
[22] G. M. Wishart, *Biochem. J.*, *14*, 406–417 (1920).
[23] T. Yamamoto, *J. Biochem.* (*Japan*), *38*, 147–155 (1951).
[24] T. Yamamoto, *J. Biochem.* (*Japan*), *38*, 277–287 (1951).
[25] O. Rosenheim, *J. Physiol.*, *40*, xiv–xvi (1910).

which facilitates contact between the water-soluble enzyme and the water-insoluble substrate. In line with this, Glick and King[26,27] have shown that the activating effect of a compound on lipase is proportional to the lowering in surface tension which it produces. Substances which increase the action of pancreatic lipase were found to inhibit liver esterase to a proportional degree.[27,28] Glick and King interpreted their data as indicative of the fact that two opposing forces operate when a foreign substance is added to a solution containing an enzyme and a substrate. The first effect is to inhibit the enzyme, either by a physical or a chemical union between the foreign compound and the enzyme, or by a combination with the substrate. The opposing influence is to activate the enzyme by rendering it and the substrate more accessible to each other; this may readily be accomplished by reducing the interfacial tension. Sagar,[29] using a 1:100 mixture of olive oil in mineral oil for his tests, noted that the extent of lipolysis was a function of the degree of emulsification. Surface-active emulsifying agents such as monostearyl and distearyl glycerides were superior to bile in promoting lipolysis. It was shown that, in the breakdown of neutral fat, active monoglyceride was formed at the oil-water border. It is postulated that, in the biological decomposition of fat, monoglycerides are formed which aid in the emulsification.

Fodor[30] studied inhibition of the hydrolysis of esters as effected by various propylene glycol mono- and di-esters. The inhibitory effect of isoamylisobutyrate and of methylhexylcarbinol isobutyrate on the enzymatic hydrolysis of methylbutyrate was found to be independent of the enzyme concentration.[31] The inhibitory effect is markedly increased by prior unification of the inhibitor and the enzyme. On subsequent addition of the substrate, a substrate-inhibitor-enzyme complex is formed which effectively blocks the action of the enzyme on the substrate. This type of inhibition is unlike that brought about by formol, which acts on the enzyme protein itself, or on the enzyme protein-water interface. The action of bile salts on lipase is due to a similar action. Minard[32] also reported that another emulsifying agent, Tween (polyoxyethylene sorbitan) inhibits the action of pancreatic lipase on corn oil. In this case, a less reactive substrate (Tween) inhibits the enzyme action on a more reactive substrate (corn oil), since the Tweens

[26] D. Glick and C. G. King, *J. Biol. Chem.*, *97*, 675–684 (1932).
[27] D. Glick and C. G. King, *J. Biol. Chem.*, *94*, 497–505 (1931–1932).
[28] D. Glick, and C. G. King, *J. Biol. Chem.*, *95*, 477–482 (1932).
[29] C. A. Sagar, *Biochem. Z.*, *321*, 44–51 (1950).
[30] P. J. Fodor, *Arch. Biochem.*, *28*, 274–280 (1950).
[31] P. J. Fodor, *Arch. Biochem.*, *35*, 311–320 (1952).
[32] F. N. Minard, *J. Biol. Chem.*, *200*, 657–660 (1953).

are slowly hydrolyzed by the enzyme in this case; the inhibition can be reversed by the addition of bile salts.

(*c*) *Synthetic Action of Pancreatic Lipase.* Although it was early recognized that fat is broken down to fatty acids and glycerol by the action of steapsin, Pottevin,[33] in 1903, was the first to demonstrate the synthetic activity of the enzyme. This investigator was able to prove a 33% combination of oleic acid and glycerol in 50 hours at 38°C. in the presence of steapsin, while Artom and Réale[34] obtained an esterification of over 50% of the starting materials when the reaction was catalyzed by fat-free pancreas powder.

(*d*) *Properties of Pancreatic Lipase.* Pancreatic lipase,[35] although soluble in water, is quite unstable in aqueous solution. It is relatively stable in glycerol. It has an optimum temperature of 40°C., but retains some activity at a temperature as low as 0°C. The enzyme is partially destroyed by subjecting solutions for ten minutes to a temperature of 45°C., while it is completely destroyed when kept at 55°C. for this time interval. Steapsin is most effective in bringing about hydrolysis of triglycerides; it attacks diglycerides and monoglycerides to a progressively lesser degree. Of the simple triglycerides, trilaurin and triolein are the most readily hydrolyzed by pancreatic lipase. Steapsin is believed to be a protein.

(*e*) *Specificity of Pancreatic Lipase.* Fodor[36] pointed out that glycerated homogenates of hog pancreas present a distinct difference in heat stability with respect to the hydrolysis of esters composed of the shorter-chain acids and monovalent alcohols, on the one hand, and that of glycerol esters on the other hand. It was later shown[37] that the fraction which was active in hydrolyzing glycol esters, short-chain triglycerides, and monovalent alcohols was more readily destroyed by crystalline trypsin and by alkali, and more thermolabile than was the true pancreatic lipase (steapsin), which acts principally on triglycerides. Thus, Fodor[37] postulates the coexistence of at least two ester-hydrolyzing enzymes in pancreatic juice. Hofstee[38] demonstrated two esterases (ali-esterases) in pancreatic juice distinct from lipase and cholinesterase. These are called Esterase I and Esterase II. Both hydrolyze valeryl salicylate, while lipase does not bring about this breakdown. The action of sodium choleate was found to be

[33] H. Pottevin, *Compt. rend.*, *136*, 767–769, 1152–1155 (1903).
[34] C. Artom and L. Réale, *Bull. soc. chim. biol.*, *18*, 959–978 (1936).
[35] W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.
[36] P. J. Fodor, *Arch. Biochem.*, *25*, 223–225 (1950).
[37] P. J. Fodor, *Arch. Biochem.*, *26*, 307–315 (1950).
[38] B. H. J. Hofstee, *J. Biol. Chem.*, *199*, 357–364 (1952).

more pronounced in augmenting the hydrolysis of triglycerides than in increasing that of the monovalent alcohol monocarboxylic acid esters.[39]

Gomori[40] reported that water-soluble unsaturated fatty acid esters are hydrolyzed almost exclusively by the lipase of the pancreatic type (true lipase), while similar esters containing saturated acids are attacked by esterases of both the hepatic and the pancreatic types. Moreover, Ravin and Seligman[41] stated that the specificity of a substrate for lipase is dependent not only upon the chain length of the acyl group but also upon the number of ester groups present in the substrate. The structure of the lipid-water interface of the emulsions formed by the esters is believed to be correlated in a regular manner with their chemical structure; this acts as an additional determinant of the specificity of lipase, by offering steric hindrance to ali-esterase. Tributyrin, which is widely used for the determination of "serum lipase," does not have a sufficiently high degree of specificity to fulfill the necessary criteria. On the other hand, 2-naphthyl laurate has a high specificity for lipase, and this is satisfactory as a substrate for the determination of serum lipase.[41]

c. Intestinal Lipase. The intestinal walls have been shown to produce a lipase which is likewise activated by bile. Schiff[42] found a lipase in the intestinal contents of depancreatized dogs. However, it is possible that the hydrolysis produced by this preparation may have been due to the effect of gastric lipase. On the other hand, it was later reported that intestinal juice obtained from dogs having a Thiry-Vella fistula has a weakly hydrolytic action.[43,44] Kalaboukoff and Terroine,[45] using glycerol, were able to prepare an active lipase from the intestinal mucosa, the activity of which was accelerated by the bile acids.

d. Serum Lipase. The lipolytic action of serum is ascribed in part to the presence of a lipase and partly to that of esterase. The lipase originates from the pancreas, while the blood esterase is manufactured in the liver. The difference in origin of these enzymes was beautifully demonstrated by Hiruma,[46] who found that ligation of the pancreatic duct increased blood lipase; Cherry and Crandall[47] also noted that, although lipase is normally absent from the blood, it appears after ligation of the pancreatic

[39] P. J. Fodor, *Arch. Biochem.*, *30*, 197–201 (1951).
[40] G. Gomori, *Proc. Soc. Exptl. Biol. Med.*, *72*, 697–700 (1949).
[41] H. A. Ravin and A. M. Seligman, *Arch. Biochem. Biophys.*, *42*, 337–354 (1953).
[42] M. Schiff, *Arch. physiol. norm. path.*, [*5*], *4*, 699–702 (1892).
[43] W. Boldyreff, *Zentr. Physiol.*, *18*, 457–460, 460–461 (1904).
[44] W. Boldyreff, *Z. physiol. Chem.*, *50*, 394–413 (1907).
[45] L. Kalaboukoff and E. F. Terroine, *Compt. rend. soc. biol.*, *59*, 617–619 (1907).
[46] K. Hiruma, *Biochem. Z.*, *139*, 336–341 (1923).
[47] I. S. Cherry and L. A. Crandall, Jr., *Am. J. Physiol.*, *100*, 266–273 (1932).

duct, while no concomitant rise in blood esterase obtains. On the other hand, Glotzer and Seligman[48] reported that the blood of normal dogs contains an appreciable concentration of lipase. By the end of the first week after pancreatectomy, serum lipase showed a marked decrease, which was maintained over several weeks, after which some increase was observed.

e. Ricinus Lipase (Castor-Bean Lipase). As early as 1890, Green[49] and also Sigmund[50] discovered that the germinating seeds of the castor-bean plant (*Ricinus communis*), of the summer rape (*Brassica napus annua*), and of the oil-producing winter rape, or colza (*B. napus oleifera*), possess a powerful lipase. It was first believed that ricinus lipase occurs in the form of a zymogen, and that it becomes activated in the germinating seeds of the castor-bean by the formation of acid. This idea was refuted by Connstein and collaborators,[51] who showed that castor-bean preparations which had been acidified and then neutralized possessed no greater lipolytic action than did control preparations treated with distilled water. This enzyme was shown to be a true lipase, inasmuch as its main action is on neutral fats. Although it has been reported to have little action on the lower esters,[52,53] Willstätter and Waldschmidt-Leitz[54] stated that the enzyme exerts an equally effective action as a lipase and as an esterase. Falk and Sugiura[55] had demonstrated earlier that the lipase and esterase properties of the castor-bean enzymes could be separated by virtue of differences in solubility. Both fractions were found to be proteins. Cholesterol esters apparently cannot be hydrolyzed by the lipase preparation from castor-bean.[56]

Willstätter and Waldschmidt-Leitz[54] denied the possibility that an esterase and a lipase can be separated into two components; they state that their lipase, which possesses both triglyceride- and ester-hydrolyzing properties, is a pure preparation. Longenecker and Haley[57] reported that ricinus lipase exhibits no specificity insofar as the triglyceride linkage is concerned, and that it brings about a similar degree of lipolysis regardless of the chain length of the fatty acids involved. In the case of natural fats,

[48] P. Glotzer and A. M. Seligman, *Am. J. Physiol.*, *164*, 486–489 (1951).
[49] J. R. Green, *Proc. Roy. Soc. London*, *48*, 370–392 (1890).
[50] W. Sigmund, *Monatsh.*, *11*, 272–276 (1890).
[51] W. Connstein, E. Hoyer, and H. Wartenberg, *Ber.*, *35*, 3988–4006 (1902).
[52] H. E. Armstrong, *Proc. Roy. Soc. London*, *B76*, 606–608 (1905).
[53] K. G. Falk, *J. Am. Chem. Soc.*, *35*, 1904–1915 (1913).
[54] R. Willstätter and E. Waldschmidt-Leitz, *Z. physiol. Chem.*, *134*, 161–223 (1923–1924).
[55] K. G. Falk and K. Sugiura, *J. Am. Chem. Soc.*, *37*, 217–230 (1915).
[56] F. E. Kelsey, *J. Biol. Chem.*, *130*, 199–202 (1939).
[57] H. E. Longenecker and D. E. Haley, *J. Am. Chem. Soc.*, *57*, 2019–2021 (1935).

the decreasing order in percentage hydrolysis of the oils under certain standardized conditions was as follows: peanut, castor, corn, cottonseed, soybean, rape, olive, linseed, neatsfoot, peach-kernel, coconut, whale, fish, and sperm oils.

Ricinus lipase has an optimum *p*H of 4.7 to 5.0,[54] depending somewhat upon the buffer employed. During germination, the activity is caused by the production of acid; however, the enzyme exists in the resting seeds, since its action can be demonstrated if the medium is acidified. The optimum temperature is 35°C.; at 60°C., the enzyme is largely destroyed, except in the presence of substrate. Under this latter condition, ricinus lipase will show resistance to destruction at temperatures up to 160°C.[35] In contradistinction to steapsin, ricinus lipase is insoluble in water; in fact, in the absence of fat, it is inactivated by water.[58] As in the case of steapsin, castor-bean lipase has been shown to catalyze the synthesis of triglycerides.[59,60] Ricinus lipase may be readily extracted from the ground castor-bean seeds by dilute alkali; methods of purification have been described by a number of workers.[54,57,61]

Lipases have been reported from a number of other plant sources, for example from the seeds of the sunflower (*Helianthus annuus*),[62] from the greater celandine (*Chelidonium majus*),[63,64] and from preparations of papain derived from the green fruit of the papaw (*Carica papaya*).[65]

According to Singer and Hofstee,[66] a lipase could be obtained in soluble form and purified from wheat germ. This enzyme was shown to act on a large number of water-soluble simple esters, as well as on mono- and triglycerides, and on Tweens 20 and 40. It is believed that this is a single enzyme; it was shown to be inhibited by all reagents commonly employed for testing —SH-containing enzymes. Calcium ions did not affect its activity. In the presence of excess substrate of either glycerides or simple esters, the enzyme was found to follow a zero order reaction, while inactivation with heat proceeded as a second order reaction.[67] The optimum temperature was found to be 38°C., and the optimum *p*H 6.8 to 7.4.[67]

[58] K. G. Falk, *The Chemistry of Enzyme Actions*, 2nd ed., Chemical Catalog Co., New York, 1924, p. 137.

[59] H. E. Armstrong and H. W. Gosney, *Proc. Roy. Soc. London, B88*, 176–189 (1914).

[60] A. Morel and L. Velluz, *Bull. soc. chim. biol., 10*, 478–488 (1928).

[61] E. Hoyer, *Z. physiol. Chem., 50*, 414–435 (1906–1907).

[62] F. Traetta-Mosca and F. Milletti, *Ann. chim. applicata, 13*, 27–288 (1923); *Chem. Abst., 18*, 2357 (1924).

[63] K. Bournot, *Biochem. Z., 52*, 172–205 (1913).

[64] K. Bournot, *Biochem. Z., 65*, 140–157 (1914).

[65] M. Sandberg and E. Brand, *J. Biol. Chem., 64*, 59–70 (1925).

[66] T. P. Singer and B. H. J. Hofstee, *Arch. Biochem., 18*, 229–243 (1948).

[67] T. P. Singer and B. H. J. Hofstee, *Arch. Biochem., 18*, 245–259 (1948).

Kvamme *et al.*[68] reported that wheat-germ lipase is inhibited by concentrations of sodium 2,4-dichlorophenoxyacetate greater than 0.009 M; this inhibition was shown to be 400 times as effective with castor-bean lipase as with that from wheat germ.[68] The primary type of inhibition was shown to be noncompetitive. Longenecker[69] has reviewed the earlier literature on plant esterases, and Sullivan[70] has summarized the information on the relationship of esterases to milling and baking.

f. Lipases in Molds and Bacteria. The presence of lipase has been noted in molds, such as the saprophytic fungus (*Aspergillus niger*[71]) and in yeasts.[35] Shipe[72] found that the lipases produced by *Aspergillus niger* (A) and the green cheese mold (*Penicillium roqueforti*) (P) are quite distinct. Thus, the A-lipase hydrolyzed tricaprylin, tributyrin, tricaproin, and tripropionin, in decreasing order, while the order of activity of the P-lipase on these triglycerides was tributyrin > tricaproin > tricaprylin > tripropionin. Neither of these lipases was able to hydrolyze triacetin. The optimum pH was 5.0 to 5.5 for both enzymes, while the optimum temperatures were 30 to 35°C. for P and 35 to 40°C. for A. Calcium chloride accelerated the action of both enzymes, while both were inhibited by acetone, ethanol, formaldehyde, dioxane, toluene, and diethyl ether, although not to the same degree. Ramakrishnan and Banerjee[73] were able to prove the presence of lipase in the green mold found in dairy products (*Penicillium chrysogenum* S_1), which had been grown on sesame (*Sesamum indicum*). In this case, the optimum pH was found to be 6.2 to 6.8. The lipase was able to effect the synthesis of n-butyl oleate. In the case of the lipase from the saprophytic "imperfect" fungus, which causes flax wilt (*Fusarium lini* Bolley), Fiore and Nord[74] reported an optimum pH of 7.0 at 37°C. This enzyme was found to be intracellular. It was very stable in the dry state, but unstable in aqueous solution. There was no dissociable prosthetic group. The instability in water was believed to be due to proteolysis. This *Fusarium* lipase was shown to be capable of hydrolyzing triacetin. Yeast lipase has been reported to have an optimum pH of 6.6 to 6.8, and an optimum temperature of 30°C.[75]

[68] C. J. Kvamme, C. O. Clagett, and W. B. Treumann, *Arch. Biochem.*, *24*, 321–328 (1949).

[69] H. E. Longenecker, "Esterases," in J. A. Anderson, ed., *Enzymes and Their Role in Wheat Technology*, Chap. IV, Interscience, New York and London, 1946, pp. 127–152.

[70] B. Sullivan, "Esterases in Relation to Milling and Baking," in *Enzymes and Their Role in Wheat Technology*, Chap. V, 153–174.

[71] R. Schenker, *Biochem. Z.*, *120*, 164–196 (1921).

[72] W. F. Shipe, Jr., *Arch. Biochem.*, *30*, 165–179 (1951).

[73] C. V. Ramakrishnan and B. N. Banerjee, *Arch. Biochem.*, *37*, 131–135 (1952).

[74] J. V. Fiore and F. F. Nord, *Arch. Biochem.*, *26*, 382–400 (1950).

[75] G. Gorbach and H. Güntner, *Monatsh.*, *61*, 47–60 (1932).

Bacteria which tolerate low temperatures, such as strains of *Achromobacter* and of the saprophytic, flagellated bacterium, *Pseudomonas*, as well as certain bacilli, may contain lipases.[76,77] Rosenfeld[78] confirmed the existence of bacteria which effect anaerobic lipolysis; these include sulfate reducers. The activity of these lipases is quite general.

(2) *Esterases*

Although the expression "esterases" should be considered as an all-embracing term to include not only lipases but also a number of ester-splitting enzymes which act on general and specific types of esters, or, in fact, on individual esters, its usual connotation is solely to indicate the non-specific group of enzymes whose behavior is of a general nature. The esterases which attack aliphatic compounds have been called "ali-esterases" by Richter.[79]

a. Origin of Esterases. Esterases are widely distributed in animal tissues, but the highest concentration occurs in the liver. There is convincing evidence that the liver is the site of origin of the esterases, since the amount present in this organ diminishes after hepatic injury brought about by phosphorus or by chloroform.[80] Prewitt[81] likewise demonstrated that this enzyme can be partially washed out of the organ by perfusion with blood.

Blood esterase would appear to have its origin in the liver rather than in the pancreas. On the one hand, no decrease in the level of esterase in the blood obtains after pancreatectomy[82] while, on the other hand, blood esterase values rise concomitantly with their decrease in the liver caused by such hepatic poisons as phosphorus or chloroform.[80]

b. Distribution of Esterases. In addition to the liver, the lung and the kidney contain an extremely active esterase,[83] while in small intestine and spleen, the activity of the esterase preparations decreases progressively.[35,84] The esterases of brain and muscle exhibit only slight activity. Rat carcinoma esterase appears to have a feeble action. As far as species is con-

[76] L. B. Jensen and D. P. Grettie, *Food Research*, *2*, 97–120 (1937).

[77] J. R. Vickery, *J. Council Sci. Ind. Research*, *9*, 107–112, 196–198 (1936); *Chem. Abst.*, *30*, 6779 (1936).

[78] W. D. Rosenfeld, *Arch. Biochem.*, *11*, 145–154 (1946).

[79] D. Richter, *Biochem. J.*, *36*, 746–757 (1942).

[80] J. W. Jobling, A. A. Eggstein, and W. Petersen, *J. Exptl. Med.*, *22*, 706–712 (1915).

[81] P. V. Prewitt, *Am. J. Physiol.*, *65*, 287–294 (1923).

[82] C. L. von Hess, *J. Biol. Chem.*, *10*, 381–398 (1911).

[83] K. G. Falk and G. McGuire, *J. Biol. Chem.*, *108*, 61–71 (1935).

[84] H M. Noyes, K. G. Falk, and E. J. Baumann. *J. Gen. Physiol.*, *9*, 651–675 (1925–1926).

cerned, the relative potency is highest for the rat, followed in order by rabbit, beef, and human tissues.[35] Esterases have been found in high concentration in all tissues of the carp; here again, the largest amount was found in the liver.[85] Gomori,[86] using a histochemical method, reported that adipose tissue of the rat and rabbit possessed lipolytic activity. Renold and Marble[87] later demonstrated that lipolysis obtained not only in the subcutaneous adipose tissue of rats, but also in that of man. The rate of lipolysis in intra-articular fat was only one-third that of the subcutaneous fat in the case of man. The levels of lipase were higher in females than in males; in diabetic males, the concentration of enzyme was only one-fourth that found in diabetic females. No correlation between sex, age, or food intake and lipolytic activity of adipose tissue was observed in rats.

(*a*) *The Presence of Several Esterases in Liver and Pancreas.* Falconer and Taylor[88] were able to demonstrate that pig liver contained two different esterases. Hofstee[89] reported two esterases in pancreatic tissue, designated Esterase I and Esterase II, both of which are distinct from steapsin. Both esterases were shown to be best adapted to esters with a carbon chain not shorter than 4 and not longer than 8 atoms. In the case of Esterase I, the "affinity" constants from the C_5 to C_8 compounds are about the same, and the activity drops sharply beyond these limits. The affinity constant of Esterase II doubles with each additional carbon between C_5 and C_8.

(*b*) *Serum Esterase.* The presence of lipase has been demonstrated in blood serum. It has been shown that esterase resembling liver esterase also occurs in this fluid. Hanriot[90] was the first to discover "lipase" in serum which hydrolyzed monobutyrin. Aldridge[91] indicates that the serum esterases of many species may be separated into two distinct types, which he designates as *A*-Esterase and *B*-Esterase. Whereas the *A*-type is not inhibited by E600 (diethyl-*p*-nitrophenyl phosphate) and hydrolyzes *p*-nitrophenylacetate more rapidly than *p*-nitrophenylbutyrate, the *B*-enzyme is inhibited by a concentration of 10^{-7} to 10^{-8} M of E600; moreover, the speed of hydrolysis of the *p*-nitrophenyl esters is reversed.

Serum esterase acts principally as a tributyrinase. The tributyrinase activity of intestinal lymph has been shown by Flock and Bollman[92] to be

[85] J. C. Kernot and H. W. Hills, *Z. physiol. Chem.*, *208*, 33–39, 39–42 (1932).
[86] G. Gomori, *Arch. Pathol.*, *41*, 121–129 (1946).
[87] A. E. Renold and A. Marble, *J. Biol. Chem.*, *185*, 367–375 (1950).
[88] J. S. Falconer and D. B. Taylor, *Biochem. J.*, *40*, 831–834 (1946).
[89] B. H. J. Hofstee, *J. Biol. Chem.*, *199*, 365–371 (1952).
[90] Hanriot, *Compt. rend. soc. biol.*, *48*, 925–926 (1896).
[91] W. N. Aldridge, *Biochem. J.*, *53*, 110–117 (1953).
[92] E. V. Flock and J. L. Bollman, *J. Biol. Chem.*, *185*, 903–908 (1950).

less than that of plasma. However, Brauer and Hardenbergh[93] found that liver lymph contains about 90% of the esterase activity of plasma and an identical proportion of esterase to protein, irrespective of whether the plasma esterase has been elevated by treatment of the animals with carbon tetrachloride, or reduced by the injection of concentrated albumin solutions. These results indicate that the lymph esterase originates from the plasma esterase. This hypothesis is supported further by the finding that the level of plasma tributyrinase is markedly reduced when the lymph is drained externally.[92]

The lymph esterases vary somewhat with the site from which they are obtained. Thus, based upon protein content, cervical lymph has only 49% of the activity of plasma. Thoracic and mesenteric lymph esterases show a composition intermediate between that of cervical and that of liver esterase. Finally, liver lymph has been shown to possess 90% of the activity of plasma.[93] The esterase from plasma thus passes into the lymph less readily than do other globulin components of the plasma.

c. Properties of Esterases. The optimum *p*H of liver esterases varies between 6.7 and 8.2, depending upon the buffer, the method of preparation, and the source of the enzyme.[94] Esterases of different origin present variations in activity according to the substrate used. In earlier studies, ethyl acetate and ethyl butyrate were usually employed,[95,96] and such differences in behavior were not noted; when more complex esters were used, many variations in activity could be demonstrated. Thus, while kidney esterase acted 50 to 100% more rapidly on ethyl than on methyl esters, lung esterase hydrolyzed both types equally well.[83] In spite of the fact that liver esterase hydrolyzed ethyl and methyl benzoate at an identical rate, the more rapid splitting of ethyl acetate as compared with methyl acetate was noted.

Esterases also yield markedly different results when diesters of the dicarboxylic acids serve as substrates. When diethyl malonate (C_3) and diethyl succinate (C_4) were treated with hog liver esterase, hydrolysis proceeded rapidly, and equilibrium was reached when one ester group per molecule had been removed.[97,98] On the other hand, when the substrate was diethyl glutarate (C_5) or diethyl adipate (C_6), both ester groups were removed by the time that equilibrium was reached.

[93] R. W. Brauer and E. Hardenbergh, *Am. J. Physiol.*, *150*, 746–753 (1947).
[94] H. Sobotka and D. Glick, *J. Biol. Chem.*, *105*, 221–230 (1934).
[95] J. H. Kastle and A. S. Loevenhart, *Am. Chem. J.*, *24*, 491–525 (1900).
[96] J. H. Kastle, M. E. Johnston, and E. Elvove, *Am. Chem. J.*, *31*, 521–550 (1904).
[97] E. C. Hyde and H. B. Lewis, *J. Biol. Chem.*, *56*, 7–15 (1923).
[98] D. A. McGinty and H. B. Lewis, *J. Biol. Chem.*, *67*, 567–577 (1926).

The action of liver esterase in hydrolyzing triacetin is stimulated to the extent of 50% by the addition of amino acids such as L-aspartic, L-glutamic, or L-histidine, while the hydrolysis of butylbutyrate is not augmented by these amino acids.[99] Moreover, Yamamoto[99] showed that the free amino group of L-aspartic acid is the significant group in accelerating the hydrolysis of triacetin. The liver esterases (ali-esterases) are highly sensitive to inhibition by various alkyl phosphate derivatives such as E600. They can be selectively inhibited by tri-*o*-cresyl phosphate.[100] In the latter case, Mendel and Myers[101] reported that the order of sensitivity of esterases is as follows: serum tributyrinase > pseudocholinesterase > true cholinesterase > brain tributyrinase.

Myers and Mendel[100] have reported that the ali-esterases of liver and phospholipase are inhibited to the extent of 60 to 70% by 10^{-2} *M* atoxyl, while the lipase activity is not significantly affected either by the phosphate derivatives or by atoxyl in concentrations which inhibit ali-esterase activity. The atoxyl-resistant part of serum lipase varies in different animals, having a maximum of approximately 80%. Highest values were found for sera of the rat, horse, and guinea pig and the lowest in that of man.[102] Irrgang and Dustmann[102] suggest that a rise in atoxyl-resistant lipase in human serum is an indication of resistance toward carcinoma. Resistance to quinine, which is a characteristic of esterases rather than of lipases, was also found to be as high as 82% for lipase in some species. The extent of atoxyl-resistant and of quinine-resistant lipase is characteristic of species.[102]

Nachlas and Seligman[103] reported that kidney and liver esterases are inhibited by eserine, sodium arsenilate, and fluoride. Of these, only eserine inhibits lipase. Taurocholate was also shown to decrease esterase activity slightly, while it augments that of lipase. Liver esterase is more resistant to alkali treatment at *p*H 9.2 to 11.0 than lipase, which is destroyed under these conditions.[99] Connors and associates[104] reported that a highly concentrated liver esterase preparation had a *p*H optimum of 8.0 in borate with methyl butyrate as a substrate; the temperature coefficient was found to be 1.28, the energy of activation 4430 calories per mole and the Michaelis-Menten constant 0.022 *M*.

d. Factors Altering the Action of Esterases. Esterases are most effective in splitting simple esters of short-chain acids.[103] The length of the

[99] T. Yamamoto, *J. Biochem.* (*Japan*), *38*, 335–341 (1950).
[100] D. K. Myers, and B. Mendel, *Biochem. J.*, *53*, 16–25 (1953).
[101] B. Mendel and D. K. Myers, *Biochem. J.*, *53*, xvi (1953).
[102] K. Irrgang and I. Dustmann, *Biochem. Z.*, *322*, 520–525 (1952).
[103] M. M. Nachlas and A. M. Seligman, *J. Biol. Chem.*, *181*, 343–355 (1949).
[104] W. M. Connors, A. Pihl, A. L. Dounce, and E. Stotz, *J. Biol Chem.*, *184*, 29–36 (1950).

acid chain is of more importance in affecting the activity of esterase than is the type of alcohol to which it is attached.[105] Alper and collaborators[106] reported that the action of serum esterase is determined by chain length and by type of glyceride as follows:

Triacetin > Diacetin > Monoacetin
Tripropionin > Monopropionin
Tributyrin > Monobutyrin
Monoolein > Triolein
Tripropionin > Tributyrin > Triacetin

Alper *et al.*[106] state that serum esterase resembles liver esterases more than it does pancreatic lipase.

The optical activity of the substrate esters, likewise, is a factor by which the specificity of esterases can be demonstrated. Beef liver esterase and that from carp liver show a preferential splitting of L-tartaric acid esters over those of the D-form.[85] On the other hand, esterase from pig liver hydrolyzes preferentially the esters of *d*-mandelic acid in a mixture of D- and L-esters; pancreatic esterase attacks mainly the esters of L-mandelic acid.[107]

Serum lipase was shown to decrease in rats on the first to third day after the administration of alloxan.[108] It is not affected by thyroidectomy in the rabbit.[109] A decreased level of tributyrinase, as compared with the normal, was reported in hypertensive and arteriosclerotic human males, but the level of this enzyme was not altered in females suffering from these conditions.[110] In the nutritional disease of children known as "kwashiorkor," subnormal plasma esterase and plasma lipase values were reported by Srinivasan and Patwardhan.[111] Komarov *et al.*[112] were unable to note any correlation between tributyrinase and lipolytic activity in different specimens of pancreatic secretion and of blood sera.

e. Preparation of Esterases. Liver esterase has been prepared by a variety of procedures. The original preparation of Dakin[113] was a crude press juice of pig liver ground with kieselguhr. Peirce[114] carried out a

[105] A. K. Balls, M. B. Matlack, and I. W. Tucker, *J. Biol. Chem.*, *122*, 125–137 (1937–1938).
[106] C. Alper, P. P. Polakoff, Jr., and E. Alexander, *Federation Proc.*, *12*, 167–168 (1953).
[107] R. Willstätter and F. Memmen, *Z. physiol. Chem.*, *138*, 216–253 (1924).
[108] J. Tuba and R. Hoare, *Science*, *110*, 168 (1949).
[109] G. Weber and K. Drechsler, *Am. J. Physiol.*, *162*, 289–292 (1950).
[110] A. Bernhard and A. Rothenberg, *Proc. Soc. Exptl. Biol. Med.*, *78*, 533–535 (1951).
[111] P. R. Srinivasan and V. N. Patwardhan, *Lancet*, *263*, 864–866 (1952).
[112] S. Komarov, H. Shay, and C. Zislin, *Federation Proc.*, *12*, 80 (1953).
[113] H. D. Dakin, *J. Physiol.*, *32*, 199–206 (1904).
[114] G. Peirce, *J. Biol. Chem.*, *16*, 1–3 (1913).

purification by dialysis followed by precipitation with ammonium sulfate while, in the method of Kraut and Rubenbauer,[115] a protein-free preparation was obtained by employing adsorption following the dialysis step. The preparation of Baker and King[116] was likewise a highly purified product. The liver has, in general, been the source of preparations of relatively pure esterase, since the enzyme is abundant there; when attempts were made to obtain purified products from other tissues, the same general procedures were employed as were used for liver. Falconer and Taylor[88] obtained a solution of pig liver esterase 10 to 15% purer than whole liver. Connors *et al.*[104] prepared an acetone-dried powder from horse liver in which the esterase was concentrated by a factor of 270. In the latter case, a combination of ammonium sulfate and acetone fractionations was employed, together with heat and heavy metal denaturations and dialysis.

f. Esterases *vs.* Lipases. All the evidence points to the fact that the mechanism involved in splitting the ester linkages in triglycerides is different from that of the esters of monohydric alcohols. Lipases which will catalyze the breakdown of triglycerides are impotent in bringing about hydrolysis of simple esters. On the other hand, esterases which accelerate the hydrolysis of simple esters are ineffective on the ester linkages in triglycerides.

The marked differences between pancreatic lipase and liver esterases are shown in a variety of ways. Thus, there is a marked contrast in the relative velocity constants of their action on triglycerides and simple esters.[117] As has already been noted, bile salts and other organic compounds which activate lipases actually depress the esterases.[26] Finally, the kinetics of the enzyme-substrate reactions differ for these two classes of enzymes.[117] While the pancreas produces a true lipase, it also elaborates at least two different esterases. On the other hand, no lipase is produced in the liver, although this organ does synthesize two esterases. Although the lipolytic action of plasma undoubtedly originates in part from the pancreatic lipase, a portion of it has the property of an esterase.

(3) *Other Lipid-Hydrolyzing Enzymes*

In addition to the enzymes included in the general classification of esterases, a number of specific enzymes, which act on the ester linkage in a limited variety of compounds, are of importance in various phases of lipid

[115] H. Kraut and H. Rubenbauer, *Z. physiol. Chem.*, *173*, 103–117 (1928).
[116] Z. Baker and C. G. King, *J. Am. Chem. Soc.*, *57*, 358–361 (1935).
[117] S. S. Weinstein and A. M. Wynne, *J. Biol. Chem.*, *112*, 641–648 (1936).

metabolism. In the present section, specific esterases concerned with the lipids will be discussed, irrespective of whether their primary action is in the gastrointestinal tract or in other parts of the animal, or whether they may be of vegetable or of animal origin.

a. Cholesterol Esterase. Although it has long been recognized that enzyme systems exist in the animal body for the hydrolysis of cholesterol esters, and also for the synthesis of cholesterol esters from free cholesterol and a fatty acid molecule, the sites of formation of such enzymes are not well known. Moreover, we have no information as to whether or not the hydrolytic and synthetic mechanisms are mediated by the same or by different enzymes. The work of Nieft and Deuel[118] seems to indicate that different enzyme systems are involved in the hydrolytic and synthetic reactions.

(*a*) *Hydrolytic Action of Cholesterol Esterases.* About 75% of the cholesterol in the blood is in the form of the ester. In 1911, Abderhalden[119] demonstrated that blood cholesterol is increased when cholesterol is fed, either in the form of the free alcohol or as the ester. This suggests that both hydrolyzing and esterifying enzymes are present in the gut. Thannhauser *et al.*[120] were the first to report the presence of cholesterol esterase in pancreatic and intestinal juices. Cholesterol esterase has been found in blood corpuscles.[121]

Cholesterol esterase was demonstrated in the liver of the horse and ox by Kondo[121] as early as 1910. Schultz[122] performed the first valid experiments which proved that cholesterol esters can be split by liver extracts. Although Mueller[123] failed to confirm these results, Klein[124] adduced strong evidence of the existence of a cholesterol-ester-hydrolyzing enzyme in the liver. The latter conclusion was borne out by the data of Nieft and Deuel.[118] Sperry and Brand[125] later demonstrated that cholesterol esterase could exert both a synthetic and a hydrolytic action in liver preparations of the enzyme, depending upon the conditions.

The presence or absence of a hydrolyzing cholesterol esterase in the blood is still a controversial subject. Although Kondo[121] did report this active

[118] M. L. Nieft and H. J. Deuel, Jr., *J. Biol. Chem.*, *177*, 143–150 (1949).
[119] E. Abderhalden, *Biochemisches Handlexikon*, Vol. III, Springer, Berlin, 1911, p. 178.
[120] S. J. Thannhauser, *Deut. Arch. klin. Med.*, *141*, 290–292 (1923); and W. Fleischmann, *ibid.*, 292–296; and Ecarius, *ibid.*, 297–300; and P. V. Miller, H. Schaber, and C. Moncorps, *ibid.*, 300–311.
[121] K. Kondo, *Biochem. Z.*, *26*, 243–251 (1910); *27*, 427–435 (1910).
[122] J. H. Schultz, *Biochem. Z.*, *42*, 255–261 (1912).
[123] J. H. Mueller, *J. Biol. Chem.*, *25*, 561–565 (1916).
[124] W. Klein, *Z. physiol. Chem.*, *254*, 1–17 (1938).
[125] W. M. Sperry and F. C. Brand, *J. Biol. Chem.*, *137*, 377–387 (1941).

enzyme in the blood, Nomura[126] denied that cholesterol esters are hydrolyzed in the blood; however, the latter worker did demonstrate that such a change could be mediated by a number of tissues other than blood. Shope[127] reported that cholesterol is hydrolyzed in the blood, but Sperry[128] questions the results, since adequate control tests for a concomitant synthetic action were not made. However, Sperry and Brand[125] later demonstrated that only the hydrolytic activity on the part of cholesterol esterase could occur in the blood; the particular effect which was noted was found to depend upon the experimental conditions.

In view of the demonstration of cholesterol esterase in pancreatic juice,[120] it is only natural that one would expect to find it in the pancreas itself. Yamamoto *et al.*[129] demonstrated this enzyme in commercial pancreatin, while Fodor[130] reported the presence of both the hydrolytic and the synthetic cholesterol esterases in hog pancreas homogenates. Optimum *p*H values for the hydrolytic and synthetic activities were 6.3 and 5.2, respectively. Both types of cholesterol esterases have likewise been demonstrated by Swell, Byron, and Treadwell[131] in rat intestinal mucosa. Optimum activities of the hydrolytic and synthetic intestinal cholesterol esterases were given for *p*H 6.5 and 6.2, respectively. Since the amount of these enzymes in the mucosa is markedly reduced in depancreatized rats, it is suggested[131] that the pancreas is the major or sole source of the cholesterol esterase in the mucosa.

The presence of a cholesterol ester-hydrolyzing enzyme has been reported in a number of tissues of the guinea pig, by Shope[127]; in addition to the liver, kidney and muscle were reported to contain the highest amounts. Klein[124] found that 70 to 85% of cholesterol esters were hydrolyzed in 15 hours at a *p*H of 5.3 by preparations of the liver, spleen, kidney, adrenal, and intestinal mucosa. At this *p*H, the synthetic phase of the reaction would be almost completely inhibited.

Nieft[132] has shown that cholesterol esterase (which causes hydrolysis of cholesterol esters), prepared from intestinal mucosa of the rat, consists of two factors. The first of these is a globulin type protein which is heat-labile and is precipitated by 50% saturation with ammonium sulfate.

[126] T. Nomura, *Tôhoku J. Exptl. Med.*, *4*, 677–684 (1924).
[127] R. E. Shope, J. Biol. Chem., *80*, 127–132 (1928).
[128] W. M. Sperry, *J. Biol. Chem.*, *111*, 467–478 (1935); *113*, 599–606 (1936).
[129] R. S. Yamamoto, N. P. Goldstein, and C. R. Treadwell, *J. Biol. Chem.*, *180*, 615–621 (1949).
[130] P. J. Fodor, *Arch. Biochem.*, *26*, 331–336 (1950).
[131] L. Swell, J. E. Byron, and C. R. Treadwell, *J. Biol. Chem.*, *186*, 543–548 (1950).
[132] M. L. Nieft, *J. Biol. Chem.*, *177*, 151–156 (1949).

The other component, the so-called "co-factor," is a heat-stable, non-dialyzable substance soluble in saturated ammonium sulfate solution. The optimum *p*H was found to be 6.7 to 7.1. Yamamoto *et al.*[129] reported the optimum *p*H of the cholesterol ester-hydrolyzing enzyme in pancreatin as 6.6. It is destroyed by a temperature of 60°C., alkali treatment to a *p*H of 10, or by digestion with purified trypsin.[130]

(*b*) *Synthetic Action of Cholesterol Esterases.* It has been recognized for a long time that the animal must possess a mechanism for bringing about esterification of cholesterol. Not only will cholesterol absorbed as the free alcohol be later found as the ester, but there is every reason to believe that the cholesterol synthesized *de novo* in the tissues can undergo esterification. The nature and site of action of the enzyme system responsible for such a reaction have only recently been investigated.

Sperry[128] was the first to demonstrate in a clear-cut fashion that blood serum contains a cholesterol-esterifying enzyme. He stated that the enzyme has an optimum *p*H of 8.0; its activity is destroyed when serum is heated to 55 to 60°C. Bile salts were shown to decrease the synthetic action of cholesterol esterase and, when present in sufficient amount, to inhibit it completely.[55] Swell and Treadwell[133] demonstrated that free cholesterol of human and dog sera was esterified during incubation and that the reaction was inhibited by bile salts. They suggest that the synthetic reaction is catalyzed by an enzyme of very low activity, or is non-enzymatic. The pancreas is believed to be the sole source of the serum cholesterol esterase in the case of the dog.[134]

Another possible site of the synthetic-acting cholesterol esterase is the intestinal wall. Mueller[135] demonstrated that the lymph of cholesterol-fed dogs contained cholesterol and cholesterol ester in a constant ratio, irrespective of whether the sterol was fed as an ester or as the free alcohol. Frölicher and Süllmann[136] observed a marked absolute increase in the cholesterol esters in rabbit chyle after feeding free cholesterol in triolein, although the proportions of free and of esterified cholesterol showed wide variations. The presence of a cholesterol-esterifying system in the intestinal wall, as well as in the liver, has been demonstrated by Nieft and Deuel.[118] This system requires the concomitant presence of phosphate and of a source of fatty acid. The enzyme exhibits maximum activity at a *p*H of 6.5.

The pancreas also mediates the esterification of cholesterol. Schramm

[133] L. Swell and C. R. Treadwell, *J. Biol. Chem.*, *185*, 349–355 (1950).
[134] L. Swell and N. C. Kramer, *Proc. Soc. Exptl. Biol. Med.*, *82*, 197–198 (1953).
[135] J. H. Mueller, *J. Biol. Chem.*, *27*, 463–480 (1916).
[136] E. Frölicher and H. Süllmann, *Biochem. Z.*, *274*, 21–33 (1934).

and Wolff[137] reported that a pancreatic esterase was able to esterify not only cholesterol but also dehydroandrosterone and dihydrocholesterol with equal rapidity. Three plant sterols, namely sitosterol, stigmasterol, and ergosterol, were also esterified by this enzyme, but at a slower rate than that of the animal sterols. Swell and Treadwell,[138] using as a substrate cholesterol and oleic acid, demonstrated that the esterifying enzyme in pancreas required bile and a source of fatty acids for activity. It was equally active in the presence of phosphate or of citrate buffer. Optimum pH was 6.2; the enzyme was inactivated by heating for 15 minutes at 65°C. Swell[139] reported that the action of pancreatic cholesterol esterase was augmented by the bile salts in the following order: taurocholate > cholate > glycocholate > desoxycholate > lithocholate. The first three bile salts were the most active, which is interpreted to mean that the —OH groups are the active ones in bile salts. Since cholate with glycine or taurine was no more active than the cholate by itself, it is suggested that sodium taurocholate and sodium glycocholate do not undergo splitting but rather function as a unit. Maximum activity was noted when one bile salt molecule was present with one cholesterol and one oleic acid molecule.

The esterifying and hydrolyzing systems occur side by side in the blood, in the liver, in the pancreas, and in the intestinal wall; however, it is not believed that these two systems are aspects of a reversible process.

b. Lecithinases. Although most lipases presumably are able to act on lecithins as well as on neutral fat, Thiele[140] was the first to report the presence of an enzyme in blood capable of hydrolyzing lecithin but which did not act on neutral fat. After examination of the enzymes in many tissues, Porter[141] concluded that lecithinases and lipases act independently. Contardi and Ercoli,[142] in 1933, postulated that four types of lecithinases exist, depending upon the particular bonds which are attacked.

(*a*) *Lecithinase A.* This enzyme liberates one fatty acid from lecithin with the formation of a lysolecithin.[142] Lecithinase A has been reported in cobra venom[143–145]; it splits a single unsaturated acid from the lecithin molecule. According to Hanahan and collaborators,[146] lecithinase A from

[137] G. Schramm and W. Wolff, *Z. physiol. Chem.*, *263*, 73–77 (1940).
[138] L. Swell and C. R. Treadwell, *J. Biol. Chem.*, *182*, 479–487 (1950).
[139] L. Swell, *Federation Proc.*, *12*, 278 (1953).
[140] F. H. Thiele, *Biochem. J.*, *7*, 275–286, 287–296 (1913).
[141] A. E. Porter, *Biochem. J.*, *10*, 523–533 (1916).
[142] A. Contardi and A. Ercoli, *Biochem. Z.*, *261*, 275–302 (1933).
[143] P. Kyes, *Berl. klin. Wochschr.*, *40*, 956–959, 982–984 (1903).
[144] C. Delezenne and S. Ledebt, *Compt. rend.*, *155*, 1101–1103 (1912).
[145] C. Delezenne and E. Fourneau, *Bull. soc. chim. France*, *15*, 421–434 (1914)
[146] D. J. Hanahan, L. D. Turner, and M. A. Rodbell, *Federation Proc.*, *12*, 214 (1953).

snake venom and from commercial pancreatin splits purified dipalmitoleyl-L-α-lecithin to a lysolecithin (monopalmitoleyl) and a single molecule of palmitoleic acid. Lecithinase A, called "Phospholipase" by Fairbairn,[147] also converts cephalins to lysocephalins.

A crystalline lecithinase A, prepared from the venom of the rattlesnake (*Crotalus terrificus*), and a preparation from pancreas, were shown to inactivate succinoxidase in rat liver homogenates and in rat liver mitochondria.[148] The inactivation is not due to an inhibiting action or to the lytic effect of the lysolecithin. Cytochrome oxidase and succinic dehydrogenase are not appreciably affected by lecithinase A under conditions which completely inactivate succinoxidase. It is suggested that lecithin may be part of a component linking succinic dehydrogenase and cytochrome *c*.

(*b*) *Lecithinase B*. This enzyme hydrolyzes both fatty acid residues from lecithin,[142] with the formation of glycerylphosphorylcholine. Lecithinase B has been demonstrated to be a component of rice hulls,[142] of the molds, *Aspergillus oryzae* (rice mold)[142] and *Penicillum notatum* (clay mold). Fairbairn[149] refers to this enzyme as "lysophospholipase." Lecithinase B also acts on phosphatidylethanolamine to produce glycerylphosphorylethanolamine. Lecithinase B is highly specific; it is inactivated by heat at a slightly alkaline reaction, by HCN, and less readily by ions of heavy metals. The optimum *p*H is at 4.0.

(*c*) *Lecithinase C*. This enzyme splits off choline from lecithin,[142] by rupturing the ester linkage between choline and phosphoric acid. Phosphatidic acid is the compound formed. Hanahan and Chaikoff[150] prepared this enzyme from the carrot; they proved that it was specific for the ester linkage between the nitrogenous base and the phosphoric acid. Maximum activity of this enzyme was in the *p*H range from 5.2 to 5.9 in a 0.05 *M* phosphate buffer. It exhibited a high degree of thermostability and was not completely inactivated when exposed to a temperature of 95°C. for 15 minutes. The existence of lecithinase C in carrots has been confirmed by Acker and his co-workers.[151] The activity of this enzyme depends to a considerable degree on the ripeness of the carrots. A similar enzyme is present in germinated wheat, but not in pressed spinach juice.[151] Acker *et al.*[151] reported that the cleavage of choline proceeded as a first-order reaction. The *p*H optimum was found to be 5.8 and the optimum tempera-

[147] D. Fairbairn, *J. Biol. Chem.*, *157*, 633–644 (1945).
[148] A. P. Nygaard and J. B. Sumner, *J. Biol. Chem.*, *200*, 723–729 (1953).
[149] D. Fairbairn, *J. Biol. Chem.*, *173*, 705–714 (1948).
[150] D. J. Hanahan and I. L. Chaikoff, *J. Biol. Chem.*, *169*, 699–705 (1947).
[151] L. Acker, W. Diemair, and R. Jäger, *Biochem. Z.*, *322*, 471–485 (1952).

ture is 25 to 35°C. The enzyme is described as a specific phosphodiesterase; it is not identical with glycerophosphatase.

(*d*) *Lecithinase D.* This enzyme is a glycerophosphatase which acts on the ester linkage between phosphoric acid and glycerol. The end-products are phosphorylcholine and a diglyceride. The enzyme was discovered by Macfarlane and Knight in 1941 in *Clostridium welchii*[152] (gas bacillus). A similar enzyme has been reported in *Clostridium edematis* (*oedematiens*)[153] (isolated from war wounds), and in *Cl. hemolyticum* toxin (hemolytic anaerobe found in the blood and tissues of cattle),[154] by Macfarlane, and in *Cl. bifermentans*[155] by Lewis and Macfarlane.

Hemolysis of the red cells of horse and sheep blood by toxins of *Cl. welchii* and *Cl. oedematiens* was always preceded, according to Macfarlane,[156] by decomposition of some of the phospholipids in the cells. Hemolysis, therefore, is to be attributed to the action of the lecithinases present in the toxin. The rates of hydrolysis of the phospholipids in intact erythrocytes by lecithinases from different sources vary. Three immunologically distinct lecithinases D are the *Cl. welchii* α-toxin, the *Cl. oedematiens* γ-toxin, and the *Cl. oedematiens* β-toxin.[156] Zamecnik *et al.*[157] found that the injection of the purified total lipids obtained from erythrocytes, plasma, and liver had a protective effect against *Cl. welchii* toxin in mice and dogs. It is suggested that the lecithin in the injected lipids protects the lecithin present in the animal from destruction by the lecithinase D.[157]

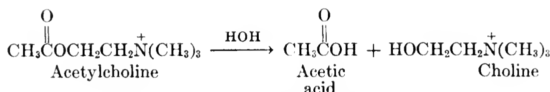
Although the lecithinase D preparations do not decompose cephalin, Macfarlane[158] showed that sphingomyelin was slowly destroyed by this agent. Hydrolysis of the latter proceeds more slowly than that of lecithin; like the hydrolysis of lecithin, that of sphingomyelin is activated by Ca and inhibited by NaF. The end-products of sphingomyelin hydrolysis were found to be phosphorylcholine together with a P-free product approaching the composition of lignocerylsphingosine.

Considerable work has been done on the undifferentiated lecithinases, in which the production of soluble phosphate is employed as an index of their activity.[159,160] King[159] reported that these enzymes were widely distri-

[152] M. G. Macfarlane and B. C. J. G. Knight, *Biochem. J.*, *35*, 884–902 (1941).
[153] M. G. Macfarlane, *Biochem. J.*, *42*, 590–595 (1945).
[154] M. G. Macfarlane, *Biochem. J.*, *47*, 267–270 (1950).
[155] G. M. Lewis and M. G. Macfarlane, *Biochem. J.*, *54*, 138–142 (1953).
[156] M. G. Macfarlane, *Biochem. J.*, *47*, 270–278 (1950).
[157] P. C. Zamecnik, J. Folch, and L. Brewster, *Proc. Soc. Exptl. Biol. Med.*, *60*, 33–39 (1945).
[158] M. G. Macfarlane, *Biochem. J.*, *42*, 587–590 (1948).
[159] E. J. King, *Biochem. J.*, *25*, 799–811 (1931).
[160] E. J. King, *Biochem. J.*, *28*, 476–481 (1934).

buted in nature. In the animal, the highest concentration was found in the kidney, with a decreasing activity in the following order: small intestine, spleen, liver, testes, pancreas, large intestine, brain, ovary, bone, suprarenals, lung, blood vessels, cardiac muscle, and skeletal muscle.[159] The optimum pH was found to be 7.5, and the optimum temperature[159] about 37°C. It was reported that artificial hydrolecithins were broken down as readily as was the parent lecithin.[160] For a more complete discussion of lecithinases A, B, C, and D, the reader is referred to Volume I of this work.

c. Cholinesterases. (*a*) *Introduction.* Cholinesterases are enzymes, present exclusively in animal tissues, which are capable of catalyzing the breakdown of acetylcholine into acetic acid and choline:



$$\underset{\text{Acetylcholine}}{CH_3\overset{\overset{O}{\|}}{C}OCH_2CH_2\overset{+}{N}(CH_3)_3} \xrightarrow{HOH} \underset{\text{Acetic acid}}{CH_3\overset{\overset{O}{\|}}{C}OH} + \underset{\text{Choline}}{HOCH_2CH_2\overset{+}{N}(CH_3)_3}$$

The Hydrolysis of Acetylcholine with Cholinesterase

The subject of cholinesterase has been reviewed by Ammon,[161] Brown,[162] Werle,[163] Zeller[164] and most recently by Nachmansohn and Wilson[165] and for a second time by Ammon.[166] The role of cholinesterase and of acetylcholine in the transmission of nerve impulses across the neuromuscular junction was discussed at a conference (1951–1952) of the Muscular Dystrophy Association of America.[167]

(*b*) *Discovery of Acetylcholine and Cholinesterases.* Although acetylcholine was discovered as early as 1867 by Baeyer,[168] it was not known until 58 years later that this substance has a widespread distribution in animal tissues, and that it plays a most dramatic role in the functioning of nervous tissue. The discovery of cholinesterase followed the demonstration of acetylcholine. Abderhalden and Paffrath[169] announced the presence of this ester in the intestine of the horse and pig.

Loewi[170] in 1921, first reported that an active compound is set free from

[161] R. Ammon, *Ergeb. Enzymforsch.*, *4*, 102–110 (1935).

[162] G. L. Brown, *Physiol. Revs.*, *17*, 485–513 (1937).

[163] E. Werle, *Fermentforschung*, *17*, 230–257 (1943).

[164] E. A. Zeller, *Advances in Enzymology*, Vol. VIII, Interscience, New York and London, 1948, pp. 459–495.

[165] D. Nachmansohn and I. B. Wilson, in *Advances in Enzymology*, Vol. XII, Interscience, New York and London, 1951, pp. 259–339.

[166] R. Ammon, *Ergeb. Enzymforsch.*, *9*, 35–69 (1943); *Chem. Zentr.*, *1943*, *II*, 1719.

[167] D. Nachmansohn, *Proc. 1st & 2nd Med. Conf. Muscular Dystrophy Association of America*, New York, Apr. 14–15 1951, May 17–18, 1952, pp. 2–15.

[168] A. Baeyer, *Ann.*, *142*, 322–326 (1867).

[169] E. Abderhalden and H. Paffrath, *Fermentforschung*, *8*, 299–307 (1925).

[170] O. Loewi, *Arch. ges. Physiol.* (*Pflüger's*), *189*, 239–242 (1921).

heart muscle when the latter organ is stimulated by the vagus nerve. Plattner and Bauer[171] offered confirmation for the identity of this substance by proving that the "Vagusstoff" (as acetylcholine was called) and pure acetylcholine were destroyed in an analogous manner by mammalian and by frog bloods. Dale and Dudley,[172] in 1929, were the first to succeed in isolating acetylcholine from animal tissues, *i.e.*, the spleen of horse and ox. Bach[173] demonstrated the production of acetylcholine as the result of stimulation of the vasodilator fibers of the dorsal nerve roots of the rabbit.

It is now recognized that the presence of acetylcholine is related to the humoral mechanism responsible for the transmission of nerve impulses across synapses, as well as from the nerve endings to the muscles innervated by such fibers. According to the classical concept, acetylcholine is set free only at the nerve endings. However, Nachmansohn[174] suggested, in 1945, that acetylcholine is produced everywhere on the neuron surface, and that it is related to electrical changes during activity, and hence to nerve impulse. This concept is further supported by the demonstration that choline acetylase, an enzyme which catalyzes the synthesis of acetylcholine, is present in the nerve axon, *i.e.*, in that part of the neuron which does not contain nerve endings and cell bodies; hence acetylcholine may also be important for the transmission of the nerve impulse along the axon.[175] Although it is known that the so-called "cholinergic" nerves, which are largely composed of the parasympathetic group, employ acetylcholine to conduct the nerve impulses across the synapses or to muscle cells, it seems probable that all nerve cells have the capacity to synthesize acetylcholine to a greater or lesser extent.

Although Dale[176] suggested, in 1914, that an enzyme exists in animal tissue capable of destroying acetylcholine, Abderhalden and Paffrath[169] first demonstrated the presence of this ester 11 years later. Shortly thereafter, Loewi and Navratil[177] found that the physiological action of acetylcholine is inhibited by extracts of heart tissue; this inactivation of acetylcholine was later proved to result from the action of an enzyme.[178] In the course of their studies on the esterases in horse serum and in pig liver, Stedman and collaborators[179] confirmed the fact that acetylcholine is

[171] F. Plattner and R. Bauer, *Arch. ges. Physiol.* (*Pflüger's*), *220*, 180–182 (1928).
[172] H. H. Dale and H. W. Dudley, *J. Physiol.*, *68*, 97–131 (1929).
[173] L. M. N. Bach, *Am. J. Physiol.*, *144*, 478–482 (1946).
[174] D. Nachmansohn, *Vitamins and Hormones*, *3*, 337–377 (1945).
[175] D. Nachmansohn, H. M. John, and M. Berman, *J. Biol. Chem.*, *163*, 475–480 (1946).
[176] H. H. Dale, *J. Pharmacol. Exptl. Therap.*, *6*, 147–190 (1914).
[177] O. Loewi and E. Navratil, *Arch. ges. Physiol.* (*Pflüger's*), *214*, 678–688 (1926).
[178] E. Engelhart and O. Loewi, *Arch. Exptl. Pathol. Pharmakol.*, *150*, 1–13 (1930).
[179] E. Stedman, E. Stedman, and L. H. Easson, *Biochem. J.*, *26*, 2056–2066 (1932).

destroyed by serum, a fact which had been reported earlier by Plattner.[180] The rate of destruction of acetylcholine by blood obtained from different species of mammals was found to decrease in the following order: man, pig, cattle, dog, horse, rabbit, and cat.[181] On the basis of inhibition experiments, Stedman *et al.*[179] were led to the conclusion that the acetylcholine-destroying principle is an esterase; hence they gave this enzyme the name "choline esterase." In the more recent literature the term has been abbreviated to "cholinesterase," as used throughout this volume, or to "Ch E."

It is obvious that the cholinesterase mechanism is one which limits the duration of life of acetylcholine, and hence of the nerve impulse. Were it not for the presence of cholinesterase in the tissues, acetylcholine would be a dangerous poison. Agents which inhibit the activity of cholinesterases, therefore, are toxic, since they prevent the degradation of the acetylcholine which is normally being produced. Torda and Wolff[182] called attention to the fact that most of the convulsion-inducing agents cause a rapid accumulation, or sudden increase, in acetylcholine, either by augmenting the rate of synthesis of acetylcholine (pentamethylene tetrazol and picrotoxin) or by decreasing the extent of hydrolysis of acetylcholine (strychnine, morphine, etc.). When acetylcholine is simultaneously administered, the effect is further potentiated.

(c) *Types of Cholinesterases.* In their original report on the enzymes of horse serum, Stedman and co-workers[179] noted that not only acetylcholine but also ethyl acetate and tributyrin were hydrolyzed by these sera. They question "whether one enzyme is responsible for the hydrolysis of all three types of substrate or whether different enzymes are involved." However, Easson and Stedman[183] subsequently concluded, on the basis of studies on human sera, that cholinesterase exerts a specific action, even though they admitted the possibility that it may likewise act to a slight extent on other esters such as tributyrin. On the other hand, the cholinesterase obtained from the blood of guinea pigs differs from that present in human sera in that it is insensitive to the inhibiting action of eserine. This finding led Easson and Stedman[183] to the conclusion that a "second esterase" might exist which had the capacity to inactivate acetylcholine.

At least three cholinesterases are now recognized, which are as follows:

1. *"True" or "specific" cholinesterase* which hydrolyzes only choline

[180] F. Plattner, *Arch. ges. Physiol. (Pflüger's)*, *214*, 112–129 (1926).
[181] O. Galehr and F. Plattner, *Arch. ges. Physiol. (Pflüger's)*, *218*, 506–513 (1928).
[182] C. Torda and H. G. Wolff, *Am. J. Physiol.*, *151*, 345–354 (1947).
[183] L. H. Easson and E. Stedman, *Biochem. J.*, *31*, 1723–1729 (1937).

esters. Augustinsson and Nachmansohn[184] proposed that the term "acetylcholinesterase" be used to designate the specific acetylcholine-splitting enzyme. Zeller and Bissegger[185] have referred to the "true" type as the "*e*-type," because it is present in erythrocytes.

2. *"Pseudo"*[186] *or "non-specific" cholinesterase*, which hydrolyzes not only acetylcholine but compounds closely related to acetylcholine, as well as simple esters such as methyl butyrate, is the designation of a second cholinesterase. Alles and Hawes[187] consider this terminology inadvisable. This type of cholinesterase is referred to by Zeller and Bissegger[185] as "*s*-type," because it is present in serum. Moreover, these workers consider that the *s* and *e* nomenclature is preferable to "serum" and "erythrocyte," since these enzymes are found in tissues other than the aforementioned. On the other hand, the letter designations *s* and *e* remind one of the original source of the respective enzymes, *i.e.*, human blood.

3. *"Ophiocholinesterase," "colubercholinesterase"* (coluber = snake) L. or simply the "*c*" type.[164] This cholinesterase, obtained from cobra venom, apparently differs from the *e* and *s* types.

There is some question as to whether or not the number of types of cholinesterase should be limited to three. The variations in activity of the enzyme prepared from various tissues may be due to the protein moiety of the enzyme in question. Zeller[164] indicates that these proteins may well be specific not only with regard to species but also with relation to organs.

As an example of such variations in connection with the *s*-enzyme, Zeller[188] found that cholinesterases from human, horse, and guinea pig sera presented different degrees of inhibition in response to such inhibitors as sulfonamides, papaverine, and percaine. In another case, the action of the enzyme prepared from the flatworm, *Planaria dorotocephala*, was inhibited to a lesser degree by eserine and by other amines than was that from human red cells; however, in other respects the *Planaria* enzyme acted as a "true" cholinesterase.[189] Augustinsson[190] showed that the cholinesterase of the Roman land-snail (*Helix pomatia*) did not fit into either of the two aforementioned categories. Further proof of the fact that at least two enzymes exist which are capable of hydrolyzing choline esters is to be found in the

[184] K. B. Augustinsson and D. Nachmansohn, *J. Biol. Chem.*, *179*, 543–559 (1949).
[185] E. A. Zeller and A. Bissegger, *Helv. Chim. Acta*, *26*, 1619–1630 (1943).
[186] B. Mendel and H. Rudney, *Biochem. J.*, *37*, 59–63 (1943).
[187] G. A. Alles and R. C. Hawes, *Science*, *100*, 75 (1944).
[188] E. A. Zeller, *Helv. Physiol. Pharmacol. Acta*, *2*, C23–C24 (1944).
[189] R. D. Hawkins and B. Mendel, *J. Cellular Comp. Physiol.*, *27*, 69–85 (1946).
[190] K. B. Augustinsson, *Biochem. J.*, *40*, 343–349 (1946).

TABLE 2

COMPARISON OF THE GENERAL PROPERTIES OF CHOLINESTERASES DERIVED FROM THE ERYTHROCYTES AND SERUM OF HUMAN BLOOD AND FROM COBRA VENOM

Property	Erythrocyte-e-type "True"	Serum-s-type "Pseudo"	Coluber-c-type
Optimal pH[a]	7.5–8.0	8.0	
Activation by NaCl[a]	+	0	
Inhibition by high substrate concentrations	+[a,b]	0[a,b]	+[c]
Inhibition by high substrate concentrations + alcohol[d]	0	0	
Optimum concentration for inhibition[e]	0.25×10^{-6} M	$>0.25 \times 10^{-4}$ M	
Hydrolysis of:			
Acetyl-β-methylcholine	+[f]	0[f]	+[g]
Benzoylcholine	0[f]	+[f]	0[g,h]
Butyrylcholine	0[i]	+[i]	0[h]
Tributyrin	0[e,j]	+[e,g,j]	0[h]
Triacetin[k]	+	0	+
Ethylchloroacetate[l]	+	+	
Ethylchloroacetate + eserine[l]	0	+	
β-Ethylchloroacetate[l]	++	+	
β-Ethylchloroacetate + eserine[l]	0	+	
Ethoxyethanol acetate[m]	++	+	
Inhibition by:			
Eserine	+[l]	+[l]	+[l]
β,β-Dichlorodiethyl-N-methylamine[n]	+	0	
Nu-1250[o,p]	+	0	

[a] G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, *133*, 375–390 (1940); R. C. Hawes and G. A. Alles, *J. Lab. Clin. Med.*, *26*, 845–853 (1941).
[b] K. B. Augustinsson, *Acta Physiol. Scand.*, *15*, Suppl. 52, 1–182 (1948).
[c] E. A. Zeller and A. Maritz, *Helv. Physiol. et Pharmakol. Acta*, *3*, C 19-C 20 (1945).
[d] K. P. Fellowes, J. P. Rutland, and A. Todrick, *Biochem. J.*, *47*, xx (1950).
[e] E. A. Zeller and A. Bissegger, *Helv. Chim. Acta*, *26*, 1619–1630 (1943).
[f] B. Mendel, D. B. Mundell, and H. Rudney, *Biochem. J.*, *37*, 473–476 (1943).
[g] F. Bovet-Nitti, *Experientia*, *3*, 283–286 (1947).
[h] L. A. Mounter, *Biochem. J.*, *49*, xlv-xlvi (1951).
[i] F. Kalsbeek, J. A. Cohen, and B. R. Bovens, *Biochim. et Biophys. Acta*, *5*, 548–560 (1950).
[j] B. Mendel and H. Rudney, *Biochem. J.*, *37*, 59–63 (1943).
[k] P. Holton, *Biochem. J.*, *43*, xiii (1948).
[l] R. A. McNaughton and E. A. Zeller, *Proc. Soc. Exptl. Biol. Med.*, *70*, 165–167 (1949).
[m] E. A. Zeller, G. A. Fleischer, R. A. McNaughton, and J. S. Schweppe, *Proc. Soc. Exptl. Biol. Med.*, *71*, 526–529 (1949).
[n] D. H. Adams and R. H. S. Thompson, *Biochem. J.*, *42*, 170–175 (1948).
[o] Physostigmine analogue, N-p-chlorophenyl-N-methylcarbamate of m-hydroxyphenyltrimethylammonium bromide (Hoffmann-LaRoche, Nu-1250).
[p] R. D. Hawkins and B. Mendel, *Biochem. J.*, *44*, 260–264 (1949).

work of Alles and Hawes,[191,192] Richter and Croft,[193] Mendel and Rudney,[186] and of Zeller and associates.[185,194]

(*d*) *Properties of Cholinesterases.* a'. General Properties of Cholinesterases: Marked variations in properties obtain between the different types of cholinesterases. The differences between the *e*-type and the *s*-type are summarized in Table 2.

It is known that true cholinesterase is inhibited by an excess of substrate while the pseudo form is not affected. When an increasing concentration of salt is added to the medium, the optimum acetylcholine concentration is shifted to higher levels. Although the higher salt levels decrease the affinity of the enzyme for acetylcholine, at the same time they potentiate the hydrolytic activity of the enzyme toward acetylcholine. According to Myers[195] this behavior is explained on the basis of a dispositional enzyme active center, which may be either its "anionic" grouping, or its "ester" grouping, or both.[196] In the case of pseudocholinesterase, the active center is also dipositional; however, the affinity of acetylcholine for the pseudo-enzyme appears to depend mainly upon the ester structure of the molecule.[195] Elley and Stone[197] have suggested that, since acetylcholine is a "low-energy" compound, the main point of adsorption of pseudoesterase is on the ester group of the acetylcholine molecule. They base their conclusion on the demonstration of a similarity between the non-enzymic hydrolysis of acetylcholine bromide and of non-ionic esters such as methyl acetate.

Augustinsson,[198] likewise, pointed out that the effect of substrate concentration varies with the type of cholinesterase. Thus, the cholinesterases present in all conductive tissues tested, and in erythrocytes, show a rather sharp optimum concentration of acetylcholine. The optimum substrate concentrations are quite similar, for most choline esters, although, in some cases, they may differ.[198] On the other hand, the cholinesterases present in serum exhibit the usual dissociation curve. Triacetin, for example, is split at a slow rate in low concentration and at a high rate when present in high concentration. The velocity of the reaction with the pseudo-enzyme decreases with the increases in molecular weight of the

[191] G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, *133*, 375–390 (1940).
[192] R. C. Hawes and G. A. Alles, *J. Lab. Clin. Med.*, *26*, 845–853 (1941).
[193] D. Richter and P. G. Croft, *Biochem. J.*, *36*, 746–757 (1942).
[194] E. A. Zeller, *Helv. Chim. Acta*, *25*, 1099–1110 (1942).
[195] D. K. Myers, *Arch. Biochem.*, *37*, 469–487 (1952).
[196] D. K. Myers, *Arch. Biochem.*, *31*, 29–40 (1951).
[197] D. D. Elley and G. S. Stone, *Biochem. J.*, *49*, xxx (1951).
[198] K. B. Augustinsson, *Arch. Biochem.*, *23*, 111–126 (1949)

homologues.[199] Lévy and Tchoubar[199] showed that methyl-2-propionylcholine, $CH_3CH(CH_3)COOCH_2CH_2\overset{+}{N}(CH_3)_3(\overline{OH})$, is hydrolyzed by the *e* enzyme, while butyrylcholine, $CH_3CH_2CH_2COOCH_2CH_2\overset{+}{N}(CH_3)_3(\overline{OH})$, is not hydrolyzed. The latter substance is considered to be an inhibitor of the enzyme, as are also methyl-3-butyrylcholine, $CH_3CH(CH_3)CH_2$-$COOCH_2CH_2\overset{+}{N}(CH_3)_3(\overline{OH})$, and ethyl-2-butyrylcholine, $CH_3CH_2CH(C_2H_5)$ $COOCH_2CH_2\overset{+}{N}(CH_3)_3(\overline{OH})$.

e-Cholinesterases were shown by Zeller *et al.*[200] to be capable of catalyzing not only the hydrolysis of ethoxyethanol acetate ($C_2H_5OC_2H_4OCOCH_3$), but also that of desoxycorticosterone acetate ($C_{21}H_{29}O_3OCCH_3$), ketopropanol acetate ($CH_3COCH_2OCOCH_3$) and acetylsalicylic acid (HO-$C_6H_4COOCCH_3$).

Drying greatly increases the resistance of cholinesterase *e* to organic solvents[201]; the lipids extracted by light petroleum ether or by acetone were shown to be unessential for the functioning of the enzyme. The optimum *p*H for stability of serum cholinesterase was reported by Goldstein and Doherty[202] as 6.0, which differs from the *p*H of optimum activity, 8.5. It was found that thermal denaturation proceeded more rapidly at low ionic strength than at physiologic salt concentration. The loss of stability resulting from dilution could be prevented by the addition of albumin, as well as by the addition of the reversible inhibitors, prostigmine and methylene blue. It was also noted by these investigators[202] that the thermal denaturation of the enzyme does not proceed as a first-order reaction, but diminishes to a very great extent with time; this is not altered by albumin. It is suggested that the active enzyme is stabilized by a denatured enzyme, or by denatured impurities in the fractions employed. Stadie *et al.*[203] found that cholinesterase was not destroyed by the application of oxygen under pressure to its solution or of other oxidizing agents such as *o*-iodosobenzoate.

Cholinesterases from the yellow-banded krait (*Bungarus fasciatus*) were found to be heat-labile, since crude and purified preparations lost their potency[204] after heating to 60–70°C. The reaction of cobra cholin-

[199] J. Lévy and B. Tchoubar, *Compt. rend.*, *231*, 1262–1264 (1950).
[200] E. A. Zeller, G. A. Fleischer, R. A. McNaughton, and J. S. Schweppe, *Proc. Soc. Expt. Biol. Med.*, *71*, 526–529 (1949).
[201] K. Bullock, *Biochem. J.*, *49*, vii–viii (1951).
[202] A. Goldstein and M. E. Doherty, *Arch. Biochem.*, *33*, 22–34 (1951).
[203] W. C. Stadie, B. C. Riggs, and N. Haugaard, *J. Biol. Chem.*, *161*, 175–180 (1945).
[204] B. N. Ghosh, *Oesterr. Chem.-Ztg.*, *43*, 158–163 (1940).

esterase was inhibited by eserine and by diisopropyl fluorophosphate (usually designated as DFP),[205] as well as by caffeine and morphine.[206]

The *turnover number* (or turnover rate) of cholinesterase gives some indication as to the rapidity of its action. Since the enzyme was not available in pure form, it was impossible to express the turnover rate in terms of molecules; consequently, Berry[207] employed the active centers for the evaluation. In the case of partially purified *e*-cholinesterase (obtained from human red blood cells), the value was reported as 162,000 molecules of acetylcholine per minute. In a later report, Berry[208] observed that the turnover rate of cholinesterase varied, not only with species, but also with individuals of any one species.

b′. Specificity of Cholinesterases: The *e*-type of cholinesterase is generally considered to be extremely specific, in that it will not act on non-choline esters.[193] On the other hand, the *s*-type of cholinesterase acts not only on acetylcholine, but also on methyl butyrate and tributyrin. Richter and Croft[193] have described ali-esterases in the sera of several species, which act on methyl butyrate or tributyrin but not on acetylcholine. Adams and Whittaker[209] have ascribed the specificity of the erythrocyte (*e*) cholinesterase to at least two factors, *i.e.*, the acyl group and the alcohol group of the substrate. In contradistinction to the behavior of the *e*-cholinesterase, the plasma (*s*) cholinesterase accommodates preferentially the larger acyl groups such as propionyl and butyryl. Chain-branching causes variations in the action of the two types of cholinesterase.

Although it is generally agreed that the cholinesterases prepared from the sera of the several species examined are non-specific, it has been reported that preparations from erythrocytes of several species other than man were able to split non-choline esters. However, Mendel and Rudney[186] did find that *purified* erythrocyte cholinesterase preparations, regardless of the source of the cells, were unable to hydrolyze methyl butyrate or tributyrin. Mounter and Whittaker[210] reported that a *partially* purified preparation of horse erythrocyte cholinesterase had the capacity to hydrolyze a number of aliphatic esters; the rates of hydrolysis increased as the structure of the substrate approached that of acetylcholine. Thus, it was found that the carbon analogue of acetylcholine (3,3-dimethylbutylacetate,

[205] F. Bovet-Nitti, *Experientia*, *3*, 283–286 (1947).

[206] E. A. Zeller, unpublished data cited in E. A. Zeller, in *Advances in Enzymology*, Vol. VIII, Interscience, New York and London, 1948, pp. 459–495.

[207] W. K. Berry, *Biochem. J.*, *47*, xxi (1950).

[208] W. K. Berry, *Biochem. J.*, *49*, 615–620 (1951).

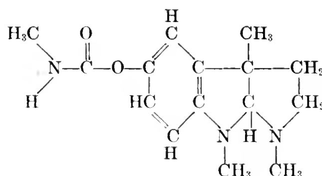
[209] D. H. Adams and V. P. Whittaker, *Biochem. J.*, *43*, xiv–xv (1948).

[210] L. A. Mounter and V. P. Whittaker, *Biochem. J.*, *47*, 525–530 (1950).

$CH_3COOCH_2CH_2C(CH_3)_2CH_3$) had the highest rate of hydrolysis of any simple aliphatic ester tested.

Corresponding specificity or non-specificity has been reported for the cholinesterases from tissues other than blood. Thus, brain cholinesterase has been shown to be of the "true" type,[186] while that prepared from dog pancreas resembled that of the serum (*i.e.*, the pseudo variety).[211]

c′. Inhibition of Cholinesterases: (a′) General Description of Inhibitors. —The most characteristic property of cholinesterase is the ability of a large number of compounds to inhibit its action. One of the best known inhibiting agents is eserine, or physostigmine. This is an alkaloid derived from the seeds of the *Physostigma venenosum*, or deadly Calabar bean. The empirical formula is $C_{15}H_{21}O_2N_3$, and it has the following structure shown below:



Eserine or Physostigmine

The specificity of the action of eserine in potentiating the effect of stimulation of cholinergic nerves was proved by Dale and Gaddum[212] in their studies of denervated voluntary muscles. These authors conclude that "the effects of eserine further limit our choice . . . (of a chemical transmitter) to a choline ester readily hydrolyzed by the tissues. Acetylcholine, the only choline ester which has been shown to exist in the animal body, is preeminent in physiological activity . . . and in its liability to the hydrolytic destruction which eserine specifically inhibits." It is now generally considered that the eserine effect on cholinesterase is so specific that a powerful induction of a nerve impulse by this drug is an evidence that acetylcholine plays a role in the transmission of this impulse. Eserine forms salts with most of the common acids.

In addition to eserine, and the several compounds listed in Table 2, many other substances are known to act as inhibiting agents for cholinesterases. These have been classified by Ammon[166] into the following 5 groups listed in Table 3.

[211] B. Mendel and D. B. Mundell, *Biochem. J.*, *37*, 64–66 (1943).
[212] H. H. Dale and S. H. Gaddum, *J. Physiol.*, *70*, 109–144 (1930).

TABLE 3
CLASSIFICATION OF THE INHIBITORS OF CHOLINESTERASES[a]

Group	Components of group
Quaternary and tertiary ammonium bases	Eserine (physostigmine), miotine, urethane, prostigmine, choline, arsenocholine, acetyl-β-methylcholine, ethoxycholine, butoxyformocholine, carbaminoylcholine (carbachol, U.S.P.); methylene blue, safranine, Nile blue; eseroline, eserine methyliodide, etc.; bufotenine; muscarine; curare; endoiodine[b]; esmodil (N-trimethyl-2-methoxy-2,3-propenylammonium bromide).
Amines and amides	Ergotamine, ergobasine; cocaine, novocaine, and other local anesthetics; benzyl alcohol, saligenin[c]; morphine, apomorphine, and other emetics; atropine; strychnine; veratrine; hordenine; strophanthin; pilocarpine; *p*-aminobenzoic acid, sulfanilamide; sympathol; racedrine (*dl*-ephedrine); pervitine; veritol (paredrine); hexeton,[b] cardiazol (metrazol), coramine (nikethamide); thiamine; nicotinic acid; urea, histamine,[d] thyroxine.[d]
Thiol reagents	Iodoacetic acid; maleic acid; alloxan; glutathione; copper.
Anions	Fluoride; oxalate; citrate; arsenite; pyrophosphate; atoxyl; potassium cyanide.
Miscellaneous	Bile acids; diphtheria and tetanus toxins; vitamin C; methyl alcohol, ethyl alcohol; chloral hydrate; ether; chloroform; caffeine; paraldehyde; phenol; sodium chloride.

[a] Data from R. Ammon, *Ergeb. Enzymforsch.*, *9*, 35–69 (1943).
[b] $(CH_3)_3N(I)CH_2CHOHCH_2N(I)(CH_3)_3$.
[c] From a chemical standpoint, these do not belong in this group; however, Ammon classifies them here on a pharmacological basis.
[d] It is questionable whether these should be included, since their action is principally on the central nervous system.

The inhibition of cholinesterases is brought about by several substances of physiological importance. Thus, it has been shown that vitamin B_1 (thiamine)[213,214] and nicotinic acid[166] have an inhibitory effect, but that such a high concentration is required to produce inhibition that these substances are of no importance from a physiological standpoint. On the other hand, Sadhu[215] has shown that thiamine can counteract the fatiguing action of acetylcholine. It is suggested that thiamine competes with choline for the acetyl radicle resulting in the formation of acetylthiamine and choline. Sobotka and Antopol[216] first called attention to the antagonistic effect of a

[213] D. Glick and W. Antopol, *J. Pharmacol. Exptl. Therap.*, *65*, 389–394 (1939).
[214] R. Ammon, unpublished work cited by R. Ammon, *Ergeb. Enzymforsch.*, *9*, 35–69 (1943).
[215] D. P. Sadhu, *Am. J. Physiol.*, *147*, 233–236 (1946).
[216] H. Sobotka and W. Antopol, *Enzymologia*, *4*, 189–191 (1937).

number of bile salts (cholic, desoxycholic, glycocholic, dehydrocholic, and dehydrodesoxycholic acids as their sodium salts) on the activity of cholinesterase. This inhibiting action may explain the fact that cholinesterase is absent from swine bile.[217] Ammon[166] likewise confirmed the partial blocking of the cholinesterase activity of human, horse, and snail sera by bile acids. The results obtained with human serum are supported by the demonstration of Antopol, Schifrin, and Tuchman[218] that the cholinesterase effect is decreased in jaundice.

At least one hormone has been shown to be important as an inhibitor. Benson[219] reported that adrenalin inhibited the ability of lyophilized preparations of *e*- and *s*-types of cholinesterase to bring about the hydrolysis of acetylcholine. It was later shown by Benson and Meek[220] that adrenalin in concentrations of 0.00015 to 0.006 *M* inhibits the ability of cholinesterase to hydrolyze choline esters in a concentration of 0.003 *M*. Both the *e*- and *s*-type are inhibited to the same extent. When methylcholine is used as the substrate for the specific esterase, the degree of inhibition by adrenalin is comparatively high, while the hydrolysis of benzoylcholine by the non-specific cholinesterase (*s*) is inhibited to only a slight extent.

The inhibition of cholinesterases possesses some degree of specificity. This can, in some cases, be used as a method for the differentiation of the type of cholinesterase.[221,222] Zeller reported that, in the case of unpurified enzymes, the degree of inhibition varied according to the tissue and species from which the cholinesterase was obtained. Thus, human serum cholinesterase was more sensitive to 4-isopropyl-antipyrine than was that prepared from the human central nervous system.[194] On the other hand, the cholinesterases of the human brain and erythrocytes were found to be inhibited to a similarly slight degree, while that prepared from serum presented a markedly different behavior, in that it was strongly inhibited.[185]

Table 4 gives data as to the inhibition of several preparations of cholinesterases by certain common drugs, while Table 5 compares the action of a variety of chemical inhibitors on the two types of cholinesterases.

(b′) Comparative Mechanism of Inhibition.—The inhibitors function differently in counteracting the several types of cholinesterases. Thus the alkaloids inhibit human plasma cholinesterase by competing with

[217] D. Glick, A. Lewin, and W. Antopol, *Proc. Soc. Exptl. Biol. Med.*, *40*, 28–32 (1939).
[218] W. Antopol, A. Schifrin, and L. Tuchman, *Proc. Soc. Exptl. Biol. Med.*, *38*, 363–366 (1938).
[219] W. M. Benson, *Proc. Soc. Exptl. Biol. Med.*, *68*, 598–601 (1948).
[220] W. M. Benson and W. J. Meek, *Am. J. Physiol.*, *158*, 327–331 (1949).
[221] E. A. Zeller, *Helv. Chim. Acta*, *25*, 216–229 (1942).
[222] E. A. Zeller, *Verhandl. Ver. schweiz. Physiol.*, *19*, 35–36 (1941); *20*, 51–52 (1942).

TABLE 4
INHIBITION, BY CERTAIN DRUGS, OF CHOLINESTERASES PREPARED FROM SERUM, ERYTHROCYTES, AND BRAIN OF MAN[a]

Inhibitors		Percent inhibition of cholinesterase		
Compound	Concn., M	*s*-Type	*e*-Type	Brain cholinesterase
Percaine (Nupercaine)[b]	0.006	94	25	12
Irgamide[c]	0.006	46	4	3
4-Isopropylantipyrine	0.002	65	9	18
Morphine	0.006 (satd.)	66	76	66
Caffeine	0.006	4	42	40

[a] Data from A. E. Zeller and A. Bissegger, *Helv. Chim. Acta*, *26*, 1619–1630 (1943).
[b] 2-Butoxy-*N*(2-diethylaminoethyl)-cinchoninamide hydrochloride.
[c] *N*′-Dimethylacroyl-*p*-aminobenzenesulfonamide (*N*′-senecioylsulfanilamide).

TABLE 5
THE INHIBITION OF CHOLINESTERASES BY DIFFERENT INHIBITORS

Inhibitor	*e*-Type cholinesterase	*s*-Type cholinesterase
Pyrazolones[a,b]	0[c]	+
Local anesthetics (percaine)[a,d]	0[c]	+
Sulfonamides[a,e,f]	0[c]	+
Methylhydroxy purines[a,d]	+	0
Triorthocresyl phosphate[g]	0[c]	+
Diisopropyl fluorophosphate[h]	0[c]	+
Dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide[i,j]	0[c]	+
Antipyrine[k]	0	+

[a] E. A. Zeller and A. Bissegger, *Helv. Chim. Acta*, *26*, 1619–1630 (1943).
[b] E. A. Zeller, *Helv. Chim. Acta*, *25*, 1099–1110 (1942).
[c] This represents a markedly lower sensitivity as compared with the other type.
[d] D. Nachmansohn and H. Schneemann, *J. Biol. Chem.*, *159*, 239–240 (1945).
[e] E. A. Zeller, *Helv. Chim. Acta*, *25*, 216–229 (1942).
[f] E. A. Zeller, *Verhandl. Ver. schweiz. Physiol.*, *19*, 35–36 (1941).
[g] B. Mendel and H. Rudney, *Science*, *100*, 499–500 (1944).
[h] R. D. Hawkins and B. Mendel, *Brit. J. Pharmacol.*, *2*, 173–180 (1947).
[i] R. D. Hawkins and J. M. Gunter, *Biochem. J.*, *40*, 192–197 (1946).
[j] R. D. Hawkins and B. Mendel, *J. Cellular Comp. Physiol.*, *27*, 69–85 (1946).
[k] E. A. Zeller, *Verh. Ver. schweiz. Physiol.*, *20*, 51–52 (1942).

acetylcholine for the enzyme surface.[223] Urethanes related to eserine (physostigmine) and prostigmine also inhibit competitively, but these also combine with and dissociate from the enzyme extraordinarily slowly, so that competitive displacement by substrate is also very slow. Gold-

[223] A. Goldstein, *Arch. Biochem.*, *34*, 169–188 (1951).

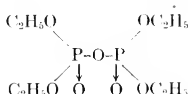
stein[223] suggests that these differences in kinetic behavior indicate that alkaloids in general combine with only one locus of an enzyme active center containing two loci, but that the urethane-like substrates interact with both loci.

In the case of DFP, Nachmansohn and his co-workers[224] have reported that the enzyme inhibition results from a stoichiometric reaction with the inhibitor. Michel and Krop,[225] using DFP with radiophosphorus, demonstrated that the inhibition of cholinesterase is associated with the binding of DFP phosphorus by the enzyme. According to Jansen *et al.*,[226] when a preparation of purified plasma cholinesterase was inhibited by radioactive DFP, the phosphorus of the inhibitor was introduced into the inhibited enzyme. The amount of P introduced into the still impure cholinesterase was 0.0023%.

Aldridge[227] has postulated that, since organophosphorus inhibitors are esters, they can attach themselves to the active centers of cholinesterase in the same way as do carboxylic esters. The inhibitor is then hydrolyzed, but the enzyme remains phosphorylated, instead of both products passing into solution. The enzyme phosphate so formed has its own stability to hydrolysis, and this is dependent upon the groups attached to the P atom. According to this hypothesis, the potency of the inhibitors is a reflection not of an especially high affinity for the enzyme, but rather of the fact that one active center is inactivated after reaction with one inhibitor molecule.

The dimethylcarbonate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide has been shown by Myers[228] to be a reversible inhibitor which combines with the enzyme active center of pseudocholinesterase on an equimolecular basis.

The explanation of Wilson[229] for inhibition caused by tetraethyl pyrophosphate:



abbreviated as TEPP, differs somewhat from the other hypotheses of the action of inhibitors. Acetylated cholinesterase, which is an intermediate

[224] D. Nachmansohn, M. A. Rothenberg, and E. A. Feld, *J. Biol. Chem.*, *174*, 247–256 (1948).

[225] H. O. Michel and S. Krop, *J. Biol. Chem.*, *190*, 119–125 (1951).

[226] E. F. Jansen, R. Jang, and A. K. Balls, *J. Biol. Chem.*, *196*, 247–253 (1952).

[227] W. N. Aldridge, *Biochem. J.*, *54*, 442–448 (1953).

[228] D. K. Myers, *Biochem. J.*, *51*, 303–311 (1952).

[229] J. B. Wilson, *J. Biol. Chem.*, *190*, 111–117 (1951).

in the hydrolysis of acetylcholine, reacts readily with water to regenerate the enzyme; however, the reaction between water and the corresponding phosphorylated enzyme, produced by the addition of TEPP, is manifestly slow, but not immeasurably so. It was shown that hydrolysis, with the simultaneous regeneration of the enzyme, could be effected if the excess TEPP was removed by hydrolysis or dialysis. Since the acylated enzyme reacts readily with hydroxylamine or choline, these compounds have also been used as regenerating agents for the TEPP-inhibited enzyme. It is postulated that the enzyme and alkyl phosphate interact on the basic group in the site of esterase activity on the enzyme surface, so that this position is phosphorylated. This phosphorylated enzyme would then react with the nucleophilic reagents (hydroxylamine or choline), resulting in the regeneration of the enzyme.

Nachmansohn *et al.*[224] point out that there is a difference between the kinetics of inhibition by the alkaloids and by DFP. At higher concentrations, DFP was found to be more effective than were the alkaloids, while the opposite condition obtains at lower concentrations. Moreover, Mackworth and Webb[230] reported that the inhibition by DFP, unlike that by eserine, is progressive; it is not affected by substrate concentration, and it cannot be prolonged by dialysis.

Myers[231] noted a marked difference in the effect of electrolytes on cholinesterase inhibition as affected by eserine and DFP. In the case of the alkaloidal inhibitors such as eserine and prostigmine, the affinity for the cholinesterase is decreased by increasing the concentration of electrolytes in the medium. On the other hand, alterations in salt concentration fail to affect the affinity of cholinesterases for DFP appreciably.

Prostigmine loses its inhibiting power during the course of acetylcholine cleavage.[232] Goldstein and Hamlish[233] reported that the cholinesterase inhibitors, eserine and prostigmine, are destroyed enzymatically by human serum, by a human plasma fraction highly purified with respect to cholinesterase, and by the same purified fraction partially denatured by heat. The rate of destruction of the inhibitor parallels the uninhibited activity toward acetylcholine in all three preparations. On the basis of these findings, it is postulated that the inhibitors are destroyed by cholinesterase. The ratio of the inhibitor turnover number to that of acetylcholine is 5.5×10^{-7} for eserine and 1.2×10^{-7} for prostigmine. These results confirm the

[230] J. F. Mackworth and E. C. Webb, *Biochem. J.*, *42*, 91–95 (1948).
[231] D. K. Myers, *Arch. Biochem.*, *27*, 341–347 (1950).
[232] E. H. Maier and H. G. Bammer, *Biochem. Z.*, *322*, 85–105 (1951).
[233] A. Goldstein and R. E. Hamlish, *Arch. Biochem.*, *35*, 12–22 (1952).

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earlier conclusions that cholinesterase inhibitors of the urethane group are actually competitive substrates with exceedingly low turnover numbers.

Mercuric chloride causes inactivation of cholinesterases by two independent reactions. Goldstein and Doherty[234] reported that, at high concentrations, it appears to combine rapidly and reversibly at the enzyme active center, causing inhibition which is competitive with respect to substrate. This enzyme active center apparently does not contain an active sulfhydryl (—SH) group, and the mercury must be assumed to react with some other group. Secondly, mercuric chloride apparently also combines elsewhere on the enzyme surface (possibly through —SH), thereby initiating a slow and irreversible denaturation. The latter process is most obvious at a low concentration of mercuric chloride.

(c′) Inhibition by Diisopropyl Fluorophosphate.—Diisopropyl fluorophosphate (DFP) is the most important synthetic inhibitor of cholinesterase; it has proved useful in the investigation of the nature of this enzyme. DFP has the following formula:

```
CH3          O          CH3
   \H        ↑        H/
    C—O—P—O—C
   /         |         \
CH3          F          CH3
```

The effectiveness of fluorophosphates as anticholinesterases was first established by Mackworth[235] in 1942. Richards and Gilman[236] reported that the fluorophosphates as a group have a cholinesterase-inhibiting action. This property is shared by sodium fluorophosphate; however, the effectiveness is greatly enhanced if alkyl groups are substituted for the hydrogens. The product thus becomes lipid-soluble. DFP is one of the most effective of these anticholinesterases, and it has the added characteristic that the inhibition is irreversible.[236] Thus, it is only by the synthesis of new enzyme that the ability to hydrolyze acetylcholine can be restored.

Nachmansohn and Feld[237] emphasize the high specificity of the action of DFP, and its powerful effect on cholinesterase in low concentration. The coincidence of death and inactivation of cholinesterase, therefore, suggests that the toxicity of the compound is due to its action on the enzyme. Of the alkyl fluorophosphonates examined by Mackworth and Webb[230] as inhibitors of horse serum cholinesterase, the diisopropyl compound was the

[234] A. Goldstein and M. E. Doherty, *Arch. Biochem.*, *33*, 35–49 (1951).

[235] J. F. Mackworth, personal communication to A. Mazur and O. Bodansky, *J. Biol. Chem.*, *163*, 261–276 (1946), p. 261.

[236] A. N. Richards and A. Gilman, *Federation Proc.*, *5*, 285–292 (1946).

[237] D. Nachmansohn and E. A. Feld, *J. Biol. Chem.*, *171*, 715–724 (1947).

most active; it was found to have 30 times the activity of eserine. The most potent alkyl fluorophosphonate inhibitors were the esters with short branched-chain alkyl groups. The order of inhibitory power of the various esters was roughly the same as that for toxicity and myotic power. Webb[238] reported that brain cholinesterase (type *e*) was inhibited by an alkyl fluorophosphonate, but not to the same extent as was serum cholinesterase. Liver esterases, human milk lipase, and kidney phosphatase were likewise sensitive to fluorophosphonate, but the amount required was much greater than in the case of cholinesterase. No correlation was shown to exist between the sensitivity of the non-choline esterases toward fluorophosphonate, toward eserine, and toward sodium fluoride. For example, liver esterase is highly sensitive to fluorophosphonate but relatively insensitive to eserine.

Different amounts of DFP are required to effect the inhibition of cholinesterase in both *in vivo* and *in vitro* tests. For example, Nachmansohn *et al.*[239] state that, while inhibition of cholinesterase *in vitro* requires only micrograms of DFP, the inhibition of cholinesterase in axonal conduction requires milligrams. These workers explain this discrepancy by the fact that a lipid membrane surrounds the axon and acts as a barrier to the entrance of DFP; a considerable difference exists in the concentration of the inhibitor inside and outside the membrane at the time of action. Similar variations in sensitivity of DFP *in vitro* and *in vivo* were noted earlier by Mazur and Bodansky.[240]

Inhibition with DFP is reversible for a certain period, depending upon temperature and upon the concentration of the inhibitor. A close parallelism was observed in the interval required in different nerve preparations, at several temperatures, between the irreversible destruction of cholinesterase and the irreversible abolition of conduction. Freedman and co-workers[241] reported that the rate of regeneration of non-specific plasma cholinesterase after large doses of DFP is more rapid than is that of the specific cholinesterases in the erythrocytes and brain. A lag in the regeneration of the cholinesterase activity in the erythrocyte occurs for twenty-four to forty-eight hours after the injection of DFP, during which time brain cholinesterase regenerates rapidly. Subsequently, the rate of regeneration of red blood cell cholinesterase becomes more rapid, and exceeds that of the brain. These workers[241] observed that a close relationship appears to exist between the degree of toxicity of DFP and the level of brain cholinesterase

[238] E. C. Webb, *Biochem. J.*, *42*, 96–98 (1948).

[239] D. Nachmansohn, M. A. Rothenberg, and E. A. Feld, *Arch. Biochem.*, *14*, 197–211 (1947).

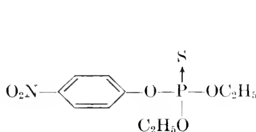
[240] A. Mazur and O. Bodansky, *J. Biol. Chem.*, *163*, 261–276 (1946).

[241] A. M. Freedman, A. Willis, and H. E. Himwich, *Am. J. Physiol.*, *157*, 80–87 (1949).

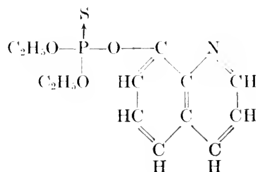
during the regeneration period. The relationship between the toxic signs and the level of *e*-cholinesterase is less exact than in the case of the brain cholinesterase; no relationship was noted between the level of serum cholinesterase (*s*) and the extent of toxicity. Freedman and Himwich[242] likewise observed a correlation between the clinical signs of DFP poisoning in rats and the level of brain cholinesterase. The more severe symptoms were noted when the cholinesterase level in the brain was greatly depressed.

Anti-inhibitors are known which can protect cholinesterase from DFP. Cohen, Warringa, and Bovens[243] reported that butyrylcholine prevented the irreversible inactivation of cholinesterase by DFP. It was shown that butyrylcholine is probably a competitive inhibitor of true cholinesterase; it acts reversibly. However, it was impossible to alter the lethal effects of DFP in rats by administering butyrylcholine before or after DFP. Wright and Mendel[244] reported the presence of a substance in incubated heart preparations which had the ability to increase the activity of cholinesterase on fresh heart pulp. It is suggested that this effect could result from the formation of an activator, or from the production of a substance which neutralizes the action of a naturally-occurring cholinesterase inhibitor. Resistance to the action of DFP in rats is increased with age,[242] up to 120 days.

(d′) Miscellaneous Inhibitors.—Bis(isopropylamine)-fluorophosphine oxide has been shown by Callaway *et al.*[245] to be a potent cholinesterase inhibitor both *in vitro* and *in vivo*. While recovery of enzyme activity was complete in 40 days after poisoning with DFP, only a 60% recovery was obtained after a similar time interval in animals injected with bis(isopropylamino)-fluorophosphine oxide. It is believed that these two inhibitors exhibit different modes of action.



(I)



(II)

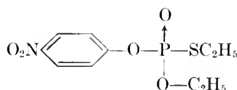
[242] A. M. Freedman and H. E. Himwich, *Am. J. Physiol.*, *153*, 121–126 (1948).

[243] J. A. Cohen, M. G. P. J. Warringa, and B. R. Bovens, *Biochim. et Biophys. Acta*, *6*, 469–476 (1951).

[244] M. Wright and B. Mendel, *J. Biol. Chem.*, *165*, 389–390 (1946).

[245] S. Callaway, D. R. Davies, and J. E. Risley, *Biochem. J.*, *50*, xxx (1952).

p-Nitrophenyl diethylthiophosphate (I) is an inhibitor which acts on cholinesterase, with a characteristic bimolecular reaction.[246] It is also referred to in the literature as parathion (E 605). On the other hand, 8-quinolyl diethylthiophosphate (II) brings about inhibition by a unimolecular reaction.[246] Aldridge and Davison[247] reported that the inhibition of sheep red-cell cholinesterase (*e*-type) by 6 substituted diethylphenyl phosphate inhibitors produced either a first order or a bimolecular reaction. In the case of dimethyl-*p*-nitrophenyl phosphate, Aldridge[227] demonstrated that the inhibition of erythrocyte cholinesterase reverses at a measurable rate. According to Diggle and Gage,[248] parathion itself has a low inhibitory action; however, the *S*-ethyl isomer (III), with which parathion is usually



(III)

contaminated, is a highly inhibitory substance. The power of parathion to inhibit cholinesterase is proportional to its content of the *S*-ethyl isomer.

Paludrine, $ClC_6H_4NH \cdot C(:NH)NHC(:NH)NHCH(CH_3)_2$, has been shown to be only weakly effective as a cholinesterase inhibitor. Blaschko *et al.*[249] showed that this drug has little affinity for the cholinesterase in the central nervous system; this accounts for its low toxicity. However, some of the other cholinesterases are believed to be more strongly inhibited by paludrine. However, there was no interference in the hydrolyses of methyl butyrate or tributyrin when extracts of rabbit pancreas were treated with a 10^{-3} M concentration of paludrine.

Bain[250] has reported on the inhibitory action of a series of β-chlorinated amines on the cholinesterase system of rat brain. The enzyme was shown to combine with two moles of acetylcholine to form an inactive complex with a dissociation constant of 1.6×10^{-2} M. The structure necessary for the inhibitory action of β-chlorinated amines was shown to be correlated with that required for convulsant activity. Although the inhibitory action of strychnine and nicotine was confirmed, metrazol and picrotoxin were not found to be inhibitors. Moreover, the anticonvulsant drugs, phenobarbital, trimethadione, phenacetylurea, diphenylhydantoin, and atropine,

[246] W. N. Aldridge, *Biochem. J.*, *46*, 451–459 (1950).
[247] W. N. Aldridge and A. N. Davison, *Biochem. J.*, *51*, 62–70 (1952).
[248] W. M. Diggle and J. C. Gage, *Biochem. J.*, *48*, xxv (1951); *49*, 491–494 (1951).
[249] H. Blaschko, T. C. Chow, and I. Wajda, *Biochem. J.*, *40*, lxvii (1946).
[250] J. A. Bain, *Am. J. Physiol.*, *160*, 187–194 (1950).

had no effect on cholinesterase, nor did they reverse the effect of any of the inhibitors studied. Cysteine was found to be an anti-inhibitor for the β-chlorinated amines.

Both arsenical and non-arsenical vesicants have been proved by Thompson[251] to be inhibitors of brain cholinesterase. When mustard gas or either of two other nitrogen-containing vesicants was applied to the skin of rats, cholinesterase activity was inhibited in the injured skin area. Moreover, a significant reduction in *s*-cholinesterase was observed in guinea pigs after heavy contamination with mustard gas. It is suggested that some of the systematic effects produced by certain of the vesicants may be due to the inhibition of cholinesterase at the cholinergic nerve endings.

Alcohols effect the hydrolysis of acetylcholine by rat brain in two different ways. Fellowes and co-workers[252] demonstrated that activation occurred at low concentrations. However, above the optimum concentration, the activation fell off rapidly, until complete inactivation occurred. A maximal degree of activation resulted from *n*-butanol. Hydrolysis of acetylcholine by serum cholinesterase (*s*-type) was found to be inhibited by butanol.

(e) Cell Permeability in Relation to Cholinesterases. In addition to their main function of destroying acetylcholine, cholinesterases bring about certain related reactions which are of importance. One of these, which has been widely investigated, is the effect on cell permeability. Greig and Holland[253] first pointed out that changes in the permeability of erythrocytes were produced by inhibition of the cholinesterase system, situated in the cell membrane. Thus, it was shown that both methadon and eserine produced changes in the permeability of dog erythrocytes. The effect of drugs was shown to be influenced by the Na^+ and K^+ content of the medium, by the pH, and by the presence of acetylcholine. Thus, according to Holland and Greig,[254] when acetylcholine was added to a suspension of dog erythrocytes in a medium containing potassium, there was a decrease in the rate of swelling and in the permeability of the red blood cell to potassium. The addition of eserine in an amount sufficient to inhibit the activity of cholinesterase 60 to 80% resulted in a reversal of the permeability produced by acetylcholine. A number of esters were shown to be effective in maintaining the integrity of the red blood cell; these were, in decreasing order of activity, the following: acetylcholine, triacetin, acetyl-β-methylcholine,

[251] R. H. S. Thompson, *J. Physiol., 105*, 370–381 (1947).
[252] K. P. Fellowes, J. P. Rutland, and A. Todrick, *Biochem. J., 47*, xx (1950).
[253] M. E. Greig and W. C. Holland, *Arch. Biochem., 23*, 370–384 (1949).
[254] W. C. Holland and M. E. Greig, *Arch. Biochem., 26*, 151–155 (1950).

ethyl propionate, and benzoyl choline.[255] This is the same order in which the cholinesterase of the red blood cells is able to split the esters.[256] On the other hand, the hydrolytic products of acetylcholine in amounts up to 1.5×10^{-2} *M* had little or no effect on the rate of hemolysis of the red blood cells of the dog.[255] It is concluded that a remarkable correlation exists between the activity of the enzyme and the ability of the cell to maintain its selective permeability.

In a further study of this problem, Holland and Greig[257] reported that changes in permeability occurred only when the inhibitors (eserine, prostigmine, caffeine, and choline) were present in sufficient amounts to produce at least a 50% inhibition of the activity of the cholinesterases. Under these conditions, the resistance of the dog erythrocytes to hemolysis could be considerably increased by the addition of acetylcholine in amounts of 10^{-2} to 10^{-5} *M;* the magnitude of the effect varied directly with the concentration of the drug. Holland and Greig[258] later showed that the Na^+ in the rabbit erythrocyte is controlled by the acetylcholine-cholinesterase system in the same way as K^+ is regulated in the dog and cat erythrocytes by this system. In the rabbit, Na^+ largely replaces K^+ in the red blood cells. It was also reported that inhibition of the cholinesterase system with eserine increases the permeability of the rabbit erythrocyte to Na^+. The diffusion of potassium ions in dog and cat blood cells[258] and in human erythrocytes[259] is likewise controlled by the cholinesterase system.

In addition to the effect of cholinesterase on the permeability of blood cells, similar relationships have been reported in other tissues. Thus, Holland *et al.*[260] noted that changes in the rate of metabolism of acetylcholine by cholinesterase may be correlated with changes in the permeability of isolated guinea pig auricles to K^+ and Na^+. The hydrolysis of acetylcholine was shown to be accompanied by the release of K^+ from the guinea pig auricle; the K^+ lost was replaced by Na^+. Potent inhibitors of cholinesterase partially or completely reverse the effect of acetylcholine, depending upon the concentration of substrate employed.

In a later publication, Greig *et al.*[261] demonstrated that the potassium could be replaced in both dog and human erythrocytes against a concen-

[255] M. E. Greig and W. C. Holland, *Am. J. Physiol., 164*, 423–427 (1951).
[256] D. H. Adams, *Biochim. et Biophys. Acta, 3*, 1–14 (1949).
[257] W. C. Holland and M. E. Greig, *Arch. Biochem., 32*, 428–435 (1951).
[258] W. C. Holland and M. E. Greig, *Am. J. Physiol., 162*, 610–615 (1950).
[259] P. E. Lindvig, M. E. Greig, and S. W. Patterson, *Arch. Biochem., 30*, 241–250 (1951).
[260] W. C. Holland, C. E. Dunn, and M. E. Greig, *Am. J. Physiol., 168*, 546–556 (1952).
[261] M. E. Greig, J. S. Faulkner, and T. C. Mayberry, *Arch. Biochem. Biophys., 43*, 39–47 (1953).

tration gradient when the cells were incubated with acetylcholine, and the cholinesterase activity was maintained, provided that the cell had previously lost part of its complement of K^+. This reaction could be inhibited by eserine. Replacement of K^+ in cells during the metabolism of glucose was shown to be blocked in a similar manner by eserine. It is concluded that a single method exists for the replacement of potassium in blood cells, irrespective of whether the substrate is glucose or acetylcholine, and that the mechanism depends upon active cholinesterase.

An understanding of the relationship of cholinesterase to cell permeability has been somewhat extended by the experiments of Taylor, Weller, and Hastings,[262] who used radioactive K^+. It was shown that cholinesterase inhibitors such as eserine and diisopropyl fluorophosphate (DFP) caused a loss of K^+ from the cells, principally as a result of a decrease in the rate at which K^+ entered the cells from the plasma. No direct relationship was found, however, between the inhibition of cholinesterase activity and potassium leakage from the cell. When choline acetylase inhibitors (methylene blue or 2-methyl-1,4-naphthoquinone) were employed, a loss of K^+ from the cells was also observed; in this case, however, the increase in the rate at which K^+ left the cell was responsible for the phenomenon. It is not known whether or not changes in permeability may account for the finding of Robertson *et al.*[263] that topical application of acetylcholine to the pyloric mucosa causes gastrin to be released, resulting in stimulation of acid secretion by the fundic glands. Acetylcholine has been reported by McDowall[264] to act as a stimulant to heart muscle. In contradistinction to adrenalin, acetylcholine acts upon the force of the heart rather than upon its frequency.

(*f*) *Physiological and Pathological Factors Altering the Amount of Cholinesterases.* Age and species have an important effect upon cholinesterase activity. Thus, in newborn humans, rats, rabbits, and cats, the cholinesterase activity of the serum tended to be near the adult level, but rose shortly after birth to approximately twice that of the adult.[265] McCance *et al.*[265] reported that the cholinesterase activity of the colostrum of man and of the cat was negligible, but that the level of this enzyme was very high in the colostrum of the bitch. This latter fact may explain why the

[262] I. M. Taylor, J. M. Weller, and A. B. Hastings, *Am. J. Physiol.*, *168*, 658–665 (1952).

[263] C. R. Robertson, K. Langlois, C. G. Martin, G. Slezak, and M. I. Grossman, *Am. J. Physiol.*, *163*, 27–33 (1950).

[264] R. J. S. McDowall, *J. Physiol.*, *104*, 392–403 (1946).

[265] R. A. McCance, A. O. Hutchinson, R. F. A. Dean, and P. E. H. Jones, *Biochem. J.*, *45*, 193–196 (1949).

cholinesterase activity in puppies was found to rise to 25 times the level at birth within three days. Although proteinaceous materials such as enzymes are generally considered to be destroyed in the gastrointestinal tract, and one would not expect orally ingested enzymes to reach the blood serum, it is recognized that a considerable amount of unaltered proteins can be absorbed from the colostrum during the early days of life. Schafer and Maier[266] also conclude that the cholinesterase content of the blood depends upon age; these workers state that the minimal level is reached at 30 years.

Age, also, has been found to have a profound effect on the cholinesterase activity of several tissues. Thus, Bayliss and Todrick[267] reported that the activity of true cholinesterase increased in the whole brain from the third to the twentieth day at a rate of slightly more than 300 units per day, after which a decrease in rate occurred. On the other hand, the concentration of pseudocholinesterase was found to remain constant at 150 to 160 units per unit weight from the third to the tenth day. An increase followed, which continued to the twenty-fourth day, when a level of 300 units was reached. The period of rapid change in the cholinesterase content of the brain coincides with the rapid increase in the number of capillaries. Craigie[268] reported that the differences between the capillary content in the newborn and adult rat brain become established between the tenth and the twenty-first day. Since Koelle,[269] on the basis of histochemical evidence, suggested that the pseudocholinesterase of the rat brain is located principally in the walls of the capillaries, in the muscle fibers of the arterioles and venules, and in some gliocytes, there would seem to be some justification for associating the rise in capillary content of the brain with an increase in pseudocholinesterase. Risley and Davies[270] likewise noted that the cholinesterase content is four times as high in adult brain as in this organ of the newborn rat. Similar changes were noted in the hydrolytic action of brain on acetyl-β-methylcholine, benzoylcholine, propionylcholine, and butyrylcholine.

In the case of spinal cord, no significant change per unit weight was found to occur between the eighth and the twenty-second days, but both enzymes

[266] H. Schafer and E. Maier, *Biochem. Z.*, *319*, 420–438 (1949).

[267] B. J. Bayliss and A. Todrick, *Biochem. J.*, *54*, xxix (1953).

[268] H. E. Craigie, in *The Circulation of the Brain and Spinal Cord, Research Publs. Assoc. Research Nervous Mental Disease*, *18*, Chap. I. "The Comparative Anatomy and Embryology of the Capillary Bed of the Central Nervous System," pp. 3–28, Williams and Wilkins, Baltimore, 1938, p. 23.

[269] G. B. Koelle, *J. Pharmacol. Exptl. Therap.*, *106*, 401 (1952).

[270] J. E. Risley and D. R. Davies, *Biochem. J.*, *54*, xxx (1953).

were initially present in higher concentration in this tissue than in brain.[26] This result is diametrically opposed to that of Risley and Davies,[270] who noted a constant increase from birth to a maximum at four weeks. This was followed by a steady decrease to a value, in the adult, of one-half that at birth and one-third of the maximal level.

The cholinesterase content of rat skeletal muscle obtained from the leg has been shown to reach a maximum seven days after birth. A slight decrease in activity then ensues. The level in the adult rat decreases to only 10% or less of that observed in the muscle during the first week of life. During the first week of life, the muscle is able to hydrolyze propionylcholine much more rapidly than acetylcholine. It was also reported that the cholinesterase activity of the kidney decreases in the rat with increasing age.

Species is another factor which can result in variations in blood cholinesterase. Thus, variations in the cholinesterase of the colostrum, related to species,[265] have already been described. Moreover, guinea pigs, in contradistinction to other newborn, did not exhibit the rapid increase in cholinesterase shortly after birth.[265] Galehr and Plattner[181] found that the rate of destruction of acetylcholine (and hence the activity of cholinesterases) in blood obtained from different species decreased in the following order: man, pig, cattle, horse, rabbit, and cat. Davies *et al.*[271] noted that the activity of sheep tissues against acetylcholine was much lower than that of rat tissues. The ratios of activity of sheep tissues are considerably less constant than in the rat. Thus, the relative activity toward propionylcholine:butyrylcholine varies only from 1.4 to 2.0 in the rat, but the values recorded for sheep are from 0.4 to 3.0. The ratios of activity of butyrylcholine:benzoylcholine and of propionylcholine:benzoylcholine are irregular in the ruminant, in contrast to their relative regularity in the case of the rat. Few sheep tissues except kidney were found to hydrolyze butyrylcholine.[271]

Sex is another factor which may alter cholinesterase content. Although Davies and Rutland[272] and Harrison and Brown[273] were unable to demonstrate a sex difference in the content of *s*-cholinesterase, in man, and the findings were the same for the dog,[273,274] the situation is entirely different in the rat. Sawyer and Everett[275] reported higher levels of the enzyme in the livers and gonads of female rats than was the case in males. Although

[271] D. R. Davies, J. E. Risley, and J. P. Rutland, *Biochem. J.*, *53*, xv (1953).
[272] D. R. Davies and J. P. Rutland, *Biochem. J.*, *47*, xxi–xxii (1950).
[273] M. F. Harrison and L. M. Brown, *Biochem. J.*, *48*, 151–154 (1951).
[274] R. W. Brauer and M. A. Root, *Am. J. Physiol.*, *149*, 611–625 (1947).
[275] C. H. Sawyer and J. W. Everett, *Am. J. Physiol.*, *148*, 675–683 (1947).

the liver and blood contained both the true and the pseudocholinesterases, the sex variation apparently was confined to the *s*-variety. Since castrates of either sex respond to the administration of estrogen by elevations in liver and blood *s*-enzyme, it was concluded that the liver is the site of synthesis of pseudocholinesterase. Harrison and Brown[273] also reported that livers of female rats exhibited a very high cholinesterase activity which was maintained by the ovarian hormones. The greater part of this increased content of cholinesterase in the female disappeared when the normal functions of the liver had been disturbed by fasting for two days, or by poisoning. The liver of the male was shown to have a much lower initial enzyme content; by the end of the second day of fasting, however, the difference had largely disappeared. These workers conclude that, in the female, there exists in the liver a considerable cholinesterase activity which is labile, sex-linked and, in some way, different from the slight basal activity common to both sexes and closely associated with liver proteins. The decrease in serum cholinesterase during fasting is believed to reflect the fact that the serum cholinesterase originates in the liver; when the liver content is reduced, a concomitant decrease in level would be expected in the serum.

A number of workers[276-278] have demonstrated that the *s*-cholinesterase in mouse and rat livers is influenced by sex hormones. It has likewise been proved that the same sex hormones have a marked effect on the level of serum cholinesterase of man, rats, cats, and guinea pigs.[278-282]

Nutritional status likewise has an important bearing on the level of cholinesterases. Davies and Rutland[272] found that the mean cell cholinesterase content of a group composed of service men who were in excellent physical condition was significantly higher than that of the regular blood donors. However, Saunders *et al.*[283] are of the opinion that the level of this enzyme is of dubious value as an index of nutritional status. In examinations on 119 children, no useful correlations could be found between serum

[276] H. Birkhäuser and E. A. Zeller, *Helv. Chim. Acta*, *23*, 1460–1464 (1940).

[277] E. A. Zeller and H. Birkhäuser, *Helv. Chim. Acta*, *24*, 120–126 (1941).

[278] E. A. Zeller, H. Birkhäuser, H. v. Wattenwyl, and R. Wenner, *Helv. Chim. Acta*, *24*, 1465–1470 (1941).

[279] E. A. Zeller, H. Birkhäuser, H. v. Wattenwyl, and R. Wenner, *Helv. Chim. Acta*, *24*, 962–968 (1941).

[280] H. v. Wattenwyl, A. Bissegger, A. Maritz, and E. A. Zeller, *Helv. Chim. Acta*, *26*, 2063–2070 (1943).

[281] J. W. Everett and C. H. Sawyer, *Endocrinology*, *39*, 323–343 (1946).

[282] C. H. Sawyer and J. W. Everett, *Endocrinology*, *39*, 307–322 (1946).

[283] J. P. Saunders, H. R. Sandstead, R. E. Butler, and O. Mickelsen, *J. Nutrition*, *47*, 191–201 (1952).

cholinesterase levels and hemoglobin, serum carotene, degree of underweight, physical signs indicative of malnutrition, or general appearance from a nutritional standpoint.

In studies of liver diseases in man, Faber[284] found *s*-cholinesterase low in the blood; the enzyme content rises after recovery. Wescoe and coworkers[285] also reported that serum cholinesterase in patients with liver disease was subnormal. Moreover, the regeneration rate of serum cholinesterase was significantly lower than the normal after the administration of DFP to patients with liver disease. These facts led Wescoe *et al.* to conclude that the liver is the primary site of formation of serum cholinesterase. Benard *et al.*[286] noted that, in the case of livers damaged experimentally with carbon tetrachloride, serum tributyrinesterase (*s*-cholinesterase?) first increases, and then drops back to a value lower than the original level, while cholinesterase (*e*-type?) remains relatively unchanged. Brauer and Root[274] likewise reported an increased plasma cholinesterase in male dogs poisoned with carbon tetrachloride, while a decrease in the level followed transfusion. In the presence of a functioning liver, but not after hepatectomy, the serum cholinesterase returned to the normal level. The liver is considered to be a storehouse of cholinesterase; the total quantity is 5 to 7 times that in the blood. Brauer and Root[274] reported a significant degree of correlation between the liver and plasma cholinesterase. These experimental data are to be interpreted as further evidence of the hepatic origin of serum cholinesterase.

There is no definite conclusion as to what role folic acid plays in maintaining cholinesterase levels. Davis and Hamilton[287] presented evidence that folic acid (pteroylglutamic acid) increases the cholinesterase activity of the plasma of dogs and human subjects. Moreover, Davis[288] reported that folic acid caused a remission of hyperchromic anemias (induced by acetylcholine) in dogs; this was accompanied by reticulocyte responses. An analogous effect was produced by liver extract. In the latter case, serum cholinesterase was increased twelve-fold. Likewise, in some instances, incubation of sera with liver extract or with folic acid increased their cholinesterase activity. It is concluded that the important action of

[284] M. Faber, *Acta Med. Scand.*, *114*, 59–71, 72–91 (1943).

[285] W. C. Wescoe, C. C. Hunt, W. F. Riker, and I. C. Litt, *Am. J. Physiol.*, *149*, 549–551 (1947).

[286] H. Benard, A. Gajdos, and M. Gajdos-Török, *Compt. rend. soc. biol.*, *142*, 1372–1374 (1948).

[287] J. E. Davis, *Proc. Soc. Exptl. Biol. Med.*, *63*, 287–290 (1946); and W. M. Hamilton, *Federation Proc.*, *6*, 95 (1947).

[288] J. E. Davis, *Am. J. Physiol.*, *147*, 404–411 (1946).

folic acid or liver extract in the treatment of anemia probably consists in its role in increasing the cholinesterase activity in the body. However, neither Hawkins,[289] who studied the effect of folic acid on the plasma of dogs, rats, and men, both *in vitro* and *in vivo*, nor Kunkel *et al.*,[290] who tested liver extract and folic acid in normal dogs or in those depleted of plasma cholinesterase, also by both *in vitro* and *in vivo* procedures, have been able to substantiate the claim that folic acid stimulates increased cholinesterase levels in the plasma.

Hemorrhage is another condition which causes increased levels of cholinesterase. Pritchard[291] noted that repeated hemorrhages in rats produce a progressive increase in erythrocyte cholinesterase. By separating out the newly formed red blood cells by centrifugation it was possible to demonstrate that the younger cells have a greatly increased cholinesterase content, which accounts for the average general increase noted. Moreover, in anemias due to severe blood loss, the elevated level of cholinesterase in the red blood cells can be accounted for by the shift in cell population to younger red cells.

Febrile conditions did not increase brain cholinesterase above normal values.[292] Increased levels of cholinesterase were noted in hyperthyroidism,[293] while decreased values have been recorded in pregnancy,[294] in neoplastic diseases,[294] but not in pulmonary tuberculosis.[294] A decrease has also been noted in such experimental tumors[295] as Walker 256 rat adenocarcinoma, Murphy-Sturm lymphosarcoma, and Crocker mouse sarcoma 180.

Levine and Hoyt[296] noted a high cholinesterase level in albuminuria. This phenomenon provides an exception to the finding that the levels of serum albumin and serum cholinesterase almost always run parallel in various pathological conditions.

Early and collaborators[297] found that, in 31 out of 55 cases of psychotic males, an elevated *e*-cholinesterase obtained. No correlation was evident

[289] R. D. Hawkins, *Arch. Biochem.*, *17*, 97–104 (1948).

[290] A. M. Kunkel, S. Krop, and W. C. Wescoe, *Am. J. Physiol.*, *152*, 309–313 (1948).

[291] J. A. Pritchard, *Am. J. Physiol.*, *158*, 72–76 (1949).

[292] C. N. Peiss, J. Field, and V. E. Hall, *Am. J. Physiol.*, *155*, 56–59 (1948).

[293] I. Gitman, I. L. Greenblatt, and N. Mitchell, *Proc. Soc. Exptl. Biol. Med.*, *71*, 179–180 (1949).

[294] M. G. Levine and R. E. Hoyt, *Proc. Soc. Exptl. Biol. Med.*, *70*, 50–53 (1949).

[295] W. M. Govier, E. S. Feenstra, H. G. Petering, and A. J. Gibbons, *Arch. Biochem.*, *39*, 276–280 (1952).

[296] M. G. Levine and R. E. Hoyt, *Science*, *111*, 286–287 (1950).

[297] D. F. Early, R. E. Hemphill, M. Reiss, and E. Brummel, *Biochem. J.*, *45*, 552–556 (1949).

between pseudocholinesterase and true cholinesterase levels in the blood; moreover, it was impossible to demonstrate any correlation between the cholinesterase in serum and in the cerebrospinal fluid. Although, in some cases, the serum cholinesterase level was increased after electroconvulsive treatment, repeated shocks always resulted in a decreased level.

In traumatic shock, the intravenous injection of cholinesterases caused restoration of the blood pressure to normal. Schachter[298] suggests that this effect may be due to a shift of fluid from the tissue to the blood stream, with a resultant increase in plasma volume, or to the destruction of any increased acetylcholine which might result in dilatation to such an extent that fluid would leave the vascular bed. Burn *et al.*[299] reported that the cholinesterase content falls by 40 to 50% in the jejunum and ileum forty-eight hours after x-irradiation.

It has been suggested by Vincent and Parant[300] that the ratio of true to pseudocholinesterase in serum may be of importance in various diseases, especially those of the nervous or mental type. The highest level of true cholinesterase in the serum was reported in a case of generalized cancer.

(*g*) *Distribution of Cholinesterases.* Cholinesterase is widely distributed throughout the animal body. Most tissues contain both the *e* (true) and the *s* (pseudo) variety of cholinesterases. Whittaker[301] has recently summarized the distribution of cholinesterase in various tissues, with special emphasis on the specific varieties of the enzyme present.

a′. Cholinesterases in Blood: The blood ordinarily contains two types of cholinesterase, the *s*-variety in the serum and the *e*-type in the erythrocytes. While the *s*-type has been demonstrated in the sera of man, guinea pig, and the horse, it is absent from that of the ruminants.[302] Adams and Whittaker,[303] using a method based upon comparing inhibition with DFP and with di-(2-chloroethyl)methylamine hydrochloride (DDM), evidence was adduced for the presence of a second enzyme in plasma, DFP- and DDM-insensitive, which accounts for 5 to 20% of the aliphatic esterase activity of the plasma, and which also hydrolyzes triolein. Sturge and Whittaker[304] found that cholinesterase in horse plasma resembles that in human plasma in hydrolyzing a large number of aliphatic esters. It was concluded that cholinesterase and ali-esterase, in spite of their close associa-

[298] R. J. Schachter, *Am. J. Physiol.*, *143*, 552–557 (1945).
[299] J. H. Burn, P. Kordik, and R H. Mole, *J. Physiol.*, *116*, 5P–6P (1952).
[300] D. Vincent and M. Parant, *Compt. rend. soc. biol.*, *143*, 1093–1095 (1949).
[301] V. P. Whittaker, *Physiol. Revs.*, *31*, 312–343 (1951).
[302] J. M. Gunter, *Nature*, *157*, 369 (1946).
[303] D. H. Adams and V. P. Whittaker, *Biochem. J.*, *44*, 62–70 (1949).
[304] L. M. Sturge and V. P. Whittaker, *Biochem. J.*, *47*, 518–525 (1950).

tion and physical similarity, have quite distinct patterns of action. Myers[228] recorded marked variations in the action of serum cholinesterase in a number of different species. Thus, the ratio of activity of serum cholinesterases toward 0.06 *M* acetylcholine:0.006 *M* benzoylcholine diminished progressively in mouse, rat, horse, man, and dog from 8.2 to 1.6. In a later study, Myers[305] reported that, in 8 of the 13 species of animals (10 mammals, 3 birds) examined, the pseudocholinesterases of the sera were butyrocholinesterases; in four cases, they were shown to be propriono-cholinesterases. The enzyme from pig serum could not be classified on the basis of substrate specificity alone. Since human serum is able to induce a slight hydrolysis of acetyl-β-methylcholine, Vincent and Parant[300] concluded that it contained a small amount of the *e*-variety of cholinesterase.

e-Cholinesterase was found in the erythrocytes of all animals which have been investigated. Mounter and Whittaker[210] reported that the cholinesterase of horse erythrocytes closely resembles that prepared from the red blood cells of human blood in specificity and physical properties. Vitamin E deficiency has been shown to reduce the erythrocyte cholinesterase of rats.[306] When phenothiazine was added to the diet, the proportion of cholinesterase activity was shown to increase almost to the original level. The erythrocytes of the tocopherol-deficient rats became susceptible to hemolysis by dialuric acid; this sensitivity was removed by the addition of phenothiazine to the diet.

b′. Cholinesterases in Brain and Nervous Tissue: Brain and nervous tissue is an important site for cholinesterases. This is to be expected because of the prominent part which acetylcholine plays in the functioning of this tissue. The *e*-type of cholinesterase has generally been accepted as the main variety, if not the only type of cholinesterase, in the brain tissue of all species of animals investigated to date.[185,186,189,206,307–311] However, Ord and Thompson[312] noted that cholinesterases, prepared from different areas of human and other mammalian brains, were able in all cases to effect a measurable degree of hydrolysis of benzoylcholine and of butyrylcholine. These workers suggest that a "butyrocholinesterase" occurs in human nervous tissue which closely resembles the pseudocholinesterase in

[305] D. K. Myers, *Biochem. J.*, *55*, 67–79 (1953).

[306] H. B. Collier and E. E. Dellert, *XIXth Intern. Congress, Abst.*, Montreal, 1953, pp. 274–275.

[307] B. Mendel and H. Rudney, *Science*, *98*, 201–202 (1943).

[308] D. Nachmansohn and M. A. Rothenberg, *J. Biol. Chem.*, *158*, 653–666 (1945).

[309] V. P. Whittaker, *Biochem. J.*, *44*, xlvi–xlvii (1949).

[310] J. M. Little, *Am. J. Physiol.*, *153*, 436–443 (1948).

[311] J. M. Little, *Am. J. Physiol.*, *155*, 60–63 (1948).

[312] M. G. Ord and R. H. S. Thompson, *Biochem. J.*, *51*, 245–251 (1952).

the plasma. More of this pseudocholinesterase was found in the white fiber tracts of the human cerebrum than in the grey matter. Burgen and Chipman[313] also reported both the true and the pseudoenzyme in dog brain. Whittaker[309] reported that the enzyme from pigeon brain closely resembles erythrocyte cholinesterase, except that the rate of hydrolysis of the aliphatic esters, where acetyl-β-methylcholine is used as a standard, is one-half to three-fourths of the value obtained with the erythrocyte enzyme. It is suggested that pigeon brain, like human erythrocytes, contains a small amount of ali-esterase. Little[310] has shown that the specific cholinesterase activity in mouse brain homogenate can be separated into two fractions by centrifugation. The fraction present in the precipitate was found to be more heat-labile than that in the supernatant. The distribution was shown to be a real one and not the result of intact cells in the homogenate (*i.e.*, it did not represent the presence of intra- and extracellular fractions). In the later report, Little[311] demonstrated that dog brain homogenate could be separated into these two cholinesterase fractions. In this case, also, the cholinesterase in the precipitate was the more heat-labile. It is suggested that the fraction in the supernatant may represent a larger proportion of cholinesterase from the nerve fibers.

In a comprehensive study of the cholinesterase content of the central nervous system of eight different species representing three classes of vertebrates, Lindeman[314] concluded that the cholinesterase content per cell, per unit of whole mass, and per unit nuclear surface, showed a consistent correlation between the activity of the enzyme and the general motor ability of the animal. Tower and Elliott[315] reported a similar study on samples of cerebral cortex obtained from normal unanesthetized adult mice, rats, guinea pigs, rabbits, cats, dogs, monkeys, cattle, and man. It was found that all components of the acetylcholine system (acetylcholine content, cholinesterase activity, rate of production of free and bound acetylcholine) decreased fairly regularly in the animals, in accordance with an ascending order on the phylogenetic scale. It was related to the average total brain weight of each species, and was the same function of the brain weight for all types of activity measured. The decrease in the average number of neurons per unit volume of cortex with increasing brain weight was shown to run parallel to the decrease in activity of the acetylcholine system.

Wide variations in cholinesterase are to be noted in different parts of the brain; this phenomenon has been reported for human, elephant, ox, dog,

[313] A. S. V. Burgen and L. M. Chipman, *J. Physiol.*, *114*, 296–305 (1951).
[314] V. F. Lindeman, *Am. J. Physiol.*, *143*, 687–691 (1945).
[315] D. B. Tower and K. A. C. Elliott, *Am. J. Physiol.*, *168*, 747–759 (1952).

and rabbit brain.[206,316–318] Some portions of the brain were found to have twenty times the concentration of cholinesterase found in other areas of the organ. The highest levels have been reported in the central grey matter (*i.e.* putamen) and the lowest values in the cortex and white matter. The concentration of cholinesterase parallels that of monoamine oxidase, the function of which is to destroy epinephrine and other monoamines.[318,319] Burgen and Chipman[313] noted that the highest cholinesterase content was to be found in the cerebellar hemispheres, and the lowest amount in the anterior spinal roots.

Both true and pseudocholinesterases have been reported in peripheral nerves, by Sawyer and Hollinshead[320] and by Sawyer[321] alone. According to Nachmansohn,[174] cholinesterase is concentrated at the neuron surface. A very high level occurs in the motor end-plates of the neuromuscular junctions, and in the synapses. When the sciatic nerves of the guinea pig were sectioned, Sawyer[321] found no change in the pseudocholinesterase during Wallerian degeneration, but there was a 60% loss of the true enzyme. On regeneration of the nerve, a considerable increase in true cholinesterase obtained. It was concluded that as much as two-thirds of the true cholinesterase is secreted by the axis cylinders, while the rest of the *e*-cholinesterase is produced by some other element, possibly by the sheath cells. In contradistinction to this finding, the pseudo-enzyme is probably resident in the connective tissue. Kalsbeek and co-workers[322] report that human cerebrospinal fluid contains both pseudo- and true cholinesterase, but no ali-esterase. Thus, not only acetylcholine and acetyl-β-methylcholine were hydrolyzed by cerebrospinal fluid, but also butyrylcholine and sometimes tributyrin.

c′. Cholinesterases in Glandular Tissues: Cholinesterase of the nonspecific type (*s*) has been reported in mouse and rat livers, where the activity is influenced by sex hormones.[276–278] Zachs and Welsh[323] have recently demonstrated the presence of pseudocholinesterase in the mitochondria and microsomes of rat liver. Specific cholinesterase was also found in rat liver, but it was of a lower order than the pseudo type. It is suggested

[316] D. Nachmansohn, *Bull. soc. chim. biol.*, *21*, 761–796 (1939).

[317] G. Pighini, *Riv. sper. frenatria med. legale delle alienazioni mentali*, *62*, 439–465 (1938).

[318] H. Birkhäuser, *Helv. Chim. Acta*, *23*, 1071–1086 (1940).

[319] H. Langemann, *Helv. Physiol. Pharmacol. Acta*, *2*, 367–375 (1944).

[320] C. H. Sawyer and W. H. Hollinshead, *J. Neurophysiol.*, *8*, 137–153 (1945).

[321] C. H. Sawyer, *Am. J. Physiol.*, *146*, 246–253 (1946).

[322] F. Kalsbeek, J. A. Cohen, and B. R. Bovens, *Biochim. et Biophys. Acta*, *5*, 548–560 (1950).

[323] S. I. Zachs and J. H. Welsh, *Am. J. Physiol.*, *165*, 620–623 (1951).

that the attraction of the enzyme receptor groups for the alkylated quaternary nitrogen groups of Janus Green B and similar basic dyes may be responsible for the staining of the mitochondria of liver and motor endplates by these dyes.

According to Langemann,[324] and Vincent and Lagreu,[325] the *s*-type of cholinesterase is present in dog pancreas. As would be expected from its presence in the pancreatic gland, pseudocholinesterase likewise occurs in pancreatic juice, in which it may be present in a high concentration.[326] The amounts of cholinesterase secreted appear to vary according to the stimulus employed to elicit its flow.[326] The salivary glands, which have a close histological relationship to pancreatic tissue, are another source of cholinesterase. While the *s*-type of cholinesterase has been reported in the parotid glands of the pig and guinea pig, the *e*-type has been found in these glands of the cow and rabbit, and a mixture of the two types has been recorded for the parotid glands of the cat and dog.[307,327] McCance and Brown[326] have confirmed the high rate of hydrolysis of acetylcholine by parotid saliva. Cholinesterase of the *s*-type has been recorded in the human ovary,[324] while Sekine[328] reported the presence of the specific type of this enzyme in the seminal plasma, where it is about one-third as active as in human serum. This worker also reports that the motion of pig spermatozoa is activated by cholinesterase and depressed by eserine. Some confusion would seem to exist as regards the type of the cholinesterase, as it does not split benzoylcholine but does hydrolyze butyrylcholine at a higher rate than is the case with the brain enzyme. Placenta,[329] like the red blood cells, provides a second example of nerve-free tissue which contains almost exclusively the true cholinesterase usually associated with nervous activity.

d′. Cholinesterases in Miscellaneous Tissues: In an extensive study of the type and distribution of cholinesterases in rat tissues, Ord and Thompson[330] demonstrated the presence of the *s*- and *e*-types in stomach, liver, lung, and submaxillary gland; in the case of heart auricle and ventricle, intestinal muscle and mucosa, Harderian gland, and skin, cholinesterase-*s* predominated. Brooks and Myers[331] found that no increase in true cholinesterase occurred in the muscle tissue of guinea pigs whose body weight had

[324] H. Langemann, *Helv. Physiol. Pharmacol. Acta, 2,* C17–C18 (1944).
[325] D. Vincent and R. Lagreu, *Bull. soc. chim. biol., 31,* 1043–1045 (1949).
[326] R. A. McCance and L. M. Brown, *Nature, 168,* 788–789 (1951).
[327] C. H. Sawyer, *Science, 101,* 385–386 (1945).
[328] T. Sekine, *J. Biochem. (Japan), 38,* 171–179 (1951).
[329] M. G. Ord and R. H. S. Thompson, *Nature, 165,* 927–928 (1950).
[330] M. G. Ord and R. H. S. Thompson, *Biochem. J., 46,* 346–352 (1950).
[331] V. B. Brooks and D. K. Myers, *J. Physiol., 116,* 158–167 (1952).

increased from 250 to 500 grams. The total content of this enzyme was likewise unchanged by denervation; however, the concentration was doubled when the muscle weight was reduced to half by atrophy. Pseudocholinesterase was shown to account for a maximum of only 5% of the total esterase activity of either normal or denervated muscle. The content of tributyrin ali-esterase decreased roughly in proportion to the loss in weight of the atrophic muscle.[331]

Anfinsen[332] reported that a high degree of cholinesterase activity obtains in areas of bovine retina which are rich in synaptic material. Cholinesterase in this tissue would be expected, in view of the fact that the retina is phylogenetically derived from the brain. Rat and human skin, also, has been shown by Thompson and Whittaker[333] to contain true (*e*) cholinesterase. Esterases which hydrolyze tributyrin and methyl butyrate are also present in rat skin.

e′. Cholinesterases in Snake Venom: Many snake venoms are potent sources of cholinesterases. Iyengar and his co-workers,[334] in 1938, were the first to note that cobra venom contains an acetylcholine-splitting enzyme. The enzyme was found to be 100 times more active than that found in the electric organs of the electric ray (*Torpedo ocellata*)[335] and of the electric eel (*Electrophorus* (*Gymnotus*) *electricus*),[336] which had previously been considered to be the most concentrated source. Zeller[164] showed that cobra cholinesterase is a separate type, which he describes as the *c*-type (see page 29). The specificity of cobra cholinesterase has recently been described by Mounter.[337,338] Chaudhuri[339] and Ghosh[204] have demonstrated the presence of cholinesterase in the toxic secretion of the yellow-banded krait (*Bungarus fasciatus*), as well as in a wide variety of snakes of the *Colubridae* family. The potencies of the cholinesterase isolated from various members of the *Colubridae* are listed in Table 6.

Cholinesterase, however, has been shown to be entirely absent from the venom of the rattlesnake (*Crotalus terrificus*),[204] the sand-burrowing viper (*Echis carinatus*),[204] the "daboia" or Russell's chain-viper of India (*Vipera*

[332] C. B. Anfinsen, *J. Biol. Chem.*, *152*, 267–278 (1944).
[333] R. H. S. Thompson and V. P. Whittaker, *Biochem. J.*, *38*, 295–299 (1944).
[334] N. K. Iyengar, K. B. Sehra, B. Mukerji, and R. N. Chopra, *Current Sci.* (*India*), *7*, 51–53 (1938).
[335] A. Marnay, *Compt. rend. soc. biol.*, *126*, 573–574 (1937).
[336] D. Nachmansohn, R. T. Cox, C. W. Coates, and A. L. Machado, *J. Neurophysiol.*, *5*, 499–515 (1942).
[337] L. A. Mounter, *Biochem. J.*, *49*, xlv–xlvi (1951).
[338] L. A. Mounter, *Biochem. J.*, *50*, 122–128 (1951).
[339] D. K. Chaudhuri, *Ann. Biochem. Exptl. Med.* (*India*), *4*, 77–86 (1944).

TABLE 6

CHOLINESTERASE CONCENTRATIONS IN THE VENOM OF VARIOUS MEMBERS OF THE *Colubridae*[a]

Systematic name	Common name	Q_{ChE}[b]	Q_{MCh}[c]	$\frac{Q_{ChE}}{Q_{MCh}}$	Ref.
Acanthophis antarcticus	"Death adder"	9240	7080	1.3	*d*
Bungarus caeruleus	Dark-blue-banded krait	24900	15300	1.6	*d*
Bungarus fasciatus	Yellow-banded krait	18700	22000	0.9	*d–f*
Demansia textilis	Brown whipsnake	140	—	—	*d*
Dendraspis angusticeps	South African mamba	250	—	—	*g*
Denisonia superba	Australian copperhead	11000	9000	1.2	*d*
var. from high altitudes		3300	—	—	*d*
Elaps corallinus	South African venomous coral snake	680	—	—	*d*
Naja bungarus	King cobra, hamadryad	4800	4740	1.0	*d*
Naja flava	Yellow cobra	7200	—	—	*d*
Naja haye	Hooded African cobra, "spy-slange," asp	>1020	—	—	*d*
Naja (Coluber) melanoleuca	Black-lipped cobra	27900	20000	1.4	*d*
Naja (Vel) naja tripudians	Indian spectacled cobra	>13000	—	—	*d–m*
Naja nigricollis	Black-necked cobra	40	—	—	*d*
Notechis scutatus	Tiger snake	3300	2900	1.1	*d*
Notechis scutatus niger	Black tiger snake	4260	—	—	*d*
Notechis (white venom var.)	Tiger snake	3180	—	—	*d*
Pseudechis australis	Australian black-snake (mulga)	90	—	—	*d*
Pseudechis porphyriacus	Australian brown adder	140	—	—	*d*
Sepedon haemachates	South African hooded "ringhals"	6750	4360	1.5	*d*

[a] Adapted from E. A. Zeller, in *Advances in Enzymology*, Vol. VIII, Interscience, New York and London, 1948, p. 470.

[b] Q_{ChE} = microliters of CO_2 per mg. dried venom with acetylcholine.

[c] Q_{MCh} = microliters of CO_2 per mg. dried venom with acetyl-β-methylcholine.

[d] E. A. Zeller, *Experientia*, *3*, 375–376 (1947).

[e] D. K. Chowdhury, *Ann. Biochem. Exptl. Med.* (*India*), *4*, 77–86 (1944).

[f] B. N. Ghosh, *Oester, Chem.-Ztg.*, *43*, 158–163 (1940).

[g] E. A. Zeller, unpublished data; cited from E. A. Zeller, *loc. cit.* above.[a]

[h] F. Bovet-Nitti, *Experientia*, *3*, 283–286 (1947).

[i] F. Bovet and D. Bovet, *Ann. Inst. Pasteur*, *69*, 309–312 (1943).

[j] D. K. Chaudhuri, *Science and Culture* (*Calcutta*), *8*, 238 (1942); *Chem. Abst.*, *37*, 1458 (1943).

[k] N. K. Iyengar, K. B. Sehra, B. Mukerji, and R. N. Chopra, *Current Sci.* (*India*), *7*, 51–53 (1938).

[l] B. B. Sarkar, S. R. Maitra, and B. N. Ghosh, *Indian J. Med. Research*, *30*, 453–466 (1942).

[m] E. A. Zeller and A. Maritz, *Helv. Physiol. Pharmacol. Acta*, *3*, C19-C20 (1945).

russelli),[204,334] the southwest European viper (*Vipera aspis*),[340] as well as from a large additional group of less common members of the *Vipera* family.[341] Marnay and Nachmansohn[342] noted the presence of cholinesterase in the muscles of the foot, leg, and tail of the green lizard (*Lacerta viridis*).

f′. Acetylesterase in Citrus: An enzyme which hydrolyzes acetic acid esters in oranges, lemons, and grapefruit has been described by Jansen and co-workers.[343] The highest concentration of this acetylesterase occurred in the yellow outside coat (flavedo). It decreased progressively toward the center of the fruit. The distribution was similar to that of phosphatase but differed from that of pectinesterase. Although acetylesterase hydrolyzes acetylcholine, it cannot be considered to be a cholinesterase, since it is not inhibited by eserine. The rate of hydrolysis of tributyrin was only 4% of that of triacetin, while monobutyrin was split at a rate which was only 1% of that of monoacetin. All aliphatic acetate esters were hydrolyzed. However, olive oil was not attacked. The enzyme was found to be unstable at a *p*H under 4.0 and was inactivated at temperatures above 35°C.

(*h*) *Preparation of Cholinesterases.* Aldridge[246] described a convenient method for the preparation of true cholinesterase. In preparing this type of enzyme from erythrocytes, Paléus[344] reported that cholinesterase is so firmly bound to the membrane that it is extremely difficult to elute it to any significant degree. Mentha and co-workers[345] noted that, when pseudoagglutination is brought about by treating erythrocytes with dilute alkali, the maximum esterase extraction can be accomplished with a minimum hemolysis. Ord and Thompson[346] prepared soluble cholinesterases from mammalian heart and brain. Bodansky,[347] in his review on cholinesterase, cites Mendel and Rudney,[186] who reported the preparation of a cholinesterase from the brain of the dog. Mendel and Mundell[211] described a procedure for the purification of cholinesterase from dog pancreas. A method for the purification of pseudocholinesterase from horse serum has been outlined by Strelitz.[348] The cholinesterase present in the electric organ of *Electrophorus electricus* (electric eel) was prepared in a highly

[340] E. A. Zeller, V. Kocher, and A. Maritz, *Helv. Physiol. Pharmacol. Acta*, *2*, C63–C64 (1944).
[341] E. A. Zeller, *Experientia*, *3*, 375–376 (1947).
[342] A. Marnay and D. Nachmansohn, *Compt. rend. soc. biol.*, *125*, 489–490 (1937).
[343] E. F. Jansen, R. Jang, and L. R. MacDonnell, *Arch. Biochem.*, *15*, 415–431 (1947).
[344] S. Paléus, *Arch. Biochem.*, *12*, 153–154 (1947).
[345] J. Mentha, H. Sprinz, and R. Barnard, *J. Biol. Chem.*, *167*, 623 (1947).
[346] M. G. Ord and R. H. S. Thompson, *Biochem. J.*, *49*, 191–199 (1951).
[347] O. Bodansky, *Ann. N. Y. Acad. Sci.*, *47*, 521–547 (1946).
[348] F. Strelitz, *Biochem. J.*, *38*, 86–88 (1944).

purified state by Rothenberg and Nachmansohn.[349] All the esterase in the electric tissue appears to be cholinesterase. The cholinesterases of cobra venom and of that of the yellow-banded krait (*Bungarus fasciatus*) have been concentrated twenty-fold and eleven-fold, respectively, as judged by the nitrogen content of the original and of the purified samples.[339,350] The preparations of this enzyme from the *Colubridae* are among the most potent hitherto known. Other preparations of cobra venom cholinesterase include those of Bovet and Bovet[351] and of Bovet-Nitti.[205]

(*i*) *Acetylcholine in Tissues.* A number of workers have made quantitative estimates of the acetylcholine content of tissues. Marquardt and Hirsch[352] reported a total of 1 to 4 μg. % of acetylcholine in ox blood, in which it occurs mostly on the surface of the erythrocyte. Davis[353] noted values of 10 to 70 μg. % in human cerebrospinal fluid, with an average of 28 μg. %, which was approximately four times the concentration which he found in normal blood serum.

The amount of acetylcholine in the whole rat brain was found to remain relatively constant after excision; the quantity in the cortex decreased in time.[354] Freezing in liquid air caused a considerable loss in acetylcholine; this loss did not take place in tissues from eserinized animals.[354]

Virtually all acetylcholine in the brain is in the "bound" or combined form. Acid, mechanical disturbance, freezing, suspension in hypotonic solutions and medium and high K^+ concentrations accelerate liberation from the bound form.[354] Mann, Tennenbaum, and Quastel[355] concluded some years ago that the synthesis of acetylcholine takes place through the intermediate formation of combined acetylcholine. The rate of formation of this complex form of acetylcholine is more rapid than is that of free acetylcholine. The rate of formation of the complex form was not found to be increased in the presence of eserine, which prevents the destruction of free acetylcholine.[355] The addition of potassium ions to eserinized respiring brain tissue slices increased the rate of formation of acetylcholine, but high concentrations of K^+ were inhibitory. An equilibrium was shown to exist between the free and the combined forms. Potassium and ammonium

[349] M. A. Rothenberg and D. Nachmansohn, *J. Biol. Chem.*, *168*, 223–231 (1947).

[350] D. K. Chaudhuri, *Science and Culture* (*Calcutta*), *8*, 238 (1942); *Chem. Abst.*, *37*, 1458 (1943).

[351] F. Bovet and D. Bovet, *Ann. Inst. Pasteur*, *69*, 309–312 (1943).

[352] P. Marquardt and H. H. Hirsch, *Z. physiol. Chem.*, *289*, 131–153 (1952).

[353] J. E. Davis, *Am. J. Physiol.*, *162*, 616–618 (1950).

[354] K. A. C. Elliott and N. Henderson, *Am. J. Physiol.*, *165*, 365–374 (1951).

[355] P. J. G. Mann, M. Tennenbaum, and J. H. Quastel, *Biochem. J.*, *32*, 243–261 (1938).

ions caused an increase in the free form at the expense of the combined form.[356] Synthesis of acetylcholine was inhibited by NH_4^+.

Elliott and Henderson[354] found that a low concentration of free acetylcholine may be maintained in brain in the absence of an anticholinesterase (inhibitor). Bound acetylcholine in brain slices is considerably increased when the tissues are incubated anaerobically in the presence of glucose, whether or not anticholinesterase is present. The increase is less marked in the presence of a high potassium ion concentration, especially when anticholinesterase is absent. It is suggested that the release of acetylcholine from the bound form is reversible.

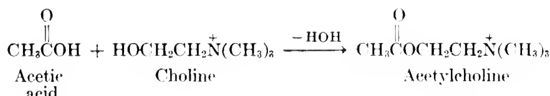
The acetylcholine content of rat brain was shown to vary with the intensity of activity. Thus, Richter and Crossland[357] found that the level during anesthesia is 300% above that during convulsions. Elliott *et al.*[358] also noted that the administration of nembutal to the cat, as well as to the rat, caused brain acetylcholine to increase greatly. The level of acetylcholine decreases as the anesthesia wears off, but it can be raised again by a new administration of the drug.[358] Other anesthetics such as metrazol or picrotoxin increase acetylcholine to some extent in unnarcotized cats, although the administration of these anesthetics decreases the brain acetylcholine level in cats given nembutal.[358] Richter and Crossland[357] observed that the fall in acetylcholine which follows electrical stimulation is transient, and is rapidly reversed on the cessation of the stimulation. The rate of resynthesis of acetylcholine under these conditions was found to be 7 μg. per gram of brain per minute.

d. Choline Acetylase. (*a*) *Introduction.* Although it is generally assumed that the same enzymes catalyze both the synthetic and the hydrolytic reaction, the enzymes responsible for the synthesis and degradation of acetylcholine have generally been considered to be separate entities. While those which effect hydrolysis of this compound are called cholinesterases, those which promote the synthesis of choline and acetic acid to the ester are referred to as choline acetylase. Choline acetylase can be separated from cholinesterase by means of acetone; this solvent completely inactivates the cholinesterase.[359]

(*b*) *Mechanism of Action.* Nachmansohn and John[359] and Nachmansohn and Machado[360] were the first to demonstrate that the homogenized brain tissue synthesizes acetylcholine anaerobically in the presence of adenosine

[356] P. J. G. Mann, M. Tennenbaum, and J. H. Quastel, *Biochem. J.*, *33*, 822–835 (1938).
[357] D. Richter and L. Crossland, *Am. J. Physiol.*, *159*, 247–255 (1949).
[358] K. A. Elliott, R. L. Swank, and N. Henderson, *Am. J. Physiol.*, *162*, 469–474 (1950).
[369] D. Nachmansohn and H. M. John, *J. Biol. Chem.*, *158*, 157–171 (1945).
[360] D. Nachmansohn and A. L. Machado, *J. Neurophysiol.*, *6*, 397–403 (1943).

triphosphate (ATP) and choline, according to the following reaction (1).



The Synthesis of Acetylcholine in the Presence of Choline Acetylase (1)

Feldberg and Mann[361] confirmed the results of Nachmansohn and collaborators; they also demonstrated that practically no synthesis of acetylcholine occurred under aerobic conditions. Reduced glutathione and cysteine strongly activated the aerobic synthesis of acetylcholine. On the other hand, oxidized compounds were found to inhibit the ester synthesis. Glucose, fructose, and certain phosphohexoses blocked acetylcholine synthesis, since the sugars esterified the labile phosphate groups of ATP. Finally, it was reported that K^+ enhanced the synthetic reaction and Ca^{++} inhibited it. Feldberg[362] demonstrated that cell-free powdered dried brain, when suspended in saline solution with eserine and ether, was able to effect a synthesis of acetylcholine at room temperature greater than under any other conditions so far observed.

According to Lipmann and collaborators,[363,364] pantothenic acid is required as a coenzyme for the synthesis of acetylcholine, in addition to the specified enzyme and ATP. Balfour and Hebb[365] have shown that the synthesis of acetylcholine depends upon the action of coenzyme A (CoA). This explains why pantothenic acid acts as a coenzyme, inasmuch as this vitamin is a component of the CoA molecule. For a complete discussion of CoA, the reader is referred to Volume III. In conjunction with CoA, it was shown that the synthesis of acetylcholine could be accelerated by acetate as well as by citrate, both of which could act as acetyl-donors to the system.[365] Lipton and Barron[366] had reported several years earlier that citrate was a suitable substrate, as well as *cis*-aconitate and acetoacetate. These workers recognized that acetylation of choline required the presence of "active" acetate.

In the light of the newer understanding of the mechanism of action of

[361] W. Feldberg and T. Mann, *J. Physiol., 104*, 8–20 (1945).
[362] W. Feldberg, *J. Physiol., 103*, 367–402 (1945).
[363] F. Lipmann, N. O. Kaplan, G. D. Novelli, L. C. Tuttle, and B. M. Guirard, *J. Biol. Chem., 167*, 869–870 (1947).
[364] N. O. Kaplan and F. Lipmann, *J. Biol. Chem., 174*, 37–44 (1948).
[365] W. E. Balfour and C. Hebb, *J. Physiol., 118*, 94–106 (1952).
[366] M. A. Lipton and E. S. G. Barron, *J. Biol. Chem., 166*, 367–380 (1946).

choline acetylase, Korey *et al.*[367] redefined it as "the enzyme apparently specifically concerned with the transfer of acetate from the intermediate product to choline." According to Nachmansohn and co-workers,[368] this "intermediate product" is presumably acetyl coenzyme. These workers reported that an enzyme prepared from pigeon liver extracts was able, in the presence of ATP, to form acetyl coenzyme. The term "acetylkinase" is proposed. Thiolacetate was shown to function in the full system in place of ATP-acetate. In later investigations by Korkes and associates,[369] it was demonstrated that a partially purified choline acetylase, prepared from squid head ganglia, brought about the synthesis of acetylcholine from acetyl-CoA and choline, associated with the appearance of a stoichiometric amount of sulfhydryl groups.

(*c*) *Distribution of Choline Acetylase.* Nachmansohn and Berman[370] reported, in 1946, that choline acetylase was present only in nerve tissue; they were able to prove its occurrence, not only in the vertebrate nervous tissue, but also in the nerves of invertebrates, in sensory nerves, and in electric tissue. In contradistinction to this, the presence of the coenzyme (acetyl-CoA) was demonstrated in brain, liver, heart, and skeletal muscle.

Choline acetylase was obtained from the sciatic nerve of the rabbit by Nachmansohn, John, and Berman.[175] A solution prepared from one gm. of these nerve fibers caused the synthesis of 70 to 90 μg. of acetylcholine per hour, as compared with that of 150 to 200 μg./hr. when comparable preparations from rat or guinea pig brains were employed. The active enzyme was shown to be in that part of the neuron which does not contain the nerve endings and cell bodies. This is considered as offering support for the hypothesis that acetylcholine may be essential not only for the transmission of the nerve impulse across the synapse but also for its propagation along the axon.

Later investigations demonstrated that choline acetylase is present in tissues other than nervous tissue. Nachmansohn and co-workers[371] reported the presence of the enzyme in pigeon breast muscle, in skeletal muscle of the guinea pig, and in cardiac muscle of the rabbit. In no case was any choline acetylase observed in liver or kidney extracts. Comline

[367] S. R. Korey, B. de Braganza, and D. Nachmansohn, *J. Biol. Chem.*, *189*, 705–715 (1951).

[368] D. Nachmansohn, I. B. Wilson, S. R. Korey, and R. Berman, *J. Biol. Chem.*, *195*, 25–35 (1952).

[369] S. Korkes, A. del Campillo, S. R. Korey, J. R. Stern, D. Nachmansohn, and S. Ochoa, *J. Biol. Chem.*, *198*, 215–220 (1952).

[370] D. Nachmansohn and M. Berman, *J. Biol. Chem.*, *165*, 551–563 (1946).

[371] D. Nachmansohn, M. Berman, and M. S. Weiss, *J. Biol. Chem.*, *167*, 295–296 (1947).

and Whatley[372] reported the presence of the acetylcholine-synthesizing enzyme in spleen, when flavine adenine dinucleotide was also present. Comline[373] noted the occurrence of the enzyme in placenta. Holland and Greig[374] found an active choline acetylase in human red blood cells. The presence of this enzyme in red blood cells, as well as the previous demonstration of its occurrence in placenta, both of which tissues have no innervation, proves that the formation of acetylcholine is not necessarily associated with nerve function. Holland and Greig[374] suggest that the formation and breakdown of acetylcholine, as reflected by the enzymes present in the particular tissues involved, is related to some general phenomenon such as control of permeability changes, which is a property of living cells, whether or not they are innervated.

(*d*) *Factors Altering the Activity of Choline Acetylase.* The activity of choline acetylase is dependent upon the presence of the coenzyme and of other products. The presence of magnesium and manganese were shown by Nachmansohn and Berman[370] to enhance the activity of the enzyme. McLennan and Elliott[375] report that the synthesis of acetylcholine by rat brain slices is augmented by a high potassium ion concentration and inhibited by lack of calcium ions. These workers also confirm the similar but less marked effect of magnesium ions. The synthesis of acetylcholine by supplemented brain extracts is strongly dependent upon the ATP concentration.[376] Because of the action of apyrase, respiring brain suspensions contain virtually no ATP.[376] As early as 1943, Nachmansohn and collaborators[377] reported that L(+)-glutamic acid increased the rate of acetylcholine formation four to five times, while D(+)-glutamic acid had practically no effect. It was suggested that L(+)-glutamic acid might be a coenzyme of choline acetylase.

The extent of acetylcholine synthesis was found to be decreased by 44% in the brains of hypophysectomized rats, as compared with the rate in sham-operated controls.[378] The administration of adrenocorticotropic hormone (ACTH) to such hypophysectomized rats induced an increase in the rate of synthesis of acetylcholine in the brain. In a later study, Torda and Wolff[379] showed that a parallelism exists between acetylcholine syn-

[372] R. S. Comline and F. R. Whatley, *Nature, 161,* 350–351 (1948).
[373] R. S. Comline, *J. Physiol., 105,* 6P–7P (1946).
[374] W. C. Holland and M. E. Greig, *Arch. Biochem., 39,* 77–79 (1952).
[375] H. McLennan and K. A. C. Elliott, *Am. J. Physiol., 163,* 605–613 (1950).
[376] H. McLennan and K. A. C. Elliott, *Arch. Biochem., 36,* 89–96 (1952).
[377] D. Nachmansohn, H. M. John, and H. Waelsch, *J. Biol. Chem., 150,* 485–486 (1943).
[378] C. Torda and H. G. Wolff, *Am. J. Physiol, 161,* 534–539 (1950).
[379] C. Torda and H. G. Wolff, *Am. J. Physiol., 169,* 140–149 (1952).

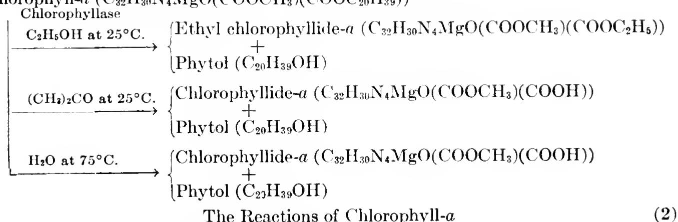
thesis and neuromuscular function. Administration of ACTH increased the ability of the brain of non-operated as well as of hypophysectomized rats to synthesize acetylcholine. These workers[379] suggest that the pituitary gland functions in maintaining the amplitude of the action potential in the nerve unaltered for a period of time during repeated stimulation; it also brings about an optimal acetylcholine synthesis by the release of ACTH. Neither cortisone nor compound F (17-hydroxycorticosterone-21-acetate) was found to duplicate fully the effects of ACTH on acetylcholine synthesis.[380]

The action of choline acetylase was found to be strongly inhibited by methylnaphthoquinone, which has a strong vitamin K action, and also by 2-methyl-1,4-naphthoquinone-8-sulfonic acid.[370] Beiler *et al.*[381] reported that vitamin P and certain other compounds with a flavonoid structure inhibit the choline acetylase action *in vitro*. It is suggested in this case that the inhibition may be due to the quinone-forming properties of these compounds.

e. Chlorophyllase. Although chlorophyllase is not present in the secretions of the gastrointestinal tract, this enzyme is very widely distributed in the plant kingdom, being present in all green-leaved plants. When consumed in uncooked, green, leafy foods, it may reach the stomach and intestine in an active form. Inasmuch as the optimum *p*H for its activity is between 6 and 7, the conditions of acidity in the small intestine may be favorable for its action at some periods.

The enzyme, chlorophyllase, is a relatively specific esterase which hydrolyzes the phytol ester linkage in chlorophyll-*a* to yield phytol and chlorophyllide-*a*. According to Weast and MacKinney,[382] the reaction varies somewhat with the solvent used, according to (2).

Chlorophyll-*a* ($C_{32}H_{30}N_4MgO(COOCH_3)(COOC_{20}H_{39})$)



The Reactions of Chlorophyll-*a* (2)

[380] C. Torda and H. G. Wolff, *Am. J. Physiol.*, *169*, 150–158 (1952).
[381] J. M. Beiler, R. Brendel, M. Graff, and G. J. Martin, *Arch. Biochem.*, *26*, 72–76 (1950).
[382] C. A. Weast and G. MacKinney, *J. Biol. Chem.*, *133*, 551–558 (1940).

The action of chlorophyllase in producing the so-called "crystalline chlorophylls" (chlorophyllides *a* and *b*), was first explained by Willstätter and Stoll.[383] The method of hydrolysis in hot water was discovered by B. E. Lesley and J. W. Shumate (see Weast and MacKinney).[382] Mayer[384] reported that, in a 66% acetone solution, chlorophyllase had an optimum temperature of 25°C. and an optimum *p*H of 6.0 to 6.2. This is the same optimal temperature as that reported for an 80% alcohol solution. The acetone concentrations could be varied between 40 and 70% with no change in activity, but the limits for optimum activity with ethyl alcohol were only from 70 to 80%. Although the optimum temperature for aqueous solutions of chlorophyllase is 75°C., the enzyme may act equally effectively at 50°C. when the leaf has been previously frozen. Apparently the accessibility of the substrate is of prime importance in determining the extent of hydrolysis.

According to Mayer,[384] chlorophyll-*a* is split by chlorophyllase at a rate 1.8 times that of chlorophyll-*b*. This worker reported that great variations in chlorophyllase obtained at different seasons; KCN was shown to have little effect on this esterase. Weast and MacKinney[382] likewise confirmed the fact that potassium cyanide was without effect on chlorophyllase; moreover, sodium fluoride caused only a slight change in activity, but mercuric nitrate ($Hg(NO_3)_2$) in 0.1% concentration produced a complete inactivation of the *Scrophularia* enzyme in alcohol. Finally, these latter workers demonstrated that the chlorophyllase activity of several species of green plants varied widely according to the solvent employed. The rate of hydrolysis in prickly-seed spinach (*Spinacia oleracea*), was highest in water, but there was little activity in acetone or alcohol. In the case of California figwort (*Scrophularia californica*), a very high activity was observed in alcohol but none in water. No chlorophyllase could be demonstrated in the wild oat (*Avena fatua*). These data are summarized in Table 7.

It has recently been shown that chlorophyllase also acts to hydrolyze bacteriophaeophytin-*a* to bacteriophaeophorbid-*a*.[385,386] The former compound is obtained from bacteriochlorophyll-*a* by treatment with acid[386]; in the presence of methanol, bacteriomethylphaeophorbid-*a*, $C_{36}H_{38}N_4O_6 \pm 2H$, is formed.[387] Bacteriochlorophyll-*a*, the commoner of the green pig-

[383] R. Willstätter and A. Stoll, *Untersuchungen über Chlorophyll*, Springer, Berlin, 1913.

[384] H. Mayer, *Z. wissensch. Biol., Abt. E. Planta, 11*, 294–330 (1930).

[385] H. Fischer and K. Bauer, *Ann., 523*, 235–284 (1936).

[386] E. Schneider, *Ber. deut. botan. Ges., 52*, 96–100 (1934).

[387] H. Fischer and J. Hasenkamp, *Ann., 515*, 148–164 (1934).

TABLE 7

PERCENT HYDROLYSIS OF CHLOROPHYLL BY CHLOROPHYLLASE FROM DIFFERENT SOURCES, IN ACETONE (40–70%, 25°C.), ETHANOL (80%, 25°C.), AND WATER (75°C.)[a]

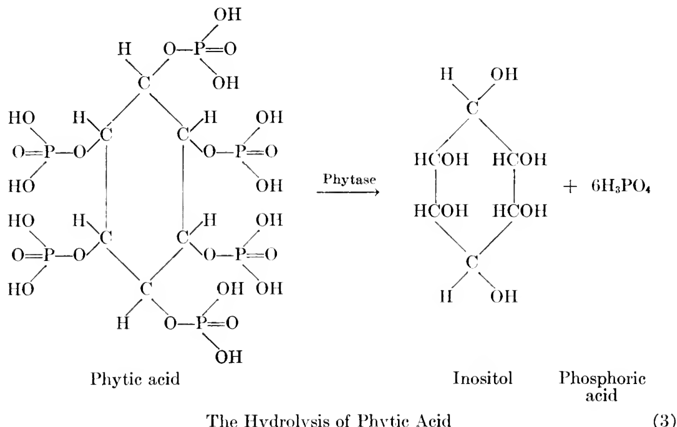
Hydrolysis medium (time)[b]	Month or Season			
	Spinach (*Spinacia oleracea*)			
	March	May	August	November
Ethanol	Trace (24)	0 (24)	0 (24)	68 (27)
Acetone	80 (24)	10 (24)	20 (24)	58 (27)
Water (20 min.)	100	5	75	31.5
	Sunflower (*Helianthus annuus*)			
	July	October	August	September
Ethanol	75 (24)	50 (24)	90 (24)	75 (24)
Acetone	25 (24)	90 (24)	—	100 (1)
Water (20 min.)	50	30	50	100
	Cow-parsnip (*Heracleum lanatum*)			
	March	May		
Ethanol	90 (24)	58 (2)		
Acetone	100 (24)	71 (2)		
Water (20 min.)	10	20		
	Figwort (*Scrophularia californica*)			
	May	May[c]	December	
Ethanol	100 (24)	78 (2)	60 (3)	
Acetone	25 (24)	76 (2)	54 (3)	
Water (20 min.)	0	0	0	
	Wild oat (*Avena fatua*)			
	Spring	Summer		
Ethanol	0	0		
Acetone	0	0		
Water (20 min.)	0	0		

[a] Data from C. A. Weast and G. MacKinney, *J. Biol. Chem.*, *133*, 551–558 (1940).
[b] Values in parentheses are hours of incubation.
[c] Mature leaves previously frozen.

ments in purple bacteria, has been shown by Fischer and Hasenkamp[387] to be closely related to chlorophyll-*a*. It is presumably the photosynthetic principle in these bacteria. As far as can be judged, the green pigment is identical in *Rhodobacillus palustris* (purple bacillus found in swamps), *Rhodovibrio* ("purple" bacteria), and *Thiocystis violacea* (violet-colored sulfur bacterium).[387]

f. Phytase. Phytase was first found in rice bran by Suzuki and Yoshi-

mura.[388] It splits phytic acid into inositol and phosphoric acid according to equation (3).



The Hydrolysis of Phytic Acid (3)

Although the substrate, phytic acid, is widely distributed in plants, this compound assumes added biological importance because of its presence in animal tissues. Rapoport[389] was the first to isolate phytic acid from an animal source, namely the erythrocytes of chicken blood; in this study, it was also shown that in all probability the acid was present in turtle blood. In a later study, Rapoport *et al.*[390] reported that phytic acid occurred in the goose and pigeon.

The enzyme, phytase, has a wide distribution in plant materials.[391,392] McCollum and Hart[393] were the first to note the occurrence of this enzyme in the animal kingdom; these workers reported the presence of phytase in the liver and blood of calves. On the other hand, neither Plimmer,[394] Martland *et al.*,[395] nor Lowe and Steenbock[396] were able to demonstrate this

[388] U. Suzuki and K. Yoshimura, *Tokyo Imp. Univ., Coll. Agr. Bull.*, *1*, 495–502 (1907); with M. Takaishi, *Ibid.*, *1*, 503–512 (1907).
[389] S. Rapoport, *J. Biol. Chem.*, *135*, 403–406 (1940).
[390] S. Rapoport, E. Leva, and G. M. Guest, *J. Biol. Chem.*, *139*, 621–632 (1941).
[391] R. J. Anderson, *J. Biol. Chem.*, *20*, 493–500 (1915).
[392] L. Adler, *Biochem. Z.*, *70*, 1–36 (1915).
[393] E. V. McCollum and E. B. Hart, *J. Biol. Chem.*, *4*, 497–500 (1908).
[394] R. H. A. Plimmer, *Biochem. J.*, *7*, 43–71 (1913).
[395] M. Martland, F. S. Hansman, and R. Robison, *Biochem. J.*, *18*, 1152–1160 (1924).
[396] J. T. Lowe and H. Steenbock, *Biochem. J.*, *30*, 1126–1134 (1936).

enzyme in extracts from the intestine, pancreas, kidney, bones, or in the liver and blood of several species of animals examined (including human blood). However, Patwardhan[397] reported phytase activity in the intestine of the rat; little if any activity was noted in the intestine of the rabbit and guinea pig, or in the bone extracts of any animals studied.

In the later studies of Rapoport *et al.*,[390] phytase was found in the plasma of fowl (goose, pigeon, chicken, and duck), snakes (moccasin and brown water-snakes), fishes (bullhead and black bass), the turtle, and the bullfrog. The presence of this enzyme in the plasma of man was so slight as to be questionable, while none was found in guinea pig, beef, or calf blood. Phytase was found in the erythrocytes only in those cases in which phytic acid was present, namely, goose, chicken, pigeon, and turtle. In these cases, it occurred in an extremely constant amount, in contradistinction to the wide variations of the enzyme in the various plasma samples. On the basis of the properties of the cell and the plasma phytase, Rapoport *et al.*[390] concluded that they are one and the same enzyme.

Phytase is a separate entity which is distinct in nature from the other phosphatases. The addition of the end-products, *i.e.*, inositol and phosphoric acid, has little effect on the activity of the enzyme, but it is inhibited by fluoride ions and, to a greater extent, by oxalate ions. Although Patwardhan[397] reported activation of the rat intestinal phytase by magnesium ions, Rapoport *et al.*[390] could demonstrate no increase in activity on the addition of such ions. When phytase was heated at 50°C. for ten minutes, its activity was depressed, while the enzyme was almost completely destroyed (87%) when subjected to a temperature of 60°C. The action of phytase was highest at a temperature of 37.5°C. The level for optimum *p*H was found to be 6.6 for plasma phytase from the goose, pigeon, turtle, and frog, and 7.0 for that from the chicken.[390]

3. The Role of Bile in Lipid Absorption

While bile contains no lipases, it does play an essential role in the absorption of neutral fats and in that of other lipids. In obstructive jaundice, under which condition bile fails to reach the intestine, there is an immediate marked decrease in the extent of lipid absorption. However, this drop in utilization may not be confined solely to the fats and other lipids, but may also be reflected in the case of proteins and carbohydrates. This latter effect is to be traced only indirectly to the failure of fat to be absorbed. It is believed that the presence of the unabsorbed fat in the gut contents

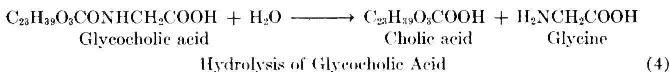
[397] V. N. Patwardhan, *Biochem J.*, *31*, 560–564 (1937).

prevents the water-soluble proteases and amylases from coming into contact with their substrates. Since proteins and carbohydrates are utilizable only after having been hydrolyzed into their simplest units, any factors which retard this hydrolysis will at the same time decrease absorption from the gastrointestinal tract.

(1) The Nature, Distribution, and Properties of the Bile Acids

The most effective components of the bile which facilitate the absorption of fats and of other lipids are the bile acids. Since the bile is normally alkaline, such acids are usually in the form of their salts; hence the term "bile salts" is a more accurate designation. However, in discussions of the chemical nature of these substances and their transformations in the animal, they are almost universally considered to be acids. An especially complete summary of the chemistry of the bile acids is to be found in Sobotka[398] and Fieser and Fieser.[399]

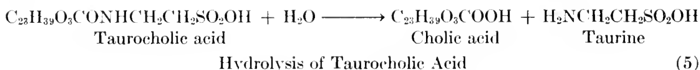
a. Distribution of the Bile Acids. The bile salts having the widest distribution in the animal kingdom are sodium glycocholate and sodium taurocholate. Cholic acid is a component of both of these compounds, but the bile salts differ from each other in that one has glycine conjugated with the cholic acid while, in the second case, taurine replaces glycine. The nature of these conjugated cholic acid compounds becomes evident when they are hydrolyzed. Thus, when glycocholic acid is split, the reaction proceeds as in (4).



$$\underset{\text{Glycocholic acid}}{C_{23}H_{39}O_3CONHCH_2COOH} + H_2O \longrightarrow \underset{\text{Cholic acid}}{C_{23}H_{39}O_3COOH} + \underset{\text{Glycine}}{H_2NCH_2COOH}$$

Hydrolysis of Glycocholic Acid (4)

The reaction occurs in a similar manner in the case of taurocholic acid (5).



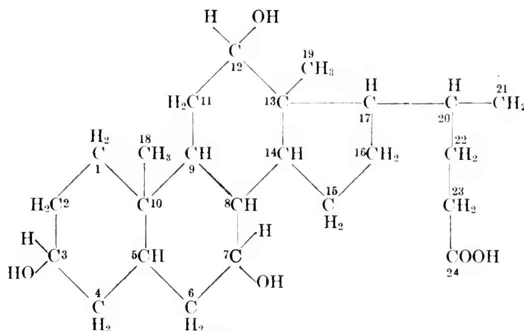
$$\underset{\text{Taurocholic acid}}{C_{23}H_{39}O_3CONHCH_2CH_2SO_2OH} + H_2O \longrightarrow \underset{\text{Cholic acid}}{C_{23}H_{39}O_3COOH} + \underset{\text{Taurine}}{H_2NCH_2CH_2SO_2OH}$$

Hydrolysis of Taurocholic Acid (5)

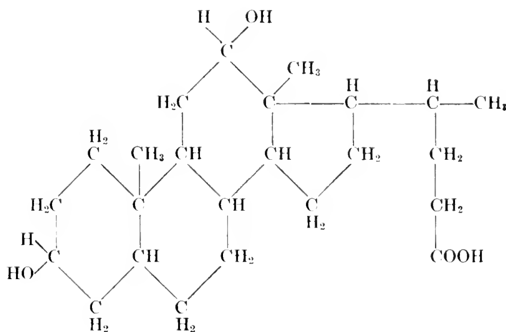
Cholic acid (Gr. χολη′, bile) is 3,7,12-trihydroxycholanic acid. It is derived from cholesterol, and possesses the cyclopentanophenanthrene ring. Instead of belonging to the C_{27} series as is the case with cholesterol, cholic acid contains only 24 carbons. This is because the aliphatic side

[398] H. Sobotka, *The Chemistry of the Sterids,* Williams and Wilkins, Baltimore, 1938.
[399] L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene,* 3rd ed., Reinhold, New York, 1949.

chain located on carbon 17 has only 5 carbons rather than the 8 which occur in cholesterol. The structural formulas for cholic acid (including the numbering system) and for the closely related desoxycholic (or deoxycholic) acid (3,12-dihydroxycholanic acid) are given in formulas IV and V.



(IV) Cholic Acid



(V) Desoxycholic Acid

Glycine or taurine is connected with the cholic or desoxycholic acid through the carboxyl group located on carbon 24.

A number of bile acids are now known which are less widely distributed in nature than are cholic and desoxycholic acid. Chenodesoxycholic acid

(Gr. χη'ν, goose) was discovered as early as 1849 by Marsson[400] in goose bile. It is now known to be 3,7-dihydroxycholanic acid. Chenodesoxycholic acid was isolated from goose bile by Windaus,[401] simultaneously with the preparation of anthropodesoxycholic acid (Gr. ανθρωπος, man) from human bile.[402] These two acids were shown to be identical, and the name used earlier, *i.e.*, chenodesoxycholic acid, is now exclusively used for both. It is an isomer of desoxycholic acid. Ursodesoxycholic acid (Latin, *ursus*, bear), which was first isolated from the bile of the polar bear by Hammarsten in 1901,[403] as the taurine conjugate, and which was later studied by Shoda,[404] has been shown to be a stereoisomer of chenodesoxycholic acid.[405] Lettré[406] found that the isomerism occurs on C_7; the hydroxyl on this carbon atom is *cis* to the C_{10}-methyl in chenodesoxycholic acid and *trans* to the C_{10}-methyl group in ursodesoxycholic acid. Ursodesoxycholic acid has recently been shown to occur in conjugation with glycine,[407] as well as with taurine.[404] Brigl and Benedict[408] have reported the presence of an isomer of glycocholic acid, namely glyconutriacholic acid, in the bile of the water-rat, or swamp beaver (*Myocaster coypus*). The finding of nutriacholic acid has recently been confirmed by Haslewood and Wootton.[409]

3-Hydroxy-6-ketoallocholanic acid is an example of a bile acid containing a ketone group. It was first isolated from hog bile by Fernholz,[410] and its presence in the bile of this species has been confirmed by a number of other workers.[411–414] Trickey[415] has recently reported that the bile acids of hog bile contain approximately 20% of keto acids, most of which consist of 3α-hydroxy-6-ketoallocholanic acid. In reviewing this work, Schoenheimer and Evans[416] suggested that the keto acid probably exists in hog bile as the cholanic acid derivative, but that its conversion to the *allo*

[400] T. Marsson, *Arch. Pharm.*, *108* ([*2*], *58*) 138–148 (1849); *Ann.*, *72*, 317–318 (1849).
[401] A. Windaus, A. Bohne, and E. Schwarzkopf, *Z. physiol. Chem.*, *140*, 177–185 (1924).
[402] H. Wieland and G. Revery, *Z. physiol. Chem.*, *140*, 186–202 (1924).
[403] O. Hammarsten, *Z. physiol. Chem.*, *32*, 435–466 (1901); *36*, 525–555 (1901).
[404] M. Shoda, *J. Biochem.* (*Japan*), *7*, 505–517 (1927).
[405] T. Iwasaki, *Z. physiol. Chem.*, *244*, 181–193 (1936).
[406] H. Lettré, *Ber.*, *68*, 766–767 (1935).
[407] S. Miyazi, *Z. physiol. Chem.*, *250*, 34–36 (1937).
[408] P. Brigl and O. Benedict, *Z. physiol. Chem.*, *220*, 106–112 (1933).
[409] G. A. D. Haslewood and V. Wootton, *Biochem. J.*, *47*, 584–597 (1950).
[410] E. Fernholz, *Z. physiol. Chem.*, *232*, 202–205 (1935).
[411] M. Anchel and R. Schoenheimer, *J. Biol. Chem.*, *124*, 609–611 (1938).
[412] R. Schoenheimer and C. G. Johnston, *J. Biol. Chem.*, *120*, 499–501 (1937).
[413] G. Sugiyama, *J. Biochem.* (*Japan*), *25*, 157–165 (1937).
[414] I. Ido and R. Sakurai, *J. Biochem.* (*Japan*), *29*, 51–55 (1939).
[415] E. B. Trickey, *J. Am. Chem. Soc.*, *72*, 3474–3477 (1950).
[416] R. Schoenheimer and E. A. Evans, Jr., *Ann. Rev. Biochem.*, *6*, 139–162 (1937).

series occurs during its isolation. Wieland and Kishi[417] reported the presence of a keto acid in cattle bile, namely 3-hydroxy-12-ketocholanic acid. Other mono-keto acids, which have been demonstrated to be components of bile, include 3α-hydroxy-7-ketocholanic acid in guinea pig bile,[418] 3,12-dihydroxy-7-ketocholanic acid[419] and 7,12-dihydroxy-3-ketocholanic acid[419]; the last two acids are present in cow bile.

The keto acids may be regarded as intermediates in the formation of the polyhydroxy acids. Thus, on the one hand, it has been demonstrated that the organism can reduce the carbonyl group of the bile acids to the hydroxyl groups,[420,421] or *vice versa*.[422] As a specific example, one may cite the *in vitro* conversion of 3-hydroxy-7-ketocholanic acid to a mixture of ursodesoxycholic and chenodesoxycholic acid reported by Miyazi.[423] On the other hand, the keto acids may originate by oxidation of the polyhydroxy acids. Hoehn, Schmidt, and Hughes[424] have shown that cholic acid (3,7,12-trihydroxycholanic acid) gives rise to a monoketo and a diketo acid during bacterial oxidation by the non-pathogenic intestinal bacterium, *Alcaligenes faecalis* (*Bacterium fecalis alcaligenes*), to triketocholanic acid. The acids which were found are 3,12-dihydroxy-7-ketocholanic acid and 3-hydroxy-7,12-diketocholanic acid. Oxidation takes place in the same order when it is brought about by chromic acid. 3,12-Dihydroxy-7-ketocholanic acid has also been isolated from the bile of the reticulated python (*Python reticulatus*) or boa of Indo-China by Kuroda and Arata[425]; they consider it to be an intermediate metabolite between cholic and desoxycholic acid.

Lithocholic acid (Gr. λιθος, stone) is another bile acid reported by a number of workers. Hans Fischer[426] first isolated it in 1911 from a gallstone obtained from an ox, while Schenck[427] more recently reported its presence in a stone obtained from the gall bladder of a hog. Its presence has also been reported in ox bile,[428] as well as in minute amounts in human

[417] H. Wieland and S. Kishi, *Z. physiol. Chem.*, *214*, 47–58 (1933).
[418] I. Imai, *Z. physiol. Chem.*, *248*, 65–68 (1937).
[419] G. A. D. Haslewood, *Biochem. J.*, *40*, 52–54 (1946).
[420] K. Yamasaki and K. Kyogoku, *Z. physiol. Chem.*, *233*, 29–35 (1935).
[421] K. Yamasaki and K. Kyogoku, *Z. physiol. Chem.*, *235*, 43–46 (1935).
[422] T. Fukui, *J. Biochem.* (*Japan*), *25*, 61–69 (1937).
[423] S. Miyazi, *Z. physiol. Chem.*, *250*, 31–33 (1937).
[424] W. M. Hoehn, L. H. Schmidt, and H. B. Hughes, *J. Biol. Chem.*, *152*, 59–66 (1944).
[425] M. Kuroda and H. Arata, *J. Biochem.* (*Japan*), *39*, 225–226 (1952).
[426] H. Fischer, *Z. physiol. Chem.*, *73*, 204–239 (1911).
[427] M. Schenck, *Z. physiol. Chem.*, *256*, 159–168 (1938).
[428] H. Wieland and P. Weyland, *Z. physiol. Chem.*, *110*, 123–142 (1920).

bile.[429] Lithocholic acid has been shown to be 3-hydroxycholanic acid in which the hydroxy on C_3 has assumed the *trans* position.[430] This *trans* position of the hydroxyl in lithocholic acid is thus similar to that found for the corresponding group in cholic, desoxycholic, and chenodesoxycholic acids.

Hyodesoxycholic acid (Gr. ὗς swine) was originally discovered in hog bile, as early as 1847, by Gundelach and Strecker.[431] More recently its presence in bile from this animal has been reaffirmed.[414,415] It has been found to be present in two isomeric forms, described as the α- and β-acids. Trickey[415] has reported that 40% of the bile acids in hog bile consist of hyodesoxycholic acid.

α-Lagodesoxycholic acid, which was discovered by Kishi[432] in rabbit bile, was considered by this investigator to be a C_{12} epimer of desoxycholic acid. It is now believed to have a different configuration, since the authentic 12-epidesoxycholic acid obtained from 3-hydroxy-12-ketocholanic acid differs in melting point from the natural α-lagodesoxycholic acid.[433]

Haslewood and Wootton[434,435] reported that the bile of three species of *Boidae*, namely the *Boa constrictor occidentalis* (Western boa), *Python molurus* (Indian "tiger" python), and *Python sebae* (African python),[409] contains a hitherto unknown bile acid, which is called pythocholic acid and which has an empirical formula of $C_{24}H_{40}O_5$. This was shown to be readily converted into pythocholic lactone, $C_{24}H_{38}O_4$, by the loss of one molecule of water. In earlier communications,[434] these compounds were referred to as "pythonic acid" and "pythonic lactone." In the case of the African python (*Python sebae*), pythocholic acid, conjugated with taurine, formed the main constituent of the bile salts. Kuroda and Arata,[425] also, isolated pythocholic lactone from the bile of the reticulated python (*Python reticulatus*). Haslewood[436] has confirmed the presence of this lactone in the reticulated python, as well as in an additional species of *Boidae*, namely the anaconda or aquatic boa (*Eunectes murinus*).

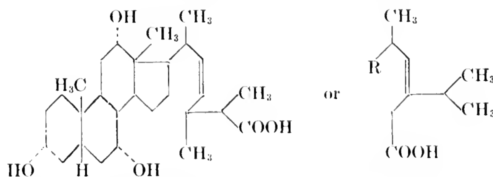
Pythocholic acid was shown to make up 50 to 70% of the total bile acids in seven species of *Boidae*.[435] The acid has three potential hydroxyl groups, *i.e.*, 3, 12, and 16 or 15.[435,436] The configuration of the hydroxyls

[429] H. Sobotka and E. Bloch, *Ann. Rev. Biochem.*, *12*, 45–80 (1943).
[430] L. Ruzicka and M. W. Goldberg, *Helv. Chim. Acta*, *18*, 668–675 (1935).
[431] C. Gundelach and A. Strecker, *Ann.*, *62*, 205–232 (1847); A. Strecker and C. Gundelach, *Ann. chim. phys.*, [*3*], *22*, 38–59 (1848).
[432] S. Kishi, *Z. physiol. Chem.*, *238*, 210–220 (1936).
[433] B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, *25*, 918–935 (1942).
[434] G. A. D. Haslewood and V. M. Wootton, *Biochem. J.*, *46*, x (1950).
[435] G. A. D. Haslewood and V. M. Wootton, *Biochem. J.*, *49*, 67–71 (1951).
[436] G. A. D. Haslewood, *Biochem. J.*, *49*, 718–720 (1951).

in positions 3 and 12 was established as α.[436] Pythocholic acid could be converted to ordinary desoxycholic acid (3α,12α-dihydroxycholanic acid). It was suggested that the presence of pythocholic acid in the bile of the *Boidae* may be the result of a genetic mutation in snakes producing cholic acid, or that the *Boidae* may have retained the ability to produce it from the limbed reptiles. The only other lactone-forming bile acid has been isolated from the chelonian reptiles, *Amyda japonica* (fresh-water alligator turtle) and *Emys orbicularis* (European pond-tortoise).[435]

Although the bile acids most frequently encountered are all members of the C_{24} series, several examples of bile acids have been found which contain 26 to 30 carbons. It is possible that these acids, containing more than 24 carbon atoms, represent intermediates between the parent sterol and the C_{24}-bile acids. Thus, Tukamoto and Kataoka[437] suggested that the tetrahydroxynorsterocholanic acid found in the bile of several fishes is to be regarded as an intermediate between 7-dehydrocholesterol and the C_{24}-bile acids. Wieland and Kishi[417] isolated an acid from cattle bile having the empirical formula, $C_{28}H_{46}O_4$. Because of its possible relation to the sterols, it was given the name, *sterocholic acid*.

Several interesting acids have been obtained from the bile of the toad. The first of these is trihydroxybufosterocholenic acid (Formula VI), which was first isolated by Shimizu and Oda.[438] It has the empirical formula, $C_{28}H_{46}O_5$, and appears to be closely related to sterocholic acid. On the basis of its degradation on ozonization to bisnorcholic acid, Shimizu and Kazuno[439] proved that the hydroxyl groups are on carbons 3, 7, and 12. The second acid isolated from toad bile is trihydroxyisobufosterocholenic acid.[440] This acid is isomeric with trihydroxybufosterocholenic acid; the isomerism has been ascribed to the position of the carboxyl group, which is believed to be on the next to the last or second from the last carbon

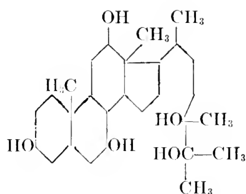


(VI) Postulated Structure of Trihydroxybufosterocholenic Acid

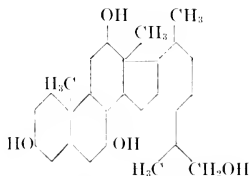
[437] M. Tukamoto and Y. Kataoka, *J. Biochem. (Japan)*, *32*, 473–475 (1940).
[438] T. Shimizu and T. Oda, *Z. physiol. Chem.*, *227*, 74–83 (1934).
[439] T. Shimizu and T. Kazuno, *Z. physiol. Chem.*, *244*, 167–172 (1936).
[440] T. Shimizu, T. Oda, and T. Kazuno, *Z. physiol. Chem.*, *239*, 67–75 (1936).

atom, respectively, on the side chain, instead of occupying the terminal position.[441]

Toad bile acids contain several polyhydroxy derivatives having a steroid nucleus which should be included in this category, although they are not strictly bile acids. One of these (formula VII) is called pentahydroxybufostane ($C_{28}H_{50}O_5$), and it appears to be 3,7,12,24,25-pentahydroxyergostane.[442] It occurs in toad bile as the sulfuric acid ester. The second representative of this group is tetrahydroxynorbufostane (formula VIII),[443] ($C_{27}H_{48}O_4$). Since both of these compounds have been isolated from the winter bile of the hibernating Japanese toad (*Bufo vulgaris japonica*), it is possible that they are products resulting from the depressed metabolism which would not appear in the bile of a toad under normal metabolic conditions.



(VII) Pentahydroxybufostane (?)



(VIII) Tetrahydroxynorbufostane (?)

Several other related compounds which have been found in toad bile include two tetrahydroxycholestanes (or tetrahydroxycholanes), one of which gives 3,7,12-triketo-20,22-epoxycholane on oxidation with chromic acid, while the second one, which occurs as the sulfuric acid ester, is dehydrated during hydrolysis to trihydroxycholene.[429]

The bile acids of the frog have been shown to resemble those of the toad. Haslewood[444,445] reported that the chief bile acid of the European brown frog, *Rana temporaria*, is apparently a sulfate ester of an alcohol which they call "ranol," and which has the empirical formula, $C_{27-28}H_{43-45}(OH)_5$. The molecule is believed to contain a primary alcohol group and secondary hydroxyls on carbons 3, 7, and 12. Trihydroxybisnorsterocholanic acid, $C_{26}H_{44}O_5$, has also been reported by Kurauti and Kazuno[446] in the bile of

[441] T. Shimizu and T. Kazuno, *J. Biochem.* (*Japan*), *25*, 245–247 (1937).
[442] T. Kazuno, *Z. physiol. Chem.*, *266*, 11–30 (1940).
[443] H. Makino, *Z. physiol. Chem.*, *220*, 49–54 (1933).
[444] G. A. D. Haslewood, *Biochem. J.*, *50*, xxxv (1952).
[445] G. A. D. Haslewood, *Biochem. J.*, *51*, 139–143 (1952).
[446] Y. Kurauti and T. Kazuno, *Z. physiol. Chem.*, *262*, 53–60 (1939).

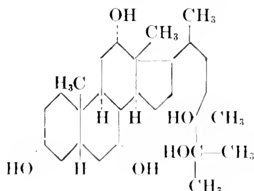
the North American bullfrog (*Rana catesbiana*). Kuroda[447] isolated three kinds of tetrahydroxyhomocholane sulfate from the bile of *Rana catesbiana* Shaw. Upon hydrolysis, three different trihydroxyhomocholenes ($C_{25}H_{42}O_3$) melting at 177°, 196°, and 238°C., respectively, were obtained. Each of the homocholenes had one double bond and an α-configuration of the hydroxyl group on C_3. The sulfate radical was shown to be combined with a tertiary hydroxyl. Komatsubara and Nakanura[448] isolated two isomeric C_{26}-acids from the spotted frog (*Rana nigromaculata nigromaculata*), namely α- and β-trihydroxybisnorsterocholanic acids, which also have an empirical formula of $C_{26}H_{44}O_5$. These acids were proved to exist partly as free acids and, in a larger proportion, conjugated with taurine.

Haslewood[444] reported that the bile of the North American alligator (*Alligator mississippiensis*) contained a mixture of acids, one of which had the formula, $C_{27}H_{46}O_5$, and a melting point[449] of 172°C. Bridgewater and Haslewood[449] converted this naturally occurring acid to "stem acid," $C_{27}H_{46}O_2$, which had a melting point of 104°C. These workers[449] succeeded in partially synthesizing this latter acid from cholanic acid. Another atypical bile acid derivative, namely the lactone of trihydroxysterocholanic acid ($C_{28}H_{46}O_5$), was isolated by Kim[450] from the bile of the European pond-tortoise (*Emys orbicularis*); it is isomeric with trihydroxybufosterocholenic acid. The finding that the same atypical acids occur in the bile of these amphibia as in the case of frogs and toads suggests that they are of an equally primitive evolutionary type.

The type of bile acids, or the component in bile corresponding to the acids, differs in the elasmobranch and in the teleost fishes. Instead of the ordinary bile acids found in the *Teleostei*, acids having empirical formulas of $C_{27}H_{46}O_6$ or $C_{28}H_{48}O_6$ have been reported in a shark from the Chosen Sea[451]; in the case of the blue skate (*Raia batis*), and of the grey Atlantic dogfish (*Squalus acanthias*), a polyhydroxy-derivative, scymnol, was found in place of the bile acids[452]; it was conjugated with sulfuric acid. Hammarsten[453] had reported the presence of "α" and "β" scymnol in the bile of a viviparous species of Greenland shark (*Scymnus* (*Laemargus*) *borealis* Scoresby, also given as *Somniosus microcephalus*, Schneider) a number of years earlier. Although the structure of scymnol has been the object of a

[447] M. Kuroda, *J. Biochem.* (*Japan*), *40*, 169–174 (1953).
[448] T. Komatsubara and H. Nakanura, *J. Biochem.* (*Japan*), *39*, *Abst.*, 8 (1952).
[449] R. J. Bridgewater and G. A. D. Haslewood, *Biochem. J.*, *51*, xxiv (1952).
[450] C. H. Kim, *J. Biochem.* (*Japan*), *30*, 247–249 (1939).
[451] K. Ohta, *J. Biochem.* (*Japan*), *29*, 241–245 (1939).
[452] J. W. Cook, *Nature*, *147*, 388 (1941).
[453] O. Hammarsten, *Z. physiol. Chem.*, *24*, 322–350 (1898).

number of investigations,[454–457] it remains unsolved. However, Fieser and Fieser[399] have postulated the structure shown by IX. Hammarsten[458]



(IX) Scymnol

isolated what proved to be an unusual bile acid from the seals, *Phoca barbata, P. groenlandica* (harp seal), *P. foetida,* and *Cystophora cristata* (hooded or "bladder-nose" seal) and from the walrus (*Odobaenus,* spp.). He called it "β-phocaecholic" acid; it is believed to be 3,7,23-trihydroxycholanic acid,[459] but the configuration of the hydroxyls is not known.

Haslewood[460] reported that, in addition to the regular occurrence of cholic acid in the *Teleostei,* chenodesoxycholic acid was present in many species. A tetrahydroxy acid was reported in four different biles; in all cases, the bile acids were conjugated with taurine.[460] Tukamoto and Kataoka[437] found that the bile acids in two marine fishes, namely the goby or haze (*Acanthogobius flavimanus*) and the tobihaze or walking fish (*Periphthalmus cantonensis*) consisted of cholic and chenodesoxycholic acids. However, Ohta[461] and Isaka and Azato[462] reported a C_{27}-acid having the empirical formula of $C_{27}H_{46}O_6$, which is 3, 6, 12, 24-tetrahydroxynorsterocholanic acid (formula X), in the bile of the "gigi" or bone-fish (*Pelteobagrus nudiceps, recte Pimelodus fulvidracus*), while Isaka and Azato[462] isolated this acid from the "aigo" or tang (*Siganus fuscescens* Hauttyn) and the "fugu" or globe-fish (*Tetrodon porphyleus* Sieb.). Strangely enough, Yamasaki[463] recorded the presence of this same tetrahydroxynorsterocholanic acid in the bile of the hen.

[454] A. Windaus, W. Bergmann, and G. König, *Z. physiol. Chem., 189,* 148–154 (1930).
[455] R. Tschesche, *Z. physiol. Chem., 203,* 263–271 (1931).
[456] H. Asikari, *J. Biochem. (Japan), 29,* 319–324 (1939); *Chem. Zentr., 110, II,* 2666 (1939).
[457] W. Bergmann and W. T. Pace, *J. Am. Chem. Soc., 65,* 477–478 (1943).
[458] O. Hammarsten, *Z. physiol. Chem., 61,* 454–494 (1909); *68,* 109–118 (1910).
[459] A. Windaus and A. van Schoor, *Z. physiol. Chem., 173,* 312–320 (1928).
[460] G. A. D. Haslewood, *Biochem. J., 47,* liv-lv (1950).
[461] K. Ohta, *Z. physiol. Chem., 259,* 53–61 (1939).
[462] H. Isaka and M. Azato, *J. Biochem. (Japan), 32,* 241–247 (1940).
[463] K. Yamasaki, *J. Biochem. (Japan), 38,* 93–98 (1951).

(X) 3,6,12,24-Tetrahydroxynorsterocholanic Acid

Komatsubara and Nakamura[448] isolated a new neutral bile acid conjugated with sulfuric acid, having the empirical formula $C_{30}H_{35}O_5SO_4Na$, and melting at 192°C., from the bile of the eel-tailed loach or weather-fish (*Misgurnus anguillicaudatus*). On hydrolysis, this acid gave rise to a hexahydrosteroid, $C_{30}H_{56}O_6$, which melted at 242–243°C.

Sapocholic acid, which is one of the weaker acids in ox bile, was first assigned the formula $C_{29}H_{46}O_3$ by Wieland and Hanke,[464] but this was later revised to $C_{30}H_{48}O_3$ by Wieland and Seibert.[465] It is now believed that sapocholic acid is not a bile acid but rather a triterpene related to hederagenin and oleanic acid. It probably is of dietary origin.[464] Pearlman[466] identified "compound B," which occurs occasionally in ox bile, as allopregnandiol-3(β),20(β).

b. Conjugation of the Bile Acids. The cholanic acids are usually conjugated with glycine, NH_2CH_2COOH, or taurine, $NH_2CH_2CH_2SO_2OH$. The hog is the only animal in which no taurine is found, and glycine is the sole amino acid conjugated with the cholanic acid. The glyco-acids predominate over the tauro-acids in man, cattle, kangaroo, hippopotamus, and musk-ox.[467] On the other hand, the bile acids of all other species contain a much larger proportion of the tauro-derivatives. Bensch,[468] as early as 1848, reported that the sulfur of crude bile salts exceeds 5%. Pure taurocholic acids have a theoretical content of only about 6% of sulfur. Thus, all carnivorous animals belong in the tauro-group, and many herbivorous and omnivorus animals are also included in this category.[467]

The presence of bile acids in a non-conjugated form has been reported in several instances. As early as 1850, Enderlin[469] discovered the presence

[464] H. Wieland and G. Hanke, *Z. physiol. Chem.*, *241*, 93–99 (1936).

[465] H. Wieland and W. Seibert, *Z. physiol. Chem.*, *262*, 1–19 (1939).

[466] W. H. Pearlman, *J. Biol. Chem.*, *166*, 473–476 (1946).

[467] H. Sobotka, *Physiological Chemistry of the Bile*, Williams and Wilkins, Baltimore, 1937.

[468] A. Bensch, *Ann.*, *65*, 194–203 (1848).

[469] C. Enderlin, *Ann.*, *75*, 162–165 (1850).

Table 8. Composition of Bile Salts Present in the Bile of Various Species[a]

Systematic and (common) names	Acid component in bile: Cholic	Desoxycholic	Chenodesoxycholic	Special acids	Conjugation product: Glycine	Taurine
Primates						
Homo sapiens (man)[b,c]	+[b]	+[c]	+[d]		+	+
Carnivora						
Ursus spp. (bear)[e,f]				*e,f*	+	+
Canis familiaris (dog)[f,g]	+	+[a]				+
Lutra vulgaris (fish otter)[h]	+	+				
Vulpes pecculio sus, Kishida (fox)[h]	+	+				
Felis leopardus (leopard)[e]	+					+
Felis leo (lion)[i]	+					+
Martes melampus melampus (marten)[j]	+	+				+
Thalassarctos (*Ursus*) *maritima* (polar bear)[k]	+			*k*		+
Otaria ursina (Northern fur seal)[l]			+	*l*		
Nyctereutes viverrinus (Japanese raccoon-dog)[j]	+	+				+
Mustela itali (weasel)[h]	+					
Ungulata						
Bos spp. (cattle (ox))[b,q]	+[b]	+[m]	+[d]	*n-q*	+	+
Hippopotamus amphibius (hippopotamus)[r]	+				+	+
Sus spp. (hog)[t]	+		+	*s-x*		
Ovis spp. (sheep)[f,y,z]	+[y]	+[y]	+[y]	*x*		+
Rodentia						
Cavia cobaya (guinea pig)[aa]			+	*aa*		
Citellus (*Dauricus*) *mongolicus*, var. *ramosus* (Manchurian pouched ground-squirrel or souslik)						*y*
Leporidae fam. (rabbit)[ac]	+			*ab*		
Marsupialia						
Macropus giganteus (bush-kangaroo, wallaby)[ad,ae]	+	+	+		+[ae]	+
Aves						
Anas domestica (domestic duck)[af]	+		+			
Mareca penelope (wild duck, widgeon)[af]	+		+			
Meleagris gallipavo (turkey)[af]	+		+			
———(chicken)[ag–ai]	+[ag]		+	*y*		+
Phasianus colchicus karpowi (pheasant "kizi")[aj]						+

Table continued

TABLE 8 (*continued*)

Systematic and (common) names	Acid component in bile				Conjugation product	
	Cholic	Desoxy-cholic	Cheno-desoxy-cholic	Special acids	Gly-cine	Tau-rine
Reptilia						
Amyda japonica (fresh-water or alligator tortoise)[ak]				*ak*		
Emys orbicularis (European pond-tortoise)				*al*		
Elaphe carinata (chicken-snake)[am]	+					+
Bungarus multicinctus (many-banded krait)[am]	+					
Amphibia						
Bufo vulgaris japonica (common toad (Japan))	+			*an-ap*		+
Rana catesbiana (American bull-frog)				*aq*		

[a] Adapted from H. Sobotka, *Physiological Chemistry of Bile*, Williams and Wilkins, Baltimore, 1937, and H. Sobotka and E. Bloch, *Ann. Rev. Biochem.*, *12*, 45–80 (1943).
[b] L. J. Thenard, *Ann. Chim.* [*1*] *64*, 103–112 (1807); H. Demarçay, *Ann. chim. phys.* [*2*] *67*, 177–203 (1838); *Compt. rend.*, *6*, 199–201 (1838); *Ann.*, *27*, 270–291 (1838).
[c] Lassar-Cohn, *Ber.*, *27*, 1339–1346 (1894).
[d] H. Wieland and G. Reverey, *Z. physiol. Chem.*, *140*, 186–202 (1924).
[e] Ursodesoxycholic acid, S. Miyazi, *Z. physiol. Chem.*, *250*, 34–36 (1937); cited by T. Kimura, *J. Biochem.* (*Japan*), *26*, 327–331 (1937).
[f] Ursodesoxycholic acid, A. Bensch, *Ann.*, *65*, 194–203 (1848).
[g] O. Hammarsten, *Z. physiol. Chem.*, *43*, 127–144 (1904).
[h] C. H. Kim, T. S. Sihn, and K. Takahashi, *J. Biochem.* (*Japan*), *29*, 35–40 (1939).
[i] K. Tanaka, *Z. physiol. Chem.*, *213*, 199–200 (1931).
[j] K. Ohta, *J. Biochem.* (*Japan*), *29*, 31–34 (1939).
[k] Ursodesoxycholic acid, O. Hammarsten, *Z. physiol. Chem.*, *32*, 435–466 (1901); *36*, 525–555 (1902).
[l] β-Phocaecholic acid, T. Mori, *J. Biochem.* (*Japan*), *28*, 161–164 (1938).
[m] P. Latschinoff, *Ber.*, *18*, 3039–3047 (1885).
[n] Lithocholic acid, H. Wieland and P. Weyland, *Z. physiol. Chem.*, *110*, 123–142 (1920).
[o] 3-Hydroxy-12-ketocholanic acid, H. Wieland and S. Kishi, *Z. physiol. Chem.*, *214*, 47–58 (1933).
[p] Sterocholic acid, H. Wieland and G. Hanke, *Z. physiol. Chem.*, *241*, 93–99 (1936).
[q] Sapocholic acid, H. Wieland and W. Seibert, *Z. physiol. Chem.*, *262*, 1–19 (1939).
[r] O. Hammarsten, *Z. physiol. Chem.*, *74*, 123–141 (1911).
[s] Lithocholic acid, M. Schenck, *Z. physiol. Chem.*, *256*, 159–168 (1938).
[t] Hyodesoxycholic acid, C. Gundelach and A. Strecker, *Ann.*, *62*, 205–232 (1847); A. Strecker and C. Gundelach, *Ann. chim. Phys.*, *22*, 38–59 (1848).
[u] 3-Hydroxy-6-ketoallocholanic acid, E. Fernholz, *Z. physiol. Chem.*, *232*, 202–205 (1935).
[v] 3-Hydroxy-6-ketoallocholanic acid, M. Anchel and R. Schoenheimer, *J. Biol. Chem.*, *124*, 609–611 (1938).
[w] 3-Hydroxy-6-ketoallocholanic acid, R. Schoenheimer and C. G. Johnston, *J. Biol. Chem.*, *120*, 499–501 (1937).
[x] 3-Hydroxy-6-ketoallocholanic acid; dehydroanthropodesoxycholic acid (sheep), T. Ido and R. Sakurai, *J. Biochem.* (*Japan*), *29*, 51–55 (1939).
[y] $C_{27}H_{46}O_6$ acid (hen), K. Yamasaki, *J. Biochem.* (*Japan*), *38*, 93–98 (1951).
[z] A. Strecker, *Ann.*, *70*, 149–197 (1849).

Table footnotes continued

TABLE 8 (*footnotes continued*)

[aa] 3-Hydroxy-7-ketocholanic acid, I. Imai, *Z. physiol. Chem.*, *248*, 65–68 (1937).
[ab] Lithocholic acid; α- and β-lago-desoxycholic acid, S. Kishi, *Z. physiol. Chem.*, *238*, 210–220 (1936).
[ac] N. Ishino, *J. Biochem. (Japan)*, *28*, 133–136 (1938).
[ad] J. Schlossberger, *Ann.*, *110*, 244–245 (1859).
[ae] T. Kimura, *J. Biochem. (Japan)*, *26*, 327–331 (1937).
[af] T. Ishihara and T. Mori, *Arb. med. Fakultät Okayama*, *5*, 538–541 (1935–1938).
[ag] K. Yamasaki, *J. Biochem. (Japan)*, *18*, 323–324 (1933).
[ah] S. Yonemura, *J. Biochem. (Japan)*, *6*, 287–296 (1926).
[ai] K. Takahashi, *Z. physiol. Chem.*, *255*, 277–280 (1938).
[aj] K. Ohta, *Arb. med. Fakultät Okayama*, *6*, 193–195 (1939).
[ak] Tetrahydroxysterocholanic lactone; trihydroxysterocholanic lactone; bile acids unconjugated, K. Yamasaki and M. Yuuki, *Z. physiol. Chem.*, *244*, 173–180 (1936).
[al] Trihydroxysterocholanic lactone, C. H. Kim, *J. Biochem. (Japan)*, *30*, 247–249 (1939).
[am] H. Imamura, *J. Biochem. (Japan)*, *31*, 21–22 (1940).
[an] Bufodesoxycholic acid, T. Okamura, *J. Biochem. (Japan)*, *8*, 351–360 (1928); *10*, 5–9 (1928).
[ao] Trihydroxysterocholenic acid, T. Shimizu and T. Oda, *Z. physiol. Chem.*, *227*, 74–83 (1934).
[ap] Tetrahydroxycholene, pentahydroxybufostane, T. Kazuno, *Z. physiol. Chem.*, *266*, 11–30 (1940).
[aq] Tetrahydroxycholane; trihydroxy-bis-norsterocholanic acid, Y. Kurauti and T. Kazuno, *Z. physiol. Chem.*, *262*, 53–60 (1939).

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of free cholic acid in cadaver bile, while Salkowski[470] demonstrated a nitrogen-free bile acid in human bile. The occurrence of such non-conjugated bile acids is apparently related to a decrease in functional activity of the hepatic tissue. Thus, when the reticuloendothelial system of this organ is temporarily blocked by the injection of India-ink, a decrease in the conjugated bile acid concentration in the secretion is noted, according to Yonemura.[471] Moreover, Schönheimer *et al.*,[472] as well as Colp and Doubilet,[473] were able to isolate large amounts of cholic and desoxycholic acids from the bile of a patient with hepatic disease, although only a minimum content of *conjugated* bile acids could be demonstrated by gasometric methods. Free chenodesoxycholic acid has also been found in abnormal human bile.[467] Since the non-conjugated bile acids have been detected chiefly under pathological conditions, the question which one naturally poses is whether the non-conjugated acids ever occur normally in bile. Colp and Doubilet[473] are of the opinion that normally only about 80% of the bile acids are conjugated. Josephson and Jungner[474] reported the presence of unconjugated acids in the gall-bladder bile of several species; it was especially high in rabbit bile and in one sample of guinea pig bile. Maeda[475]

[470] E. Salkowski, *Berl. klin. Wochschr.*, *54*, 63–64 (1917).
[471] S. Yonemura, *J. Biochem. (Japan)*, *7*, 101–116 (1926).
[472] R. Schönheimer, E. Andrews, and L. Hrdina, *Z. physiol. Chem.*, *208*, 182–184 (1932).
[473] R. Colp and H. Doubilet, *Arch. Surg.*, *33*, 913–925 (1936).
[474] B. Josephson and G. Jungner, *Biochem. J.*, *30*, 1953–1959 (1936).
[475] K. Maeda, *Arb. med. Fakultät Okayama*, *6*. 101–102 (1938).

TABLE 9
TELEOST FISHES HAVING CHOLIC ACID AND TAURINE IN THEIR BILE

Systematic name	Common name	Ref.
Anago anago	"Ginanago," anago fish, Conger eel	*a*
Carassius auratus	"Funa," goldfish	*a*
Cyprinus carpio	"Koi," carp	*a*
Muraenesox cinereus	"Hamo," moray	*b*
Mugil cephalus	"Bora," striped grey mullet	*c*
Pagrosomus major	"Tai-fish," pagrus, porgy	*b*
Plecoglossus altivelis	"Ayu," sweetfish	*d*
Salmo milktschish (Oncorhyncus masou)	"Masu," Pacific quinnat salmon	*e*
Scomberemorus niphonius	"Sawara," kingfish (mackerel)	*f*
Seriola quinqueradiata	"Buri," amberfish (amberjack)	*g*
Stereolepis ishinagi	"Ishinagi"	*h*
Tetrodon porphyleus Sieb.	"Fugu-fish," globefish (puffer, sea-hedgehog)	*i*
Thunnus thynnus	"Maguro," blue-fin tuna	*h*

[a] T. Hatakeyama and T. Okamura, *J. Biochem. (Japan), 9,* 333–335 (1928).
[b] T. Hosokawa, *Okayama-Igakkai-Zasshi, 39, No. 446,* 311–314 (1927).
[c] S. Miyazi and T. Kimura, *J. Biochem. (Japan), 26,* 337–339 (1937).
[d] T. Kobayasi, *Okayama-Igakkai-Zasshi, 39, No. 449,* 923–928 (1927).
[e] T. Fukui, *Arb. med. Fakultät Okayama, 5,* 201–204 (1937).
[f] M. Schoda, *Okayama-Igakkai-Zasshi, 39, No. 447,* 443–445 (1927).
[g] H. Makino, *J. Biochem. (Japan), 19,* 249–251 (1934).
[h] T. Shimada, *J. Biochem. (Japan), 26,* 181–185 (1937).
[i] M. Teraoka, *J. Biochem. (Japan), 8,* 341–350 (1928).

isolated non-conjugated chenodesoxycholic acid from normal chicken bile. No free acid has been isolated from normal human bladder bile.

Although under normal conditions most, if not all, of the bile acids are conjugated, this situation may be reversed if large amounts of non-conjugated bile acids are administered. According to Foster, Hooper, and Whipple[476] and Whipple alone,[477] free cholic acids given to an animal with a bile fistula are conjugated to the extent of the glycine or taurine available. It was postulated by these workers that only after the exhaustion of all available glycine and taurine would the free cholic acid appear in the bile. That this concept must be modified is evident from the more recent tests of Josephson, Jungner, and Rydin,[478] who injected comparatively large doses of cholic acid (250 mg.) into cats and rabbits. A cannula was placed in the common bile duct of the animal, and the gall bladder was ligated.

[476] M. G. Foster and C. W. Hooper, *J. Biol. Chem., 38,* 355–366 (1919); with G. H. Whipple, *Ibid., 38,* 367–377; 379–392; 393–411; 413–420; 421–433 (1919).
[477] G. H. Whipple, *Physiol. Revs., 2,* 440–459 (1922).
[478] B. Josephson, G. Jungner, and A. Rydin, *Acta Med. Scand., 97,* 237–253 (1938).

Table 10
Teleost Fishes Having Cholic and Chenodesoxycholic Acids and Taurine in Their Bile

Systematic name	Common name	Ref.
Conger myriaster	"Maanago," eel	*a*
Euthynnus pelamis	"Katsuwo," little tuna	*b*
Monacanthus cirrhifer	"Kawahagi," file-fish, trigger-fish	*c*
Nibea mitsukurii, Jordan and Snyder	"Nibe," corvina	*d*
Paralichthys olivaceus	"Hirame," flat-fish, sole	*e*
Engraulis japonica, Temminck and Schlegel	"Iwashi," anchovy	*f*
Sebastodes inermis	"Mebaru," rock-fish	*c*
Sebastodes matsubarae, Hilgendorf	"Ako," rock-fish	*g*
Siganus fuscescens, Houttuyn	"Aigo" (Nagasaki), tang	*h*
Parasilurus asotus (*Silurus pararulus*)	"Namazu," sheat-fish, cat-fish, shad, wels	*a*
Sparus macrocephalus	"Kurodae," sea-bream	*i*
Theragra chalcogramma	"Suketo," North Pacific wall-eye pollack	*e*

[a] K. Takahashi and T. Mori, *Arb. med. Fakultät Okayama*, *6*, 358–360 (1940).
[b] G. Sugiyama, *Arb. med. Fakultät Okayama*, *6*, 175–177 (1939).
[c] H. Ashikari, C. H. Kim, and T. S. Sihn, *Arb. med. Fakultät Okayama*, *6*, 136–140 (1938).
[d] T. S. Sihn and K. Maeda, *Arb. med. Fakultät Okayama*, *5*, 542–544 (1938).
[e] T. S. Sihn and C. H. Kim, *Arb. med. Fakultät Okayama*, *6*, 49–53 (1938).
[f] N. Takeuti, *Arb. med. Fakultät Okayama*, *5*, 319–322 (1937).
[g] T. Ishihara, *Arb. med. Fakultät Okayama*, *5*, 535–537 (1938).
[h] T. Fukui, *Arb. med. Fakultät Okayama*, *5*, 201–204 (1937).
[i] T. Hasagawa, *Arb. med. Fakultät Okayama*, *6*, 84–86 (1938).

During the 30-minute interval following the injection, an enormous amount of bile salts was excreted, and most of the salts were free. In the succeeding periods, a larger proportion of the bile salts was excreted in conjugated form, and less free cholate appeared. The experiments were later confirmed on human subjects.[479]

Josephson[480] suggested an alternative hypothesis to explain his results. The time required after the injection of free cholate before conjugated bile acids appear in the bile may be the interval necessary for mobilizing or producing the glycine or taurine needed for conjugation. The reaction is similar to that involved in the formation of hippuric acid after benzoic acid is administered; in fact, Josephson[480] considers it as not improbable

[479] B. Josephson and H. Larsson, *Acta Med. Scand.*, *99*, 140–146 (1939).
[480] B. Josephson, *Physiol. Revs.*, *21*, 463–486 (1941).

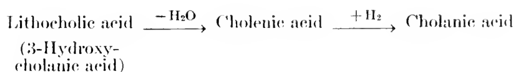
that the same enzymatic mechanism mediates both syntheses. The diminished excretion of hippuric acid observed in the Quick test after benzoate is given to individuals having parenchymatous liver disorders is believed to result from a failure in the glycine supply rather than from an impediment in the enzymatic mechanism. Similar reasoning would apply to the appearance of free cholic acid in the bile after the administration of large doses of free cholic acid.

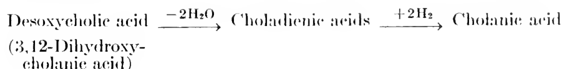
The distribution of acids and related derivates in the bile of different species of animals, as well as the component used in conjugation, are summarized in Table 8 (page 80). Tables 9 and 10, on pages 83 and 84, give data on the composition of the bile of teleost fishes.

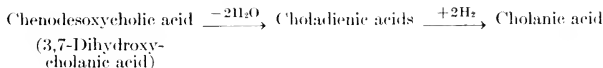
c. Properties of the Bile Acids. The bile acids are found under natural conditions largely in the conjugated form. They occur as water-soluble acids in which the carboxyl of the steroid acid is combined in peptide linkage with either glycine or taurine. The greatest variations in properties are to be found in the non-conjugated (nitrogen-free) acids obtained by hydrolysis of the conjugated compounds.

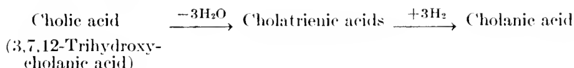
The ring systems, as is evident from the structural formulas already presented, are characteristic of the steroids. The configuration at C_5 is similar to that in coprostanol (A/B = *cis*) while the rest of the molecule corresponds to the natural sterols.

One important reaction of the nitrogen-free bile acids is their tendency to lose water when heated in a vacuum. The alcoholic groups are removed, unsaturated acids are formed, all of which are convertible to the same saturated cholanic acid on hydrogenation (6).









Conversion of Bile Acids to Cholanic Acid (6)

TABLE 11. CONFIGURATION OF THE HYDROXYL GROUPS, MELTING POINTS, SPECIFIC ROTATION AND SOURCES OF VARIOUS NATURAL AND SYNTHETIC BILE ACIDS[a]

Position of hydroxyls		Name of acid	M.p., °C.	α	Source
Fieser[a]	Author				
3α	—	Lithocholic[b]	184–186	+32°	Man,[c] ox,[b,d] rabbit[e]
3β	—	3β-Hydroxycholanic	176–177	+26°	Synthetic[f,g]
6α	6β	6α-Hydroxycholanic	221–222	+ 8.5°	Synthetic[h]
7α	—	7α-Hydroxycholanic	96–102	—	Synthetic[i,j]
11β	11α	11β-Hydroxycholanic	85–86[k]	+50°[k]	Synthetic[l]
12α	12β	12α-Hydroxycholanic	90–95	+43.5°	Synthetic[m,n]
12β	12α	12β-Hydroxycholanic	110–116	+38°	Synthetic[n]
3α,6α	—	"α"-Hyodesoxycholic[o]	196–197	+ 8°	Hog, boar; synthetic[p]
3α,6β	—	3α,6β-Dihydroxycholanic	205–208[o]	+37°	Synthetic[o,p]
3β,6α	—	"β"-Hyodesoxycholic[q]	189–190	+ 5°	Hog; synthetic[p]
3β,6β	—	3β,6β-Dihydroxycholanic	250[r]; 258[p]	—	Synthetic[p,r]
3α,7α	—	Chenodesoxycholic[s,t]	140[t]; 153–154[v]	+11°	Man, ox, goose, hen, bear,[u] guinea pig; synthetic[v]
3α,7β	—	Ursodesoxycholic[u]	203[u]; 199[v]	+57°	Bear; synthetic[v]
3α,11α	—	3α,11α-Dihydroxycholanic	147	+22°	Synthetic[w]
3α,11β	—	3α,11β-Dihydroxycholanic	200	+55°	Synthetic[x]
3β,11β	3β,11α	3β,11β-Dihydroxycholanic	139–140[y]	+50°[y]	Synthetic[z]
3α,12α	—	Desoxycholic[aa,ab]	176–177	+53°	Man, ox, deer, goat, dog, sheep, antelope, rabbit[a]
3β,12β	—	12-Epidesoxycholic	186	+38°	Synthetic[ab]
7α,12α	—	Isodesoxycholic	210–211	+27°	Synthetic[ac,ad]
—	—	7,12-Dihydroxycholanic[ae]	226–227	—	Synthetic[af]
11α,12α	—	11,12-Dihydroxycholanic[ag]	211–214	+ 3.2°	Synthetic[ah]
11β,12β	—	11,12-Dihydroxycholanic[ai]	170.5–174.5	+56°[ai]	Synthetic[aj]
—	—	"α"-Lagodesoxycholic[e]	156–157	+80°	Rabbit
—	—	"β"-Lagodesoxycholic[e]	213	+37°	Rabbit
3α,7α,12α	—	Cholic[a]	196–198	+37°	Man, ox, goat, sheep, antelope
3α,11α,12α	—	3α,11α,12α-Trihydroxycholanic	173–175	+31°	Synthetic[ak]
3α,11α,12β	—	3α,11α,12β-Trihydroxycholanic	164–166	+45°	Synthetic[ak]
3α,11β,12α	—	3α,11β,12α-Trihydroxycholanic	145–146	+54°	Synthetic[ak]
3α,11β,12β	3α,11β,12α	3α,11β,12β-Trihydroxycholanic	177	+43°	Synthetic[al]
3,7,23	—	"β"-Phocaecholic[am]	222–223	+27°[a]	Walrus, seal
—	—	Nutriacholic[an]	198	—	Beaver

[a] Adapted from L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949. The terminology "α" and "β" is based upon the configuration of cholestanol.

[b] H. Wieland and P. Weyland, *Z. physiol. Chem.*, *110*, 123–142 (1920).

[c] H. Wieland and R. Jacobi, *Z. physiol. Chem.*, *148*, 236–244 (1925).

[d] H. Fischer, *Z. physiol. Chem.*, *73*, 204–239 (1911).

[e] S. Kishi, *Z. physiol. Chem.*, *238*, 210–220 (1936).

[f] F. Reindel and K. Niederländer, *Ber.*, *68*, 1243–1246 (1935).

[g] E. Fernholz, *Z. physiol. Chem.*, *232*, 97–100 (1935).

[h] W. M. Hoehn, J. Linsk, and R. B. Moffett, *J. Am. Chem. Soc.*, *68*, 1855–1857 (1946). Subsequent work by these authors has demonstrated the α configuration for this compound (R. B. Moffett and W. M. Hoehn, *J. Am. Chem. Soc.*, *69*, 1995–1996 (1947).

[i] H. Wieland and E. Dane, *Z. physiol. Chem.*, *210*, 268–281 (1932).

[j] H. Wieland and W. Kapitel, *Z. physiol. Chem.*, *212*, 269–277 (1932).

[k] Methyl ester.

[l] H. Reich and T. Reichstein, *Helv. Chim. Acta*, *26*, 562–585 (1943).

[m] J. Barnett and T. Reichstein, *Helv. Chim. Acta*, *21*, 926–938 (1938).

[n] M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, *26*, 2097–2101 (1943).

[o] R. B. Moffett and W. M. Hoehn, *J. Am. Chem. Soc.*, *69*, 1995–1996 (1947).

[p] M. Tukamoto, *J. Biochem. (Japan)*, *32*, 451–460, 467–472 (1940).

[q] T. Kimura, *Z. physiol. Chem.*, *248*, 280–284 (1937).

[r] J. S. Moffatt, *J. Chem. Soc.*, *1947*, 812–815.

[s] A. Windaus, A. Bohne, and E. Schwarzkopf, *Z. physiol. Chem.*, *140*, 177–185 (1924).

[t] H. Wieland and G. Reverey, *Z. physiol. Chem.*, *140*, 186–202 (1924).

[u] T. Iwasaki, *Z. physiol. Chem.*, *244*, 181–193 (1936).

[v] M. Tukamoto, *J. Biochem. (Japan)*, *32*, 461–465 (1940).

[w] W. P. Long and T. F. Gallagher, *J. Biol. Chem.*, *162*, 511–519 (1946).

[x] R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie, and E. C. Kendall, *J. Biol. Chem.*, *166*, 345–365 (1946).

[y] Methyl ester of 3-acetate.

[z] J. Press, P. Grandjean, and T. Reichstein, *Helv. Chim. Acta*, *26*, 598–606 (1943).

[aa] T. Reichstein and M. Sorkin, *Helv. Chim. Acta*, *25*, 797–805 (1942).

[ab] B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, *25*, 918–935 (1942).

[ac] R. Grand and T. Reichstein, *Helv. Chim. Acta*, *28*, 344–349 (1945).

[ad] S. Kuwada and S. Morimoto, *Bull. Chem. Soc. (Japan)*, *17*, 147–152 (1942).

[ae] Referred to by author as 7,13-dihydroxycholanie.

[af] H. Wieland, E. Honold, and J. Pascual-Vila, *Z. physiol. Chem.*, *130*, 326–337 (1923).

[ag] Configuration established on basis of *cis*-addition of oxidation reagent and on the basis of optical rotation.

[ah] H. B. Alther and T. Reichstein, *Helv. Chim. Acta*, *25*, 805–821 (1942); this may be identical with 11,12-dihydroxycholanic acid (m. p., 204–208°C.) prepared by R. E. Marker, A. C. Shabica, E. E. Jones, H. M. Crooks, Jr., and E. L. Wittbecker, *J. Am. Chem. Soc.*, *64*, 1228–1229 (1942).

[ai] Methyl ester.

[aj] H. Reich, *Helv. Chim. Acta*, *29*, 581–586 (1946).

[ak] T. F. Gallagher, *J. Biol. Chem.*, *162*, 539–548 (1946).

[al] O. Wintersteiner, M. Moore, and K. Reinhardt, *J. Biol. Chem.*, *162*, 707–723 (1946).

[am] A. Windaus and A. van Schoor, *Z. physiol. Chem.*, *173*, 312–320 (1928).

[an] P. Brigl and O. Benedict, *Z. physiol. Chem.*, *220*, 106–112 (1933).

The alcoholic groups in general have an α-orientation.[481] The hydroxyl on C_3 is not in the correct position to be precipitated by digitonin. Data on the melting point, specific rotation, and configuration of the hydroxyl groups are summarized in Table 11.

d. Synthesis of the Bile Acids. As is evident from Table 11, an increasing number of cholanic acids, related to the natural bile acids, have been obtained synthetically. One of the successful procedures has been the reduction of the corresponding diketo compounds. Thus, when Tukamoto[482] applied the Meerwein-Pondorf reduction[483,484] to 3,6-diketocholanic acid, he was able to prepare the following amounts of the four possible dihydroxycholanic acids[482]: $3\alpha,6\alpha$- (hyodesoxycholic acid),[485] 15%; $3\alpha,6\beta$-, 2%; $3\beta,6\alpha$-("β" hyodesoxycholic acid), 1.5%; and 3β, 6β-, 8%. Tukamoto[486] prepared $3\alpha,6\beta$- and $3\beta,6\beta$-dihydroxyallocholanic acids by the application of the same reduction procedure to 3,6-diketoallocholanic acid.

Although Iwasaki[405] was able to prepare only $3\alpha,7\alpha$-dihydroxycholanic acid (chenodesoxycholic acid) by catalytic hydrogenation of 3,7-diketocholanic acid, Miyaji[423] reported the production of the two C_7 isomers on hydrogenation of 3α-hydroxy-7-ketocholanic acid, namely chenodesoxycholic acid ($3\alpha,7\alpha$-dihydroxycholanic acid) and ursodesoxycholic acid ($3\alpha,7\beta$-dihydroxycholanic acid). Tukamoto,[486] likewise, was able to synthesize the $3\alpha,7\alpha$- and $3\alpha,7\beta$-dihydroxycholanic acids by reduction of the corresponding diketone.

The production of four enantiomorphs of the 3,6-dihydroxycholanic acids has been carried out readily by the use of the 3α- or 3β-hydroxy acid having a keto group in position 6. Tukamoto[487] obtained $3\alpha,6\alpha$-(hyodesoxycholic acid), and $3\alpha,6\beta$-dihydroxycholanic acids in 30 and 9.3% yield, respectively, by reduction of 3α-hydroxy-6-ketocholanic acid; similarly, $3\beta,6\beta$- and $3\beta,6\alpha$-dihydroxycholanic acids were prepared in yields of 33 and 10%, respectively, when the 3β-hydroxy 6-ketone was reduced.

e. Interrelations of the Bile Acids. The fact that certain specific bile acids are characteristic of the bile of different species of animals would

[481] The terminology "α" and "β" is in accordance with a system proposed by L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949, which is based upon the configuration of cholestanol.

[482] M. Tukamoto, *J. Biochem. (Japan)*, *32*, 451–460 (1940).

[483] H. Meerwein and R. Schmidt, *Ann.*, *444*, 221–238 (1925).

[484] H. Meerwein, B. v. Bock, B. Kirschnick, W. Lenz, and A. Migge, *J. prakt. Chem.*, *147*, 211–225 (1937).

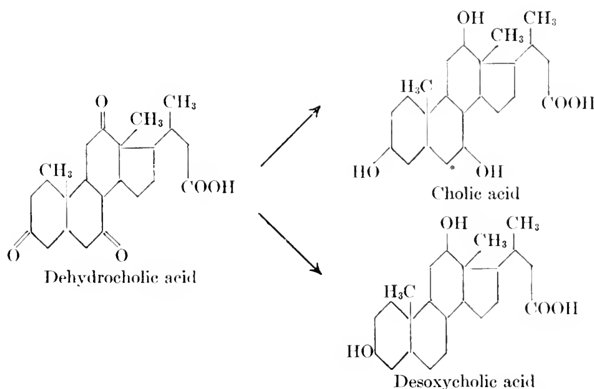
[485] A. Windaus and A. Bohne, *Ann.*, *433*, 278–287 (1923).

[486] M. Tukamoto, *J. Biochem. (Japan)*, *32*, 461–465 (1940).

[487] M. Tukamoto, *J. Biochem. (Japan)*, *32*, 467–472 (1940).

argue that the animal body mediates reactions which favor the production of its own characteristic type of bile acid. The nature of some of these reactions can be demonstrated by following the changes of various types of bile acids in different species of animals.

(*a*) *Reduction of Ketocholanic Acids.* Most animals possess the ability to reduce a triketo bile acid completely to the corresponding trihydroxy compound, or partially to the dihydroxy compound, with the simultaneous loss of an oxygen molecule. Takamori[488] demonstrated that staphylococci and pneumococci reduced dehydrocholic acid (3,7,12-triketocholanic acid) not only at C_3 and C_7 but also on C_{12} to produce cholic acid (7). As an example of a less complete reduction, Kim[489] reported that desoxycholic acid resulted after the intravenous injection of dehydrocholic acid in rabbits. Although desoxycholic acid is not a normal constituent of the bile of guinea pigs,[418,490] Sasaki[490] was able to demonstrate the presence of small amounts of it in the bile of this species after massive doses of dehydrocholic acid.



Conversion of Dehydrocholic Acid to Desoxycholic Acid after Feeding of Massive Doses to Guinea Pigs (7)

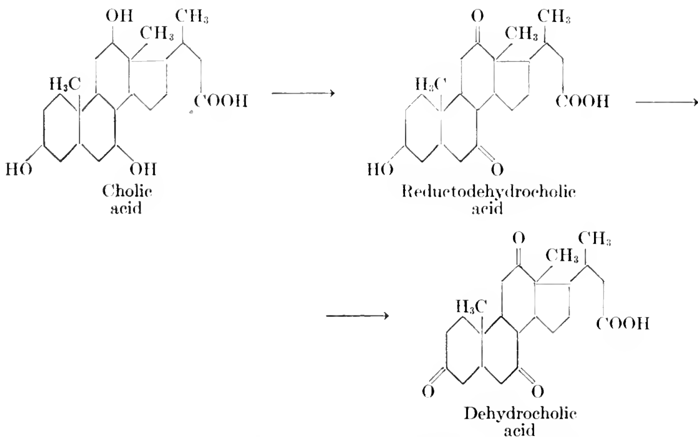
When reductodehydrocholic acid (3α-hydroxy-7,12-diketocholanic acid) is given to guinea pigs, desoxycholic acid is formed, which is excreted in the bile as the free desoxycholic acid or as the glycodesoxycholic acid.[491]

[488] M. Takamori, *J. Biochem. (Japan)*, *39*, 255–258 (1952).
[489] C. H. Kim, *Z. physiol. Chem.*, *255*, 267–270 (1938).
[490] T. Sasaki, *J. Biochem. (Japan)*, *32*, 87–90 (1940).
[491] T. Sasaki, *J. Biochem. (Japan)*, *32*, 81–86 (1940).

When 3-hydroxy-12-ketocholanic acid was given parenterally to rabbits, Mori[492] was able to isolate considerable amounts of desoxycholic acid (3,12-dihydroxycholanic acid). Desoxycholic acid was likewise demonstrated in the bile after the feeding of 3α-hydroxy-6-ketocholanic acid[493]; this unusual finding is explained by Tukamoto on the hypothesis that, when foreign bile acids are fed, the normal pathways for conversion of the bile acids are inhibited.

(*b*) *Removal of Hydroxyl Groups.* Not only may the number of hydroxy groups be decreased, along with a concomitant reduction in ketone groups, but this may also occur in cholic acid (3,7,12-trihydroxycholanic acid) from which ketone groups are absent. Thus, Kim[494] proved that, in the case of guinea pigs, desoxycholic acid is excreted both in the bile and in the urine after the administration of cholic acid.

(*c*) *Oxidation of Hydroxy- to Keto-Acids.* Under certain conditions the animal organism is able to bring about the reverse change to that illustrated in (*a*), namely the oxidation of hydroxycholanic acids to the corresponding keto acids. Sasaki[491] reported that, after the incubation of guinea pig liver brei with cholic acid (3,7,12-trihydroxycholanic acid), he was able to



Conversion of Cholic Acid and Reductodehydrocholic Acid to Dehydrocholic Acid in the Guinea Pig (8)

[492] T. Mori, *Z. physiol. Chem.*, *258*, 143–146 (1939).
[493] M. Tukamoto, *Z. physiol. Chem.*, *260*, 210–216 (1939).
[494] C. H. Kim, *Z. physiol. Chem.*, *261*, 97–102 (1939).

isolate the corresponding triketo acid, namely dehydrocholic acid. On the other hand, he was unable to demonstrate a similar oxidation with the liver brei of cattle. It was also demonstrated that the triketo acid originates after reductodehydrocholic acid is given to guinea pigs (8); under such conditions the product is excreted in the urine.

Another example of oxidation of the hydroxy- to the keto-bile acids is the conversion of ursodesoxycholic acid (3,7-dihydroxycholanic acid) to 3,7-diketocholanic acid. Isaka[495] demonstrated this oxidation in tissue brei of both human and rat cancer. In the case of rat carcinoma (Flexner), an intermediate product was also identified, namely 3-hydroxy-7-keto-cholanic acid. It is concluded that the reduction process or the oxidation process may occur in the case of the bile acids, depending upon the oxygen supply in the particular tissue involved.

(*d*) *Change in Isomeric Form.* Kyogoku[496] has shown that, when 3α-hydroxy-7,12-diketocholanic acid was administered to toads, its epimeric form, 3β-hydroxy-7,12-diketocholanic acid, could be detected in the urine. A similar phenomenon was observed by Tukamoto,[493] who found 3β-hydroxy-6-ketoallocholanic acid in the urine of rabbits after the parenteral administration of 3α-hydroxy-6-ketocholanic acid. In this case not only did an epimerization occur at the 3 position, but the steroid structure assumed an allo configuration.

(*2*) *The Action of Bile in Lipid Absorption*

It has long been recognized that the presence of bile in the intestine is a necessary concomitant of the absorption of fats and other lipids. Rachford[497] first demonstrated that pancreatic lipase was activated by bile, and this observation has since been confirmed many times. According to Loevenhart and Souder,[498] the amount of bile required for the activation of pancreatic lipase varies with the type of substrate, and is a function of the bile salt concentration. In the case of triglycerides composed of long-chain fatty acids, 2–4% of bile salts are required to produce the maximum activation of the lipase; in simple esters the optimum concentration of bile salts amounts to only 0.1%. However, a fair degree of lipolytic activity is known to obtain even in the complete absence of the bile salts.

Another action of bile which facilitates the digestion and absorption of

[495] H. Isaka, *J. Biochem.* (*Japan*), *32*, 131–135 (1940).
[496] K. Kyogoku, *Z. physiol. Chem.*, *246*, 99–105 (1937); *250*, 253–257 (1937).
[497] B. K. Rachford, *J. Physiol.*, *12*, 72–94 (1891).
[498] A. S. Loevenhart and C. G. Souder, *J. Biol. Chem.*, *2*, 415–425 (1906–1907).

fats is its ability to produce emulsification of fats.[499] Haller first recognized this emulsifying property of bile many years ago, although unrecorded observations must frequently have been made of this behavior before the time of Haller. It is now known that, in addition to the bile acids, the phospholipids and soaps in bile contribute to the emulsifying activity. However, the action of bile salts does not seem to be due merely to an improved emulsification, inasmuch as Tidwell and Nagler,[500] as well as Annegers,[501] demonstrated that other emulsifiers are ineffective in improving absorption.

However, the most important action of bile in facilitating the absorption of lipids from the gastrointestinal tract is to be traced to the production of water-soluble coordination compounds of the fatty acids with the bile salts. Such "choleic acids" enable the fatty acids to diffuse through the walls of the mucosa cells lining the gut into the aqueous medium existing there.

A hitherto unsuspected action on the part of the bile salts on intestinal digestion is their effect on intestinal motility. Meyer and McEven[502] reported that conjugated bile acids, desoxycholic acid and cholic acid, as well as the choline salts of these latter two acids, and choline itself, exert analogous effects on the small and large intestine of the guinea pig, when applied to the internal surface. Low doses were shown to cause stimulation of the rhythmic movements, while higher doses tended to produce an increased tonus, thus counteracting the wave movements. Bile salts had a greater tendency to stimulate the oscillatory movements, while choline had a stronger effect on tonus. Conjugated bile salts were shown to have the weakest action, cholic acid a somewhat stronger influence, while desoxycholic acid produced the strongest effect. While choline itself tended to produce a spastic arrest of the gut movements, the choline salts of the bile acids principally maintained a slow longitudinal and peristaltic movement at a high tonus. It is not known whether or not these effects are mediated by the bile salts under ordinary conditions.

a. Choleic Acids and Related Coördination Compounds. The solvent action of bile salts on the fatty acids was demonstrated by Moore and Rockwood[503] in 1896. It was shown that, whereas oleic acid was soluble to the extent of 4–5% in ox bile, stearic acid was much less soluble. How-

[499] C. G. Lehmann, *Lehrbuch der physiologische Chemie*, Leipzig, 1850, 2nd ed., translated by J. C. Morris, Blanchard & Lea, Philadelphia, 1856, pp. 173–174, 254.

[500] H. C. Tidwell and M. E. Nagler, *Proc. Soc. Exptl. Biol. Med.*, *81*, 12–15 (1952).

[501] J. H. Annegers, *Proc. Soc. Exptl. Biol. Med.*, *81*, 277–278 (1952).

[502] A. E. Meyer and J. P. McEven, *Am. J. Physiol.*, *153*, 386–392 (1948).

[503] B. Moore and D. P. Rockwood, *Proc. Roy. Soc. London*, *60*, 438–442 (1896).

ever, when stearic and oleic acids were mixed in a 1:1 ratio, 15% of the mixture (7.5% of each acid) could be brought into solution in bile.[504] The solubility could be doubled by adding an equivalent amount of sodium carbonate. The solvent action of bile acids was found to be much less in the case of the neutral fats.[505]

The unique ability of certain bile acids to form stable coordination compounds must, in large part, be responsible for their solvent action which allows them to transport the fatty acids into the mucosa cells. Cholic acid possesses the property of crystallization. The solvent is retained most tenaciously, and can be removed from the crystals only by prolonged heating under reduced pressure. This unusual property of cholic acid is magnified many times in desoxycholic acid.

(*a*) *Discovery.* The explanation for the behavior of cholic and desoxycholic acid in bringing about a solution of fatty acids and other lipids is largely based upon the brilliant research of Wieland and Sorge[506] on the substance known as "choleic acid." This term had been coined in 1838 by Demarçay[507]; for several years it had been applied to a number of acidic substances of biliary origin. However, in 1885, Latschinoff[508] applied this name to a nitrogen-free acid which occurred with cholic acid in varying proportions, and which appeared to resemble the recently discovered desoxycholic acid.[509] The designations "choleic" and "desoxycholeic" were used more or less synonymously by a number of workers[510,511] until the studies of Wieland and Sorge.[506]

The circumstances which led to the discovery of the nature of choleic acid are of considerable interest. In investigating its composition, the acid was subjected to dehydration by vacuum distillation by a method analogous to that previously applied to desoxycholic acid. Instead of obtaining a choladienic acid which was isomeric with that obtained from the desoxycholic acid, it was found that the two unsaturated acids were identical. However, it was observed that a small amount of palmitic or stearic acid was formed as well; it was proved that the aliphatic fatty acids did

[504] E. Pflüger, *Arch. ges. Physiol. (Pflüger's)*, *88*, 299–338 (1902).

[505] W. Marcet, *Proc. Roy. Soc. London*, *9*, 306–308 (1858); *Arch. path. Anat. Physiol. (Virchow's)*, *17*, 204 (1859).

[506] H. Wieland and H. Sorge, *Z. physiol. Chem.*, *97*, 1–27 (1916).

[507] H. Demarçay, *Ann.*, *27*, 270–291 (1838); *Ann. chim. phys.*, [2], *67*, 177–203 (1838); *Compt. rend.*, *6*, 199–201 (1838).

[508] P. Latschinoff, *Ber.*, *18*, 3039–3047 (1885).

[509] F. Mylius, *Ber.*, *19*, 369–379; 2000–2009 (1886).

[510] V. Wahlgren, *Z. physiol. Chem.*, *36*, 556–567 (1902).

[511] A. Gullbring, *Z. physiol. Chem.*, *45*, 448–458 (1905).

not arise through a rupture of the original ring by pyrolysis, but that the acid was in a molecular combination with desoxycholic acid.

(*b*) *General Properties.* Choleic acid was found to contain 8 molecules of desoxycholic acid associated with one molecule of fatty acid. The complex was shown to dissolve in alkali without decomposition. A similar compound could be produced synthetically by allowing crystallization to occur from an alcoholic solution of desoxycholic acid and stearic acid. It has a sharp melting point (186°C.), which is appreciably higher than that of desoxycholic acid or of stearic acid. The stearic acid was found to be bound so firmly that it could be split off only with difficulty as, for example, by transformation of the complex into products which were dehydrated or oxidized. There is no explanation as to how this change can be mediated in the animal body.

According to von Fürth and Minibeck,[512] Chiray and Cuny have determined that the ratio of bile acids to fat, fatty acids, and phosphatides varies between 1:8 and 1:24 in the case of children, between 1:6 and 1:20 in cats, and from 1:1.5 to 1:5 in rats. According to these results, the bile salts are very economically used in the intestinal absorption of these lipids. It has been suggested that the bile acids function by forming an adsorption layer on the intestinal membranes which acts like a turnstile in admitting the fatty acids through the membrane. Such a conception would explain the relatively small amount of bile salts used in the absorption of large quantities of the lipids.[512] Verzár and Kúthy[513] believe that they have proved the existence of choleic acids in solution. Although the minimum *p*H values for obtaining clear soap solutions with oleate, palmitate, and stearate were found to be 8.1, 9.1, and 9.0 respectively, this condition could be effected in the presence of conjugated bile salts at *p*H values of 6.18, 6.35, and 6.16, respectively. Thus, conjugated bile acids are able to maintain solution and diffusion on the acid side of neutrality in a *p*H range where the unconjugated bile acids do not dissolve.

(*c*) *Occurrence and Types of Choleic Acids.* There is every reason to believe that the "choleic acid principle" extends to lipids other than the fatty acids.[514] It is known that the absorption of ingested cholesterol as well as of that secreted by the intestinal mucosa requires bile.[515–517] Desoxycholic acid is an excellent solvent for cholesterol; it was shown that a

[512] O. von Fürth and H. Minibeck, *Biochem. Z.*, *237*, 139–158 (1931).

[513] F. Verzár and A. Kúthy, *Biochem. Z.*, *205*, 369–379 (1929); *210*, 265–280 (1929).

[514] J. A. Gardner and H. Gainsborough, *Quart. J. Med.*, *23*, 465–483 (1930).

[515] M. A. Rothschild and A. O. Wilensky, *Am. J. Med. Sci.*, *156*, 239–247 (1918).

[516] H. Salomon and L. L. Silva, *Arch. Verdauungskrankh.*, *36*, 353–359 (1926); *Biol. Abst.*, *1*, 468 (1927).

[517] H. Beumer and F. Hepner, *Z. ges. exptl. Med.*, *64*, 787–797 (1929).

desoxycholic acid and cholesterol combination dialyzed through a collodion membrane.[506] The passage of cholesterol through plates of agar and gelatin was shown to be aided by bile salts,[518] although Breusch[519] does not subscribe to the idea that cholesterol in bile salts can diffuse. Schönheimer[520] was able to demonstrate that the speed of absorption of cholesterol from the intestines of mice was accelerated by the presence of desoxycholic acid, while Loeffler[521] found that cholesterol storage in the liver of rabbits was greatly increased when cholesterol was fed along with the bile acids.

In addition to fatty acids and cholesterol, many other organic compounds can combine with desoxycholic acid. Sobotka[398] named these compounds "*acholic*" constituents of choleic acids. This group includes aliphatic alcohols, ethers, ketones, esters, and hydrocarbons, as well as cyclic alcohols, ketones, and hydrocarbons. Huntress and Phillips[522] described 34 paraffin hydrocarbons which form with desoxycholic acid (in methanol) definite molecular compounds which are easily reproducible. It was found that one mole of hydrocarbon is combined with 2 to 8 molecules of the bile acids.

A combination of β-carotene and desoxycholic acid in a molecular ratio of 1:1 has recently been prepared from an alcohol and carbon tetrachloride solution.[523] Almquist and Klose[524] described a choleic acid compound of vitamin K, melting at 185°C. and containing about 10% of the acholic derivative; the bile salt:vitamin K ratio was reported to be about 8:1 in this preparation. Stable additive compounds have also been prepared with alkaloids. It is therefore obvious that a large number of choleic acids exist. These vary in composition not only according to the acholic constituent but also in the ratio of desoxycholic acid to the acholic component in the molecule. The term, choleic acid, should therefore be considered as referring solely to a group of compounds rather than to a specific substance. The choleic acids containing palmitic, stearic, or oleic acids which have been prepared from bile are sometimes designated as "natural choleic acids." A list of the choleic acids, including their melting points and coordination numbers, is given in Tables 12 to 14.

(*d*) *Coordination Number.* It was assumed by Wieland and Sorge[506] that the choleic acids contain one bile acid for each $-CH_2CH_2-$ unit.

[518] O. von Fürth and R. Scholl, *Biochem. Z.*, *222*, 430–456 (1930).
[519] F. L. Breusch, *Biochem. Z.*, *293*, 280–294 (1937).
[520] R. Schönheimer, *Biochem. Z.*, *147*, 258–263 (1924).
[521] K. Loeffler, *Z. physiol. Chem.*, *178*, 186–191 (1928).
[522] E. H. Huntress and R. F. Phillips, *J. Am. Chem. Soc.*, *71*, 458–460 (1949).
[523] G. Milazzo and G. Giacomello, *Gazz. chim. ital.*, *70*, 73–86 (1939).
[524] H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc.*, *61*, 745–746 (1939).

TABLE 12

MELTING POINTS AND COÖRDINATION NUMBERS OF CHOLEIC ACIDS WITH ALIPHATIC ACIDS, ALCOHOLS, ETHERS, HYDROCARBONS, OR KETONES AS THE ACHOLIC COMPONENTS[a]

Acholic component	Number of carbons[b]	M.p., °C.	Coördi-nation number
Acids, fatty:			
Formic[c]	1	[d]	—
Acetic[e]	2	140–145	1
Propionic[c,f]	3	*ca.* 168	3(?)
Butyric[f]	4	—	2
Butyric[c,f]	4	*ca.* 170	4
Isobutyric[f]	1,3	—	2
Valeric[f]	5	—	4
Isovaleric[f]	1,4	—	2
Methyl-ethyl-acetic[f,g]	1,2,2	—	2
Trimethyl-acetic[f]	1,1,1,2	—	2
Caproic[h]	6	—	(4)
Heptylic[i]	7	169	4
Caprylic[i]	8	*ca.* 171	4
Pelargonic[i]	9	173	6
Capric[i]	10	174–175	6
α-Butyl-caproic[j]	4,6	147–148	4
α-Ethyl-caprylic[j]	2,8	*ca.* 154	2
β-Methyl-pelargonic[j]	1,9	170.5	6
Undecylic[i]	11	176	6
Lauric[i]	12	176–177	6
α-Ethyl-capric[j]	2,10	176	4
α-Methyl-undecylic[j]	1,11	179	4
Tridecylic[i]	13	177–178	6
Myristic[i]	14	181–182	6
Pentadecylic[i]	15	183.5	8
Palmitic[c,i]	16	184–185	8
Heptadecylic[i]	17	185.5–186.5	8
Stearic[c,i]	18	186–187	8
α-Ethyl-palmitic[j]	2,16	178–179	8
Arachidic[i]	20	188–189	8
Behenic[i]	22	189–189.5	8
Lignoceric[i]	24	193–193.5	8
Cerotic[i]	26	193–194	8
α-Dodecyl-myristic[j]	12,14	—	4,8
Cetyl-octyl-acetic[i]	2,8,16	188–188.5	8
$C_{27}H_{54}O_2$[i]	27	194.5	8
Montanic	28	196	8(?)
Acids, monoethenoid:			
Crotonic[f]	4	—	3
Tiglic[i]	1,4	171.5–172	4

Table continued

TABLE 12 (*continued*)

Acholic component	Number of carbons[b]	M.p., °C.	Coordination number
Undecylenic[i]	11	171–171.5	6
Oleic[a,k]	18	185–186; 188	8
Elaidic[k]	18	187–188	8
Erucic[k]	22	193.5–194.5	8
Brassidic[i,k]	22	184; 193–194	8
Acid, diethenoid:			
Sorbic[i]	6	171–172	4
Acids, ethynoic:			
Sterolic[i]	18	174	8
Behenolic[i]	22	183–183.5	8
Acids, substituted:			
Dibromobehenic[i]	22	177	8
Acids, alicyclic:			
Hydnocarpic[l]	16	183	8
Chaulmoogric[l]	18	185–186	8
Dihydrohydnocarpic[e]	16	182–183	8
Dihydrochaulmoogric[e]	18	186	8
Acids, dicarboxylic[f]:			
Succinic	4	171	2
Succinic	4	169	4(?)
Glutaric	5	[d]	0
Adipic	6	165.5	3
Pimelic	7	[m]	4
Suberic	8	172.5	4
Azelaic	9	172	4
Sebacic	10	181	4
Undecanedioic	11	172	4
Dodecanedioic	12	183.5	6
Brassylic	13	181	6
Tetradecanedioic	14	186.5	6
Thapsic	16	188	6
Octadecanedioic	18	191.5	6
Eicosanedioic	20	192	6
Alcohols:			
Methyl[a]	1	[d]	0
Ethyl[a]	2	125	1
	2	—	2
Caprylyl[n]	8	169.5–170	4
Myristyl[n]	14	183.5–184.5	6
Chaulmoogryl[e]	18	185–186	8
Dihydrochaulmoogryl[e]	18	186–187	8
Ethers:			
Diethyl	2,2	153[o]	—
Diethyl	2,2	153–155[e]	1
Dioxane[p]	4	173.5–174.5	(?)

Table continued

TABLE 12 (*continued*)

Acholic component	Number of carbons[b]	M.p., °C.	Coordination number
Hydrocarbons, saturated:			
Dichloro-ethylene[a]	2	—	2
Hexamethylethane[p]	4,4	[d]	0
Undecane[q]	11	183	6
Dodecane[p]	12	186–187[p]	(?)
Pentadecane[q]	15	189.5–190	8
Hexadecane	16	192–193[p]	8[q]
Pentatriacontane[q]	35	201.5	8
Tritetracontane[q]	43	*ca.* 201	8
Hydrocarbons, unsaturated:			
Cetene[q]	16	190	8
Δ^{13}-Heptacosene[q]	27	195.5–196.5	8
Ketones:			
Acetone[o]	3	160–162	1
Acetyl acetone[r]	5	162	3

[a] Most of the data are adapted from H. Sobotka, *The Chemistry of the Sterids*, Williams and Wilkins, Baltimore, 1938, p. 112.
[b] Where more than one number is listed, the numerals represent the numbers of carbons in each branched chain. The value for the main carbon chain is listed last.
[c] H. Wieland and H. Sorge, *Z. physiol. Chem.*, *97*, 1–26 (1916).
[d] No choleic acid formed.
[e] E. Vahlen, *Z. physiol. Chem.*, *23*, 99–108 (1897).
[f] H. Sobotka and A. Goldberg, *Biochem. J.*, *26*, 555–568 (1932).
[g] H. Sobotka and A. Goldberg, *Biochem. J.*, *26*, 905–909 (1932).
[h] Not prepared.
[i] H. Rheinboldt, H. Pieper, and P. Zervas, *Ann.*, *451*, 256–273 (1927).
[j] E. Chargaff and G. Abel, *Biochem. J.*, *28*, 1901–1906 (1934).
[k] W. Marx and H. Sobotka, *J. Org. Chem.*, *1*, 275–279 (1936).
[l] N. P. Buu-Hoi, *Z. physiol. Chem.*, *278*, 230–235 (1943).
[m] By diagram only.
[n] H. Reinboldt, O. König, and R. Otten, *Ann.*, *473*, 249–259 (1929).
[o] A. W. Downie, L. Stent, and S. M. White, *Brit. J. Exptl. Pathol.*, *12*, 1–9 (1931).
[p] L. F. Fieser and M. S. Newman, *J. Am. Chem. Soc.*, *57*, 1602–1604 (1935).
[q] H. Rheinboldt, P. Braun, E. Flume, O. König, and A. Lauber, *J. prakt. Chem.*, *153*, 313–336 (1939).
[r] H. Sobotka and J. Kahn, *Biochem. J.*, *26*, 898–904 (1932).

However, such a regular proportionality has not been found to exist in the extensive studies summarized in Tables 12 and 13. Only those coordination numbers could be verified in the choleic acids investigated which had previously been recognized as fitting in with the general structural arrangement of inorganic coordination compounds according to the work of Werner. The number of units must be such that a symmetrical arrangement can obtain around a pivotal atom, molecule, or ion. In the case of inorganic coordination compounds, the numbers most commonly encountered are 4, 6, and 8, although 2 or 3 may occasionally occur. There is no way in

TABLE 13
MELTING POINTS AND COORDINATION NUMBERS OF CHOLEIC ACIDS WITH SIMPLE ALIPHATIC ESTERS AS ACHOLIC COMPONENTS[a]

Acholic component	M.p., °C.	Coordination number[b]		
		E	Al	Ac
Formate, hexyl	167.5	4	4	0
Heptyl	168–169	4	4	0
Octyl	170.5–171	6	6	0
Dodecyl	179	6	6	0
Tetradecyl	185	8	8	0
Cetyl	187.5	8	8	0
Acetate, methyl	*ca.* 145	3	0	1
Ethyl	*ca.* 140–145	3	1	1
Propyl	*ca.* 142–146	4	?	1
Hexyl	168–169	4	4	1
Heptyl	169	4	4	1
Octyl	172–173	6	6	1
Dodecyl	180.5	6	6	1
Tetradecyl	185.5–186	8	8	1
Cetyl	189	8	8	1
Propionate, methyl	*ca.* 144–148	3	0	?
Ethyl	*ca.* 145–149	4	1	?
Heptyl	170–171	4	4	?
Octyl	173.5–174.5	6	6	?
Dodecyl	182	6	6	1
Cetyl	187	8	8	1
Butyrate, methyl	*ca.* 148–152	4	0	4
Ethyl[c]	—	2,4	1	4
Octyl	176–176.5	6	6	4
Dodecyl	183.5	6	6	4
Cetyl	189	8	8	4
Valerate, butyl	169–170	6	4	4
Octyl	176–177	6	6	4
Caproate, amyl	173.5	6	4	4
Octyl	181–181.5	6	6	4
Heptylate, methyl	169–170	4	0	4
Hexyl	177–177.5	6	4	4
Octyl	182	6	6	4
Caprylate, methyl	169–170	4	0	4
Heptyl	180.5–181	6	4	4
Octyl	181–181.5	6	6	4
Pelargonate, methyl	174–175	6	0	6
Heptyl	182.5–183	6	4	6
Octyl	185	8	6	6
Caprate, nonyl	186.5–187	8	6	6

Table continued

TABLE 13 (*continued*)

Acholic component	M.p., °C.	Coordination number[b] E	Al	Ac
Laurate, methyl	180	6	0	6
Ethyl	182	6	1	6
Propyl	183.5–184	6	?	6
Butyl	184–184.5	6	4	6
Hexyl	185.5	6	4	6
Octyl	186	6	6	6
Myristate, methyl	181.5–182	6	0	6
Ethyl	183.5	6	1	6
Butyl	186	6	4	6
Hexyl	188	6	4	6
Tetradecyl	194.5–195.5	8	8	6
Hexadecyl	197–198	8	8	6
Pentadecylate, methyl	184.5–185	8	0	8
Palmitate, cetyl[d]	195	8	8	8
Behenate, ethyl[d]	189–189.5	8	1	8
Montanate, ethyl[d]	197–198	8	1	8(?)
Hydnocarpate, ethyl[e]	186–187	8	1	8
Dihydrohydnocarpate, ethyl[e]	185–186	8	1	8
Chaulmoograte, ethyl[e]	187	8	1	8
Dihydrochaulmoograte, ethyl[e]	188	8	1	8
Phenylacetate, methyl[e]	168–169	4	0	—
4-Phenylbutyrate, ethyl[e]	170–172	6	1	—
11-Phenylundecylate, ethyl[e]	174	8	1	—
Benzoate, butyl[e]	169–170	4	4	—

[a] Most of the data are adapted from H. Sobotka, *The Chemistry of the Sterids*, Williams and Wilkins, Baltimore, 1938, p. 112, ff. Unless otherwise noted, data are cited from H. Rheinboldt, O. König, and R. Otten, *Ann.*, *473*, 249–259 (1929).
[b] E = ester; Al = alcohol component; Ac = acid component.
[c] Determination also by H. Sobotka and A. Goldberg, *Biochem. J.*, *26*, 555–568 (1932).
[d] H. Rheinboldt, H. Pieper, and P. Zervas, *Ann.*, *451*, 256–273 (1927).
[e] N. P. Buu-Hoi, *Z. physiol. Chem.*, *278*, 230–235 (1943).

which a symmetrical arrangement can obtain with 5 or 7 units; according to Hüttig,[525] symmetrical arrangements can also occur with coordination numbers of 12 and 20.

In studies of the various acids, the coordination numbers most frequently noted were likewise 4, 6, and 8, but values of 2 and 3 were found in the case of some short-chained acids, and a value of 1 was noted for acetic acid. In no case was any evidence adduced for the occurrence of choleic acids with 5 or 7 units, or of high molecular aggregates containing 12 or 20 units. The

[525] G. F. Hüttig, *Z. anorg. Chem.*, *114*, 24–26 (1920).

TABLE 14

MELTING POINTS AND COORDINATION NUMBERS OF CHOLEIC ACIDS WITH CYCLIC ALCOHOLS, HYDROCARBONS, AND KETONES AS ACHOLIC COMPONENTS.[a]

Acholic component	M.p., °C.	Coordination number
Alcohols:		
Phenol[a]	—	(?)
Phenylethylethanol[b]	—	(?)
Cholesterol (?)[c]	155–157	(?)
Hydrocarbons:		
Benzene[c]	161–163	(?)
Xylene[c]	180–182	(?)
Naphthalene[a]	—	2
Anthracene[d]	193	4
Phenanthrene[d,e]	184–185; 186–187	3
Acenaphthene[e]	175.5–176.5	2
1,2-Benzanthracene[e,f]	198–199	3
1,2,5,6-Dibenzanthracene[e]	223–224	4
1,2-Benzpyrene[g]	—	—
Methylcholanthrene[e]	193.5–194.5	4
Hexahydromethylcholanthrene[e]	191.5–192.5	4
Dipentene[b]	192	3
Cetylbenzene[h]	189–189.5	8
p-Hydroxyphenyloctadecane[h]	171	8
Ketones		
Benzoylacetone[i]	185	3
Dibenzoylmethane[d]	199.5–200.5	3
Cinnamoylmethane[d]	190.5–191.5	4
Dicinnamoylmethane[d]	195–196	6
Dicinnamoylacetone[d]	191.5	6
Anthrone[i]	179	4
Camphor[j]	178–179	1
Camphor[b,j]	179–180; 183	2
Estrone[k]	—	—
Benzaldehyde[a,c]	155	(?)

[a] Most of the data are adapted from H. Sobotka, *The Chemistry of the Sterids*, Williams and Wilkins, Baltimore, 1938, p. 112.

[b] H. Sobotka and A. Goldberg, *Biochem. J.*, *26*, 905–909 (1932).

[c] A. W. Downie, L. Stent, and S. M. White, *Brit. J. Exptl. Pathol.*, *12*, 1–9 (1931).

[d] W. Marx and H. Sobotka, *J. Org. Chem.*, *1*, 275–279 (1936).

[e] L. F. Fieser and M. S. Newman, *J. Am. Chem. Soc.*, *57*, 1602–1604 (1935).

[f] Also called *2,3*-benzphenanthrene.

[g] A. Winterstein and H. Vetter, *Z. physiol. Chem.*, *230*, 169–174 (1934); cited by H. Sobotka, *The Chemistry of the Sterids*, 1938, as *3,4*-benzpyrene.

[h] H. Rheinboldt, P. Braun, E. Flume, O. König, and A. Lauber, *J. prakt. Chem.*, *153*, 313–336 (1939); *Chem. Abst.*, *34*, 113 (1940).

[i] H. Sobotka and J. Kahn, *Biochem. J.*, *26*, 898–904 (1932).

[j] H. Rheinboldt, O. König, and E. Flume, *Z. physiol. Chem.*, *184*, 219–224 (1929).

[k] H. Wieland, W. Straub, and T. Dorfmüller, *Z. physiol. Chem.*, *186*, 97–103 (1929).

coordination numbers of choleic acids composed of mono- or dicarboxylic acids are illustrated in Figure 1.

In the fatty acid series, formic acid alone fails to yield a choleic acid, and acetic acid gives the only product having a single bile acid molecule. The normal coordination number for propionic and butyric acids appears to be 2, although choleic acids containing 3 and 4 units, respectively, have

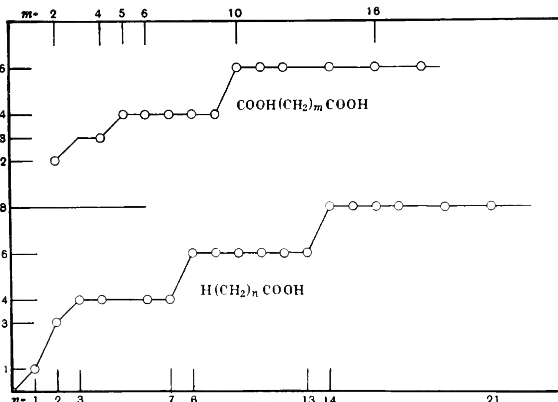


Fig. 1. Coordination numbers of choleic acids containing mono- or dicarboxylic acids.[526] The abscissae represent the number of methylene groups present in each acid, while the ordinates indicate the number of molecules of desoxycholic acid which combine with a single molecule of each respective acid.

likewise been reported.[527] All straight-chain acids from C_5 to C_8 have coordination number 4, while acids from C_9 to C_{14} combine with 6 desoxycholic acid molecules. For the remaining acids of the *n*-series, the coordination number is 8 (up to C_{27}). In the branched-chain acids, the coordination numbers are ordinarily those of the longest straight chain in the molecule.[526] The branched chain may interfere with choleic acid formation,[528] as is indicated by the fact that hexamethylethane forms no addition compound in alcohol.[529]

[526] H. Sobotka and A. Goldberg, *Biochem. J.*, *26*, 555–568 (1932).

[527] The designation "monocholeic, dicholeic, tetracholeic" acids, etc., is sometimes applied to choleic acids containing 1, 2, or 4 molecules of desoxycholic acid, respectively, to one molecule of the acholic component.

[528] E. Chargaff and G. Abel, *Biochem. J.*, *28*, 1901–1906 (1934).

[529] L. F. Fieser and M. S. Newman, *J. Am. Chem. Soc.*, *57*, 1602–1604 (1935).

Unsaturated and halogenated acids usually have the same coordination numbers as do the saturated acids having the same number of carbons. Crotonic acid, the simplest unsaturated acid studied, combines with 3 bile acids, while butyric acid, the saturated acid with the same number of carbon atoms, forms choleic acids with 2 or 4 desoxycholic acids, depending upon the method of preparation.[526] The *cis* and *trans* isomers, oleic and elaidic acids,[530] have the same coordination value (8) as stearic acid.[506,531] Such alicyclic ring acids as hydnocarpic and chaulmoogric behave in the same manner as the corresponding straight-chain acids.[532]

In the case of the dicarboxylic acids, glutaric (C_5) acid fails to form a coordination compound, although data are not available on the two simplest acids in this series (oxalic and malonic). Dicarboxylic acids having 7 to 11 carbons (5–9 $—CH_2—$ groups) combine with four bile acid molecules, while no coordination numbers higher than 6 have been observed in the higher acids (even up to C_{20}).

A large group of simple esters have been examined by Rheinboldt, alone[533] and with König and Otten.[534] These investigators found that an alcohol with n carbons has the same coordination number as does an acid with $(n + 1)$ carbon atoms. In the alkyl esters of the aliphatic acids, the coordination number corresponds to the value characteristic of the longer of the two chains. In most cases where the acyl and alkyl groups have the same coordination number, the ester will have the next higher coordination value. Thus, in the case of butyl valerate and amyl caproate, in which both alkyl and both acyl groups, when present as free alcohols or acids, have a coordination number of 4, there are six bile acid components in the choleic acid molecules of the esters. Triolein has been shown to yield choleic acid[535] melting at 184–185°C., but the coordination number is uncertain. Sobotka and Kahn[536,537] reported a figure of 3 for the coordination value of the choleic acid derivative prepared from ethyl acetoacetate, which melts at 154°C.

It is surprising that choleic acid derivatives of many hydrocarbons, both aliphatic and cyclic, can be readily prepared. A coordination number of 8

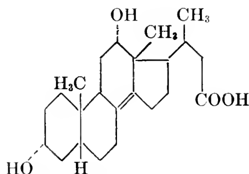
[530] W. Marx and H. Sobotka, *J. Org. Chem.*, *1*, 275–279 (1939).
[531] H. Rheinboldt, H. Pieper, and P. Zervas (with M. Kircheisen), *Ann.*, *451*, 256–273 (1927).
[532] A. P. Buu-Hoi, *Z. physiol. Chem.*, *278*, 230–235 (1943).
[533] H. Rheinboldt, *Ann.*, *451*, 256–273 (1926).
[534] H. Rheinboldt, O. König, and R. Otten, *Ann.*, *473*, 249–259 (1929).
[535] A. W. Downie, L. Stent, and S. M. White, *Brit. J. Exptl. Pathol.*, *12*, 1–9 (1931).
[536] H. Sobotka and J. Kahn, *Biochem. J.*, *26*, 898–904 (1932).
[537] H. Sobotka and J. Kahn, *Ber.*, *65*, 227–232 (1932).

appears to be the maximum, even in the case of tritetracontane, which contains 43 carbons.[531]

The ability to form choleic acids is apparently related to the solubility of the complex in the solvent employed. Ethyl alcohol is usually used with desoxycholic acid because it forms a labile complex from which the acid can be recovered by prolonged drying in a vacuum. Xylene gives a similar complex with desoxycholic acid which is more stable than the alcohol complex; in fact, the xylene will displace the ethyl alcohol from the choleic acid. Finally, the acetic acid choleate is more stable than is xylene-choleic acid; acetic acid will displace xylene from its combination.

Cyclic hydrocarbons form compounds with desoxycholic acid, but the number of molecules added is much smaller than is the case when straight-chain hydrocarbons having the same number of carbons are employed. For example, naphthalene and acenaphthene[529] form compounds with a coordination value of 2, phenanthrene[529] one of 3, and anthracene[530] one of 4. On the other hand, Fieser and Newman[529] did not obtain choleic acids when chrysene, 1,2-benzpyrene or several related hydrocarbons were employed in alcoholic solutions.

(*e*) *Apocholic Acid Complexes.* Desoxycholic acid is the only one of the natural bile acids which possesses the ability to form choleic acids. However, Boedecker alone[538] and in association with Volk[539] discovered that apocholic acid, an unsaturated derivative of desoxycholic acid, is able to combine with acids and hydrocarbons in a manner analogous to that of desoxycholic acid. Two forms of apocholic acid were shown to be active,



(XI) Apocholic Acid

α- (or simply apocholic) and β-apocholic acid. The mild dehydration of cholic acid with anhydrous zinc chloride in acetone solution gives a 75% yield[540] of apocholic acid; when carried out in acetic acid solution the yield

[538] F. Boedecker, *Ber.*, *53*, 1852–1862 (1920).
[539] F. Böedecker and H. Volk, *Ber.*, *54*, 2489–2492 (1921).
[540] A. W. Devor and H. W. Marlow, *J. Am. Chem. Soc.*, *68*, 2101 (1946).

of the unsaturated acid was only 24%.[541] The structure of apocholic acid (XI) has been established by Callow[542] and others.[543,544]

Table 15 summarizes the known data concerning the melting points and coordination numbers of various apocholeic acids.

TABLE 15
MELTING POINTS AND COORDINATION NUMBERS OF VARIOUS APOCHOLEIC ACIDS[a]

Acholic component	M.p., °C.	Coordination number[a]
Cetyl alcohol[b]	177.5	8
Acetone[c]	[d]	—
Acetic acid[c]	*ca.* 135	1
Butyric acid[c]	*ca.* 170	4
Palmitic acid[b,c]	184–185	8
Stearic acid[b,c]	185–186	8
Tiglic acid[e]	168–168.5	4
Sorbic acid[e]	170–171	4
Ethyl acetate[c]	[d]	—
Hexadecyl acetate[f]	189	8
Benzene[c]	[d]	—
Naphthalene[c]	173–174	2
Xylene[c]	171–172	2
Camphor[c]	179–180	1
" [g]	176.5–177.5	—
" [g]	178–179	2
Benzaldehyde[c]	156	—

[a] Most of the data are adapted from H. Sobotka, *The Chemistry of the Sterids*, Williams and Wilkins, Baltimore, 1938, p. 121.
[b] H. Rheinboldt, *Z. physiol. Chem.*, *180*, 180–186 (1928).
[c] F. Boedecker, *Ber.*, *53*, 1852–1862 (1920).
[d] Unstable.
[e] H. Rheinboldt and M. Kircheisen, *J. prakt. Chem.*, *113*, 199–211 (1926).
[f] H. Rheinboldt, O. König, and R. Otten, *Ann.*, *473*, 249–259 (1929).
[g] H. Rheinboldt, *Z. physiol. Chem.*, *182*, 255–258 (1929).

(*f*) *Physical Properties of Choleic Acids.* The melting point of the choleic acid is frequently higher than that of either component. This offers a convenient way to determine the composition of choleic acid by the use of the melting point diagram.[526,531,545] Various mixtures of the desoxycholic acid and the acholic compound are fused together; the melting point is determined after complete homogeneity of the product is attained. In

[541] P. L. Plattner, L. Ruzicka, and S. Holtermann, *Helv. Chim. Acta*, *28*, 1660–1669 (1945).
[542] R. K. Callow, *J. Chem. Soc.*, *1936*, 462–469.
[543] D. H. R. Barton, *J. Chem. Soc.*, *1946*, 1116–1123.
[544] E. Berner, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, *30*, 1542–1553 (1947).
[545] H. Rheinboldt, *J. prakt. Chem. (II)*, *111*, 242–272 (1925).

the case of most substances which form a choleic acid, a maximum point can be shown on the melting point diagram. When no compound results, no maximum point can be noted. The thawing point diagram is used when the melting point curve shows no sharp maximum.[545]

Figures 2 and 3 illustrate how the composition of the choleic acid derivative can be demonstrated from the melting point curves.

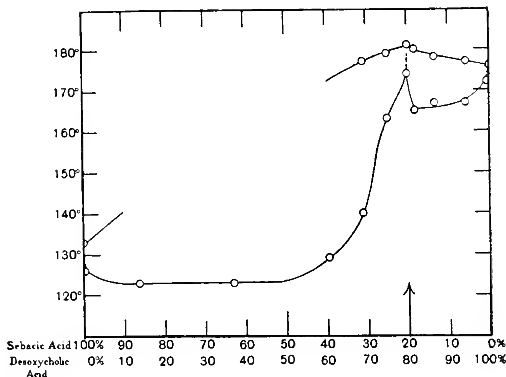


Fig. 2. Melting point and thawing point diagram of the system sebacic acid + desoxycholic acid.[526] The abscissae give molecular percentages, the ordinates give the melting points (upper curve) and thawing points (lower curve). The melting point of the molecular compound, 4 molecules desoxycholic acid + molecule sebacic acid, is higher than that of either of its constituents.

Senise[546] has pointed out that, in carrying out the determination of the "thaw" diagrams, different choleic acids are able to form an unbroken series of mixed crystals with each other. Thus, it was found that choleic acids composed of the following pairs of acholic components gave an unbroken series of mixed crystals: stearic acid, palmitic acid; stearic acid, myristic acid; stearic acid, lauric acid; arachidic acid, lauric acid; naphthalene, anthracene; naphthalene, phenanthrene; naphthalene, acenaphthene; anthracene, phenanthrene; anthracene, acenaphthene; phenanthrene, acenaphthene; stearic acid, naphthalene; stearic acid, anthracene; and stearic acid, phenanthrene. The apocholeic acids behaved in the same manner, as is shown by the following pairs: naphthalene-apocholeic acid,

[546] P. Senise, *Bol. faculdade filosof., ciênc. e letras, Univ. São Paulo, 14, Quím., No. 1,* 35–73 (1942).

stearic acid-choleic acid; and naphthalene-apocholeic acid, myristic acid-choleic acid. Desoxycholic and apocholic acids gave a similar unbroken curve. It is believed that this ability to form an unbroken series of mixed crystals indicates that the crystal structures of the different choleic acids are analogous, in spite of the unlike nature of their constituents and the differences in the proportion of the acholic component.

The x-ray patterns of the various choleic acids composed of the fatty acids from propionic to cerotic acids have been investigated by Kratky

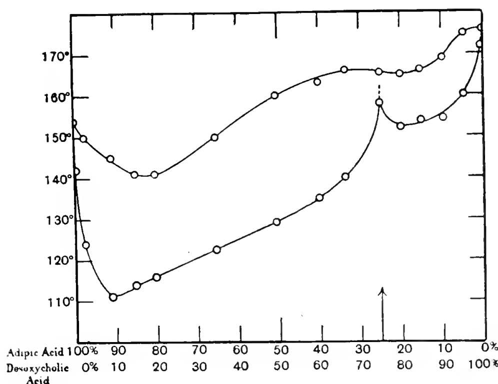


Fig. 3. Melting point and thawing point diagram of the system adipic acid + desoxycholic acid.[526] The abscissae show molecular percentages, the ordinates the melting points (upper curve) and thawing points (lower curve). The melting point of the molecular compound, 3 molecules desoxycholic acid + 1 molecule adipic acid, is situated between the melting points of its components.

and Giacomello.[547] These complexes show identical interference and intensity values. It is suggested that the fatty acids are oriented in canals and surrounded by a network of desoxycholic acid. However, this does not explain why no coordination values higher than 8 are found with acids, paraffins, and aralkyl compounds, nor why the polynuclear aromatic hydrocarbons have very low coordination values. More recently, Caglioti and Giacomello[548] and Giacomello alone[549] have investigated the palmitic acid-

[547] O. Kratky and G. Giacomello, *Monatsh., 69,* 427–436 (1936).
[548] V. Caglioti and G. Giacomello, *Gazz. chim. ital., 69,* 245–254 (1939).
[549] G. Giacomello, *Atti accad. naz. Lincei, Ser. 6, Classe sci. fis. mat e nat., 27,* 101–108 (1938).

choleic acid lattice structure, and report that the results confirm those of the earlier investigations.

(*g*) *Factors Involved in the Formation of Choleic Acid.* Desoxycholic and apocholic acids are the only bile acids which share in the formation of the choleic acids. On the other hand, cholic, lithocholic, and cholanic acids, which are completely ineffective, have residual valences scattered in various directions from the main longitudinal axis of the molecule, which lies between the hydroxyl group on C_3 and the carboxyl group on C_{24}. In desoxycholic and apocholic acids, the hydroxyl on C_{12} would appear to direct the residual affinities to the opposite front of the molecule. Sobotka[398] suggests that this side of the desoxycholic acid molecule presents an "aliphatic" aspect along the chain of carbons *3, 4, 6, 7, 8, 14, 15, 16, 17, 20, 22, 23, 24*. A double bond inhibits this chain in dihydroxycholenic acid; in the case of chenodesoxycholic and hyodesoxycholic acids, an analogous affinity does not occur on the other side of the molecule, since it is interrupted by methyl groups.

Moreover, the conjugated desoxycholic acids, also, are known to lose their ability to form choleic acids.[550] Desoxycholic acid loses its power to form choleic acid when the hydroxyl groups have been formylated, or when the acid has been esterified.[550] The acholic component also acquires different properties from that exhibited by the free compound. Benzaldehyde, for example, is no longer subject to auto-oxidation when present as benzaldehydecholeic acid.[399]

(*h*) *Types of Coordination Compounds of Bile Acids Other Than Choleic Acids.* Although cholic acid cannot form choleic acid derivatives, it can combine with various alcohols such as methyl and allyl, with glycol and with ethyl mercaptan.[509,551] It likewise forms unstable compounds with nitrobenzene, *m*-toluidine, aniline, benzaldehyde, and triolein.[552] Bile acids form coordination compounds with each other. Thus, chenodesoxycholic acid and 3-hydroxy-12-ketocholanic acid, both of which occur in human bile, form a coordination compound in a molecular ratio of 1:1.[417]

(*i*) *Physiological Importance of Choleic Acid Formation.* The hypothesis that the formation of choleic acid complexes is involved in the absorption of fats is most appealing. One is naturally led to the supposition that a connection must obtain between the beneficial action of bile salts on lipid absorption and the highly specific property of the reaction which involves

[550] F. Cortese and L. Bauman, *J. Biol. Chem.*, *113*, 779–785 (1936).
[551] F. Mylius, *Ber.*, *20*, 683–688; 1968–1989 (1887).
[552] S. Minovici and M. Vanghelovici, *Bul. Soc. Chim. Romania*, *12*, 5–13 (1930); *Chem. Abst.*, *25*, 521 (1931).

the synthesis of choleic acid complexes. However, there are several considerations which prompt the question as to whether or not such a mechanism can have a direct application *in vivo*.

In the first place, although desoxycholic and apocholic acids do readily form coordination compounds with a wide variety of lipids acting as the acholic components, these are the only two bile acids in which such an *in vitro* reaction can be demonstrated. Actually, desoxycholic acid is a minor constituent in the bile of most animals, and apocholic acid is a synthetic product never found as such in normal bile. Cholic acid, which has a wide distribution in the bile of various species and which, quantitatively, is usually the most important of the bile acids, is completely impotent in forming choleic acids.

Secondly, while *free* desoxycholic acid can combine with fatty acids and other lipids *in vitro* to form the choleic acids, conjugated desoxycholic acids do not react in this way. Wieland and Stender[553] reported, in 1919, that taurodesoxycholic acid lacked the capacity of choleic acid formation, while Cortese and Bauman[550] later noted the inability of glycodesoxycholic acid to participate in such a reaction.

On the other hand, Verzár and McDougall,[554] in their monograph on absorption from the intestine, emphasized the fact that the conjugated cholic acids facilitate the solution of fatty acids. This property is referred to as *hypertrophism*. Hypertrophic substances are defined as compounds which can render water-insoluble substances water-soluble.[555] Their action cannot be explained by any influences on membrane permeability.

Using drop formation as measured with the Traube stalagmometer as the criterion, Verzár and Kúthy[556] have shown that oleic, palmitic, and stearic acid solutions in glycocholic acid have a markedly depressed surface tension within the physiological range of *p*H 8 to 6. The effect on surface tension is not observed from *p*H 8 to 11, where the fatty acids are present as the alkaline soaps, or below *p*H 6 where the complex is again broken down to free fatty acids and the bile salt. Since the smaller the difference in surface tension occurring at the interface of two liquids, the higher is the solubility of one in the other, it is believed that "water-insoluble substances can be dissolved in an aqueous solution of a hydrotropic substance, owing to the surface force between them being remarkably decreased."[554] The effect

[553] H. Wieland and H. Stender, *Z. physiol. Chem.*, *106*, 181–189 (1919).

[554] F. Verzár and E. J. McDougall, *Absorption from the Intestine*, Longmans, Green, London and New York, 1936.

[555] C. Neuberg, *Biochem. Z.*, *76*, 107–176 (1916).

[556] F. Verzár and A. Kúthy, *Biochem. Z.*, *210*, 281–285 (1929).

of pH on the surface tension of several fatty acids in sodium glycocholate solutions is illustrated in Figure 4.

Verzár and Kúthy[513] subscribed to the importance of choleic acid formation in the case of desoxycholic and apocholic acids, and postulated an analogous behavior in the case of glycocholic acid. It was shown that fatty acids give a clear solution in the presence of conjugated bile salts when the concentration is low but that, when a greater concentration of fatty acid obtains, the solution becomes milky. These results are interpreted as indicating that conjugating bile acids form definite molecular complexes at low

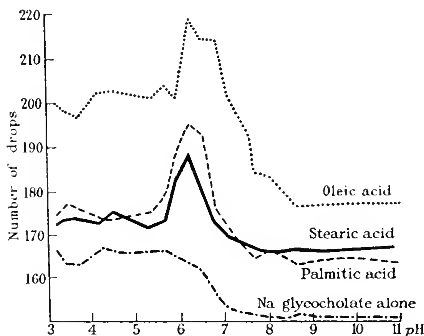


Fig. 4. Surface tension of different fatty acids in glycocholic acid solution as influenced by pH.[554]

concentrations of fatty acids, while at higher concentrations of the fatty acids the turbid solutions result, since larger complexes are formed in addition. The solution will be complete so long as the bile acid molecules entirely engulf the fatty acid molecule. It is believed that, at the lower concentration, this condition is satisfied by a ratio of 4 bile salt molecules to one fatty acid molecule; in the higher complexes, the ratio may be 9:4. Thus, it would appear that the choleic acid principle may well be of considerable importance in explaining fat absorption. The mere fact that crystalline coordination compounds cannot be demonstrated in the case of conjugated bile acids, as they can with desoxycholic and apocholic acids, is not sufficient justification for discarding the entire hypothesis. The observations of the effect of paired (or conjugated) bile salts on the solution of fatty acids may well be explained by the formation of a complex similar

to choleic acids but having a different ratio of components. Fowweather[557] reported the presence of 90% choleic acid together with 10% cholic acid in two enteroliths removed from the intestine of a woman. He concludes that the formation of choleic acid stones requires the concurrence of a number of factors, *viz.*, a mechanical condition which is capable of allowing the retention of the precipitated acid, bile which contains more desoxycholic acid than is usually present, and also bile in which a considerable proportion of the desoxycholic acid is in the unconjugated form. Hyperchlorhydria may be an additional factor.

b. Circulation of the Bile Acids. It has been recognized for a long time that an enterohepatic circulation of bile salts obtains. The amount of bile salts produced *de novo* would be far too small to account for that needed for the absorption of an appreciable amount of fat if these acids were not used over and over again. Berman and co-workers[558] and Irvin *et al.*[559] have concluded that the regulation of the final level of circulating bile acids is accomplished by a state of equilibrium between the rate of synthesis and the rate of loss of these acids. This subject has been reviewed recently by Josephson,[480] who considered the circulation of the bile acids in connection with their production, conjugation, and excretion.

Since Hoppe-Seyler,[560] in 1863, was able to find only a very small amount of bile salts in the feces, he concluded that they were resorbed by the intestine. The choleretic effect of bile, when given by mouth or when introduced directly into the intestine, was explained by Schiff[561] as the result of the circulation of the bile. The experiments of Tschernoff[562] and of Stadelmann,[563] carried out somewhat later, proved that the bile acids are absorbed from the intestine and are reexcreted through the liver.

(*a*) *Absorption of the Bile Acids.* When the bile salts, combined with fatty acids as the soluble choleic acids, are absorbed from the lumen of the intestine, they are dissociated almost immediately in the mucosa cells. The fatty acids set free are recombined to form neutral fat, after which they are transported to the liver *via* the lymph. When bile salts are combined with other lipids, they are also set free within the mucosa cell.

The bile acids set free may remain for some time in the intestinal wall

[557] F. S. Fowweather, *Biochem. J.*, *44*, 607–610 (1949).

[558] A. L. Berman, E. Snapp, A. C. Ivy, and A. J. Atkinson, *Am. J. Physiol.*, *131*, 776–782 (1940).

[559] J. L. Irvin, C. G. Johnston, and E. A. Sharp, *Am. J. Physiol.*, *146*, 293–306 (1946).

[560] F. Hoppe-Seyler, *Arch. path. Anat. Physiol.* (*Virchow's*), *26*, 519–537 (1863).

[561] M. Schiff, *Arch. ges. Physiol.* (*Pflüger's*), *3*, 598–624 (1870).

[562] W. Tschernoff, *Arch. path. Anat. Physiol.* (*Virchow's*), *98*, 231–293 (1884).

[563] E. Stadelmann, *Z. Biol.*, [2], *16* (34), 1–64 (1896).

itself, where it is assumed that they may act continuously as transporters of fat. However, these bile acids are gradually carried to the liver by way of the portal blood supply, rather than by the lymphatic route. In support of this hypothesis, Josephson and Rydin[564] have shown that a higher level of cholate occurs in the portal blood than in the systemic blood of rabbits and cats (2.5 to 5 mg./100 ml. in portal blood compared with 1 to 2 mg./100 ml. in blood from the heart). The discrepancy between the content of bile acids in the portal and in the systemic blood is increased when bile salts are introduced into the intestine,[565] while no difference obtains when there is no bile in the intestine, due to previous ligation of the bile duct.[564] Similar results were obtained on dogs by Josephson and Kaunitz;[566] in normal dogs, the lymph never contained cholates in concentrations high enough to be determined, even when a considerable absorption of bile salts was taking place. On the other hand, the cholate concentration in the portal blood was markedly augmented during the absorption of bile. Jenke and Graff[567] reported similar results. The absorption of the bile is believed to take place chiefly in the ileum, although sodium glycocholate can be absorbed in the jejunum. The absorption of bile salts in the duodenum is zero,[568] or at least minimal.[569]

The absorption of bile acids from the intestine was demonstrated by Johnston and Irvin[570] in patients with choledochostomy drainage over periods of 10 to 20 postoperative days, before the resumption of synthesis of bile acids had occurred. During this period, a considerable fraction of the acids was unconjugated; the excretion of the dihydroxycholanic acids occurred before that of cholic acid.

When desiccated hog bile was given orally, not only was an excretion of hyodesoxycholic acid observed in the drainage bile, but also the ratio of bile acids conjugated with glycine to those combined with taurine increased. It was suggested that glycohyodesoxycholic acid was absorbed and re-excreted unchanged in the drainage bile. When ox bile was administered, the cholic and desoxycholic acids in the drainage bile were increased in proportion to the amount of these acids present in the ox bile. These re-

[564] B. Josephson and A. Rydin, *Biochem. J.*, *30*, 2224–2228 (1936).

[565] C. H. Greene, M. Aldrich, and L. G. Rowntree, *J. Biol. Chem.*, *80*, 753–760 (1928).

[566] B. Josephson and H. Kaunitz, *Z. ges. exptl. Med.*, *102*, 195–201 (1937).

[567] M. Jenke and U. Graff, *Klin. Wochschr.*, *18*, 125–127 (1939).

[568] H. Tappeiner, *Sitz-Ber. Akad. Wiss., Wien, Math.-Naturw. Klasse*, *77*, *Abt. II*, 281–304 (1878).

[569] E. Frölicher, *Biochem. Z.*, *283*, 273–279 (1935–1936).

[570] C. G. Johnston and J. L. Irvin, *J. Clin. Invest.*, *26*, 802–814 (1947).

sults furnish cogent support for the absorption and reexcretion of the bile salts.

(*b*) *Reexcretion of the Bile Acids.* Whipple and Smith[571,572] confirmed the earlier work of Tschernoff,[562] Stadelmann,[563] and Greene *et al.*[565] by demonstrating the marked effect of removal of the bile salts from the gastrointestinal tract on the excretion of these acids in the bile. Dogs with bile fistulas were shown to produce only 100 mg. of bile salts per kilogram body weight per day when they were deprived of their bile. This figure has also been reported by Magee *et al.* for cholate[573]; the latter workers suggest that a reduced secretion of cholate is indicative of liver dysfunction, and that a significant correlation exists between the mean deficit and the bromsulphthalein retention. On the other hand, when the excreted bile salts were fed back to the dogs, the daily production of these components rose to 800 mg./kg./day. It was calculated that a 10 kg. dog keeps about 7 to 8 g. of bile salts in circulation by resorption and by reexcretion, and that the time required for the circulation of this amount is 8 to 16 hours. These results are in line with those obtained by Schmidt and collaborators.[574] The bile salt secretion can be stepped up to 15 to 17 g. per day if the animal is fed more bile salts than have been excreted. When higher amounts than this quantity are given, the surplus is lost.

There is some question as to the extent of the loss of bile salts in the course of their recirculation through the liver. Schmidt *et al.*[574] estimated that, when bile is given orally in physiological amounts, about 10% is lost. Irvin and associates[559] reported that, during ten hours of circulation of foreign conjugated bile salts in the hog, 10% of the amount circulated was lost; on the other hand, unconjugated cholate was handled less efficiently. It was also shown that the disappearance of hyodesoxycholic acid was comparable to the loss of the conjugated bile acids. The average equilibrium level was 600 mg./kg., and this total amount was found to be unchanged by the administration of cholate. In fact, no evidence for the conversion of cholic acid to the dihydroxycholanic acids such as hyodesoxycholic acid was demonstrated. Moreover, cholates did not appear in the peripheral circulation of normal hogs. However, when the bile flow was obstructed, and cholates were administered either by the duodenal or the

[571] G. H. Whipple and H. P. Smith, *J. Biol. Chem.*, *80*, 697–707 (1928).
[572] G. H. Whipple and H. P. Smith, *J. Biol. Chem.*, *89*, 727–738 (1930).
[573] D. F. Magee, K. S. Kim, V. C. Pessoa, and A. C. Ivy, *Am. J. Physiol.*, *169*, 309–316 (1952).
[574] C. R. Schmidt, J. M. Beazell, A. L. Berman, A. C. Ivy, and A. J. Atkinson, *Am. J. Physiol.*, *126*, 120–135 (1939).

intravenous route, cholic acid could be shown to enter the peripheral circulation.

Some bile acids may be normally excreted by the kidney.[461] Friedman and collaborators[575] reported that, while some bile acids continued to be discharged into the blood stream from the liver after obstruction of the bile duct of the rat, the levels of plasma bile acids were increased 5 times above this basal level if bilateral nephrectomy was performed simultaneously with duct ligation. Moreover, it has been well known that, in man, bile acids are eliminated by the kidney in jaundice or after their injection in large amounts.[576] Yamasaki and Miyashita[577] reported that urinary excretion of dehydrocholate by rabbits, after its administration, was markedly increased when the liver was poisoned by carbon tetrachloride. Simultaneous administration of vitamin B_{12} or methionine, together with the dehydrocholate, reduced its excretion to a normal level.

A slight destruction of bile acids may occur in the liver.[578,579] Licht[580] noted that some decomposition of the bile acids was brought about by intestinal bacteria. Berman *et al.*[581] suggested that the oxidation of the bile acids to keto acids may account for the loss of these compounds. However, the main pathway for loss is *via* the intestine, a fact which was proved by Hoppe[582] and by Hoppe-Seyler[560] many years ago. Josephson[480] and Sobotka[467] agree that "since catabolic destruction of bile acids by the animal organism is exceedingly doubtful, and since their elimination from the body is almost entirely confined to the intestinal route, equilibrium is maintained by synthesis of bile acids paralleling the rate of fecal losses." Josephson[480] calculates that, in man, the bile salts are recirculated about 3 times before being broken down. The endogenous bile salt production of bile fistula dogs has been found to be about 100 mg./kg./day.[571]

The mechanism of synthesis of the bile acids will be discussed in the section on the intermediary metabolism of cholesterol in Volume III. It is of interest to note here that Magee *et al.*[583] demonstrated the occurrence of a mean daily increase of 25% in cholate production when the 10 essential

[575] M. Friedman, S. O. Byers, and F. Michaelis, *Am. J. Physiol.*, *164*, 786–788 (1951).

[576] S. S. Lichtman, *Am. J. Physiol.*, *117*, 665–671 (1936).

[577] K. Yamasaki and N. Miyashita, *J. Biochem.* (*Japan*), *39*, 8 (1952).

[578] F. Rosenthal, L. Wislicki, and H. Pommernelle, *Arch. exptl. Pathol. Pharmakol.*, *122*, 159–183 (1927).

[579] J. Bollman and F. Mann, *Arch. Pathol.*, *16*, 304 (1933).

[580] H. Licht, *Biochem. Z.*, *153*, 159–164 (1924).

[581] A. L. Berman, E. Snapp, A. C. Ivy, A. J. Atkinson, and V. S. Hough, *Am. J. Digestive Diseases*, *7*, 333–346 (1940).

[582] F. Hoppe, *Arch. path. Anat. Physiol.* (*Virchow's*), *25*, 181–183 (1862).

[583] D. F. Magee, K. S. Kim, and A. C. Ivy, *Am. J. Physiol.*, *169*, 317–325 (1952).

amino acids present in casein and glycine were given. Lysine, glycine, and the D-amino acids were not involved in this effect; the ketogenic amino acids (leucine, tyrosine, and phenylalanine) were found to provoke a cholepoietic response, which was augmented by the addition of methionine, threonine, and valine. Vitamin B_{12} produces a transient increase in cholate output, but cortisone acetate and testosterone propionate do not have this effect.

4. The Pathways for Lipid Absorption

(1) *The Sites of Absorption in the Gastrointestinal Tract*

In order that a substance may be absorbed from the gastrointestinal tract, it is necessary that the material be present in water-soluble form, that it be allowed to remain in contact with the surface of the alimentary canal for an adequate length of time, and finally, that the anatomical structure of the gastrointestinal tract be suitable for the process of absorption.

a. Mouth. Under normal conditions, no absorption whatsoever occurs in the mouth. In the first place, the fats are not in solution at this stage. No fat-splitting enzymes are present in saliva, and there is no mechanism whereby emulsification of the fat may occur. However, the mucosa of the mouth and esophagus is capable of absorbing small amounts of water-soluble material if the substance is retained in the oral cavity. Meltzer[584] reported that, following tying of the esophagus, rats were quickly poisoned if phenol or nicotine was placed in their mouths. It has also been reported by Mendell[585] and by Mendel *et al.*[586] that, in man, codeine, morphine, atropine, strophanthin, medinal, nitroglycerol, and pyramidon can be absorbed from the mouth if the drug is kept there for a sufficient period. There is also evidence that strychnine can be absorbed fairly readily from the pharynx and esophagus of the dog.[587]

b. Stomach. No appreciable absorption of lipids normally occurs in the stomach. Although fats may be emulsified to a considerable extent in this organ, the hydrolysis of the fat molecules is kept at a minimum. The presence of lipase has been postulated in gastric juice; however, its action is weak at the *p*H existing in the gastric cavity. Highly emulsified fats

[584] S. J. Meltzer, 1899; cited by F. Verzár and E. J. McDougall, *Absorption from the Intestine*, Longmans, Green, London and New York, 1936, p. 4, and by F. Mendell, *Münch. med. Wochschr.*, *69*, 1593–1595 (1922); *70*, 1526 (1923).

[585] F. Mendell, *Münch. med. Wochschr.*, *69*, 1593–1595 (1922); *70*, 1526 (1923).

[586] B. Mendel, A. Wittgenstein, and E. Wolffenstein, *Klin. Wochschr.*, *3*, 470–472 (1924).

[587] S. J. Meltzer, *Am. J. Med. Sci.*, *118*, 560–570 (1899).

such as that in egg yolk and in milk may undergo some hydrolysis. Regurgitation of intestinal contents, likewise, may aid in gastric lipolysis, both by changing the *p*H of the medium to one nearer the optimum for gastric lipase, and by supplementing the gastric enzyme with the pancreatic lipase.

Some absorption of foodstuffs has been demonstrated in the stomach of animals following ligation of the pylorus, but such substances would ordinarily leave the stomach quickly if the pylorus were functioning normally, and digestion would not have proceeded far enough, under usual conditions, to allow any absorption. Klemperer and Scheurlen[588] demonstrated that no fat was absorbed during a period of three to six hours in dogs, when a ligature had been placed around the intestine just below the pylorus. Moreover, the stomach mucosa is not adapted to absorption.

However, several workers have demonstrated, by histological examination, the presence of fat droplets in the gastric epithelium after fatty meals. Weiss[589] believed that this absorption occurred only in young animals, but Greene and Skaer[590] noted similar fat droplets in the gastric mucosa of old dogs, as well as in the mucosa of young animals. The histological picture of the gastric mucosa resembled, to a considerable extent, that of the intestinal mucosa during fat absorption. After the fat had left the stomach, the cycle was reversed, and the fat disappeared from the mucosa. Mendel and Baumann[591] found some fat droplets in gastric mucosa, as determined histologically and chemically, but they could detect no concomitant change in the level of blood fat. Inouye,[592] however, was able to demonstrate a slight increase in the fat content of thoracic lymph when fat was present in the stomach, although the rise was a minor one.

Finally, one must conclude that, normally, the stomach is not the site of absorption of fat, but that the gastric mucosa can take up fat temporarily in a manner similar to that of the intestinal mucosa. However, since the stomach mucosa is not adapted to complete the absorption, the fat droplets disappear when the food fat has passed into the intestine. The chief function of the stomach seems to be to free the fat from protein by digestion of the latter, to bring about a small amount of hydrolysis of the fat with the formation of fatty acid, and thus to provide ready material for the formation of enough soap to aid in prompt emulsification when the chyme passes into the small intestine. Apparently, the stomach also regulates the entry

[588] G. Klemperer and E. Scheurlen, *Z. klin. Med.*, *15*, 370–378 (1889).
[589] O. Weiss, *Arch. ges. Physiol. (Pflüger's)*, *144*, 540–543 (1912).
[590] C. W. Greene and W. F. Skaer, *Am. J. Physiol.*, *32*, 358–368 (1913).
[591] L. B. Mendel and E. J. Baumann, *J. Biol. Chem.*, *22*, 165–190 (1915).
[592] T. Inouye, *Am. J. Physiol.*, *69*, 116–124 (1924).

of the fat into the intestine to a rate which will prevent flooding the small intestine with a difficultly absorbable foodstuff.

c. Small Intestine. The small intestine is the main site of absorption of fats and other lipids. As soon as the fats pass into the intestine, the chyme comes in contact with the pancreatic juice, which contains the lipolytic enzyme, steapsin. It is also almost immediately mixed with the bile; emulsification of the fat promptly occurs, and the action of the steapsin in bringing about hydrolysis of the fat is greatly accelerated. Whether the fat must be broken down completely to fatty acids before absorption can occur is a moot question which will be discussed later (see page 137).

Little absorption occurs in the upper part of the small intestine. After the admixture of pancreatic juice, bile, and intestinal juice in the duodenum, the process of digestion continues rapidly. Within three to four hours, the liquid mass has reached the lower part of the ileum, and absorption is proceeding with great rapidity. By the time the foodstuff has reached the end of the ileum, almost all of the ingested fat has been removed from the chyme. After five to eight hours, the intestinal contents have passed into the cecum. The splenic flexure fills as early as the tenth hour, although in some cases this is delayed to the twelfth to fifteenth hour. The remnants of the food reach the rectum by the fourteenth to eighteenth hour. It is therefore evident that within ten hours all ingested foodstuffs have passed out of the small intestine.

d. Large Intestine. Practically all absorption of fat has occurred before the intestinal contents pass through the ileocecal valve into the large intestine. The chyme at this point contains almost no absorbable material, and it has a constant water content. In man, the intestinal residues remain in the large intestine for from twenty-six to thirty-eight hours. During this interval, water especially is absorbed; the water content is decreased to about one-third of that which obtained when the chyme was poured into the large intestine. The material at this stage has assumed a fecal character.

(2) Anatomical Features of the Small Intestine of Importance in Absorption

a. The Surface Structure of the Small Intestine. The small intestine is especially adapted to absorption because of its large surface area. In man, it has a length of about 27 feet, and the total surface area is estimated at about 8 to 10 square meters. The length and area of the small intestine of the lower animals is of a magnitude proportionate to those in man.

Thus, in the case of the adult rat, the small intestine is approximately 2 feet long. Although the length of the intestine is not a good index of its surface area, since the latter is likewise a function of the diameter of this organ, it is interesting that this figure more nearly corresponds to body surface than to body weight. The length of the small intestine is about twelve to fifteen times as great in the adult man as it is in the adult rat. On the other hand, the surface area of the body will be about forty times as large, and the body weight two hundred times the magnitude when the values of rat and of man are compared.

The extremely large value calculated for the area of the small intestine is due to the fact that there are many folds in it, and especially to the fact that the surface is covered with millions of finger-like processes called *villi*. According to Verzár and McDougall,[554] there are 18 to 40 villi per square millimeter in man, and these have a height of 0.2 to 1.0 mm. The total number of villi is calculated as 5,000,000 in man. In dogs, the villi have an average height of 0.5 to 0.6 mm., a breadth of 0.2 to 0.25 mm. and a surface of 0.43 sq. mm.

The surface area of the small intestine in the rat, without allowing for the area occupied by the villi, has been calculated as 6727 sq. mm. There are, on an average, 6537 villi per square centimeter, which have a surface area of 38,765 sq. mm. This represents a total surface area of 45,486 sq. mm. for the small intestine of the rat; only about one-seventh of the total is contributed by the superficial area of the intestine, while six-sevenths are traced to the area represented by the villi.

In the case of birds, there is a considerable variation in the type of villi

TABLE 16

SURFACE AREA OF VARIOUS PORTIONS OF PIGEON'S INTESTINE[a]

Segment of intestine	Length, mm.	Surface area, sq. mm.			Villi	
		Without villi	Villi alone	Total	Length, mm.	Breadth, mm.
Duodenum	140	1960	21029	22989	0.84	0.36
Jejunum						
1st third	200	2400	13708	16108	0.84	0.18
2nd third	200	2000	9072	11072	0.42	0.27
3rd third	200	1800	7387	9187	0.36	0.27
Ileum	220	880	3564	4444	0.27	0.45
Colon	70	490	510	1000	—	—
Total	*1030*	*9530*	*55,270*	*64,800*		

[a] Adapted from F. Verzár and E. J. McDougall, *Absorption from the Intestine*, Longmans, Green and Co., London and New York, 1936. Figures for the totals are corrected.

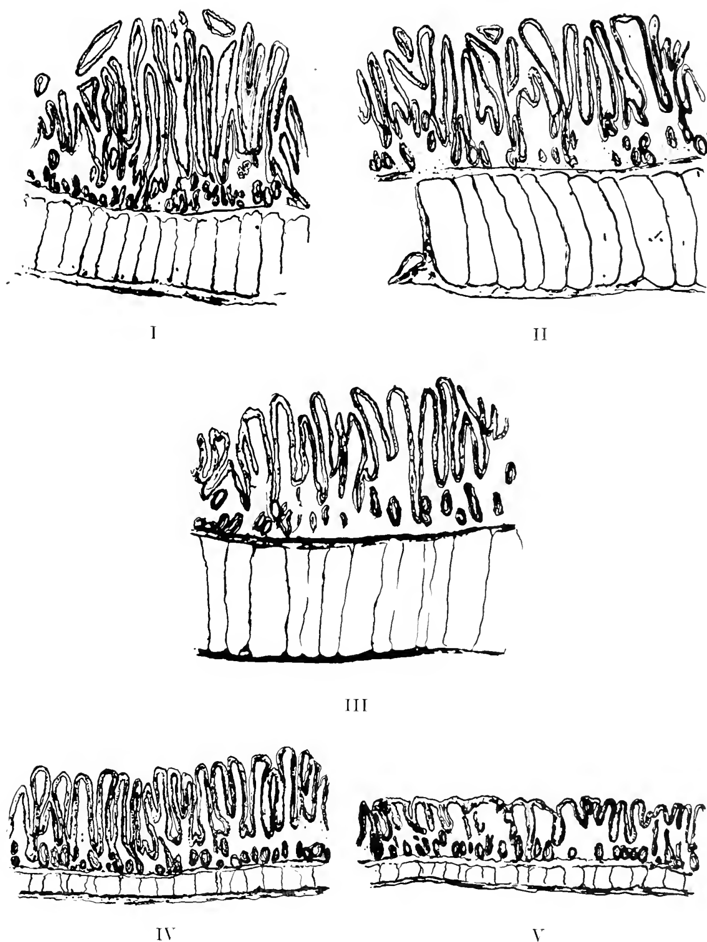


Fig. 5. Transverse sections through the small intestine of the pigeon at the following locations: I, duodenum; II, upper part of jejunum; III, medial part of jejunum; IV, lower part of jejunum; and V, ileum.[554] See text p. 120.

at the different levels of the small intestine. This contrasts with the situation in mammals, where all of the small intestine presents a more or less uniform structure. A comprehensive study of variations in structure of the pigeon's intestine has been made by Verzár and McDougall.[554] Marked differences in the structure of the duodenum, jejunum, and ileum can be noted, not only macroscopically but also microscopically. The variations in anatomy are evident from the calculations summarized in Table 16 (p. 118).

About one-third of the total intestinal surface occurs in the duodenum while, in the jejunum, the surface continually decreases, so that the last third has only about one-half of that of the first third; the ileum, which is

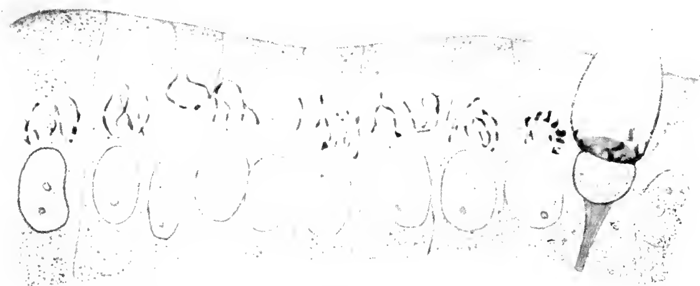


Fig. 6. Intestinal epithelium from duodenum of a man 44 years of age. This illustrates the trophosphongium (Golgi apparatus) and also a mucus-secreting cell (at right).[593]

equal in length to one-third of the total jejunum, has less than one-half the surface of the last third of this portion of the intestine.

The villi vary in structure with the portion of intestine in which they are found. Thus, in the duodenum, they are extremely long, narrow, finger-like processes, which show strong pumping movements. In the jejunum, the villi are shorter, but they also appear to undergo pumping movements. The villi present in the ileum are short, tongue-shaped structures, closely packed together; in contrast to the villi higher up in the small intestine, those located in the ileum do not present the contractions described as pumping. Figure 5 (p. 119) illustrates the variations in structure noted in various sections of the small intestine of the pigeon.

b. The Histological Structure of the Intestinal Mucosa. The mucosa lining the intestinal cavity is of great importance in the absorption of food-

stuffs. The epithelium of the mucosa is made up of cylindrical cells which measure 22–68 μ by 6–9 μ. A homogeneous basal membrane lies under it. On its free side, the surface is covered by cilia-like processes which were formerly supposed to show ameboid movements related to absorption. This concept has now been discarded. At present, the membrane is believed either to contain fine pores or to be composed of material having a different chemical nature which is arranged in the form of pillars. In the non-absorbing state, an alveolar region can be seen in the upper part of the epithelial cells. Parallel fibrillar structures can also be noted which are the so-called mitochondria of Benda (see Figure 6). It has been suggested that changes occur in these mitochondria during absorption.

The *trophospongium* or the *Holmgren apparatus* is another well defined structure in the epithelial cell.[593–595] This is considered by Corti[596] to be identical with the Golgi apparatus.[554] It is a glomerular structure lying over the nucleus. Although several investigators were of the opinion that this structure does not change during absorption, Mottram *et al.*[597] and Cramer and Ludford[598] have reported that certain alterations do take place coincident with absorption (see Figure 7).

Leucocytes are present in great numbers between the cells and in the subepithelial tissue. They can apparently pass through the mucosa to the lumen of the intestine by ameboid movements, and are believed to be concerned with fat absorption. It is also possible that the leucocytes provide a protective mechanism against the masses of bacteria in the lower intestine, or against toxic substances produced by them.

Lymphatic folliculi occur in the lamina propria of the intestinal wall, where they are found as small solitary folliculi or as large Peyer's patches. The lymphatic network, which is called the "Teichman net," surrounds these patches, but does not enter them. The lymphatic vessels, which provide a route for the transport of fat, begin in each villus, with one or more round knotty swellings[554]; under the villi, a second large plexus occurs, as well as an additional one in the submucosal tissue. In the submucosal plexus, there are valves which allow the lymph to flow only in one direction. The small lymphatics from the villi eventually form larger vessels which

[593] F. Kopsch, *Z. mikroskop. anat. Forsch.*, *5*, 221–284 (1926).

[594] E. Holmgren, *Anat. Anz.*, *20*, 433–440 (1902); *21*, 477–484 (1902).

[595] A. Guilliermond, *Les constituants morphologiques du cytoplasme. III. Le système vacuolaire, ou vacuome*, Hermann, Paris, 1934, pp. 63 ff.

[596] A. Corti, *Richerche morf.*, *4*, 313–422 (1924).

[597] J. C. Mottram, W. Cramer, and A. H. Drew, *Brit. J. Exptl. Pathol.*, *3*, 179–181 (1922).

[598] W. Cramer and R. J. Ludford, *J. Physiol.*, *60*, 342–346 (1925).

carry the *chyle* from the intestines to the *cisterna chyli*, and eventually to the thoracic duct, from which the lymph is poured into the blood stream.

In addition to the lymphatics, arteries also enter the villi. Only a single artery is involved with each villus in the case of man and dog; 2 or 3 arteries occur in each villus in the case of the rat and the rabbit. The artery extends to the top of the villus, where it divides, part of it to form a capillary

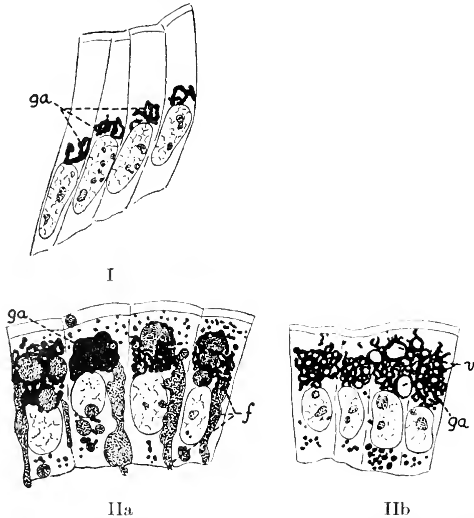


Fig. 7. The Golgi apparatus (trophospongium) in the intestinal epithelium of rats during fasting (I) and after a meal containing fat (II)[598] (*f*, fat droplets; *ga*, Golgi apparatus; *v*, vacuole).

network and part to establish direct connection with the main vein. In the fasting animal, the blood passes only through the arteriovenous connection. In man, there are fifteen to twenty capillaries which pass directly under the epithelial layer; little nests of veins are also found in the submucosal layer. These veins provide a direct connection with the portal blood supply, by way of which sugars, amino acids, salts, and other water-soluble components are believed to pass to the liver; only minimal amounts of lipids are transported by this route.

In addition to the mechanism whereby absorption of the foodstuffs is effected, the intestinal mucosa also functions to produce an intestinal secre-

tion. In the so-called Lieberkühn crypts, which are not concerned with absorption, erepsin, enterokinase, lipase, and sodium carbonate are produced. Mucus-forming cells are scattered throughout the intestinal epithelium.

5. Methods for the Study of Fat Absorption and Lipid Absorption in General

The most satisfactory procedures for the investigation of lipid absorption from a quantitative standpoint are those which involve a determination of the rate of disappearance of the substance under consideration from the gastrointestinal tract. Such procedures do not afford any information as to the form in which the component is absorbed or as to the pathways involved in the transport of the material from the intestine. On the other hand, methods of study of fat absorption which will reveal the latter data, such as thoracic cannulation, are less adapted to reveal quantitative relationships. One would expect the best results from those procedures which involve the use of unanesthetized animals.

(1) Thiry-Vella Fistulas

The intestinal loop devised almost a hundred years ago by Thiry (also spelled "Thiery"),[599] and modified by Vella,[600] has been the classical method for the study of absorption of foodstuffs since it was originally described. The simple Thiry fistula involves the excision of a section of the intestine. The exposed ends remaining after the removal of the section are connected to form an anastomosis. One end of the excised section is fastened to the abdominal wall. The rest of the separated portion of gut is allowed to remain in the peritoneal cavity after the open end is tied off with a suitable ligature, to prevent drainage of the intestinal juice into the peritoneal cavity. During all operative procedures, the blood and nerve supply to the loop are kept intact, so that they will continue to function normally after the operation is completed and the wound has healed. In the Vella modification of the operation, both ends of the excised loop are brought to the abdominal surface. About 3 to 4 cm. of the gut are allowed to project above the surface to take care of the sloughing off of the injured portion of the intestinal section adjacent to the area where it has been cut.

The double fistula appears to be more satisfactory than the single one,

[599] L. Thiry, *Sitz.-Ber. Akad. Wiss., Wien, Math.-naturw. Klasse, 50, Abt.* I, 77–96 (1865).
[600] S. Vella, *Untersuch., Naturlehre (J. Moleschott), 13,* 40–74 (1883).

especially when the ends are brought out at a higher and at a lower point on the abdominal wall. The length of the loop employed by Bosio and Giaume,[601] for their quantitative studies was approximately 20 cm. The chief objection to either the Thiry or the Thiry-Vella procedure is the inability to prevent leakage of the intestinal contents from the opening. Several modifications have been proposed to obviate this difficulty. Gumilewski[602] employed two rubber balloons, one at each opening of the Thiry-Vella loop. The first was inflated and served as a plug. The second balloon was penetrated by a short rubber tube which permitted the filling and emptying of the loop.

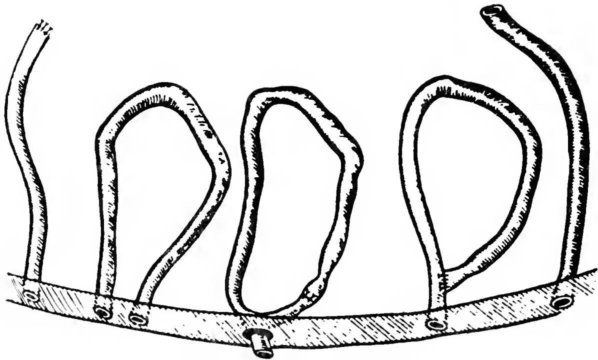


Fig. 8. Different forms of the Thiry-Vella fistulas.[554]

There are several disadvantages in the Gumilewski technic. In the first place, a single balloon has a tendency to be sucked into or expelled from the opening, due to the peristaltic waves which continue to occur in the intestine after the preparation of the fistula. It is difficult to anchor balloons sufficiently to ensure an effective seal. Second, the use of a short rubber tube is complicated by the fact that fluid collects in segments of the loop, which can be removed only with considerable difficulty. A disadvantage of the original Thiry procedure is the tendency of the loop to prolapse through the fistulous opening. Modifications of the Gumilewski technic have been proposed by Nagano,[603] by Cobet,[604] and by White and Rabino-

[601] P. Bosio and C. Giaume, *Pathologica, 20,* 504–509 (1928).
[602] Gumilewski, *Arch. ges. Physiol. (Pflüger's), 39,* 556–592 (1886).
[603] J. Nagano, *Arch. ges. Physiol. (Pflüger's), 90,* 389–404 (1902).
[604] R. Cobet, *Biochem. Z., 114,* 33–57 (1921).

witch,[605] but none of these is entirely free from all of the objections enumerated above. Figure 8 gives a diagrammatic representation of some of the variations in the Thiry-Vella fistulas.

In 1933, Johnston[606] described a modification of the Thiry loop designed to overcome the objection of leakage and to maintain normal peristalsis over a period as long as seven months. Absorption rates for glucose and sodium chloride after the seven-month interval were within the limits of error of experiments made many months earlier. The rate of secretion was maintained at a constant level and enzyme activity was noted in the

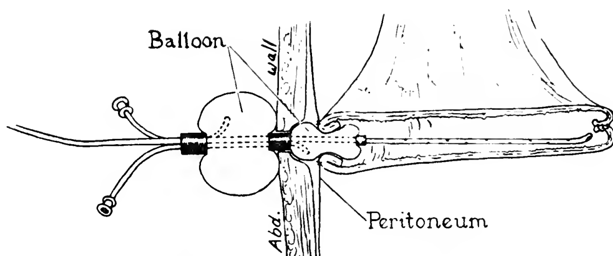


Fig. 9. The fistula as devised by Johnston[606] for quantitative studies of intestinal absorption.

samples obtained seven months after the fistulas were prepared. Histological sections made after this period showed that no microscopic changes had developed in any of the structures. These data led to the conclusion that the intestinal loops had remained normal after continued usage, and that with ordinary care they will continue to function satisfactorily over a prolonged interval.

In the Johnston technic, a Thiry fistula is first made from a section of intestine 20 to 50 cm. in length. After the wound is allowed to heal, which requires about two weeks, the loop is provided with a catheter to which two balloons are attached and which will prevent leakage. One of these is fastened in such a position that it lies within the loop, while the outer one is fixed in a position partly within the fistulous tract and partly exposed on the outside of the abdominal wall. When these are properly inflated, the inner balloon closes the proximal end of the loop, and the outer one, which lies entirely upon the abdominal wall, prevents the catheter from being

[605] H. L. White and J. Rabinowitch, *J. Biol. Chem.*, *74*, 449–454 (1927).
[606] C. S. Johnston, *Proc. Soc. Exptl. Biol. Med.*, *30*, 193–196 (1932–1933).

drawn into the fistula by the peristaltic movements. Fluids can be introduced and recovered quantitatively. Figure 9 gives a representation of this type of fistula. Although most of the earlier fistulas have been adapted only to dogs, Johnston[606] states that the new procedure is suitable for any animal, when loop studies are desired.

(2) Cori Technic

The most physiological procedure, and obviously the one which should give the closest approximation to that normally taking place in the gastrointestinal tract, involves the use of unoperated, unanesthetized rats. Such prerequisites are met in the technic which was first employed by Cori[607] for the study of the rate of absorption of sugars from the gastrointestinal tract of rats. The same procedure was used later by this investigator for amino acids,[608] for lactic acid,[609] and for ethyl alcohol.[610] In the Cori procedure, accurately measured amounts of solutions of the substances under investigation are given by stomach tube to unanesthetized, fasted rats. The animals are sacrificed after several time intervals, the gastrointestinal tract is removed intact, and the amount of the component under study still remaining in the gut is determined by analysis. The difference between the amount fed and the amount recovered represents that absorbed during the interval under study. Corrections must usually be applied to the analyses of the intestinal contents based upon blank values obtained with control rats, which are fasted for the same intervals but which are given no supplements. Care should be taken to avoid frightening the animals, as this may influence the emptying time of the stomach as well as the intestinal movements. Any such variations will obviously have the greatest influence on experiments of short duration. When the animals will eat spontaneously, some of these difficulties may be avoided, but the time of ingestion of the food cannot be established with as much precision as when the material is given by stomach tube. In order to obtain consistent results, it is important that the animals be of the same stock, age, weight, and also the same sex. Cori expressed the absorption in terms of the *absorption coefficient,* which is the number of milligrams of the substance absorbed per 100 g. of body weight per hour. Modifications of the Cori technic have been employed in the study of the absorption of fats and fatty acids, by Irwin, Steenbock, and Templin,[611] and by Deuel, Hallman, and Quon.[612]

[607] C. F. Cori, *J. Biol. Chem.,* *66,* 691–715 (1925).
[608] C. F. Cori, *Proc. Soc. Exptl. Biol. Med.,* *24,* 125–126 (1926).
[609] G. T. Cori, *J. Biol. Chem.,* *87,* 13–18 (1930).
[610] C. F. Cori, E. F. Villiaume, and G. T. Cori, *J. Biol. Chem.,* *87,* 19–25 (1930).

a. Method of Irwin, Steenbock, and Templin.[611] In this procedure only adult male rats, four to seven months of age and weighing 200 to 400 g., were used, following a forty-eight hour fast prior to the absorption tests. The rats were lightly anesthetized with ether, and the test fat was delivered directly into the stomach, by means of a 2 ml. syringe connected with a rubber catheter. Although doses of several amounts were tested, a standard 1.5 ml. dose was used in the tests with various fats. Different groups of rats were sacrificed by decapitation at several periods after feeding, and the intact gastrointestinal tract was removed. The stomach, cecum, and the intestine, divided into two sections, were all filled with distilled water, and the washings were emptied into beakers after ten minutes; this was followed by the introduction of petroleum ether into the several portions of the intestinal tract for a second ten-minute interval. The stomach and cecum were then opened and washed thoroughly with a jet of water and petroleum ether, while the intestine was stripped manually to remove the last trace of food. After extraction of the fat from the aqueous washings with additional petroleum ether, the combined petroleum ether extracts were dried with anhydrous sodium sulfate, the extract was filtered, and the petroleum ether was removed in a vacuum oven. When this technic was tested on rats which were sacrificed immediately after the feeding of the fat, recoveries of 97 to 98% were obtained. Since these workers could demonstrate no relationship between absorption rate on the one hand, and body weight, surface area, or length of the intestines on the other hand, the results were expressed in percentage of administered fat absorbed in a given time.

b. Method of Deuel, Hallman, and Quon.[612] This procedure is quite similar in many respects to that of Irwin *et al.*[611] However, because of the adjustment of the dosage to the size of the animal, it was possible to use the procedure for animals with a somewhat wider range of body weights. The animals were not anesthetized for the fat feeding, but were given amytal just before being sacrificed, at the end of the absorption period. At the termination of the test period, after the rat was under the influence of the anesthetic, the gastrointestinal tract was carefully removed; the fat remaining in the gastrointestinal tract was removed by flushing it with petroleum ether under some positive pressure. This was accomplished by attaching to the esophagus a syringe containing the ether and connected with a large blunt needle. Pressure was applied manually to force the ether into the gastrointestinal tract at a rate sufficient to produce the de-

[611] M. H. Irwin, H. Steenbock, and V. M. Templin, *J. Nutrition, 12,* 85–101 (1936).
[612] H. J. Deuel, Jr., L. F. Hallman, and S. Quon, *J. Biol. Chem., 128,* xix–xx (1939).

sired distention. During the flushing process, the stomach and cecum were constantly massaged to remove any solid material. Usually 50 to 75 ml. of ether were used. When the rats were sacrificed immediately after the administration of the fat, about 95% could be recovered. The amount of material soluble in petroleum ether which could be removed from the gastrointestinal tract of rats previously fasted was only 3 mg. for female rats

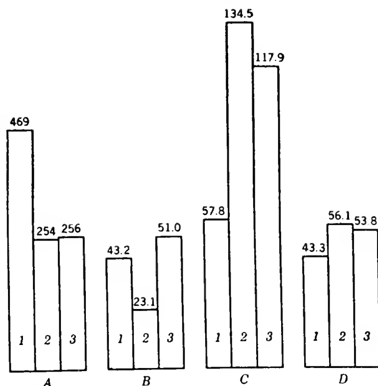


Fig. 10. Comparison of the different procedures for expressing fat absorption in male rats.[613]

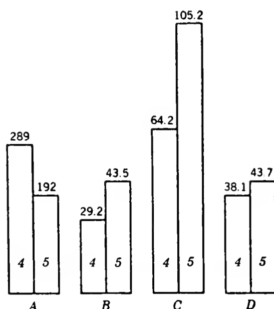


Fig. 11. Comparison of the different procedures for expressing fat absorption in female rats.[613]

Series *A* represents the total fat (margarine) absorbed, expressed in milligrams; Series *B* gives percentage of ingested fat absorbed; Series *C* lists the milligrams of fat absorbed per 100 g. body weight per hour; and Series *D* gives the milligrams of fat absorbed per 100 sq. cm. per hour.

and 12 mg. for male rats. When diethyl ether was employed in place of petroleum ether, the control value for intestinal lipids[614] was about 26 mg.

The chief difference between the procedure of Deuel *et al.*[612,614] and that of Irwin *et al.*[611] is the manner of expressing absorption. It was shown that the best method for comparing the rate of absorption of different fats is on the basis of body surface area.[613] Apparently the application of surface area is not limited to basal metabolism; it has also been shown to be the most satisfactory index for the evaluation of the absorption of glucose.[615]

[613] H. J. Deuel, Jr., in A. E. Bailey, ed., *Cottonseed and Cottonseed Products,* Interscience, New York and London, 1948, pp. 763–811 (p. 786).

[614] H. J. Deuel, Jr., L. Hallman, and A. Leonard, *J. Nutrition, 20,* 215–226 (1940).

[615] E. M. MacKay and H. C. Bergman, *J. Biol. Chem., 101,* 453–462 (1933).

Moreover, it is known that a proportionality exists between the body surface area and that of the intestine. Since the rate of absorption must be a function of the available area of the small intestine, it is only logical that any biometric measurement related to this value would be useful in the correlation of such data on fat absorption.

Deuel, Hallman, and Leonard[614] have compared the results on absorption of margarine fat when fed at a standard dosage to adult male rats (Group *1*) and to adult female rats (Group *4*); also, at a standard dosage, to young males (Group *2*) and young females (Group *5*); and at a high dosage to young males (Group *3*). The results are graphically illustrated in Figures 10 and 11, in which the rate of absorption is compared by several procedures.

On the basis of the above tests, it would seem quite definite that the most constant results can be obtained with animals of varying size if the rate of absorption is expressed on the basis of milligrams absorbed per 100 sq. cm. of body surface per hour. This procedure has been used in the tests on absorption referred to in a later section. It has also been the usual practice to feed the fat in such tests on the basis of surface area (usually in a dosage of 300 mg. per 100 sq. cm.).

(*3*) *Other Methods for the Study of Fat Absorption*

In addition to the direct method of estimating fat absorption by analysis of the gastrointestinal contents, a number of indirect procedures have been employed. As mentioned earlier, such methods have the advantage of giving data on the pathways of absorption and the form in which the substances are carried to the tissues.

a. Cannulation of the Thoracic Duct. The most important procedure for the study of fat absorption involves the cannulation of the thoracic duct. Since a large portion of the fatty substances travel *via* the lymphatic route from the intestine, a collection of this lymph before it is poured into the blood stream will furnish a qualitative as well as a rough quantitative method for following fat absorption. It is a relatively simple operative procedure to introduce a cannula into the thoracic duct. While the amount of lymph passed into the blood stream *via* this route is small in the fasted animal, the quantity is immediately increased, in a large measure, within a few hours after a fatty meal has been ingested. The lymph becomes milky from the fat globules; it is then referred to as chyle. Such lymph may contain as much as 5 to 15% of emulsified fat. Some criticism has been directed at experiments in which studies have been made on thoracic lymph, since this represents lymph not only from the intestinal area but also from other organs.

Süllmann and Wilbrandt[616] employed a technic on rabbits whereby intestinal lymph can be collected separately from systemic lymph. The large lymphatic vessels to the *cisterna chyli* can easily be found, especially if the left innominate vein is ligated and if the animal has previously been fed fat. If the lymphatic vessels are then cut with fine scissors, the lymph will collect in a little lake, and this can be continually pipetted off over a prolonged period. In their later work, Frölicher and Süllmann[136] modified

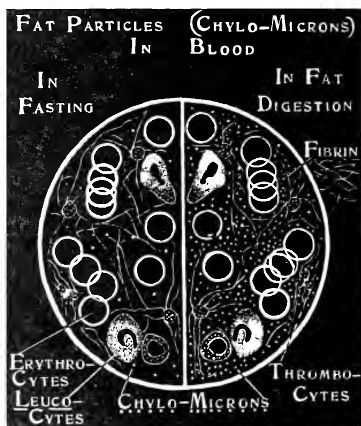


Fig. 12. Appearance of fresh blood under the high-power dark-field microscope to demonstrate the chylomicrons. The field at the left represents the appearance of blood obtained after a 16-hour to 24-hour fast. The field pictured at the right is for blood obtained 3 to 4 hours following a generous fat meal.[617]

the technic by omitting ligation of the innominate vein; thus, the lymph from other parts of the body could flow in the usual channels and not be shunted back into the intestinal lymphatics. This was considered to give a much purer "enteric lymph." A procedure for the separate collection of intestinal and thoracic duct lymph in the rat has been devised by Bollman and associates.[618]

b. Chylomicron Method. This procedure was first described in the classical paper of Gage and Fish[617] published in 1924. These investigators

[616] H. Süllmann and W. Wilbrandt, *Biochem. Z.*, *270*, 52–62 (1934).

[617] S. H. Gage and P. A. Fish, *Am. J. Anat.*, *34*, 1–85 (1924).

[618] J. L. Bollman, J. C. Cain, and J. H. Grindlay, *J. Lab. Clin. Med.*, *33*, 1349–1352 (1948).

found that the microscopic droplets, originally referred to in 1846 by Gulliver[619] as the "molecular base of chyle" and later by Munk and Rosenstein[620] as "fat dust," are a measure of fat absorption. They proposed the term *chylomicron* (pl. *chylomicrons* or *chylomicra*) from the Greek, χυλός, meaning chyle, and μιχρός indicating small. The chylomicrons are thus designated as originating from the chyle; they have an average size of about one micron.

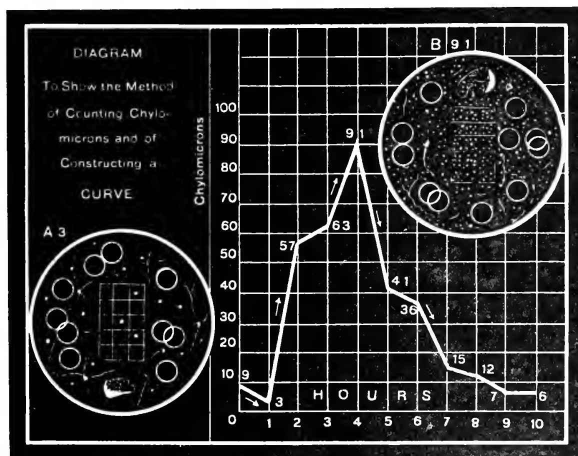


Fig. 13. A chylomicron curve obtained from a fasting individual before feeding and at hourly intervals up to 10 hours after the feeding of a fat meal. Two sample microscopic fields are included. The numbers along the curve show the chylomicron population present at the different hours.[617]

A comparative diagrammatic representation of the chylomicron population in the blood of fasted individuals and of one who had been fed a generous fat meal three or four hours previously is shown in Figure 12.

For the test on absorption, Gage and Fish[617] recommend that the subject be fasted for fifteen to twenty-four hours. This will reduce the chylomicron count for the net-micrometer field to approximately 0. A blood

[619] G. Gulliver, in introduction and notes of article by: W. Hewson, "On the Blood," *Philos. Trans.*, *60*, 368–383, 384–397, 398–413 (1770–1771); *63*, 303–323 (1773). W. Hewson, *Works*, Sydenham Soc., 1846; cited by S. H. Gage and P. A. Fish, *Am. J. Anat.*, *34*, 1–85 (1924), p. 13.

[620] I. Munk and A. Rosenstein, *Arch. path. Anat. Physiol.* (*Virchow's*), *123*, 230–279, 484–518 (1891).

sample is taken for chylomicron count before the fat meal is started. The fat is then administered with practically fat-free food such as bread, cereal, or especially with boiled rice. The dose of fat which was found to be the most satisfactory for adult human subjects was 20 g.; when larger amounts of fat were given, the chylomicron count was so high at the height of digestion and absorption that an accurate estimation of their number was quite difficult. Finger blood is usually employed in tests on human subjects, while ear blood from large animals and tail blood from the rat are satisfactory. In addition to the fasting sample, blood samples are withdrawn at intervals of one hour or less until the chylomicron count has returned to the preprandial level. This usually requires eight to ten hours. For the counting of the chylomicrons, a high-power dark-field microscope is employed, with homogeneous oil between the top of the condenser and the under surface of the slide, as well as upon the top of the cover glass. The slide contains a micrometer net in half-millimeters, the entire net containing 15 small squares. The usual practice is to count the chylomicrons in all 15 squares in five different areas. The results for a sample experiment are shown in Figure 13.

Irwin, Steenbock, and Templin[611] employed the Gage-Fish procedure for the study of the rate of absorption of fats after feeding 0.5 ml. of the product under investigation to fasted rats. Tail blood was used. However, these authors reported that this method "had little quantitative value." Frazer and Stewart,[621,622] using a carefully standardized technic, were able to demonstrate that the neutral fat content of the blood runs parallel to that of the chylomicrograph, whereas blood cholesterol yields a value quite divergent from it. The timing of the basic levels and peaks of the chylomicrons and neutral fat content coincides exactly. Frazer[623] states, however, that "the changes demonstrated by this method are sufficiently great to be significant, but the quantitative relationships can only be regarded as relative at present." Cooper and Lusk[624] reported that the chylomicron count is a reliable index of blood lipids. More recently, Nhavi and Patwardhan[625] used the chylomicron procedure with some success in studying the absorption of fats in human subjects.

c. Hemolipokrit Method. Rückert[626] has developed a procedure based on the analysis of the fat in the serum from 3 ml. samples of blood by a

[621] A. C. Frazer and H. C. Stewart, *J. Physiol.*, *90*, 18–30 (1937).
[622] A. C. Frazer and H. C. Stewart, *J. Physiol.*, *95*, 21P–23P (1939).
[623] A. C. Frazer, *Physiol. Revs.*, *20*, 561–581 (1940).
[624] R. R. Cooper and H. Lusk, *Am. J. Digestive Diseases*, *9*, 395–396 (1942).
[625] N. G. Nhavi and V. N. Patwardhan, *Indian J. Med. Research*, *34*, 49–58 (1946).
[626] W. Rückert, *Klin. Wochschr.*, *10*, 1853–1858 (1931).

modified Babcock technic.[627] Using this procedure, Irwin *et al.*[611] were able to obtain concordant results which were much more satisfactory than were those yielded by the chylomicron method. However, the results found by either of these indirect procedures did not parallel the data obtained from the direct absorption tests. Herrmann and co-workers[627] published a detailed report on the hemolipokrit method.

d. Studies on the Portal Blood. Limited information on fat absorption can be obtained by the examination of the portal blood, especially when the composition is compared simultaneously with that from the systemic circulation. By such studies it has been shown that some of the fat may pass into the portal blood, in place of being transported by the lymphatics. However, the concentration of the substance under study in the blood is influenced by so many factors that such a procedure cannot be used as a criterion of the rate of absorption. The technic of angiostomy introduced by London,[628] by which blood can be repeatedly obtained from different vessels without anesthetization, should give results which are more normal; they also offer the opportunity for following changes over a long period of time.

e. Roentgenologic Method for the Study of Fat Absorption. Groen[629] devised a new procedure for following the course of fat absorption which involves the determination of the rate of disappearance of the contrast shadow from x-ray films taken at regular intervals after the oral administration of "lipiodol." This product is an iodized fat made by treating poppyseed oil with iodine. The extent of excretion of iodine in the urine and stools confirms the x-ray evidence, although the latter data cannot be employed to determine the *rate* but only the *completeness* of absorption. This procedure is obviously of value only for determining abnormalities in absorption, and is of no use in assessing the behavior of natural non-iodized oils, since these yield no shadows under the x-ray.

f. The Use of Radioactive Iodinated Fat. Stanley and Thannhauser[630] employed unsaturated fats iodinated with I^{131} to study the rate of absorption and of utilization of fats. The proportion of the administered radioactive iodine present in the thyroid gland and excreted in the urine, as well as that in the water-soluble portion of the serum, was shown to give an index to the extent of utilization of the fat. Whereas, in normal subjects, a degradation of 50 to 73% of the orally administered iodinated fat was

[627] L. G. Herrmann, A. Ames, and R. J. Tapke, *J. Lab. Clin. Med.*, *19*, 411–421 (1934).

[628] E. S. London, *Angiostomie und Organestoffwechsel*, All-Union Inst. Exptl. Med., Moscow, 1935.

[629] J. Groen, *Am. J. Med.*, *4*, 814–826 (1948).

[630] M. M. Stanley and S. J. Thannhauser, *J. Lab. Clin. Med.*, *34*, 1634–1639 (1949).

shown to take place within twenty-four hours, subjects with "idiopathic" hyperlipemia and the nephrotic syndrome were found to utilize the labeled lipid more slowly. Kirchmair[631] employed a similar method for the determination of lipid absorption in rabbits, based upon the rate of excretion of ordinary iodine in the urine. A method for preparing iodized fat is described by Rutenburg *et al.*[632]

g. Elaeostearic Acid as a Tracer for the Study of Fat Absorption. Fillerup and Mead[633] have proposed that small amounts of β-elaeostearic acid (prepared from tung oil) be incorporated in the fat under investigation. The amount of total fat absorbed can be calculated spectrophotometrically from the rate of disappearance of the conjugated acid. The rate of absorption, in mice, of methyl oleate (mixed with 5% methyl-β-elaeostearate) was shown to be identical when calculated by the standard Cori technic, or by the use of the elaeostearate tracer. However, the incorporation of the elaeostearate into the fat increased the rate of absorption. The procedure has likewise been used in a human subject for the indirect determination of fat absorption by following the levels of elaeostearate in the blood.[634]

6. The Digestion of Fats

The main changes involved in the digestion of foodstuffs in the gastrointestinal tract are due to hydrolysis. In the case of proteins and carbohydrates, these hydrolytic changes are so extensive that the end-products have only a fraction of the molecular weight of the original components in the food. These hydrolyses not only bring about a greater solubility of the products, but they also permit wider flexibility in the substances which can be synthesized from these building blocks. It is, of course, obvious that the simpler the unit, the more diverse are the products which can be synthesized from it.

The scope of the hydrolyses which take place in the digestion of fat is extremely minor compared with that in the digestion of proteins and carbohydrates. At most, only three linkages are capable of hydrolysis in the triglyceride molecule, and there is some dispute as to whether all of these possible points of splitting are actually attacked before absorption occurs.

[631] H. Kirchmair, *Klin. Wochschr.*, *27*, 588–589 (1949).

[632] A. M. Rutenburg, A. M. Seligman, and J. Fine, *J. Clin. Invest.*, *28*, 1105–1109 (1949).

[633] D. L. Fillerup and J. F. Mead, *Absorption and Distribution Studies Using Eleostearic Acid*, UCLA-148, Atomic Energy Project, Aug. 7, 1951.

[634] J. F. Mead, D. L. Fillerup, A. B. Decker, and L. R. Bennett, *J. Nutrition*, *46*, 499–513 (1952).

In the case of lipids such as the esters of vitamins A and D, only a single hydrolysis can occur; in hydrocarbons, as for instance carotene or lycopene, no hydrolysis whatsoever is possible. Although other reactions, such as reduction, can take place in the lower gastrointestinal tract, these are not usually concerned with absorbable material, most of which has left the gastrointestinal tract prior to reaching areas where such changes can take place.

(1) *Digestion in the Stomach*

Although some lipase may be secreted in gastric juice, it is a water-soluble enzyme which can come in contact with only an infinitesimal amount of fat unless there is some factor causing an increase in the water-fat interface. Those fats, such as milk fat and egg-yolk fat, which are already highly emulsified when ingested, provide a large surface area for attack by water-soluble enzymes. Although similar fat emulsions can readily be produced from ordinary fats when soap is present, the acidity of the gastric contents is seldom sufficiently low to permit the formation of such soap emulsions.

The formation of fat emulsions, and the action of lipase on such emulsions are, however, favored by one physiological reaction. When any considerable amount of fat is present in the food, the period of time during which the food remains in the stomach is markedly increased. According to Tangl and Erdélyi[635] and von Fejér,[636] the emptying time of the stomach depends upon the melting point and viscosity of the fat ingested; the presence of large amounts of fat may delay the emptying time of the stomach for several hours. The secretion of pepsin and of acid by the gastric mucosa is likewise inhibited when fat is present in the stomach. The pancreatic juice and bile may pass into the stomach from the intestine as the result of antiperistaltic movements.[637,638] The regurgitation of intestinal contents into the stomach may be sufficiently pronounced to reduce the acidity of the gastric contents to a significant extent. All of these factors will tend to act independently and collectively to produce some lipolysis in the stomach. As the chyme is passed into the intestine, enough fatty acid is already present in the free state to produce a soap emulsion immediately on alkalinization of the medium.

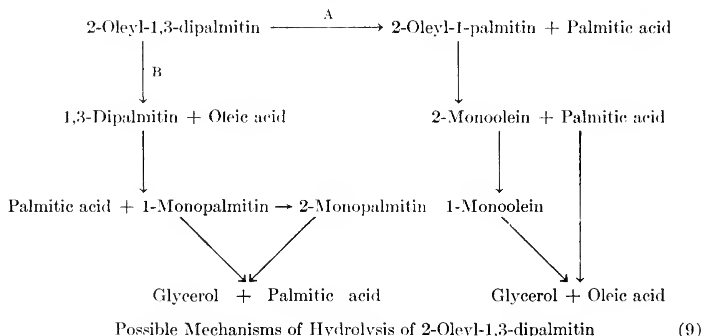
[635] F. Tangl and A. Erdélyi, *Biochem. Z.*, *34*, 94–110 (1911).
[636] A. von Fejér, *Biochem. Z.*, *53*, 168–178 (1913).
[637] W. Boldyreff, *Arch. ges. Physiol. (Pflüger's)*, *121*, 13–53 (1908).
[638] W. Boldyreff, *Ergeb. Physiol.*, *11*, 121–217 (1911).

(2) *Digestion in the Small Intestine*

Conditions are all normally favorable for a rapid digestion of fats in the small intestine. The chyme reaches the small intestine from the stomach in small installments. When the food being digested is low in fat, the quantities of chyme passed through the pylorus are rather large and the passage into the intestine occurs at frequent intervals; the small percentage of fat in the mixture prevents overtaxing of the fat-digesting and fat-absorbing mechanisms. On the other hand, when the quantities of fat in the food are high, the passage of the chyme into the small intestine is extended over a long period, and the quantities of chyme passing through the pylorus at any one time are small and the amount of fatty material present in the small intestine is not large enough to overburden the organism.

a. The Action of Lipases. Lipase is supplied not only by the pancreatic juice but likewise by the intestinal secretion. The intestinal lipase is sufficiently potent so that, even in the absence of the more powerful pancreatic lipase, steapsin, most of the fat will be digested. In fact, Angelico and Ligori[639] report that the external pancreatic secretion does not influence the intestinal absorption of lipids. As was indicated earlier, emulsification is a prerequisite for fat splitting. Since bile salts apparently have the property of lowering surface tension, the formation of an emulsion is facilitated by the presence of these substances.

It is thought that, as a result of the lipolytic action of the fat-splitting enzymes, a series of intermediate products may arise, namely the di- and monoglycerides. One might assume that the breakdown of 2-oleyl-1,3-dipalmitin could take place in the following stages:



Possible Mechanisms of Hydrolysis of 2-Oleyl-1,3-dipalmitin (9)

[639] R. Angelico and M. G. Ligori, *Bull. soc. chim. biol., 31,* 540–543 (1949).

Thus, if the hydrolysis of triglycerides were a random reaction, one might expect that the intestinal contents, during the early stages of digestion of a mixed triglyceride, would include not only undigested triglyceride, but also mixtures of at least two diglycerides, at least two monoglycerides, glycerol, and the several fatty acids composing the mixed triglyceride molecule. However, according to Mattson *et al.*,[640] the action of the pancreatic lipase is not a random one but proceeds according to pathway A.

If the medium is alkaline, one may anticipate finding that the soaps replace the fatty acids; if soluble calcium compounds are available, the soaps will tend to consist largely of the calcium salts. In the absence of calcium, sodium and potassium soaps predominate. However, most evidence points to the fact that the reaction in the intestine is such that fatty acids rather than soaps are present in the small intestine. For example, Kostyál,[641] in a study of the *p*H in the intestines of rats, dogs, guinea pigs, and pigeons, found that it was almost never above 7.0, and was usually on the acid side of neutrality. Robinson[642] also reported a *p*H of 6.5 in the duodenum of dogs and rats, and a value of 7.5 to 8.0 at the ileocecal valve. Schulte[643] noted that the glycerides formed from fatty acids produced synthetically by oxidation of hydrocarbons were acted upon by pig pancreatic lipase. Cleavage occurred in the same manner as in the case of the regular dietary fats.

b. The Extent of Hydrolysis of Fats. There is a difference of opinion as to how completely the triglycerides are hydrolyzed in the small intestine. Verzár and McDougall,[554] and Bloor[35,644] believe that a complete hydrolysis to glycerol and fatty acids is a prerequisite for fat absorption. Since it is known that fats are largely digestible, if one accepts the Lipolytic Theory, one must postulate that an equally complete hydrolysis of the triglycerides must have obtained prior to absorption. The fact that glycerol and fatty acids (or soaps) are rapidly removed from the medium by absorption will permit the hydrolysis of the triglyceride to continue, without inhibition due to the accumulation of end-products, until the splitting reaction has proceeded to completion.

On the other hand, Frazer[645] questioned whether or not the complete

[640] F. H. Mattson, J. H. Benedict, J. B. Martin, and L. W. Beck, *J. Nutrition, 48,* 335–344 (1952).

[641] L. Kostyál, *Magyar Orvosi Archivum, 27,* 276–281 (1926); *Chem. Abst., 21,* 126 (1927).

[642] C. S. Robinson, *J. Biol. Chem., 108,* 403–408 (1935).

[643] K. E. Schulte, *Biochem. Z., 318,* 220–226 (1948).

[644] W. R. Bloor, *Physiol. Revs., 2,* 92–115 (1922).

[645] A. C. Frazer, *Physiol. Revs., 26,* 103–119 (1946).

hydrolysis of the triglycerides is a necessary prerequisite to fat absorption. This investigator has amassed a great deal of evidence which suggests that unhydrolyzed fat can be absorbed in a finely emulsified form. The formation of di- or monoglycerides is considered merely as an aid to fat absorption in that it provides a medium conducive to the formation of an emulsion of fat droplets sufficiently fine to be capable of absorption.

Although the initial stage of the lipolysis proceeds rapidly, it comes practically to a stop before 30% of the potential fatty acids are set free. Addition of more lipase will change the extent of the hydrolysis in a given time, although the final percentage is not elevated above the limiting figure. It is not believed that the amount of lipase available is increased markedly as digestion proceeds, since only the initial flow of pancreatic juice contains appreciable amounts of lipase, while the later secretion consists largely of water and salts.[646] When a potent lipase preparation is added to a standard meal, the postabsorptive lipemia is suppressed. This probably indicates an increase in the extent of hydrolysis. The natural assumption is that the amount of lipase furnished to the digesting mixture in the intestine is normally small, and that this fact accounts for the partial hydrolysis which occurs. If this quantity of lipase is increased by artificial means, then the whole process of digestion and absorption of the foodstuff proceeds by a different course.

When pancreatic lipolysis is carried out *in vitro* under the conditions which obtain in the small intestine, without the introduction of excessive quantities of activators or an abnormal *p*H level, no free glycerol can be demonstrated over a five-hour period. Control experiments with glycerol demonstrate that this polyhydric alcohol is not destroyed, although it does disappear when the digestion period is prolonged much beyond the five-hour period. Although a considerable proportion of fatty acids is set free during the five-hour period of lipolysis, these originate from the conversion of triglycerides to di- or monoglycerides, rather than from complete cleavage of the fatty acids from the triglyceride molecule. The presence of di- and monoglycerides is inferred from the fact that an increased content of free hydroxyl groups occurs, as is shown by the marked rise in the acetyl value. Since no free glycerol is present, and no hydroxy acids are produced, the increased content of free hydroxyl groups can be attributed only to the di- and monoglycerides.[647] Support for the partial hydrolysis concept is afforded by the isolation of monoglyceride from the intestinal contents obtained from *in vivo* tests with rats.[647]

[646] J. Mellanby, *J. Physiol.*, *60*, 85–91 (1925).
[646] A. C. Frazer and H. G. Sammons, *Biochem. J.*, *39*, 122–128 (1945).

It is well known that the duration of the absorption period of moderate doses of fat is only five hours. Frazer[645] states the crux of his argument as follows:

"If the end-products of hydrolysis during the first five hours are fatty acids and glycerides, and over 90% of the fat is absorbed during this period, it is obvious that a significant proportion of the fat must be absorbed as unhydrolysed or partly hydrolysed triglyceride."

A number of investigators have confirmed the demonstration by Frazer and Sammons[647] that only a partial hydrolysis of fat occurs in the small intestine. Desnuelle *et al.*[648] reported that the hydrolysis of coconut oil, peanut and sunflower oils, when catalyzed *in vitro* by pancreatic lipase, gives rise to only a minimum quantity of free fatty acids. The action of pancreatic lipase on triglycerides was shown to be a three-stage reaction.[649,650] First, a rapid formation of diglycerides obtains, while the second and third stages, which involve the removal of the second and third fatty acid from glycerol, proceed very slowly. It is suggested that the affinity of lipase for the glycerides diminishes with the appearance of free hydroxyl groups. The digestion mixture present, when triglycerides and pancreatin are incubated over a period of one to four hours, in the absence of calcium, consists largely of unattacked triglycerides, of diglycerides, of small amounts of monoglycerides, and of traces of glycerol.[651] Artom and Réale were the first to make the observation that mono- and diglycerides are formed in both the digestion[652] and the synthesis[34] of fats by pancreatic lipase. Kuhrt and associates[653] reported that 37 to 50% of the lipids in the intestinal lumen of human subjects after fatty meals consisted of monoglycerides.

On the other hand, Desnuelle *et al.*[654,655] reported that the presence of calcium ions in the gastrointestinal tract of rats and dogs augments the proportion of monoglycerides at the expense of the diglycerides. The same ratio of acids and partial glycerides was shown to be present when the

[648] P. Desnuelle, M. Naudet, and J. Rouzier, *Arch. sci. physiol.*, *2*, 71–79 (1948).

[649] P. Desnuelle, M. Naudet, and J. Rouzier, *Compt. rend. soc. biol.*, *141*, 1242–1244 (1947).

[650] P. Desnuelle, M. Naudet, and M. J. Constantin, *Compt. rend. soc. biol.*, *144*, 1182–1183 (1950).

[651] P. Desnuelle, M. Naudet, and J. Rouzier, *Biochim. et Biophys. Acta*, *2*, 561–574 (1948).

[652] C. Artom and L. Réale, *Arch. sci. biol. (Italy)*, *21*, 368–380 (1935).

[653] N. H. Kuhrt, E. A. Welch, W. P. Blum, E. S. Perry, W. H. Weber, and E. S. Nasset, *J. Am. Oil Chemists' Soc.*, *29*, 271–278 (1952).

[654] P. Desnuelle and M. J. Constantin, *Biochim. et Biophys. Acta*, *9*, 531–537 (1952).

[655] P. Desnuelle, M. Naudet, and M. J. Constantin, *Biochim. et Biophys. Acta*, *5*, 561–568 (1950).

fat was treated with lipase *in vitro* and *in vivo*, in the case of the intraluminar lipids. In a later report, Desnuelle and co-workers[656] stated that about one-third of the fat is completely hydrolyzed by pancreatic lipase while the remaining two-thirds is in the form of mono- and diglycerides.

Several workers have investigated the extent of hydrolysis of fats, using tagged glycerol. Thus, Favarger *et al.*[657] found that trielaidin containing deuterioglycerol was hydrolyzed to the extent of only 5% in rats, as determined from the proportion of glycerol incorporated into intestinal fats and blood. Reiser and associates[658] noted that 25 to 45% of the ingested glycerides are completely hydrolyzed during absorption, while the remaining glycerides are converted to monoglycerides. The hydrolyzed glycerol was not used for the resynthesis of fat, but it followed an independent metabolic pathway. It was shown that about 50% of the phospholipid present in lymph formed from ingested fat utilized the hydrolyzed fatty acids and endogenous glycerol. Morehouse and Skipski,[659] using dioleyl-deuteriostearin containing C^{14}-labeled glycerol, demonstrated that some breakdown of lipid material took place in the gastrointestinal tract, with a subsequent recombination. The possibility that unchanged neutral fat may be absorbed was indicated.

A quantitative evaluation of the nature of the intestinal contents after a fat meal has been made possible by the separation of the mixture into its several components by the use of a countercurrent analytical procedure, by Mattson and his associates.[640] The results of these investigators support the Frazer hypothesis. The mono- and diglycerides isolated from the intestinal contents of rats four hours after fat feeding accounted for 16 and 36% of the total lipids. Moreover, the hydrolysis of the fatty acids from the glycerol moiety was found to proceed by a definite pathway rather than in a random fashion. Thus, evidence was adduced that the diglycerides formed were of the 1,2-(α,β) variety. The monoglycerides initially formed consisted practically entirely of the 2-isomer, although this compound was found to be labile and readily changed to the 1-(α) isomer by the manipulative procedures. Confirmatory evidence for this sequence in hydrolysis has been obtained in *in vitro* studies with tripropionin. Schønheyder and Volqvartz[660] demonstrated that, when liver esterase or pancreatic lipase

[656] P. Desnuelle, M. Naudet, and M. J. Constantin, *Biochim. et Biophys. Acta*, *7*, 251–256 (1951).

[657] P. Favarger, R. A. Collet, and E. Cherbuliez, *Helv. Chim. Acta*, *34*, 1641–1654 (1951).

[658] R. Reiser, M. S. Bryson, M. J. Carr, and K. A. Kuiken, *J. Biol. Chem.*, *194*, 131–138 (1952).

[659] M. G. Morehouse and W. Skipski, *Federation Proc.*, *12*, 248–249 (1953).

[660] F. Schønheyder and K. Volqvartz, *Biochim. et Biophys. Acta*, *8*, 407–415 (1952).

acted on a homogeneous solution of tripropionin, 1,2-dipropionin was first formed, followed later by monopropionin, which was proved to be mainly the 2-isomer. Whereas the first hydrolysis, namely tripropionin → 1,2-dipropionin, represented a fairly rapid reaction, the next change, *viz.*, 1,2-dipropionin → 2-monopropionin, proceeded at an extremely slow rate. The presence of free alcoholic groups, especially of primary groups, was found to greatly decrease the rate of hydrolysis of the *in vitro* reaction in a manner similar to that of its inhibition of the *in vivo* hydrolysis. Peers[661] reported experiments with tributyrin which would seem to indicate that the action of lipase on tributyrin is only partial. When this triglyceride was treated with a purified cat lipase, only one fatty acid was split off, and the hydrolysis did not proceed further.

Loncin[662] reported a confirmation of the stepwise degradation of triglycerides based upon an entirely different approach. This worker observed that palm oil, on storage, undergoes a partial hydrolysis by the process of "spontaneous autocatalytic hydrolysis." The fact that the speed of hydrolysis increases with the elevation of the temperature to fairly high levels precludes an enzymatic or bacterial mechanism. The first phase of the reaction involves the formation of diglycerides and free fatty acids from the palm oil; after the acidity has reached a certain degree, monoglycerides and free fatty acids tend to be formed from the diglycerides. No free glycerol could be demonstrated until more than 18% of the fatty acids had been liberated. After 115 days of storage at 70°C., the following percentage composition was noted: triglycerides, 22.2%; diglycerides, 26.4%; monoglycerides, 10.85%; and free fatty acids, 40.6%.

c. The Bile Requirement. Irrespective of which of the theories best explains the mechanism of the absorption of fats, there is no question that bile plays an essential role in promoting both the digestive and the absorptive phases of this physiological phenomenon. We have discussed earlier how the action of bile can be largely attributed to the bile salts present therein. These bile salts help to activate steapsin, and in this manner accelerate the digestion of the fat molecule. The bile salts also have a solubilizing effect on the neutral fats, di- and monoglycerides, as well as on the fatty acids. In the case of incompletely digested fats, this behavior will allow a more intimate contact between the water-soluble lipases and the fatty substrates.

There are many explanations for this so-called "solubilizing" effect.

[661] F. G. Peers, *Nature, 171*, 981–982 (1953).

[662] M. Loncin, *L'Hydrolyse spontanée des huiles glyceridiques et en particulier de l'huile de palme*, Couillet, Brussels.

It may be attributed in part to the decreased surface tension which obtains in the aqueous phase; such a phenomenon would likewise cause a lowering of the interfacial tension between the aqueous and the oily phases. When this occurs, the formation of emulsions is favored, and the solution of one phase in the other is facilitated.

Verzár and McDougall[554] suggested that the bile salts act as hydrotropic agents to bring water-insoluble fatty acids into solution. These workers were able to demonstrate that fatty acids could be brought into a soluble and even into a diffusible form *in vitro* in acid solution when bile salts were present in the medium. The possible mechanism for accomplishing this condition is the formation of water-soluble bile salt + fatty acid complexes known as "choleic acids"; these complexes are carried into the epithelial cells, where they are broken down. For a discussion of the circulation of bile salts, see page 111. Although these choleic acid complexes are readily demonstrable in the case of the fatty acids, their formation with mono-, di-, and triglycerides has not been proved *in vitro*. However, it is quite probable that such coordination compounds might occur *in vivo*, since the acholic portion of the choleic acids varies considerably in composition. Thus, it would seem possible that this mechanism might be an essential part of the absorption cycle, whether the fatty acids or the glycerides themselves are the components transported into the epithelial cells.

7. The Absorption and Transport of Fats

(1) *The Absorption of Fats from the Gastrointestinal Tract*

a. Introduction. After the hydrolysis of fat has proceeded to the di- and monoglyceride stage or, according to the Bloor-Verzár theory, to glycerol and fatty acids, absorption of these intermediates occurs. It has long been known that the split products of the triglyceride molecule are readily absorbable. The fact that mono- and diglycerides are either absorbed as such or after hydrolysis is shown by the complete utilization of these compounds.[663–665]

Proof that the glycerol moiety is absorbable has been afforded by the results reported by a number of workers. Thus it has been noted [666–669] that ingested glycerol reduces the ketonuria in fasting or diabetic men.

[663] H. C. Tidwell, *J. Biol. Chem.*, *182*, 405–414 (1950).

[664] W. Q. Braun and C. L. Shrewsbury, *Oil and Soap*, *18*, 249–250 (1941).

[665] J. R. Ames, M. P. O'Grady, N. D. Embree, and P. L. Harris, *J. Amer. Oil Chemists' Soc.*, *28*, 31–33 (1951).

[666] F. Hirschfeld, *Z. klin. Med.*, *28*, 176–209 (1895).

[667] G. Satta, *Beitr. chem. Physiol. Pathol.*, *6*, 376–391 (1905).

[668] R. M. Lang, *Biochem. J.*, *9*, 456–478 (1915).

[669] H. M. Thomas, *Bull. Johns Hopkins Hosp.*, *35*, 201–206 (1924).

or in rats having an exogenous ketonuria.[669a] Voegtlin, Dunn, and Thompson[670] showed that glycerol can replace glucose in counteracting the hypoglycemic and toxic effects resulting from insulin injection. One must predicate that glycerol is absorbed from the intestine and is converted to glucose before it is able to produce either of the above effects.

Cremer[671] has carried the proof of absorption one step further by accounting for 40% of the glycerol given to phlorhizinized dogs as "extra-glucose" in the urine, while Lüthje[672] reported essentially similar results for depancreatized animals. Chambers and Deuel[673] were able to demonstrate a practically quantitative conversion of glycerol to glucose in phlorhizinized dogs when the polyhydric alcohol was given orally, and a 70% transformation after subcutaneous administration. Glycerol also yields glycogen.[669a]

The absorption of the fatty acid moiety from the intestine was demonstrated as early as 1868 by Radziejewski,[674] who fed the acids in the form of soaps. Perewoznikoff[675] reported that a mixture of soap and glycerol was absorbed and synthesized into fat; he showed that, after this mixture was fed, the lacteals had the same milky appearance as after a fat meal, and also that the epithelial cells contained fat globules. Many other workers have confirmed the early results with fatty acids and soaps.[620,676,677] Jeker[677] reported the presence of free fatty acid in the mucosa within ten to twenty minutes after fat feeding, as determined histologically; by the sixth hour the free fatty acid had disappeared and had been replaced by neutral fat. Terroine[678] also reported that the rate of hydrolysis of fats by pancreatic juice *in vitro* has a direct relationship with the speed of absorption of fat.

Another indication of the absorption of the free fatty acids is obtained from the studies with simple esters of fatty acids. Thus, it was demonstrated that fatty acids were absorbed when given as ethyl esters,[679] as amyl esters,[620] or as the optically-active mannite esters.[680] Frank[679] found

[669a] I. Shapiro, *J. Biol. Chem.*, *108*, 373–387 (1935).

[670] C. Voegtlin, E. R. Dunn, and J. W. Thompson, *Am. J. Physiol.*, *71*, 574–582 (1924–1925).

[671] M. Cremer, *Münch. med. Wochschr.*, *49*, 944 (1902).

[672] H. Lüthje, *Deut. Arch. klin. Med.*, *80*, 98–104 (1904).

[673] W. H. Chambers and H. J. Deuel, Jr., *J. Biol. Chem.*, *65*, 21–29 (1925).

[674] S. Radziejewski, *Arch. path. Anat. Physiol. (Virchow's)*, *43*, 268–286 (1868); *56*, 211–219 (1872).

[675] A. Perewoznikoff, *Zentr. med. Wiss.*, *14*, 851–852 (1876).

[676] I. Bang, *Biochem. Z.*, *91*, 111–121 (1918).

[677] L. Jeker, *Arch. ges. Physiol. (Pflüger's)*, *237*, 1–13 (1936).

[678] E. F. Terroine, *Ann. sci. nat., Zool.*, [*10*], *4*, 1–397 (1920).

[679] O. Frank, *Z. Biol.*, [*2*], *18* (*36*), 568–593 (1898).

[680] W. R. Bloor, *J. Biol. Chem.*, *11*, 141–159, 429–434 (1912).

that the feeding of monoglycerides resulted in the formation of triglycerides, a fact which was interpreted as indicative of complete hydrolysis of the monoglyceride preliminary to absorption.

Finally, the more recent studies with fatty acids and soaps have confirmed the earlier investigations in demonstrating the absorbability of these substances. These studies included an investigation of the relationship between calcium salts and the absorption of fatty acids,[681–684] a comparison of the absorption of fatty acids with that of their triglycerides,[685] as well as a consideration of the role of the adrenal cortex in the absorption of fatty acids.[686,687] For a further discussion of the absorption of fat in the form of neutral fat, di- or monoglycerides, or as fatty acids or soaps, see page 151, where the evidence for the several theories on fat absorption is discussed.

b. Theories of Fat Absorption. The mechanism by which the ingested fat passes into the wall of the intestine and from there into the lymph or portal blood has been the object of much experimental work and of considerable conjecture. The appearance of the fine emulsions in the intestinal contents, as well as in the chyle from the thoracic duct, would seem to offer some evidence that the process of absorption of fat involves the passage of this substance in sufficiently fine droplets through the wall of the gut.

(*a*) *Early Theories on Fat Absorption.* Schäfer[688] was one of the first to advance proof that fat is absorbed in particulate form. He believed that the leucocytes played an important role in the process, and aided in the transfer of fat into the epithelial cells. It was recognized that, during absorption, there is no rigid cell membrane on the open (lumen) side of the columnar epithelium. The surface could readily be indented, and it was believed that leucocytes might work their way into the cells as well as between them. Fat globules of various sizes were observed within the epithelial cells during absorption. Usually the largest globules are between the nucleus and the outer border of the cell (toward the lumen), while those near the basement membrane are small, as a rule. In some instances the majority of the fat was found in the outer portion of the cell, while, in other

[681] M. H. Givens, *J. Biol. Chem.*, *31*, 441–444 (1917).

[682] A. W. Bosworth, H. H. Bowditch, and L. A. Giblin, *Am. J. Diseases Children*, *15*, 397–407 (1918).

[683] O. F. Boyd, C. L. Crum, and J. F. Lyman, *J. Biol. Chem.*, *95*, 29–41 (1932).

[684] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949).

[685] R. Hoagland and G. G. Snider, *J. Nutrition*, *26*, 219–225 (1943).

[686] L. A. Bavetta and H. J. Deuel, Jr., *Am. J. Physiol.*, *136*, 712–715 (1942).

[687] L. A. Bavetta, *Am. J. Physiol.*, *140*, 44–46 (1943).

[688] E. A. Schäfer, *Intern. Monatsschr. Anat. Histol.*, *2*, 6–29 (1885).

cases, the fat globules were largely in the inner part of the cells. It is now believed that these two pictures may represent different stages in fat absorption. According to the Schäfer concept, fat is carried into the cells by the leucocytes; it is presumably deposited there temporarily, and gradually works its way to the basement membrane of the cell. During its passage through the length of the cells, the large droplets gradually disappear, to be replaced by much finer ones, preliminary to their removal from the cell through the basement membrane.

In 1888, Heidenhain[689] proposed a slightly modified hypothesis to explain fat absorption. The main difference from the Schäfer theory lay in the fact that he ascribed a minor role to the leucocytes in the fat absorption process. Heidenhain cites numerous data to prove that no parallelism exists between the leucocyte population and fat absorption. For example, in newborn puppies, leucocytes are seldom present in the intestinal epithelium during active fat absorption, although they are found in large numbers during fasting. Again, many leucocytes which apparently contain fat are present in the glands of Lieberkühn; there is no logical explanation, insofar as absorption is concerned, to account for their presence in this area. Finally, granules which stain black with osmic acid may be found in fasting animals, near and within Lieberkühn's gland. Although osmic acid is generally accepted as a stain which is specific for fat, it has been suggested that some substance, other than fat, accounts for the stained particles found in these experiments.

The results of Clark and Clark[690] throw further light on the functions of the leucocytes in fat absorption. These workers have confirmed Zawarykin's findings[691] that leucocytes are present in large numbers in the intestinal mucosa during fat absorption. However, they are only rarely found in the lumen of the intestine, but occur chiefly in the lacteals[692,693] and around the epithelial cells. These data lead one to the conclusion that the leucocytes are not concerned with the first stage in fat absorption, namely, the transfer of fat from the lumen of the gut to the epithelial cells. The leucocytes would exert their chief function in the second stage of fat absorption, *i.e.*, the transport of the fats from the epithelial cells to the lacteals.

Munk,[694,695] in 1884, was more definite in his hypothesis that fat was

[689] R. Heidenhain, *Arch. ges. Physiol.* (*Pflüger's*), *43*, suppl., 1–103 (1888).
[690] E. R. Clark and E. L. Clark, *Am. J. Anat.*, *21*, 421–448 (1917).
[691] T. Zawarykin, *Arch. ges. Physiol.* (*Pflüger's*), *31*, 231–239 (1883).
[692] T. Zawarykin, *Arch. ges. Physiol.* (*Pflüger's*), *35*, 145–157 (1885).
[693] R. Zipkin, *Anat. Hefte*, *23*, 113–186 (1904).
[694] I. Munk, *Ergeb. Physiol.*, *1*, 296–329 (1902).
[695] I. Munk, *Arch. path. Anat. Physiol.* (*Virchow's*), *95*, 407–467 (1884).

absorbed in a fine emulsion. Although he recognized that as much as 12% of the ingested fat could be split in the intestine,[695] he favored the concept that the bulk of the fat was absorbed in the form of finely emulsified droplets of neutral fat. This was the first definite statement of the current particulate theory of fat absorption. The chief support for this concept advanced by Munk was the fact that fat appears as neutral fat in the lymphatic system,[696] and hence is presumed to pass from the intestine through the cells in fine droplets,[694] without chemical alteration. No good explanation of the motivating force which brings this about was suggested.

The method by which fat gains admission into the cells is one of the most puzzling questions left unanswered by Schäfer, Heidenhain and Munk. One suggestion was that the striated outer border of the cells can form ameboid protrusions into the intestines whereby the cells are enabled to engulf fat particles in the intestinal lumen and transfer them into the cell. However, there is little histological evidence that these cells act as phagocytes, although Wotton and Zwemer[697] obtained photographs which appear to show fat-like globules in the process of passing through the outer membranes of the epithelial cells. However, Bloor[35] criticizes this conclusion by stating that no proof is offered as to whether the droplets pictured represent unsplit fat or fatty acids.

Another of the earlier theories to explain fat absorption is that of Pflüger.[698] This investigator suggested that fats were hydrolyzed to fatty acids and glycerol by lipase, that the fatty acids were neutralized by the alkali present in bile and pancreatic juice, and that the sodium soaps so formed and the glycerol, both of which are water-soluble, were easily transported across the mucosal wall. At some stage before the fat droplets entered the lymphatics of the villi, a synthesis of glycerol and soaps to neutral fat obtained. However, since the disclosure that the sodium soaps are not stable at a *p*H below 8 and that the small intestine is practically never alkaline,[641,642] the soap theory has lost much of its appeal.

(*b*) *Lipolytic Theory* (*Verzár*). According to the Lipolytic Theory,[644] fats must be hydrolyzed to glycerol and fatty acids in the small intestine before they can be absorbed. The greatest support for this hypothesis has been brought forward in recent years by Verzár and his colleagues; the work was summarized in the monograph of Verzár and MacDougall[554] and later by Verzár.[699] This theory has gained wide acceptance over much

[696] I. Munk, *Arch. Physiol.*, *1879*, 371–374.
[697] R. M. Wotton and R. L. Zwemer, *Anat. Record*, *75*, 493–503 (1939).
[698] E. Pflüger, *Arch. ges. Physiol.* (*Pflüger's*), *82*, 303–380 (1900).
[699] F. Verzár, *Arch. sci. physiol.*, *2*, 43–63 (1948).

of the twentieth century; it has been thrown open to question only comparatively recently by Frazer,[700] who pointed out certain phenomena which cannot be explained by such an hypothesis.

a′. The Hydrotropic Action of Bile Salts: According to the Verzár theory, the fatty acids are brought into diffusible form by the hydrotropic action of the bile salts. It was postulated that the fatty acids form combinations with the bile salts similar to the choleic acids demonstrated by Wieland and Sorge[506]; these complexes are conveyed through the intestinal wall, whereupon the bile salt plus fatty acid combination is broken up within the cell, and the bile salts are set free, to be excreted and to aid in the absorption of more fatty acids. It is suggested that the bile salts may be adsorbed to the surface of the epithelial cells, where they are able to dissolve more fatty acid molecules and to transport them into the cells.

Although the importance of choleic acid may be somewhat overemphasized, since such complexes have been isolated only for desoxycholic and apocholic acids in a non-conjugated form, convincing experiments indicate the importance of the conjugated bile salts in solubilizing the fatty acids. Verzár and Kúthy[513] were able to obtain clear soap solutions with oleate, palmitate, and stearate at *p*H values of 8 to 9; however, clear solutions of these soaps could be formed with conjugated bile salts at *p*H values of 6.18, 6.35, and 6.16, respectively. Thus, it was possible to obtain the fatty acids in soluble and diffusible form *in vitro*, on the acid side of neutrality, in a *p*H range at which the unconjugated bile acids do not dissolve.

The role of the bile acids in aiding in fat absorption has likewise been proved by the *in vivo* experiments of Verzár and Laszt.[701,702] When olive oil alone was added to isolated intestinal loops of dogs, no absorption occurred. The same results were found when taurocholic acid was added to the olive oil. Negative results were likewise noted when the fat and an active lipase preparation were placed in the intestinal loop. Positive results were obtained only when a mixture of fat, lipase, and taurocholic acid was employed. Thus, hydrolysis alone is not enough to effect absorption. Moreover, bile salts are unable to dissolve unhydrolyzed fat. This would seem to offer cogent proof for the lipolysis theory. However, the results do not completely negate the hypothesis of Frazer[645] that a minimum amount of hydrolysis occurs, with the resultant formation of di- and monoglycerides, which are required to render possible the formation of a satisfactory emulsion. This criticism could be adequately disproved if it

[700] A. C. Frazer, *Arch. sci. physiol.*, *2*, 15–41 (1948).
[701] F. Verzár and L. Laszt, *Biochem. Z.*, *270*, 35–43 (1934).
[702] F. Verzár and L. Laszt, *Biochem. Z.*, *270*, 24–34 (1934).

could be shown by a test that an emulsified fat mixture containing some mono- and diglycerides was not absorbed when introduced into the isolated loop along with taurocholic acid, but without lipase.

b′. Phosphorylation and Absorption: According to the Verzár hypothesis, phosphorylation is of considerable importance in fat absorption, possibly because of its function in aiding in the resynthesis of fat. Sinclair[703] believed that the synthesis of neutral fat in the epithelial cells resulted by condensation of the fatty acids into a phospholipid, followed by a breakdown of the phospholipid and the formation of neutral fats and the phosphoric acid base complex residue. The latter recombined with new fatty acids to form phospholipids, which continued to assist in the resynthesis of the fatty acids into fat. Under such conditions, the phospholipids would play a commanding role in the resynthesis of neutral fats, although the content in the intestinal mucosa would undergo only slight variations during the process.

On the other hand, Süllmann and Wilbrandt reported a considerable increase in phospholipids during the absorption of fat.[616] It was also found[702] that, although oleic acid was absorbed from the intestinal loop of a rat in the presence of taurocholate at a fairly rapid rate, the speed of absorption was considerably accelerated when glycerol and phosphate or, especially, glycerophosphate was added to the intestinal loop. This finding has been confirmed by Cera and Bellini.[704] Glycerol or phosphate alone had little effect in altering the rate of absorption of the oleic acid. The beneficial effects of the glycerophosphate on absorption are explained by its stimulation of fat resynthesis in the epithelial cells. Since the mucosa of the epithelial cells do not have the capacity to store at one time more than a small part of the total fat ingested, when generous amounts of this foodstuff are fed, anything which will cause a rapid transfer of the fat through the cells and into the lymphatic channels will increase the speed of absorption. Since free fatty acids apparently cannot pass into the lymph and remain in the cells until converted to neutral fat, any mechanism which will speed up the resynthesis of neutral fat in these cells will help to prevent a clogging of this portion of the fat-absorption cycle.

Other experiments of the Verzár group can be interpreted as offering further evidence that the resynthesis mechanism is essential (and indirectly that lipolysis is a necessary prerequisite for absorption). Thus, it was shown that the absorption of olive oil was completely inhibited when sodium

[703] R. G. Sinclair, *J. Biol. Chem.*, *82*, 117–136 (1929).

[704] B. Cera and L. Bellini, *Pathologica*, *32*, 375–377 (1940); *Chem. Abst.*, *35*, 1474 (1941).

iodoacetate or phlorhizin was given.[701] Since these substances inhibit the phosphorylation of hexoses, it was assumed that they behaved in a similar manner toward the fatty acids. In the absence of phosphorylation of these compounds, a resynthesis of neutral fat did not take place, and the absorption of the fatty acids was effectively blocked. Verzár and Laszt[705,706] have likewise found that fat absorption is slowed down in adrenalectomized animals. This is interpreted to mean that the mechanism controlling phosphorylation is upset. The livers were not poisoned by phosphorus after extirpation of the adrenals, and fat could not be removed from the fatty livers under such conditions. Laszt and Verzár[707] attribute this to a failure of the phosphorylating mechanism to function. All these conditions were cleared up by adrenocortical extract.[708]

Bavetta *et al.*[709] confirmed the findings of the Verzár group by establishing the fact that fat absorption is decreased in rats following removal of the adrenal cortex, and that the deficiency is cleared up by the prefeeding of adrenocortical extract. The failure of Barnes *et al.*[710] to observe a decrease in the rate of fat absorption following adrenalectomy has been ascribed to the fact that old animals were used, in which case the absence of the adrenal cortex is less critical than in young animals.[709]

In addition to the results of the Verzár group, other evidence of the importance of the phosphorylating mechanism has continued to accumulate. Thus, Artom and Peretti[711] found iodized fatty acids in the phospholipid fraction of intestinal mucosa after feeding large doses of iodized fat to rats. Similar results were obtained with cats by Sinclair and Smith,[712] who found that the intestinal phospholipids contained as much as 35% of elaidic acid after the unnatural fat, trielaidin, had been fed. Perlman *et al.*[713] found that larger amounts of P^{32} were present in the intestinal phospholipid when the radioactive phosphate was given with cod-liver oil than when it was administered without fat.

According to Favarger *et al.*,[714] the site of the most active synthesis of

[705] F. Verzár and L. Laszt, *Biochem. Z.*, *276*, 11–16 (1935).
[706] F. Verzár and L. Laszt, *Biochem. Z.*, *278*, 396–400 (1935).
[707] L. Laszt and F. Verzár, *Biochem. Z.*, *285*, 356–367 (1936).
[708] L. Laszt and F. Verzár, *Biochem. Z.*, *288*, 351–355 (1936).
[709] L. A. Bavetta, L. Hallman, H. J. Deuel, Jr., and P. O. Greeley, *Am. J. Physiol.*, *134*, 619–622 (1941).
[710] R. H. Barnes, E. S. Miller, and G. O. Burr., *J. Biol. Chem.*, *140*, 241–246 (1941).
[711] C. Artom and G. Peretti, *Arch. intern. physiol.*, *42*, 61–94 (1935).
[712] R. G. Sinclair and C. Smith, *J. Biol. Chem.*, *121*, 361–372 (1937).
[713] I. Perlman, S. Ruben, and I. L. Chaikoff, *J. Biol. Chem.*, *122*, 169–182 (1937–1938).
[714] P. Favarger, R. A. Collet, and P. Veraguth, *Bull. soc. chim. biol.*, *31*, 384–388 (1949).

phosphatides is at the border of the intestinal villi. It is believed that the synthesis of phospholipids from glycerylphosphorylcholine and fatty acids is promoted by pancreatic lecithinase. The suggestion is made that the glycerylphosphorylcholine does not leave the cellular membrane, where it is bound to proteins, but that, through continual synthesis of phospholipids from it, and subsequent hydrolysis *in situ* to glycerylphosphorylcholine and fatty acids, the latter compounds are transported through the cell membrane. This hypothesis presents an explanation for the mechanism of phospholipid in fatty acid absorption analogous to that of Schramm and Wolff,[715] which ascribes this same role to cholesterol, and to that of Verzár,[554] who suggested a similar behavior on the part of the bile salts.

The results of Williams[716] also indicate that phospholipid synthesis is one of the most important considerations involved in fat absorption. On the basis of the histological investigation of frozen sections from different levels of the intestine of the rat, obtained after olive oil had been fed, it was shown that the first evidence of fat absorption was the deposition of phospholipids between the nucleus and the free border of the columnar epithelial cells. It was reported that, immediately beneath the free border, there was a clear zone containing phosphatase; this is presumably the site of phosphorylation. The next stage in fat absorption involves the enlargement of the vacuoles of the Golgi complex and the deposition on them of the phospholipids. They are then transformed into fats and fatty acids. As the fat increases in quantity, it breaks away from the Golgi complex. The cytoplasm becomes filled with fine globules, which apparently coalesce and pass into the core of the villus.

In spite of these positive results, which indicate the importance of phosphate in the absorption of fats, the recent studies of Zilversmit, Chaikoff, and Entenman[717] have cast some doubt on the correctness of this assumption. These workers found that, in the dog, neither the amount nor the rate of turnover of intestinal phospholipids was influenced by the absorption of cream, corn oil, or corn oil fatty acids. Although some increases in the rate of turnover of the phospholipid fraction were noted in the rat during fat absorption, they were too small in relation to all the fat passing through this stage. These results lead the authors to state that "fat can pass through the intestinal wall without involving phospholipide as an obligatory intermediate."

[715] G. Schramm and A. Wolff, *Z. physiol. Chem.*, *263*, 61–72 (1940).

[716] T. D. Williams, *J. Physiol.*, *108*, 30P (1949).

[717] D. B. Zilversmit, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *172*, 637–650 (1948).

The subject of phospholipid metabolism has been reviewed by Sinclair,[718,719] while Bloor[720] discussed its role in fat transport. Chaikoff[721] considered the application of labeling agents to the study of phospholipid metabolism.

(c) *Partition Theory (Frazer).* Although Frazer is rightfully credited with the postulation and development of the so-called "Partition Theory," several earlier workers suggested that the absorption of unhydrolyzed fat occurs when the latter is present in a finely emulsified state. These include Schäfer,[688] Heidenhain,[689] Munk,[694] Kitagawa,[722] and Onozaki.[723] The details of the Partition Theory were first suggested[724] in 1938; it has recently been reviewed in a comprehensive manner by Frazer.[623,645,725,726]

According to this authority, only part of the ingested fat is hydrolyzed in the intestine; this partially hydrolyzed fraction, consisting of mono- and diglycerides, assists in reducing the bulk of the fat still in the unhydrolyzed form to a fine emulsion. The neutral fat passes through the intestinal membrane in a finely divided emulsion of negatively charged particles which measure less than 0.5 μ in diameter. Frazer believes that any fatty acids present are absorbed either as soluble compounds or as complexes. More detailed information on the Partition Theory, and a discussion of its relation to the Lipolytic Theory, are given below for the several phases of the digestion process.

The main points of difference between the lipolytic and the partition hypotheses, insofar as the changes in the lumen are concerned, are summarized in Table 17.

a′. Emulsification: In order that an emulsion system may function in the absorption of fat as such, it must be one in which the particles average less than 0.5 μ. It must be produced spontaneously without undue agitation, and should be stable for at least three hours.

One of the most important considerations which influences the ease with which an emulsion is formed, and likewise the stability of the emulsion system, once it is formed, is the *p*H. Systems which will form a practically permanent emulsion when the medium is alkaline will be immediately re-

[718] R. G. Sinclair, *Physiol. Revs., 14,* 351–403 (1934).
[719] R. G. Sinclair, *Biol. Symposia, 5,* 82–98 (1941).
[720] W. R. Bloor, *Physiol. Revs., 19,* 557–577 (1939).
[721] I. L. Chaikoff, *Physiol. Revs., 22,* 291–317 (1942).
[722] R. Kitagawa, *Tôhoku J. Exptl. Med., 24,* 329–349 (1934).
[723] T. Onozaki, *Tôhoku J. Exptl. Med., 29,* 224–243 (1936).
[724] A. C. Frazer, *Analyst, 63,* 308–314 (1938).
[725] A. C. Frazer, *Bull. soc. chim. biol., 33,* 961–967 (1951).
[726] A. C. Frazer, *The Mechanism of Fat Absorption,* in R. T. Williams, *Lipid Metabolism,* Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 5–13 (1952).

solved into their separate components when an acid reaction obtains. Although it has generally been considered that the reaction in the small intestine is alkaline, more recent results have indicated that the contents of the duodenum and jejunum have an acid reaction,[641,642] the *p*H averaging about 6.5. Although a more alkaline medium may exist in the lower ileum, an emulsifying system, to be effective in the intestine, must be stable at a *p*H of 6.5.

TABLE 17

COMPARISON OF THE EXPLANATIONS FOR CHANGES OCCURRING IN THE LUMEN OF THE INTESTINE AS POSTULATED IN THE LIPOLYTIC AND PARTITION THEORIES[a]

	Lipolytic theory	Partition theory
Emulsification:		
1. Mechanism	Acid soap	Triple combination: fatty acid/bile salt/lower glyceride[b]
2. Function	To promote hydrolysis	To disperse unhydrolyzed fat preparatory to absorption
Hydrolysis:		
1. Extent	Complete	Partial
2. End-products	Fatty acids and glycerol	Fatty acids, di- and monoglycerides, and later glycerol[c]
3. Function	Essential preliminary to absorption	Provides 2/3 of components of emulsifying system. Partitions fall into fatty acid and glyceride fractions

[a] A. C. Frazer, *Physiol. Revs.*, *26*, 103–119 (1946).
[b] A. C. Frazer, J. H. Schulman, and H. C. Stewart, *J. Physiol.*, *103*, 306–316 (1944).
[c] A. C. Frazer and H. G. Sammons, *Biochem. J.*, *39*, 122–128 (1945).

Frazer[645] has analyzed possible mechanisms for promoting emulsification which will function under the conditions listed above. Bile salts, sometimes considered to be the only essential requirement for producing an emulsion, form only a crude emulsion, with low stability compared with that known to exist in the lumen of the gut. In the second place, soap has been considered as the component in the intestinal contents which renders possible the production of a stable emulsion.[727] It is true that soap is an excellent emulsifying agent in an alkaline solution, but it will not function at a *p*H on the acid side of 7.5. It has been suggested that so-called "acid soaps" cause emulsification, but Frazer[645] discounts the effectiveness of such compounds, and indicates their complete ineffectiveness in the presence of calcium. Although the presence of cholesterol may enhance the emulsify-

[727] J. Mellanby, *J. Physiol.*, *64*, vP–viP (1927).

ing action of soap by the formation of soap/cholesterol complexes,[728] such changes will not alter the *p*H-conditioned character of the soap emulsion.

In seeking the answer as to what systems might be satisfactory within the acid range, Frazer and co-workers[729] examined bile salts, fatty acids (soaps), cholesterol, and monoglycerides, singly, in double, or in triple combination. The only combination which met all the requirements, *i.e.*, stability over a long period of time at a *p*H on the acid side of 7.0, was the triple combination fatty acid/bile salt/monoglyceride. The presence of monoglycerides in intestinal contents had been proved earlier by Frazer and Sammons,[647] who showed that glycerol was absent from the *in vitro* digest of pancreatic lipase and olive oil over a five-hour period, although the acetyl value, which is indicative of the free alcohol groups, rose from 5 to 64 during that interval. Similar proportions of the lower glycerides were obtained in material recovered from the intestines of rats sixteen hours after large doses of fat had been administered.

In further investigations of the nature of the emulsions, Elkes, Frazer, *et al.*[730] analyzed their compositions. The presence of fatty acids, triglycerides, diglycerides, and monoglycerides in intestinal emulsions could be demonstrated, but no phospholipid was present.[731] The latter finding was supported by the fact that the lecithinase D obtained from *Clostridium welchii* (gas bacillus) did not affect the emulsion. The flocculation pattern was that of simple negatively charged particles.[621,731] These later studies would seem to add further support to the Frazer Partition Theory by furnishing new evidence that the fatty acid/bile salt/monoglyceride complex is the only emulsion system which will function under the conditions existing in the small intestine.

b′. Hydrolysis: The most crucial part of the Partition Theory is the assumption that the hydrolysis of triglycerides in the intestine is a partial one. Without supporting evidence for this fact, the whole hypothesis would be most insecure.

In considering the effectiveness of lipase in bringing about the splitting of triglycerides, the most important consideration is the time factor. Although it is frequently stated that the peak of fat absorption occurs six hours after a meal containing fat is taken, this is true only when excessive amounts of this foodstuff are consumed. Ordinarily, with an intake of 30 g., the maximum lipemia obtains within 2.5 to 3 hours, and the normal

[728] J. H. Schulman and E. G. Cockbain, *Trans. Faraday Soc.*, *36*, 651–661 (1940).

[729] A. C. Frazer, J. H. Schulman, and H. C. Stewart, *J. Physiol.*, *103*, 306–316 (1944).

[730] J. J. Elkes, A. C. Frazer, J. H. Shulman, and H. C. Stewart, *Proc. Roy. Soc. London*, *A184*, 102–115 (1945).

[731] J. J. Elkes and A. C. Frazer, *J. Physiol.*, *102*, 24P–25P (1943).

preprandial pattern of blood lipids is reestablished in 4.5 hours.[621] The delay noted for the maximum blood level after a large intake of fat is largely the result of the prolongation of the period during which the food remains in the stomach. Evidence that unhydrolyzed triglycerides, diglycerides, and monoglycerides, rather than glycerol and free fatty acids, are chiefly present four hours after fat is given, is summarized on page 137.

c′. The Passage of Fat through the Outer Border of the Intestinal Cell: The main points of difference between the Lipolytic and Partition Theories insofar as they refer to the passage of fat through the outer (free) border of the intestinal cell are included in Table 18.

TABLE 18

COMPARATIVE EXPLANATIONS FOR THE MECHANISM OF THE PASSAGE OF FAT THROUGH THE OUTER MEMBRANE OF THE INTESTINAL CELL, AS POSTULATED IN THE LIPOLYTIC AND PARTITION THEORIES[a]

	Lipolytic theory	Partition theory
1. Structure of brush border	Solid pavement	Canal structure[b]
2. Fatty acids	Pass through the membrane as soluble complexes with bile salts[c]	Pass through the membrane either as soluble compounds or as complexes
3. Glycerides	Do not pass through the membrane	Pass through the membrane as finely dispersed emulsion of negatively charged particles less than 0.5 μ in diameter[d]
4. Adrenal cortex	Not concerned here	Controls normal electrolyte balance, which is closely related to the absorption of charged particles

[a] A. C. Frazer, *Physiol. Revs.*, *26*, 103–119 (1946).
[b] J. R. Baker, *Quart. J. Microscop. Sci.*, *84*, 73–103 (1942).
[c] F. Verzár and A. Kúthy, *Biochem. Z.*, *205*, 369–379 (1929); *210*, 265–280, 281–285 (1929); *230*, 451–457 (1931).
[d] A. C. Frazer, J. H. Schulman, and H. C. Stewart, *J. Physiol.*, *103*, 306–316 (1944).

In developing his theory for the absorption of triglycerides as discrete particles having a diameter under 0.5 μ, Frazer[623] first considered the possibility of an anatomical structure suitable for accomplishing this transfer. One of the early and frequently repeated statements contrary to the hypothesis of particulate absorption was to the effect that no one had ever observed the histological picture of a globule of fat in the process of passing through the cell membrane. However, this statement cannot be made now without qualification, in view of the fact that Wotton and Zwemer [697] have recently reported an observation of this nature.

By histological examination of the structure of the outside cell membrane,

referred to as the "brush-border," Baker[732] detected the presence of a system of fine canals, running at right angles to the surface, which might allow particles having a diameter of less than 0.5 μ to pass. These canals were visible when the tissues were examined in transverse or longitudinal sections. Although Baker's observations were made on the intestine of amphibia, they do offer a suggestion as to the possibility of an anatomical basis for the Partition Theory in the higher animals.

One should be able to prove that, if this canalicular structure is able to function, materials can be absorbed when present in a sufficiently fine emulsion, whereas they are not absorbable when present in unemulsified or coarsely emulsified form. The best substance on which to test this hypothesis is paraffin. There are contradictory statements in the literature as to whether or not paraffin is absorbed (see Chap. IV), inasmuch as these earlier experiments were not controlled as regards the size of the emulsion particles. The only way to answer the question satisfactorily is to introduce an emulsion of known size directly into the small intestine, since passage of an emulsion through the stomach may largely alter the size of its particles. Frazer, Schulman, and Stewart[729] demonstrated that, when paraffin emulsions having an average particle diameter of less than 0.5 μ were introduced intraduodenally into rats, absorption was comparable with that existing when a similar olive oil emulsion was given. This phenomenon was repeatedly confirmed by these workers, and by Frazer alone,[733] using large numbers of rats, by the use of histological examination and residual analyses. Bernhard and Scheitlin[734] supplied additional evidence of particulate absorption by demonstrating that finely emulsified mineral oil is taken up by the intestinal mucosa. Moreover, Bernhard *et al.*[735] reported further confirmation of particulate absorption by the finding that a series of hydrocarbons from C_8 to C_{18}, labeled with deuterium in the 1,2 or 2,3 position, were absorbed and metabolized.

The mechanism of absorption of fats through the intestinal wall has been shown to have an analogy with the passage of fat through the cell membrane of the soybean. Heupke and Rost[736] demonstrated that duodenal juice is able to liberate the fat from the plant cells; it could be dissolved under these conditions by human or ox bile at a *p*H of 6.5. Bile salts and fatty acids were present in a 1:1 ratio. This dissolved fat readily passed through the cell wall in either direction.

[732] J. R. Baker, *Quart. J. Microscop. Sci.*, *84*, Part I, 73–103 (1942).
[733] A. C. Frazer, *Biochem. J.*, *51*, xiii (1952).
[734] K. Bernhard and E. Scheitlin, *Helv. Physiol. et Pharmacol. Acta*, *10*, 54–61 (1952).
[735] K. Bernhard, U. Gloor, and E. Scheitlin, *Helv. Chim. Acta*, *35*, 1908–1913 (1952).
[736] W. Heupke and G. Rost, *Z. physiol. Chem.*, *284*, 204–210 (1949).

On the other hand, Berry and Ivy[737] were unable to demonstrate the presence of mineral oil in the lymph obtained from the thoracic duct of dogs, when various emulsions of mineral oil ranging in particle size from 200 to 0.5 μ and less were introduced into the alimentary tract. In spite of this negative result, the positive findings are sufficiently numerous to suggest strongly that a mechanism may exist in the intestinal membrane which will allow the absorption of particulate matter having a diameter of less than 0.5 μ.

According to the Partition Hypothesis, fatty acids pass through the membrane, as do other water-soluble compounds or complexes. However, in view of the fact that fats are only partially hydrolyzed and remain largely in the form of glycerides, it is necessary to consider the mechanism for their absorption. One possibility would be that they are dispersed in water in the form of phosphorylated compounds. Under such conditions, an anatomical structure for particulate absorption would not be required. However, since the phospholipids appear to be broken down in the intestine and are resynthesized in the intestinal wall, passage through the intestinal membrane in this form would appear to be impossible. Since absorption of triglyceride fat in molecular dispersion is ruled out, Frazer[645] considers that the alternative is passage of the fat through the intestinal membrane in particulate form.

d′. Adrenalectomy and Fat Absorption: The alterations in fat absorption brought about by adrenalectomy can be readily explained by the Partition Theory. Since the cortical hormones control normal electrolyte balance, they should likewise control the absorption of fat particles which carry a negative charge.[729] Verzár and Laszt,[705,706] Bavetta *et al.*,[709] and Frazer[645] agree that fat absorption is depressed following adrenalectomy. According to Verzár and Laszt,[705,706] the decreased absorption is to be ascribed to interference with phosphorylation, which they consider to be an essential process in fat absorption; this process is controlled by the cortical hormones. However, according to Frazer,[645] other workers failed to confirm the fact that phosphorylation is a prerequisite for absorption of fat,[738] carbohydrate,[739–741] or vitamins.[742] Since the normal fat absorption was

[737] I. M. Berry and A. C. Ivy, *Am. J. Physiol.*, *162*, 80–87 (1950).

[738] N. Stillman, C. Entenman, E. Anderson, and I. L. Chaikoff, *Endocrinology*, *31*, 481–485 (1942).

[739] H. J. Deuel, Jr., L. F. Hallman, S. Murray, and L. T. Samuels, *J. Biol. Chem.*, *119*, 607–615 (1937).

[740] W. G. Clark and E. M. MacKay, *Am. J. Physiol.*, *137*, 104–108 (1942).

[741] T. L. Althausen, E. M. Anderson, and M. Stockholm, *Proc. Soc. Exptl. Biol. Med.*, *40*, 342–344 (1939).

[742] W. G. Clark, *Endocrinology*, *28*, 545–554 (1941).

reestablished by the administration of corticosterone, and since it was also improved by the administration of salt solution,[709,710,743] Frazer[645] does not believe that phosphorylation is a prerequisite for fat absorption. Other observations,[738,744] likewise, are considered to support the view that the adrenal cortex is not concerned with phosphorylation in the intestine. Frazer[645] found that the absorption of triglycerides was depressed to 40% following adrenalectomy, while that of fatty acid was practically normal from a quantitative standpoint. If fat is absorbed largely in the form of triglycerides, according to the Partition Hypothesis, then the question of phosphorylation is not relevant.

The observations of Bavetta and Deuel[686] that a normal absorption of tributyrin occurs following adrenalectomy appear to be in conformity with the idea of particulate absorption. These lower triglycerides are probably completely hydrolyzed to fatty acids and glycerol, and as such would not be subject to particulate absorption.

Finally, Frazer[645] postulates that at least 60% of the fat absorbed passes through the outer membrane of the epithelial cells in particulate form. This figure is based upon the following considerations: Munk and Rosenstein[620] were able to account for only 60% of ingested fat in the chyle obtained from a thoracic fistula. The fat being absorbed by this route is believed to be derived from material absorbed in particulate form. Secondly, the interference with particulate absorption after adrenalectomy results in a depression to 40% of the normal and, thirdly, lipolysis proceeds to only 30% of completion under the conditions which obtain in the intestine.

(2) Changes in Fat in the Intestinal Cells

In considering fat absorption from the small intestine, it is necessary to separate the two phases of the process, namely the transfer of the material from the lumen of the intestine into the intestinal cells, and the discharge of the fat from these epithelial cells into the lacteals and capillaries. Unless both phases of these mechanisms are functioning, absorption of fat may be seriously delayed.

The actual amount of fat which can be retained at one time in all the intestinal cells is only a fraction of the total absorbed during several hours after a meal containing a generous quantity of fat. The speed with which fat can be absorbed from the lumen of the small intestine will therefore

[743] W. G. Clark and A. N. Wick, *Proc. Soc. Exptl. Biol. Med.*, *42*, 336–338 (1939).
[744] S. Ochoa and R. J. Rossiter, *J. Physiol.*, *97*, 1P–2P (1940).

depend not entirely upon the rate at which this foodstuff can be transferred through the cell wall into the epithelial cells, but also upon the speed with which these cells become cleared of fat so that additional quantities can be received from the lumen of the gut. The rate of absorption will thus be controlled by the slower of these two processes.

a. The Breakdown of the Fatty Acid + Bile Salt Complex in the Intestinal Cells. As already discussed in an earlier section (see pages 91 to 114), according to the Lipolytic Theory, the fatty acid is carried into the epithelial cells as a fatty acid + bile salt complex. If this theory is the correct one, it is necessary that this combination be broken down to allow the fatty acid to be present in such a form as to facilitate its conjugation with glycerol to form fat.

Although there is no certain evidence as to the mechanism which may accomplish this reaction, Verzár and Kúthy[745,746] suggested two possibilities. In the first place, the fatty acid + bile salt complex would be expected to disintegrate if the interior of the mucosal cells had an acid reaction (*i.e.*, below a *p*H of 6.2). A clear oleic acid/glycocholic acid solution can be shown by *in vitro* tests to become turbid when the *p*H is reduced to 6 by a few drops of 0.1 *N* HCl. A second possibility to account for the disintegration of the bile salt + fatty acid complex within the cells is that the complex is negatively charged; the fatty acids may be precipitated by positively charged ions within the cells. Hemoglobin has been shown to precipitate oleic acid at a *p*H below 6.7, since it has a positive charge. A mechanism of this type is apparently related to the so-called "coacervation" of fatty substances.[746] On the disintegration of the bile salt complex, the bile acid is presumably returned to the intestine for further functional activity in fat absorption.

b. The Synthesis of Phospholipids in the Intestinal Mucosa. All evidence points to the fact that phospholipids are readily synthesized in the intestinal cells. This synthesis has been demonstrated by histological methods[747] as well as by the use of labeled phosphate[713,748–752] or fatty acids.[702,711,713,753–755]

[745] F. Verzár and A. Kúthy, *Biochem. Z.*, *225*, 267–277 (1930).
[746] A. Kúthy, *Klin. Wochschr.*, *14*, 308–309 (1935).
[747] L. Lison, *Histochimie animale*, Gauthier-Villars, Paris, 1936.
[748] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab, Biol. Medd.*, *15*, No. 4, 1–60 (1940).
[749] C. Artom, G. Sarzana, and E. Segré, *Arch. intern. physiol.*, *47*, 245–276 (1938).
[750] C. Entenman, S. Ruben, I. Perlman, F. W. Lorenz, and I. L. Chaikoff, *J. Biol. Chem.*, *124*, 795–802 (1938).
[751] B. A. Fries, S. Ruben, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, *123*, 587–593 (1938).

According to Fries *et al.*,[751] who employed phosphate containing P^{32}, the intestine is the most active gastrointestinal site for phosphorylation. In the bird, a greater deposition of P^{32} likewise occurs in the intestinal cells[750] than in the gizzard, proventriculus, cecum, or colon. After the oral administration of phosphate-containing P^{32}, Artom and co-workers[749] found that the specific activity of phospholipid phosphorus in the intestine exceeded that in the liver. Recovery of P^{32} was found to be greater in the small intestine when the phosphate was given by stomach tube than when it was administered subcutaneously.[751] The feeding of fat has been shown to increase the deposition of phospholipid in the intestine.[749] Hevesy and Hahn[748] reported that P^{32} was incorporated in the cephalin molecule to the largest degree at short intervals after the administration of the P^{32}; however, Chargaff[752] has shown that lecithin contains a larger proportion of the radioactive phosphorus after twenty-four hours than does cephalin.

Another experimental procedure which has been employed to prove the synthesis of phospholipid in the intestinal wall is the feeding of fats containing fatty acids which can be identified. Thus, Artom and Peretti[711] were able to demonstrate that intestinal phospholipid contained iodized fatty acids after iodized fats had been fed to rats; Sinclair and Smith[712] noted that fatty acids in the intestinal phospholipids of cats contained as much as 35% of elaidic acid after trielaidin had been fed. Collet and Favarger[756] reported that, when labeled elaidic acid, palmitic acid, or tripalmitin was fed to monkeys, a significant proportion of the fatty acids were incorporated into the phospholipid of the intestinal mucosa; on the other hand, very little of the tagged glycerol fed was found to become incorporated into the intestinal phospholipid.

According to the Bloor hypothesis, as soon as the fatty acids are absorbed in the epithelial cells, molecules of phospholipid react with them to form triglycerides and a residual phosphoric acid + base complex. The latter combines with new fatty acids and glycerol to form new phospholipid which, in turn, passes through the same cycle with a new supply of fatty acids. This hypothesis is based upon the experiments of Sinclair[703] who reported that, although the total amount of phospholipid in the intestine did not change during fat absorption, the fatty acids combined in the phospholipid did change in response to the fatty acids which were being absorbed. How-

[752] E. Chargaff, *J. Biol. Chem.*, *128*, 587–595 (1939).

[753] R. G. Sinclair, *J. Biol. Chem.*, *115*, 211–220 (1936).

[754] L. Hahn and G. Hevesy, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, *22*, 188–192 (1938).

[755] G. Hevesy, *Enzymologia*, *5*, 138–157 (1938).

[756] R. A. Collet and P. Favarger, *Helv. Physiol. et Pharamcol. Acta*, *9*, C61–C62 (1951).

ever, if phosphorylcholine is the residual portion of lecithin set free to serve as the matrix for synthesis of a new phospholipid molecule, the hypothesis has little support. Riley[757] found that administered phosphorylcholine was readily converted to inorganic phosphate. It should not be inferred, therefore, that the phosphoric acid + nitrogen base complex enters and leaves the phospholipid molecule as a stable unit.

(a) *Phospholipids as Obligatory Components in Fat Synthesis.* Although there is no question that the synthesis of phospholipids is an important function of the intestinal mucosa, it is still debatable whether the newly synthesized phospholipids represent obligatory intermediates in the formation of the triglyceride molecule from fatty acids and glycerol (or from mono- or diglycerides), or whether the phospholipids are to be considered to be terminal products which are necessary in the transport and further metabolism of fat.

There have been several reports which suggest that phosphorylation is a prerequisite in the synthesis of triglycerides.[554,702,758] Reiser,[759] using mono- and triglycerides in which both the fatty acids and glycerol were tagged with C^{14}, interprets his experiments as indicating that fats are converted to monoglycerides, from which phospholipids are formed in the intestinal mucosa; the phospholipids so formed are then converted to triglycerides. However, Frazer[645] states that, irrespective of whether or not resynthesis occurs in the intestinal cell, the concept that phosphorylation activates it is open to question. For example, the reversible nature of lipolysis has been demonstrated *in vitro*, and neither change requires an intermediate phosphorylation. Moreover, Frazer[645] questions the validity of proof of the importance of phosphorylation in fat absorption based upon the interference in the utilization of this foodstuff caused by monoiodoacetate or phlorhizin.[701] The doses of the inhibiting agents were believed to be excessively large, and were reported to produce extensive destruction of intestinal mucosa.[760–762]

Moreover, although phospholipid synthesis does occur in the intestinal mucosa, recent experiments have indicated that it does not proceed at a sufficient rate to account for the synthesis of all the fat passing through the intestinal cells. Although the phospholipid content of intestinal and tho-

757 R. F. Riley, *J. Biol. Chem.*, *153*, 535–549 (1944).

758 R. G. Sinclair, *Oil and Soap*, *15*, 70–74 (1938).

759 R. Reiser, *Federation Proc.*, *12*, 257 (1953).

760 Simon and White, personal communication to K. A. Klinghoffer, *J. Biol. Chem.*, *126*, 201–205 (1938), p. 204.

761 K. A. Klinghoffer, *J. Biol. Chem.*, *126*, 201–205 (1938).

762 R. Öhnell and R. Höber, *J. Cellular Comp. Physiol.*, *13*, 161–174 (1939).

racic lymph is increased during fat absorption,[717,763] the fatty acids in the phospholipids account for only a fraction of the total absorbed. This does not rule out a continued turnover of phospholipid within the intestinal cell or the transfer of extra phospholipid to the liver in the portal blood. However, more recent studies render these explanations questionable.

A number of reports are available which demonstrate an increase in the specific activity of intestinal phospholipid phosphorus, during fat absorption, after the injection of P^{32} into rats. The recent findings of Schmidt-Nielsen[764] and of Zilversmit *et al.*[717] indicate that this augmentation of the specific activity of phospholipid phosphorus is too small to account for the conversion of the absorbed fat to phospholipid unless the phosphorylation process is limited to the epithelial cells. In the case of the tests on dogs, no increase whatsoever was noted in the relative specific activities of the phospholipids in the villi during fat absorption.[717] Another experimental finding which supports this thesis is that of Artom and Cornatzer,[765] who noted that fat absorption proceeded without an increase in phospholipid formation in the intestine in choline-deficient rats. All of these results are interpreted by Zilversmit and collaborators[717] as indicating that phospholipids are not obligatory intermediates in the passage of absorbed fat through the intestinal wall.

(*b*) *Functions of Phospholipids Other Than in Fat Synthesis.* Even if phospholipids are not obligatory intermediates in the resynthesis of fats in the intestinal mucosa, there are a number of reasons why the synthesis of phospholipids may assist in fat utilization. The phospholipids are convenient forms for the transportation of fat, and they may be more suitable than the triglycerides for metabolic transformations. The most important function of the phospholipids is as an essential component in stabilizing the film on the circulating fat droplets in the blood. Without a stabilizer, negatively charged fat droplets flocculate in the presence of plasma proteins and 1% sodium chloride[645,730] at a *p*H of 7.5. Soap will not prevent the flocculation under the above-mentioned conditions. The protective film on these chylomicrons contains phospholipid, as can be shown by its instability to the lecithinase D produced by *Clostridium welchii* (gas bacillus). Frazer[645] suggests that the phospholipid may be added to the fat particles in the intestinal cell, where it may be formed *in situ* at the oil/water interface. Another possibility is that the monoglyceride absorbed may serve as

[763] E. V. Flock, W. C. Cain, J. H. Grindlay, and J. L. Bollman, *Federation Proc.*, *6*, 252 (1947).

[764] K. Schmidt-Nielsen, *Acta physiol. Scand.*, *12*, suppl., *37*, 3–83 (1946).

[765] C. Artom and W. E. Cornatzer, *J. Biol. Chem.*, *165*, 393–394 (1946).

a precursor for the phospholipid.[731] Finally, it is possible that the phospholipid may be preformed in the intestinal cell and spread as a film on the oil globule. In any event, the phospholipids in the intestinal cells would be considered as terminal products rather than as intermediates in the synthesis of fats.

c. The Synthesis of Fats. The essential differences in the reactions occurring in the intestinal cells as postulated in the Lipolytic and Partition Theories are summarized in Table 19.

TABLE 19

COMPARATIVE EXPLANATIONS OF CHANGES OCCURRING IN THE INTESTINAL CELLS AS POSTULATED IN THE LIPOLYTIC AND PARTITION THEORIES[a]

	Lipolytic theory	Partition theory
1. Resynthesis	Essential part of mechanism. All fatty acid reconverted to triglyceride	Not essential part of absorptive mechanism
2. Phosphorylation	A stage in resynthesis of triglyceride fat[b,c]	Not concerned with resynthesis. Occurs at oil/water interface, as an essential change in interfacial structure and probably elsewhere[d]
3. Adrenal cortex	Essential for normal phosphorylation[e]	Not concerned in phosphorylation[f]

[a] A. C. Frazer, *Physiol. Revs.*, *26*, 103–119 (1946).
[b] R. G. Sinclair, *J. Biol. Chem.*, *82*, 117–136 (1929).
[c] R. G. Sinclair, *Oil & Soap*, *15*, 70–74 (1938).
[d] J. J. Elkes and A. C. Frazer, *J. Physiol.*, *102*, 24P–25P (1943).
[e] F. Verzár and L. Laszt, *Biochem. Z.*, *276*, 11–16; *278*, 396–400 (1935).
[f] N. Stillman, C. Entenman, E. Anderson, and I. L. Chaikoff, *Endocrinology*, *31*, 481–485 (1942).

The question as to whether or not triglycerides are normally synthesized from fatty acids (or soaps) and glycerol in the intestinal cells is one of the controversial phases of the problem of fat absorption. This concept must be accepted if one agrees with the Lipolytic Theory of Bloor and Verzár that fat is carried into the intestinal cell only after a complete hydrolysis of the triglyceride molecule has occurred in the small intestine. On the other hand, if one believes the Partition Theory of Frazer, in which it is postulated that fat is absorbed in particulate form, largely as triglyceride, there is no need to assume a triglyceride synthesis in the epithelial cells.

Frazer[645] discounts the fact that evidence of the presence of triglycerides in the chyle after monoglycerides, fatty acids, or esters have been con-

sumed[679,766–768] necessarily proves the synthesis of triglycerides in the intestinal cells. It is suggested that the synthesis may as well be assumed to take place in the lumen of the intestine under the influence of lipase as within the epithelial cell. Several investigators[33,34,59] have proved the *in vitro* synthesis of fat from glycerol and fatty acid in the presence of lipase; it would therefore be reasonable to assume a similar *in vivo* synthesis.[645] The evidence[677] of the appearance of fatty acids in the intestinal cells within 30 minutes after absorption starts, followed after six hours by the presence of neutral fat alone in these cells, might as well be indicative of an immediate absorption of fatty acids followed by a later absorption of neutral fat as of the conversion of the fatty acids to triglycerides within the cell during this period.[623] On the other hand, Reiser and Williams[768] reported that 73% of the monoglycerides administered were hydrolyzed; they suggested that the hydrolysis of monoglycerides and the synthesis of triglycerides both occur in the intestinal mucosa. These workers[768] observed that palmitoxyhydroxyacetone and monopalmitin were hydrolyzed in the gut in a similar manner. The fatty acid esters of dihydroxyacetone did not appear in the lymph as such, but only after reduction of the ketone group and conversion of the molecule to a triglyceride. It is postulated that esterification of the fatty acid with dihydroxyacetone, followed by its reduction and esterification, may be the normal method of triglyceride synthesis during the absorption of fatty acids. An enzyme which oxidizes glycerol to dihydroxyacetone was isolated from the intestinal bacillus, *Escherichia coli*, by Asnis and Brodie.[769] This glycerol dehydrogenase is relatively heat-stable, and requires diphosphopyridine nucleotide.

Even if one accepts the Particulate Theory as the explanation for fat absorption, one must still assume the synthesis of triglycerides in the epithelial cell under certain conditions. For example, mono- and diglycerides, which are formed in the small intestine and which are presumably absorbed as such, must be reconverted to triglycerides. The experiments of Argyris and Frank[766] demonstrate that the change occurs before these compounds pass into the lacteals; these workers isolated triglycerides from the chyle after the feeding of monoglycerides. It has already been shown that, when soaps, fatty acids, and ethyl esters of the fatty acids are fed, they are converted to triglycerides (see page 154), inasmuch as no free fatty acids are detected in the chyle or in the depot fats. The intestinal cell would seem to be the most logical site for this transformation.

[766] A. Argyris and O. Frank, *Z. Biol.*, [*2*], *41* (*59*), 143–164 (1912).
[767] J. Müller and H. Murschhauser, *Biochem. Z.*, *78*, 63–96 (1917).
[768] R. Reiser and M. C. Williams, *J. Biol. Chem.*, *202*, 815–819 (1953).
[769] R. E. Asnis and A. F. Brodie, *J. Biol. Chem.*, *203*, 153–159 (1953).

(3) *The Transfer of Fat to the Interior of the Villi*

a. Introduction. Less information is available as to the mechanism by which fat passes from the epithelial cells to the interior of the villi than is known concerning its passage into these cells. Fat accumulates in the intestinal cells during fat absorption, but a continual stream of this foodstuff must pass into the chyle, and thence into the circulation, during the entire period of fat absorption; the lipemia occurring for the period of four to six hours after fat has been ingested can only be a reflection of the fat which has passed the barriers of the intestinal cells and gained access to the lymph and blood stream. Several theories have been proposed to account for the transfer of the fat particles from the cell to the interior of the villus.

b. Theories for the Mechanism of Transfer. (*a*) *Theory of Schäfer.*[688] This hypothesis recognizes that the leucocytes play an important role in the transport of fat. According to Schäfer's observations, the leucocytes in the intestinal wall are always full of fat, irrespective of whether little or much fat is being absorbed. It was considered that the function of the intestinal cells is to receive the hydrolysis products of fat, to synthesize them into triglycerides, and to store them until the leucocytes can transport them into the lacteals. The experiments of Clark and Clark[690] are interpreted as offering support to the Schäfer hypothesis. In these tests, it was shown that leucocytes were instrumental in removing fat injected into the musculature of the tail of the tadpole. When drops of fat 30 to 70 μ in diameter were introduced, leucocytes were observed to pass through the walls of the blood vessels and to wander in the direction of the fat droplets. On reaching one of these, the leucocyte flattened out and formed a ring around the globule, becoming pigmented as it engulfed it. The lymph vessels gradually extended out to the oil and remained in contact with the leucocytes and the oil until the latter was dissipated.

In the case of fat transport in the intestine, it has been suggested that the leucocytes engulf the small fat particles either in the epithelial cells or after they have been extruded through the basement membrane of the intestinal cells; these leucocytes then carry the fat droplets through the intercellular spaces within the villus to the lacteal. When the leucocytes pass inside these lacteals, they disgorge the fat, which then passes into the lymphatic channels. Presumably, a somewhat similar mechanism may explain the passage of the fat into the capillaries.

(*b*) *Theory of Heidenhain.*[689] Heidenhain agrees with Schäfer that the fat from the epithelial cells is transferred to the lacteals without alteration, except in the size of the particles. However, this latter investigator believed that the contraction of the protoplasm was responsible for the trans-

ference of fat droplets from the cells to the interior of the villi. It is believed that this is accomplished by pumping movements produced by lengthening and shortening of the musculature.[770] Within the villus, the fat remains in relatively coarse globules, and it does not form the dust-like particles until it reaches the chyle.

(c) *Hydrolysis-Resynthesis Theory of Loevenhart.*[771] According to this hypothesis, fat is hydrolyzed whenever it passes in or out of the intestinal cells. It is assumed that, wherever the fat globules are required to pass through a cell membrane, similar hydrolysis and resynthesis occur. Loevenhart based his theory upon the fact that lipase is found to be widely distributed in all tissues, especially in those which are most concerned in fat metabolism. However, there is some question as to whether lipase or esterase was the enzyme studied by this worker, as the substrate employed was ethyl butyrate.[772,773] Moreover, Bradley[774] was unable to confirm Loevenhart's demonstration of lipases in the mammary tissues and in fat depots.

(4) *The Transport of Fat to the Liver and Tissues*

There are two pathways by which fat may be transported from the intestine to the liver and tissues. One of these routes is by way of the lacteals, the intestinal lymphatics, and the thoracic duct into the blood stream. The second pathway originates in the capillary network in each villus, from which the fat ultimately reaches the liver by way of the portal circulation. Although this latter route is presumably of limited importance insofar as fat is concerned, it is of major significance in the transport of water-soluble substances to the body tissues. Accurate methods have not been available until recently to trace the course of fat after absorption; however, the use of fat having fatty acids tagged with radiocarbon has apparently rendered possible a much more quantitative evaluation than was possible before this technic was employed.

a. The Transport of Fat in the Lymph. In the classical experiments of Munk and Rosenstein,[620] which were carried out on a patient suffering from elephantiasis who developed a lymphatic fistula in the left leg, it was possible to account for only 60% of the ingested fat in the lymph. The recoveries of ingested fat reported by later investigators have been of a much

[770] F. Verzár, *Ergeb. Physiol.*, *32*, 391–471 (1931).
[771] A. S. Loevenhart, *Am. J. Physiol*, *6*, 331–350 (1901–1902).
[772] M. Arthus, *J. physiol. path. gén.*, *4*, 455–461 (1902).
[773] C. Foá, *Arch. ital. biol.*, *63*, 229–238, 239–258 (1915).
[774] H. C. Bradley, *J. Biol. Chem.*, *13*, 407–418, 431–439 (1913).

lower order. For example, Eckstein,[775] working with anesthetized dogs, was able to account for only 21% of ingested olive oil in the thoracic duct lymph in twelve hours, or 17% in a six-hour period. The recoveries were still less when oleic or palmitic acid was fed in the form of the free acid. Eckstein pointed out that the fat absorption would probably have been better under normal conditions if anesthesia had been avoided. Little and Robinson[776] reported quite similar low recoveries in thoracic lymph after fat feeding in the case of dogs.

Bloom and his associates[777] recently reported a series of experiments on rats which were fed palmitic acid containing C^{14} in the carboxyl group. The lymph was collected from the unanesthetized animals by means of a new technic devised by Bollman, Cain, and Grindlay[618] for collecting lymph from the thoracic duct or intestinal lymphatics. When palmitic acid was fed as the triglyceride, or as the free acid, 70 to 92% of the absorbed fatty acid was recovered from the thoracic duct lymph in nineteen to twenty-four hours in nine of the ten tests, while 69 to 84% of the absorbed palmitic acid-C^{14} was accounted for in the intestinal lymph.[777] This is by far the largest proportion of fat which has been proved to be transported by the lymphatic pathway.

It is possible that the palmitic acid-C^{14} not recovered may have traveled *via* the portal vein. However, Bloom *et al.*[777] call attention to the possibility that additional amounts of the fatty acid over and above that recovered from the intestinal or thoracic fistulas may have entered the lymphatic circulation in the intestine, to be subsequently lost to the systemic circulation by lymphatic-venous anastomoses. Although such anastomoses were first described over forty years ago,[778–780] their role in fat transport has never been investigated.

The fact that small amounts of fat containing C^{14} were shown to be present in the liver of rats in which thoracic duct cannulas had been continuously present indicates some absorption of this foodstuff by the portal system; however, the findings have not disproved the hypothesis that the tagged fat found in the liver may represent material absorbed into the lymphatic system which has reached the systemic circulation *via* the lymphatic-venous anastomoses.

[775] H. C. Eckstein, *J. Biol. Chem.*, *62*, 743–757 (1925).
[776] J. M. Little and C. S. Robinson, *Am. J. Physiol.*, *134*, 773–780 (1941).
[777] B. Bloom, I. L. Chaikoff, W. O. Reinhardt, C. Entenman, and W. G. Dauben, *J. Biol. Chem.*, *184*, 1–8 (1950).
[778] F. C. Lea, *Bull. Johns Hopkins Hosp.*, *33*, 21–31 (1922).
[779] T. T. Job, *Am. J. Anat.*, *24*, 467–491 (1918).
[780] C. F. Silvester, *Am. J. Anat.*, *12*, 447–471 (1912).

Bollman and associates[781] adduced direct evidence that lipids are transported primarily *via* the thoracic duct. The concentration of lipids was found to increase enormously in the intestinal and thoracic duct lymph of the dog after the feeding of a fatty meal or of one containing free fatty acid or oleic acid; the increase could be traced largely to neutral fat. On the other hand, the concentration of fatty acids in the hepatic lymph was lower than that from the thoracic duct or from the intestine; moreover, it did not increase after fat feeding.

(*a*) *The Comparative Composition of Ingested and Lymph Fat.* It has already been shown that the lymph may contain triglycerides after fatty acids or monoglycerides have been fed.[768] There is also evidence that changes in the nature of the fatty acids may likewise obtain in the lymph fat as contrasted with the acids fed. It is suggested by Bloor[35] that these conditions may be traced to: (1) a selective absorption of certain fractions (usually the lower-melting) by the intestinal cells, (2) other changes in the nature of the additions of body fat, or (3) chemical changes such as saturation or desaturation which may alter the composition.

Several examples of the different compositions of food and lymph fats may be cited. Munk[782] found a higher melting point in the fecal fat than in the original food fat. When cetyl palmitate was given, the chyle fat was shown to consist of one part of triolein and 6 parts of tripalmitin with a melting point of 36°C.[620] Frank reported 36% of olein in chyle fat[679] after the feeding of ethyl palmitate, while, after the feeding of mutton tallow (m.p., 51.7°C.), the chyle fat melted at 38°C.[783] On the other hand, Bloor[784,785] obtained chyle fat having a higher melting point than the ingested food fat. After the administration of olive oil in which the fatty acids melted at 16°C., fat melting at 30°C. was obtained from the chyle. Similar results are reported by Raper.[786]

According to Bollman *et al.*,[781] the phospholipids increased three-fold in lymph as the result of fat feeding; this was associated with the maximal increase found in neutral fat. Since the increase in phospholipids occurs in the intestinal and thoracic duct lymph, and not in the hepatic lymph, it is suggested that this fact affords proof of the synthesis of this material

[781] J. L. Bollman, E. V. Flock, J. C. Cain, and J. H. Grindlay, *Am. J. Physiol.*, *163*, 41–47 (1950).

[782] I. Munk, *Arch. path. Anat. Physiol.* (*Virchow's*), *122*, 302–325 (1890).

[783] O. Frank, *Arch. Physiol.* (*Du Bois-Reymond's*), *1894*, 297–308.

[784] W. R. Bloor, *J. Biol. Chem.*, *15*, 105–117 (1913).

[785] W. R. Bloor, *J. Biol. Chem.*, *16*, 517–529 (1914).

[786] H. S. Raper, *J. Biol. Chem.*, *14*, 117–134 (1913).

in the intestinal mucosa. Bloom *et al.*,[787] using C^{14}-labeled palmitic acid, also showed that, during the absorption of fat, the intestinal lymph contributes phospholipid to the plasma. It was believed that these phospholipids were synthesized in the small intestine. Borgstrom[788] noted that the neutral fat comprised 90% and phospholipids 10% of the total lipids, irrespective of whether the fats were fed as triglycerides or as free fatty acids. No free fatty acids or soaps occurred in the lymph. Little change in the cholesterol, as a result of fat feeding, was observed in lymph obtained from any of these sources.[781]

The proportion of ingested fatty acids which is transported by the lymph has been shown to vary with the fatty acid component of the triglyceride. Thus, Bloom *et al.*[777] found that practically all of the palmitic acid administered as triglyceride to rats could be accounted for in the thoracic lymph. Chaikoff *et al.*[789] reported similar findings for pentadecanoic acid. In a later study,[790] it was reported that C^{14}-labeled stearic and myristic acid could also be recovered practically quantitatively in the lymph. It is concluded that the lymph is the major if not the exclusive pathway for the absorption of fats containing long-chain acids. When similar experiments were carried out with lauric and decanoic acids, the recovery in the lymph was only 15–55% and 5–19%, respectively. This is interpreted as evidence of the transport of these acids *via* the portal route (see following section).

b. The Transport of Fat in the Portal Circulation. In spite of the fact that the bulk of the fat is carried from the intestine in the lymph, one must still accept the possibility that a portion of it travels by way of the portal circulation. Numerous investigators[791–796] have shown an increase in the fat of the portal blood during fat absorption. Cantoni[795] reported that portal blood contained more fatty acids than did arterial blood, and Eck-

[787] B. Bloom, I. L. Chaikoff, W. O. Reinhardt, and W. G. Dauben, *J. Biol. Chem., 189*, 261–267 (1951).

[788] B. Borgstrom, *Acta Chem. Scand., 5*, 643–646 (1951).

[789] I. L. Chaikoff, B. Bloom, B. P. Stevens, W. O. Reinhardt, and W. G. Dauben, *J. Biol. Chem., 190*, 431–435 (1951).

[790] B. Bloom, I. L. Chaikoff, and W. O. Reinhardt, *Am. J. Physiol., 166*, 451–455 (1951).

[791] G. D'Errico, *Arch. fisiol., 4*, 513–522 (1907).

[792] H. Roger and L. Binet, *Compt. rend. soc. biol., 86*, 79–80 (1922).

[793] W. W. L. Glenn, S. L. Cresson, F. X. Bauer, F. Goldstein, O. Hoffman, and J. E. Healy, Jr., *Surg. Gynecol. Obstet., 89*, 200–208 (1949).

[794] J. A. Sicard, R. Fabre, and G. Forestier, *Bull. soc. chim. biol., 5*, 413–425 (1923).

[795] O. Cantoni, *Boll. soc. ital. biol. sper., 3*, 1278–1282 (1928).

[796] S. W. Nedswedski, *Arch. ges. Physiol. (Pflüger's), 214*, 337–342 (1926).

stein[797] noted a definite increase in blood fatty acids during fat absorption, following a diversion of the thoracic duct lymph. However, other workers such as Zucker,[798] Winter and Crandall,[799] Little and Robinson,[776] and Brockett, Spiers, and Himwich,[800] have presented opposite results.

The possibility of an alternative pathway for absorption, *i.e.*, the portal vein, is indicated by certain data. The failure to recover approximately 40% of fat in the lymphatics as demonstrated in the classic experiments of Munk,[694] and the disappearance of 70% of the fat from the intestine in a case of idiopathic steatorrhea, without an appreciable increase in the fat of the systemic system, could be explained by assuming a partial absorption by this alternative route.

There seems to be general agreement that the volatile fatty acids may be absorbed *via* the portal vein, as suggested by Raper.[786] It is also in accord with the report that, although tributyrin is absorbed, none can be detected in the chyle[801] or in the depot fat.[802] Moreover, Eckstein[802] demonstrated that tributyrin was not stored by the rat, while Davis[803] obtained similar results with chickens. However, the latter investigator was able to detect small amounts of tributyrin in the body fat when this triglyceride was injected subcutaneously or intraperitoneally. The finding of Bavetta and Deuel,[686] that tributyrin is absorbed in a normal manner by the rat after adrenalectomy, in contradistinction to the depressed absorption of the fats having long-chain fatty acids, has been interpreted by Frazer[645] as indicating that the butyrate is absorbed after hydrolysis; such water-soluble components are believed to take the portal route rather than that *via* the lymphatics. In later work, Bavetta[687] proved that all water-soluble fatty acids up to caprylic acid are absorbed as satisfactorily after removal of the adrenal glands as when these glands are present; however, all of the higher fatty acids which are insoluble in water are dependent upon the adrenocortical hormone. Bloom *et al.*[790] supplied circumstantial evidence that, in addition to the volatile acids (acetic, butyric, and caproic), decanoic acid may also be transported by the portal route, and, to a lesser extent, lauric acid. Although the acids were not isolated from the portal blood, it was shown that their disappearance from the gut was not the result of

[797] H. C. Eckstein, *J. Biol. Chem.*, *62*, 737–739 (1924–1925).
[798] T. F. Zucker, *Proc. Soc. Exptl. Biol. Med.*, *17*, 89–91 (1920).
[799] I. C. Winter and L. A. Crandall, Jr., *J. Biol. Chem.*, *140*, 97–104 (1941).
[800] S. H. Brockett, M. A. Spiers, and H. E. Himwich, *Am. J. Physiol.*, *110*, 342–347 (1934).
[801] R. H. Hughes and E. J. Wimmer, *J. Biol. Chem.*, *108*, 141–144 (1935).
[802] H. C. Eckstein, *J. Biol. Chem.*, *81*, 613–628 (1929).
[803] R. E. Davis, *J. Biol. Chem.*, *88*, 67–75 (1930).

bacterial degradation, and that they could not be recovered in appreciable amounts from the lymph. However, it was later shown by Kiyasu and co-workers[804] that the ratio of C^{14}-labeled fatty acids in portal blood to that in the inferior vena cava was high after tagged decanoic acid was fed, in contrast to a 1:1 ratio following the administration of C^{14}-palmitic acid. Frazer[645] has accepted as part of his theory the absorption of the short-chain triglycerides after hydrolysis *via* the portal circulation.

In addition to the transport of the water-soluble fatty acids by the portal route, it is probable that other water-soluble hydrolysis products of lipids may also be absorbed in this manner. On the other hand, de la Huerga and Popper[805] reported that choline is changed by bacteria, in the intestinal tract, into trimethylamine; this is absorbed as such and excreted in the urine, mainly as the oxide. No significant amounts of choline were found to be present in the stools. In another study,[806] these workers noted that about two-thirds of the ingested choline appeared in the urine as trimethylamine and trimethylamine oxide. No choline is normally present in urine. Although the transformation of choline to trimethylamine can be largely ascribed to intestinal bacteria, the liver also plays a role. In liver disease the elimination of trimethylamine in the urine after the administration of choline was found to be delayed or decreased.

According to Rohse and Searle,[807] choline is transported from the intestine *via* the portal vein rather than *via* the intestinal lymphatics, in the case of the dog.

One fact which is not explained in the Frazer Theory is the site of the resynthesis of fatty acids to neutral fat. Most workers agree that the free fatty acid content of the blood and lymph is low. If free fatty acids which are absorbed from the intestine pass through the intestinal cells as such, then one would expect the synthesis into triglyceride to take place in the liver.

c. The Route of Distribution of Fats *vs.* Fatty Acids. One of the unique methods of approach to the question of fat absorption has been a study of the ultimate fate of the absorbed fat when given as fatty acid or as triglyceride. A comparison of the differences in behavior of these two types of fat according to the Lipolytic and Partition Theories is summarized in Table 20.

According to the Lipolytic Theory, one should observe no differences in the disposal of the fat, irrespective of whether the substances fed were

[804] J. Y. Kiyasu, B. Bloom, and I. L. Chaikoff, *J. Biol. Chem.*, *199*, 415–419 (1952).
[805] J. de la Huerga and H. Popper, *J. Clin. Invest.*, *31*, 598–603 (1952).
[806] J. de la Huerga and H. Popper, *J. Clin. Invest.*, *30*, 463–470 (1951).
[807] W. G. Rohse and G. W. Searle, *Federation Proc.*, *12*, 118 (1953).

TABLE 20
COMPARATIVE DISTRIBUTION IN THE BODY OF ABSORBED FATTY SUBSTANCES AS POSTULATED BY THE LIPOLYTIC AND PARTITION THEORIES[a]

	Lipolytic theory	Partition theory
1. Fatty acid fraction	After resynthesis, triglycerides pass through the lacteals and the thoracic duct into the systemic circulation Negligible amounts pass up the portal vein to the liver	Mainly passes by the portal vein to the liver[b-d]
2. Glyceride fraction	Not absorbed as such. Resynthesized from fatty acid and glycerol and transported as described above	Passes to the systemic blood *via* the lacteals and thoracic duct, and so to the fat depots[d]

[a] A. C. Frazer, *Physiol. Revs.*, *26*, 103–119 (1946).
[b] A. C. Frazer, *Analyst*, *63*, 308–313 (1928).
[c] A. C. Frazer, *J. Physiol.*, *102*, 329–333 (1943).
[d] A. C. Frazer, *J. Physiol.*, *102*, 306–312 (1943).

triglycerides or fatty acids. Under either condition, the fat would reach the intestinal cells in the form of fatty acids, which would then be synthesized into the triglycerides; these would be transported to the tissues and to the liver *via* the lymphatic system, a small fraction probably passing to the liver by way of the portal circulation.

However, Frazer[808] showed that the pathway of absorption in the rat varied markedly when neutral fat was fed, as compared with the situation which obtained when fatty acids and glycerol were given. When olive oil was administered, the intestinal cells were filled with fat particles, the lacteals had a distinctive milky appearance, a characteristic postabsorptive lipemia obtained, and Sudan-colored oil could be traced to the fat depots. No appreciable change in the portal blood fat could be noted, and only a slight deposition of Sudanized fat could be observed in the liver.

On the other hand, when fatty acids and glycerol were fed, the picture was completely reversed. The intestinal cells had a granular appearance, the milkiness in the lacteals was absent, no postabsorptive lipemia was noted, and no deposition of dye was found in the tissues when Sudan IV was added to the fatty acids in the intestine. Moreover, an increase in the fatty material was noted in portal blood, and an extensive deposition of Sudan-containing fat was observed in the liver. When an excess of lipase was administered to an animal receiving the triglyceride, the picture was

[808] A. C. Frazer, *J. Physiol.*, *102*, 306–312 (1943).

shown to approach that obtained in the fatty acid tests. When lipolysis was inhibited by hexadecyl sulfate, the opposite effect was observed. Frazer[809] had shown earlier that the administration of lipase to human subjects caused a considerable reduction in the postabsorptive lipemia.

Auld and Needham[810] have recently recorded a similar result in the case of a patient with non-traumatic chylothorax. When fats stained with Sudan III were fed to this man, a substantial amount of the dye was recovered from the chylothorax. On the other hand, when Sudanized fatty acid was given together with glycerol, no dye was recovered in one instance, and only a small amount in a second test. These results are interpreted as supporting the Frazer hypothesis. Although this evidence would appear to be quite convincing in establishing different pathways for the absorption of triglycerides and fatty acids, opposite results have been obtained recently on rats by Bloom and associates,[787,790] by Borgstrom,[788] as well as by Reiser and Bryson.[811]

(5) Current Status of Theories of Fat Absorption

Undoubtedly certain aspects of fat absorption are best explained by the Lipolytic Theory, and other features of fat utilization seem to support the Partition Theory. Both of these hypotheses have merit, and it seems probable that eventually a new theory will be propounded which includes the salient features of both concepts.

8. The Rate of Absorption of Common Fats

The rate of absorption of fat is an important index of its nutritional value. Although no differences may be observed between the total quantities of two fats which can be utilized, as determined from digestibility studies, when the fats are taken in moderate amounts, an entirely different picture may be obtained if they are fed in large doses. Thus, the maximum amount of a fat which may be taken without causing a digestive disturbance varies widely. If the fat is one which is slowly absorbed, diarrhea may occur on the administration of relatively small quantities; in the case of a fat having a more rapid rate of absorption, a greater tolerance obtains before the onset of diarrhea. It is therefore evident that absorption rates and digestibility coefficients, although related, afford information on independent physiological responses to fat.

[809] A. C. Frazer, *J. Physiol.*, *102*, 329–333 (1943).
[810] W. H. R. Auld and C. D. Needham, *Lancet*, *260*, 991–993 (1951).
[811] R. Reiser and M. J. Bryson, *J. Biol. Chem.*, *189*, 87–91 (1951).

TABLE 21
MEAN PERCENTAGES OF FATS ABSORBED BY PREVIOUSLY FASTED MALE RATS AT SEVERAL PERIODS AFTER THE FEEDING OF 1.5 ML. OF THE VARIOUS FATS BY STOMACH TUBE[a]

Fat fed	Absorption time[b]				
	2 hrs.	4 hrs.	6 hrs.	8 hrs.	12 hrs.
Butterfat...	36.2 ± 1.6	60.3 ± 1.2	77.2 ± 2.0	91.2 ± 1.1	97.4 ± 0.4
Butter oil...	37.4 ± 2.3	71.0 ± 1.2	86.4 ± 1.7	95.6 ± 1.0	—
Cod-liver oil.	40.8 ± 1.4	67.7 ± 1.9	79.7 ± 1.5	89.2 ± 0.7	98.2 ± 0.4
Corn oil....	28.9 ± 0.8	58.3 ± 0.9	71.4 ± 2.1	94.4 ± 0.7	97.9 ± 0.3
Halibut liver oil...	39.4 ± 1.6	70.2 ± 2.0	78.1 ± 1.3	85.4 ± 0.9	—
Lard.......	24.1 ± 0.8	57.0 ± 1.5	67.5 ± 1.5	92.3 ± 0.9	97.8 ± 0.4
Shortening A	26.6 ± 1.5	53.8 ± 1.6	68.5 ± 1.7	86.0 ± 1.3	98.6 ± 0.3
Shortening B	27.1 ± 1.8	52.8 ± 2.4	71.1 ± 1.5	85.6 ± 1.2	99.6 ± 0.1

[a] H. Steenbock, M. H. Irwin, and J. Weber, *J. Nutrition*, *12*, 103–111 (1936).
[b] Including Probable Error of the Mean.

Although considerable data are found in the literature on the completeness of the digestibility of fats, there is a paucity of information as to conditions obtaining where the time relations have been studied. Moreover, much of the information cannot be compared, since different units have been employed to express absorption rates. The index of absorption rate as used by Steenbock, Irwin, and Weber[812] is the percentage of ingested fat which is absorbed during a four-hour period. As long as the size of the animals, the time of absorption, and the dosage employed are uniform, one would expect to obtain fairly consistent results by this procedure. However, this index is not of value when any of these conditions are varied.

A second procedure for comparing absorption rates is to base them on terms of the quantity absorbed per unit surface area of the body per hour.[614] In employing this method of evaluation, Deuel, Hallman, and Leonard[614] noted that fairly constant results are obtained with rats of widely varying sizes, and with different dosages over various time intervals. The use of the index proposed by Steenbock *et al.*[812] was shown to yield inconsistent results under such conditions (see pages 128 and 129).

The absorption rates of some common fats in the case of adult male rats at two-hour intervals up to twelve hours after feeding are summarized in Table 21. The animals were fed 1.5 ml. of the oil or melted fat, and the absorption rate is expressed in percentages of ingested fat absorbed. In Table 22, the results of the four-hour tests were calculated on the basis of surface area as well.

[812] H. Steenbock, M. H. Irwin, and J. Weber, *J. Nutrition, 12*, 103–111 (1936).

TABLE 22

FAT ABSORBED AS PERCENTAGE OF THAT FED AND AS MILLIGRAMS PER 100 SQ. CM. PER HOUR BY PREVIOUSLY FASTED MALE RATS OVER A FOUR-HOUR PERIOD AFTER THE FEEDING OF 1.5 ML. OF THE VARIOUS FATS BY STOMACH TUBE[a]

Kind of fat fed	Number of rats in group	Fat fed, mg.[b]	Fat absorbed in 4 hrs.		Fat absorbed, mg./100 sq. cm./hr.[c]
			Per cent	Weight	
Animal fats:					
Butter oil	10	1402	71.0 ± 1.2	996	62.3
Halibut liver oil	10	1390[d]	70.2 ± 2.0	975	60.8
Cod-liver oil	10	1390	67.7 ± 1.9	940	58.8
Whale oil	9	1390[d]	62.1 ± 1.3	864	54.0
Butterfat	10	1402	60.3 ± 1.2	847	53.0
Lard	10	1404	57.0 ± 1.5	800	50.0
Lard (rancid)	10	1404	53.8 ± 1.6	755	47.2
Oleo-stock	10	1402[e]	35.8 ± 1.0	503	31.4
Vegetable fats:					
Linseed oil (raw)	9	1398	67.0 ± 0.9	937	58.5
Olive oil	10	1370	63.4 ± 1.8	868	54.4
Soybean oil	10	1388	58.5 ± 1.4	812	50.7
Corn oil	10	1385	58.3 ± 0.9	808	50.5
Peanut oil	9	1372	58.3 ± 1.7	800	50.0
Shortening A	10	1382[f]	53.8 ± 1.6	744	46.5
Cottonseed oil	10	1380	53.7 ± 1.6	742	46.4
Shortening B	10	1382[f]	52.8 ± 2.4	732	45.7
Cocoa butter	9	1440	47.9 ± 1.1	689	43.1
Coconut oil	9	1390	47.4 ± 1.6	659	41.2
Palm oil	10	1378	37.4 ± 1.5	516	32.2

[a] Data adapted from H. Steenbock, M. H. Irwin, and J. Weber, *J. Nutrition, 12*, 103–111 (1936).
[b] Calculated from specific gravity of fat or oil.
[c] Based on rat having surface area of 400 sq. cm.
[d] Based on specific gravity of cod liver oil.
[e] Based on specific gravity of lard.
[f] Based on specific gravity of cottonseed oil.

In Tables 23 and 24, which report the absorption rates of natural fats and hydrogenated fats, respectively, for rats of both sexes, the dosages of fat given and the calculation of the rate of absorption are based upon the procedures of Deuel and his co-workers.[612,614]

The rates of absorption of butterfat, coconut, corn, and cottonseed oils and prime steam lard (Table 23), and of margarine fat (Table 24), are in the same range in the six- or eight-hour tests, although, in the studies at the three-hour interval, butter appears to be absorbed somewhat more readily. On the other hand, the absorption rate of rapeseed oil is definitely slower than that of other limpid oils.

In the case of the hydrogenated fats other than margarine fat, Crisco, a hydrogenated cottonseed oil melting at 46°C., and a hydrogenated lard melting at 48°C., are absorbed at a slightly slower rate. On the other hand, the absorption rates of the hydrogenated lard melting at 55°C. and of the hydrogenated cottonseed oil having a melting point of 54°C. are markedly lower than those of other fats.

In spite of the wide discrepancies in absorption rate, most of the fats are ultimately equally well digested, and only small amounts are lost in the feces. An exception to this is the low digestibility of rapeseed oil in the rat[813] but not in man,[814,815] of hydrogenated cottonseed oil with a higher melting point (54°C.),[816] and of the higher melting hydrogenated lard (55°C.).[817]

In a number of instances, the rate of absorption appears to be highest in the shorter periods, while in other instances it apparently remains fairly constant throughout the entire absorption period. The most marked differences obtaining between the absorption rate at three and at six hours are in the case of butterfat and rapeseed oil, while the other fats exhibit a fairly consistent absorption rate. In the case of cottonseed oil also, where tests were made at two hours, the rate was considerably higher than that noted for the later periods.

There are at least two possible explanations for these discrepancies in absorption rate at different periods after administration. In the first place, it is known that the rate of absorption of different triglycerides varies. Where a considerable portion of the fat contains triglycerides with short-chain fatty acids like butyric acid, the rapid rate of absorption of this fraction will be reflected in the early hours. As the digestion and absorption proceed, the longer-chain fatty acid triglycerides will tend to disappear at a slower but constant rate. In the case of rapeseed oil, the triglycerides containing C_{18} acids will be absorbed first. As these gradually are removed, the slower absorption noted in the later periods must reflect the retardation occasioned by the presence of large amounts of erucic acid, which represents approximately 50% of the total fat.[818]

The second explanation for the slowing up in absorption rate might be

[813] H. J. Deuel, Jr., A. L. S. Cheng, and M. G. Morehouse, *J. Nutrition, 35*, 295–300 (1948).

[814] H. J. Deuel, Jr., R. M. Johnson, C. E. Calbert, J. Gardner, and B. Thomas, *J. Nutrition, 38*, 369–379 (1949).

[815] A. D. Holmes, *U. S. Dept. Agric. Bull. No. 687*, 1–20 (1918).

[816] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition, 33*, 177–186 (1947).

[817] M. Crockett and H. J. Deuel, Jr., *J. Nutrition, 33*, 187–194 (1947).

[818] T. P. Hilditch, T. Riley, and N. L. Vidyarthi, *J. Soc. Chem. Ind., 46*, 457–462; 462–464T (1927).

TABLE 23

SUMMARY OF RESULTS OF ABSORPTION OF NATURAL FATS WHEN FED TO RATS AT A LEVEL OF 300 MG. PER 100 SQ. CM. OF BODY SURFACE

Fat fed	Sex	Absorption, mg./100 sq. cm./hr.[a]			
		2 hrs.	3 hrs.	6 hrs.	8 hrs.
Butterfat[b]	M	—	49.6 ± 2.7 (9)	42.6 ± 2.1 (12)	—
	F	—	52.6 ± 5.1 (6)	41.6 ± 2.1 (5)	—
Coconut fat[b]	M	—	42.0 ± 4.5 (8)	43.2 ± 2.0 (7)	—
Corn oil[c]	F	—	—	—	44.9 ± 1.6 (9)
Cottonseed oil[b,d]	M	—	39.8 ± 2.9 (10)	39.7 ± 1.5 (10)	—
	F	47.8 ± 2.3 (11)	38.5 (6)	—	—
	F	—	—	43.3 ± 1.3 (8)	—
Prime steam lard[e]	F	—	35.0 ± 1.9 (10)	38.3 ± 2.0 (10)	—
Rapeseed oil[b]	M	—	38.5 ± 3.0 (11)	30.0 ± 1.8 (11)	—
	F	—	37.0 ± 1.5 (5)	26.2 ± 1.9 (10)	—

[a] Including Standard Error of Mean. Figures in parentheses indicate the number of tests included in the average.
[b] H. J. Deuel, Jr., L. Hallman, and A. Leonard, *J. Nutrition, 20*, 215–226 (1940).
[c] L. Bavetta and H. J. Deuel, Jr., *Am. J. Physiol., 136*, 712–715 (1942).
[d] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition, 33*, 177–186 (1947).
[e] M. Crockett and H. J. Deuel, Jr., *J. Nutrition, 33*, 187–194 (1947).

TABLE 24

SUMMARY OF RESULTS ON ABSORPTION OF HYDROGENATED FATS WHEN FED TO RATS AT A LEVEL OF 300 MG. PER 100 SQ. CM. OF BODY SURFACE

Fat fed	M.p., °C.	Sex	Absorption, mg./100 sq. cm./hr.[a] 3 hrs.	6 hrs.	8 hrs.	Ref.
Crisco	43	F	37.1 ± 2.0 (10)	34.3 ± 0.6 (10)	—	*b*
Hydrogenated cotton-seed oil	46	F	26.5 ± 1.8 (10)	24.7 ± 2.0 (11)	—	*c*
	54	F	18.0 ± 1.8 (9)	8.5 ± 0.9 (9)	—	*c*
Hydrogenated lard	48[d]	F	34.5 ± 1.7 (10)	31.4 ± 1.6 (10)	—	*b*
	55	F	20.7 ± 3.5 (12)	21.6 ± 3 5 (10)	—	*b*
Margarine fat	34	M	44.5 ± 3.3 (10)	46.1 ± 1.9 (10)	—	*e*
	34	M	41.7 (10)	36.5 (9)	—	*f*
	34	F	36.3 ± 1.0 (17)	—	—	*g*
	34	F	—	39.7 ± 1.8 (10)	—	*e*
	34	F	43.2 (10)	36.0 (10)	—	*f*
	34	F	—	—	42.8 ± 1.4 (12)	*h*

[a] Including Standard Error of Mean. Figures in parentheses indicate the number of tests included in the average.
[b] M. Crockett and H. J. Deuel, Jr., *J. Nutrition*, *33*, 187–194 (1947).
[c] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[d] Bland lard. This is a mixture of unhydrogenated lard and lard hydrogenated to a melting point above 48°C.
[e] H. J. Deuel, Jr., L. Hallman, and A. Leonard, *J. Nutrition*, *20*, 215–226 (1940).
[f] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951). Values listed at three hours are actually for four-hour tests.
[g] L. Bavetta, L. Hallman, H. J. Deuel, Jr., and P. O. Greeley, *Am. J. Physiol.*, *134*, 619–622 (1941).
[h] L. Bavetta and H. J. Deuel, Jr., *Am. J. Physiol.*, *136*, 712–715 (1942).

that it is related to the amount of fat present in the intestine. As the quantity of fat decreases, the rate of absorption decreases in a parallel manner. While there is some indication that an increased rate may obtain when excessively large amounts of fat are fed to rats,[614] this probably is not a valid explanation for the differences noted when fat is fed at the level of 300 mg. per 100 sq. cm. of body surface. Another fact which argues against this second explanation is that, in a number of fats, the rate of absorption is relatively constant over a long time interval.

The results obtained when the fat absorption is determined on the percentage basis[611] would seem to be in the same range as when it is calculated on the surface area basis.[612,614] However, the figures calculated in Table 22 for absorption in mg. per 100 sq. cm. per hour are mere assumptions. Since the weights of the rats are not given, it is impossible to calculate surface area. However, Irwin *et al.*[611] state that rats weighing from 200 to 400 g. can be used. Our calculations are based upon rats weighing approximately 300 g. If the average weight of the rats was 200 g., then the calculated values are about 33% too low; if, on the other hand, the body weights averaged 400 g., the calculated values in Table 22 are 25% too high.

It is a moot question as to what relationship exists between the rate of absorption and the nutritional value of fat. In the first place, when a fat is absorbed at a rapid rate, large amounts can be tolerated without producing diarrhea. On the other hand, this rapid absorption of fats might more readily bring about an alimentary lipemia.

In the case of fats which are more slowly absorbed, one can argue that the presence of the fat in the gastrointestinal tract for a longer period has a beneficial effect in that it may extend the period of absorption and thus entail less of a burden on the organism. It is only when the rate of absorption of the fat is so slow that this process cannot be completed before the fat reaches the lower gut (and hence is lost in the feces) that the rate of absorption of fat can be considered a function of its nutritional value.

9. Factors Affecting the Rate of Absorption of Fats

(1) *Age and Sex*

Irwin *et al.*[611] report that, within the limits of their experiments, covering four to seven months, age was not found to be a factor in fat absorption in rats. However, due to the technic employed by these workers, namely the administration of 1.5 ml. of fat irrespective of the size of the animal, a marked discrepancy in the percentage of fat absorbed in a given time is

noted when very young rats are compared with adult animals.[614] Thus, it was shown by Deuel and collaborators[614] that, when approximately 1100 mg. of fat were fed to male rats weighing 270 g., 43.2% was absorbed in three hours, while in young rats with an average weight of 65 g. the percentage absorbed was only 23.1% after the feeding of a similar dose. However, when the absorption rate was calculated on body surface, the relative rates were much closer, namely, 43.3 and 56.1 mg. per 100 sq. cm. per hour for the adult and young rats, respectively. Moreover, when a standard dosage of 300 mg. per 100 sq. cm. was employed, the comparative values for males (expressed in mg./100 sq. cm./hr.) were 43.3 ± 1.8 (270 g.) and 53.8 ± 2.6 (74 g.); the results obtained with females were 38.1 ± 3.2 (150 g.) and 43.7 ± 2.1 (64 g.). One can conclude that the rate of absorption of fats is not influenced by the size of the rat (and hence the age) over a fairly wide range if the results are expressed in terms of body surface; the size of the dosage of fat would appear to be of less consequence, although a dosage having a constant proportion to surface area is probably the best.

In the case of man, recent work has indicated that extreme variations in age do influence the rate of absorption. Sobel and co-workers,[819] as well as Tidwell, Holt *et al.*,[820] demonstrated that newborn infants and babies under one year of age absorb fats quite inefficiently, as compared with older children. On the other hand, the data of Becker *et al.*[821] indicate that fat may be absorbed or metabolized much more slowly in the aged than in younger subjects. This is indicated by the chylomicron count, which remains at an elevated level for a prolonged period after the feeding of fats to aged subjects.

Sex does not appear to be a factor in determining the rate of fat absorption. This conclusion was postulated by Irwin and co-workers,[611] while the results of the Deuel group recorded in Tables 23 and 24 support this statement. Pregnancy was likewise reported to be without effect on the rate of absorption of fats in the case of rats.[611] Cordier and Piery[822] reported extremely wide variations in the speed of absorption of peanut oil in different rats; figures varying from 8 to 97% were cited for ninety-minute tests. These authors suggest that the amount of absorption may be affected by the amount of oil which passes into the intestine, and may be related to the amount of enterogastrone set free. Extreme variations in absorption be-

[819] A. E. Sobel, L. Besman, and B. Kramer, *Am. J. Diseases Children*, *77*, 576–591 (1949).

[820] H. C. Tidwell, L. E. Holt, Jr., H. L. Farrow, and S. Neale, *J. Pediat.*, *6*, 481–489 (1935).

[821] G. H. Becker, J. Meyer, and H. Necheles, *Gastroenterology*, *14*, 80–90 (1950).

[822] D. Cordier and Y. Piery, *Compt. rend. soc. biol.*, *145*, 730–731 (1951).

come modified after longer periods. The author has found that much smaller variations are to be observed in tests lasting for three or four hours.

(2) *The Nature of the Fat*

There is no doubt that the nature of the fat itself is of prime importance in establishing the rate of absorption. If one subscribes to the Frazer hypothesis of fat absorption, then variations in physical properties may well be the factor responsible for differences in absorption rate. On the other hand, if fats must be split, as Verzár assumes, the differences between several fats may reflect variations in the rate of hydrolysis, as well as in the rate of absorption of the resultant fatty acids.

a. The Rate of Absorption of Synthetic Simple Triglycerides. Because all natural fats contain a wide variety of fatty acids, and consist chiefly of mixed rather than of simple triglycerides, information concerning the effect of the length of the fatty acid chain can more readily be obtained with simple triglycerides than with natural fats. These data, however, can be obtained only for the triglycerides with fatty acids lower than C_{12}, since the high melting point of the longer-chained compounds practically precludes their administration. Moreover, such high-melting fats are digested and absorbed only to a minor extent.

TABLE 25

SUMMARY OF RESULTS ON ABSORPTION OF SYNTHETIC SIMPLE TRIGLYCERIDES IN FASTING MALE RATS OVER THREE-HOUR PERIODS[a]

Even-chain fats			Odd-chain fats		
Fat fed	Number of carbons	Absorption, mg./100 sq. cm./hr.[b]	Fat fed	Number of carbons	Absorption, mg./100 sq. cm./hr.[b]
Triacetin	2	68.1 ± 1.4 (12)			
			Tripropionin	3	31.4 ± 2.1 (12)
Tributyrin	4	65.0 ± 2.5 (10)			
	4	69.1 ± 2.7 (11)[c]			
Triisovalerin	5	45.7 ± 2.5 (6)			
			Trivalerin	5	32.9 ± 2.3 (9)
Tricaproin	6	54.5 ± 1.5 (9)			
			Triheptylin	7	28.0 ± 1.6 (10)
Tricaprylin	8	45.9 ± 4.1 (8)			
Tricaprin	10	[d]			
Trilaurin	12	21.9 (5)			

[a] H. J. Deuel, Jr. and L. Hallman, *J. Nutrition*, *20*, 227–232 (1940).
[b] Including Standard Error of Mean. Figures in parentheses indicate the number of tests included in average.
[c] L. Bavetta and H. J. Deuel, Jr., *Am. J. Physiol.*, *136*, 712–715 (1942). Female rats were used in these tests.
[d] Diarrhea in all 20 tests.

Table 25 gives a summary of absorption tests on simple triglycerides composed of C_2 to C_{12} acids.

Two interesting variations are immediately evident from the results with the simple triglycerides. In the first place, the most rapid absorption rate is found in the case of triacetin and tributyrin. As the number of carbons on the acid component increases, a regular decrease in absorption occurs when one compares the values for the fats having C_6, C_8, and C_{12} acids. The second striking phenomenon is the marked retardation in absorption rates of the triglycerides having the odd-chain carbon fatty acids as compared with the triglycerides having the even-chain acids. However, tripropionin (C_3), trivalerin (C_5), and triheptylin (C_7) all have practically identical absorption rates, and do not show the decrease noted between tributyrin (C_4) and tricaprylin (C_8). Triisovalerin gives results considerably higher than those for trivalerin, but lower than the figures for tributyrin. It is provisionally classified with the even-chain fats, but might be considered as belonging to the odd-chain series.

b. The Rate of Absorption of Fatty Acids. The variations in the rate of absorption of the simple triglycerides might be the result of differences in the rate of hydrolysis of the triglycerides or in the rate of absorption of the resultant fatty acids. The results in Table 26 (p. 182) on the rate of absorption of fatty acids (or their salts in the case of the short-chain acids) would seem to indicate that the rate of absorption of the triglycerides is a function of the rate of absorption of the fatty acids. This finding does not support either the Lipolytic or the Partition Theory, inasmuch as both Verzár and Frazer postulate the hydrolysis of the triglycerides which contain water-soluble acids.

The results in general support the thesis that the variations in absorption rate of the simple triglycerides are related to the speed of absorption of their constituent fatty acids. Some alterations are brought about in the case of the short-chain fatty acids by the use of their sodium salts rather than of the free acids, which are too irritating to be used. This may account for the poor showing for acetate, since sodium acetate is known to be an intestinal irritant.[823] The reduced rate of absorption of the lower odd-chain fatty acids, as compared with the even-chain acids, can be noted for propionic, valeric, and heptylic acids when they are fed as their sodium salts.

c. The Absorption of Short-Chain Acids. There has been some question as to the fate of the volatile fatty acids when ingested by animals. In spite of the fact that acetic, propionic, and butyric acids disappear from the

[823] H. J. Deuel, Jr., and A. T. Milhorat, *J. Biol. Chem.*, *78*, 299–309 (1928).

TABLE 26

SUMMARY OF RESULTS OF ABSORPTION OF LOWER FATTY ACIDS BY FASTING FEMALE RATS[a]

Fatty acid fed	Absorption, mg./100 sq. cm./hr.[b] 1 hr.	3 hrs.
Acids fed as sodium salts in doses of 100 mg./100 sq. cm.		
Acetic	26.6 ± 1.0 (10)	—
Propionic	21.4 ± 1.2 (16)	—
Butyric	39.7 ± 1.5 (18)	—
"	45.0 ± 2.6 (16)[c]	—
Valeric	23.3 ± 1.9 (11)	—
Caproic	38.0 ± 1.7 (11)	—
Heptoic	25.8 ± 1.5 (20)	—
Acids fed free in doses of 200 mg./100 sq. cm.		
Caprylic	37.3 ± 1.5 (23)	46.0 ± 1.7 (10)
Nonylic	34.4 ± 2.3 (24)	32.8 ± 1.3 (23)
Capric	19.2 ± 1.5 (22)	22.6 ± 2.1 (11)
Undecylic	21.3 ± 3.4 (15)	21.4 ± 0.5 (9)
Lauric	2.7 ± 1.2 (10)	3.8 ± 0.7 (10)
Tridecylic	20.8 ± 3.2 (15)	[d]

[a] H. J. Deuel, Jr., L. Hallman, and A. Reifman, *J. Nutrition, 21,* 373–382 (1941).
[b] Including Standard Error of Mean. Figures in parentheses indicate number of tests included in the average.
[c] L. Bavetta and H. J. Deuel, Jr., *Am. J. Physiol., 136,* 712–715 (1942).
[d] Diarrhea developed in 16 of 18 rats before 3 hours.

gut when fed to rats as their sodium salts,[686] or as their triglycerides,[824] practically no information has been available until recently as regards the pathway by which they are absorbed. Acetate has been shown to be utilized by the phlorhizinized dog,[823] although no clue as to its fate was obtained other than to prove that it does not give rise to "extra" sugar. However, the demonstration that C^{14}-labeled acetic acid appears in the long-chain fatty acids of milk after its administration to lactating goats is presumptive evidence of absorption and transport to the mammary tissue.[825] This subject is discussed more completely in Volume III of this treatise. The proof that propionic acid, when fed as the sodium salt, gives rise to liver glycogen[826–828] is sufficient to establish the absorption of this short-

[824] H. J. Deuel, Jr., and L. Hallman, *J. Nutrition, 20,* 227–232 (1940).
[825] G. Popják, *Fat Synthesis from Small Molecules,* in R. T. Williams, *Lipid Metabolism,* Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 37–51, 1952.
[826] H. J. Deuel, Jr., J. S. Butts, L. F. Hallman, and C. H. Cutler, *J. Biol. Chem., 112,* 15–23 (1935–1936).
[827] H. C. Eckstein, *J. Biol. Chem., 102,* 591–594 (1933).
[828] J. S. Butts, H. Blunden, W. Goodwin, and H. J. Deuel, Jr., *J. Biol. Chem., 117,* 131–133 (1937).

chain acid. Moreover, tripropionin exhibits a similar property.[829] In the case of butyric acid, it has been repeatedly observed that an exogenous ketonuria results after the administration of sodium butyrate to fasting rats. This would seem to constitute proof of the ready absorption of the butyrate ion. The subject of exogenous ketonuria is discussed *in extenso* in Volume III. Probably more definite proof of the absorption of butyrate can be derived from the results of Morehouse,[830] who demonstrated the appearance of deuterio-β-hydroxybutyrate in the urine of fasting rats after the oral administration of sodium dideuteriobutyrate.

Direct proof of the absorption of the volatile fatty acids from the gastrointestinal tract was produced by Barcroft and co-workers,[831] who noted that the concentration of volatile fatty acids in the blood draining the large intestine of the horse, pig, rabbit, and sheep, as well as that of the rumen, of the reticulum, and to a lesser extent of the omasum of sheep, is higher than the proportion of these acids in blood from the general circulation. On the basis of these studies, it was concluded that sodium acetate is rapidly absorbed, sodium propionate is utilized at a somewhat lower rate, while sodium butyrate is absorbed at the slowest rate of the three volatile acids. Using differences between the concentration in carotid and in portal blood as the criterion for absorption, Schambye and Phillipson[832] concluded that both volatile fatty acids and glucose are absorbed from the alimentary tract in appreciable amounts, but that the uptake of the former is greater than that of the glucose. Masson and Phillipson[833] reported that, when equimolecular concentrations of the volatile acids were present in the rumen of sheep, the concentration in the blood leaving the rumen was in decreasing order: acetate, propionate, butyrate. While acetate was found in arterial blood, propionate was not present and, in most cases, butyrate was also absent from the arterial blood, even when these compounds were fed. It was later reported[834] that, although the mixture of acetic and butyric acids present in the portal blood contains more acetic and less butyric acid than the mixture present in the rumen, when a correction is made for the acetate in the arterial blood, the concentrations of acetate in the portal blood and in the rumen, respectively, are similar, while less butyrate occurs in the blood from the latter area.

[829] H. J. Deuel, Jr., J. S. Butts, H. Blunden, C. H. Cutler, and L. Knott, *J. Biol. Chem.*, *117*, 119–129 (1937).

[830] M. G. Morehouse, *J. Biol. Chem.*, *129*, 769–779 (1939).

[831] J. Barcroft, R. A. McAnally, and A. T. Phillipson, *Biochem. J.*, *38*, iii (1944).

[832] P. Schambye and A. T. Phillipson, *Nature*, *164*, 1094–1095 (1949).

[833] M. J. Masson and A. T. Phillipson, *J. Physiol.*, *113*, 189–206 (1951).

[834] P. Kiddle, R. A. Marshall, and A. T. Phillipson, *J. Physiol.*, *113*, 207–217 (1951).

In conformity with the hypothesis of Frazer[645] that the volatile acids are transported *via* the portal route rather than by way of the lymphatics, Kiddle *et al.*[834] found that the volatile acid content of lymph drawn from the thoracic duct is not greater than that of the arterial blood.

It has been reported that fatty acids are absorbed more rapidly than the corresponding anions,[835] and, in fact, that no absorption at all occurs when the *p*H exceeds 7.0[835] When alkaline solutions of acetate, propionate, or butyrate are introduced into the rumen, the reaction rapidly approaches neutrality[833]; Cl^- and CO_2 both appear in the rumen when aqueous solutions of the short-chain fatty acids are placed in the rumen. Gray[836] reported that the rate at which the free acid disappears from the rumen increases with the lengthening of the hydrocarbon chain.

The absorption of the short-chain acids differs from that of the long-chain acids in that it is not controlled by the adrenocortical hormones. Thus, in spite of the fact that adrenalectomy retards the absorption of fats having C_{16} and C_{18} fatty acids, Bavetta and Deuel[686] showed that no change in the absorption rate of tributyrin resulted from removal of the adrenal glands. In a later study, Bavetta[687] found that no significant depression in absorption of tricaproin (C_6) or tricaprylin (C_8) resulted from adrenalectomy. Sodium butyrate[686] and sodium caproate[687] were also absorbed at a normal rate in operated rats. However, the rates of absorption of caprylic (C_8) and capric (C_{10}) acids, when fed as the free acids, were considerably slower after adrenal extirpation. It was concluded that the adrenal cortex is concerned only with the absorption of the longer-chain fatty acids insoluble in an aqueous medium. Frazer and associates[837] confirmed the fact that adrenalectomy depresses the absorption of long-chain fats but not of tributyrin.

d. The Effect of the Melting Point of the Fat. Probably the most important physical property of fat which influences the rate of absorption and, in fact, its digestibility also, is the melting point. Few observations on the rate of absorption of fats melting above 50°C. are available because of the difficulty in administering the test material in a liquid form without killing the animal. A number of tests on digestibility of higher melting fats are described later (see Chap. III). However, in the absorption tests listed in Table 24, some decrease in the rate of absorption was observed for a blended hydrogenated fat (bland lard) melting at 48°C., while a some-

[835] F. V. Gray, *J. Exptl. Biol.*, *25*, 135–144 (1948).
[836] F. V. Gray, *J. Exptl. Biol.*, *24*, 1–10 (1947).
[837] A. C. Frazer, J. M. French, and H. G. Sammons, *Abstr. Commun. 1st Intern. Congr. Biochem.*, Cambridge, England, 1949, 12–13; *Chem. Abst.*, *45*, 4328 (1951).

what greater decrease was noted for straight hydrogenated cottonseed oil melting at 46°C. A decrease in absorption rate to 20% of that of the value for the limpid oils was observed with hydrogenated cottonseed oil melting at 55°C. Hydrogenated lard having an identical high melting point showed a somewhat smaller depression in absorption rate.

(3) The Presence of Emulsifying Agents

It has recently been shown that the presence of emulsifying agents will increase the rate of absorption and the coefficient of digestibility of difficultly absorbed fats. Although these emulsifiers also appear to increase the speed of absorption of readily absorbed fats such as limpid cottonseed oil, they cannot improve the coefficient of digestibility, in view of the fact that these fats, without the emulsifier, are practically completely digested. The common emulsifying agents present in foods include lecithin, mono- and diglycerides, polyoxyethylene sorbitan monooleate (PSM, marketed under the trade name of "Tween 80"), and isopropyl and stearyl citrates. Not only do these substances have an effect on the absorption of difficultly digested triglycerides, but they also are quite effective in improving the utilization of the fat-soluble vitamins, particularly vitamin A and carotene.

a. The Effect of Lecithin on the Absorption of Fat. Commercial lecithin has been widely employed as an emulsifying agent in a variety of food products over the past decade. The presence of lecithin in a fat-water mixture greatly aids in the emulsification of fat, and assists in the production of very fine fat droplets. The beautiful fine emulsion which exists in egg yolk is an excellent example of an emulsion of a natural fat which is stabilized by lecithin.

The formation of such a stable fine emulsion should increase the absorbability of fat, irrespective of whether the Lipolytic or the Partition Hypothesis represents the actual process by which fat is absorbed. The advocates of the Lipolytic Theory can explain any improved absorbability when lecithin is present as a result of the production of a finer emulsion. This will increase the ability of the fat to be hydrolyzed by the pancreatic lipase, steapsin, since a greatly enlarged fat surface area is available to the enzyme. On the other hand, if fat is absorbed largely in the form of droplets of neutral fat, as is postulated in the Partition Theory, any agent which will increase the capacity for the production of an emulsion, or which will assist in the development of an emulsion system composed of finer droplets, will aid in the absorption of fat.

Adlersberg and Sobotka[838] reported that both fat and vitamin A are ab-

[838] D. Adlersberg and H. Sobotka, *J. Nutrition, 25*, 255–263 (1943).

TABLE 27.
EFFECT OF LECITHIN ON THE RATE OF ABSORPTION OF LIMPID COTTONSEED OIL AND HYDROGENATED COTTONSEED OIL IN FASTING FEMALE RATS[a]

Fat fed	Duration of test, hrs.	Average absorption, mg./100 sq. cm./hr.[b]	
		Fat without lecithin (A)	Fat with lecithin (B)[c]
Cottonseed oil	2	47.8 ± 2.3 (11)	61.8 ± 3.7 (14) *3.21*
	3	38.5 (6)	59.9 (8)
Hydrogenated cottonseed oil (m. p., 46°C.)	3	26.5 ± 1.8 (10)	48.9 ± 3.0 (10) *3.56*
	6	24.7 ± 2.0 (11)	36.7 ± 2.2 (9) *4.12*
Hydrogenated cottonseed oil (m. p., 54°C.)	3	18.0 ± 1.8 (9)	30.1 ± 2.8 (8) *3.58*
	6	8.5 ± 0.9 (6)	21.8 ± 1.5 (6) *7.65*

[a] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[b] Including the Standard Error of the Mean. The figures in parentheses indicate the number of experiments included in the average.
[c] Mean Difference: Standard Error of the Mean Difference of results on "A" compared with "B" (in italics). When this exceeds 3, the results are considered to be significant.

sorbed at a faster rate by man if lecithin is added to the diet. On the other hand, the prolonged ingestion of lecithin was shown to result in a marked decrease in serum cholesterol.[839] Similar results were recorded by Steiner and Domanski,[840] although a higher level of blood cholesterol recurred after four to five weeks, in spite of continued lecithin feeding. However, Urbach[841] maintained persistently lower levels of blood cholesterol when a diet low in fat and carbohydrate was given along with lecithin. Similar results were reported by Gross and Kesten.[842]

All of the studies reported above employed an indirect approach, that is, they were based upon a change in the level of the constituent under investigation in the blood. Augur *et al.*,[816] on the other hand, employed a direct procedure for the study of the effect of lecithin on fat absorption, namely by following the rate of disappearance of the triglyceride from the intestine. The results of these investigators are summarized in Table 27.

[839] D. Adlersberg and H. Sobotka, *J. Mount Sinai Hosp.*, *9*, 955–956 (1943).
[840] A. Steiner and B. Domanski, *Proc. Soc. Exptl. Biol. Med.*, *55*, 236–238 (1944).
[841] E. Urbach, *Skin Diseases, Metabolism and Nutrition*, Grune & Stratton, New York, 1946, p. 542.
[842] P. Gross and B. Kesten, *Arch. Dermatol. and Syphilol.*, *47*, 159–174 (1943).

Another experimental procedure which has been employed to demonstrate the effect of lecithin on the absorption of fat is a test of the susceptibility of rats to diarrhea after the feeding of large doses of fat. This effect is summarized in Table 28. In the lecithin tests, fat containing 20% of crude lecithin (or 12% of phospholipids) was used.

TABLE 28

INCIDENCE OF DIARRHEA IN FASTING FEMALE RATS AFTER BEING FED FATS WITHOUT OR WITH LECITHIN[a]

Length of period, hrs.	Dose, mg./100 sq. cm.	Fat fed alone			Fat fed with lecithin		
		Number of tests	Diarrhea		Number of tests	Diarrhea	
			Number	Per cent		Number	Per cent
Tests with cottonseed oil							
3	328	3	1	33	—	—	—
3	410	4	3	75	7	0	0
2	410	23	5	22	16	1	6
2[b]	615	10	4	40	10[b]	2	20
Tests with hydrogenated cottonseed oil							
6	337	10	7	70	7[c]	1	14

[a] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[b] Male rats used in these tests.
[c] Dosage in lecithin group was 374 mg./100 sq. cm.

In a grand total of fifty tests in which fat was fed without lecithin, diarrhea developed in twenty cases; this is an incidence of 40%. On the other hand, only four rats of the total of forty used in the tests with the lecithin-containing fat presented diarrhea. This corresponds to 10%, which is only one-fourth of the incidence noted in the tests on the fat without added lecithin.

Another possible explanation for the augmentation of the rate of fat absorption as caused by lecithin may be its choline content. Tidwell[663] reported that choline administered orally or parenterally was active in accelerating the absorption of fat. He suggested that choline might be the limiting factor in the resynthesis of phospholipid in the intestinal cells. This process was considered to be essential, in accordance with the Lipolytic Theory of fat absorption. In a later report by Tidwell and Nagler,[500] the rate of absorption of fats from the intestine of normal orally fed rats was found to be unaffected by 6% or 20% supplements of a number of exogenous emulsifiers. It is suggested that the action of lecithin in promoting fat absorption may best be explained by some property other than its emulsifying action.

b. The Effect of Diglycerides and Monoglycerides on the Absorption of Fat. Since it has been demonstrated that diglycerides and monoglycerides are normal components of the intestinal contents during the digestion of fats,[640,647] and since Frazer[645] postulated that these compounds are essential for the formation of the fine emulsions of fat required for absorption according to the Particulate Theory, it should be possible to demonstrate an improved absorption in the small intestine when such components are added to fat. Huff *et al.*,[843] employing the level of plasma fat as an indirect index of absorption, found that the addition of 0.5% of glycerol monostearate to hydrogenated cottonseed oil dispersed by homogenization increased the rate of absorption.

c. The Effect of Isopropyl and Stearyl Citrates on the Absorption of Fat. Although isopropyl citrate and stearyl citrate have found application chiefly as anti-flavor-reversion agents,[844] they should also be classed as emulsifiers. Isopropyl citrate is prepared commercially largely in the form of the monoester; it is fat-dispersible but not fat-soluble. Preparations of the stearyl ester consist chiefly of distearyl citrate. These latter esters are readily soluble in fat.

Table 29 gives a summary of the effect of these substances on the absorption of margarine fat.

TABLE 29
EFFECT OF ISOPROPYL CITRATE OR STEARYL CITRATE ON THE ABSORPTION OF MARGARINE FAT[a]

Citrate used	Citrate present, %	Sex	Number of rats	Duration of test, hrs.	Fat absorbed, mg./100 sq. cm./hr.
Isopropyl...........	0	M	10	4	41.7
	3.33	M	9	4	45.5
	0	M	9	6	36.5
	3.33	M	9	6	33.2
Stearyl.............	0	F	10	4	43.2
	5	F	8	4	43.5
	0	F	10	6	36.0
	5	F	10	6	37.5

[a] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research, 16,* 294–305 (1951).

d. The Effect of Polyoxyethylene Sorbitan Monooleate (PSM) on the Absorption of Fat. Another type of emulsifier which has been shown to

[843] J. S. Huff, R. K. Waugh, and G. H. Wise, *J. Dairy Sci., 34,* 1056–1063 (1951).
[844] H. J. Deuel, Jr., S. M. Greenberg, C. E. Calbert, R. Baker, and H. R. Fisher, *Food Research, 16,* 258–280 (1951).

improve the absorption of fat, particularly in cases where an impaired utilization exists, is exemplified by polyoxyethylene sorbitan monooleate, the so-called Tween 80.

Although no figures on the effect of Tween 80 on the rate of absorption of fat are available, Jones *et al.*[845] demonstrated a marked decrease in fat loss in the feces when 1.5 g. of the emulsifier were added to each meal. Whereas the average fat content of stools amounts to not more than 4% of the ingested fats,[846] the loss of fat in the feces may amount to as much as 40 to 50% of the ingested fat in sprue, in certain disorders of the pancreas, as a result of infections of various parts of the gastrointestinal tract, or after partial removal of the stomach.[847] Thus, Jones and co-workers[845] were able to improve greatly the fat absorption of a group of patients with nutritional difficulties secondary to subtotal gastrectomy carried out to correct duodenal ulcer, as well as in a case of sprue. In a recent review of this work,[848] it is stated that "on the basis of these observations it appears that the agent, polyoxyethylene sorbitan monooleate, actually has an important influence on the absorption of fat." It is believed that the improvement in absorption is accomplished through its effect on surface tension, which facilitates the production of a fat emulsion composed of finer droplets than would otherwise be the case. According to Krantz,[849] Tween 80 exhibits no toxic effects when fed to animals over several generations.

(4) The Role of Adrenocortical Hormones

It is now known that hormones are important in fat absorption. As early as 1910, Falta[850] noted that fats were not absorbed normally in Basedow's disease. Similar abnormalities were observed by Schmidt and von Noorden[851] in 1921, and by Westerlund,[852] as occurring in Addison's disease. However, it was not realized until later that the deficiency in fat absorption, in this and analogous conditions, was related to hypofunction of the adrenal cortex.[852,853]

[845] C. M. Jones, P. J. Culver, G. D. Drummey, and A. E. Ryan, *Ann. Int. Med.*, *29* 1–10 (1948).

[846] E. E. Wollaeger, M. W. Comfort, and A. E. Osterberg, *Gastroenterology*, *9*, 272–283 (1947).

[847] E. E. Wollaeger, M. W. Comfort, J. F. Weir, and A. E. Osterberg, *Gastroenterology*, *6*, 93–104 (1946).

[848] Anonymous, *Nutrition Revs.*, *7*, 205–207 (1949).

[849] J. C. Krantz, Unpublished observations cited in *Nutrition Revs.*, *7*, 205–207 (1949).

[850] W. Falta, *Z. klin. Med.*, *71*, 1–22 (1910).

[851] A. Schmidt and C. von Noorden, *Klinik der Darmkrankheiten*, 2nd ed., Bergmann, Munich and Wiesbaden, 1921, Vol. 5, pp. 317 ff.

[852] E. Westerlund, *Klin. Wochschr.*, *18*, 856–858 (1939).

[853] T. E. Hess-Thaysen, *Non-tropical Sprue*, Oxford Univ. Press, London, 1932.

Verzár and Laszt[705,706] were the first to demonstrate the influence of the adrenal glands on fat absorption. Judovits and Verzár[854] also pointed out a somewhat similar mechanism to explain the absorption of carbohydrate. In an extensive series of investigations, Verzár and Laszt demonstrated that fat absorption was inhibited by adrenalectomy in rats, and also that the normal function could be restored by the administration of cortical extract, but not by epinephrine or ascorbic acid. These substances would be expected to restore fat absorption to normal if the adrenal medulla were the portion of the gland important in controlling fat absorption. It was postulated that the hormones secreted by the adrenal cortex controlled the phosphorylation of fat in the intestinal mucosa, a process which was believed to be essential in bringing about the absorption of fat. This mechanism was also related to the lack of riboflavin (vitamin B_2), since the activity of the adrenal cortex was found to be diminished in riboflavin deficiency. Verzár and McDougall[554] suggest that, in all cases of diminished activity of the adrenal cortex (experimental vitamin B_2-avitaminosis, pellagra, tropical and non-tropical sprue, and Addison's disease), disturbances in fat absorption occur concomitantly.[855]

On the other hand, Barnes *et al.*[856,857] failed to note any decrease in the absorption of the methyl esters of the fatty acids of corn oil, or of the corn oil itself, after removal of the adrenal glands, if the rats were maintained on salt solution. Deuel and co-workers[739] had concluded earlier that the role of the adrenal cortex in glucose absorption is a secondary one; if dehydration and the consequent circulatory disturbances were avoided following adrenalectomy, by the administration of Rubin-Krick or sodium chloride solution, no disturbance in carbohydrate absorption was noted after adrenal extirpation.

However, the results of Bavetta and associates[709] do support those of the Verzár group in demonstrating a decrease of fat absorption following adrenalectomy. The drop in absorption rate amounted to 38% of that of the normal in the untreated adrenalectomized rats, while it was only 24% of the normal in the salt-treated animals from which the adrenals had been extirpated. The reduction in absorption was associated with an accumulation of fatty acids in the intestine. Both of the deficiencies were corrected by the feeding of cortin.

[854] N. Judovits and F. Verzár, *Biochem. Z.*, *292*, 182–188 (1937).

[855] F. Verzár, *Schweiz. med. Wochschr.*, *65*, 1093–1097 (1935).

[856] R. H. Barnes, A. N. Wick, E. S. Miller, and E. M. MacKay, *Proc. Soc. Exptl. Biol. Med.*, *40*, 651–655 (1939).

[857] R. H. Barnes, E. S. Miller, and G. O. Burr, *Am. J. Physiol.*, *126*, P 427 (1939).

Barnes, Rusoff and Burr[858] later reported that emulsified hydrogenated cottonseed oil did show a decreased absorption rate in adrenalectomized rats, but that no deviation from the normal could be noted in the absorption of corn, olive, hydrogenated cottonseed oil, or mutton tallow which could be related to ablation of the adrenals. Bavetta and Deuel[686] were also unable to confirm this later work of Barnes *et al.*,[858] and suggested that the failure of the latter workers to note differences in their operated rats may have been due to the fact that large (and probably old) rats were used; it is well known that cortical deficiency is much more critical in younger animals.

In spite of the fact that the adrenocortical hormones are essential for the efficient absorption of the common fats, apparently they do not function in a similar manner in the case of triglycerides having water-soluble fatty acid components. Thus, Bavetta and Deuel[686] noted that the rate of absorption of tributyrin was not influenced by adrenalectomy. Bavetta[687] later found that the same was true in the case of triglycerides containing other water-soluble acids, as well as of the acids themselves when given as their sodium salts.

(5) *The Effect of Inhibitors on Fat Absorption*

Substances such as monoiodoacetic acid and phlorhizin, which presumably inhibit phosphorylation, have been shown to decrease the rate of absorption of fat or of fatty acid in rats.

a. Monoiodoacetic Acid. Although there seems to be little question that iodoacetate interferes with the absorption of glucose, galactose, and fructose, but not of the pentoses, xylose, and arabinose,[859] it appears to be questionable whether a similar interference obtains in fat absorption. Verzár and McDougall[554] report experiments on rats given 3.5 ml. of olive oil without (*A*) or with (*B*) monoiodoacetic acid in a dosage of 0.07 to 0.1 mg. per g. body weight. After six hours, the following comparative results were obtained: fat absorbed, *A*, 1 to 1.4 g., *B*, 0 g.; fat in stomach *A*, 1.5 g., *B*, 3 g.; fat in intestine, *A*, 0.5 g., *B*, 0.5 g. Failure in absorption is not due to the fact that fat largely remained in the stomach in the iodoacetate-injected rats, as is shown by the inability of such animals to absorb the fat when it is introduced directly into the intestine. Moreover, monoiodoacetic acid was shown to have no inhibitory action on lipase.[860]

[858] R. H. Barnes, I. I. Rusoff, and G. O. Burr, *Proc. Soc. Exptl. Biol. Med.*, *49*, 84–87 (1942).
[859] W. Wilbrandt and L. Laszt, *Biochem. Z.*, *259*, 398–417 (1933).
[860] F. Barth. *Biochem. Z.*, *270*, 63–65 (1934).

In addition, half of the fat in the intestine was found to be in the form of fatty acids, in the case of monoiodoacetate-treated rats.

On the other hand, the studies with monoiodoacetate were criticized by Klinghoffer[761] and by Öhnell and Höber,[762] on the grounds that this substance is very toxic and produces bleeding and irreversible damage to the intestinal mucosa. It was pointed out that the animals subjected to iodoacetate also presented a decrease in the absorption of NaCl, as well as of xylose and of the hexoses. Although the decreased absorption of hexoses is in agreement with the theory of Verzár that monoiodoacetate inhibits phosphorylation (and hence prevents absorption of hexoses), one is unable to answer the other criticisms on this basis.

b. Phlorhizin. Verzár and Laszt[861] were the first to report that inhibition of fat absorption was brought about by the injection of the glucoside, phlorhizin. This substance was shown by von Mering,[862] in 1886, to produce an intense glycosuria; however, the polyuria resulting from this compound is reversible, inasmuch as it ceases as soon as phlorhizin has been eliminated from the system.

Phlorhizin (also spelled phlorhidzin, phlorrhizin, and phlorizin) is obtained from apple and pear trees, where it is most abundant at the tip of the shoots; it occurs in progressively smaller quantities toward the base. The concentration is about twice as great in the pear tree as in the apple tree. In the early summer, when the glucoside is present in the greatest quantity, it may comprise 20% of the total solids, or 5 to 6% of the fresh weight.[863] It has been widely used to produce a temporary glycosuria, largely for the study of the intermediary metabolism. Lusk,[864] more than anyone else, is responsible for standardizing and developing the quantitative basis for the use of phlorhizin in metabolic experiments. When it is injected subcutaneously into a dog, in the amount of 1 g. daily in oil, a complete diabetes obtains over a 24-hour period; thereafter the glucosuria gradually wears off unless the dose is repeated. Deuel, Wilson, and Milhorat[865] adduced evidence that phlorhizin exerts its effect by primary action on the kidneys; the accompanying diabetic symptoms, such as ketosis, exaggerated glucose tolerance curves, and excretion of administered glucose, are secondary effects which can be avoided if the primary action on the kidneys can be prevented. Nash[866] has written a comprehensive review on phlorhizin diabetes.

It was found that, when phlorhizin was given to rats, absorption was practically zero over a six-hour period, irrespective of whether the fat was

[861] F. Verzár and L. Laszt, *Biochem. Z.*, *276*, 1–10 (1935).

[862] J. von Mering, *Deut. Gesell. inn. Med., Verhandl. 5ter Congr. inn. Med., Wiesbaden, 1886,* 185–189; cited by L. V. Beck, *J. Biol. Chem.*, *143*, 403–415 (1942), p. 403.

[863] E. M. Harvey, *Oregon Agr. College, Exptl. Sta. Bull. No. 215*, 5–23 (1925).

[864] G. Lusk, *Ergeb. Physiol.*, *12*, 315–392 (1912).

[865] H. J. Deuel, Jr., H. E. C. Wilson, and A. T. Milhorat, *J. Biol. Chem.*, *74*, 265–297 (1927).

[866] T. P. Nash, Jr., *Physiol. Revs.*, *7*, 385–430 (1927).

given by stomach tube or was injected into the intestine.[701] Moreover, not only was the absorption of fat prevented, but a similar inhibition in the absorption of fatty acids was noted. In this case one cannot level the same criticism as regards toxicity as applies to monoiodoacetate, since phlorhizin is much less toxic.[867]

As in the case of monoiodoacetate, the inhibitory activity of phlorhizin has been ascribed to its interference with phosphorylation, which Verzár[554] considers an essential step in the absorption of fatty acids. However, the interference with fat absorption in the case of both monoiodoacetate and phlorhizin is not concerned with inability to transfer the fatty acid to the intestinal mucosa, but rather with the absence of synthesis of neutral fat within the cell. Verzár[554] assumes that the change of the fatty acid into the triglyceride involves an intermediate phosphorylation, and that this reaction cannot occur when inhibitors are present. Frazer,[645] on the other hand, discounts the validity of the experiments with phlorhizin and with monoiodoacetate, on the basis that the doses used were excessively large and caused extensive damage to the intestinal mucosa.[761]

(6) *Miscellaneous Factors Affecting Fat Absorption*

Frazer[868] reported that the rate at which fat was absorbed was accelerated if choline was given. After olive oil and water were administered, the intestinal cells were filled with large globules of fat, while very little appeared to have passed through into the areolar tissue of the villi or into the lacteals. When choline was added, masses of fat could be seen in the areolar tissue of the villi, and the cells were more rapidly cleared. The fat remaining in the cells appeared to be more finely dispersed. It is possible that this increase in rate of absorption is related to the similar effect noted when lecithin was included in the diet.[816]

Starup[869] reported that the absorption rate was augmented in rabbits subjected to prolonged exposure to reduced atmospheric pressure, as indicated by a marked lipemia; however, MacLachlan and Thacker[870] were unable to demonstrate any interference with the absorption of fat within ranges of anoxia compatible with life. Although x-radiation was shown to bring about an increased tone and motility in the gastrointestinal tract of rats, Mead *et al.*[871] found that the absorption rate of fats was essentially

[867] L. V. Beck, *J. Biol. Chem.*, *143*, 403–415 (1942).
[868] A. C. Frazer, *Nature*, *157*, 414 (1946).
[869] U. Starup, *Biochem. Z.*, *270*, 74–92 (1934).
[870] P. L. MacLachlan and C. W. Thacker, *Am. J. Physiol.*, *143*, 391–395 (1945).
[871] J. F. Mead, A. B. Decker, and L. R. Bennett, *J. Nutrition*, *43*, 485–499 (1951).

normal. Clarke, Ivy, and Goodman[872] observed that resection of the mesenteric lymph nodes had no effect upon the level of fecal fat. A rapid reestablishment of anatomic and functional continuity of the interrupted mesenteric lymphatics was shown to occur, with a return to normal values six to twelve days after the operation. Dubouloz and Fondarai[873] demonstrated that peroxidation of ethyl linoleate did not prevent it from being absorbed in the intestinal tract of the rat; the peroxidized compound was subsequently found in different tissues.

[872] B. G. Clarke, A. C. Ivy, and D. Goodman, *Am. J. Physiol.*, *153*, 264–267 (1948).
[873] P. Dubouloz and J. Fondarai, *Compt. rend. soc. biol.*, *141*, 1066 (1947).

CHAPTER III

THE DIGESTIBILITY OF FATS

1. Introduction

To many people, the terms "absorption" and "digestibility" are synonomous, and connote the same physiological phenomenon. However, in the eyes of the biochemist and physiologist, absorption and digestibility constitute two distinctly different properties of foodstuffs, although they are often related to each other.

By "absorption" one refers to the process by which foodstuffs are removed from the small intestine. It is usually considered to be a function of time. On the other hand, the term, "digestibility," signifies the overall utilization of a foodstuff in the gastrointestinal tract. It cannot be expressed on a rate basis, but it is usually considered to be an index of the completeness with which the material is removed from the intestine during its passage through the body.

Substances which are slowly absorbed frequently have a low digestibility. However, the latter condition may not be attained unless the slowly absorbed material is ingested in large amounts. Fructose and cottonseed oil are two substances which are somewhat slowly removed from the intestine. However, both of these foodstuffs are practically completely absorbed under normal conditions. In the comparison of the rates of absorption recorded in Chapter II, rather wide variations between the absorption rates of a number of fats have been noted. However, it will be found that in most instances the fats have a similar practically complete digestibility, which bears no relationship to the rate of absorption.

The *coefficient of digestibility* is the term used to indicate the extent of digestibility. It represents the percentage of the ingested foodstuff which gains entrance into the body and hence is not lost in the feces. In the case of fats, the coefficient of digestibility is expressed by the following ratio:

$$\frac{[\text{Fat ingested}] - [\text{Fat excreted (corrected for metabolic fat)}]}{\text{Fat ingested}} \times 100$$

2. Methods for the Study of Digestibility of Lipids

Most procedures for the study of digestibility are quite similar and involve variations in the type of diet employed, the length of the test period, or the method of analysis of feces. Several of the more widely employed procedures for the study of the digestibility of fat and of other lipids are described below.

(1) Procedure of Atwater Used in U.S.D.A. Studies

The methods employed in a long series of tests on a wide variety of fats by Langworthy and Holmes, on human subjects, are typical of those in general use. These workers followed the procedure originally described by Atwater[1] for testing feces by means of various markers.

The tests were carried out on young adult male subjects over three-day periods. This interval was considered to be of sufficient length for an accurate collection of feces, but not too long for the diet to become monotonous. During this period, the subjects partook exclusively of the test diet, which was given to them *ad libitum*. The weight of each food constituent eaten at each meal was recorded; from this figure the weight of the test substance ingested could be calculated.

The diet employed in the tests of fat digestibility consisted of several common foods which are practically fat-free, together with a cornstarch blanc-mange pudding into which the test fat was incorporated. The diet included oranges, whole-wheat biscuits, sugar, and coffee or tea without cream. The blanc-mange was prepared with cornstarch, sugar, skimmed milk, caramel (to mask the flavor of the fat) and the fat under investigation. After cooling, the blanc-mange was thoroughly mixed and put through a potato ricer to insure equal distribution of the fat throughout the mixture.

The feces passed during the diet period were separated by the use of markers. The markers consisted of non-absorbable materials which imparted a characteristic color to the feces. Charcoal is the substance most frequently employed for such tests, while carmine or vital red may also be used. The subjects were instructed to take one or two gelatin capsules containing the marker with the first meal of the test diet, and with the first meal following the conclusion of the experiment. The feces showing the color of the first marker, and all material excreted until the

[1] H. C. Atwater, *U. S. Dept. Agr. Office Expt. Sta., Bull. No. 143,* 12–13, 58–60, 66–77 (1904).

color of the second marker appeared, were retained for analyses, and were considered to represent total feces for the experimental period.

The following procedure was used for the analysis of the feces. The material was first dried to a constant weight in an oven at 95°C. The total dried weight of the feces was recorded, and they were ground so that uniform samples might be prepared for analysis. In the U.S.D.A. studies, fat determinations were made on aliquot samples of the dried feces in the Soxhlet extractor, using diethyl ether as the solvent.

a. Correction for Metabolic Fat or Metabolic Lipid. In order to make allowance for ether-soluble materials in the feces other than that arising from the fat ingested, a correction must be made for the so-called "metabolic fat" or "metabolic lipid." This consists of the fatty materials present in bacterial residues, digestive juices and secretions, and in the epithelial cells of the stomach and intestinal tract, which will be present in the feces along with any undigested food fat. Since it is impossible to separate the undigested food fat from the metabolic lipid, an indirect method is necessary to estimate its quantity.

To determine the factor to be employed to correct for metabolic lipid, Langworthy and Holmes[2] carried out tests with a fat-free basal diet similar to that used in the other studies except that the blanc-mange contained no added fat. Under such conditions, the lipid in the stools, after correction was made for the minimal quantities of undigested fat[3] in the whole-wheat crackers (10%) and milk fat (5%) in the blanc-mange, was considered to be metabolic lipid. The figures for total fecal fat and metabolic lipid, respectively, in the four subjects were 11.8 and 9.3 g., 8.3 and 6.7 g., 16.8 and 14.9 g., and 11.7 and 9.9 g. Expressed in percentage of the weight of the water-free feces, the values were 6.84, 7.70, 16.02, and 9.00%, respectively; the average correction figure for metabolic lipid was 9.89 g. per 100 g. of dried feces.

(2) *Procedure of Deuel, Johnson et al.*[4]

The procedure employed by Deuel, Johnson *et al.*[4] is quite similar to that used in the earlier U.S.D.A. tests, with several modifications designed to give greater accuracy in the collection of feces and to render the diets more palatable.

[2] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 310,* 1–22 (1915).

[3] W. O. Atwater and A. P. Bryant, *State of Connecticut, Twelfth Ann. Report Storrs Agr. Expt. Sta.,* Storrs, Conn., 73–110, 111–123, 124–141 (1899); also cited by C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 310* (1915), p. 19.

[4] H. J. Deuel, Jr., R. M. Johnson, C. E. Calbert, J. Gardner, and B. Thomas, *J. Nutrition, 38,* 369–380 (1949).

TABLE 1. COMPOSITION OF THE DIFFERENT MENUS EMPLOYED IN THE DIGESTIBILITY TESTS OF FAT IN HUMAN SUBJECTS[a]

Breakfast		Lunch		Dinner	
Food	Amt., g.	Food	Amt., g.	Food	Amt., g.
Menu I					
Strained orange juice	183	Mashed potatoes	166	Vegetable soup	157
Cornflakes	16	Gravy	65	Cottage cheese	100
Graham date gems	89	String beans	150	Peach (half)	100
Sugar	22	Hot rolls	58	Lettuce	20
		Lettuce with	50	Mayonnaise	15
		French dressing	10	Bread	30
		Apricot tapioca	161	Prune whip	112
Menu II					
Strained orange juice	183	Spanish rice	176	Cottage cheese	100
Rice Krispies	30	Glazed carrots	113	Apricots	40
Cinnamon rolls	65	Cornbread	65	Orange	60
Sugar	22	Lemon sponge pudding	116	Pear in syrup	60
				Lettuce	20
				Mayonnaise	10
				Muffins	53
				Brown sugar tapioca	112
Menu III					
Prunes	100	Chili and beans	207	Creamed peas on	164
Cornflakes	16	Raw chopped onions	20	Steamed potatoes	150
Bread	48	Bread	48	Sliced tomato	100
Sugar	18	Lettuce	50	Cornbread	90
		Beets	30	Pear in Jello	152
		Celery	25		
		Green pepper	8		
		French dressing	10		
		Shortcake	70		
		Cherries	50		

Additional food served: all meals—skimmed milk, 200 g.; breakfast—black coffee, 180 g.

[a] H. J. Deuel, Jr., R. M. Johnson, C. E. Calbert, J. Gardner, and B. Thomas, *J. Nutrition*, *38*, 369–380 (1949).

In the first place, in order to insure greater accuracy, the experiments were continued over a longer interval. The test period was nine days instead of the three-day interval used in the earlier experiments. Moreover, the experimental period followed a two-day orientation during which the diet was the same as that used throughout the nine-day period.

In the second place, no markers were used. Since the subjects were trained to have a bowel movement each morning, all stool samples were collected except that evacuated on the morning on which the test began. The collection included the sample excreted on the morning of the tenth day following the start of the experimental period. Because of the relatively long test period, any inaccuracy entailed in the separation of the feces is considered to be minimal.

Another variation in the procedure employed by Deuel, Johnson *et al.*[4] involves the use of a much more varied diet, which was designed to overcome the monotony. Actually, three different menus were employed, which were rotated. The composition of typical menus is given in Table 1.

Although the diets contained some extraneous fat other than the test fat, about 88% of the total eaten represented the fat under investigation. The amount of fecal fat originating from this extraneous fat was disregarded in the calculations.

Another modification in the present method involves the estimation not only of neutral fat and fatty acids but also of that portion of the fat excreted as soaps. Although this results in a considerably larger figure for the total fecal fats, it apparently does not appreciably alter the figure for digestibility, since the correction figure for metabolic lipids is likewise increased when one includes the value for metabolic soaps. Deuel, Johnson *et al.*[4] estimate this value as 19.8% of the total dry weight of the stools, as compared with the figure of 9.89% employed by Langworthy and Holmes.[2]

(3) *Methods for the Study of Digestibility of Lipids in Rats*

a. General Procedures. The procedures used for the tests with rats are quite similar to those employed in the studies on human subjects. The lipid under test is fed as a component of a standard diet. As in the case of the second procedure described for man, a two-day orientation period is used. During these two days the diet containing the fat under study is fed, but the feces are not collected. Beginning on the morning of the third day, a record is kept of the weight of the food given the rat, and all feces on the pan of the cage are discarded. Typical diets employed in the rat tests are listed in Table 2.

TABLE 2

TYPICAL DIETS USED IN DIGESTIBILITY EXPERIMENTS WITH RATS

Food component	Augur *et al.*[a] (Fat-free), %	Augur *et al.*[a] (Hydrogenated cottonseed oil), %	Deuel *et al.*[b] (Rapeseed oil), %	Cheng *et al.*[c] (Various triglycerides), %	Savage and Deuel[d] (Jojoba oil), %	Calbert *et al.*[e] (Margarine), %
Commercial casein	18	18	18	18	9.0	18
Test fat	0	15	15	15	14.8	15
Glucose	71	56	56	61	—	—
Sucrose	—	—	—	—	26.0	46
Starch	—	—	—	—	38.0	—
Salt mixture[f]	7	7	7	5[g]	4.0	7
Yeast[h]	1	1	1	1	8.0	4
Liver extract[i]	3	3	3	—	—	—
Isopropyl or stearyl citrate	—	—	—	—	—	10
Fat-soluble vitamin mixture	—	—	—	—	0.2	—

[a] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[b] H. J. Deuel, Jr., A. L. S. Cheng, and M. G. Morehouse, *J. Nutrition*, *35*, 295–300 (1948).
[c] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949).
[d] E. E. Savage and H. J. Deuel, Jr., Unpublished results, 1952.
[e] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).
[f] T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, *32*, 309–323 (1917).
[g] Calcium- and magnesium-free salt mixture employed.
[h] Anheuser-Busch, Strain G.
[i] Wilson and Co., 1:20 concentrate.

The length of the experimental period can be adjusted according to the convenience of the experimenter. However, the shorter the experimental period, the greater the danger of error due to inaccuracies in the separation of the feces. In our laboratory, the duration of the tests has usually been eight or nine days, in addition to the two-day orientation period. An accurate record is kept of the total food consumed during the eight- or nine-day period; the last weighing of the food is done on the morning of the ninth or tenth day, respectively. Any food which is spilled during the test is collected and weighed; the record of food consumed is corrected by this factor. Total fat or lipid can be estimated from the food consumption, and the analysis for the lipid is based upon determinations of several portions of diet.

The feces are collected at frequent intervals during the experimental period, care being taken to separate them mechanically from any adherent food particles. The feces collection is terminated on the morning of the ninth or tenth day, coincident with the last weighing of the food cups.

The feces are dried to constant weight in a vacuum oven at 80°C., or in an ordinary oven at 100°C. They are ground to a powder, from which aliquots are taken for the determination of fecal fat (see Section (6)).

b. Determination of Metabolic Lipid. In order to calculate digestibility, it is necessary also, in the case of the rat, to make allowance for metabolic lipid. One should realize that this correction may likewise be considered as a correction factor for metabolic lipid when applied to experiments in which the digestibility of lipids other than fats is being tested. To determine metabolic lipid, the same technic is applied, in the case of the rat, as in the determination of the digestibility of fat (two-day orientation period followed by eight- or nine-day experimental period), except that the fat in the diet is completely replaced by sucrose. Since the interval is relatively short and the rats are in a good state of nutrition so far as the fat-soluble vitamins are concerned, the vitamin supplements are omitted during the test period.

Table 3 summarizes typical results which have been reported for metabolic lipid in rats.

TABLE 3
METABOLIC LIPID AS DETERMINED BY EXPERIMENTS ON RATS

Investigator	Augur *et al.*[a]	Deuel *et al.*[b,c]	Cheng *et al.*[c]	Calbert *et al.*[d]
Number of rats	10	10	10	8
Average weight of rats, g.	209	170	166	215
Average weight of dried stools, g.	5.35	3.83	1.53	2.82
Average neutral fat fraction in stools, mg.	181	119	149	107
Average soap fraction in stools, mg.	87.5	122	72	81
Total average feces fat, mg.	268	241	221	188
Metabolic lipid in feces, mg./g. dried feces	50.5	65.0	148.0	66.0

[a] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[b] H. J. Deuel, Jr., A. L. S. Cheng, and M. G. Morehouse, *J. Nutrition*, *35*, 295–300 (1948).
[c] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949). (Fat-free diet in which Ca and Mg salts were omitted from the salt mixture.)
[d] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).

With the exception of the results of Cheng *et al.*,[5] which were carried out on rats fed a fat-free diet in which the calcium and magnesium salts were omitted from the salt mixture, the results in the other three series of tests are extremely constant, giving the average value for metabolic lipid

[5] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949).

as 60.5 mg. per gram dried feces. The high value obtained for the calcium-low and magnesium-low diets is to be attributed to the very low weight of feces which resulted, rather than to any increase in the excretion of metabolic lipid.

(4) *Methods for the Study of the Digestibility of Lipids in Dogs*

Procedures similar to those used in the digestibility tests with human subjects and with rats have likewise been employed in the tests with dogs. Most dogs can be trained to consume a synthetic diet, such as that proposed by Cowgill,[6] in which the test fat can be incorporated in the ration at a constant level. A dietary regimen of this nature was employed by Calbert *et al.*[7] for a study of the absorption of margarine fat and the extent to which it was altered by the simultaneous administration of isopropyl citrate or stearyl citrate. The composition of the diets fed is given in Table 4.

TABLE 4
COMPOSITION OF DIETS USED IN THE DIGESTIBILITY TESTS WITH DOGS[a]

Food component	Composition of diets, %		
	Diet 1	Diet 2	Diet 3
Commercial casein	20.0	20.0	20.0
Sucrose	35.0	35.0	35.0
Dextrin	12.2	12.2	12.2
Margarine fat[b]	18.3	18.24	15.3
Cod liver oil (U.S.P.)	5.0	5.0	5.0
Cellu flour[c]	2.5	2.5	2.5
Salt mixture[d]	2.0	2.0	2.0
Dried brewer's yeast[e]	5.0	5.0	5.0
Isopropyl citrates plus vehicle[f]	—	0.06	—
Stearyl citrates[g]	—	—	3.0

[a] Data from C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).
[b] Without additives.
[c] Added for bulk. Preparation of cellulose obtained from Chicago Dietetics Supply House, Chicago, Illinois.
[d] L. G. Wesson, *Science*, *75*, 339–340 (1932).
[e] Strain G. Anheuser-Busch Brewing Co., St. Louis, Mo.
[f] Chiefly monoisopropyl citrate in 1:1 mixture of mono- and diglycerides.
[g] Chiefly distearyl citrate.

[6] G. R. Cowgill, *J. Biol. Chem.*, *56*, 725–737 (1923).
[7] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).

(5) The Use of Inert Chemical Substances as Indices of Digestibility

In addition to the several methods listed above for the determination of digestibility, which involve a timed collection of feces, it is likewise possible to obtain essentially the same information from random samples of feces when the substance which is being tested for digestibility is fed concomitantly with a completely indigestible substance. One needs only to know the proportion of the test substance and of the indigestible material in the food and in a sample of feces to calculate the coefficient of digestibility of the test material.

There are several prerequisites if a substance is to be used as the indigestible component of the diet. In the first place, the material must be completely indigestible under all dietary conditions. Furthermore, it must not be toxic, and it is essential that it be completely inert insofar as the test substance is concerned. An indigestible substance which causes diarrhea, or which produces an increased peristalsis, would obviously affect the extent of digestibility of substances concomitantly present in the intestine. Moreover, a substance such as mineral oil would be unsatisfactory for fats, since it would dissolve most fatty material and so alter the extent of digestibility. This behavior on the part of mineral oil has been repeatedly demonstrated with β-carotene; this carotenoid becomes almost entirely ineffective as a provitamin A when fed concomitantly with mineral oil.

a. Ferric Oxide as an Inert Material in the Determination of Digestibility. The use of iron oxide (Fe_2O_3) as an inert substance in the determination of digestibility was first proposed by Bergeim.[8] Heller and associates[9] were able to demonstrate that, when rats were used as the experimental animals, the iron oxide method gave fairly accurate results. However, Moore and Winter,[10] as well as Knott and co-workers,[11] reported that the iron oxide procedure yielded unsatisfactory results with cattle. This finding was confirmed by Hale, Duncan, and Huffman,[12] who ascribed the irregular results to variations in the amount of iron oxide present in various parts of the gastrointestinal tract. The results of Bell and Crampton[13] with swine were satisfactory, on the whole, except

[8] O. Bergeim, *J. Biol. Chem.*, *70*, 29–33 (1926).

[9] V. G. Heller, C. H. Breedlove, and W. Likely, *J. Biol. Chem.*, *79*, 275–282 (1928).

[10] L. A. Moore and O. B. Winter, *J. Dairy Sci.*, *17*, 297–305 (1934).

[11] J. C. Knott, H. K. Murer, and R. E. Hodgson, *J. Agr. Research*, *53*, 553–556 (1936).

[12] E. B. Hale, C. W. Duncan, and C. F. Huffman, *Proc. Amer. Soc. Animal Production*, *32*, 389–393 (1939).

[13] J. M. Bell and E. W. Crampton, *Sci. Agr.*, *27*, No. 1, 42–49 (1947).

with some feeds which, because of their physical nature, were unsuitable for the use of this marker.

b. Lignin as an Inert Material in the Determination of Digestibility. There have been quite diverse reports as to the applicability of lignin as an indicator substance in digestibility tests. Hale *et al.*[12] reported that the use of lignin ratios was an unsatisfactory method for determining digestibility. The results of Forbes and associates[14] on cattle might likewise be interpreted as proving the non-applicability of lignin. These latter investigators were able to demonstrate instances in which the coefficient of digestibility was 29, although their results showed wide variations which, in some cases, gave negative values. Crampton and Jackson[15] likewise found that the use of lignin yielded unreliable results.

On the other hand, a number of workers have reported excellent results with this polysaccharide. Ellis, Matrone, and Maynard[16] found that lignin was not digested to any significant degree, while Swift and collaborators[17] considered that, in ruminants, the use of lignin produced highly valid results. Moreover, Forbes and Garrigus[18] applied the lignin technic successfully for the determination of the nutritive intake of grazing animals. A later report of Kane, Jacobson, and Moore[19] has shown that lignin is not absorbed, and that its use "shows promise of saving much of the time, labor, and expense involved in the present cumbersome method of conducting digestion trials." The recovery of ingested lignin in the feces of cows was as follows (6 experiments in each case): cow 65, 100.3% (103.4–97.4); cow 54, 99.0% (103.4–96.4); cow 10, 97.2% (100.3–95.4). This gave a grand average of 98.8% for recovery, which is certainly within the range of experimental error. The comparison of results obtained by the use of lignin and of chromic oxide is illustrated in Table 5 (p. 207).

c. Silica as an Inert Material in the Determination of Digestibility. Silica (silicon dioxide, SiO_2) is another material which has been proposed as the inert substance for the calculation of digestibility. However, it has been claimed that dust in barns or dirt on food may be sufficient to invalidate the results when silica is used as the indicator. Moreover,

[14] E. B. Forbes, R. W. Swift, J. W. Bratzler, A. Black, E. J. Thacker, C. E. French, L. F. Marcy, R. F. Elliott, and H. P. Moore, *Penn. State College, School Agr., Agr. Expt. Sta., Bull. No. 452,* 1–34 (1943).

[15] E. W. Crampton and I. R. C. Jackson, *J. Animal Sci., 3,* 333–339 (1944).

[16] G. H. Ellis, G. Matrone, and L. A. Maynard, *J. Animal Sci., 5,* 285–297 (1946).

[17] R. W. Swift, E. J. Thacker, A. Black, J. W. Bratzler, and W. H. James, *J. Animal Sci., 6,* 432–444 (1947).

[18] R. M. Forbes and W. P. Garrigus, *J. Animal Sci., 7,* 373–382 (1948).

[19] E. A. Kane, W. C. Jacobson, and L. A. Moore, *J. Nutrition, 41,* 583–596 (1950).

Gallup and Kuhlman[20] stated that approximately 15% of silica was metabolized; this would preclude its use for accurate experimental work. Furthermore, Druce and Willcox[21] reported unsatisfactory results when silica was used as an index in digestibility studies, since its recovery in the feces was too variable. On the other hand, Skulmowski and co-workers[22] found good agreement between the digestibility figure based upon quantitative collection of the feces and one based upon the silica ratio procedure.

d. Chromic Oxide as an Inert Material in the Determination of Digestibility. Chromic oxide (Cr_2O_3) appears to be a compound which meets all the prerequisites as an indicator substance. The use of this material for determining digestibility was proposed as early as 1918 by Edin[23]; the method was later referred to as "Edin's indicator method."

A number of workers have demonstrated the applicability of the chromium oxide method under a variety of conditions, as well as with a number of species. Its application for cattle was confirmed by Edin and associates,[24] by Anderson alone [25] and with Frederiksen,[26] and by Kane, Jacobson, and Moore.[19] Hamilton, Mitchell, and co-workers[27] were the first to employ chromic oxide in the United States. They showed that it gave fairly satisfactory agreement in sheep as compared with the conventional method (quantitative collection of feces), provided that the collections were carried out for at least three days. The results on sheep were confirmed by Barnicoat[28] and by Crampton and Lloyd;[29] Skulmowski *et al.*[22] reported similar findings for horses. However, Crampton and Lloyd found that a period of five days must elapse from the start of

[20] W. D. Gallup and A. H. Kuhlman, *J. Agr. Research, 52,* 889–894 (1936).

[21] E. Druce and J. S. Willcox, *Empire J. Exptl. Agr., 17,* 188–192 (1949).

[22] J. Skulmowski, A. Szymański, and T. Wyszynski, *Ber. Landw. Forschungsanstalt General-Gouvernments, 1,* 76–104 (1943); *Chem. Zentr., 114, I,* 2460 (1943); *Chem. Abst., 38,* 4716 (1944); cited by E. A. Kane, W. C. Jacobson, and L. A. Moore, *J. Nutrition, 41,* 583–596 (1950), p. 584.

[23] H. Edin, *Medd. Centralanstalt. försöks-väsendet jordbruks., No. 105* (1918); cited by A. F. Schürch, L. E. Lloyd, and E. W. Crampton, *J. Nutrition, 41,* 629–636 (1950), p. 630.

[24] H. Edin, G. Kihlin, and S. Nordfeldt, *Lantbrukshögskol. Ann., 12,* 166–171 (1944); cited by E. A. Kane, W. C. Jacobson, and L. A. Moore, *J. Nutrition, 41,* 583–596 (1950), p. 584; *Chem. Abst., 39,* 4358–4359 (1945).

[25] A. C. Anderson, *Skand. Arch. Physiol., 69,* 33–58 (1934).

[26] A. C. Anderson and L. Frederiksen, *Biedermann's Zentr., Abt. A, 5,* 334 (1935).

[27] T. S. Hamilton, H. H. Mitchell, C. H. Kick, and G. G. Carman, *Ill. Agr. Expt. Sta., 41st Ann. Report, Livestock Investigations,* 119–121 (1927–1928); cited by A. F. Schürch, L. E. Lloyd, and E. W. Crampton, *J. Nutrition, 41,* 629–636 (1950), p. 630.

[28] C. R. Barnicoat, *New Zealand J. Sci. Technol., 27, Sect. A.* 202–212 (1945).

[29] E. W. Crampton and L. E. Lloyd, *J. Nutrition, 45,* 319–327 (1951).

the experiment until the samples of feces may be collected for the test. Moreover, it was noted that the results were reliable only when the ratio included ground feed with which the Cr_2O_3 could be premixed; when the chromic oxide was added to an unground all-roughage ration, it was partially retained, leading to unreliable and low estimates of digestibility. It was found that, when these precautions were observed, the results were satisfactory provided they were based upon analyses of a composite sample prepared from random samples taken over four days. The chromic acid method has been employed successfully in the case of horses,[22,30] pigs,[28] and calves.[28] Kreula,[31,32] Virtanen,[33] and Irwin and Crampton[34] found that the chromic acid procedure gives satisfactory results with human subjects. Kreula[31] was able to recover as much as 97.2% of the chromium oxide fed. Finally, Schürch, Lloyd, and Crampton[35] carefully evaluated the use of chromic oxide as an indicator substance in the rat. It was found that identical results for digestibility were obtained when analyses of random samples obtained at various periods of the day were used for the calculation.

The recovery of chromic oxide has been proved to be quantitative in cows. Thus, Kane, Jacobson, and Moore[19] observed the following recoveries of ingested Cr_2O_3 in cows: Cow 65, 100.0% (101.8–97.8); Cow 54, 100.4% (102.1–97.3); and Cow 10, 99.3% (101.0–96.7). This gave a grand average recovery of 99.9% for all tests with cows.

e. Indigestible Chromogenic Substances as Inert Materials in the Determination of Digestibility. Reid *et al.*[36,37] used indigestible chromogenic materials as indicators for digestibility in ruminants. The feed and feces were extracted with 85% acetone, and the concentration of chromogenic material in the resulting colored extract was determined by means of a spectrophotometer at 406 mμ. Anthraquinone violet is one chro-

[30] N. Olsson, G. Kihlen, and W. Cagell, *Lantbrukshögskol. Husjürsförsökanstalt., Medd., 36* (1949); cited by A. F. Schürch, L. E. Lloyd, and E. W. Crampton, *J. Nutrition, 41,* 629–636 (1950), p. 630.

[31] M. S. Kreula, *Biochem. J., 41,* 269–273 (1947).

[32] M. S. Kreula, "Die Resorption des pflanzlichen Carotins aus dem Darmkanal des Menschen," *Univ. Helsinki, Agr.-Forstwissensch. Fakultät* (1950); cited by M. I. Irwin and E. W. Crampton, *J. Nutrition, 43,* 77–85 (1951).

[33] A. I. Virtanen, Personal communication; cited by A. F. Schürch, L. E. Lloyd, and E. W. Crampton, *J. Nutrition, 41,* 629–636 (1950), p. 630.

[34] M. I. Irwin and E. W. Crampton, *J. Nutrition, 43,* 77–85 (1951).

[35] A. F. Schürch, L. E. Lloyd, and E. W. Crampton, *J. Nutrition, 41,* 629–636 (1950).

[36] J. T. Reid, P. G. Woolfolk, C. R. Richards, R. W. Kaufmann, J. K. Loosli, K. L. Turk, J. I. Miller, and R. E. Blaser, *J. Dairy Sci., 33,* 60–71 (1950).

[37] J. T. Reid, P. G. Woolfolk, W. A. Hardison, C. M. Martin, A. L. Brundage, and R. W. Kaufmann, *J. Nutrition, 46,* 255–269 (1952).

mogenic material which has been suggested as an indicator by Corbin and Forbes.[38] When this material was fed to lambs, it could be quantitatively recovered from the feces, and so should serve as an indirect means for estimating digestibility. However, this substance was shown to be subject to the same limitation as chromic oxide. It was found that the dye was not excreted uniformly; morning and evening samples of feces contained less dye than did samples excreted at noon.

f. Comparison of Different Technics for the Determination of Digestibility. On the basis of a comprehensive study of the digestibility of dry matter, crude protein, crude fiber, nitrogen-free extract and ether extract of alfalfa silage, field-cured and barn-cured alfalfa, Kane and co-workers[19] showed that both the chromic oxide and the lignin methods are satisfactory insofar as the cow is concerned. The comparative values for digestibility of the several fractions of the diet as calculated by total collection, chromic oxide method, and lignin method are summarized in Table 5.

TABLE 5

SUMMARY TABLE SHOWING THE COEFFICIENT OF DIGESTIBILITY OF VARIOUS DIETARY FRACTIONS IN COWS, AS DETERMINED BY THE TOTAL COLLECTION OF FECES OR BY THE USE OF CHROMIC OXIDE OR LIGNIN AS AN INDICATOR[a]

Indicators	Dry matter	Crude protein	Crude fiber	N-free extract	Ether extract
Alfalfa silage[b]					
Total collection	61.2	63.8	43.2	72.4	66.1
Cr_2O_3 method	61.4	64.0	43.4	72.4	66.2
Lignin method	61.8	64.2	43.8	72.7	66.6
Field-cured alfalfa[b]					
Total collection	59.5	61.8	44.9	70.7	55.9
Cr_2O_3 method	59.1	61.4	44.4	70.5	55.4
Lignin method	58.7	61.0	43.8	70.2	55.0
Barn-cured alfalfa[b]					
Total collection	63.6	68.6	46.7	74.0	52.7
Cr_2O_3 method	63.8	68.8	46.9	74.1	52.9
Lignin method	62.4	67.6	45.0	73.2	51.2

[a] Data adapted from E. A. Kane, W. C. Jacobson, and L. A. Moore, *J. Nutrition*, *41*, 583–596 (1950).
[b] Three cows, six tests per group.

(6) *Determination of Fecal Lipids*

a. Methods Employing Dried Feces. In the extensive program on fat digestibility carried out by the U.S.D.A. on human subjects, and in most

[38] J. E. Corbin and R. M. Forbes, *J. Animal Sci.*, *10*, 574–580 (1951)

TABLE 6

COMPARISON OF METHODS FOR DETERMINATION OF LIPIDS IN FECES OBTAINED FROM RATS FED A DIET CONTAINING MARGARINE FAT OR BLENDED HYDROGENATED PEANUT OIL[a]

Method[b]	Sample used, g.	Fat in sample, mg.	Soap in sample, mg.	% total fat in feces		Fecal lipids		Average coeff. of digestibility[c]
				Uncorrected	Corrected for glyceride	Total, g.	Corrected for metabolic lipid, g.	
Experiments on margarine fat (total fat consumed = 179 g.; total weight dried feces = 39.2 g.)								
1	1.62	164	348	31.6	—	12.4	8.8	95.2 (96.0–94.1)
2	1.58	127	276	25.7	—	10.1	8.5	95.2 (96.0–94.8)
3	1.58	—	—	33.3	34.4	13.5	8.2	95.4 (95.5–95.0)
4	1.54	—	—	27.3	28.7	11.2	8.5	95.2 (95.5–94.1)
Experiments on blended hydrogenated peanut oil (total fat consumed = 222 g.; total weight dried feces = 63.2 g.)								
1	1.74	147	732	50.6	—	32.2	26.3	88.2 (88.6–87.9)
2	1.73	156	581	42.8	—	27.3	24.7	88.7 (89.1–88.4)
3	1.71	—	—	48.7	49.8	31.7	23.0	89.6 (89.8–89.3)
4	1.69	—	—	44.8	46.9	29.8	25.4	88.5 (88.8–88.4)

[a] Data from E. J. Severance, *The Digestibility of Some Peanut Oils in the Rat,* Thesis, Univ. of So. Calif., Dept. of Biochem. Nutrit., January, 1952, pp. 25, 26.

[b] The methods were as follows: (1) Goldfisch extractor, diethyl ether; (2) Goldfisch extractor, petroleum ether; (3) saponification, diethyl ether; and (4) saponification, petroleum ether.

[c] Values in parentheses give range in 5 determinations.

of the rat experiments reported recently, the determination of lipids has been made on the dried feces. Several objections have been raised to this procedure in comparison with the use of methods in which the wet feces are employed directly. These are: (1) that, during drying, some hydrolysis of fat may take place, and that unsaturated acids may become oxidized; (2) that volatile acids might escape during drying; and (3) that the procedures in which drying is required are quite time-consuming.

There are a number of modifications of the technic for lipid determination in dried samples. In the first place, aliquots of the dried feces may be extracted with diethyl ether, as carried out in the U.S.D.A. studies[1] (I), or with petroleum ether (II). Another general method involves saponification of the stools with alcoholic potash, extraction of the acidified solution with diethyl ether or petroleum ether, drying of the ethereal solution with anhydrous sodium sulfate or with an adequate drying agent, followed by removal of the solvent (III). It is obvious that, by the use of Methods I or II, only neutral fat, fatty acids, and components of the non-saponifiable fraction are extracted, leaving the soap with the residue. In Method III, all neutral fats, fatty acids, soaps, and the non-saponifiable fraction are extracted; however, the glycerol has previously been removed from the tri-, di-, and monoglycerides by the saponification procedure, and one cannot determine definitely how large a proportion of fat was originally present in the form of free fatty acids or soaps. One can correct for the loss of glycerol by the addition of a calculated weight based upon the total fatty acid content. It is of course evident that the corrected value becomes progressively less accurate as the proportion of fatty acid derived from triglyceride is decreased.

Augur *et al.*[39] have modified Method I by a two-stage Soxhlet extraction, so that the soap fraction can be included. The neutral fats, fatty acids, and unsaponifiable extract are first removed, the fecal residue is acidified by the dropwise addition of 50% sulfuric acid, and the acidified mixture is subjected to a second Soxhlet extraction. As indicated earlier, a metabolic soap exists which must be used as a correction factor in the calculation of the digestibility of a fat when this analytical procedure is used.

The method of Folin and Wentworth[40] likewise includes the soaps in the analysis. The air-dried feces are extracted for twenty hours with diethyl ether containing 10% hydrochloric acid. After removal of the diethyl ether, the extract is again subjected to extraction with petroleum

[39] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition, 33*, 177–186 (1947).
[40] O. Folin and A. H. Wentworth, *J. Biol. Chem., 7*, 421–426 (1909–1910).

ether; the fatty material remains after evaporation of the petroleum ether.

Severance[41] reported that identical data on digestibility were obtained by the use of several procedures, provided, of course, that the appropriate factors were used in each case as a correction for metabolic lipid. Her results are summarized in Table 6 (p. 208).

Holt *et al.*[42] reported a practical procedure for the determination of fat in dried feces which makes use of the Roese-Gottlieb method employed in determining fat in dried and condensed milks, as reported by Patrick and Boyle.[43]

b. Methods Employing Wet Feces. These include a large number of procedures which have been suggested for the determination of lipids, chiefly in feces, but also in tissues, in which the wet sample is employed.[42–57] Most of these are used clinically for the determination of fat, but some of them may be sufficiently precise for research.

According to van de Kamer and associates,[44] estimations of fat based upon microscopical examination are unreliable, and fail to be quantitative. Likewise, the calculation of fat excretion from the weight of a random sample of dried feces is equivocal, since considerable variations occur in the proportion of dry matter in feces, irrespective of the fat present.[44,57,58]

[41] E. J. Severance, *The Digestibility of Some Peanut Oils in the Rat,* Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., Jan., 1952.

[42] L. E. Holt, A. M. Courtney, and H. L. Fales, *Am. J. Diseases Children, 17,* 38–42 (1919).

[43] G. E. Patrick and M. Boyle, *U. S. Dept. Agr., Bu. Chem., Bull. No. 105, Proc. 23rd Annual Convention Assoc. Official Agr. Chemists,* Washington, Nov., 1906, pp. 106–109 (1907).

[44] J. H. van de Kamer, H. ten Bokkel Huinink, and H. A. Weyers, *J. Biol. Chem., 177,* 347–355 (1949).

[45] L. von Liebermann and S. Székely, *Arch. ges. Physiol. (Pflüger's), 72,* 360–366 (1898).

[46] E. Polenske, *Arb. kaiserl. Gesundh., 33,* 563–579 (1910–1911).

[47] G. J. Saxon, *J. Biol. Chem., 17,* 99–102 (1914).

[48] F. S. Fowweather, *Brit. J. Exptl. Pathol., 7,* 7–14 (1926).

[49] F. S. Fowweather and W. N. Anderson, *Biochem. J., 40,* 350–351 (1946).

[50] G. Rosenfeld, *Biochem. Z., 200,* 280–288 (1928).

[51] P. Muller, *Nederl. Tijdschr. Geneesk., 79,* 3960–3962 (1935).

[52] H. C. Tidwell and L. E. Holt, Jr., *J. Biol. Chem., 112,* 605–613 (1935–1936).

[53] F. C. Gephart and F. A. Csonka, *J. Biol. Chem., 19,* 521–531 (1914).

[54] G. Nielsen, *Acta Paediat., 31,* 225–234 (1943).

[55] M. Kumagawa and K. Suto, *Biochem. Z., 8,* 212–347 (1908).

[56] R. Inaba, *Biochem. Z., 8,* 348–355 (1908).

[57] R. Goiffon, *Manuel de coprologie clinique,* 5th ed., Masson, Paris, 1949.

[58] W. T. Cooke, J. J. Elkes, A. C. Frazer, J. Parkes, A. L. P. Peeney, H. G. Sammons, and G. Thomas, *Quart. J. Med., n.s., 15,* 141–155 (1946).

Van de Kamer *et al.*[44] have proposed two procedures by which the fat can be measured within thirty-five to forty-five minutes, with an error which does not exceed 2%. The procedures are modifications of the methods of von Liebermann and Székely[45] and of Saxon.[47] In the first procedure (Method *A*), feces are saponified with concentrated potassium hydroxide in ethanol; HCl is added to acidify the solution, additional ethanol is added, and the fatty acids are extracted with petroleum ether. The concentration of ethanol is so chosen that the petroleum ether and ethanol solutions readily separate. This can be expedited by the addition of sodium chloride and amyl alcohol. The separation usually requires five to ten minutes. The total fatty acids are estimated by titration of an aliquot sample of the petroleum ether extract with alkali, using thymol blue as an indicator. This entire determination requires only approximately thirty-five minutes.

By means of the second procedure of van de Kamer *et al.*[44] (Method *B*) a determination of split and of unsplit fat can be made separately. In this case, the sample of feces is not treated with alkali; instead it is boiled for one minute with hydrochloric acid to convert the soaps into free fatty acids, as in the method of Saxon.[47] Ethanol, sodium chloride, and amyl alcohol are then added, and the solution is extracted with petroleum ether as in Method A. An aliquot sample of the petroleum ether extract is evaporated to dryness, and the free fatty acids are estimated by titration with 0.1 *N* isobutyl alcoholic KOH. Thereupon, an excess of the same solution is added, and the unsplit fat is hydrolyzed by boiling. The excess alkali remaining is determined by back titration with 0.1 *N* hydrochloric acid, again using thymol blue as an indicator. It is, of course, obvious that neither Method *A* nor Method *B* of van de Kamer and collaborators[44] is useful in the determination of fecal lipids other than fats.

(7) *Methods for the Determination of Inert Chemical Substances*

a. Lignin. Lignin can be accurately determined by the procedure of Ellis, Matrone, and Maynard.[16] This involves the four-hour extraction of a 1 g. sample of food or feces ground to pass through a forty-mesh screen, with an ethanol-benzene mixture (32 parts of 95% ethanol to 68 parts of benzene by weight); a coarse alundum thimble is used. After two washings, each with 95% ethanol and with diethyl ether (using suction), the material is dried, transferred to a 50 ml. stoppered Erlenmeyer flask, and digested overnight at 40°C. with a 1% pepsin solution in 0.1 *N* HCl. After the residue has been thoroughly washed with hot water,

making use of a filter stick, approximately 150 ml. of 5% sulfuric acid (by weight) are added. In the course of this addition, the material adhering to the filter stick is washed into the flask; the mixture is then refluxed for one hour and filtered. The residue is again washed with hot water, ethanol, and finally with diethyl ether, and is dried while remaining in the flask. Following this, 20 ml. of 72% sulfuric acid are added to the flask, and it is allowed to stand for two hours, with occasional stirring. Then 125 ml. of water are added; the residue is filtered and is washed with 15 to 20 ml. of hot water. Again the residue from the filter stick is returned to the flask with the aid of 3% sulfuric acid; the volume is made up to 150 ml. and the mixture is refluxed for two hours. After filtering into a Gooch or alundum crucible, the residue is washed with hot distilled water until free from acid. Lignin is determined by the loss of weight of the substance (dried at 105° to 110°C.) on ignition at 600°C.

b. Chromic Oxide. Several methods have been employed in the determination of chromic oxide in food or feces. These include the method of Paloheimo and Paloheimo,[59] Edin *et al.*,[24] the "reference-substance" method of Barnicoat,[28] that of Kane and associates,[19] and of Schürch, Lloyd, and Crampton.[35]

According to the method of Schürch *et al.*, a sample of 1 to 2 g. of food or feces, containing 20 to 50 mg. of chromic oxide, is ashed in 75 ml. nickel crucible at about 600°C. When this is cooled, about 1 g. of sodium peroxide is added, it is well mixed with the ash by swirling the crucible, and the mixture is fused at a low red heat until liquid. The heating is continued for five minutes at low red heat, during which time the mixture in the crucible is swirled occasionally. After cooling, the crucible is placed in a 500 ml. beaker. Cold distilled water is added to the crucible; after an interval of five to ten minutes, to allow for dissolving the residue, the solution is poured into the beaker and the crucible is washed several times with hot distilled water. After the solution has stood for thirty minutes in the beaker, it is filtered. The residue is washed with warm distilled water, this filtrate is added to the original solution, and the extract is made up to 500 ml. The concentration can be determined on a photoelectric colorimeter, using a 440 mμ filter or a spectrophotometer. The concentration of Cr_2O_3 is then read from a calibration curve.

(8) Special Methods for the Calculation of Digestibility of Lipids

When the diets contain large amounts of indigestible material, the cal-

[59] L. Paloheimo and I. Paloheimo, *Biedermanns Zentr., Abt. B., Tierernähr.*, 7, 317–324 (1935).

culation of digestibility by the use of correction factors based upon the weight of the feces may yield quite erroneous results. Under such conditions, it is necessary first to correct the weight of the feces by subtracting the weight of the indigestible fraction, which has previusly been determined by an independent analysis. The correction factor for metabolic lipid is obtained by multiplying the corrected weight of the feces by the appropriate value.

Table 7 illustrates the procedures used by Calbert *et al.*[7] in the calculation of the total stearyl citrate and stearyl alcohol excreted in the feces by dogs previously fed stearyl citrate. After the determination of these values, it is possible to use them for the calculation of the digestibility of fat and stearyl citrate, as shown in Table 7. "N.S.F." is the abbreviation used in Tables 7 and 8, and subsequently in the text, for the nonsaponifiable fraction.

a. General Formulas for the Calculation of the Digestibility of Higher Alcohols, Hydrocarbons, or Waxes. It is possible to calculate the digestibility of higher alcohols or hydrocarbons, as well as of fats fed concomit-

TABLE 7

DETERMINATION OF PROPORTION OF STEARYL CITRATE AND OF STEARYL ALCOHOL EXCRETED IN FECES AFTER ADMINISTRATION OF STEARYL CITRATE[a]

Category	Results on dog 10
Stearyl alcohol excreted:	
(*a*) N.S.F., total, mg.	219
(*b*) N.S.F., control value, mg.	33
(*c*) Stearyl alcohol, total, mg. ($a - b$)	186
Citric acid excreted:	
(*d*) As free acid (before hydrolysis), mg.	0
(*e*) Combined (after hydrolysis), mg.	49
Distribution of stearyl alcohol:	
(*f*) Total, mg. (*c*)	186
(*g*) Combined, mg. (Combined citric acid (*e*) × 281 (540/192))	138
(*h*) Free, mg. ($f - g$)	48
Calculation of stearyl citrate hydrolyzed:	
(*i*) Total stearyl alcohol as stearyl citrate, mg. (Corrected stearyl alcohol × 1.29 (696/540))	240
(*j*) Unhydrolyzed stearyl citrate, mg. (Combined citric acid (*e*) × 3.63 (696/192))	178
(*k*) Hydrolyzed, mg. ($i - j$)	62
(*l*) Hydrolyzed, % ($k/i \times 100$)	25.8
Calculation of stearyl citrate and stearyl alcohol in feces:	
(*m*) N.S.F., total, g. (by analysis)	16.1
(*n*) N.S.F., corrected, g. (Control N.S.F. (17.2% dried feces) subtracted from *m*)	13.2
(*o*) Stearyl alcohol and stearyl citrate in feces, g. ($n \times l$) + (100 − l) × n × (696/540)	16.0

[a] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research, 16,* 294–305 (1951).

TABLE 8

TESTS WITH DOGS ILLUSTRATING CALCULATION OF THE DIGESTIBILITY OF FAT AND STEARYL CITRATE WHEN FED TOGETHER[a]

Category	Results on dog 10
Food eaten, g.	968.0
Fat eaten, g.	196.5
Stearyl citrate eaten, g.	29.1
Feces:	
Dry weight, g.	103.7
Corrected dry weight, g. (Corrected by subtracting weight of stearyl citrate plus stearyl alcohol (item *o*, Table 6) from total weight of dried feces	87.7
Lipid excreted:	
Neutral fat, g.	35.8
Soaps, g.	12.1
Total, g.	47.9
Corrected for stearyl citrate and stearyl alcohol only, g.	31.9
Corrected for stearyl citrate, stearyl alcohol, and metabolic fat, g.	14.5
Coefficients of digestibility	
Total lipid	75.6
Fat	
Corrected for stearyl citrate, and stearyl alcohol only, g.	83.8
Corrected for stearyl citrate, stearyl alcohol, and metabolic fat, g.	92.6
Stearyl citrate alone	45.0

[a] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).

antly, if the undigested residue of the test substance can be determined in the N.S.F. Similarly, the same procedure can be applied to waxes if the proportion of the wax which has been hydrolyzed can be ascertained.

The steps in the calculation of the digestibility of the higher alcohol (stearyl alcohol) have been summarized by Calbert *et al.*[7] as follows:

A. Calculation of digestibility of total lipid by the usual methods[39]
B. Calculation of digestibility of fat alone
  a. Determination of total fat fed
  b. Total fat excreted = total fecal lipids − [metabolic lipid[a] + N.S.F. (corr.)[b]]
  c. Digestibility of fat alone = (a − b)/a × 100
C. Calculation of digestibility of alcohol[c] alone
  d. Determination of total alcohol fed
  e. Alcohol excreted = total N.S.F. excreted − control N.S.F. excreted
  f. Digestibility of alcohol = (d − e)/d × 100

[a] Metabolic lipid is determined by total lipid excretion (neutral fat + soap) on fat-free diet.
[b] Non-saponifiable fraction (corr.) = total fecal N.S.F. − N.S.F. of control groups.
[c] Applies only to higher alcohols which are not water-soluble.

The calculation to be used for determining the digestibility of the fat separately, or of the higher alcohol alone, is similar if a higher hydro-

carbon is tested. On the other hand, when the higher alcohol is fed as an ester, the calculation becomes somewhat more involved. The estimation of the digestibility of stearyl citrate and of the fat fed along with it is summarized by Calbert and associates[7] as follows:

D. Calculation of digestibility of total lipid by the usual methods[39]
E. Calculation of digestibility of fat alone
  g. Determination of total fat fed
  h. Total fat excreted (corr.) = total fecal lipids $- \left[\text{metabolic fat}^a + \frac{\text{N.S.F. (corr.)}^b}{x^c}\right]$
  i. Digestibility of fat alone = (g − h)/g × 100
F. Calculation of digestibility of wax
  j. Determination of total wax fed
  k. Wax excreted $= \frac{\text{total N.S.F. excreted} - \text{control N.S.F. excreted}}{x^c}$
  l. Digestibility of wax = (j − k)/j × 100

[a] Metabolic lipid is determined by total lipid excretion (neutral fat + soap) on fat-free diet.
[b] Non-saponifiable fraction (corr.) = total fecal N.S.F. − N.S.F. of control groups.
[c] Factor for converting alcohol to ester (stearyl alcohol to stearyl citrate).

3. Digestibility Studies on Fats, Oils, and Fatty Acids

(1) *Studies on Human Subjects*

a. Digestibility of Vegetable and Animal Fats Melting Under 50°C. In general, vegetable and animal fats which melt below 50°C. are almost completely utilized by man. In most cases, the coefficients of digestibility have been found to exceed 95. The chief exceptions to this rule are fats which are irritating to the gastrointestinal tract, such as castor oil and croton oil; these oils may fail to be appreciably utilized, while simultaneously preventing the efficient absorption of other foodstuffs. The most complete study of digestibility of fats in man is undoubtedly that made by the Office of Home Economics of the United States Department of Agriculture, results of which have been summarized by Langworthy.[60] Data based upon the studies on vegetable fats are given in Table 9, while those on animal fats are recorded in Table 10.

The foregoing data indicate that vegetable and animal fats melting under 50°C. are, in general, equally well digested and absorbed by normal men when taken in amounts of 50 to 100 g. daily. Of the thirty-four vegetable fats examined, all were digested to the extent of 94% or better,

[60] C. F. Langworthy, *Ind. Eng. Chem.*, *15*, 276–278 (1923).

TABLE 9

AVERAGE COEFFICIENTS OF DIGESTIBILITY OF DIFFERENT VEGETABLE FATS AND OILS IN HUMAN SUBJECTS[a]

Fat or oil	Number of expts.	Av. daily fat intake, g.	Coeff. of digestibility
Almond[b]	4	70	97.1
Apricot kernel[c]	4	70	98.4
Avocado[d]	3	100	87.8
Black walnut[b]	4	56	97.5
Brazil nut[b]	3	81	96.3
Butternut[b]	3	43	95.4
Charlock[e]	4	60	98.9
Cherry kernel[c]	4	57	98.0
Cocoa butter[f]	11	51	94.9
Coconut[f,g]	12	65	97.9, 88.7
Cohune palm[d]	4	52	99.1
Corn[e,g]	7	82	96.9
Cottonseed[f]	12	86	97.8
Cottonseed[h]	8	67	96.5
Cupuassú[d]	4	41	94.1
English walnut[b]	3	78	97.6
Hempseed[d]	3	57	98.5
Hickory nut[b]	4	95	99.3
Japanese mustard seed[e]	3	79	98.8
Java almond[i]	2	60	97.0
Melon seed[c]	3	41	98.2
Olive[f]	10	73	97.8
Palm kernel[d]	3	100	98.0
Peach kernel[c]	3	62	96.6
Peanut[f]	5	98	98.3
Pecan[b]	4	104	96.8
Poppyseed[d]	7	50	96.3
Pumpkin seed[c]	2	75	98.2
Rapeseed[e]	4	82	98.9
Rapeseed[h]	8	60	99.0
Sesame[f]	5	90	98.0
Soybean[e,g]	7	80	97.5, 93.7
Sunflowerseed[e]	4	90	96.5
Teaseed[i]	1	50	91.2
Tomato seed[c]	3	57	95.8
Watermelon seed[i]	3	30	94.8

[a] Adapted from H. J. Deuel, Jr., in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. II, Interscience, New York-London, 1951.

[b] A. D. Holmes, *U. S. Dept. Agr., Bull. No. 630*, 1–19 (1916).

[c] A. D. Holmes, *U. S. Dept. Agr., Bull. No. 781*, 1–16 (1919).

[d] A. D. Holmes and H. J. Deuel, Jr., *J. Biol. Chem., 41*, 227–235 (1920).

[e] A. D. Holmes, *U. S. Dept. Agr., Bull. No. 687*, 1–20 (1918).

[f] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 505*, 1–18 (1917).

[g] L. E. Holt, Jr., H. C. Tidwell, C. M. Kirk, D. M. Cross, and S. Neale, *J. Pediat., 6*, 427–480 (1935). These second experiments were on normal infants.

[h] H. J. Deuel, Jr., R. M. Johnson, C. E. Calbert, J. Gardner, and B. Thomas, *J. Nutrition, 38*, 369–380 (1949).

[i] H. J. Deuel, Jr., and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 1033*, 1–15 (1922).

TABLE 10
AVERAGE COEFFICIENTS OF DIGESTIBILITY OF DIFFERENT ANIMAL FATS AND OILS FED TO HUMAN SUBJECTS[a]

Fat or oil	Number of expts.	Av. daily fat intake, g.	Coeff. of digestibility
Bacon[b]	—	289	96.7
Beef (m.p., 45°C.)[c]	10	100	93
Brisket[d]	7	80	97.4
Butter[c]	8	100	97
Butter[e]	—	214	97
Chicken[d]	8	95	96.7
Cod-liver[f]	4	47	97.7
Cream[d]	7	78	96.9
Egg yolk[d]	6	83	93.8
Fish[d]	3	60	95.2
Goat's butter[g]	4	45	98.4
Goose[d]	7	95	95.2
Hard palate (m.p., 34°C.)[g]	3	90	93.7
Horse[g]	3	65	93.9
Kid[g]	3	62	95.3
Lard[c]	9	90	97
Oleo[g]	8	59	96.8
Ox marrow[g]	4	87	93.5
Ox tail[g]	3	77	96.6
Turtle[g]	4	49	98.6

[a] H. J. Deuel, Jr., in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. II, Interscience, New York-London, 1951.
[b] K. Blunt and M. G. Mallon, *J. Biol. Chem.*, *38*, 43–48 (1919).
[c] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr.*, *Bull. No. 310*, 1–20 (1915).
[d] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr.*, *Bull. No. 507*, 1–19 (1917).
[e] M. Rubner, *Z. Biol.*, *15*, 115–202 (1879); cited by R. P. Cook, *Comparative Aspects of Lipid Absorption and Excretion*, in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 14–29 (1952), p. 15.
[f] H. J. Deuel, Jr., and A. D. Holmes, *U. S. Dept. Agr.*, *Bull. No. 1033*, 1–15 (1922).
[g] A. D. Holmes, *U. S. Dept. Agr.*, *Bull. No. 613*, 1–27 (1919).

with the exception of avocado fat (87.8) and teaseed oil, for which the result of a single test gave a coefficient of digestibility of 91.2. All of the eighteen animal fats studied had coefficients of digestibility of 93 or better.

The results of the tests reviewed by Langworthy[60] may be open to some question, in view of the fact that the method employed for the determination of fecal lipids accounts for only neutral fat, fatty acid, and non-saponifiable components, but does not include soaps. It is well known that soaps may comprise a considerable proportion of the stools, especially in the case of subjects on high calcium diets and when abnormalities in fat absorption obtain. Holmes and Kerr[61] recognize this as a possible criticism of the U.S.D.A. work. However, when the

[61] A. D. Holmes and R. H. Kerr, *J. Biol. Chem.*, *58*, 377–381 (1923–1924).

calculation of the coefficient of digestibility was based upon the fecal lipid without soaps, as determined in the U.S.D.A. studies, the values were actually somewhat lower than when the method of Folin and Wentworth,[40] which includes soaps, was employed for the determination of fecal lipids. In the tests of Deuel *et al.*[4] on cottonseed and rapeseed oils, as shown in Table 9, the fecal fat was determined by a method which included the soaps. The results of tests with these two oils by the two analytical procedures were found to be identical. Moreover, the results obtained on rats with a number of natural fats,[39,62] by analytical procedures which included the soap fraction, were substantially the same as if the analysis had been made without acidification of the feces. The failure to change the coefficient of digestibility when the fecal soaps were included is explained by the fact that the increased lipid excreted as soap was almost exactly counterbalanced by an increased metabolic "soap" fraction. On the basis of these findings, it seems quite likely that the U.S.D.A. results are substantially correct insofar as normal individuals and fats with melting points below 50°C. are concerned.

Somewhat lower results are reported by Holt *et al.*[63] for the digestibility of coconut oil (88.7 and 89.8) in normal infants than are reported for normal men; the latter averaged 97.9.[64] On the other hand, Tidwell and collaborators[65] observed that both olive oil and soybean oil were more efficiently absorbed by premature infants than was butterfat.

b. Digestibility of High-Melting Fats. It has long been recognized that fats having melting points considerably above body temperature are less completely digested than are those fats with lower melting points. Langworthy and Holmes[2] stated, in 1915, that "it seems fair to conclude that of those tested the fats of low melting points are capable of a more complete assimilation than are those which have a high melting point." This same opinion was expressed later by Holmes and Deuel,[66] who stated that an inverse relationship obtains between the melting point and the coefficient of digestibility. The results of experiments on animal fats and on hydrogenated vegetable fats having high melting points are summarized in Table 11.

The tests reported in Table 11 were carried out by employing methods

[62] M. E. Crockett and A. J. Deuel, Jr., *J. Nutrition*, *33*, 187–194 (1947).

[63] L. E. Holt, H. C. Tidwell, C. M. Kirk, D. M. Cross, and S. Neale, *J. Pediat.*, *6*, 427–480 (1935).

[64] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr.*, *Bull. No. 505*, 1–20 (1917).

[65] H. C. Tidwell, L. E. Holt, Jr., H. L. Farrow, and S. Neale, *J. Pediat.*, *6*, 481–489 (1935).

[66] A. D. Holmes and H. J. Deuel, Jr., *J. Biol. Chem.*, *41*, 227–235 (1920).

TABLE 11

AVERAGE COEFFICIENTS OF DIGESTIBILITY, IN HUMAN SUBJECTS, OF SOME HIGH-MELTING FATS AND SOME HYDROGENATED FAT MIXTURES[a]

Fat or oil	M.p., °C.	Number of expts.	Av. daily fat intake, g.	Coeff. of digestibility
Mutton[b]	50	7	53	88
Oleostearin[c]	50	3	68	80.1
Deer[d]	51.4	3	46	81.7
Hydrogenated fats[e]				
Cottonseed	35	5	84	96.8
	46	3	89	94.9
Peanut	37	5	76	98.1
	39	3	78	95.9
	43	5	91	96.5
	50	4	59	92.0
	52.4	3	62	79.0
Corn	33	5	78	94.7
	43	5	74	95.4
	50	5	44	88.5
Blended hydrogenated fats[d,f]				
Cottonseed				
(12.5:87.5)[g]	45.8	2	52.6	96.4
(18.8:81.2)	47.8	4	76.3	94.2
(23.5:76.5)	48.1	2	49.4	94.4
(22.1:77.9)	50.0	3	56.9	87.0
Peanut				
(6.2:93.8)	43.0	5	73.8	96.6
(9.1:90.9)	43.2	4	79.7	97.4
(33.3:66.7)	51.1	4	90.3	92.8
Corn				
(9.1:90.9)	39	4	102.9	95.2
(25.0:75.0)	49	3	105.4	93.3
(30.8:69.2)	54	3	92.5	91.5

[a] H. J. Deuel, Jr., in K. S. Markley, ed., *Soybeans and Soybean Products,* Interscience, New York-London, 1951.

[b] C. F. Langworthy and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 310,* 1–22 (1915).

[c] A. D. Holmes, *U. S. Dept. Agr., Bull. No. 613,* 1–27 (1919).

[d] H. J. Deuel, Jr., and A. D. Holmes, *U. S. Dept. Agr., Bull. No. 1033,* 1–15 (1922).

[e] A. D. Holmes and H. J. Deuel, Jr., *Am. J. Physiol., 54,* 479–488 (1921). In these tests, all of the oil was subjected to partial hydrogenation to yield fats of the varying melting points.

[f] A portion of the fats was almost completely saturated with hydrogen and blended with sufficient untreated oil to give mixtures of the melting points indicated.

[g] The values in parentheses indicate the respective proportions of completely saturated fat and untreated oil mixed in the fat blend.

for the determination of fecal fat which did not include the soaps. Some question has arisen, as a result of the investigations of Crockett and

Deuel[62] on rats, as to whether or not lower coefficients of digestibility would have been obtained in the tests on human subjects had the soap fraction been included in the calculation. In the latter tests with rats, the digestibility of hydrogenated lard melting at 55°C. was 63.2, while that of the lard melting at 61°C. was found to be 21.0. If no allowance had been made in the calculations for the soap fraction, the figures for digestibility would have been 100 and 91, respectively. In the light of these studies on rats, further tests on human subjects are obviously needed to determine whether or not a similar phenomenon applies to man.

c. Digestibility of Oleomargarines. All evidence points to the conclusion that oleomargarines are as completely utilized as are the natural fats melting below 50°C. This high digestibility was recorded by Holmes[67] for the margarines containing mixtures of animal and vegetable fats, in current use during the period from 1910 to 1920, as well as for a sample prepared by modern technics using hydrogenated vegetable oils.[68] These data are summarized in Table 12.

TABLE 12
AVERAGE COEFFICIENTS OF DIGESTIBILITY OF SOME OLEOMARGARINES IN HUMAN SUBJECTS

Composition	Number expts.	Av. fat eaten, g.	Coeff. of digestibility
59% Oleo oil, 7% lard, 22% vegetable oils (cottonseed, peanut), 12% milk fat[a]	9	—	97.2
41% Oleo oil, 32% lard, 24% vegetable oil (peanut), 3% milk fat[a]	7	80	93.4
67% Oleo oil, 33% vegetable oil (cottonseed), 0.1% milk fat[a]	4	—	96.8
Vegetable oil margarine (m.p., 94–95°F. (Wiley))[b]	7	86.5	96.7

[a] A. D. Holmes, *Boston Med. Surg. J.*, *192*, 1210–1212 (1925).
[b] H. J. Deuel, Jr., *J. Nutrition*, *32*, 69–72 (1946).

Although the experiments on man were carried out without consideration of the soap fraction excreted in the feces, there is no reason to question the high digestibility of oleomargarine. In these experiments, there was no indication of a large bulk of feces which would normally accompany any considerable excretion of soap. Moreover, there are considerable data in the literature indicating the practically complete digestibility of margarine in other species. For example, confirmatory tests by Crockett and Deuel,[62] with rats, demonstrated that the average coefficient of

[67] A. D. Holmes, *Boston Med. Surg. J.*, *192*, 1210–1212 (1925).
[68] H. J. Deuel, Jr., *J. Nutrition*, *32*, 69–72 (1946).

digestibility was 97.0; these tests included the soap fraction. Severance[41] also reported a correspondingly high figure (95.2) for the digestibility of margarine in rats when the total fecal lipids were determined by a variety of procedures. Finally, Calbert *et al.*[7] reported a number of series of tests on rats with margarine fat alone at a level of 15, 22.5, or 25% of the diet; the digestibility of the margarines in all cases exceeded 95%. Corresponding figures indicating a high digestibility were likewise noted in tests in which isopropyl citrate was also present in the diet. The digestibility was somewhat depressed in rat tests when stearyl citrate was included in the diet at high levels; this effect, however, was not observed in dogs, and is believed to be the result of an abnormal condition produced by the inclusion of excessive quantities of a high-melting wax. Calbert and associates[7] were likewise able to prove the high digestibility of margarine fat in dogs (99.2 to 100%) when the fecal soaps were included in the calculations.

The complete utilization of the modern margarines would be expected because of the additives included in these preparations. Thus, it is believed that the absorption is facilitated by the presence of monoglycerides, diglycerides, and lecithins contained by practically all margarines. In fact, it has been reported[69] that margarine is also an excellent vehicle for the feeding of β-carotene, since a higher provitamin A potency was found than might be expected if the carotenoid were administered in a fat devoid of the additives. This augmentation in effect is believed to be related to a more satisfactory absorption of the carotenoid when administered in margarine.

(2) *Studies on Animals Other Than Man*

a. Digestibility of Natural Fats. There is general agreement that, in most cases, the pattern of digestibility of fats is similar in man, dog, and the rat. On the other hand, McCay and Paul[70] reported that guinea pigs digest some of the high-melting vegetable and animal fats poorly. Cook[71] and also Cook and Thomson[72] have confirmed the low digestibility of several fats in guinea pigs. On the other hand, rabbits and sheep were shown by Paul and McCay[73] to have higher coefficients of digestibility for

[69] H. J. Deuel, Jr., S. M. Greenberg, E. E. Savage, and D. Melnick, *J. Nutrition, 43*, 371–387 (1951).
[70] C. M. McCay and H. Paul, *J. Nutrition, 15*, 377–382 (1938).
[71] R. P. Cook, *Biochem. J., 51*, xiii (1952).
[72] R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol., 36*, 61–74 (1951).
[73] H. Paul and C. M. McCay, *Arch. Biochem., 1*, 247–253 (1942).

Table 13. Average Coefficients of Digestibility of Some Fats in Several Species of Animals[a]

Fat fed	M.p., °C.	Coefficients of digestibility: Guinea pig[b]	Rat	Rabbit	Sheep	Dog
Beef tallow	—	72.0	—	—	—	—
Butter	34.5	91.0	88.3[c]	—	—	—
	34.5	—	90.7[d]	—	—	—
	—	—	97.4[e]	—	—	—
	—	—	98.0[f]	—	—	—
Castor oil	—	96.2[g]	98[g]	92.1[h]	99[h]	—
Cacao butter	28	—	63.3[c]	—	—	—
	28	—	81.6[d]	—	—	—
Coconut oil	26	94.0	98.9[c]	—	—	—
	26	—	96.5[d]	—	—	—
Cod-liver oil	—	93.8	98.2[e]	—	—	—
Corn oil	—	86.5	97.5[c]	—	—	99[i]
	—	—	98.3[d]	—	—	—
	—	—	97.9[e]	—	—	—
Cottonseed oil	—	87.4	97.4[b]	91.2[h]	94[h]	99[i]
	—	—	99.1[f]	—	—	—
	—	—	94.8[j]	—	—	—
Cottonseed oil, hydrogenated	38	—	91[k]	—	—	—
	46	—	83.8[l]	—	—	—
	54	—	68.7[l]	—	—	—
	62	—	38[k]	—	—	—
	63	—	24.0[l]	—	—	—
Crisco	43	73.8	94.8[b]	91.0[h]	94[h]	—
	43	—	97.3[m]	—	—	—
Lard	—	75.2	97.8[e]	—	—	97[n]
	37	75.2	96.6[m]	—	—	98[i]
Lard, bland	48	—	94.3[m]	—	—	—
Lard, hydrogenated	55	—	63.2[m]	—	—	—
	61	—	21.0[m]	—	—	—
Margarine fat	34	—	97.0[m]	—	—	99.7[o]
	34	—	97[p]	—	—	—
Mutton tallow	47	79.8	74.6[c]	—	—	—
	47	—	84.8[d]	—	—	—
Neat's foot oil	—	93.5	—	—	—	—
Oleo stock	48	—	74.0[c]	—	—	—
	48	—	86.7[d]	—	—	—
Olive oil	—	94.5	98.4[q]	—	—	98[n]
	—	77[r]	92[r]	94[r]	—	—
Peanut oil, crude	—	—	97.6[j]	—	—	—
Peanut oil, refined	—	91.8	96.4[j]	—	—	—

Table continued

TABLE 13 (*continued*)

Fat fed	M.p., °C.	Coefficients of digestibility				
		Guinea pig[b]	Rat	Rabbit	Sheep	Dog
Peanut oil, hydrogenated (blended)	39	—	92.6[j]	—	—	—
	39	—	91.4[l]	—	—	—
Perilla oil (hydrogenated)........	67.5	—	6[k]	—	—	—
Rapeseed oil................	—	—	82[p]	—	—	—
Salmon oil..................	—	94.0	—	—	—	—
Sardine oil..................	—	—	98.3[s]	—	—	—
Shortening A..................	—	—	98.6[e]	—	—	—
Shortening B..................	—	—	99.6[e]	—	—	—
Soybean oil..........	—	94.5	98.5[c]	—	—	—
	—	—	98.3[d]	—	—	—
Tobacco-seed oil..........	—	—	97.9[f]	—	—	—
Tropical fats[t]..................	—	—	—	—	—	—
Aceituna, unrefined[u]..........	—	—	93.5	—	—	—
Cacao volador[v]............	—	—	96.9	—	—	—
Corozo[w]..................	—	—	97.0	—	—	—
Morro[x]..................	—	—	96.4	—	—	—
Sapayulo[y]..................	—	—	92.2	—	—	—
Tambor[z]..................	—	—	94.5	—	—	—

[a] Soaps were included in the determination in all reports except in references *b*, *e*, *g*, *h*, and *s*.
[b] C. M. McCay and H. Paul, *J. Nutrition*, *15*, 377–382 (1938), except where noted otherwise.
[c] R. Hoagland and G. G. Snider, *J. Nutrition*, *25*, 295–302 (1943). Fat fed at 5% level.
[d] Reference *c* but fat fed at 15% level.
[e] H. Steenbock, M. H. Irwin, and J. Weber, *J. Nutrition*, *12*, 103–111 (1936).
[f] K. E. Rapp, J. T. Skinner, and J. S. McHargue, *J. Nutrition*, *31*, 273–282 (1946). Average of experiments in which fat was fed at 5, 15, or 30%.
[g] W. C. Stewart and R. G. Sinclair, *Arch. Biochem.*, *8*, 7–11 (1945).
[h] H. Paul and C. M. McCay, *Arch. Biochem.*, *1*, 247–258 (1942).
[i] E. W. Rockwood and P. B. Sivickes, *J. Am. Med. Assoc.*, *71*, 1649–1650 (1918).
[j] E. J. Severance, *The Digestibility of Some Peanut Oils in the Rat.* Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., Jan., 1952.
[k] H. M. Evans and S. Lepkovsky, *J. Biol. Chem.*, *96*, 165–177 (1932).
[l] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[m] M. E. Crockett and H. J. Deuel, Jr., *J. Nutrition*, *33*, 187–194 (1947).
[n] L. Arnschink, *Z. Biol.*, *26* (*n.s. 8*), 434–451 (1890).
[o] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).
[p] H. J. Deuel, Jr., A. L. S. Cheng, and M. G. Morehouse, *J. Nutrition*, *35*, 295–300 (1948).
[q] R. Hoagland and G. G. Snider, *J. Nutrition*, *26*, 219–225 (1943).
[r] R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol.*, *36*, 61–74 (1951). Fed at 2.7 and 3.0 g./day.
[s] S. Lassen, E. K. Bacon, and H. J. Dunn, *Arch. Biochem.*, *23*, 1–7 (1949).
[t] R. L. Squibb, H. T. Love, and M. K. Wyld, *J. Nutrition*, *44*, 547–552 (1951).
[u] *Simaruba glauca* (paradise-tree). [v] *Virola guatemalensis* (virola).
[w] *Orbignya cohune* (cohune palm). [x] *Crescentia alata* (cross-leaf calabash tree).
[y] *Calocarpum mammosum* (*sapota*) (sapote). [z] *Omphalea oleifera* (navelspurge).

hard and soft fats than did guinea pigs. In fact, Cook[71] states that, although mammals in general have high digestibilities (90–98%), low coefficients of digestibility obtain in guinea pigs, particularly when high levels of fat are given.

Table 13 records the results of digestibility tests on several species.

Although the coefficients of digestibility in man and in the lower animals generally show agreement, several differences are immediately apparent. One of the most striking variations is in the behavior of castor oil. Thus, castor oil is readily digested in rabbits, rats, sheep, and guinea pigs, and exerts no cathartic effect; this is in striking contrast to its action in man, where its utilization may be slight, even when a sufficient amount is taken to produce catharsis. It is not known to what extent castor oil is utilized in man when taken in amounts too low to produce diarrhea; one might expect that utilization takes place to some extent under such conditions. Stewart and Sinclair,[74] who reported that castor oil was digested to the extent of 98% in rats, were unable to find any trace of its principal fatty acid, ricinoleic acid, in the phospholipids of the small intestine, liver, or muscle, or in the triglycerides of the liver. These results were interpreted as indicative of the rapid metabolism of this hydroxy acid.

A second difference between man and the rat as regards the digestibility of fat has been noted in the case of rapeseed oil. Deuel, Cheng, and Morehouse[75] found that crude rapeseed oil was digested to the extent of only 77% in the rat, while the digestibility of the refined oil was found to be 82%. This is the lowest digestibility which has been reported for a fat, liquid at ordinary temperatures, other than waxes or fats which contain intestinal irritants. This finding is in line with the earlier observation[76] that the rate of absorption of rapeseed oil by the rat is the lowest of any for the common liquid fats. The low digestibility would likewise explain the poor showing of rapeseed oil in the growth tests on rats reported by Boer *et al.*[77]

The poor utilization of rapeseed oil by the rat may well be related to its composition. This fat consists of 40 to 50% of trierucin and not more than 5% of glycerides of saturated acids; the balance is made up of triolein and trilinolein, as well as of mixed triglycerides.[78] Since no failure of

[74] W. C. Stewart and R. G. Sinclair, *Arch. Biochem.*, *8*, 7–11 (1945).

[75] H. J. Deuel, Jr., A. L. S. Cheng, and M. G. Morehouse, *J. Nutrition*, *35*, 295–300 (1948).

[76] H. J. Deuel, Jr., L. Hallman, and A. Leonard, *J. Nutrition*, *20*, 215–226 (1940).

[77] J. Boer, B. C. P. Jansen, and A. Kentie, *J. Nutrition*, *33*, 339–358 (1947).

[78] T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947.

lipolysis was noted in the tests by Deuel *et al.*,[75] the low digestibility was ascribed to the failure to absorb erucic acid.

On the other hand, Holmes[79] had reported earlier that the digestibility of rapeseed oil in man was 99%. This high figure for utilization cannot be attributed to the fact that the investigator failed to take into consideration the excretion of fat in the form of soaps inasmuch as, in later experiments of Deuel and associates,[4] a coefficient of digestibility was found for human subjects identical with that reported by Holmes. In these later tests,[4] the excretion of fecal soap was determined. One must conclude that a fundamental difference in the utilization of rapeseed oil obtains between the rat and man; at the present time, there is no adequate explanation for this variation.

Another difference in fat utilization is noted when one compares the digestibility of fats by the guinea pig and by other species. Although cod-liver, olive, and soybean oils are utilized approximately as well in the guinea pig as in other species, the coefficient of digestibility of corn, cottonseed, and peanut oils appears to be somewhat depressed in this rodent.[70] Moreover, Cook and Thomson[72] reported a coefficient of digestibility for olive oil of only 77 in the guinea pig when this vegetable oil was fed at a comparatively high level (3 g. per day). Lard was found to be digested only to the extent of 75.2% by the guinea pig,[70] as contrasted with 96.6% in the rat[62] and 97.0% in man.[2]

b. Digestibility of Simple Triglycerides and Fatty Acids. A number of pertinent studies on the digestibility of simple triglycerides and of fatty acids have been made for several species. These are recorded in Table 14.

Several interesting factors are noted when one considers the digestibility of the higher simple triglycerides. In the first place, when no other fats were present in the diet, trilaurin was found to be practically completely digested, while trimyristin, tripalmitin, and tristearin were progressively less efficiently utilized in the rat.[5] In the experiments of Hoagland and Snider,[80] in which the triglycerides were fed in 5 or 10% solutions in olive oil, trilaurin and trimyristin were highly utilized and only about 15% of the tripalmitin failed to be absorbed. The coefficients of digestibility of the entire fat mixtures when the triglycerides made up 5 or 10% of the total fat, respectively, were as follows: trilaurin, 98.2 and 99.9; trimyristin, 96.4, 99.0; tripalmitin, 95.8, 95.6; and tristearin, 92.7 and 89.2. However, the digestibility of tristearin was not increased by its solution in olive oil. The coefficients of digestibility calculated for this triglyc-

[79] A. D. Holmes, *U. S. Dept. Agr., Bull. No. 687,* 1–20 (1918).
[80] R. Hoagland and G. G. Snider, *J. Nutrition, 26,* 219–225 (1943).

TABLE 14

AVERAGE COEFFICIENTS OF DIGESTIBILITY OF SIMPLE TRIGLYCERIDES AND FATTY ACIDS IN SEVERAL SPECIES OF ANIMALS

Triglyceride or fatty acid fed	Melting point, °C.		Coefficient of digestibility		
	Pure substance	Mixture fed	Guinea pig	Rat	Dog
Trilaurin	49	—	—	97.3[a]	—
Trimyristin	56	—	—	76.6[a]	—
Tripalmitin	66.5	—	—	27.9[a]	95[d]
	—	45	—	84.0[b]	—
	—	51	—	82.0[c]	—
Tristearin	70	—	—	18.9[a]	10[e]
	—	55	—	6.0[b]	—
	—	59	—	8.0[c]	—
+ triolein (2:1)	—	—	—	39.4[f]	—
+ triolein (1:2)	—	—	—	68.6[f]	—
Lauric acid	44	32	—	81.5[g]	—
Myristic acid	53	44	—	81.9[g]	—
Palmitic acid	63	—	—	35.6[a]	82[d]
	—	37	—	39.6[b]	—
	—	44	—	37.1[c]	—
	—	48	—	31.2[h]	—
	—	53	—	23.8[g]	—
Stearic acid	69	—	—	15.8[a]	—
	—	43	—	9.4[b]	—
	—	51	—	13.3[c]	—
	—	55	—	21.0[h]	—
	—	59	—	19.6[g]	—
Oleic acid	—	—	95.4[i]	95.4[i]	—
Elaidic acid	—	—	55.6[i]	95.6[i]	—

[a] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949).
[b] R. Hoagland and G. G. Snider, *J. Nutrition*, *26*, 219–225 (1943). The fat mixture consisted of 5% of simple triglyceride or fatty acid and 95% olive oil.
[c] Reference *b*, but fat mixture consisted of 10% of simple triglyceride or fatty acid and 90% olive oil.
[d] J. F. Lyman, *J. Biol. Chem.*, *32*, 7–11 (1917).
[e] L. Arnschink, *Z. Biol.*, *26* (*n.s. 8*), 434–451 (1890).
[f] K. F. Mattil and J. W. Higgins, *J. Nutrition*, *29*, 255–260 (1945).
[g] Reference *b*, but fat mixture consisted of 25% fatty acid and 75% olive oil.
[h] Reference *b*, but fat mixture consisted of 15% fatty acid and 85% olive oil.
[i] H. Paul and C. M. McCay, *Arch. Biochem.*, *1*, 247–253 (1942).

eride were 6.0 and 8.0 when it was dissolved in olive oil at a level of 5 and 10%, respectively. Although the results of Lyman[81] indicate a high absorption for tripalmitin in the dog, Arnschink[82] found that practically no tristearin could be utilized by this species (9–13.8%).

[81] J. F. Lyman, *J. Biol. Chem.*, *32*, 7–11 (1917).
[82] L. Arnschink, *Z. Biol.*, *26* (*n.s. 8*), 434–451 (1890).

In general, the higher saturated fatty acids are poorly absorbed, even when fed in solution in olive oil. Both lauric and myristic acids were found to be only approximately 80% utilized by the rat, while the coefficients of digestibility of palmitic and stearic acids are approximately the same when they are fed alone and when they are administered in 5 to 25% solutions in olive oil. The high digestibility reported by Lyman[81] for palmitic acid in the dog is in line with the high utilization of tripalmitin in this species, but it stands out in sharp contrast to the results on rats.

Paul and McCay[73] demonstrated an interesting species variation in the utilization of oleic acid and its *trans* isomer, elaidic acid. Whereas the rat is able to utilize both of these acids to the extent of 95%, the guinea pig can digest only the oleic acid efficiently. Elaidic acid was found to have the low coefficient of digestibility of 56 in the latter species.

The hen presents an interesting species variation in the case of tributyrin. Although digestibility experiments of the usual type have not been recorded in rats, it has been found that 100% of tributyrin fed had disappeared from the gut of the rat within eight hours after feeding.[83] In all probability, this triglyceride is practically completely absorbed by the rat. Davis[84] reported an average coefficient of digestibility of 91.9 for tributyrin in hens, except in two tests in which a coefficient of 86.9 was found; in these latter cases toxicity apparently obtained. McClure and Carr[85] have found that the lower fatty acids are also toxic for pigeons. Cook[86] has reviewed the comparative aspects of lipid absorption and excretion.

4. Normal Factors Altering the Digestibility of Fats

(*1*) *The Effect of Age*

No systematic studies have been made to determine the effect of age on the digestibility of fats. However, it is evident from the studies of Holt and collaborators[87,88] that the digestibility of fat is less efficient in

[83] H. J. Deuel, Jr., J. S. Butts, H. Blunden, C. H. Cutler, and L. Knott, *J. Biol. Chem.*, *117*, 119–129 (1937).

[84] R. E. Davis, *J. Biol. Chem.*, *88*, 67–75 (1930).

[85] F. J. McClure and R. H. Carr, *Am. J. Physiol.*, *74*, 70–78 (1925).

[86] R. P. Cook, "Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 14–29 (1952).

[87] L. E. Holt, A. M. Courtney, and H. L. Fales, *Am. J. Diseases Children*, *17*, 241–250 (1919).

[88] L. E. Holt, A. M. Courtney, and H. L. Fales, *Am. J. Diseases Children*, *18*, 107–126 (1919).

infants and in young children than it is in older children and adults. In the case of premature infants, Tidwell and associates[65] noted a poor absorption of fats; olive and soybean oils were more readily digested than was butter. Gordon and McNamara[89] likewise reported a high excretion of fat in the feces of premature infants. Holt *et al.*[63] obtained coefficients of digestibility of 95 for olive oil, 97 for corn oil, 89 for butter, 88.7 for coconut oil, and 83 for hydrogenated corn oil in normal infants less than ten months old. The authors concluded that fats containing a high percentage of unsaturated fatty acids are more easily absorbed by infants than are the more saturated fats. Williams and co-workers[90] reported that the fecal fat excretion of children four to twelve years of age was slightly but significantly higher for the older and larger children than for the younger ones. The increased excretion was confined to the soap fraction. Macy[91] noted that the coefficient of digestibility of children four to twelve years of age on a mixed diet was 97 when an average of 79.30 g. of fat was being ingested. Harrison and Sheldon[92] have also recorded a lower digestibility of fat in children than in adults.

(2) The Effect of Sex

Although it is generally accepted that the digestibility of fats is independent of sex, Severance[41] has obtained some evidence that female rats exhibit slightly higher coefficients of digestibility when fed peanut oil or hydrogenated peanut oil than do male rats of the same age. The variations in values obtained, although consistent, were quite small.

(3) The Effect of Species

Considerable variation has been noted in the coefficients of digestibility of fats with high melting points, of castor oil, and of lard, in guinea pigs, rats, and man. Moreover, the guinea pig digests olive oil rather poorly when it is fed at a high level. These variations can be noted when the results listed in Tables 9, 10, 11, and 14 are compared.

In addition to the digestibility studies on guinea pigs, rats, rabbits, sheep, and dogs, which are summarized in Table 13, tests have been made

[89] H. H. Gordon and H. McNamara, *Am. J. Diseases Children, 62,* 328–345 (1941).

[90] H. H. Williams, E. N. Endicott, M. L. Shepherd, H. Galbraith, and I. G. Macy, *J. Nutrition, 25,* 379–387 (1943).

[91] I. G. Macy, *Nutrition and Chemical Growth in Children.* Vol. I, *Evaluation,* C. C Thomas, Springfield, Ill., 1942, pp. 92, 126.

[92] G. A. Harrison and W. P. H. Sheldon, *Arch. Disease in Childhood, 2,* 338–348 (1927).

on a number of other species. According to Schneider,[93] soybean seed, with a fat content of from 15 to 17%, has the following coefficients of digestibility: cattle, 98; sheep and goats, 84–95; and pigs, 84. Palm kernel oil, present to the extent of 20% in the meal, was digested by sheep and goats to the same extent as was soybean oil. Cottonseed oil, found in the cottonseed cake to the extent of 7%, was shown[86] to have a coefficient of digestibility of 96. Soybean and peanut oils are reported to be well utilized by the pig.[94] Güntherberg[95] found that ordinary fats are well absorbed by the hen. Even the trout, which has a very low body temperature, has been shown by McCay[96] to utilize relatively high-melting fats to the extent of 57%, without any apparent injury.

(4) *The Effect of Melting Point*

In general, an inverse relation exists between melting point and digestibility for fats melting at temperatures above 50°C.[2,97] Cheng and her associates[5] reported that this inverse relationship exists in the absorption of simple triglycerides in the rat. Figure 1 shows the relationship between the percent of unabsorbed fat and the melting point of the fat.

In analyzing the relationship of melting point of a fat to its digestibility in rats, Deuel[98] has shown that the greatest increase in fat excretion occurs in the soap fraction. A summary of these results is given in Table 15.

Paul and McCay[73] state that the melting point of a fat is important in determining its utilization by guinea pigs, but that this is not the case with rabbits and sheep.

On the other hand, Hoagland and Snider[99] have pointed out that their experiments do not support the thesis that a definite relationship exists between melting point of a fat and digestibility. Thus, they found that mutton tallow (m.p., 47°C.) had a higher coefficient of digestibility than

[93] B. H. Schneider, *Feeds of the World, West Va. Agr. Expt. Sta.*, Morgantown, West Va. (1947); cited by R. P. Cook, "Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 14–26 (1952), p. 16.

[94] N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, *69*, 219–238 (1926).

[95] K. Güntherberg, *Wiss. Arch. Landwirtsch., Abt. B, Arch. Tierernähr. u. Tierzucht*, *3*, 339–367 (1930); *Chem. Abst.*, *25*, 5451 (1931).

[96] C. M. McCay, *Ann. Rev. Biochem.*, *6*, 445–468 (1937).

[97] A. D. Holmes and H. J. Deuel, Jr., *Am. J. Physiol.*, *54*, 479–488 (1921).

[98] H. J. Deuel, Jr., "Nutritional Value of Soybeans and Soybean Products," in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. II, Interscience, New York-London, 1951.

[99] R. Hoagland and G. G. Snider, *J. Nutrition*, *25*, 295–302 (1943).

cacao butter (m.p., 28°C.). Moreover, cacao butter, butterfat, and coconut oil, each melting below body temperature, had widely different coefficients of digestibility. These authors[99] suggest that some factor other than melting point, possibly the stearic acid content, determines

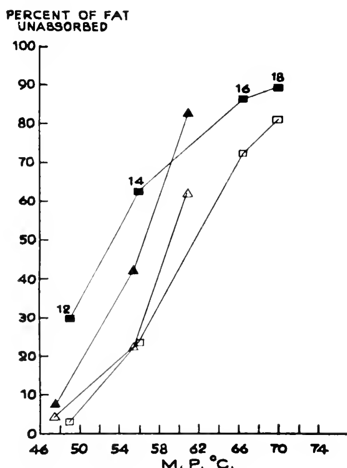


Fig. 1. The relationship of melting point to digestibility of simple triglycerides (■), and of samples of hydrogenated lard (▲) when calcium and magnesium were present in the diet. The result of tests with calcium-magnesium low diets is represented in each case by similar characters which are not filled in. The numbers beside each character indicate the number of carbon atoms in the fatty acid component of the simple triglycerides.[5]

digestibility. However, the experiments of Mattil and Higgins,[100] quoted below, do not indicate that the stearic acid content is the factor which governs the digestibility of fats.

(5) *The Effect of Structural Configuration*

Natural fats are known to exist largely in the form of mixed triglycerides, rather than as simple triglycerides. Mattil and Higgins[100] reported that, when identical amounts of oleic and stearic acid were fed to rats as mix-

[100] K. F. Mattil and J. W. Higgins, *J. Nutrition*, *29*, 255–260 (1945).

TABLE 15
COMPARISON OF EFFECT OF MELTING POINT ON DIGESTIBILITY OF SOME NATURAL AND HYDROGENATED FATS IN RATS, AND DISTRIBUTION OF EXCRETED FAT BETWEEN NEUTRAL FATS AND SOAPS[a]

Fat in diet	M.p., °C.	Number of expts.	Fat intake, g.	Neutral fat + fatty acid in stool, g.	Soap in stool, g.	Coeff. of digestibility
Fat-free diet[b]	—	10	0	0.18	0.088	—
Margarine fat[c]	34	20	10.7	0.24	0.30	97.0 ± 0.4
Crisco[c]	43	20	12.3	0.23	0.38	97.3 ± 0.3
Bland lard[c]	48	18	12.8	0.34	0.68	94.3 ± 1.8
Prime steam lard[c]	37	16	12.2	0.21	0.48	96.6 ± 1.4
Hydrogenated cottonseed oil[b]	46	10	14.9	0.73	2.09	83.4 ± 1.4
	54	9	12.3	0.62	3.80	68.7 ± 2.7
	63	10	15.7	6.31	6.93	24.0 ± 2.6
Hydrogenated lard[c]	55	18	13.5	0.58	4.90	63.2 ± 1.2
	61	20	13.2	2.21	8.89	21.0 ± 2.6

[a] Adapted from H. J. Deuel, Jr., in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. II, Interscience, New York-London, 1951.
[b] V. Augur, H. S. Rollman, and H. J. Deuel, Jr., *J. Nutrition*, *33*, 177–186 (1947).
[c] M. E. Crockett and H. J. Deuel, Jr., *J. Nutrition*, *33*, 187–194 (1947).

tures of simple triglycerides or as mixed triglycerides, the mixed triglycerides were better utilized than were the simple triglycerides. These data are summarized in Table 16.

In some cases, branched-chain acids have been found to be less efficiently utilized than straight-chain acids. Thus, Weitzel[101] investigated the behavior of α-substituted myristic acids when fed to dogs as the triglyc-

TABLE 16
DIGESTIBILITY OF STEARIC ACID FED AS A SIMPLE AND AS A MIXED TRIGLYCERIDE[a]

Fat in diet	Fat, g. In food	Fat, g. In feces	Coeff. of digestibility
Tristearin, 10%, triolein, 5%	51.0	29.5	42.2
	79.2	49.4	37.6
Distearomonoolein, 15%	94.2	39.7	57.9
	24.5	9.0	63.3
Tristearin, 5%, triolein, 10%	87.5	27.4	68.7
	35.9	11.3	68.5
Monostearodiolein, 15%	89.4	23.3	72.8
	14.6	3.7	73.3

[a] K. F. Mattil and J. W. Higgins, *J. Nutrition*, *29*, 255–260 (1945).

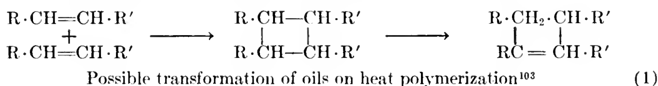
[101] G. Weitzel, *Z. physiol. Chem.*, *287*, 254–296 (1951).

erides or as ethyl esters. No increased output of fecal fat was observed after α-methylmyristic acid was fed. However, after the administration of α-ethylmyristic acid, there was a significant increase in fecal fat, while the administration of α-propylmyristic acid produced an added augmentation in fecal fat. The effect resulting from α-butyl acids was similar to that of the α-propyl acids.

(6) *The Effect of Polymerization*

It is known that, when fats are heated at a high temperature, polymerization occurs. The characteristic odor and taste of the unsaturated triglycerides may be removed by such treatment. Polymerization is undoubtedly associated with profound changes, not only in the physical properties but also in the chemical structure. On extensive heat treatment of oils, an increased viscosity develops; this change in physical property is related to a chemical alteration, as is demonstrated by the fact that the degree of unsaturation, determined from the iodine number, is markedly decreased.

According to Bradley,[102] the most profound change in unsaturated fats during heating centers around the double bonds. Brocklesby[103] has pictured the reaction as involving, first of all, the formation of an unstable 4-carbon ring which ruptures and rearranges as shown in (1).



Possible transformation of oils on heat polymerization[103] (1)

This type of reaction is believed to occur between two unsaturated acid residues which are present on the same glyceride, as well as on different molecules. Lassen *et al.*[104] are of the opinion that the latter hypothesis is the more probable one. The relation between coefficient of digestibility and percent of polymerization is shown in Figure 2.

Crampton, Farmer, and Berryhill[105] reported a marked decrease in the weight gains per 1000 Cal. of food ingested, in the case of rats fed oils which had been heated at 275°C. The decreased efficiencies were noted with heated corn, herring, linseed, peanut, rapeseed, and soybean oils.

[102] T. F. Bradley, *Ind. Eng. Chem.*, *29*, 440–445 (1937).

[103] H. N. Brocklesby, *The Chemistry and Technology of Marine Animal Oils, with Particular Reference to Those of Canada, Fisheries Research Bd., Canada, Bull. No. 59,* Sect. 5, *Chemical and Physical Properties of Fats and Oils,* 107–174 (1941).

[104] S. Lassen, E. K. Bacon, and H. J. Dunn, *Arch. Biochem.*, *23*, 1–7 (1949).

[105] E. W. Crampton, F. A. Farmer, and F. M. Berryhill, *J. Nutrition*, *43*, 431–440 (1951).

The most marked effect was noted for peanut oil; the gain in weight/1000 Cal. decreased from 87 for the unheated oil to 30 for the oil heated for thirty hours. Treatment of herring oil at 275°C. for only ten hours reduced this efficiency index from 64 to 28. When linseed oil previously heated for twelve hours was added to the diet at a 20% level, the index dropped from 80 to 4. On the basis of later work from this same laboratory,[106] it was concluded that the primary cause of the reduction in nutritive value of the diets which contained the thermally polymerized linseed

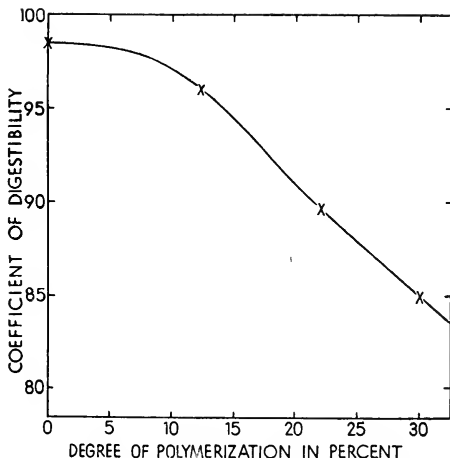


Fig. 2. The relationship between the degree of polymerization in % and the coefficient of digestibility of sardine oil.[104] The amount of polymerization was calculated by the following formula:

$$\frac{\text{Iodine No. unpolymerized oil} - \text{Iodine No. polymerized oil}}{\text{Iodine No. unpolymerized oil}} \times 100$$

oil was the presence of one or more dimeric fatty acid radicals; these are believed to act in some way inimical to the well-being of the animals. Crampton *et al.*[107] later suggested that the destruction of some of the essential fatty acids, which occurs during polymerization, may act to aggra-

[106] E. W. Crampton, R. H. Common, F. A. Farmer, F. M. Berryhill, and I. Wiseblatt *J. Nutrition*, *44*, 177–189 (1951).

[107] E. W. Crampton, R. H. Common, F. A. Farmer, A. F. Wells, and D. Crawford, *J. Nutrition*, *49*, 333–346 (1953).

vate any deleterious effects. Evidence that the effect of polymerization on digestibility is a general one which applies equally to animal and to vegetable fats has recently been advanced by Roy.[108] The progressive nature of the effect of heating is manifest from the data cited in Table 17.

TABLE 17
EFFECT OF THERMAL TREATMENT ON PHYSICAL CONSTANTS AND COEFFICIENTS OF DIGESTIBILITY OF GHEES, LARD, AND HYDROGENATED PEANUT OIL[a]

Substance	Temperature (°C.) to which fat was subjected				
	Control	200	250	275	300
Cow ghee					
Sapon. value	220	220	218	220	216
Iodine value	35	35	33	31.5	28.6
Reichert value	20	20	18.6	17.3	16.4
Coeff. of digestibility	98	97.6	97	93	89
Buffalo ghee					
Sapon. value	227	226	225.8	226	222
Iodine value	29.5	26.8	26.6	24.2	21.5
Reichert value	26	26.2	24.6	22.7	21.6
Coeff. of digestibility	95	95	94.6	90.2	87.6
Lard					
Sapon. value	196	195.3	192	194	192.6
Iodine value	60	61.5	60.8	59.6	56.8
Coeff. of digestibility	97	97	96.4	92	90.4
Hydrogenated peanut oil					
Sapon. value	191.5	190	190.6	189.6	187.5
Iodine value	66.8	66.8	66.0	65.3	62.6
Coeff. of digestibility	97	97	95.2	93.0	88.2

[a] Adapted from A. Roy, *Ann. Biochem. Exptl. Med. (India)*, *4*, 71–72 (1944).

On the other hand, one must realize that a higher level of heat than that to which food fats ordinarily are subjected must be applied to bring about polymerization. No decreased growth rate of rats or decreased efficiency in utilization of the diet could be demonstrated when diets containing margarine fat at a 40% level were given, irrespective of whether unheated fat was used or fat heated at 205°C. for eight hours (during which successive batches of potato chips were fried).[109] Moreover, potato chips fried in this fat possessed no inhibitory effects on rat growth.

[108] A. Roy, *Ann. Biochem. Exptl. Med.*, *4*, 71–72 (1944).
[109] H. J. Deuel, Jr., S. M. Greenberg, C. E. Calbert, R. Baker, and H. R. Fisher, *Food Research*, *16*, 258–280 (1951).

(7) *The Effect of Emulsifying Agents*

The extent of digestibility of fats has been shown to be improved when emulsifying agents are present. Although it is impossible to improve the digestibility of highly digested fats by the use of emulsifying agents, Augur and co-workers[39] were able to show a marked elevation in the coefficients of digestibility of hydrogenated cottonseed oils when lecithin was added to the diets. The coefficients of digestibility without and with lecithin were as follows: sample melting at 46°C., 83.8 and 87.9; sample melting at 54°C., 68.7 and 82.8; and the sample melting at 65°C., 24.0 and 44.2.

"Tween 80" (PSM or polyoxyethylene-sorbitan monooleate) is an especially effective emulsifying agent. When this was added to the diet of patients having a poor fat digestibility, Jones *et al.*[110] were able to demonstrate a marked increase in the coefficients of digestibility of fat. Tween 20 (polyoxyethylene sorbitan monolaurate) was found by Johnson *et al.*[111] also to increase the fat retention to some extent in the case of premature infants. Thus, in over 50% of the cases tested, the administration of this drug slightly increased the coefficient of digestibility of fat. The effect of lecithin and PSM on the rate of fat absorption has been described earlier (page 185).

(8) *The Effect of Foodstuffs Concomitantly Present with Ingested Fat*

There are a number of ways in which the digestibility of fat may be influenced by other materials present in the stomach and intestines. The most important substances, which may produce a decrease in fat absorption, are the salts which form insoluble soaps with the fatty acids, and hence may prevent the absorption of a large proportion of these acids. The length of stay in the intestine may be so reduced, when intestinal irritants are present, as to prevent the complete utilization of the fat. The accompanying foodstuff may change the conditions in the intestine by altering the *p*H, increasing the flow of bile, pancreatic juice, or intestinal juice, or by a variety of other changes.

a. The Effect of Calcium and Magnesium Salts. Although Cheng and her associates[5] reported that no appreciable effect was exerted on the high digestibility of bland lard when calcium salts were present in the diet, the

[110] C. M. Jones, P. J. Culver, G. D. Drummey, and A. E. Ryan, *Ann. Internal Med.*, *29*, 1–10 (1948).

[111] A. L. Johnson, R. B. Scott, and L. H. Newman, *Am. J. Diseases Children*, *80*, 545–550 (1950).

TABLE 18

EFFECT OF INCLUSION OF CALCIUM AND MAGNESIUM SALTS ON DIGESTIBILITY OF FATS[a]

Fat fed	M.p., °C.	Diets free from Ca and Mg					Diets containing Ca and Mg				
		No. of tests	Fat fed, g.	Neutral fat + fatty acid in stool, g.	Soap in stool, g.	Coeff. of digestibility	No. of tests	Fat fed, g.	Neutral fat + fatty acid in stool, g.	Soap in stool, g.	Coeff. of digestibility
Bland lard	47.8	5	12.2	0.39	0.54	95.8	5	13.3	0.25	1.16	92.4
Blended lard[b]	47.8	5	12.5	0.38	0.57	95.6	5	14.1	0.24	1.43	90.7
Blended lard[c]	55.2	5	9.1	1.33	1.16	80.0	5	10.7	1.06	2.97	66.2
Hydrogenated lard[d]	55.4	5	9.2	1.17	1.40	77.9	5	11.8	0.51	4.66	58.0
Hydrogenated lard[d]	61.0	5	10.6	5.98	1.99	38.0	5	11.0	3.78	6.03	17.3
Trilaurin	49	5	8.8	0.16	0.29	97.3	6	8.9	0.25	2.72	70.5
Trimyristin	56	5	9.7	1.49	1.33	76.6	5	12.8	1.12	7.46	37.7
Tripalmitin	66.5	5	9.8	7.51	1.27	27.9	5	9.1	6.40	2.27	12.8
Tristearin	70	5	9.0	7.97	0.88	18.9	5	9.5	7.50	1.90	10.8
Palmitic acid	63	5	9.1	4.98	2.29	35.6	5	9.7	3.89	4.60	19.8
Stearic acid	71.8	5	9.6	6.92	2.78	15.8	5	9.8	3.99	5.53	14.4
Monostearin	59.9	5	9.5	4.00	1.84	47.4	5	10.1	3.22	5.55	20.7

[a] A. L. S. Cheng, M. G. Morehouse, and H. J. Deuel, Jr., *J. Nutrition*, *37*, 237–250 (1949).

[b] 1 part hydrogenated to 9 parts prime steam lard. H. J. Deuel, Jr., in K. S. Markley, ed., *Soybeans and Soybean Products*, Vol. II, Interscience, New York-London, 1951.

[c] 1 part hydrogenated lard to 2 parts prime steam lard.

[d] Whole sample of lard partially hydrogenated.

TABLE 19

COMPARATIVE DIGESTIBILITY OF SEVERAL FATS IN RATS RECEIVING LOW- OR HIGH-PROTEIN DIETS

		Low-protein diet						High-protein diet					
Fat fed	Sex	No. of rats	Fat intake, g.	Wt. of feces, g.	Fecal fat, %	Fat absorbed, g.	Digestibility, %	No. of rats	Fat intake, g.	Wt. of feces, g.	Fecal fat, %	Fat absorbed, g.	Digestibility, %
Steam lard[a]	M	6	2.09	0.75	6.52	2.04	97.6	6	2.39	0.90	4.06	2.35	98.4
	M	6	1.59	0.72	16.74	1.47	91.9	7	2.07	0.95	10.20	1.98	95.4
	F	7	1.77	0.56	5.28	1.73	98.0	5	1.86	0.73	1.51	1.85	99.5
	F	6	1.82	0.57	19.11	1.71	94.0	6	1.36	0.64	7.88	1.31	96.7
Standard butter[a]	M	4	1.80	0.86	20.26	1.64	89.3	7	1.89	0.80	9.41	1.81	95.9
	F	6	1.63	0.66	18.86	1.51	92.1	5	1.61	0.58	6.09	1.57	97.7
Special butter spread[a]	M	4	2.38	1.02	17.61	2.20	92.4	7	2.33	0.92	10.41	2.24	96.2
	F	6	2.00	0.74	19.47	1.84	92.4	5	1.86	0.72	10.67	1.78	96.0
Peanut oils[b]													
Crude	M	9	2.68	0.64	25.48	2.55	95.5	9	1.96	0.61	11.89	1.92	97.6
Refined	M	8	2.64	0.61	25.98	2.51	95.5	8	2.38	0.68	14.35	2.31	97.3
Hydrogenated	M	8	2.80	0.92	47.10	2.42	86.4	7	2.19	0.80	36.70	1.93	89.4
Blended hydrogenated	M	8	2.70	0.74	38.40	2.46	90.8	7	2.34	0.81	29.10	2.14	91.7

[a] R. H. Barnes, M. F. Primrose, and G. O. Burr, *J. Nutrition*, *27*, 179–184 (1944). Digestibility is not corrected for metabolic fat.

[b] Data from E. J. Severance, *The Digestibility of Some Peanut Oils in the Rat*, Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., Jan., 1952.

extent of digestibility of most fats was markedly reduced when these salts were included in the ration. The data from these tests are summarized in Table 18 (p. 236).

The digestibility of a hydrogenated lard melting at 55°C. was reduced from 78 to 58% in the presence of calcium. Trimyristin was found to be digested to the extent of 77% in the absence of calcium and of only 36% when calcium salts were present. It was also reported that the depressing effect of calcium salts was in proportion to the quantity present. Thus, trilaurin had the following coefficients of digestibility, respectively, in diets containing 6.1, 2.5, 1.17, and 0 mg. of calcium per gram of food: 70.5, 87.2, 89.5, and 97.3.

Givens[112] was among the first to report that the excretion of fecal calcium was increased when the absorption of fat was poor. In fact, this worker reported that calcium storage was decreased under such conditions, even when the calcium intake was at a high level. The extent of soap formation was believed by Bosworth and co-workers[113] to be a function of the ionized calcium present, although the amount excreted in the feces depended upon the proportion of the soaps which were soluble. Since calcium oleate is more readily soluble in bile than calcium palmitate or calcium stearate, it will disappear in larger quantities than the two latter salts, even in the presence of an excess of calcium. Similar data are recorded by Boyd and collaborators.[114] They found that, when rats had a calcium intake of 37 to 56 mg./day, the utilization of oleate, palmitate, and stearate soaps was 90, 38, and 25%, respectively. On the other hand, when the intake of calcium was lower (20 to 32.5 mg./day), the digestibilities of palmitate and stearate were increased to 65 and 45%, respectively, while the figure for calcium oleate was 91%. Confirmation of the depressing effect of calcium on the utilization of fats containing saturated fatty acids was given by Rao and De.[114a] When coconut oil was fed to rats, no appreciable change in the digestibility coefficient of 98.4 was noted, irrespective of whether or not calcium salts were present in the diet. Furthermore, the coefficient of digestibility in the unsaturated fraction of coconut oil was not altered when calcium was present. However, it was shown that the coefficient of digestibility of the saturated fraction (m.p. 52°C.) was decreased from 89.1 to 80.0 when the diets contained calcium.

[112] M. H. Givens, *J. Biol. Chem.*, *31*, 441–444 (1917).
[113] A. W. Bosworth, H. I. Bowditch, and L. A. Giblin, *Am. J. Diseases Children*, *15*, 397–407 (1918).
[114] O. F. Boyd, C. L. Crum, and J. F. Lyman, *J. Biol. Chem.*, *95*, 29–41 (1932).
[114a] M. N. Rao and S. S. De, *Indian J. Med. Research*, *39*, 457–464 (1951).

Conversely, the amount and nature of the dietary fat is of considerable importance in relation to calcium retention. It is obvious that the calcium loss will be minimal when the fatty acids in the dietary fat consist of oleic acid and of other low-melting acids. However, when the melting point of the ingested fat exceeds 50°C., marked loss of calcium in the feces may be expected, and the loss will be proportional to the amount of fat fed.

b. The Effect of Protein. A second dietary constituent, which may modify the coefficient of digestibility of fat, is protein. It was first pointed out by Barnes, Primrose, and Burr[115] that fats are digested to a lesser extent on a low-protein diet (14%) than on a dietary regimen having a higher protein content (30%). These results have been confirmed by Savage[116] and by Severance.[41] Munk[117,118] and Rosenheim[119,120] reported that, when dogs were fed a low-protein diet for six to eight weeks, a serious derangement in fat absorption occurred, which was apparently associated with severe lesions in the gastrointestinal tract. The findings of Barnes *et al.*[115] and of Severance[41] are shown in Table 19, p. 237.

On the other hand, Jägerroos[121] and Chittenden[122] were unable to find any indication of a decreased capacity to digest fat in the case of dogs or of human subjects maintained on a low-protein diet. Coffey *et al.*[123] likewise reported that the protein level has little effect on fat digestibility.

c. The Effect of Crude Fiber. Walker[124] has observed that, on diets with the same fat content, the amount of fecal fat was detectably increased when the crude fiber in the diet was augmented. It is suggested that this fecal fat is not of dietary origin, but that it may be derived from intestinal secretions, from fatty acids produced by bacterial action on cellulose, or by bacterial synthesis. Walker believes that the wide differences in the excretion of fecal fat by different human subjects on a standard diet may be due to the variations in the non-dietary fat excreted as a result of the ingestion

[115] R. H. Barnes, M. F. Primrose, and G. O. Burr, *J. Nutrition, 27*, 179–184 (1944).

[116] E. S. Savage, *A Comparative Study of the Utilization of Jojoba and Cottonseed Oil in the Rat*, Thesis, Univ. So. California, Los Angeles, Dept. Biochem. Nutrit., 1951.

[117] I. Munk, *Arch. Path. Anat. Physiol. (Virchow's), 132*, 91–157 (1893).

[118] I. Munk, *Arch. Anat. u. Physiol., Physiol. Abt. (Du Bois-Reymond), 1891*, 338–341.

[119] T. Rosenheim, *Arch. ges. Physiol. (Pflüger's), 46*, 422–432 (1890); *54*, 61–71 (1893).

[120] T. Rosenheim, *Arch. Anat. u. Physiol., Physiol. Abt. (Du Bois-Reymond), 1891*, 341–344.

[121] B. H. Jägerroos, *Skand. Arch. Physiol., 13*, 375–418 (1902).

[122] R. H. Chittenden, *Physiological Economy in Nutrition*, Stokes, New York, 1905, pp. 9, 10.

[123] R. J. Coffey, F. C. Mann, and J. L. Bollman, *Am. J. Digestive Diseases, 7*, 141–143 (1943).

[124] A. R. P. Walker, *Nature, 164*, 825–827 (1949).

of different quantities of crude fiber, rather than to divergences in the capacity to absorb food fat.

(9) Miscellaneous Factors

Frazer[125] is of the opinion that the vitamins are not linked to the absorption of fat, although it had previously been suggested that fat absorption was influenced by deficiencies in vitamins A or B. Although, in the sprue syndrome, a deficiency in fat absorption is sometimes associated with deficiencies in vitamins A, C, D, or E, these deficiencies are slight and are probably due to difficulty in absorption. Butson[126] reported that a 4000 Cal. high-fat diet containing 40.5% of fat is well tolerated at 0°F. but not at 32°F. Just why environmental temperature influences fat digestibility is not understood.

5. Pathologic Factors Altering the Digestibility of Fats

Several abnormal conditions are recognized in man in which the digestibility of fats is markedly lowered. Whenever a deficiency of pancreatic lipase occurs in the intestine, due either to a failure of secretion of pancreatic juice or to an obstruction of the pancreatic duct, the proportion of fecal fat is considerably increased. This condition in which fatty stools occur is usually referred to as *steatorrhea*. The defective fat absorption in the case of pancreatic insufficiency is believed to result indirectly from the failure of lipolysis. On the other hand, in another disease known as sprue, the lipolysis of fat proceeds normally, but the failure in absorption represents an interference in the pathway of absorption through the intestinal wall. Although the etiology of some types of steatorrhea is known, the cause of sprue and of congenital steatorrhea remains somewhat obscure. In 1921, Schmidt and von Noorden[127] produced an excellent monograph dealing with intestinal absorption in disease, while French,[128] in 1952, surveyed the status of our knowledge concerning defective fat absorption.

(1) Methods for the Recognition of Defective Fat Absorption

a. By Chemical Estimation of Fecal Fat. According to French,[128] it has been the general practice, during the past fifty years, to base the diagnosis

[125] A. C. Frazer, *Bull. soc. chim. biol., 33,* 968–972 (1951).
[126] A. R. C. Butson, *Lancet, 258,* 993–994 (1950).
[127] A. Schmidt and C. von Noorden, *Klinik der Darmkrankheiten,* Vol. 5, 2nd ed., Bergmann, Munich and Wiesbaden, 1921.
[128] J. M. French, "Defective Fat Absorption in Man," in R. T. Williams, *Lipid Metabolism,* Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 30–36 (1952).

of defective fat absorption upon a determination of the level of fecal fat or upon histological examination of a single sample of feces. When fat is determined by one of the chemical procedures, it is expressed as the percent fat on the wet or dry basis. This index has been shown to give entirely unreliable results when compared with a more satisfactory procedure such as the fat balance.

A straight line relationship has been shown to be absent. French[128] showed that the scatter is so wide that the percentage of fecal fat is not only unreliable as an index of defective fat absorption but may lead to totally erroneous conclusions in the detection of both normal and abnormal fat absorption.

b. By the Use of the Fat Balance. Van de Kamer, Huinink, and Weyers[44] described a method of determining the state of fat absorption by the use of fat balances which has proved exceedingly useful and reliable in the diagnosis of impaired fat utilization. In this technic, a known amount of fat is introduced into the diet daily, as determined by calculation from diet tables; 50 g. is usually employed.[128] The daily output of fat is estimated by a method of total saponification on an aliquot from the entire twenty-four hour collection of feces. After saponification of the aliquot with alcoholic potassium hydroxide, the sample is acidified with hydrochloric acid and the freed fatty acids are extracted with petroleum ether. This extract is titrated with standard base and the fatty acid content expressed as stearic acid.[128] French[128] considers that an absorption of 90% or above is normal, one of 85 to 90% is borderline, while a value below 85% is to be considered definitely abnormal.

The use of the fat balance procedure gives certain advantages. In the first place, a continuous fat balance is of far greater use than a single determination. Thus, in the case of celiac disease, only variations in the trends upward or downward over a ten-day interval can be considered significant. The simple estimation of fatty acids may be carried out in thirty to forty minutes after hydrolysis by this procedure.[128] The results based upon the intake and excretion of fat over a period of twenty-four hours are likewise comparable with values in the literature.

c. By the Use of Labeled Fats. Another procedure which has been proposed for assessing the capacity for absorption of fats involves the use of fats containing Sudan III or having fatty acids in the glycerides tagged with elaidic acid, conjugated dienoic acids, or labeled with C^{14}, I^{127}, or I^{131}. However, there are certain objections to the use of this method. Since, in the case of celiac disease, there is a preferential rejection of saturated fatty acids concomitantly with a practically complete digestion of the un-

saturated acids, any method in which the label is not uniformly distributed will give erroneous results. Weijers and van de Kamer[129] have shown that unsaturated acids may be almost completely absorbed (90%) when the overall absorption of dietary fat is only 70%. Since there may not be any diminution of the hydrolytic activity of pancreatic lipase, and a delay in absorption of particulate fat occurs in celiac disease, the excessive lipolysis may result in the separation of saturated fatty acids which cannot be absorbed. These factors render the use of absorption studies on labeled fats of limited value in assessing abnormal fat absorption.

(2) *General Changes Occurring in Defective Fat Absorption*

The abnormalities in fat absorption can be determined both from the intraluminar phase and from tests of the absorptive capacity of the upper intestine. To ascertain the condition of the fat in the intestine, samples of intestinal contents obtained by the aid of a Miller-Abbott tube are examined microscopically to determine the state of emulsification after the administration of olive oil.[128] The absorptive capacity of the upper intestine may be assessed by intraduodenal administration of glucose and urea, and subsequent determination of the rise in concentration of these substances in the systemic blood.

Another factor of importance in sprue is the presence of excessive mucus in the small intestine, which interferes with absorption by mechanical action. Although the amount of mucus cannot be determined directly, it can be estimated radiographically by following the extent of flocculation which occurs after the administration of a simple suspension of barium sulfate.[128]

According to Frazer, French, and Thompson,[130] there is a rough quantitative relationship between the amount of mucus and the size of the floccule formed.

Another index for assessing whether or not abnormal fat absorption obtains is defined as the distributive phase. To determine this response, serial counts of chylomicrons of the blood are made by a standardized technic,[131] after a test dose of 24 g. of butterfat.

[129] H. A. Weijers and J. H. van de Kamer, *Fat Absorption in Normal and Abnormal Infants and Children, with Special Reference to Coeliac Disease,* Public. 113, Centraal Inst. voor Voedingsonderzoek, T. N. O., Dekker & Van de Vegt, Utrecht, 1950, pp. 9–82.

[130] A. C. Frazer, J. M. French, and M. D. Thompson, *Brit. J. Radiol., 22,* 123–136 (1949).

[131] A. C. Frazer and H. C. Stewart, *J. Physiol., 95,* 21P–23P, 23P–24P (1939).

Table 20 summarizes the variations in these general changes in fat absorption which have been found in pancreatic insufficiency and sprue.

TABLE 20

THE EFFECT OF PANCREATIC INSUFFICIENCY AND SPRUE ON THE PHYSIOLOGICAL INDICES OF FAT ABSORPTION[a]

Category	Pancreatic syndrome	Sprue syndrome
Apparent fat absorption	48% (15–68), 5 cases	73% (54–86), 24 cases
Pancreatic enzymes (amylase, lipase, trypsin)	All deficient	Normal
Emulsification	Faulty: particles > 10 μ	Normal: particles < 0.5 μ
Radiography (small intestine)	Feathery mucosal pattern (No excess mucus)	Flocculation pattern (Excess mucus)
Glucose and urea absorption (intraduodenal)	Normal absorption	Depressed and delayed
Chylomicrographs in standard field after use of:		
(a) Unemulsified oil	Flat: maximum <10 particles	Flattened: maximum < 50 particles
(b) Pre-emulsified oil	Normal: > 120 particles	—

[a] Adapted from J. M. French, *Defective Fat Absorption in Man*, in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia No. 9, Cambridge Univ. Press, 30–36 (1952), p. 34.

(3) *Steatorrhea Related to Deficiency of Pancreatic Juice*

In cases in which the pancreatic duct is occluded, the steapsin cannot be excreted into the intestine; this results in an almost complete cessation of lipolysis. When the acinar tissue of the pancreas is incapacitated, with a resultant decrease in the production of pancreatic juice, the decrease in fat digestibility is proportional to the extent to which the organ is affected. Chronic pancreatic disease is characterized by the production of large, soft, oleaginous stools which may amount to as much as 1000 g. daily. They are pale in color, due to the presence of excess fat. A considerable proportion of undigested protein is also present; this condition is known as a *creatorrhea*.

Crohn[132] reported that the coefficients of digestibility of fat may vary between 15 and 50 in cases of chronic pancreatitis with occluded ducts or in patients having malignant tumors of the pancreas. Spriggs and Leigh[133] cited a case in which 50 to 99% of the ingested fat was lost, and

[132] B. B. Crohn, "Disturbances of Metabolism Accompanying Pancreatic Disease," in L. F. Barker, *Endocrinology and Metabolism*, Vol. IV, Appleton, New York-London, 1922, pp. 657–691.

[133] E. I. Spriggs and A. J. Leigh, *Quart. J. Med.*, *9*, 11–43 (1915–1916).

in which 1000 to 2650 g. of oily feces were excreted per day. A high fat excretion has likewise been reported in acute pancreatitis. The fat may amount to over 50% of the weight of the dried feces; neutral fat makes up 40 to 70% of the total fat.

Walker[134] reported the case of a man who was subjectively in good health, in spite of the fact that he presented a persistent steatorrhea for twenty-seven years before his death at the age of ninety. Throughout this entire period he passed "butter stools." Autopsy revealed that the pancreatic duct had been blocked by calculi, and that the pancreas had been largely replaced by fat.

Cameron and Gilmour,[135] who accept the Lipolytic Theory for fat absorption, attribute the failure in fat utilization in pancreatic insufficiency entirely to the absence of lipolysis. On the other hand, if one accepts the Partition Theory, the lack of absorption can also be explained as due to the failure of partial lipolysis. Under such conditions, none of the di- or monoglycerides are formed which are essential in the formation of the fine emulsion required for absorption.

Further evidence in support of the Partition Hypothesis is afforded by the radiographic findings. A feathery pattern is noted in the radiogram formed with barium sulfate in the case of pancreatic fibrosis. However, Lowe *et al.*[136] showed that a flocculation pattern develops if pancreatin is added which is similar to that observed in normal patients.[137] Frazer *et al.*[130] reported that a flocculation pattern occurs after the administration of fatty acids.

(4) Steatorrhea Related to Deficiency of Bile

In normal feces, the fat content makes up a maximum of only 25 to 30% of the dried weight of the feces, and the neutral fat constitutes only about one-fourth of this total. When a bile deficiency occurs, the total fat may comprise as much as 70% of the total dried feces, but it consists almost entirely of fatty acids and soaps. Such a deficient fat utilization results not from a failure in lipolysis, although this is slowed up, but

[134] T. J. Walker, *Med.-Chir. Trans., Roy. Med. Chir. Soc., London*, *72*, 257–273 (1889); cited by A. E. Garrod, *Inborn Errors of Metabolism*, 2nd ed., Chap. IX, Oxford Univ. Press, 1923, p. 170.

[135] A. T. Cameron and C. R. Gilmour, *The Biochemistry of Medicine*, Wood, Baltimore, 1933.

[136] C. U. Lowe, C. D. May, H. M. Stauffer, and E. D. Neuhauser, *Am. J. Diseases Children*, *79*, 91–98 (1950).

[137] E. P. Pendergrass, I. S. Ravdin, C. G. Johnston, and P. J. Hodes, *Radiology*, *26*, 651–662 (1936).

rather from a failure to absorb the fatty acids and soaps in the absence of the solvent action of the bile salts. However, Heerschma and Annegers[138] were unable to improve the steatorrhea in bile fistula dogs by 3 g. doses of certain bile preparations, including desoxycholic acid. While 90 ml. doses of fresh ox bile reduced the steatorrhea by 50%, 6 g. doses of desiccated ox bile were only one-half as effective. In the case of obstructive jaundice, the feces are bulky and pale in color, due both to the absence of the bile pigments and to the increased proportion of fat excreted.

(5) *Non-tropical Sprue*

This disease also goes by the name of idiopathic steatorrhea. The stools are frequent and voluminous, containing a large proportion of fats, fatty acids, and soaps. This condition may lead to tetany, due to the continual loss of calcium by way of the intestine. Frazer[139] is of the opinion that this condition is due to a defect in particulate absorption of fat which may possibly be related to an enzyme deficiency. However, French[140] ascribed the reduced fat absorption in sprue to a depressed intestinal absorption. In the case of pancreatic enzyme deficiency, the impairment of fat absorption is believed to result from failure of intraluminar emulsification in the absence of lipolysis. On the other hand, in sprue, the intraluminar changes were found to be normal. The delayed glyceride absorption is believed to cause an increased fatty acid formation, which results in augmented mucus formation. It is suggested that this increased mucus secretion, together with a decrease in intestinal motility and changes in the intestinal flora, may all play a role in the sprue syndrome. In addition to non-tropical sprue, the sprue syndrome includes tropical sprue,[139,141] and celiac disease in children.[142] Annegers[143] originally pointed out that this defect in fat absorption in these conditions resembles the syndrome observed in patients and experimental animals deprived of bile. However, he[144] later expressed doubt of this viewpoint, on the basis of the fact that Tween 80 failed to reduce the fecal fat excretion in bile fistula dogs. On the other hand, much smaller doses of Tween 80 had been found to be effective in celiac disease.[145]

[138] J. R. Heerschma and J. H. Annegers, *Proc. Soc. Exptl. Biol. Med.*, *67*, 339–341 (1948).
[139] A. C. Frazer, *Brit. Med. J.*, *2*, 769–773 (1949).
[140] J. M. French, *Biochem. J.*, *51*, xiv (1952).
[141] N. H. Fairley, *Trans. Roy. Soc. Trop. Med. Hyg.*, *30*, 9–32 (1936).
[142] L. G. Parsons, *Am. J. Diseases Children*, *43*, 1293–1346 (1932).
[143] J. H. Annegers, *Quart. Bull. Northwestern Univ. Med. School*, *23*, 198–206 (1949).
[144] J. H. Annegers, *Proc. Soc. Exptl. Biol. Med.*, *81*, 277–278 (1952).
[145] H. Boyd and F. Helfrick, *J. Pediat.*, *38*, 493–497 (1951).

(6) *Congenital Steatorrhea*

This condition is believed to be caused by the absence of pancreatic lipase from the pancreatic juice; according to Garrod,[146] trypsin is present. Congenital steatorrhea is an exceedingly rare condition, in which "butter stools" are passed from birth. There are no other symptoms of pancreatic deficiency. Miller[147] suggested that this abnormality may occur in early infancy. Barrit[148] has cited an interesting case of secondary steatorrhea, following a gastroileal anastomosis, due to the exclusion of the small intestine resulting from a faulty gastroenterostomy.

[146] A. E. Garrod, *Inborn Errors of Metabolism*, 2nd ed., Chap. IX, Oxford Univ. Press, 1923, pp. 166, 167.
[147] R. Miller, *Proc. Roy. Soc. Med., Part I, Sect. Study Disease in Children, 16,* **22–24 (1923).**
[148] D. W. Barrit, *Lancet, 263,* 564–565 (1952).

CHAPTER IV

THE DIGESTION, ABSORPTION, AND DIGESTIBILITY OF LIPIDS OTHER THAN FATS

1. Introduction

Although, from a quantitative standpoint, the neutral fats occupy by far the most important position of any of the lipids, as regards digestion and absorption, the utilization of the other lipids poses individual problems in each instance. In some cases, specific enzymes are involved in the preparation of the other lipids for absorption; these usually differ from the lipases required in the preliminary stages of the absorption of the triglycerides. Such lipids as the hydrocarbons possess no reactive group through which combination may take place. In fact, there is no site in the molecule at which rupture can occur to effect a change akin to hydrolysis. All lipids are insoluble in water and are fat-soluble; they therefore encounter the same difficulties, and are subject to the corresponding limitations which have been noted for triglyceride fats.

The absorption of lipids other than fat may involve conditions entirely different from those operative when neutral fat is absorbed. However, most lipids are absorbed more readily when they are administered in a fat solution. In the case of lecithin, it is extremely difficult to distinguish its absorption from that of neutral fat when the two substances are being absorbed simultaneously, since the phospholipids have been proved to be synthesized in the intestinal mucosa during the absorption of fat, even in the complete absence of phospholipid from the food.

2. The Digestion and Absorption of Phospholipids

Most of the information on the digestion and absorption of phospholipids concerns lecithin. From a quantitative standpoint, lecithin is the most widely distributed of any of the group of phospholipids in animal and plant sources.

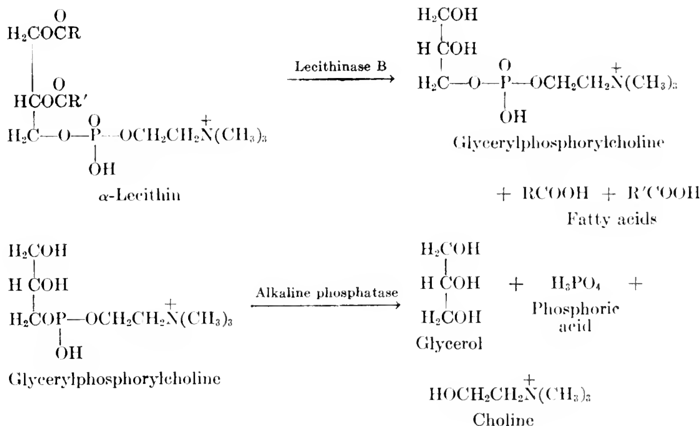
It has been generally assumed, until recently, that lecithin and other phospholipids are hydrolyzed in the gastrointestinal tract, and are absorbed in the form of the hydrolytic products. It is believed that these fragments are reconstituted into the phospholipid molecule in the intestinal mucosa.

The mechanism of the hydrolysis of lecithins in the small intestine is not certain. As early as 1877, Bókay[1] proposed that lecithin was broken down to fatty acids, choline (referred to as "neurine"), and glycerophosphate; the latter ester was believed to be split into glycerol and phosphoric acid in the intestine, by phosphatase.[2] Abderhalden and Paffrath demonstrated that choline originated when lecithin was allowed to remain in intestinal segments of the rabbit *in vitro*,[3] as well as when lecithin solutions were incubated with intestinal juice.[4] It was proved by Kahane and Lévy[5] that lecithinase B is present in rat intestine. This enzyme splits the two fatty acid residues from the lecithin molecule, leaving glycerylphosphorylcholine. Schmidt *et al.*[6] have likewise shown that lecithin disappears during the autolysis of minced rat intestine, concomitantly with the appearance of glycerylphosphorylcholine. In earlier work, Schmidt, Hershman, and Thannhauser[7] had reported that duodenal juice was without effect on lecithin or cephalin, although intestinal mucosa was capable of hydrolyzing these phospholipids. It has likewise been reported that alkaline phosphatase has the ability to convert glycerylphosphorylcholine into glycerol, phosphoric acid, and choline.[7] Lecithinase B has been found in beef pancreas.[6,7] A review of the earlier literature is given by Belfanti, Contardi, and Ercoli.[8] The mechanism for the hydrolysis of the lecithin molecule is pictured on the following page.

According to Le Breton and Pantaléon,[9] an enzyme in the pancreatic juice of dogs which is probably distinct from steapsin or other pancreatic lipase liberates fatty acids from lecithin and from cephalin.

There are several reports in the literature which demonstrate a more or less significant increase in lecithin in the lymph, following administration

[1] A. Bókay, *Z. physiol. Chem.*, *1*, 157–164 (1877).
[2] P. Grosser and J. Husler, *Biochem. Z.*, *39*, 1–5 (1912).
[3] E. Abderhalden and H. Paffrath, *Fermentforschung*, *8*, 284–293 (1926).
[4] E. Abderhalden and H. Paffrath, *Fermentforschung*, *8*, 294–298 (1926).
[5] E. Kahane and J. Lévy, *Compt. rend.*, *219*, 431–433 (1944).
[6] G. Schmidt, L. Hecht, and S. J. Thannhauser; cited by S. J. Thannhauser and G. Schmidt, *Physiol. Revs.*, *26*, 274–317 (1946), p. 310.
[7] G. Schmidt, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, *161*, 523–536 (1945).
[8] S. Belfanti, A. Contardi, and A. Ercoli, *Ergeb. Enzymforsch.*, *5*, 213–232 (1936).
[9] E. Le Breton and J. Pantaléon, *Arch. sci. physiol.*, *1*, 63–80 (1947).



The Partial and Complete Hydrolysis of Lecithin

of this phospholipid (Slowtzoff[10] and Eckstein[11]) as well as in the blood (Eichholtz[12]). Leites[13] was unable to demonstrate any increase in the portal venous blood after the feeding of lecithin, and therefore he concluded that it was transported by way of the lymph. However, somewhat the same picture has been reported by Süllmann and Wilbrandt[14] as occurring after the feeding of neutral fat and lecithin. This finding renders the interpretation of the results somewhat uncertain. On the other hand, Rewald,[15] Serejski,[16] and Heinlein[17] all reported an increased phospholipid content in the tissues, when the feeding of phospholipids was continued over a long period. Heinlein[17] further proved that this increase in tissue phospholipids does not occur when the bile is excluded from the intestine, a condition which is known to result in the impairment of phospholipid absorption. After consideration of all of the above findings, one may conclude that it is still an open question whether or not lecithin can be absorbed as such. If emulsification is a prime requisite

[10] B. Slowtzoff, *Beitr. chem. Physiol. Pathol.*, *7*, 508–513 (1906).
[11] H. C. Eckstein, *J. Biol. Chem.*, *62*, 743–757 (1925).
[12] F. Eichholtz, *Biochem. Z.*, *144*, 66–69 (1924).
[13] S. Leites, *Biochem. Z.*, *184*, 310–317 (1927).
[14] H. Süllmann and W. Wilbrandt, *Biochem. Z.*, *270*, 52–62 (1934).
[15] B. Rewald, *Biochem. Z.*, *198*, 103–110 (1928).
[16] M. Serejski, *Biochem. Z.*, *201*, 292–297 (1928).
[17] H. Heinlein, *Z. ges. exptl. Med.*, *91*, 638–682 (1933).

for the particulate absorption of fat (as postulated in the Partition Theory), then lecithin should be readily absorbed in the same manner, because of the extreme ease with which it is emulsified in water.

Artom and Swanson[18] demonstrated in a convincing manner that part of the phospholipid is absorbed without hydrolysis. This conclusion was based upon the demonstration that the content of phospholipid, labeled with P^{32}, was considerably greater in the blood and liver after the feeding of phospholipid so labeled than resulted in control rats receiving non-labeled phospholipid and P^{32} as sodium phosphate or glycerophosphate in an equivalent amount. This suggestion is of interest in relation to the early statements of Mayer.[19] While he believed that naturally occurring *d*-lecithin (now referred to as L-lecithin) is split by lipase, he stated that the unnatural form (*l*-lecithin, now called D-lecithin) is absorbed as such, since the lipases cannot attack it.

However, Artom and Swanson[18] state that phospholipids may be absorbed at various stages of hydrolysis. There appears to be definite evidence that part of the phospholipid is hydrolyzed in the gastrointestinal tract, so that inorganic phosphate or glycerophosphate is set free. The results of Artom and Swanson[18] would seem to offer an entirely independent confirmation of the Partition Theory of fat absorption as postulated by Frazer.[20]

3. The Absorption and Digestibility of Waxes

Although the waxes include a wide variety of components, from a quantitative standpoint, they comprise a much smaller proportion of the diet than is the case with the triglyceride fats. Waxes are usually considered to be simple lipids, ordinarily solid at room temperature, in which a simple fatty acid is combined with a monatomic alcohol, usually of high molecular weight. Two main classes of true waxes exist, namely those in which the fatty acid is combined with an aliphatic alcohol such as cetyl or stearyl, and those in which the alcohol component is a cyclic compound with a steroid nucleus or one containing a β-ionone residue.

(1) *Waxes Containing Aliphatic Alcohols*

Ordinarily waxes of this type are very difficult to hydrolyze in the laboratory, even after prolonged saponification. Thaysen[21] proposed a

[18] C. Artom and M. A. Swanson, *J. Biol. Chem.*, *175*, 871–881 (1948).
[19] P. Mayer, *Biochem. Z.*, *1*, 39–52 (1906).
[20] A. C. Frazer, *Physiol. Revs.*, *26*, 103–119 (1946).
[21] T. E. H. Thaysen, *Biochem. Z.*, *62*, 89–114 (1914).

differential saponification method for the separation of waxes from fats. There is little positive *in vivo* evidence indicating that the waxes containing aliphatic alcohols can be hydrolyzed by enzymes. However, some *in vivo* experiments demonstrate a partial splitting of such waxes in the gastrointestinal tract.

a. Jojoba Oil. Savage[22] reported digestibility tests on a liquid wax, commonly referred to as jojoba oil, which is obtained from the nuts of the goat-nut shrub, *Simmondsia chinensis* (*californica*). This lipid contains no glycerol.[23] According to McKinney and Jamieson,[23] the chief acid in jojoba oil is $\Delta^{11,12}$-eicosenoic ($CH_3(CH_2)_7CH:CH(CH_2)_9COOH$), which accounts for 30.30% of the total. Other acids are $\Delta^{13,14}$-docosenoic ($CH_3(CH_2)_7CH:CH(CH_2)_{11}COOH$), 14.20%, and saturated acids, 1.64%. The alcohols include $\Delta^{11,12}$-eicosenol ($CH_3(CH_2)_7CH:CH(CH_2)_9CH_2OH$), 14.6% and $\Delta^{13,14}$-docosenol ($CH_3(CH_2)_7CH:CH(CH_2)_{11}CH_2OH$), 33.7%.[24]

The results of digestibility tests on rats with jojoba oil are summarized in Table 1 (page 252).

The low digestibility of jojoba oil, which approximates 50% when it is fed at the lower dosage and 70% when the material is given at the higher level, is the lowest recorded for limpid fats in rats. Although these experiments offer no proof as to whether or not hydrolysis of the wax is a prerequisite to absorption, it was found that the largest proportion of the fecal lipids was composed of unhydrolyzed jojoba oil. However, some free alcohols were present in the feces, although no free fatty acids or soaps could be demonstrated. This would indicate that the higher alcohols may be somewhat more difficultly absorbed from the gastrointestinal tract than are the corresponding acids and soaps; the results also afford evidence that a partial hydrolysis of this wax occurs in the gastrointestinal tract of rats.

b. Cetyl Palmitate. Munk and Rosenstein[25] reported, many years ago, that cetyl palmitate ($CH_3(CH_2)_{14}CH_2OCO(CH_2)_{14}CH_3$), when fed as spermaceti, was absorbed by a human subject. These workers, using a patient with a chronic chyle fistula, were able to demonstrate that the palmitic acid moiety appeared in the chyle as tripalmitin, while no trace of the cetyl alcohol could be detected in this fluid or in the feces. The failure to find any cetyl alcohol would suggest that it was destroyed in the

[22] E. S. Savage, *A Comparative Study of the Utilization of Jojoba and Cottonseed Oil in the Rat,* Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., 1951.

[23] R. S. McKinney and G. S. Jamieson, *Oil & Soap, 13,* 289–292 (1936).

[24] T. G. Green, T. P. Hilditch, and W. J. Stainsby, *J. Chem. Soc., 1936,* 1750–1755.

[25] I. Munk and A. Rosenstein, *Arch. path. Anat. u. Physiol.* (*Virchow's*), *123,* 230–279; 484–518 (1891).

TABLE 1
DIGESTIBILITY OF JOJOBA OIL IN THE RAT[a]

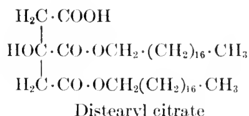
Category	Male rats				Female rats			
	High protein		Low protein		High protein		Low protein	
	Period 1	Period 2	Period 3	Period 4	Period 1	Period 2	Period 3	Period 4
Rats used	9	10	10	8	10	9	10	10
Diet fed								
Protein, %	19.8	22.0	9.0	10.0	19.8	22.0	9.0	10.0
Jojoba oil, %	15.7	6.3	14.8	5.2	15.7	6.3	14.8	5.2
Jojoba oil eaten, g.	15.86	7.68	15.54	6.62	13.59	5.66	13.58	4.64
Feces lipid excreted, mg.								
Neutral fat	4505	3180	3725	3365	3887	1812	3120	2052
Soap	901	1090	1100	862	683	653	953	644
Total uncorrected	5412	4270	4998	4200	4570	2465	3973	2696
Total corrected	5139	4049	4748	4011	4341	2341	3773	2559
Coefficient of digestibility	69.0	47.9	69.2	35.4	68.0	57.8	72.2	44.4
	±1.3	±2.7	±1.9	±4.8	±2.2	±3.6	±3.9	±3.2

[a] E. S. Savage, *A Comparative Study of the Utilization of Jojoba and Cottonseed Oil in the Rat*, Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., 1951.

gastrointestinal tract, or was converted to another substance. There is considerable experimental proof that the latter explanation is the more probable one.

The occurrence of cetyl palmitate to the extent of 90% of the total composition of spermaceti and to a considerable extent in sperm oil[26] is in sharp contrast to its absence from other animal fats. It suggests that the sperm whale (*Physeter macrocephalus* Linné) and the bottlenose whale (*Balaena rostrata*), from which spermaceti is obtained, may be able to synthesize cetyl palmitate *in situ*, since the wax is not widely distributed in marine species other than cetaceans.

c. Distearyl Citrate. Calbert *et al.*[27] studied the digestibility of a wax-like ester, distearyl citrate. This ester, which acts as an anti-flavor-reversion agent toward soybean oil, is an oil-soluble wax which has been shown to be completely innocuous when fed to rats, rabbits, and dogs.[28]



Distearyl citrate

When distearyl citrate was fed to rats in amounts of 2.5 or 10% of the diet, it was digested to the extent of only 5.7 or 19.3%, respectively. Furthermore, a concomitant decrease in fat utilization obtained; the coefficients of digestibility reported for the fats were 77.1 and 71.6 when the wax was added to the diet to the extent of 2.5 or 10%, respectively. On the other hand, when stearyl citrate comprised only 0.13% of the diet, the figure for fat digested was 94.1%, as contrasted with a control value of 95.2%.[27]

Moreover, when distearyl citrate was fed to dogs at a level of 3%, it was also digested to the extent of only 52.2%, on an average. However, under these circumstances, no appreciable interference in fat absorption obtained, since the mean coefficient of digestibility of the fats was 94.5.[27] On the other hand, isopropyl citrate ester (chiefly the mono-ester) was completely utilized by the rat and dog, and no trace of it could be demonstrated in the feces; doses as high as 10% were administered to rats, while the ester preparation made up only 0.06% of the diet fed the dogs.

[26] A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947.

[27] C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).

[28] H. J. Deuel, Jr., S. M. Greenberg, C. E. Calbert, R. Baker, and H. R. Fisher, *Food Research*, *16*, 258–280 (1951).

In the case of the dog tests, stearyl citrate was found to be only partially hydrolyzed, as determined by analysis of the feces. It was found that 67.6% of the stearyl alcohol was present as the ester in the feces, while 32.4% occurred as the free alcohol. The method employed in calculating the proportion of free and combined stearyl alcohol from the analytical values is illustrated in Table 2.

TABLE 2

DETERMINATION OF THE PROPORTION OF STEARYL CITRATE AND STEARYL ALCOHOL EXCRETED IN THE FECES OF DOGS AFTER THE ADMINISTRATION OF STEARYL CITRATE[a]

Category	Dog 9	Dog 10
Stearyl alcohol excreted:		
(*a*) Non-saponifiable fraction (N.S.F.), total, mg.	258	219
(*b*) N.S.F., control value, mg.	33	33
(*c*) Stearyl alcohol, total, mg. ($a - b$)	225	186
Citric acid excreted:		
(*d*) As free acid (before hydrolysis), mg.	0	0
(*e*) Combined (after hydrolysis), mg.	46	49
Distribution of stearyl alcohol:		
(*f*) Total, mg. (c)	225	186
(*g*) Combined, mg. ($e \times 540$ (M.W. stearyl citrate)/192 (M.W. citric acid))	129	138
(*h*) Free, mg. ($f - g$)	96	48
Calculation of stearyl citrate hydrolyzed:		
(*i*) Total stearyl citrate, mg. ($c \times (696/540)$)	290	240
(*j*) Unhydrolyzed, mg. ($e \times (696/192)$)	166	178
(*k*) Hydrolyzed, mg. ($i - j$)	124	62
(*l*) Hydrolyzed, % ($k/i \times 100$)	42.8	25.8
Calculation of stearyl alcohol and stearyl citrate in feces:		
(*m*) N.S.F., total g.	14.7	16.1
(*n*) N.S.F., corrected, g.[b]	12.3	13.2
(*o*) Stearyl alcohol in feces, g. ($n \times l$)	5.3	3.4
(*p*) Stearyl citrate in feces, g. ($n \times (100 - l) \times 696/540$)	9.1	12.6
(*q*) Total, g. ($o + p$)	14.4	16.0

[a] Adapted from C. E. Calbert, S. M. Greenberg, G. Kryder, and H. J. Deuel, Jr., *Food Research*, *16*, 294–305 (1951).
[b] Control N.S.F. (estimated as 17.2% of dried feces) subtracted from *m*.

(2) *Waxes Containing Cyclic Alcohols*

Cholesterol and other sterol esters are the chief wax-like products which contain a cyclic alcohol. These esters are hydrolyzed with more difficulty than are the neutral fats. In order to bring about hydrolysis in the test tube, special means must be employed to rupture the ester linkage, such as long continued heating or the use of sodium ethylate in ethereal

solution, as proposed by Gardner and Fox.[29] Dam[30] indicated the possibility that, when the hydrolysis of cholesterol esters is carried out with alkali, cholesterol may be so altered that it can no longer be determined by the usual methods of analysis. The separation of cholesterol esters from neutral fat in blood by the use of a differential hydrolysis technic has not proved satisfactory.

Cholesterol esterase has a wide distribution in the tissues, including the intestinal wall (see page 21); this enzyme is specific in effecting the hydrolysis of cholesterol esters, while other lipases are inactive. Reichel and Reinmuth[31] demonstrated that castor bean lipase is devoid of any action on cholesterol stearate, although it has a potent action on triglycerides. Kelsey[32] based a procedure for the separate determination of neutral fat and cholesterol esters with castor bean lipase upon this variation in enzyme composition.

The fate of cholesterol esters in the gastrointestinal tract appears to be identical with that of the free alcohol. The discussion of absorption, transformations in the gastrointestinal tract, and digestibility of both the ester and the free alcohol forms of cholesterol is included in a later section (see page 259). For a treatment of the absorption and digestibility of the carotenoid esters or vitamin A esters, the reader is referred to later sections in this chapter.

4. The Absorption and Digestibility of Higher Aliphatic Alcohols

The higher aliphatic alcohols are much less widely distributed in nature than are the sterols. Some of them apparently are natural products, the metabolism of which is closely connected with that of the corresponding fatty acids. The greatest amount of information is available as to the behavior of cetyl, stearyl, and oleyl alcohols.

(*1*) *Cetyl Alcohol*

Cetyl alcohol, $CH_3(CH_2)_{14}CH_2OH$, was first shown by Gardner[33] to be a component of feces. Schoenheimer and Hilgetag[34] were later able to isolate it from the feces of man, dogs, and cats. It was likewise isolated from the human intestinal walls, from meconium (sterile feces of the new-

[29] J. A. Gardner and F. W. Fox, *Biochem. J.*, *18*, 1058–1069 (1924).
[30] H. Dam, *Biochem. Z.*, *194*, 177–187 (1928).
[31] L. Reichel and W. Reinmuth, *Z. physiol. Chem.*, *244*, 78–80 (1936).
[32] F. E. Kelsey, *J. Biol. Chem.*, *130*, 187–193 (1939).
[33] J. A. Gardner, *Biochem. J.*, *15*, 244–273 (1921).
[34] R. Schoenheimer and G. Hilgetag, *J. Biol. Chem.*, *105*, 73–77 (1934).

born), and also from the feces of bile-fistula dogs on a fat-free diet. Sterile cysts in the small and large intestine also contained this alcohol. It is believed that it is normally secreted into the lumen of the intestine, to be excreted in the feces. It is suggested by Schoenheimer and Hilgetag[34] that endogenous cetyl alcohol may act as a natural purgative, a fact proved earlier by Macht[35] with exogenous cetyl alcohol. However, in latter work, Stetten and Schoenheimer[36] failed to note any purgative action on the part of cetyl or octadecyl alcohol in rats.

Although the presence of cetyl alcohol in feces might be interpreted as indicative of its failure to be absorbed, there is evidence that this alcohol may be taken up by the intestinal mucosa. The metabolic fate may to some extent resemble that of cholesterol, which can be absorbed by the small intestine, and which is also known to be secreted by the mucosa, particularly of the large intestine. As noted earlier, Munk and Rosenstein[25] were unable to detect cetyl palmitate or cetyl alcohol in the chyle from a patient suffering from a chronic chyle fistula, after he had been given cetyl palmitate in the form of spermaceti, although they did demonstrate the presence of some of the palmitic acid, as tripalmitin, in the chyle. This would indicate that the ester was hydrolyzed, and that at least part of it was absorbed. The experiments of Mancke[37] are somewhat more indicative in proving the absorption of cetyl alcohol, since he was able to account for only a fraction of ingested cetyl alcohol in the feces. As much as 62% of the cetyl alcohol fed to a goose failed to be recovered from the feces. However, no cetyl alcohol was found in the deposit fat or in the chyle, so there is still a possibility that the alcohol unaccounted for might have been destroyed in the gastrointestinal tract. Thomas and Flaschenträger[38] have shown that the dog absorbs cetyl alcohol rather poorly, but that its ester is more readily utilized. The authors attribute this difference in assimilability to the lower melting point of the ester. Others have demonstrated the same behavior in the rat.[36,39,40]

Channon and Collinson[40] considered that the finding of a markedly increased unsaponifiable fraction in the liver of rats following the administration of cetyl alcohol was indirect proof that the alcohol itself had been absorbed. The quantities of unsaponifiable extract were too small to afford a positive identification of the substance. However, the presence

[35] D. I. Macht, *Proc. Soc. Exptl. Biol. Med.*, *30*, 1272–1273 (1932–1933).
[36] De W. Stetten and R. Schoenheimer, *J. Biol. Chem.*, *133*, 347–357 (1940).
[37] R. Mancke, *Z. physiol. Chem.*, *162*, 238–263 (1927).
[38] K. Thomas and B. Flaschenträger, *Skand. Arch. Physiol.*, *43*, 1–5 (1923).
[39] C. L. Carter and J. Malcolm, *Biochem. J.*, *21*, 484–493 (1927).
[40] H. J. Channon and G. A. Collinson, *Biochem. J.*, *22*, 391–401 (1928).

of cetyl alcohol in a number of marine animals is a further suggestion as to its probable absorption. It is found in the livers of a number of fishes as a component of the glyceryl ether, chimyl alcohol.[41-44] Cetyl alcohol has also been shown to be present as such in some fish liver oils.[42,45]

Stetten and Schoenheimer[36] used a deuterated cetyl alcohol by which they obtained direct proof that cetyl alcohol is absorbed by the rat. Moreover, it was found that this alcohol is rapidly converted to palmitic acid in the intestinal mucosa. This rapid transformation to another product which is normally present probably accounts for the failure to detect the alcohol in the chyle in the earlier tests. These workers also demonstrated that deuteropalmitic acid was converted to deuterocetyl alcohol which, in turn, could be isolated from the feces. It is interesting that a conversion of deuterocetyl alcohol to deuterostearic acid was also indicated. The results did not determine whether the transformation of the acid to the alcohol, or the reverse change, takes place in the lumen of the gut or in the intestinal wall. However, it is believed that the latter site is the more probable one.

(2) *Stearyl Alcohol*

Stearyl or octadecyl alcohol, $CH_3(CH_2)_{16}CH_2OH$, has the same relationship to stearic acid that cetyl alcohol has to palmitic acid; it has been detected only once in feces when it had not previously been fed. Schoenheimer and Hilgetag[34] reported it in a sample of dog feces, but the amount was far less than that of cetyl alcohol. However, after the feeding of deuterostearyl alcohol to rats, its conversion to stearic acid was easily demonstrated[34]; stearic acid was likewise shown to be convertible to stearyl alcohol. The fate of stearyl alcohol is therefore believed to be quite similar to that of cetyl alcohol. When fed to normal animals, it is readily absorbed in the intestine, and is converted to the corresponding acid in the intestinal mucosa. It is likewise believed to be an intermediate in the conversion of stearic to palmitic acid.

Stearyl alcohol is a component of batyl alcohol, which is one of the glyc-

[41] M. Tsujimoto and Y. Toyama, *Chem. Umschau Gebiete Fette, Öle, Wachse u. Harze, 29*, 27–29, 35–37, 43–45 (1922).

[42] Y. Toyama, *Chem. Umschau Gebiete Fette, Öle, Wachse u. Harze, 32*, 113–115 (1925); *Chem. Abst., 19*, 2882 (1925).

[43] Y. Toyama, *Chem. Umschau Gebiete Fette, Öle, Wachse u. Harze, 31*, 61–67, 153–155 (1924); *Chem. Abst., 18*, 2613, 3733 (1924).

[44] J. C. Drummond and L. C. Baker, *Biochem. J., 23*, 274–291 (1929).

[45] Y. Toyama, *Chem. Umschau Gebiete Fette, Öle, Wachse u. Harze, 29*, 237–240, 245–247 (1922); *Chem. Abst., 17*, 892–893 (1923).

eryl ethers. The alcohol, "astrol," originally prepared by Kossel and Edlbacher[46] in 1915 from stellasterol, has recently been proved to be batyl alcohol.[47] Batyl alcohol was first recognized in fish liver oils.[41–44] However, later workers have reported its presence in such widely diverse sources as the Japanese crab (*Paralithoides camtschatica* Tilesius),[48] a reef-building gorgonia (coral) (*Plexaura flexuosa*),[49] the star fish (*Asterias rubens* and *A. forbesi*),[47] the bone marrow of cattle,[50] the spleen of the pig,[51] and arteriosclerotic arteries of man.[52]

Calbert and associates[27] investigated the utilization of stearyl alcohol in rats, as well as of an ester, distearyl citrate, in rats and dogs. When stearyl alcohol was fed to rats at a level of 1.8% of the diet, a coefficient of digestibility of 88.6 was obtained; when this alcohol made up 7.5% of the diet, the digestibility coefficient was found to be 54.8. In both cases, the digestibility of the fat given concomitantly was somewhat reduced, the values being 91.9 and 80.0%, respectively. A discussion of the digestibility tests with distearyl citrate is given earlier (see page 253).

(3) *Oleyl Alcohol*

Oleyl alcohol, $CH_3(CH_2)_7CH{:}CH(CH_2)_7CH_2OH$ has been shown by Channon and Collinson[40] to be readily absorbed in the rat. Evidence for this fact is based, not only upon the disappearance of the alcohol from the gastrointestinal tract, but also upon the appearance of an appreciable unsaponifiable fraction in the liver fat. Oleyl alcohol is known to be a component of selachyl alcohol, which is a glyceryl ether widely distributed in fish liver oils.[41–44] Oleyl alcohol also occurs free in fish liver oils,[42,45] as well as in Arctic sperm oil.[53,54]

(4) *Other Alcohols*

Phytol, $CH_3[CH(CH_3)(CH_2)_3]_3C(CH_3){:}CHCH_2OH$, has been shown to be readily absorbed by the rat.[40] The absorption was confirmed by the

[46] A. Kossel and S. Edlbacher, *Z. physiol. Chem.*, *94*, 264–283 (1915).

[47] W. Bergmann and H. A. Stansbury, *J. Org. Chem.*, *8*, 283–284 (1943).

[48] M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, *32*, 363–364 B(1929); *Chem. Abst.*, *24*, 4650 (1930).

[49] C. A. Kind and W. Bergmann, *J. Org. Chem.*, *7*, 424–427 (1942).

[50] H. N. Holmes, R. E. Corbet, W. B. Geiger, N. Kornblum, and W. Alexander, *J. Am. Chem. Soc.*, *63*, 2607–2609 (1941).

[51] V. Prelog, L. Ruzicka, and P. Stein, *Helv. Chim. Acta*, *26*, 2222–2242 (1943).

[52] E. Hardegger, L. Ruzicka, and E. Tagmann, *Helv. Chim. Acta*, *26*, 2205–2221 (1943).

[53] M. Tsujimoto, *Chem. Umschau Gebiete Fette, Öle, Wachse u. Harze*, *32*, 127–128 (1925); *Chem. Abst.*, *19*, 2882 (1925).

[54] T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, *48*, 359–364T (1929).

fact that an increased unsaponifiable fraction was noted in the liver oil after this alcohol was fed.

$\Delta^{11,12}$-Eicosenol and $\Delta^{13,14}$-docosenol, two unsaturated alcohols which occur in the wax, jojoba oil, are partially digested by the rat.[22] For a discussion of the digestibility of these alcohols when fed as the esters, see page 251.

5. The Digestion, Absorption, and Transformations of Sterols in the Gastrointestinal Tract

(1) *The Digestion and Absorption of Cholesterol*

a. Absorption Experiments with Cholesterol. The best proof of the absorption of cholesterol is afforded by the demonstration of increased cholesterol in the blood, lymph, and tissues after this alcohol is fed. As early as 1906, Pribram[55] reported an increase in the blood cholesterol of rabbits following the ingestion of cholesterol as the free alcohol or as the ester.

Gardner and co-workers,[56–58] Lehman,[59] Knudson,[60] Ssokoloff,[61] and Mjassnikov and Iljinsky[62] have all reported that the feeding of cholesterol results in an increased level in the blood. According to Gardner *et al.*,[56–58] the feeding of cholesterol either as the free alcohol or as the ester produces an augmentation of the blood cholesterol; however, it was shown that the cholesterol esters are hydrolyzed before being absorbed.

Mueller[63,64] found that the cholesterol content of chyle was increased after the administration of this sterol; the proportion of free alcohol to ester in the chyle was similar to the proportion in the blood. Moreover, the amount of chyle cholesterol increased after the administration of this substance in either the free or the ester form. These results are also in line with the observation that a cholesterol esterase system is present in the wall of the small intestine, and can bring about both hydrolysis and esterification.[65,66]

[55] H. Pribram, *Biochem. Z.*, *1*, 413–424 (1906).
[56] C. Dorée and J. A. Gardner, *Proc. Roy. Soc. London*, *B81*, 109–128 (1909).
[57] M. T. Fraser and J. A. Gardner, *Proc. Roy. Soc. London*, *B81*, 230–247 (1909).
[58] M. T. Fraser and J. A. Gardner, *Proc. Roy. Soc. London*, *B82*, 559–568 (1910).
[59] E. P. Lehman, *J. Biol. Chem.*, *16*, 495–503 (1914).
[60] A. Knudson, *J. Biol. Chem.*, *45*, 255–262 (1921).
[61] S. A. Ssokoloff, *Z. ges. exptl. Med.*, *46*, 650–655 (1925).
[62] A. Mjassnikov and B. Iljinsky, *Z. ges. exptl. Med.*, *53*, 100–106 (1926).
[63] J. H. Mueller, *J. Biol. Chem.*, *22*, 1–9 (1915).
[64] J. H. Mueller, *J. Biol. Chem.*, *27*, 463–480 (1916).
[65] M. L. Nieft and H. J. Deuel, Jr., *J. Biol. Chem.*, *177*, 143–150 (1949).
[66] E. Frölicher and H. Süllmann, *Biochem. Z.*, *274*, 21–33 (1934).

(*a*) *The Effect of Fat on Cholesterol Absorption.* The absorption of cholesterol from the small intestine apparently takes place most efficiently if it is given with fat,[67,68] although Bollman and Flock[69] agreed with the findings of Dubach and Hill,[70] who were able to demonstrate an increase in the level of plasma cholesterol after feeding the sterol without an oil carrier.

Apparently the requirement for fat to insure cholesterol absorption varies with species. On the one hand, Cook[71] reported that, if rats are fed a diet relatively free from lipid but containing cholesterol, the sterol can be recovered quantitatively from the feces. On the other hand, Popják[72] noted that guinea pigs and rabbits on a low-fat diet were able to absorb small amounts of cholesterol when it was given in a finely divided aqueous suspension. Cook and Thomson[73] found that, while rats and guinea pigs absorbed 46 to 50% of the cholesterol on a diet containing 16.6% of olive oil, rabbits utilized 82% of the sterol. Thus, the more ready absorption of cholesterol by the rabbit is not solely dependent upon the presence of fat in the diet. However, Frölicher and Süllmann[66] demonstrated that the content of free cholesterol in the intestinal lymph of rabbits is increased when fat is given; they attribute this phenomenon to a reabsorption of excreted cholesterol. Kim and Ivy[74] likewise reported that the presence of fat in the diet of rats may facilitate the absorption of exogenous cholesterol.

In the case of chickens, although hypercholesteremia developed to some extent when cholesterol was incorporated into a fat-free diet to the extent of 2% (Stamler and Katz),[75] or 1% (Peterson *et al.*),[76] a much greater degree of cholesteremia occurred when extra fat was fed with the diet, thus indicating that fat facilitates cholesterol absorption in the chick as well. It is suggested by Peterson *et al.*[76] that this effect of fat in augmenting cholesterol absorption may be associated with its action in increasing bile flow.

(*b*) *The Effect of Bile on Cholesterol Absorption.* Bile salts are an even more important factor in stimulating the absorption of cholesterol than is the solvent action of fat. Although the normal bile secretion may be

[67] S. J. Thannhauser, *Deut. Arch. klin. Med.*, *141*, 290–311 (1923).
[68] M. Sano, *Tôhoku J. Exptl. Med.*, *4*, 417–425 (1924).
[69] J. L. Bollman and E. V. Flock, *Am. J. Physiol.*, *164*, 480–485 (1951).
[70] R. Dubach and R. M. Hill, *J. Biol. Chem.*, *165*, 521–531 (1946).
[71] R. P. Cook, *Biochem. J.*, *30*, 1630–1636 (1936).
[72] G. Popják, *Biochem. J.*, *40*, 608–621 (1946).
[73] R. P. Cook and R. O. Thomson, *Biochem. J.*, *44*, li (1949).
[74] K. S. Kim and A. C. Ivy, *Am. J. Physiol.*, *171*, 302–318 (1952).
[75] J. Stamler and L. N. Katz, *Circulation*, *4*, 255–261 (1951).
[76] D. W. Peterson, E. A. Shneour, N. F. Peek, and H. W. Gaffey, *J. Nutrition*, *50*, 191–201 (1953).

sufficient to result in a minimum absorption of cholesterol from the gastrointestinal tract on a cholesterol-rich diet, a large excess of bile salts must be furnished if appreciable amounts of cholesterol are to be deposited in the liver. The rate of increase in blood and liver cholesterol in rats fed a 1% cholesterol diet with 0.5% bile salts is illustrated in the results of Alfin-Slater *et al.*[77] which are summarized in Table 3 (page 262).

Schönheimer[78] found that an optically visible lipemia could be produced in rabbits by means of a single cholesterol feeding along with bile salts. However, Member and his co-workers[79] were able to produce an increased deposition of cholesterol in the aortas of rabbits, as a result of feeding this sterol, only when cholic or glycocholic acid was fed concomitantly. The cholesterol levels in the aorta were no higher than the controls when dehydrocholic, hyodesoxycholic, or desoxycholic acid was included in the diet. A summary of these data is given in Table 4 (page 263).

Finally, Siperstein, Chaikoff, and Reinhardt,[80] employing C^{14}-labeled cholesterol, concluded that bile plays an obligatory role in the passage of cholesterol from the intestinal tract to the lymph. Although these workers found that the absorption of palmitic acid was likewise increased by the presence of bile, it was noted that small amounts of palmitic acid can be absorbed when bile is completely eliminated from the gastrointestinal tract.

The mechanism by which bile salts facilitate the absorption of cholesterol is uncertain. However, Wieland and Sorge[81] found that cholesterol is one of the substances which can be dissolved by the hydrotropic action of sodium desoxycholate and other bile acids. Moreover, Downie *et al.*[82] reported a coordination compound in which cholesterol is the acholic component. The best explanation for the absorption of cholesterol is therefore the fact that it is rendered possible by the hydrotropic action of bile salts, and that it is facilitated when cholesterol is dissolved in fat.

(*c*) *The Esterification of Cholesterol in Relation to Its Absorption.* Peterson *et al.*[76] demonstrated that phytosterols produce a depressive effect on the absorption of cholesterol in chickens. It is suggested that this may be due to inhibition exerted by the latter compounds on the cholesterol-esterifying system in the intestinal mucosa. Since it is known that the

[77] R. Alfin-Slater, M. Schotz, S. M. Greenberg, and H. J. Deuel, Jr., unpublished data, 1952.

[78] R. Schönheimer, *Biochem. Z.*, *147*, 258–263 (1924).

[79] S. Member, M. Bruger, and E. Oppenheim, *Arch. Pathol.*, *38*, 210–214 (1944).

[80] M. D. Siperstein, I. L. Chaikoff, and W. O. Reinhardt, *J. Biol. Chem.*, *198*, 111–114 (1952).

[81] H. Wieland and H. Sorge, *Z. physiol. Chem.*, *97*, 1–27 (1916).

[82] A. W. Downie, L. Stent, and S. M. White, *Brit. J. Exptl. Pathol.*, *12*, 1–9 (1931).

TABLE 3
THE EFFECT OF BILE SALTS ON THE ABSORPTION OF CHOLESTEROL IN RATS[a]

Component analyzed	Diet	Cholesterol in mg./g. or mg./100 ml.		
		After 1 day	After 3 days	After 6 days
Liver.........	Basal	3.08	2.07	2.41
	" + 0.5% bile salts	2.80	2.73	2.52
	" + 1.0% cholesterol	3.53	3.61	4.24
	" + 1.0% cholesterol + 0.5% bile salts	4.43	8.35	11.25
Plasma........	Basal	79.5	63.0	90.0
	" + 0.5% bile salts	78.0	78.0	75.0
	" + 1.0% cholesterol	63.0	84.0	69.0
	" + 1.0% cholesterol + 0.5% bile salts	63.5	100.5	118.5
Feces.........	Basal	5.85	—	3.19[b]
	" + 0.5% bile salts	3.94	—	3.91
	" + 1.0% cholesterol	28.5	65.1	91.1
	" + 1.0% cholesterol + 0.5% bile salts	26.8	56.0	81.6

[a] R. Alfin-Slater, M. Schotz, S. M. Greenberg, and H. J. Deuel, Jr., Unpublished results, 1952.
[b] Value for 8 days. Values for 12 and 14 days were 3.26 and 3.50, respectively.

TABLE 4

EFFECT OF FEEDING CHOLESTEROL, WITH OR WITHOUT BILE SALTS, ON THE CHOLESTEROL IN THE BLOOD AND AORTA OF RABBITS[a]

Supplement used 3 times weekly at 0.5 g.	Number of tests	Blood cholesterol in mg./100 ml.					% ester		Cholesterol in aorta, mg. %
		Start	2 weeks	4 weeks	6 weeks	14 weeks	Start	End	
None	8	98	126	212	345	529	15	61	1588
Dehydrocholic acid	7	112	220	277	449	675	19	58	1488
None	10	104	244	272	409	401	8	42	1398
Cholic acid	8	102	506	622	801	649	9	49	3389
			3 weeks	6 weeks	9 weeks	12 weeks			
None	6	94	227	307	734	784	16	47	1334
Hyodesoxycholic acid	6	97	322	408	676	768	20	50	1221
			3 weeks	7 weeks	11 weeks	13 weeks			
None	6	95	328	681	770	779	19	42	1061
Desoxycholic acid	9	116	387	654	728	695	26	41	1047
			2 weeks	4 weeks	6 weeks	13 weeks			
None	9	110	283	384	455	761	24	39	2932
Glycocholic acid	6	136	346	397	648	858	31	42	4799

[a] Adapted from S. Member, M. Bruger, and E. Oppenheim, *Arch. Pathol.*, *38*, 210–214 (1944).

proportion of esterified cholesterol present in the bile of dogs is constant, irrespective of whether the sterol is administered in the form of an ester or as the free alcohol,[65,66] it has been accepted that a cholesterol-esterase is present in intestinal mucosa. Swell *et al.*[83] demonstrated the presence of an enzyme in the intestinal mucosa. They concluded that the major source of this enzyme is the pancreatic juice, since the mucosa of rats which had been almost completely depancreatized was found to contain very little of the esterase. However, Stamler and Katz[75] reported that the hypercholesterolemia was greater than normal in depancreatized chicks fed cholesterol. The latter observation indicates that the pancreas is not essential for the absorption of cholesterol from the gastrointestinal tract of the chick. If an esterifying enzyme is required for such absorption, its origin apparently is not in the pancreas. It thus becomes a moot question as to whether or not esterification in the intestinal mucosa is a prerequisite to the absorption of cholesterol.

(*d*) *Other Factors Concerned with the Absorption of Cholesterol.* Lecithin was reported by one group of workers to reduce cholesterol absorption, and by another group of investigators to increase absorption of this sterol. Thus, Kesten and Silbowitz[84] noted that the level of plasma cholesterol was decreased in rabbits fed high levels of lecithin together with cholesterol. On the other hand, Stamler and co-workers[85] observed that choline and inositol increased rather than decreased the level of plasma cholesterol in chicks fed on this sterol. Peterson and associates[76] also concluded that the administration of soy lecithin concomitantly with cottonseed oil and cholesterol to chicks caused a somewhat greater increase in plasma cholesterol than was noted when the same diet was given but without lecithin. It is not known whether these conflicting findings are to be ascribed to the fact that they were obtained on different species, or whether the lack of agreement might be related to differences in the amount of the lecithin dosage employed. The effect of sterols other than cholesterol on the absorption of the latter compound is discussed on page 270.

b. The Effect of Cholesterol on the Absorption of Fatty Acids. There is some evidence that cholesterol may aid in the absorption of fatty acids. Brockett and associates[86] found that, when fat was ingested, not only did a marked rise in the total fatty acids and some increase in the lipid phosphorus in thoracic lymph occur, but also a considerably higher level

[83] L. Swell, J. E. Byron, and C. R. Treadwell, *J. Biol. Chem.*, *186*, 543–548 (1950).

[84] H. D. Kesten and R. Silbowitz, *Proc. Soc. Exptl. Biol. Med.*, *49*, 71–73 (1942).

[85] J. Stamler, C. Bolene, R. Harris, and L. N. Katz, *Circulation*, *2*, 714–721 (1950).

[86] S. H. Brockett, M. A. Spiers, and H. E. Himwich, *Am. J. Physiol.*, *110*, 342–347 (1934)

of both free and esterified cholesterol obtained. Although these results are interpreted by Bloor[87] as evidence that cholesterol aids in fat absorption, they may as readily be construed as indicative of the role of cholesterol in fat transport after the absorption of the fatty acids from the gastrointestinal tract has been completed.

c. Balance Experiments with Cholesterol. The problem of cholesterol utilization has been attacked by the use of balance experiments. The whole picture of cholesterol absorption is complicated by the fact that cholesterol may be destroyed or converted into other steroids, that it may be synthesized in the tissues, and that it is excreted into the gastrointestinal tract through the bile or *via* the intestinal mucosa.

The results of Kusumoto,[88] who could account for only 70% of the ingested cholesterol in the feces, are likewise interpreted as evidence of cholesterol absorption,[87] but it is possible to explain this 30% loss by bacterial destruction in the gastrointestinal tract. Destruction of cholesterol has been demonstrated in the chicken by Dam,[89] and by Page and Menschick in rabbits[90] and in cats.[91] The results of Schönheimer,[92] based upon the study of a case of hypercholesteremia in man, likewise indicate the probable destruction by the human subject. In a later publication, Schoenheimer and Breusch[93] demonstrated that destruction of cholesterol occurs in mice; since the extent of destruction is increased when bile acids are given, it is believed that the increased disappearance of cholesterol results from increased absorption. In other words, it is indicated that the degradation of cholesterol is a function of the tissues, and occurs there rather than in the gastrointestinal tract. Similar conclusions can also be drawn from the results of Cook.[94]

The role played by the intestinal bacteria has been the subject of extensive investigation. A number of workers[95–98] have suggested that the intestinal flora are primarily responsible for the destruction of cholesterol. Rosenheim and Webster[99] concluded that it is improbable that

[87] W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

[88] C. Kusumoto, *Biochem. Z.*, *14*, 411–415 (1908).

[89] H. Dam, *Biochem. Z.*, *232*, 269–273 (1931).

[90] I. H. Page and W. Menschick, *J. Biol. Chem.*, *97*, 359–368 (1932).

[91] W. Menschick and I. H. Page, *Z. physiol. Chem.*, *218*, 95–103 (1933).

[92] R. Schönheimer, *Z. klin. Med.*, *123*, 749–763 (1933).

[93] R. Schoenheimer and F. Breusch, *J. Biol. Chem.*, *103*, 439–448 (1933).

[94] R. P. Cook, *Biochem. J.*, *31*, 410–415 (1937).

[95] S. Bondzyński and V. Humnicki, *Z. physiol. Chem.*, *22*, 396–410 (1896–1897).

[96] R. Schönheimer, H. v. Behring, R. Hummel, and L. Schindel, *Z. physiol. Chem.*, *192*, 73–76 (1930).

[97] S. J. Thannhauser, *Klin. Wochschr.*, *13*, 161–167 (1934).

[98] H. Dam, *Biochem. J.*, *28*, 820–825 (1934).

intestinal bacteria or protozoa are responsible for the conversion of cholesterol to coprosterol; Schoenheimer and Breusch[93] are of the same opinion. However, in the experiments of Wainfan and collaborators,[100] it was demonstrated that, in mice fed on a diet containing 1.5% of cholesterol, an average of approximately 12% of the administered sterol was either destroyed or chemically modified. That this deficit is to be ascribed to bacterial action in the gut is strongly indicated by the fact that a complete recovery of ingested cholesterol obtained when 1% sulfasuxidine and 0.04% streptomycin were added to the diet. Although the synthesis of cholesterol with its resultant excretion into the intestine might compensate for some of the cholesterol destroyed, the experiments of Wainfan and her associates involved the use of much larger amounts than the physiological quantities.

Unquestionably cholesterol can be absorbed when bile salts are present; the absorption is probably aided when fat is likewise available. However, the ability of the animal to absorb cholesterol is limited; if the quantities of the sterol are appreciable, then invariably some of it fails to be absorbed. The quantity of sterol in the intestine is augmented by that contributed by the bile, as well as by that secreted by the intestinal mucosa.

Most of the cholesterol undergoes a transformation to cholestanol (dihydrocholesterol) or coprostanol (coprosterol), which are the chief sterols in the feces. However, Schoenheimer[101] has devised an analytical procedure for the separation of unsaturated from saturated sterols which is sufficiently precise as to render possible the isolation and identification of cholesterol in human feces. The various transformations of the sterols in the intestinal tract are discussed on pages 271–274.

(*a*) *Balance Experiments in Man.* Gardner and Fox[102] reported that, with low intakes of cholesterol, more was excreted in the feces than was ingested. On the other hand, when large amounts of this alcohol were fed, considerable absorption of the sterol took place. Thus, Bürger and Winterseel[103] observed absorption of 50%, 49%, and 12% of cholesterol when 5 g. was fed with 100 g. of olive oil on a mixed diet to three subjects. When a milk diet was given, the digestibility coefficient was found to be 63. Cook and Edwards[104] reported an absorption of 20% when 10 g. of crystal-

[99] O. Rosenheim and T. A. Webster, *Biochem. J.*, *37*, 580–585 (1943).
[100] E. Wainfan, G. Henkin, I. Rice, and W. Marx, *Arch. Biochem. Biophys.*, *38*, 187–193 (1952).
[101] R. Schoenheimer, *J. Biol. Chem.*, *105*, 355–357 (1934).
[102] J. A. Gardner and F. W. Fox, *Proc. Roy. Soc. London*, *B92*, 358–367 (1921).
[103] M. Bürger and W. Winterseel, *Z. physiol. Chem.*, *181*, 255–263 (1929).
[104] R. P. Cook and D. C. Edwards, *Biochem. J.*, *49*, xli (1951).

line cholesterol was added to a diet containing 50 g. of olive oil, while a figure of 60% (equivalent to 6.9 g. of cholesterol) was found when cholesterol was given as egg yolk along with 360 g. of fat.[105]

In studies on man, in which the criterion of absorption is an elevation in plasma cholesterol, rather than fecal excretion, transient increases in plasma cholesterol have been noted after the ingestion of large amounts of cholesterol in the form of eggs.[106,107] On the other hand, Gough[108] was unable to demonstrate any appreciable increase of cholesterol, either in the plasma or in the bile, when this sterol was fed as the crystalline compound or as sheep brain, in the amount of 2 g. cholesterol in 50 g. brain. In his review of comparative aspects of lipid absorption and excretion, Cook[105] states that the absorption of cholesterol in man is not marked, and that the level is kept fairly constant; the ingestion of fat alone, however, may result in a hypercholesterolemia.

(*b*) *Balance Experiments on Animals.* Cook and Thomson[109] reported that the maximum amount of cholesterol which can be absorbed per kilogram of body weight is fairly uniform in the rat, guinea pig, and rabbit. However, the coefficient of digestibility was found to be highest in the rabbit, lower in the guinea pig, and lowest in the rat. Had the cholesterol been fed at a standard dosage per kilogram body weight, it is probable that the coefficients of digestibility obtained might have been more

TABLE 5

THE COMPARATIVE CHOLESTEROL ABSORPTION IN DIFFERENT ANIMALS[a]

Category	Rat	Guinea pig	Rabbit
Cholesterol			
Fed, g./kg. body wt./day	1.05	0.55	0.37
Absorbed, g./kg. body wt./day	0.35	0.25	0.25
Absorption, %	34	47	77
Plasma cholesterol, mg./100 ml.			
Control	70	95	148
After cholesterol feeding	222	271	1720

[a] Adapted from R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol., 36,* 61–74 (1951).

[105] R. P. Cook, "Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams, *Lipid Metabolism,* Biochem. Soc. Symposia, No. 9, 14–29, Cambridge Univ. Press, 1952.

[106] R. Okey and D. Stewart, *J. Biol. Chem., 99,* 717–727 (1933).

[107] M. F. Collen, D. de Kruif, and F. Geier, *Permanente Foundation* (Oakland, Calif.) *Med. Bull., 7,* 60–66 (1949).

[108] N. Gough, *Brit. Med. J., 1943, II,* 390–391.

[109] R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol., 36,* 61–74 (1951).

uniform for the several species than was observed in these experiments. Cholesterol was apparently absorbed most readily by rabbits, as judged by the effect of cholesterol feeding on plasma cholesterol. These data are summarized in Table 5 (page 267).

(2) *The Digestion and Absorption of Sterols Other Than Cholesterol*

Although cholesterol can readily be absorbed from the small intestine if fat and bile salts are present, many other closely related sterols do not share this property. The first studies in this field were concerned with the plant sterols (phytosterols), chiefly sitosterols and stigmasterols. Schönheimer[110–112] found that the phytosterols are not absorbed even when fed with bile salts.[113] Although Fraser and Gardner[58] originally believed that they had demonstrated the absorption of phytosterols followed by their conversion to cholesterol, Gardner and Gainsborough[114] later agreed with Schönheimer that plant sterols cannot change to cholesterol in the animal body. The only evidence that phytosterols can be absorbed is that of Nikuni[115] who experimented on mice, and that of von Gierke, who reported tests on rabbits.[116]

In more recent years, considerable additional research has been carried out with individual sterols. Thus Schönheimer[117] and Breusch[118] reported that the main group of phytosterols, namely, a mixture of α, β, and γ-sitosterols, are not absorbed by mice, rats, or rabbits. Rosenheim and Webster[119] noted also that neither stigmasterol nor β-sitosterol (22-dihydrostigmasterol) is absorbed by rats; the latter was found to be converted to coprostanol. Sperry and Bergmann[120] likewise reported that no increase in liver sterols occurred in mice after sitosterol or stigmasterol was fed. Schönheimer and associates[112,113,117] reported that, not only was sitosterol not absorbed by rabbits and dogs, but furthermore

[110] R. Schönheimer, *Z. physiol. Chem.*, *180*, 1–5, 16–18, 24–32, 32–37 (1929); *185*, 119–122 (1929).

[111] R. Schönheimer and D. Yuasa, *Z. physiol. Chem.*, *180*, 6–16, 19–23 (1929).

[112] H. v. Behring and R. Schönheimer, *Z. physiol. Chem.*, *192*, 97–102 (1930).

[113] R. Schönheimer, H. v. Behring, and R. Hummel, *Z. physiol. Chem.*, *192*, 117–123 (1930).

[114] J. A. Gardner and H. Gainsborough, *Quart. J. Med.*, *23*, 465–483 (1930).

[115] J. Nikuni, *J. Agr. Chem. Soc., Japan*, *7*, 827–838 (1931); *Chem. Abst.*, *26*, 1323 (1932).

[116] E. von Gierke, *Verhandl. deut. pathol. Ges., 20th meeting*, 1925, p. 159; cited by R. Schönheimer, *Z. physiol. Chem.*, *180*, 1–5 (1929), p. 2.

[117] R. Schönheimer, *Klin. Wochschr.*, *11*, 1793–1796 (1932).

[118] F. L. Breusch, *J. Biol. Chem.*, *124*, 151–158 (1938).

[119] O. Rosenheim and T. A. Webster, *Biochem. J.*, *35*, 928–931 (1941).

[120] W. M. Sperry and W. Bergmann, *J. Biol. Chem.*, *119*, 171–176 (1937).

no increase in liver sterols could be detected after the feeding of stigmasterol or brassicasterol to mice. The latter sterol belongs to the C_{28}-series, and was first isolated from the oil of turnip.

Phytosterols possess the property of forming soluble compounds with bile salts. Thus, Kúthy[121] demonstrated that phytosterols are as readily diffusible through parchment paper, in the presence of bile acids, as is cholesterol. In spite of this finding, and also in spite of the fact that phytosterols occur to a large extent in the human diet, there is no valid evidence that they can be converted to cholesterol or that they can be absorbed as such.

Ostreasterol, which is a C_{29}-sterol obtained from oysters and other molluscs, and which occupies a position intermediate between cholesterol and the phytosterols, resembles cholesterol in that it is capable of being absorbed. Thus, Sperry and Bergmann[120] found that an appreciable increase in liver sterols occurred after ostreasterol was fed to mice.

In addition to the phytosterols, there is considerable evidence that sterols more closely related to cholesterol cannot be absorbed. Tests have been made on several of the C_{27}-group of sterols, such as β-cholestanol, coprostanol, and coprostenol (allocholesterol). According to Schönheimer and co-workers,[113] as well as to Bürger and Winterseel,[122] β-cholestanol (dihydrocholesterol), which is present in most cholesterol preparations to the extent of 1 to 2%, is not absorbed by man or by animals. Coprostanol, also, is non-absorbable.[122] The same is true of coprostenol,[118,122] which is isomeric with cholesterol[113,117,123,124]; this sterol cannot be absorbed by mice or dogs. Evans[125] reported that coprostenol could not be demonstrated in eggs after it had been fed to hens. Presumably, this sterol is not absorbed by the hen. Finally, Schönheimer[112,113,126] reported that all four isomers of cholestanol are non-absorbable. Thus, in the group of C_{27}-steroids, only cholesterol can be absorbed from the gastrointestinal tract.

Ergosterol is the sole common representative of the C_{28}-sterols to be investigated. Although Schönheimer *et al.*[127] were unable to demonstrate

[121] A. v. Kúthy; cited by F. Verzár and E. J. McDougall, *Absorption from the Intestine*, Longmans, Green, London-New York, 1936, p. 213.

[122] M. Bürger and W. Winterseel, *Z. physiol. Chem.*, *202*, 237–245 (1931).

[123] R. Schoenheimer, *Science*, *74*, 579–584 (1931).

[124] R. Schoenheimer, D. Dam, and K. von Gottberg, *J. Biol. Chem.*, *110*, 667–671 (1935).

[125] E. A. Evans, Jr., *J. Biol. Chem.*, *115*, 449–451 (1936).

[126] R. Schönheimer and H. v. Behring, *Z. physiol. Chem.*, *192*, 102–111 (1930).

[127] R. Schönheimer, H. v. Behring, and K. v. Gottberg, *Z. physiol. Chem.*, *208*, 77–85 (1932).

any deposition of ergosterol in rats, rabbits, mice, and dogs, after feeding this sterol to the animals over long periods of time, Page and Menschick[128] recorded some absorption of this sterol. These latter results were confirmed by Hanahan and Wakil[129] who found that 2 to 5% of orally administered C^{14}-ergosterol was absorbed within six hours, mainly through the lymph system. On the other hand, the evidence is more clearcut that ergosterol (provitamin D_2) when given in large amounts, can be absorbed by laying hens.[130,131] However, Bloor[87] reported that ergosterol could not be absorbed unless it had previously been irradiated (and presumably changed to vitamin D_2).

Isocholesterol, which is obtained from lanolin, and which has long been considered to be a C_{30}-sterol, is likewise nonabsorbable.[113] However, it is now recognized that agnosterol and lanosterol, of which isocholesterol is mainly composed, belong to the group of triterpenes rather than to that of the steroids.

It should be apparent from the above experiments that a selective absorption of the sterols obtains in the gastrointestinal tract; these data suggest that extremely small differences in molecular structure can largely determine the fate of a sterol. One possible explanation for the difference between cholesterol and the other sterols is the fact that the latter can apparently not be esterified after subcutaneous injection[111]; on the other hand, cholesterol is quickly esterified following subcutaneous administration. If a similar discrepancy in behavior between these two types of sterols also obtains in the intestinal mucosa, it is possible that this could account for the failure of the phytosterols and of other cholesterol esters to be absorbed.

(3) The Effect of Plant Sterols on the Absorption of Cholesterol

Although the experimental data are almost unanimous in indicating that the phytosterols are inert insofar as their absorption from the gastrointestinal tract is concerned, it has been suggested that the presence of these substances in the diet may influence the absorption of cholesterol from the small intestine. Peterson[132] was the first to show that, when soybean sterols were fed to chicks together with cholesterol, hypercholesterolemia was prevented, and the deposition of cholesterol in the liver

[128] I. H. Page and W. Menschick, *Biochem. Z.*, *221*, 6–10 (1930).
[129] D. J. Hanahan and S. J. Wakil, *Arch. Biochem. Biophys.*, *44*, 150–158 (1953).
[130] R. Schönheimer and H. Dam, *Z. physiol. Chem.*, *211*, 241–245 (1932).
[131] W. Menschick and I. H. Page, *Z. physiol. Chem.*, *211*, 246–252 (1932).
[132] D. W. Peterson, *Proc. Soc. Exptl. Biol. Med.*, *78*, 143–147 (1951).

was markedly decreased. In a later paper of Peterson, Nichols, and Shneour,[133] it was demonstrated that the inclusion of soy sterols in a high (1%) cholesterol diet, fed to chickens over a period of twenty-eight weeks, not only prevented the usual hypercholesterolemia, but also decreased the cholesterol deposition in the liver and the incidence of atherosclerosis. In a still later study of the Peterson group,[76] it was found that dietary soy sterols were able to prevent a progressive rise of plasma cholesterol and the deposition of cholesterol in the liver in chicks fed a diet containing 1% of cholesterol with 4% cottonseed oil, and that, furthermore, the addition of mixed sitosterols, β-sitosterol, stigmasterol, or ergosterol to the diet resulted in a similar inhibition. Moreover, when cholesterol was fed at a constant level and the soy sterols were introduced into the diet at varying levels, the optimum effect in depressing both plasma and liver cholesterol levels was obtained when the ratio of soy sterols to cholesterol was 2:1 or 3:1. Siperstein and co-workers[134] reported that cholestanol likewise prevents the increase of plasma cholesterol in chicks fed a high cholesterol diet.

Esterification of the soy sterols with capric acid destroyed their ability to prevent the increase in plasma and liver cholesterol caused by feeding cholesterol. Moreover, cholesteryl caprate was ineffective in producing the effects caused by the unesterified cholesterol. It was postulated that the inhibitory effect of soy sterols on cholesterol absorption may therefore be caused by an inhibition of an enzyme system concerned in cholesterol absorption which may involve the esterification of cholesterol.

(4) *Changes in Sterols in the Gastrointestinal Tract*

Although a considerable amount of ingested cholesterol may be excreted unchanged in the feces,[103] some of the sterol undergoes a transformation in the gastrointestinal tract. Popják and Beeckmans,[135] by the use of tracers, demonstrated that cholesterol is synthesized in the intestine. This sterol may be dehydrogenated in the intestine of the guinea pig to yield 7-dehydrocholesterol,[136] or it may be reduced to cholestanol, which is excreted in the feces.[137] Schönheimer and co-workers[126,138]

[133] D. W. Peterson, C. W. Nichols, Jr., and E. A. Shneour, *J. Nutrition*, *47*, 57–65 (1952).

[134] M. D. Siperstein, C. W. Nichols, Jr., and I. L. Chaikoff, *Circulation*, *7*, 37–41 (1953).

[135] G. Popják and M. L. Beeckmans, *Biochem. J.*, *47*, 233–238 (1950).

[136] M. Glover, J. Glover, and R. A. Morton, *Biochem. J.*, *51*, 1–9 (1952).

[137] A. Windaus and C. Uibrig, *Ber.*, *48*, 857–863 (1915).

[138] R. Schönheimer and L. Hrdina, *Z. physiol. Chem.*, *212*, 161–172 (1932).

have demonstrated that cholestanol cannot be absorbed from the intestine.

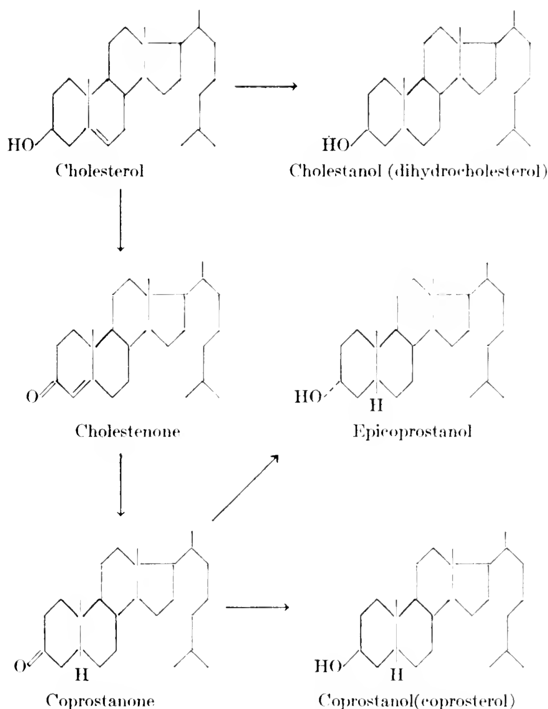
A second transformation of cholesterol in the intestinal tract is its conversion to coprostanol. Coprostanol has been known since 1862, when Austin Flint[139] separated it from human feces and showed that it differed from cholesterol. It was originally called *stercorine*,[140] and later *koprosterin*, by Bondzyński,[141] who rediscovered it in human feces. In the more recent literature it is referred to as *coprosterol*. The term *coprostanol*, which is gaining favor as the designation for this compound, is probably to be preferred, inasmuch as it connotes that the alcohol is saturated, and is isomeric with cholestanol. It has the same empirical formula as cholestanol, namely $C_{27}H_{48}O$, but it differs widely from the latter compound.

The most important evidence of the transformation of cholesterol into coprostanol is the demonstration that the amount of coprostanol in the feces is increased after the administration of cholesterol or cholestenone.[95,142–144] It was first believed that a direct reduction was mediated by intestinal bacteria, but this direct reaction was open to objection, since only cholestanol could be shown to originate.[143] Although Dam[98] recognized the importance of intestinal bacteria in coprostanol formation, he suggested that they act, not on cholesterol, but on an intermediate of this sterol.

An alternate pathway for the conversion of cholesterol to coprostanol has been postulated by Rosenheim and Webster,[145] and by Anchel and Schoenheimer.[146] This involves a conversion to cholestenone, which is reduced to coprostanone. Coprostanone can be reduced either to coprostanol or to epicoprostanol. These transformations are illustrated in the accompanying equations.

There are two pathways by which coprostanol can be synthesized in the laboratory. If coprostenol is reduced, equal amounts of coprostanol and cholestanol are formed.[147] Since no traces of coprostenol were reported in the organism by Schoenheimer *et al.*,[124] it is believed that this route must be excluded in the animal.

[139] A. Flint, *Am. J. Med. Sci.*, *44*, 305–365 (1862).
[140] A. Flint, *Z. physiol. Chem.*, *23*, 363–367 (1897).
[141] S. Bondzyński, *Ber.*, *29*, 476–478 (1896).
[142] R. Schoenheimer, D. Rittenberg, and M. Graff, *J. Biol. Chem.*, *111*, 183–192 (1935).
[143] G. Bischoff, *Biochem. Z.*, *227*, 230–236 (1930).
[144] C. Dorée and J. A. Gardner, *Proc. Roy. Soc. (London)*, *B80*, 227–239 (1908).
[145] O. Rosenheim and T. A. Webster, *Nature*, *136*, 474 (1935).
[146] M. Anchel and R. Schoenheimer, *J. Biol. Chem.*, *125*, 23–31 (1938).
[147] A. Windaus, *Ann.*, *453*, 101–112 (1927).



Some of the Biologic Transformations of Cholesterol Taking Place in the Intestine

The second method by which cholesterol yields coprostanol involves a preliminary conversion to cholestenone. The unsaturated bond of this ketone is reduced with palladium and hydrogen to yield coprostanone; the latter ketone is reduced to coprostanol with platinum and hydrogen.[148–150] This represents the biological method, as is indicated by the transformation of the intermediate products *in vivo* to coprostanol. In the first place, when cholestenone was administered to a dog on a meat diet,

[148] H. Grasshof, *Z. physiol. Chem.*, *223*, 249–251 (1934).
[149] H. Grasshof, *Z. physiol. Chem.*, *225*, 197–198 (1934).
[150] L. Ruzicka, A. Brüngger, E. Eichenberger, and J. Meyer, *Helv. Chim. Acta*, *17*, 1407–1416 (1934).

coprostanol was formed.[142] However, when the basal diet consisted of dog biscuits, cholesterol was found to be the excretion product of cholestenone.[142] In a later report, Anchel and Schoenheimer[146] demonstrated the conversion of deuteriocholestenone to deuteriocoprostanol in man. After deuteriocholestenone was fed to mice, the cholesterol contained no appreciable amount of deuterium; this may mean that cholestenone cannot be converted to cholesterol in mice, as is possible in the dog, or that the labile deuterium is lost during the reaction.

Proof that coprostanone is likewise an intermediate in the biological transformation of cholesterol to coprostanol has been obtained by the use of tagged molecules. Thus, when deuteriocoprostanone was fed to dogs[142] or to man,[146] the administration was followed by the excretion of deuteriocoprostanol in the feces. Another proof of the hypothesis that 3-ketone compounds are intermediates in the conversion of cholesterol to coprostanol is the fact that both epicoprostanol and coprostanol occur in the feces.[151]

Another change, analogous to the cholesterol → coprostanol reaction, is the conversion of β-sitosterol to 24-ethylcoprostanol.[119] Turfitt[152–154] demonstrated that a soil bacterium, *Proactinomyces* spp., especially *P. erythropolis*, oxidizes cholesterol to cholestenone; following this, the side chain is partially ruptured, resulting in the production of $\Delta^{4,5}$-3-keto-etiocholanic acid, and Ring A is split between C_3 and C_4 to yield a keto-carboxylic acid.[155] There is no indication that intestinal bacteria can bring about a similar degradation of cholesterol.

(5) *The Transport of Cholesterol from the Gut*

As early as 1916, Mueller[63,64] demonstrated that cholesterol is readily absorbed from the intestine of the dog, and that it passes into the lymphatics, where it can be detected in the thoracic duct lymph. It was found that the proportion of cholesterol esterified in the lymph is the same, irrespective of whether the cholesterol is given in free or in ester form. Biggs, Friedman, and Byers[156] reported that exogenous cholesterol is conveyed into the systemic circulation *via* the lymphatics into the

[151] R. E. Marker, E. L. Wittbecker, R. B. Wagner, and D. L. Turner, *J. Am. Chem. Soc.*, *64*, 818–822 (1942).

[152] G. E. Turfitt, *Biochem. J.*, *38*, 492–496 (1944).

[153] G. E. Turfitt, *J. Bacteriol.*, *47*, 487–493 (1944).

[154] G. E. Turfitt, *Biochem. J.*, *40*, 79–81 (1946).

[155] G. E. Turfitt, *Biochem. J.*, *42*, 376–383 (1948).

[156] M. W. Biggs, M. Friedman, and S. O. Byers, *Proc. Soc. Exptl. Biol. Med.*, *78*, 641–643 (1951).

thoracic duct. They were unable to demonstrate the transport of any appreciable quantity by way of the portal venous system. Chaikoff and co-workers[157] were able to account quantitatively for the absorbed cholesterol in the lymph of rats. Thus, when 3.5 mg. of ring-labeled cholesterol dissolved in 0.5 ml. of corn oil was given to rats by stomach tube, from 22 to 49% was shown to be absorbed. These workers were able to recover 70 to 90% of that absorbed in lacteal lymph, and 94 to 101% in the thoracic duct lymph. About 50% of the cholesterol in the lymphatics was shown to be esterified.[69,157] It is suggested that the esterification of dietary cholesterol takes place during its passage from the lumen to the site of lymph collection. Swell and collaborators[83] reported that the pancreas is the major source of the cholesterol esterase of the rat intestinal mucosa.

6. The Digestion and Absorption of Hydrocarbons

(1) *Paraffins*

In all of the earlier work on the absorption of paraffins, the experimental data were interpreted to mean that none was absorbed. This conclusion was based upon the ability to recover administered hydrocarbons practically quantitatively from the feces, and also upon the failure to find any trace of such substances in the chyle. Thus, Henriques and Hausen[158] were able to recover 95% of the vaselin administered in a vaselin-lard mixture from the feces of rats. Furthermore, Bloor[159] obtained 85 to 100% of the hydrocarbons in the feces after giving a liquid hydrocarbon mixture, and also vaselin alone or in emulsions in olive or coconut oil. Connstein[160] had earlier reported a quantitative recovery, from the feces of a dog, of 20 g. of lanolin which had previously been fed. However, lanolin consists of sterols and triterpenes, which should not be confused with the aliphatic hydrocarbons. Moreover, Bloor[159] was unable to demonstrate the presence of any absorbed hydrocarbon in the chyle. Clark and Clark[161] and Clark alone[162] reported a similar rejection of hydrocarbons when they were injected into the tails of larvae of the bullfrog (*Rana catesbiana*) and Fowler's toad (*Bufo lentiginosus Fowleri*). Fi-

[157] I. L. Chaikoff, B. Bloom, M. D. Siperstein, J. Y. Kiyasu, W. O. Reinhardt, W. G. Dauben, and J. F. Eastham, *J. Biol. Chem.*, *194*, 407–412 (1952).

[158] V. Henriques and C. Hausen, *Zentr. Physiol.*, *14*, 313–316 (1900).

[159] W. R. Bloor, *J. Biol. Chem.*, *15*, 105–117 (1913).

[160] W. Connstein, *Arch. Physiol.*, *1899*, 30–32.

[161] E. R. Clark and E. L. Clark, *Am. J. Anat.*, *21*, 421–448 (1917).

[162] E. R. Clark, *Anat. Record*, *10*, 191–192 (1915–1916); *11*, 1–17 (1916–1917).

nally, Lundbaek and Maaløe[163] were unable to detect absorption of liquid paraffin when it was injected, in an emulsified state, into the duodenum of rats fasted for twenty-four hours. Among the earlier workers, Bradley and Gasser[164] were the only ones to express the opinion that paraffin oil can be absorbed if it is well emulsified. In spite of these findings, the general opinion held until recently has been that hydrocarbons cannot be absorbed.

More recent work has forced a revision of our concepts on this subject. One is forced to conclude from the results of Channon and his collaborators[165–167] that small amounts of the paraffins can be absorbed and deposited in the tissues. Thus, it was shown that liquid paraffins were absorbed from the gastrointestinal tract of the rat and the pig, as demonstrated by the small but unmistakable increase in the non-cholesterol fraction of the unsaponifiable extract of liver fat after paraffin oil was included in the diet.[166] The experiments demonstrate the role of the liver in storing unsaponifiable substances. That this positive result was not to be attributed to the polycyclic nature of the paraffin hydrocarbons was demonstrated by El Mahdi and Channon,[167] who obtained a similar result with a purified synthetic *n*-hexadecane ($CH_3(CH_2)_{14}CH_3$), in the rat. Channon and Devine[165] were likewise able to duplicate this result with purified hexadecane in the cat; moreover, they succeeded in isolating the hydrocarbon from the omentum, perirenal fat, muscle, and skin, although they were unable to prepare it from the liver, where it was probably present only in traces. The experimental evidence of Channon and co-workers that hydrocarbons are absorbed has been given strong support by the findings of Stetten.[168] Thus, it was noted that, when *n*-hexadecane into which deuterium had been incorporated was fed to rats in doses of 83 mg./day, it was very efficiently absorbed from the gastrointestinal tract, and was partially deposited as such in the tissue lipids. It was likewise observed that the absorbed hexadecane was oxidized to fatty acid in the body. Apparently this change takes place in the liver. Additional confirmation of the absorption of liquid paraffin by rats has been obtained in the experiments of Daniel, Frazer, French, and Sammons.[169] When a 50% solution of liquid paraffin in olive oil was fed to

[163] K. Lundbaek and O. Maaløe, *Acta Physiol. Scand.*, *13*, 247–252 (1947).
[164] H. C. Bradley and H. S. Gasser, *J. Biol. Chem.*, *11*, xx (1912).
[165] H. J. Channon and J. Devine, *Biochem. J.*, *28*, 467–471 (1934).
[166] H. J. Channon and G. A. Collinson, *Biochem. J.*, *23*, 676–688 (1929).
[167] M. A. H. El Mahdi and H. J. Channon, *Biochem. J.*, *27*, 1487–1494 (1933).
[168] De W. Stetten, Jr., *J. Biol. Chem.*, *147*, 327–332 (1943).
[169] J. W. Daniel, A. C. Frazer, J. M. French, and H. G. Sammons, *Biochem. J.*, *54*, xxxvii–xxxviii (1953).

rats, over 60% of the paraffin was absorbed over a period of three hours. The hydrocarbon, fed in olive oil, could be isolated from the unsaponifiable fraction of the lipids of the intestinal mucosa. It was suggested that the paraffin was metabolized *in situ*, or that an active transport *via* the lymph occurred. The latter suggestion was supported by the demonstration of a wax in lymph after paraffin had been administered which was not present after olive oil alone was given, and by the demonstration of paraffin oil to the extent of 0.4% of the dry weight of the liver in rats fed for fifteen months on a diet containing 10% of paraffin in olive oil.

The fact that a number of saturated aliphatic hydrocarbons have been shown to form coordination compounds with desoxycholic acid would seem to substantiate our evidence that these substances are absorbed. Thus, choleic acids composed of 8 molecules of bile acid and one of the acholic component have been reported for pentadecane (C_{15}), hexadecane (C_{16}), pentatriacontane (C_{35}), and tritetracontane (C_{43}), while a coordination compound with 6 molecules of bile acids has been obtained with undecane (C_{11}).[170]

Another fact which indicates that the aliphatic hydrocarbons have a physiological significance is their widespread distribution in plant products. Thus, *n*-eicosane (C_{20}) has been found in red-berry bryony oil (*Bryonia dioica*),[171] in Grecian laurel berry fat (*Laurus nobilis*),[172] and parsley seed oil (*Petroselinum latifolium* (*sativum*)),[173] while *n*-heptacosane (C_{27}) has been reported in apple cuticle wax.[174] *n*-Nonacosane (C_{29}) has been identified in cabbage lipids,[175,176] in brussels sprouts,[177] and in the waxes of apple peel,[178,179] pear,[180] grapefruit peel,[181] and Bing cherry skin.[182] Other saturated hydrocarbons include *n*-hentriacontane (C_{31}), found in brussels

[170] H. Rheinboldt, H. Pieper, and P. Zervas, *Ann.*, *451*, 256–273 (1927).
[171] A. Étard, *Compt. rend.*, *114*, 364–366 (1892).
[172] H. Matthes and H. Sander, *Arch. Pharm.*, *246*, 165–177 (1908).
[173] H. Matthes and W. Heintz, *Ber. deut. pharm. Ges.*, *19*, 325–329 (1909).
[174] A. C. Chibnall, S. H. Piper, A. Pollard, J. A B. Smith, and E. F. Williams, *Biochem. J.*, *25*, 2095–2110 (1931); with P. N. Sahai, *Ibid.*, *28*, 2189–2208 (1934).
[175] H. J. Channon and A. C. Chibnall, *Biochem. J.*, *23*, 168–175 (1929).
[176] D. L. Collison and I. Smedley-MacLean, *Biochem. J.*, *25*, 606–613 (1931).
[177] P. N. Sahai and A. C. Chibnall, *Biochem. J.*, *26*, 403–412 (1932).
[178] K. S. Markley and C. E. Sando, *J. Biol. Chem.*, *101*, 431 (1933).
[179] K. S. Markley, S. B. Hendricks, and C. E. Sando, *J. Biol. Chem.*, *98*, 103–107 (1932).
[180] K. S. Markley, S. B. Hendricks, and C. E. Sando, *J. Biol. Chem.*, *111*, 133–146 (1935).
[181] K. S. Markley, E. K. Nelson, and M. S. Sherman, *J. Biol. Chem.*, *118*, 433–441 (1937).
[182] K. S. Markley and C. E. Sando, *J. Biol. Chem.*, *119*, 641–645 (1937).
[183] J. Ozaki, *J. Agr. Chem. Soc. Japan*, *6*, 773–782 (1930); *Chem. Abst.*, *25*, 2754 (1931).

sprouts,[177] cabbage lipids,[183] spinach lipids,[176] the waxes of grapefruit peel,[181] and in the Chinese wax insect (*Coccus ceriferus*),[184] as well as *n*-pentatriacontane (C_{35}) reported in sugar-cane wax.[185]

(2) *Unsaturated Hydrocarbons*

There are a number of unsaturated hydrocarbons which have a wide distribution in the tissues of marine animals. Whether these hydrocarbons originate in the liver of these animals, or whether they are absorbed as such and stored in the liver, is not certain.

Squalene, $C_{30}H_{30}$, is one of the commonest of the unsaturated hydrocarbons. This unsaturated hydrocarbon was first isolated in 1906 from the liver oil of a black shark of the genus *Zameus*, by Tsujimoto.[186] It was later shown to occur in the liver oils of sixteen of thirty-six species of elasmobranch fishes, all from Japanese waters.[187] Although squalene is found almost exclusively in marine organisms, it has been reported from several plant sources. Thus, Thorbjarnarson and Drummond[188] found that the non-saponifiable fraction of Palestinian, Tunisian, Spanish, and Turkish olive oils contained 31 to 64% of this hydrocarbon. It is also present in yeast fat[189] but not in tea-seed oil.[188]

Other unsaturated hydrocarbons present in fish liver oils include pristane, $C_{18}H_{38}$, from the basking shark (*Cetorhinus maximus*),[190,191] and other fishes,[192] gadusene, $C_{18}H_{32}$, from the Japanese ishinagi (*Stereolepis ishinagi*),[193] and zamene, $C_{18}H_{36}$, from the basking shark.[190] Gadusene also occurs in wheat germ oil,[194] rice germ oil, and soybean oil.[195] Peanut oil contains hypogene, $C_{15}H_{30}$, and arachidene, $C_{19}H_{38}$,[196] while at least

[184] F. J. E. Collins, *J. Soc. Chem. Ind.*, *54*, 33–35T (1935).

[185] N. L. Vidyarthi and M. Narasingarao, *J. Indian Chem. Soc.*, *16*, 135–143 (1939).

[186] M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, *9*, 953 (1906); *Ind. Eng. Chem.*, *8*, 889–896 (1916).

[187] M. Tsujimoto, *Ind. Eng. Chem.*, *12*, 63–73 (1920).

[188] T. Thorbjarnarson and J. C. Drummond, *Analyst*, *60*, 23–29 (1935).

[189] K. Täufel, H. Thaler, and H. Shreyegg. *Z. Untersuch. Lebensm.*, 72, 394–404 (1936).

[190] M. Tsujimoto, *Bull. Chem. Soc. Japan*, *10*, 144–148, 149–153 (1935); *J. Chem. Soc. Japan*, *55*, 702–741 (1934); *Chem. Abst.*, *28*, 6484 (1934).

[191] M. Tsujimoto, *Ind. Eng. Chem.*, *9*, 1098–1099 (1917).

[192] Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind. Japan*, *38*, suppl., 254–258 (1935).

[193] M. Tsujimoto, *Bull. Chem. Soc. Japan*, *6*, 237–239 (1931); *Chem. Abst.*, *26*, 612–613 (1932).

[194] J. C. Drummond, E. Singer, and R. J. MacWalter, *Biochem. J.*, *29*, 456–471 (1935).

[195] Z. Nakamiya, *Sci. Papers Inst. Phys. Chem. Research, Tokyo*, *28*, 16–26 (1935).

[196] H. Marcelet, *Bull. soc. chim.*, [5], *3*, 1156–1160, 2055–2057 (1936); *Compt. rend.*, *202*, 867–869, 1809–1811 (1936).

eight different compounds have been reported from olive oil, ranging in composition from $C_{13}H_{24}$ to $C_{28}H_{50}$.[196,197] For a more complete discussion of these hydrocarbons, the reader is referred to Volume I (pages 400–404) of this monograph.

Channon[198] has demonstrated that squalene is absorbed by the rat and appears in the liver and body fat as a component of the nonsaponifiable extract. The storage of squalene in the liver and tissues is only temporary, since it begins to decrease as early as eight days after the rats are transferred to the control diet.

(3) *Carcinogenic and Related Non-carcinogenic Hydrocarbons*

A number of hydrocarbons and related compounds have been found to be potent carcinogenic agents. Cook and Haslewood,[199] in 1934, demonstrated that methylcholanthrene is capable of initiating malignant growth in test animals. It is now generally recognized that this hydrocarbon is the most potent carcinogenic agent known. It is able to produce tumors of various types. Methylcholanthrene has been synthesized by an anomalous degradation of cholesterol.[200] It was also produced in a 5.4% yield by Fieser and Newman[201] as a degradation product of cholic acid, through dehydrocholic acid and 3,7-dihydroxy-12-ketocholanic acid; when desoxycholic acid was degraded, the yield was 4.3%. Since it is possible to synthesize it in the laboratory from such natural products as cholesterol and bile acids, Fieser and Fieser[202] suggest that this substance may arise in the body through a process of abnormal metabolism, and so may initiate cancer. However, the degradations of desoxycholic acid, cholic acid, and cholesterol have not been effected under conditions which approach physiologic ones. Moreover, there is no proof that methylcholanthrene or any other hydrocarbon carcinogen plays any role in the etiology of human cancer.[202] It is possible, however, that a trace of methylcholanthrene could produce a few malignant cells which, by cell division and without further stimulation by the proliferating agent, could initiate the abnormal metabolism resulting in the formation of considerable cancerous tissue. This might follow an induction period during which the original exciting agent had been eliminated from the body.

[197] G. Sani, *Atti accad. Lincei*, *12*, 238–242 (1930).
[198] H. J. Channon, *Biochem. J.*, *20*, 400–408 (1926).
[199] J. W. Cook and G. A. D. Haslewood, *J. Chem. Soc.*, *1934*, 428–453.
[200] W. Rossner, *Z. physiol. Chem.*, *249*, 267–274 (1937).
[201] L. F. Fieser and M. S. Newman, *J. Am. Chem. Soc.*, *57*, 961 (1935).
[202] L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949.

Acenaphthene was shown by Chang and Young[203] to be absorbed by adult white rats when fed at a 1% level in the diet. Thus, it was observed that the material was excreted in the urine either as naphthalene-1,8-dicarboxylic acid or, possibly, as naphthalic acid. It is also possible that the end-product may have been excreted as a conjugated compound which was decomposed by acid to yield naphthalic acid or naphthalic anhydride. The formation of the naphthalic acid involves the rupture of the 5-carbon ring. Since the acenaphthene structure forms an integral part of the cholanthrene molecule, the question arises as to whether or not the 5-membered ring in cholanthrene or in methylcholanthrene undergoes fission in the body. The possible intermediary changes are given below:

$H_2C—CH_2$ → HOOC COOH → O=C-O-C=O

Acenaphthene → Naphthalene-1,8-dicarboxylic acid → Naphthalene-1,8-dicarboxylic acid anhydride

Possible Metabolism of Acenaphthene in the Rat[203]

In a later study, Chang[204] investigated the digestibility of a number of carcinogenic and noncarcinogenic polycyclic hydrocarbons, both when the

TABLE 6

DIGESTIBILITY COEFFICIENTS OF CARCINOGENIC AND NON-CARCINOGENIC HYDROCARBONS IN RATS[a]

Test substance	Coefficient of digestibility[b]	
	Test substance in diet	Test substance by stomach tube
Naphthalene	100	100
Acenaphthene	94	90(87,94)
Anthracene	27(16,18,47)	31(26,36)
Phenanthrene	95(94,96)	94(93,94)
Chrysene	24(15,19,28,36)	16(13,18)
1,2,5,6-Dibenzanthracene	12(9,10,11,19)	4(0,7)
3,4-Benzpyrene	58(56,56,62)	44(42,45)
Methylcholanthrene	33(28,30,42)	32(31,32)

[a] Adapted from L. H. Chang, *J. Biol. Chem.*, *151*, 93–99 (1943).
[b] Figures in parentheses are individual determinations.

[203] L. H. Chang and L. Young, *J. Biol. Chem.*, *151*, 87–91 (1943).
[204] L. H. Chang, *J. Biol. Chem.*, *151*, 93–99 (1943).

test substances were administered by stomach tube to rats and when they were incorporated in the diet. Table 6 summarizes these data.

With the exception of 1,2,5,6-dibenzanthracene, which was not absorbed in any appreciable amount, and of chrysene, anthracene, and methylcholanthrene, which were utilized to an average extent of 20, 29, and 32%, respectively, the other polycyclic hydrocarbons tested were absorbed to a large degree from the gastrointestinal tract of the rat.

According to Weil-Malherbe and Dickens[205] the rate of elimination of 3,4-benzpyrene is affected by the solvent employed; the incidence of sarcoma was found to be dependent upon the rate of absorption. When the carcinogen was dissolved in synthetic tricaprylin in the presence of cholesterol, a higher rate of elimination and a higher tumor incidence were found to occur in mice. In the presence of phospholipids, a slower elimination of the carcinogen occurred, and the latent period of the sarcomas was extended. In a later study,[206] it was found that not only cholesterol, but also cholestanol, sitosterol and α-tocopherol caused acceleration of the elimination rate of 3,4-benzpyrene; on the other hand, ascorbyl palmitate had an inhibitory effect on the elimination of the administered carcinogen. Epicholestanol, coprostanol, epicoprostanol, stigmasterol, 7-dehydrocholesterol and ergosterol were found to be inactive. Dickens and Weil-Malherbe[207] suggest that high tumor incidence is primarily connected with the rate of oxidative metabolism of the carcinogen. The higher the rate of metabolism, the greater the carcinogenic action. A high rate of metabolism is directly related to the rate of elimination. Fat itself when used as a solvent for the subcutaneous injection of carcinogens may inhibit carcinogenesis,[207,208] although the anticarcinogenic action of mouse fat was shown to be lost after three years of storage.[207] Peacock and Beck[208] attribute the anticarcinogenic action of fat to an increased rate of elimination of the carcinogen.

Peacock[209] noted that, after the injection of the carcinogenic agents, 3,4-benzpyrene and methylcholanthrene, or of the non-carcinogenic compound, anthracene, into rabbits, guinea pigs, fowl, or mice, these agents are eliminated in the bile as fluorescent compounds or occasionally in the feces in unchanged form. Chalmers[210] proved that the bile, feces, and urine of mice contain a fluorescent compound, called BPX, after the

[205] H. Weil-Malherbe and F. Dickens, *Cancer Research*, *6*, 171–178 (1946).
[206] H. Weil-Malherbe, *Biochem. J.*, *42*, xxxiii (1948).
[207] F. Dickens and H. Weil-Malherbe, *Biochem. J.*, *39*, xxxix (1945).
[208] P. R. Peacock and S. Beck, *Brit. J. Exptl. Pathol.*, *19*, 315–319 (1938).
[209] P. R. Peacock, *Brit. J. Exptl. Pathol.*, *17*, 164–172 (1936).
[210] J. G. Chalmers, *Biochem. J.*, *32*, 271–278 (1938).

intravenous injection of colloidal solutions of 3,4-benzpyrene, methylcholanthrene, or anthracene. BPX is probably a monohydroxypyrene.

In a later study[211] it was found that none of ten related polycyclic hydrocarbons was eliminated unchanged in fowl bile in recognizable amounts after their intravenous injection. However, the following gave rise to fluorescent compounds in the bile: 1,2,5,6-dibenzanthracene, cholanthrene, methylcholanthrene, anthracene, pyrene, 2,6-dimethyl-1,2-benzanthracene, 2,7-dimethyl-1,2-benzanthracene, and fluoranthene. On the other hand, 3,4-benzphenanthrene and phenanthrene did not give rise to fluorescent compounds in the bile. Shear investigated the carcinogenic action, in mice, of isomers of cholanthrene and methylcholanthrene,[212] of 2-amino-5-azotoluene,[213] and also of a number of anthracene derivatives.[214]

7. The Digestion, Absorption, and Transformation of the Carotenoids in the Gastrointestinal Tract

The carotenoids include both those compounds which are exclusively hydrocarbons and those classed as oxycarotenoids; the latter may be alcohols, oxides, aldehydes, or ketones. Some of the members of each class are provitamins A. Thus, the hydrocarbons, α-, β-, and γ-carotenes (but not lycopene) belong in this category, while cryptoxanthin is the principal oxygen-containing carotenoid which gives rise to vitamin A.

Several types of criteria can be employed to study the absorption of the carotenoids. In the first place, the only procedure which will yield quantitative results is the direct method, in which one determines absorption by the rate at which the carotenoid leaves the lumen of the gut. The indirect procedure, in which one observes the carotenoid content of the blood following the administration of the test substance, is of some value but lacks a quantitative interpretation. Moreover, this procedure is impossible for the carotenoids which are broken down in the intestinal wall, as obviously no appreciable concentrations of such substances are found in the blood before or after feeding them.

In the case of carotenoids which are convertible to vitamin A (α-, β-, and γ-carotenes, cryptoxanthin), another criterion of absorption is possible. One can determine the increase in vitamin A in the blood and in the tissues such as the liver, or in such excretion products as milk and eggs.

[211] J. G. Chalmers and P. R. Peacock, *Biochem. J.*, *35*, 1276–1282 (1941).
[212] M. J. Shear, *J. Biol. Chem.*, *114*, lxxxix–xc (1936).
[213] M. J. Shear, *J. Biol. Chem.*, *114*, xc–xci (1936).
[214] M. J. Shear, *J. Biol. Chem.*, *123*, cviii–cix (1938).

One may likewise use a vitamin A bioassay to determine to what extent the material yields vitamin A. However, these latter procedures are of little value in determining absorption, in view of the fact that such small quantities of the compound are involved.

The various technics outlined above which concern the determination of vitamin A as the proof of absorption of the carotenoid are a measure of at least two factors; these are the rate of absorption of the carotenoid and the speed of transformation of absorbed carotenoid to vitamin A. If the synthesis of vitamin A were to occur in the lumen of the gut rather than in the intestinal wall, then these last procedures would not be of any value in proving the absorption of the provitamins A. However, the best evidence indicates that the conversion of carotenoids to vitamin A is a function of the wall of the intestine and not of its lumen. Vitamin A determinations are of no value in assessing the absorption of carotenoids which are not provitamins A.

Still another method which has been used for the estimation of carotenoid absorption is to determine the excretion of these substances in the feces. Knowing the amount of the test substance fed, one can calculate the quantity of material which has disappeared during its passage through the gastrointestinal tract, either by absorption or by decomposition. This procedure will be of no value if the carotenoid is synthesized in the intestine. Although such a phenomenon is probably extremely rare, McGillivray[215] has presented evidence that an appreciable synthesis of carotene may occur in the ileum and cecum of the sheep. If this also occurs in cattle, it would explain the earlier report of Whitnah *et al.*[216] that the excretion of carotenoids in the feces exceeded the quantity taken in the food. Finally, this last technic is not an index of *rate* of absorption but rather of *completeness of utilization* or *digestibility*.

Barrick and co-workers[217] reported that no absorption of carotene occurs in the cecum or colon. The jejunum and upper part of the ileum are the areas of most active absorption of carotenoids, although some absorption may also occur from the lower part of the ileum; McGillivray[215] reported carotene synthesis in this area, as well as in the cecum.

(1) The Absorption of Carotenoids from the Intestine

a. The Absorption of β-Carotene. Because β-carotene is the commonest and most widely distributed of the provitamins A and, in fact, of the caro-

[215] W. A. McGillivray, *Brit. J. Nutrition*, *5*, 223–228 (1951).

[216] C. H. Whitnah, W. J. Peterson, F. W. Atkeson, and H. W. Cave, *J. Agr. Research*, *58*, 343–355 (1939).

[217] E. R. Barrick, F. N. Andrews, and J. F. Bullard, *J. Animal Sci.*, *7*, 539 (1948).

tenoids as a whole, more information is available concerning its absorption and metabolism than is the case with any other carotenoid. Since carotenes are unsaturated hydrocarbons with aliphatic chains somewhat resembling those of squalene, one might expect them to be absorbed and deposited in the liver in a somewhat similar manner.

A number of observations indicate that carotene can be absorbed from the gastrointestinal tract. Thus, the color of the milk and of the butterfat made from it is to a large extent directly dependent upon the carotene present in the feed. The same is to some degree also true for egg yolk, although in this case carotenoids other than β-carotene may contribute a large proportion of the chromogenic material. The same relationship exists between dietary carotenoids and plasma carotenoids, particularly in the case of man and cattle. The abnormal condition in man referred to as *carotenemia,* which resembles jaundice in appearance, is due to the unusual deposition of carotene under the skin. This excess carotene content of the blood and tissues is directly related to an excessive absorption of the pigment due to the continued high consumption of such carotene-high vegetables as Hubbard squash and carrots.

There is likewise a considerable amount of indirect evidence of the high absorbability of β-carotene, at least when it is fed in small amounts to the rat. The fact that exceedingly small amounts of β-carotene can furnish the necessary vitamin A for the animal organism demonstrates its exceedingly efficient absorption and transformation into vitamin A. It is generally agreed that, on the weight basis, the requirement for β-carotene as compared with that for vitamin A does not exceed a factor of 2:1. Since vitamin A is known to be completely absorbed, a weight factor of 2:1 would denote absorption of a minimum of 50% of the β-carotene, provided that β-carotene were transformed quantitatively to vitamin A. However, a number of workers have suggested that a higher percentage of carotene is converted to vitamin A than would be indicated by a weight relationship of 2:1. Moreover, if one assumes that, within the organism, the maximum possible yield of vitamin A does not result from carotene, then the minimum absorption value of 50% must be appreciably increased and, in all probability, may approximate 100%.

Other indirect methods have likewise been used to study the absorption of β-carotene. These include quantitative bioassay based upon the amount of vitamin A laid down in the liver. Another procedure involves the determination of the effect of ingested carotene on the level of blood carotene. However, the latter test can be used only in the case of ani-

mals, such as man, cattle, and dogs, in which carotene is present as such in the blood.

Although it is generally agreed that carotene cannot be absorbed in the stomach, it is believed that the small intestine is the principal site for absorption. Ronning and Knodt[218] reported that a low concentration of carotene obtains in the middle third of the small intestine after a single administration of this compound to young male Holstein calves. While it is possible that this low concentration of carotene in the wall of the gastrointestinal tract may be evidence of carotene conversion to vitamin A in this location, this hypothesis must be discounted, since it was not possible to demonstrate concurrent increases in vitamin A under these conditions.

(a) *Factors That Affect the Absorption of β-Carotene.* Although the investigation of the factors that influence the absorption of carotenoids has been largely carried out with β-carotene, it seems probable that these conditions may likewise be applicable to the absorption of other carotenoids. Since these studies have been almost entirely carried out with β-carotene, they are listed under this compound.

a′. Surface Area and the Absorption of β-Carotene: Shaw and Deuel[219] have shown that the absorption of β-carotene, fed in cottonseed oil, to rats is a function of surface area. After the feeding of massive doses of approximately 4 mg. (4000 μg.) in a single dose, β-carotene was absorbed at the rate of approximately 110 μg. per 100 sq. cm. per hour. After twelve hours, less than 20% of the carotenoid could be isolated from the lumen of the gut; however, at twelve and at eighteen hours, approximately 50% of the administered dose could be recovered from the wall of the intestine. After forty-two hours, almost 20% of the carotenoid fed could still be extracted from the gut wall. In view of our more recent knowledge that carotenes are converted to vitamin A in the wall of the intestine of the rat, the high concentration in this tissue over a prolonged period is not surprising. Vavich and Kemmerer[220] reported that the size of the rats markedly influences the extent of utilization of carotene for vitamin A storage in the liver. Thus, when 60 μg. of β-carotene equivalents were fed, the combined amount of vitamin A stored in the liver and kidneys was higher in rats weighing approximately 50 g. than it was in groups of rats averaging 100 g. in weight. The fate of this carotene is discussed later (see page 303).

[218] M. Ronning and C. B. Knodt, *J. Dairy Sci.*, *35*, 283–291 (1952).
[219] R. J. Shaw and H. J. Deuel, Jr., *J. Nutrition*, *27*, 395–401 (1944).
[220] M. G. Vavich and A. R. Kemmerer, *J. Nutrition*, *40*, 605–610 (1950).

b′. The Effect of Bile on the Absorption of β-Carotene: One of the important conditions controlling absorption is the presence of bile. In any condition in which there is an hepatic dysfunction, such as an obstructive jaundice, or in any other situation in which biliary flow is impaired, the absorption of carotene and, in fact, of all other fat-soluble vitamins (except vitamin A), is greatly decreased. The bile salts have been shown to be the active agents, present in bile, effecting the absorption of carotene.

Greaves and Schmidt[221] proved that bile acids such as glycodesoxycholic and desoxycholic acids are required for the absorption of carotene in vitamin A-deficient, choledochocolostomized rats, as judged by a bioassay for vitamin A based upon the vaginal smear technic. Orally administered carotene proved to be ineffective in overcoming the vitamin A deficiency in such animals, although vitamin A itself gave positive results. When bile salts were administered in the diet of the operated rats, carotene was absorbed. In later work,[222] it was shown, in confirmation of these earlier results, that carotene could not be utilized in jaundiced rats or in those in which the liver injury was produced by phosphorus poisoning, although vitamin A was still effective. Similar convincing experiments, on isolated intestinal loops of dogs, have also been reported by Irvin *et al.*[223] Insignificant amounts of the provitamin were absorbed when carotene was introduced into the intestinal loops in cottonseed oil solution. However, when either gall-bladder bile (from ox or hog) or pancreatic lipase was given concomitantly, considerable amounts of carotene were utilized. Best results were obtained when bile salts and pancreatic lipase were both added to the intestinal loop along with the cottonseed oil solution of carotene. One may assume that the bile salts produce their effect on carotene absorption by the formation of a choleic acid-like compound with carotene.

c′. The Effect of Simultaneous Fat Feeding on the Absorption of β-Carotene: Another factor of considerable importance in effecting carotene absorption is the nature of the solvent in which the carotenoid is dissolved.[224,225] Thus, when the provitamin A is dissolved in fat, utilization may occur whereas, in the absence of fat, the carotenoid may not be absorbed from the intestine. Both rats and cats were shown by Ahmad[226]

[221] J. D. Greaves and C. L. A. Schmidt, *Am. J. Physiol.*, *111*, 492–501 (1935).
[222] J. D. Greaves and C. L. A. Schmidt, *Am. J. Physiol.*, *111*, 502–506 (1935).
[223] J. L. Irvin, J. Kopala, and C. G. Johnston, *Am. J. Physiol.*, *132*, 202–209 (1941).
[224] E. J. Lease, J. G. Lease, H. Steenbock, and C. A. Baumann, *J. Nutrition*, *17*, 91–102 (1939).
[225] H. C. Sherman, *J. Nutrition*, *22*, 153–165 (1941).
[226] B. Ahmad, *Biochem. J.*, *25*, 1195–1204 (1937).

to be unable to absorb carotene on a fat-low diet, although an almost complete utilization of the provitamin obtained when 10% of fat was incorporated in the diet. These conclusions have been confirmed, for both animal and human subjects, by a number of investigators[227–231]; Shaw and Deuel[219] observed a correlation between the quantities of fat and of carotene absorbed by rats. The same dietary requirement for the utilization of carotene also obtains for hens as for other animals, according to Russell and co-workers.[232] Fraps and Meinke[233] found that carotene fed in the form of vegetables was less effective than when given as a component of butterfat or of beef liver. Between 25 and 45% of carotene from raw spinach or grated carrots can be absorbed by man,[234] while the absorption from the cooked material was found to be considerably less, *i.e.*, 2 to 12%. It has also been reported, in the case of man, that carotene is biologically more effective when given in butter than when administered as a component of carrots.[235] On the other hand, Randoin *et al.*[236] reported that rats can utilize β-carotene and vitamin A as well on a fat-free diet as on one containing 10% of peanut oil. These workers[237] likewise reported a greater efficiency in the utilization of carotene when it was mixed in the diet than when it was given as a supplement in an oily solution. This result is interpreted to mean that some dietary component may have a protective action on carotene in the gastrointestinal tract. Thus, Molander[238] noted that carotene dissolved in corn oil or in mineral oil could be demonstrated by examination of human serum when the particle size was 0.5 μ. On the other hand, it was reported that the corn oil fatty acids do not carry carotene efficiently from the gastrointestinal tract to the tissues, but rather carry the chromogen to the liver. These experiments would seem to indicate that carotene may gain entrance to

[227] N. K. Basu, *Z. Vitaminforsch.*, *6*, 106–110 (1937).

[228] H. E. C. Wilson, S. M. Das-Gupta, and B. Ahmad, *Indian J. Med. Research*, *24*, 807–811 (1937).

[229] M. van Eekelen and W. Pannevis, *Nature*, *141*, 203–204 (1938).

[230] A. R. Kemmerer and G. S. Fraps, *J. Nutrition*, *16*, 309–315 (1938).

[231] S. Y. Thompson, R. Braude, A. T. Cowie, J. Ganguly, and S. K. Kon, *Biochem. J.*, *44*, ix–x (1949).

[232] W. C. Russell, M. W. Taylor, H. A. Walker, and L. J. Polskin, *J. Nutrition*, *24*, 199–211 (1942).

[233] G. S. Fraps and W. W. Meinke, *Food Research*, *10*, 187–196 (1945).

[234] W. van Zeben, *Z. Vitaminforsch., Sonderabdruck*, *17*, 74–84 (1946).

[235] M. Kreula and A. I. Virtanen, *Upsala, Läkarefören. Förh.*, *45*, 355–362 (1939); *Chem. Abst.*, *34*, 5897 (1940).

[236] L. Randoin, D. Hugot, and J. Causeret, *Compt. rend. soc. biol.*, *145*, 65–68 (1951).

[237] L. Randoin, D. Hugot, and J. Causeret, *Compt. rend. soc. biol.*, *145*, 68–70 (1951).

[238] D. W. Molander, *Yale J. Biol. Med.*, *21*, 201–210 (1949).

the blood stream in man in solution, in the form of fine fat particles which are absorbed as the triglyceride.

d′. The Effect of Emulsifying Agents on the Absorption of β-Carotene: The beneficial effect of fat on carotene absorption must lie in its solvent action. On the other hand, the favorable action of bile salts must be related to their action in bringing about an emulsification of the fat and thus assisting in the digestion and absorption of this foodstuff.

Emulsifying agents other than bile salts are now known to aid in the absorption of carotene. This fact was first observed by Adlersberg and Sobotka[239] in man. It was later shown by Adlersberg and associates[240] that butter which contained phospholipid was a better vehicle in bringing about the absorption of carotene than was cottonseed oil, from which phospholipid is largely absent. However, when lecithin was added to the cottonseed oil, the absorption of carotene from this modified solvent was at a higher level than was the case when butter was the solvent. Esh and Sutton[241] also reported that lecithin improves the absorption of both carotene and vitamin A in the rat. However, it was shown that choline failed to exert a beneficial effect on absorption. Slanetz and Scharf[242,243] obtained similar results with lecithin.

The enhanced provitamin A activity of carotene, when present in margarine,[244] has been attributed to the improved absorption caused by such emulsifying agents as lecithin and mono- and diglycerides; these increase the dispersibility of margarine in water, at body temperature, and thus increase the physiological availability of the provitamin, through an augmented absorption. Lecithin has likewise been shown to facilitate the absorption of high melting fats and vitamin A (see pages 185 and 317).

Tween 40 (polyoxyethylene sorbitan monopalmitate) has been widely used for producing water-soluble solutions of carotene for oral and parenteral injection. Thompson *et al.*[231] reported that an aqueous colloidal solution of carotene was approximately as efficiently utilized as when the provitamin A was given in an oil solution. Burns, Hauge, and Quackenbush[245] reported that the growth of rats fed carotene emulsified in a water

[239] D. Adlersberg and H. Sobotka, *J. Nutrition*, *25*, 255–263 (1943).

[240] D. Adlersberg, S. Kann, A. P. Maurer, K. Newerly, W. Winternitz, and H. Sobotka, *Am. J. Digestive Diseases*, *16*, 333–337 (1949).

[241] G. C. Esh and T. S. Sutton, *J. Nutrition*, *36*, 391–404 (1948).

[242] C. A. Slanetz and A. Scharf, *Proc. Soc. Exptl. Biol. Med.*, *53*, 17–19 (1943).

[243] A. Scharf and C. A. Slanetz, *Proc. Soc. Exptl. Biol. Med.*, *57*, 159–161 (1944).

[244] H. J. Deuel, Jr., S. M. Greenberg, E. E. Savage, and D. Melnick, *J. Nutrition*, *43*, 371–388 (1951).

[245] M. J. Burns, S. M. Hauge, and F. W. Quackenbush, *Arch. Biochem.*, *30*, 341–346 (1951).

suspension containing 10% of Tween was somewhat better than in rats receiving the chromogen in an oil solution. Eaton and co-workers[246] found that calves, depleted of vitamin A, had higher plasma levels of β-carotene, and greater amounts of vitamin A in the liver and lung after an aqueous suspension of carotene had been given intravenously than following its oral administration. Jacobson and co-workers[247] found that, when carotene and vitamin A were dispersed in milk and fed to calves from a bottle with a nipple, the supplements were more rapidly absorbed than when administered by stomach tube. Moreover, the rate of absorption of β-carotene and vitamin A was somewhat less rapid when the concentrates were fed in gelatin capsules than when the foregoing procedures were employed. Tomarelli and associates,[248] and more recently Bieri and Sandman,[249] reported that β-carotene can be readily utilized by rats when given parenterally, provided an aqueous suspension in Tween 80 or Tween 40 is used.

e′. The Effect of Thyroxine and Thiouracil on the Absorption of β-Carotene: When thyroxine is administered to rats, the absorption of β-carotene is improved; on the other hand, the administration of thiouracil has been found to inhibit the absorption of this provitamin.[250] A similar behavior on the part of these two drugs in causing an alteration in the coefficient of digestibility of carotene has recently been noted in the goat and lactating cow[251] (see page 302).

Although it was formerly claimed that thyroid-active materials accelerate the conversion of β-carotene into vitamin A,[252,253] more recent data have failed to confirm these earlier observations.[254–256] If the sole site of conversion of β-carotene into vitamin A is in the wall of the in-

[246] H. D. Eaton, L. D. Matterson, C. F. Helmboldt, and E. L. Jungherr, *J. Dairy Sci.*, *34*, 1073–1080 (1951).

[247] N. L. Jacobson, G. H. Wise, R. S. Allen, and O. Kempthorne, *J. Dairy Sci.*, *33*, 645–656 (1950).

[248] R. M. Tomarelli, J. Charbney, and F. W. Bernhart, *Proc. Soc. Exptl. Biol. Med.*, *63*, 108–110 (1946).

[249] J. G. Bieri and R. P. Sandman, *Proc. Soc. Exptl. Biol. Med.*, *77*, 617–619 (1951).

[250] H. R. Cama and T. W. Goodwin, *Biochem. J.*, *45*, 236–241 (1949).

[251] R. Chanda, H. M. Clapham, M. L. McNaught, and E. C. Owen, *Biochem. J.*, *50*, 95–99 (1951).

[252] T. J. Balaba, *J. Physiol.* (*U.S.S.R.*), *29*, 318–326 (1940).

[253] S. Kaplanskiĭ and T. J. Balaba, *Biokhimiya*, *11*, 327–331 (1946); *Chem. Abst.*, *41*, 507 (1947).

[254] H. R. Cama and T. W. Goodwin, *Biochem. J.*, *45*, 317–320 (1949).

[255] C. W. Lowry and J. R. Lowry, *Arch. Biochem.*, *26*, 287–290 (1950).

[256] J. G. Bieri and M. O. Schultze, *Arch. Biochem. Biophys.*, *34*, 280–284 (1951)

testine in the case of the rat,[231,257–262] and the goat,[263,264] as is now believed to be the case, it would be anticipated that any effect of thyroxine or thiouracil on the conversion of the provitamin A to vitamin A would be greatest if these substances were administered by the oral route. However, Chanda and co-workers[251] reported that the digestibility of carotene was altered as readily when thyroid preparations were given by the parenteral route as when they were administered orally. This would indicate that the beneficial effect of thyroid must be exerted on the absorption of carotene rather than on its subsequent metabolism.

f′. The Effect of Substances Fed Concomitantly with β-Carotene on Its Absorption: The beneficial effects of fats and oils on the absorption of carotene have already been discussed. Other substances, when present in the gut simultaneously, may likewise help or hinder the absorption of the provitamin A.

(a′) The Effect of Mineral Oil.—The presence of difficultly absorbed hydrocarbons such as mineral oil will have a deleterious effect on carotene utilization. Rowntree[265] was the first to report that the minimum quantities of vitamin A required for the growth of rats were inadequate when mineral oil was present in the diet. Dutcher and associates[266] found that carotene absorption was depressed by this hydrocarbon to a far greater extent than was that of vitamin A. The deleterious effect of mineral oil on carotene absorption also applies to man. Thus, it was reported that plasma carotene values and the vitamin A in the liver were increased to a lesser degree after the ingestion of carotene in the presence of mineral oil than when the latter substance was absent.[267,268] These results have been confirmed by a number of workers.[269–279]

[257] F. H. Mattson, J. W. Mehl, and H. J. Deuel, Jr., *Arch. Biochem.*, *15*, 65–73 (1947).
[258] S. Y. Thompson, J. Ganguly, and S. K. Kon, *Brit. J. Nutrition*, *1*, v (1947).
[259] S. Y. Thompson, J. Ganguly, and S. K. Kon, *Brit. J. Nutrition*, *3*, 50–78 (1949).
[260] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *41*, xiv (1947).
[261] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *43*, 512–518 (1948).
[262] A. B. McCoord and S. W. Clausen, *Abst.*, *114th Meeting, Am. Chem. Soc., Div. Biol. Chem.*, Washington, Aug. 30, 1946, 16 C.
[263] T. W. Goodwin, A. D. Dewar, and R. A. Gregory, *Biochem. J.*, *40*, lx–lxi (1946).
[264] T. W. Goodwin and R. A. Gregory, *Biochem. J.*, *43*, 505–512 (1948).
[265] J. I. Rowntree, *J. Nutrition*, *3*, 345–351 (1931).
[266] R. A. Dutcher, P. L. Harris, E. R. Hartzler, and N. B. Guerrant, *J. Nutrition*, *8*, 269–283 (1934).
[267] A. C. Curtis and E. M. Kline, *Arch. Internal Med.*, *63*, 54–63 (1939).
[268] B. Alexander, E. Lorenzen, R. Hoffmann, and A. Garfinkel, *Proc. Soc. Exptl. Biol. Med.*, *65*, 275–278 (1947).
[269] O. Andersen, *Klin. Wochschr.*, *18*, 499–502 (1939).
[270] A. C. Curtis and R. S. Ballmer, *J. Am. Med. Assoc.*, *113*, 1785–1788 (1939).
[271] D. L. Collison, E. M. Hume, I. Smedley-MacLean, and H. H. Smith, *Biochem. J.*, *23*, 634–647 (1929).

(b′) The Effect of Tocopherols.—The tocopherols are known to enhance the vitamin A effect of both carotene and vitamin A.[280] This effect is probably to be traced to a protective action on the part of tocopherol against oxidative destruction of provitamin A or vitamin A in the gastrointestinal tract and at the site of utilization within the organism, rather than to an effect on absorption. However, Major and Watts[281] were unable to demonstrate any improvement in the utilization of carotene, or any increased deposition of vitamin A in the livers of rabbits on high tocopherol diets as compared with results obtained with regimens low in this vitamin.

On the other hand, Swick and Baumann[282] demonstrated that high doses of α-tocopherol, γ-tocopherol, and α-tocopherol acetate diminish the storage of vitamin A in rats fed moderate doses of β-carotene. γ-Tocopherol was found to be nearly equal to α-tocopherol in inhibiting vitamin A deposition under these conditions, while α-tocopherol acetate was somewhat more active than the free vitamin E. Hydroquinone, vitamin C, and diamylhydroquinone failed to increase the activity of α-tocopherol, but diamylhydroquinone slightly reduced the deposition of vitamin A.

(c′) The Effect of Xanthophylls.—The question as to whether or not the xanthophylls increase or decrease the utilization of β-carotene is still uncertain. Sherman,[283] in 1947, reported that the addition of xanthophyll to the diet of rats apparently decreased the destruction of carotene, vitamin A alcohol, and vitamin A acetate in the gastrointestinal tract. However, Kemmerer *et al.*,[284] in the same year, found that xanthophylls and chlorophylls, when fed with carotene dissolved in cottonseed oil, decreased the effectiveness of carotene for liver storage of vitamin A by about 20%.

[272] R. W. Jackson, *J. Nutrition*, *7*, 607–616 (1934).
[273] A. E. Mahle and H. M. Patton, *Gastroenterology*, *9*, 44–53 (1947).
[274] H. S. Mitchell, *Proc. Soc. Exptl. Biol. Med.*, *31*, 231–233 (1933).
[275] T. Moore, *Biochem. J.*, *23*, 1267–1272 (1929).
[276] M. J. Burns, S. M. Hauge, and F. W. Quackenbush, *Arch. Biochem.*, *30*, 347–350 (1951).
[277] T. K. With, *Nord. Med.*, *3*, 2468–2470 (1939).
[278] T. K. With, *Z. Vitaminforsch.*, *10*, 1–6 (1940).
[279] T. K. With, *Vitamine u. Hormone*, *2*, 369–399 (1942); *Chem. Zentr.*, *114*, I, 1286 (1943).
[280] K. C. D. Hickman and P. L. Harris, *Advances in Enzymology*, Vol. VI, Interscience, New York and London, 1946, pp. 469–524.
[281] R. Major and B. M. Watts, *J. Nutrition*, *35*, 103–116 (1948).
[282] R. W. Swick and C. A. Baumann, *Arch. Biochem. Biophys.*, *36*, 120–126 (1952).
[283] W. C. Sherman, *Proc. Soc. Exptl. Biol. Med.*, *65*, 207–210 (1947).
[284] A. R. Kemmerer, G. S. Fraps, and J. De Mottier, *Arch. Biochem.*, *12*, 135–138 (1947).

On the other hand, α-tocopherol or sulfasuxidine did not affect the utilization of carotene in spinach, as demonstrated by liver storage tests for vitamin A.

Kelley and Day[285] reported later that, when large amounts of lutein (xanthophyll) were given to rats, the vitamin A storage in the tissues was lower after the administration of β-carotene or vitamin A than when the carotenol was absent from the diet. However, the ingestion of xanthophyll did not affect the rate of disappearance of vitamin A already present in the tissues. This is interpreted as meaning that the inhibitory action of xanthophyll is exerted in the gastrointestinal tract. This inhibitory action is not counteracted by an extra dose of vitamin E. It was likewise shown that the effect of xanthophyll is not the result of a specific impairment of the enzymatic mechanism for the transformation of β-carotene to vitamin A. In a later paper from this laboratory, High and Day[286] confirmed the earlier report, but also noted that the quantity of vitamin A stored in the liver and kidneys was *increased* when *small* amounts of xanthophyll were administered. It was also found that large amounts of phytol, squalene, and α-tocopherol acetate, when fed with carotene or vitamin A, likewise decreased the extent of storage of vitamin A. However, squalene, phytol, geraniol, and menthol were all without effect in decreasing the quantity of vitamin A already present in the liver and kidneys. Vavich and Kemmerer[287] reported that xanthophylls, when fed at levels of 300 or 600 μg. daily, did not reduce the storage of vitamin A in the livers of chicks fed 65 μg. of β-carotene daily. However, doses of 100, 300, or 600 μg. of xanthophylls did cause a reduction in the amount of vitamin A stored in the livers of chicks fed 130 μg. of carotene daily. In the case of rats, Callison, Hallman, Martin, and Orent-Keiles[288] could not demonstrate any change in carotene utilization for growth. This would indicate that the difference, in availability of the carotenes, between green and in yellow vegetables, respectively, is not explicable on the basis of the xanthophylls, which are present in large amounts in the green leaves, but which are almost wholly lacking in the yellow vegetables.

The several investigators are all in agreement that any effect which the xanthophylls may exert on the vitamin A storage is concerned with the absorptive rather than with the metabolic phases. There appears also to be some agreement in the results as indicating that the quantity

[285] B. Kelley and H. G. Day, *J. Nutrition*, *40*, 159–168 (1950).

[286] E. G. High and H. G. Day, *J. Nutrition*, *43*, 245–260 (1950).

[287] M. G. Vavich and A. R. Kemmerer, *Arch. Biochem.*, *28*, 295–298 (1950).

[288] E. C. Callison, L. F. Hallman, W. F. Martin, and E. Orent-Keiles, *Arch. Biochem. Biophys.*, *32*, 407–413 (1951).

of xanthophyll present is of prime importance in that it may enhance the deposition of vitamin A in the tissues under one condition and inhibit its deposition under other conditions.

g′. The Effect of Pteroylglutamic Acid: Low values for serum carotene have been shown to occur in sprue. Darby and co-workers[289] observed that this condition is gradually improved following therapy with pteroylglutamic acid. The fact that a typical flat curve obtains after withdrawal of the treatment during a relapse is taken as evidence of the influence of pteroylglutamic acid on the absorption of fat-soluble substances.

b. The Absorption of α- and γ-Carotenes. Although no quantitative results are available on the rate of absorption of α- or γ-carotenes, there are considerable data to prove that their absorption is in the same range as is that of β-carotene. Thus, α-carotene has been shown to be effective as a provitamin A to the extent of about 50% of the activity of β-carotene.[290] Since only one-half of the α-carotene molecule is capable of transformation to vitamin A while, from a theoretical standpoint, all of β-carotene can be transformed to vitamin A, a 50% biopotency of the α-isomer would indicate an absorption identical with that of the β-variety. The biological value of γ-carotene, which was first found[291] to be approximately 26% and later 42% of that of β-carotene,[292] would likewise justify the conclusion that the γ-isomer is as well absorbed as is β-carotene. One can make a similar statement in the case of pro-γ-carotene, which is a naturally occurring poly-*cis* variety.[292,293] The biopotency of this compound was only slightly less than 50% of that of β-carotene. Since only one-half of the γ- or of the pro-γ-carotene can yield vitamin A, a conversion to vitamin A which equals 50% of that of β-carotene must be indicative of an absorption equal to that of β-carotene.

Another proof which helps establish the absorption of α-carotene is the demonstration of its presence in animal tissues. Although data are not available on animals which store carotene as such, minimal amounts of α- and β-carotenes have been demonstrated in the liver of rats, as well as in the liver and eggs of chickens, following its administration.[294]

[289] W. J. Darby, M. K. Kaser, and E. Jones, *J. Nutrition*, *33*, 243–250 (1947).

[290] H. J. Deuel, Jr., E. Sumner, C. Johnston, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, *6*, 157–161 (1945).

[291] H. J. Deuel, Jr., C. Hendrick, E. Straub, A. Sandoval, J. H. Pinckard, and L. Zechmeister, *Arch. Biochem.*, *14*, 97–103 (1947).

[292] L. Zechmeister, J. H. Pinckard, S. M. Greenberg, E. Straub, T. Fukui, and H. J. Deuel, Jr., *Arch. Biochem.*, *23*, 242–245 (1949).

[293] H. J. Deuel, Jr., C. Johnston, E. Sumner, A. Polgár, W. A. Schroeder, and L. Zechmeister, *Arch. Biochem.*, *5*, 365–371 (1944).

[294] J. Ganguly, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *50*, 59–72 (1953).

However, the demonstration of a provitamin A activity on the part of the carotenes, as demonstrated by growth-promoting action or by the deposition of vitamin A in the liver, cannot be considered as absolute proof of the absorption of the carotenes as such. One can argue that the provitamins A are converted into vitamin A in the lumen of the gut; under such conditions, vitamin A and not the carotenes would be the component absorbed. This argument carries no weight in the case of man or of other animals in which carotene is a normal constituent of the blood and tissues.

c. The Absorption of Lycopene. Lycopene, $C_{40}H_{56}$, is a hydrocarbon in which both β-ionone rings have been ruptured; consequently it is not convertible to vitamin A. The absorption of this compound is predicated by the fact that it has been found in human fat,[295,296] human liver,[297,298] and in the blood serum of man.[299,300] Gillam and Heilbron[301] also reported the isolation of lycopene from butter. The occurrence of lycopene in tissues is believed to be adventitious and to reflect the absorption of this carotenoid from that consumed in the food.

d. The Absorption of Oxycarotenoids. Oxycarotenoids having one or more alcohol groups might be expected to be more readily absorbable, since alcohols in general are more soluble in aqueous media than are the corresponding hydrocarbons. In the case of the entire group of oxycarotenoids, only indirect methods have been employed to prove absorbability; for this reason no comparative rates of absorption can be indicated.

(*a*) *Cryptoxanthin.* Cryptoxanthin, $C_{40}H_{55}OH$, is the only common member of the oxycarotenoids which can be listed as a provitamin A. It has been reported to be a component of butter,[301] of eggs,[302] and of the blood serum of cattle.[303] In all these cases, it is believed the origin of the carotenol can be ascribed to its absorption from food.

Another proof of the absorption of cryptoxanthin is its ability to serve as a provitamin A. It is converted to vitamin A in an amount somewhat in excess of 50% of that of β-carotene.[304,305] This indicates that it is absorbed as well as β-carotene when administered in the small dosages required for bioassay studies. Since cryptoxanthin has only one intact β-ionone ring, and since it has a molecular weight slightly in excess of that

[295] L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *231*, 259–264 (1935).
[296] L. Zechmeister and P. Tuzson, *Bull. soc. chim. biol.*, *17*, 1110–1118 (1935).
[297] L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *234*, 241–244 (1935).
[298] H. Willstaedt and T. Lindqvist, *Z. physiol. Chem.*, *240*, 10–18 (1936).
[299] E. V. Dániel and G. J. Scheff, *Proc. Soc. Exptl. Biol. Med.*, *33*, 26–30 (1935).
[300] E. V. Dániel and T. Béres, *Z. physiol. Chem.*, *238*, 160–162 (1936).
[301] A. E. Gillam and I. M. Heilbron, *Biochem. J.*, *29*, 834–836 (1935).

of β-carotene, the theoretical proportion of vitamin A formed would be 48.6% of that of a corresponding amount of β-carotene, if one assumes that the latter compound yields two molecules of vitamin A. The absorption of cryptoxanthin is likewise proved by the fact that it is changed to vitamin A in the wall of the intestine in the case of the rat[306] and of the chicken.[307]

(*b*) *Lutein.* Two isomeric dihydroxycarotenoids, known also as xanthophylls, which are widely distributed in nature, have the empirical formula, $C_{40}H_{54}(OH)_2$. These carotenols are lutein and zeaxanthin. They frequently occur in mixtures in natural products, but they can be readily separated from each other by modern chromatographic technics.

The presence of lutein in the tissues and excretion products constitutes our main proof of its absorbability. As in the case of the other carotenoids, its occurrence under such conditions is the result of its presence in the food. Lutein has been reported in human fat,[295,308] in the livers obtained *post mortem* from men considered to be normal,[298] or from patients who had died from a variety of diseases.[297] The presence of this carotenol has likewise been demonstrated in human skin after the consumption of large amounts of it by partaking of winter squash (*Cucurbita maxima*)[309] over a prolonged period.[310] The highest concentration of lutein reported by Zechmeister and Tuzson[296] in human fat was in the case of a woman suffering from jaundice.

Although lutein has not been reported in the fat of cattle, it is a constant component of milk and butter,[311] in which the concentration varies with the amount of green fodder. As might be expected, lutein has also been reported in the serum of cattle.[303]

The occurrence of lutein in fowl is quite general. It has been reported in chicken fat,[308] as well as in egg yolk.[302] Brockmann and Völker[312] observed the presence of this pigment in the feathers of the wild Madeira

[302] A. E. Gillam and I. M. Heilbron, *Biochem. J.*, *29*, 1064–1067 (1935).
[303] A. E. Gillam and M. S. El Ridi, *Biochem. J.*, *29*, 2465–2468 (1935).
[304] H. J. Deuel, Jr., E. R. Meserve, C. H. Johnston, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, *7*, 447–450 (1945).
[305] S. M. Greenberg, A. Chatterjee, C. E. Calbert, H. J. Deuel, Jr., and L. Zechmeister, *Arch. Biochem.*, *25*, 61–65 (1950).
[306] S. M. Patel, J. W. Mehl, and H. J. Deuel, Jr., *Arch. Biochem.*, *30*, 103–109 (1951).
[307] J. Ganguly and H. J. Deuel, Jr., Unpublished experiments, 1952.
[308] L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *225*, 189–195 (1934).
[309] H. Suginome and K. Ueno, *Bull. Chem. Soc., Japan*, *6*, 221–228 (1931).
[310] M. Ansai, *Japanese J. Med. Sci., V. Pathology*, *1*, 175–190 (1931).
[311] A. E. Gillam, I. M. Heilbron, R. A. Morton, G. Bishop, and J. C. Drummond *Biochem. J.*, *27*, 878–888 (1933).
[312] H. Brockmann and O. Völker, *Z. physiol. Chem.*, *224*, 193–215 (1934).

canary (*Serinus canarius*) and of the yellow-hammer (*Emberizia citrinella*). Lutein occurs in the green water-frog (*Rana esculenta*),[313] as well as in a number of lower forms. The possible effect of xanthophylls (lutein) on the absorption of β-carotene and vitamin A has been discussed earlier.

(*c*) *Zeaxanthin.* Zeaxanthin and lutein have been isolated from similar sources. Zeaxanthin occurs in human fat,[308,314] in human liver,[298] in the yolk of hen's egg,[314–316] and in the egg of the Atlantic dogfish (*Squalus acanthias*).[317] This carotenol likewise occurs along with lutein in the feathers of the wild canary (*Serinus canarius*),[312] and in the green water-frog.[313]

(*d*) *Other Oxycarotenoids.* Zechmeister and Tuzson[296] demonstrated the interesting fact that capsanthin was found in the depot fat of man. These authors[308] consider the presence of the pigment to be adventitious, since it is present chiefly in the fatty tissues of Hungarian peasants who consume large quantities of paprika; the latter vegetable is the principal natural source of the capsanthin pigments.

e. Stereoisomeric Forms of Carotenoids. No direct absorption studies of the stereoisomeric *cis*-forms of the naturally occurring all-*trans* carotenoids are available. A number of the isomers have been proved to be sources of vitamin A; it is not certain, however, whether the vitamin A arises directly from the *cis* form of the carotenoid or from a portion of the natural all-*trans* compound which has been produced by isomerization in the intestine or after absorption in the tissues.

In general, the provitamin A potency of the stereoisomers varies from 15 to 50% of that of the corresponding all-*trans* forms, although pro-γ-carotene has a bioactivity equal to that of its all-*trans* isomer.

The following provitamin A activities have been recorded for the stereoisomers tested on rats, all-*trans*-β-carotene being assigned a potency of 100: neo-β-carotene B, 53%[318]; neo-β-carotene U, 38%,[319] and 24%[320]; α-carotene set (all-*trans*, 53%)[290]; neo-α-carotene B, 16%[318]; neo-α-caro-

[313] L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *238*, 197–203 (1936).

[314] P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 183. Although Karrer and Jucker[314] list zeaxanthin as a component of human fat, authorities whom they cite in support of their statement[295,308] do not mention this compound.

[315] R. Kuhn, A. Winterstein, and E. Lederer, *Z. physiol. Chem.*, *197*, 141–160 (1931).

[316] H. H. Strain, *Chromatographic Adsorption Analysis*, Interscience, New York-London, 1945.

[317] H. v. Euler and U. Gard, *Arkiv Kemi Mineral. Geol.*, *B 10*, No. 19, 1–6 (1931).

[318] H. J. Deuel, Jr., C. Johnston, E. R. Meserve, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, *7*, 247–255 (1945).

[319] H. J. Deuel, Jr., C. Johnston, E. Sumner, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, *5*, 107–114 (1944).

[320] A. R. Kemmerer and G. S. Fraps, *J. Biol. Chem.*, *161*, 305–309 (1945).

tene U, 13%[290]; γ-carotene set (all-*trans*, 42%)[292]; neo-γ-carotene P, 19%[291]; mixture of neo-γ-carotenes, 16%[291]; pro-γ-carotene, 41%[292]; cryptoxanthin set (all-*trans*, 57%)[321]; neocryptoxanthin A, 42%[322]; and neocryptoxanthin U, 27%.[323] The provitamin activity of the pro-γ-carotene is slightly higher in the chick than in the rat.[324] Kemmerer and Fraps[320] found that the carotene isolated from the gastrointestinal tract of rats four to six hours after the feeding of neo-β-carotene U had been changed to the all-*trans* form and to neo-β-carotene.

(2) *The Stability of β-Carotene in the Intestine*

Carotene has repeatedly been found to be less effective as a source of vitamin A when fed in pure solvents than when administered in crude oils. For example, β-carotene was reported to have no potency as a source of vitamin A when fed in ethyl oleate.[325] Drummond *et al.*[326] attributed the lack of vitamin A potency under such conditions to an oxidation of the carotene. After the demonstration by Mattill[327] of the protective effect of polyphenols in preventing the autoxidation of lard, Olcovich and Mattill[328] were able to demonstrate that hydroquinone exerts a stabilizing influence on β-carotene in ethyl oleate or ethyl laurate, and prevents its oxidation. A number of workers[329–332] then turned to hydroquinone as an agent for preventing oxidative destruction of the carotene. Moore[333] in 1940, and Davies and Moore[334] in 1941, were also among the first to recognize the sparing effect of tocopherol on β-carotene and vitamin A. Carotene was also shown to be destroyed in the gastrointestinal tract when fed with methyl linoleate or methyl linolenate.[225] Tomarelli and

[321] L. Zechmeister, *Bull. soc. chim. biol.*, *31*, 956–964 (1949).

[322] H. J. Deuel, Jr., E. R. Meserve, A. Sandoval, and L. Zechmeister, *Arch. Biochem.*, *10*, 491–496 (1946).

[323] H. J. Deuel, Jr., S. M. Greenberg, E. Straub, T. Fukui, A. Chatterjee, and L. Zechmeister, *Arch. Biochem.*, *23*, 239–241 (1949).

[324] S. M. Greenberg, C. E. Calbert, J. H. Pinckard, H. J. Deuel, Jr., and L. Zechmeister, *Arch. Biochem.*, *24*, 31–39 (1949).

[325] W. Dulière, R. A. Morton, and J. C. Drummond, *J. Soc. Chem. Ind.*, *48*, 316–321T (1929).

[326] J. C. Drummond, B. Ahmad, and R. A. Morton, *J. Soc. Chem. Ind.*, *49*, 291–296T (1930).

[327] H. A. Mattill, *J. Biol. Chem.*, *90*, 141–151 (1931).

[328] H. S. Olcovich and H. A. Mattill, *J. Biol. Chem.*, *91*, 105–117 (1931).

[329] C. A. Baumann and H. Steenbock, *J. Biol. Chem.*, *101*, 561–572 (1933).

[330] F. G. McDonald, *J. Biol. Chem.*, *103*, 455–460 (1933).

[331] R. G. Turner and E. R. Loew, *J. Infectious Diseases*, *52*, 102–120 (1933).

[332] F. J. Dyer, K. M. Key, and K. H. Coward, *Biochem. J.*, *28*, 875–881 (1934).

[333] T. Moore, *Biochem. J.*, *34*, 1321–1328 (1940).

[334] A. W. Davies and T. Moore, *Nature*, *147*, 794–796 (1941).

György[335] reported that rice bran extract acts synergistically with mixed tocopherols in retarding the oxidation of linoleic acid, and consequently in preserving carotene.

However, even with added hydroquinone, carotene was found to be less effective as a source of vitamin A when fed in certain oils than when administered in others.[332] Thus, it was much less active when given in butterfat[336] than when administered in cottonseed oil. Kraybill and Shrewsbury[336] found, after treating butterfat with Lloyd's reagent, that β-carotene was stable at 40°C., although it was only 50% as potent biologically as when it was fed in cottonseed oil. These workers[336] suggested that "Lloyd's reagent may have removed a factor which supplemented the vitamin A activity of the carotene." Lathbury and Greenwood[337] reported that a much lower biological response was obtained with β-carotene when it was given in coconut oil with or without hydroquinone than when it was administered in linseed oil.

Quackenbush, Cox, and Steenbock[338,339] were the first to demonstrate that tocopherol renders the action of β-carotene possible; these workers concluded that tocopherol functions by virtue of its antioxidant action on carotene in the gastrointestinal tract. On the other hand, Sherman[340] expressed the view that tocopherol was concerned with the utilization of carotene rather than with protection from oxidation, although he also showed that α-tocopherol was highly effective in preserving carotene in the presence of highly unsaturated acids.

Harris, Kaley, and Hickman[341] made further studies of the synergism exerted by the tocopherols on the biopotency of carotene. They reported that 0.5 mg. of natural mixed tocopherols provides an optimum daily dose, as a demonstration of the sparing action of vitamin E on carotene. In another study, these workers[342] reported that the three tocopherols (α-, β-, and γ-) are equally effective in sparing carotene and vitamin A, although they show marked variations as antisterility vitamins. When

[335] R. M. Tomarelli and P. György, *J. Biol. Chem.*, *161*, 367–379 (1945).

[336] H. R. Kraybill and C. L. Shrewsbury, *J. Nutrition*, *11*, 103–110 (1936).

[337] K. C. Lathbury and G. N. Greenwood, *Biochem. J.*, *28*, 1665–1673 (1934).

[338] F. W. Quackenbush, R. P. Cox, and H. Steenbock, *J. Biol. Chem.*, *140*, civ–cv (1941).

[339] F. W. Quackenbush, R. P. Cox, and H. Steenbock, *J. Biol. Chem.*, *145*, 169–177 (1942).

[340] W. C. Sherman, *Proc. Soc. Exptl. Biol. Med.*, *47*, 199–200 (1941).

[341] P. L. Harris, M. W. Kaley, and K. C. D. Hickman, *J. Biol. Chem.*, *152*, 313–320 (1944).

[342] K. C. D. Hickman, M. W. Kaley, and P. L. Harris, *J. Biol. Chem.*, *152*, 321–328 (1944).

tocopherols were fed with carotene to rats or to men, almost twice the amount of provitamin A was recoverable from the feces. Guggenheim[343] has confirmed the increased fecal excretion of carotene which results when vitamin E is fed. This worker also reports a greater utilization of carotene as demonstrated by the vitamin A storage test; it is concluded that vitamin E functions by protecting carotene and vitamin A from oxidation while in the intestine. This brings about both increased absorption and greater fecal excretion. Hebert and Morgan[344] reported that the addition of 0.5 mg. daily of α-tocopherol to the diet of partially depleted rats increased the liver storage of vitamin A when carotene was given; however, no augmentation of liver vitamin A occurred when vitamin A itself was given.

Although there seems to be adequate proof that moderate doses of α-tocopherol protect carotene from oxidation and enhance its value as a source of vitamin A, there are at least two reports which indicate that large doses of this antioxidant have the opposite effect. Thus, Johnson and Baumann[345] found that, when 5 or 10 mg. of α-tocopherol was fed simultaneously with β-carotene, the stores of vitamin A in the liver were much lower than was the case when the tocopherol was not given. Intraperitoneally injected tocopherol also interfered with the utilization of β-carotene; however, when the tocopherol was given eight hours after the carotene, no comparable interference with the storage of vitamin A was noted. The effect of high levels of tocopherol on vitamin A storage is presumably not concerned with the absorption of carotene, since the fecal excretion of this provitamin A was no greater in these cases than in control tests. High and collaborators[346] reported that large amounts of tertiary butylhydroquinone or octylhydroquinone also decreased the utilization of carotene for tissue deposition of vitamin A, whereas small amounts of octylhydroquinone were shown to augment vitamin A storage. On the other hand, large amounts of octylhydroquinone were more effective than were smaller quantities in inhibiting the oxidative decomposition of carotene in *in vitro* tests. The same situation was shown to obtain in the case of vitamin A. It is postulated that, when these substances are present in large amounts *in vivo*, the utilization of carotene may be impaired, due to interference with the enzymatic conversion of carotene to vitamin A.[346]

[343] K. Guggenheim, *Biochem. J.*, *38*, 260–264 (1944).
[344] J. W. Hebert and A. F. Morgan, *J. Nutrition*, *50*, 175–190 (1953).
[345] R. M. Johnson and C. A. Baumann, *J. Biol. Chem.*, *175*, 811–816 (1948).
[346] E. G. High, L. A. Woods, Jr., and S. S. Wilson, *J. Biol. Chem.*, *195*, 787–793 (1952).

The relative stability of β-carotene in the intestine has likewise been shown by *in vitro* tests. Thus Goodwin and Gregory[264] found that carotene was not destroyed when incubated with rabbit intestinal contents at 37°C.; in addition, Seshan and Sen[347] reported that no carotene was lost when it was incubated with cattle feces.

Gossypol has likewise been shown to be an effective antioxidant in protecting carotene both *in vitro* and *in vivo*.[348] Mattill[327] had previously shown that gossypol is an effective antioxidant in protecting lard *in vitro*. Moreover, Hove and Hove[349] reported that gossypol is able to protect carotene from destruction *in vitro* from preformed fat peroxides. In a later study, Hove[348] observed that gossypol and dianilinogossypol, when fed to rats at daily doses of 1 mg., were only slightly less effective than α-tocopherol in preserving carotene when diets containing lard or methyl linoleate were fed. Cottonseed oil meal, which contains gossypol, was shown to be equal to wheat germ in stabilizing a carotene solution in ethyl oleate *in vitro*, and much superior in this respect to several other common feeds.

(3) *The Digestibility of Carotenoids*

Most of the studies on carotenoids have been carried out with too small amounts to serve as a basis for reports on digestibility by the usual balance study methods. However, Johnson and Baumann[350] measured the recovery, from the feces of rats, of several carotenoids fed in cottonseed oil in amounts of 2 to 78 γ daily. It was found that cryptoxanthin had the highest digestibility (64%), followed by β-carotene (60%) and α-carotene (46%). The percentage excretion did not vary with the dose, nor was it influenced when lutein was given concomitantly. Fraps and Meinke[351] had previously reported digestibility coefficients in rats of 57 and 64, respectively, for β-carotene and α-carotene dissolved in oil, while "neo-β-carotene" had practically the same digestibility as β-carotene.

a. Factors Affecting the Digestibility of Carotenes. The extent of digestibility of carotene, as well as its absorption, is influenced by a number of extraneous factors.

(*a*) *The Amount of Carotene Administered.* In the case of rats, Fraps and Meinke[351] reported that the digestibility of α-carotene ranged from

[347] P. A. Seshan and K. C. Sen, *J. Agr. Sci., 32,* 194–201 (1942).
[348] E. L. Hove, *J. Biol. Chem., 156,* 633–642 (1944).
[349] E. L. Hove and Z. E. Hove, *J. Biol. Chem., 156,* 611–621 (1944).
[350] R. M. Johnson and C. A. Baumann, *Arch. Biochem., 19,* 493–501 (1948).
[351] G. S. Fraps and W. W. Meinke, *Arch. Biochem., 6,* 323–327 (1945).

47% in rats receiving 20 μg. daily to 33% for those receiving 60 μg. Kemmerer and Fraps[230] found that the digestibility of β-carotene in rats varied with the amount fed and with the vehicle. When it was fed to rats in dehydrated alfalfa at levels of 1, 10.5, or 20 parts per million, the digestibility coefficients were 43, 22, and 18 to 23, respectively. When chickens were the test animals, values of 69 and 29 were obtained for the 1 part/million and 20 part/million dosage levels, respectively.

(b) *The Effect of the Food Given Simultaneously with Carotene.* Digestibility coefficients are much higher for carotene when the provitamin is given in oil. Thus, Kemmerer and Fraps[230] reported a coefficient of digestibility of 51 when carotene was given in oil to rats, and one of 22 when it was fed as dehydrated alfalfa without added fat. Kreula[352] reported that, whereas the absorption of carotene from finely grated carrots by three human subjects on a fat-free diet was only 10%, it was increased to 50% when carotene dissolved in olive oil was taken in a single dose. When the oil solution was taken in two portions, as much as 70% was utilized.

The carotene digestibility varies considerably according to the vegetable source. Thus, Wilson and co-workers[228] observed a digestibility of 90% for carotene when it was given in raw or cooked carrots or cooked spinach with fat, but the coefficient of digestibility was only 50 when it was given without fat. On the other hand, van Eekelen and Pannevis[229] found that 99% of the carotene from carrots and 95% of the carotene from spinach was present in the feces. Kreula and Virtanen[235] likewise observed that the digestibility for carotene varied between 1 and 36%, and generally amounted to 10%. Very finely grated carrots appeared to be more favorable for utilization than were those which had been merely masticated. With[353] reported that carotene was absorbed to the extent of 30 to 60% from purées of tomatoes, carrots, spinach, and carrot meal. The Vitamin A Sub-Committee of the Accessory Food Factors Committee[354] reported a 25% utilization of carotene from carrots and a utilization of about 40% from spinach. Hume and Krebs[355] also studied the efficiency of absorp-

[352] M. S. Kreula, *Biochem. J.*, *41*, 269–273 (1947).

[353] T. K. With, *Absorption, Metabolism and Storage of Vitamin A and Carotene*, Munksgaard, Copenhagen, 1940, and Milford, London, 1940; cited by M. S. Kreula, *Biochem. J.*, *41*, 269–273 (1947), p. 269.

[354] *Report of the Vitamin A Subcommittee of the Accessory Food Factors Committee*, Lister Inst., *Rep. Med. Research Council* (*Brit.*), 1939–1945, pp. 107–109. H. M. Stationery Office, London, 1945.

[355] E. M. Hume and H. A. Krebs, *Vitamin A Requirements of Human Adults, Report of the Vitamin A Subcommittee of the Accessory Food Factors Committee, Med. Research Council* (*Brit.*), *Spec. Rep. Ser., No. 264*, H. M. Stationery Office, London, 1949.

tion of carotene in man by balance experiments carried out by a team organized by the Medical Research Council. Fraps[356] found that, when carotene, dissolved in oil, was fed with a ration containing cottonseed hulls or agar to furnish bulk, the digestibility was approximately the same as that obtained on a corn meal ration. When casein was substituted for corn meal, the apparent digestibility of carotene was increased, as was also the storage of vitamin A in the liver. In spite of considerable evidence indicating the superior digestibility of carotene when fed in oil as compared with a fat-free diet, Frey and Wilgus[357] reported that the total potential vitamin A activity of the eggs from pullets receiving alfalfa was about twice that of eggs from birds receiving carotene in oil, and three times that of birds receiving vitamin A from fish liver oil.

(*c*) *The Effect of Thyroxine and Thiouracil.* Not only is the rate of absorption of carotene controlled by thyroxine and thiouracil, but it has also been found that the digestibility of this pigment is a function of the thyroid secretion. Chanda *et al.*[251] demonstrated conclusively that the antagonism which is known to exist between thyroxine and thiouracil, insofar as metabolic effects are concerned, is also exerted decisively in controlling the absorption of carotene. Not only was it demonstrated that the digestibility of carotene was higher during the administration of thyroxine and lower during the feeding of thiouracil than the coefficient found during the basal period, but it was likewise found that the rate of reappearance of carotene in the stools after a carotene-free diet was retarded if thyroxine was given, and was accelerated when thiouracil was administered. Goats were able to digest carotene more efficiently than were cows.[251,358] This difference in digestibility is consistent with the suggestion of Schultze and Turner[359] that the thyroid gland is more active in the goat than in the cow. This variation was likewise borne out by the fact that the increase in digestibility produced by thyroxine was greater in the cow than in the goat, while the inhibition of absorption was greater in the goat than in the cow.

The comparative effects of the administration of thyroxine and of thiouracil on the digestibility of carotene in lactating cows and goats, as contrasted with those in normal, untreated animals, are given in Table 7.

[356] G. S. Fraps, *Arch. Biochem.*, *10*, 485–489 (1946).

[357] P. R. Frey and H. S. Wilgus, *J. Nutrition*, *39*, 517–528 (1949).

[358] R. Chanda, H. M. Clapham, M. L. McNaught, and E. C. Owen, *J. Agr. Sci.*, *41*, 179–186 (1951).

[359] A. B. Schultze and C. W. Turner, *Univ. Missouri Coll. Agr., Agr. Expt. Sta., Research Bull. No. 392*, 1–89 (Aug., 1945).

TABLE 7

EFFECT OF THYROXINE AND THIOURACIL ON THE DIGESTIBILITY OF CAROTENE IN LACTATING COWS AND GOATS[a]

Treatment in exptl. period	Fecal carotene (2 days), mg.			Apparent digestibility		
	Pre-basal period	Experi-mental period	Post-basal period	Pre-basal period	Experi-mental period	Post-basal period
Cows						
None	401.9	413.8	425.7	58.9	57.7	55.0
"	452.8	407.4	425.1	52.2	57.0	56.0
Thyroxine	460.2	289.1	404.0	52.7	69.8	57.8
"	450.5	262.8	432.3	53.2	73.2	54.5
Thiouracil	418.6	480.4	430.8	56.7	51.1	53.6
"	414.9	508.0	426.0	55.3	47.8	56.2
Goats						
None	30.8	27.4	20.9	63.0	62.3	64.5
"	21.1	18.9	10.2	62.9	60.7	61.3
Thyroxine	26.8	10.3	27.9	62.9	74.1	57.7
" [b]	23.8	6.2	—	66.0	73.9	—
Thiouracil	26.8	36.8	27.9	68.4	53.8	62.1
"	32.1	34.8	19.6	61.6	48.0	61.3

[a] Data adapted from R. Chanda, H. M. Clapham, M. L. McNaught, and E. C. Owen, *Biochem. J.*, *50*, 95–99 (1951).
[b] Animal died of hyperthyroidism before third period.

(4) Changes in the Carotenoids in the Intestinal Wall

The mechanism by which the carotenoids are carried into the intestinal epithelia and from there into the blood stream is not clear. It is believed that the bile salts provide a mechanism for their transfer into the intestinal cell in much the same way that they function in the absorption of fat.

The changes which take place in the carotenoids in the epithelial cells must vary according to the species of the animal. Thus, in animals whose blood and lymph contain no carotenoids, the intestinal wall is obviously one site of breakdown of these substances. Whether or not this cleavage of the carotenoids is limited to the provitamins A is not certain; here again there must necessarily be species variations. On the other hand, it has not been determined whether animals like the dog, cow, and man, which may have carotene in the blood stream, possess any power to split the carotenoids in the intestinal wall.

Other changes which take place in the epithelial cells of the intestine

include the transformation of vitamin A aldehyde (retinene) to vitamin A, and the esterification of the vitamin A formed from carotene.

a. The Transformation of Carotenoids Into Vitamin A. Until quite recently, it has been generally assumed that the liver is the organ in which β-carotene is changed to vitamin A.[360] An enzyme, referred to as "carotenase," has been credited with bringing about the cleavage.

However, Sexton *et al.*[361] were the first to suggest that the intestine is the site of the transformation of carotene to vitamin A. They state that "the conversion of carotene to vitamin A may be an extrahepatic function in the rat. The wall of the intestine is suggested as a possible site of such transformation." There are several arguments in favor of this concept. In the first place, it was shown that no appreciable amounts of β-carotene are present in the livers of rats, regardless of how large an amount of carotene is given in the diet. Moreover, it was found that carotene, injected intrasplenically, could not be utilized as a source of vitamin A. Rats died, presenting typical symptoms of avitaminosis A, in spite of the fact that large amounts of carotene were present in the livers of the animals at the time of death. No intrinsic impairment of carotene metabolism could be demonstrated in these vitamin A-deficient rats, since carotene given orally promptly relieved the symptoms of avitaminosis A. Lease *et al.*[362] likewise demonstrated that injected carotene was ineffective as a source of vitamin A.

Mattson, Mehl, and Deuel[257] demonstrated that vitamin A could be detected in the intestinal walls of vitamin A-deficient rats shortly after the feeding of β-carotene; vitamin A appeared in the intestinal wall earlier than in the liver, and remained at higher levels there for four hours. Moreover, it was shown that, when normal rats were fed large doses of vitamin A and then kept on a vitamin A-free diet for approximately two weeks, no vitamin A could be detected in the intestinal wall, in spite of the fact that a high concentration of vitamin A was present in the liver. This experiment indicates that the level of intestinal vitamin A is not a reflection of the quantity stored in the liver, and that the intestinal level may be entirely independent of that in the liver.

The results of Mattson *et al.*[257] were presented almost simultaneously with a note by Thompson, Ganguly, and Kon,[258] which was later published *in extenso*.[259] The intestinal wall has since been shown to be the site of transformation of β-carotene to vitamin A, not only in the rat,[260–262]

[360] T. Moore, *Biochem. J.*, *25*, 275–286 (1931).

[361] E. L. Sexton, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *31*, 299–320 (1946).

[362] J. G. Lease, E. J. Lease, H. Steenbock, and C. A. Baumann, *J. Lab. Clin. Med.*, *27*, 502–510 (1941–1942).

but also in the chicken,[363,364] in the guinea pig,[365] in the pig,[258,259,366] and in goats, sheep, and rabbits.[263,264] Proof that the material formed in the intestinal wall of rats after the administration of carotene actually is vitamin A was presented by Mattson.[367] The fat-soluble material from the intestinal walls of rats previously fed β-carotene was extracted, after saponification of the tissue, with Skellysolve A, and most of the vitamin A was removed by extraction of the petroleum ether solution with 90% methanol. After further purification, the active material was separated chromatographically. It was proved to be identical with vitamin A alcohol, both by spectroscopic examination and by virtue of forming a single homogeneous chromatogram when mixed with known vitamin A.

Popper and Greenberg[368] also reported experiments which can now be interpreted as indicative of the extrahepatic conversion of carotene. When the various organs of a series of vitamin A-depleted rats were examined by the use of fluorescence microscopy at intervals after the administration of β-carotene, fluorescence, due to the presence of vitamin A, appeared in the liver after the carotene was given orally, but not when it was administered by the parenteral route. This author sometimes detected fluorescence in the intestinal wall before its demonstration in the liver, but usually it was noted in the latter organ first.

Another confirmation of the extrahepatic conversion of carotene to vitamin A has been adduced by Krause and Pierce.[369] These workers found that, in rats which had undergone ligation of the portal vein, so that hepatic circulation was reduced to 10% of the normal, the amount of vitamin A in the serum increased after the oral administration of carotene. Even under such severe conditions, no carotene was found in the blood. This would indicate that the transformation of carotene to vitamin A occurs without any assistance from the liver.

In addition to the demonstration of the conversion of β-carotene to vitamin A by *in vivo* technics, Wiese *et al.*[370] succeeded in bringing about the same change by an *in vitro* procedure. Thus, it was shown that vitamin A was produced when a carotene solution in Tween was introduced into the intestine of vitamin A-deficient rats, after which the gastro-

[363] S. Y. Thompson, M. E. Coates, and S. K. Kon, *Biochem. J.*, *46*, xxx (1950).
[364] A. L. S. Cheng and H. J. Deuel, Jr., *J. Nutrition*, *41*, 619–628 (1950).
[365] L. Woytkiw and N. C. Esselbaugh, *J. Nutrition*, *43*, 451–458 (1951).
[366] M. E. Coates, S. Y. Thompson, and S. K. Kon, *Biochem. J.*, *46*, xxx–xxxi (1950).
[367] F. H. Mattson, *J. Biol. Chem.*, *176*, 1467–1468 (1948).
[368] H. Popper and R. Greenberg, *Arch. Pathol.*, *32*, 11–32 (1941).
[369] R. F. Krause and H. B. Pierce, *Arch. Biochem.*, *19*, 145–148 (1948).
[370] C. E. Wiese, J. W. Mehl, and H. J. Deuel, Jr., *Arch. Biochem.*, *15*, 75–79 (1947).

intestinal tract was immediately removed and kept under anaerobic conditions for three hours. More recently, McGillivray[371] demonstrated that highly significant increases in vitamin A occur in sections of sheep intestine incubated with colloidal carotene. Stallcup and Herman[372] have likewise proved that the small intestine is one site for the conversion of carotene to vitamin A in the case of dairy calves. However, in this species of animal, the liver was likewise shown to be capable of changing carotene to vitamin A. Rosenberg and Sobel[373] were also able to demonstrate the *in vitro* conversion of carotene to vitamin A in the isolated small intestine of the rat. In another report, Rosenberg and Sobel[374] showed that the rates of both the *in vitro* conversion in the small intestine of the rat and the *in vivo* transformation[375] are markedly diminished in alloxan diabetes. Davies[376] noted that the vitamin A storage in avian coccidiosis is decreased. It is suggested that the invasion of the intestinal wall by coccidia may result in impairment of the conversion of carotene to vitamin A.

It is reasonable to suppose that the intestinal wall is the site of the transformation of α- and γ-carotenes to vitamin A in species in which carotene is not present in the blood and tissues. That this is the case is indicated by the absence of the carotenoid from the liver of the chicken after the feeding of α-carotene,[377] and its relative absence from the liver of hens after the administration of pro-γ-carotene.[378]

One would likewise assume that cryptoxanthin would be acted on by the same enzyme system in the rat as is carotene, to yield vitamin A. That such is the case has been demonstrated by Patel and co-workers[306] for the rat, and by Ganguly and Deuel[307] for the chicken.

b. Sites for the Conversion of Carotene to Vitamin A Other Than the Small Intestine. Although there is extensive proof that the intestinal mucosa is an important site for the conversion of carotene to vitamin A, some reports would seem to indicate that the same change may be mediated in other tissues. However, the transformation may be carried out less efficiently under the latter conditions. Thus, Bieri and Sandman[249] were

[371] W. A. McGillivray, *Australian J. Sci. Research, B4,* 370–376 (1951).

[372] O. T. Stallcup and H. A. Herman, *J. Dairy Sci., 33,* 237–242 (1950).

[373] A. Rosenberg and A. E. Sobel, *Arch. Biochem. Biophys., 44,* 320–325 (1953).

[374] A. Rosenberg and A. E. Sobel, *Arch. Biochem. Biophys., 44,* 326–329 (1953).

[375] A. E. Sobel, A. Rosenberg, and H. Adelson, *Arch. Biochem. Biophys., 44,* 176–180 (1953).

[376] A. W. Davies, *Nature, 170,* 849 (1952).

[377] J. Ganguly, Personal communication, 1952.

[378] H. J. Deuel, Jr., J. Ganguly, B. K. Koe, and L. Zechmeister, *Arch. Biochem. Biophys., 33,* 143–149 (1951).

able to demonstrate growth in vitamin A-deficient rats when carotene was given in as small an amount as 1.6 μg. daily by intramuscular injection. However, for maximum growth, approximately four to six times as much carotene was required when given parenterally as when administered orally. Carotene in aqueous solution was also found to be well utilized when given subcutaneously. However, in confirmation of the results of Sexton *et al.*,[361] oil solutions of the pigment were essentially non-utilizable when given by the parenteral route.[249] In a later paper by Bieri and Pollard,[379] it was noted that an aqueous suspension of β-carotene solubilized with Tween 40 provided greater growth and longer survival when given by a single intravenous injection than when administered orally. However, the oral route was found to be superior to the intravenous when tocopherol was incorporated in the carotene preparation. Moreover, when vitamin A-deficient rats from which the small intestine had been surgically removed were injected with β-carotene, the vitamin A content of the serum four to six hours after the injection varied from 24 to 107 microgram per cent. Since this is considerably higher than the normal plasma content of vitamin A, the results are taken as evidence that a considerable formation of vitamin A from carotene can take place in tissues other than the small intestine. According to Samaras and Hingerty,[380] the conversion of carotene to vitamin A takes place in about two hours in normal rats. The reticuloendothelial system plays a role in this change; this was shown by the fact that the injection of trypan blue, which blocks the reticuloendothelial system, increased the ability of the normal rat to change carotene, administered eighteen hours after the dye, to vitamin A. In the vitamin A-deficient rat, in which this transformation of provitamin A to vitamin A is markedly diminished, the injection of trypan blue further inhibited the transformation of carotene to vitamin A. In the case of the normal rat it is believed that the trypan blue stimulated the RE system, while, in the case of the vitamin A-deficient animals, the dye caused a further deterioration of the already deficient organism. In another communication, these workers[381] proved that the vitamin A-deficient rat is unable to utilize carotene, and will die, even when carotene is available in its tissues as a source of vitamin A.

c. The Effect of the Thyroid Gland on the Transformation of Carotene to Vitamin A. In addition to playing an important role in the absorption of carotene from the gastrointestinal tract, there seems to be some evidence

[379] J. G. Bieri and C. J. Pollard, *Federation Proc.*, *12*, 409 (1953).
[380] S. C. Samaras and D. J. Hingerty, *Am. J. Physiol.*, *159*, 588–589 (1949).
[381] S. C. Samaras and D. J. Hingerty, *Am. J. Physiol.*, *159*, 588 (1949).

that the thyroid gland in some way regulates the transformation of carotene to vitamin A. As early as 1907, von Noorden[382] suggested that the condition of carotenemia may be associated with certain metabolic disorders. Anderson and Soley[383] later ascribed this metabolic upset to an alteration in thyroid function. On the basis of the poor dark adaptation of hypothyroid patients, Wohn and Feldman[384] concluded that the thyroid gland was in some way connected with carotene metabolism. Escamilla[385] and Mandelbaum *et al.*[386] demonstrated that, in the clinical condition of hypothyroidism in man, namely myxedema, carotenemia ensued. Both conditions tended to clear up under treatment with thyroid substance. Wendt[387] reported a low level of serum vitamin A in patients with hyperthyroidism (Basedow's disease), even though the carotene intake was sufficient to produce a normal value.

Studies on the relationship between the thyroid secretion and carotene metabolism have furnished considerable information. Thus, Kunde[388] noted the appearance of xerophthalmia in rabbits, eight to twenty months after total thyroidectomy. Fasold and Heidemann[389] likewise made the interesting observation that the carotene content of goat milk increased following thyroidectomy, coincident with a decrease in its vitamin A content. Von Euler and Klussmann[390] also pointed out that an antagonism exists between carotene and thyroxine. Drill and Truant[391] likewise reported that thyroidectomy in rats is followed by a depression in the carotene → vitamin A reaction, since it was found to be impossible to relieve ocular symptoms caused by vitamin A deficiency by means of daily doses of β-carotene amounting to as much as 10 μg., although preformed vitamin A afforded protection against these lesions. However, Remington *et al.*[392] reported that an oral dose of β-carotene as small as 0.6 μg. was able to bring about a cure of the xerophthalmia in vitamin A-deficient thyroidectomized rats, within seven to nine days. Goodwin[393] demonstrated that, in the hyperthyroid rat, the converse of the above

[382] C. von Noorden, *Die Zuckerkrankheit*, 4th ed., Hirschwald, Berlin, 1907; cited by V. A. Drill, *Physiol. Revs.*, *23*, 355–379 (1943), p. 359.

[383] H. H. Anderson and M. H. Soley, *Am. J. Med. Sci.*, *195*, 313–318 (1938).

[384] M. G. Wohn and J. B. Feldman, *Endocrinology*, *24*, 389–396 (1939).

[385] R. F. Escamilla, *J. Clin. Endocrinol.*, *2*, 33–35 (1942).

[386] T. Mandelbaum, S. Candel, and S. Millman, *J. Clin. Endocrinol.*, *2*, 465–467 (1942).

[387] H. Wendt, *Klin. Wochschr.*, *14*, 9–14 (1935).

[388] M. M. Kunde, *Proc. Soc. Exptl. Biol. Med.*, *23*, 812 (1926).

[389] H. Fasold and E. R. Heidemann, *Z. ges. exptl. Med.*, *92*, 53–56 (1933).

[390] H. von Euler and E. Klussmann, *Z. physiol. Chem.*, *213*, 21–34 (1932).

[391] V. A. Drill and A. P. Truant, *Endocrinology*, *40*, 259–264 (1947).

[392] R. E. Remington, P. L. Harris, and C. L. Smith, *J. Nutrition*, *24*, 597–606 (1942).

[393] T. W. Goodwin, *Biochem. J.*, *43*, xliii–xliv (1948).

occurs, namely, that carotene is converted to vitamin A more efficiently than in the animal which possesses a normal thyroid function.

There are a number of reports in the literature on the effect of hypothyroidism on carotene metabolism when the deficiency has been produced by the administration of thiouracil or thiourea. Thus, Johnson and Baumann[394] reported that very little storage of vitamin A occurred in the livers of rats treated with thiourea or thiouracil followed by the administration of carotene; thyroxine increased the ability of these animals to convert carotene to vitamin A. Kelley and Day[395] confirmed the fact that vitamin A deposition in the liver after the feeding of carotene was decreased in thiouracil-treated rats and increased when thyroid was given. However, differences recorded by these workers in the several conditions are not great. Canadell and Valdecasas[396] likewise reported that carotene was unable to relieve the ocular symptoms of a vitamin A deficiency in a thiouracil-treated animal. However, these symptoms could be cleared up if small amounts of desiccated thyroid were administered with the carotene.

Although all the above reports are in agreement in supporting the hypothesis that the carotene → vitamin A change is regulated by the thyroid gland, several reports are in disagreement. Thus, Wiese and her collaborators[397] were unable to demonstrate any differences in the liver storage of vitamin A after the administration of 348 μg. of β-carotene, irrespective of whether or not the rats had previously been treated with thiouracil. In a later study by these same investigators,[398] it was found that, although the extent of maximum growth, after vitamin A or carotene feeding, is markedly depressed by hypothyroidism, the point of 50% response to carotene or vitamin A was unaltered. These results are taken to indicate that carotene and vitamin A are equally well utilized by hypothyroid rats when fed at low levels. In these later tests, no differences were observed in the comparative amounts of vitamin A and carotene required to bring about growth or to relieve eye symptoms in vitamin A-deficient hypothyroid rats. Morgan and Arnrich[399] also concluded that the normally functioning thyroid gland is not essential for carotene utilization in rats and dogs. These workers reported that young vitamin A-

[394] R. M. Johnson and C. A. Baumann, *J. Biol. Chem.*, *171*, 513–521 (1947).

[395] B. Kelley and H. G. Day, *J. Biol. Chem.*, *175*, 863–866 (1948).

[396] J. M. Canadell and F. G. Valdecasas, *Experientia*, *3*, 35–36 (1947).

[397] C. E. Wiese, H. J. Deuel, Jr., and J. W. Mehl, *Proc. Soc. Exptl. Biol. Med.*, *66*, 213–214 (1947).

[398] C. E. Wiese, J. W. Mehl, and H. J. Deuel, Jr., *J. Biol. Chem.*, *175*, 21–28 (1948).

[399] A. F. Morgan and L. Arnrich, *Federation Proc.*, *12*, 424–425 (1953).

depleted rats stored less vitamin A after carotene than did similar animals which had been treated with thiouracil. Moreover, the preformed stores of vitamin A were found to be more slowly depleted in the hypothyroid rats than in their normal controls. After ten weeks on a vitamin A-free diet the controls retained 5% of the original vitamin A, while 17 and 14% were retained in hypothyroid rats and in pair-weighed controls.

The finding of Kaplanskiĭ and Balaba[253] that carotene can be converted to vitamin A by *in vitro* incubation of colloidal solutions of carotene with aqueous solutions of either thyroglobulin or iodinated casein has also been considered to offer support for the supposition that the thyroid hormone stimulates the conversion of carotene to vitamin A. However, neither Cama and Goodwin,[400] Lowry and Lowry,[255] nor Wiese.[401] have been able to confirm this observation. Although the bulk of evidence would seem to support the hypothesis that the thyroid secretion regulates the carotene → vitamin A reaction, more proof is needed before this theory can be accepted unequivocally. Drill[402] reviewed this subject in 1943.

8. The Digestion and Absorption of Phytofluene in the Gastrointestinal Tract

Phytofluene, a polyene closely related to the carotenoids, was first discovered by Zechmeister and Polgár.[403] It has been found to be widely distributed in plant organs which also produce carotenoid pigments.[404] Sandoval *et al.*[405] found that this pigment was absorbed to some extent by rabbits, and was deposited in the liver. However, the major portion of the phytofluene fed was destroyed; it has not been determined whether or not this destruction occurs in the gastrointestinal tract.

9. The Digestion, Absorption, and Transformations of the Fat-Soluble Vitamins

(1) Vitamin A

a. The Absorption of Vitamin A from the Intestine. Vitamin A is much more readily absorbed from the gastrointestinal tract than is its

[400] H. R. Cama and T. W. Goodwin, *Biochem. J.*, *43*, xliv (1948).

[401] C. E. Wiese, *The Site of Conversion of Carotene to Vitamin A in the Rat. The Effect of Hypcthyroidism on this Conversion.* Thesis, Univ. So. Calif., Dept. Biochem. Nutrit., June, 1948.

[402] V. A. Drill, *Physiol. Revs.*, *23*, 355–379 (1943).

[403] L. Zechmeister and A. Polgár, *Science*, *100*, 317–318 (1944).

[404] L. Zechmeister and A. Sandoval, *J. Am. Chem. Soc.*, *68*, 197–201 (1946).

[405] A. Sandoval, E. R. Meserve, H. J. Deuel, Jr., and L. Zechmeister, *Arch. Biochem.*, *11*, 373–375 (1946).

provitamin, carotene. In contradistinction to carotene, vitamin A is as well utilized when given by the parenteral route as it is when administered orally.[361] In fact, Sobel and co-workers[406] reported that, when vitamin A was administered to cows intravenously as an aqueous dispersion, the increased excretion of vitamin A in the milk was fifteen times as great as when the same quantity of vitamin A was given orally, dissolved in oil. When vitamin A in aqueous dispersion was ingested, the increased vitamin A content in the milk of cows was five times that observed when the vitamin was given in oil by the same route.

(*a*) *Hydrolysis of Vitamin A Esters as a Preliminary to Absorption.* On the basis of the experiments of Gray, Morgareidge, and Cawley,[407] it is now generally accepted that vitamin A esters must first be hydrolyzed to the alcohol form before they are absorbed from the gastrointestinal tract. It is believed that an esterase in the intestinal juice is responsible for this hydrolysis. Gray *et al.*[407] found that, when the naturally occurring ester was given to rats, a small but steady increase in the amount of free alcohol in the intestinal contents resulted as absorption proceeded. However, the decisive finding which indicated that hydrolysis of the ester must precede absorption was the proof that the vitamin A present in the intestinal wall was largely in the form of the free alcohol. In the samples of gut wall obtained from rats in the three- to six-hour period after the feeding of vitamin A ester, 82% of the total vitamin A in the tissues was found to be in the form of the free alcohol. Eden and Sellers[408,409] extended the observation of this phenomenon to calves and sheep. When the animals were slaughtered four hours after the administration of vitamin A acetate, almost complete hydrolysis had obtained in the contents of the intestinal lumen, in some cases, and only a partial hydrolysis in other cases. When vitamin A ester was fed, the ester fraction in the mucosa was 73% of the total vitamin A in the case of the calves, and 56% in the case of the sheep. On the other hand, the corresponding values for the ester fraction in the intestinal mucosa of the calves and sheep, respectively, after the administration of vitamin A alcohol, were 82 and 77%. This indicates that esterification of the vitamin A alcohol occurs after it has been absorbed into the mucosa. Further confirmation of the hydrolysis of vitamin A esters prior to absorption is to be found in the results of Clausen[410] and in those of Popper and Volk.[411]

[406] A. E. Sobel, A. Rosenberg, and E. Engel, *J. Nutrition*, *48*, 183–192 (1952).
[407] E. L. Gray, K. Morgareidge, and J. D. Cawley, *J. Nutrition*, *20*, 67–74 (1940).
[408] E. Eden and K. C. Sellers, *Biochem. J.*, *45*, xxxiii (1949).
[409] E. Eden and K. C. Sellers, *Biochem. J.*, *46*, 261–266 (1950).
[410] S. W. Clausen, *Harvey Lectures*, *38*, 199–226 (1943).
[411] H. Popper and B. W. Volk, *Arch. Pathol.*, *38*, 71–75 (1944).

Lovern and associates[412,413] suggested that the vitamin A alcohol may mediate in the absorption of the fatty acids. Although the vitamin A may act in this capacity in the case of the halibut and of other fishes in which this vitamin plays a more important role quantitatively than in the higher mammals, Gray *et al.*[407] were unable to demonstrate any such action in the rat.

(*b*) *Factors Altering the Rate of Absorption of Vitamin A.* a′. The Effect of Age: As in the case of triglycerides, age appears to be an important factor in controlling the rate of absorption of vitamin A. Thus, Sobel and his collaborators[414] reported that vitamin A was absorbed at a markedly diminished rate in the newborn baby, as compared with the rate in children over one year of age, or with that of adults, when it was administered either in an oily medium or in an aqueous dispersion. Moreover, the rapidity of absorption of vitamin A, as determined by the vitamin A tolerance test, was shown to be low in normal infants,[415] as contrasted with that in older children. Clausen[416] reported that the maximum level of vitamin A in the blood was attained three to five hours after its oral administration in older children. This is interpreted as indicating a rapid utilization at this age. On the other hand, Rafsky and Newman[417] reported that the absorption of vitamin A proceeds relatively slowly in the aged (69 to 89 years).

b′. The Effect of Concentration of Administered Vitamin A: Normally, vitamin A is rapidly absorbed by the rat. It has been shown that the concentration of the vitamin A administered is an important factor which influences the rate of utilization in this species. In the experiments of Reifman *et al.*[418] a direct proportionality was found, over a wide range, between the concentration of the material fed and the rate of absorption. Thus, the average absorption of vitamin A (calculated as I.U. per 100 sq. cm. of body surface per hour) was as follows for the different dosages: 100 I.U. dosage, 4.2 to 6.5 I.U.; 1000 I.U. dosage, 28.5 I.U.; 10,000 I.U. dosage, 369 I.U.; 100,000 I.U. dosage, 2108 I.U.; and 1,000,000 I.U. dosage, 10,140 I.U.

c′. The Effect of Bile: The importance of bile in the absorption of

[412] J. A. Lovern and R. A. Morton, *Biochem. J.*, *33*, 330–337 (1939).

[413] J. A. Lovern, T. H. Mead, and R. A. Morton, *Biochem. J.*, *33*, 338–343 (1939).

[414] A. E. Sobel, L. Besman, and B. Kramer, *Am. J. Diseases Children*, *77*, 576–591 (1949).

[415] A. E. Sobel, S. P. Gottfried, B. Kramer, and L. Besman, *Abstracts, 110th Meeting, Am. Chem. Soc., Div. Biol. Chem.*, Chicago, Sept. 11, 1946, 28B–29B.

[416] S. W. Clausen, *J. Am. Med. Assoc.*, *101*, 1384–1388 (1933).

[417] H. A. Rafsky and B. Newman, *Gastroenterology*, *10*, 1001–1006 (1948).

[418] A. G. Reifman, L. F. Hallman, and H. J. Deuel, Jr., *J. Nutrition*, *26*, 33–42 (1943).

vitamin A is much less than in the case of carotene. Schmidt and Schmidt[419] reported that vitamin A could still be efficiently absorbed by choledochocolostomized vitamin A-deficient rats.

d′. The Effect of Fat Feeding: Fat likewise plays a less important role in the absorption of vitamin A from the gastrointestinal tract than it does in the case of the carotenoids. Although Basu[227] stated that fat is required for the absorption of vitamin A and carotene, contrary results have been reported for the absorption of vitamin A in rats by De[420] as well as by Reifman and associates.[418] The presence of fats was also found to be unessential for the absorption of vitamin A in the fowl,[232] as well as in man.[228]

e′. The Effect of Mineral Oil: The presence of mineral oil in the intestine may likewise modify the absorption of vitamin A, but here also the effects are less serious than in the case of provitamin A. In fact, Moness and Christiansen,[421] as well as Alexander and co-workers,[268] failed to demonstrate any deleterious effect whatsover on the part of mineral oil on the utilization of vitamin A.[422] However, a number of other workers[269,277,423–425] reported some impairment in the absorption of vitamin A when mineral oil was present, although to a lesser degree than in the case of carotene.

f′. A Comparison of the Utilization of Vitamin A Alcohol and Vitamin A Esters: In general, the absorption of vitamin A has been believed to proceed equally well, in normal subjects, when given as the ester or as the alcohol.[426,427] However, since the hydrolysis of the vitamin A ester to the alcohol must precede its absorption, any conditions which retard or inhibit the hydrolysis of the ester should at the same time reduce the effectiveness of the ester form. Accepting this as a working hypothesis, Week and Sevigne compared the biological responses to vitamin A given in the form of natural esters, acetate or free alcohol when fed to chickens[428]

[419] W. Schmidt and C. L. A. Schmidt, *Univ. Calif. (Berkeley) Pub. Physiol.*, *7*, 211–221 (1930).

[420] N. K. De, *Indian J. Med. Research*, *24*, 751–766 (1937).

[421] E. Moness and W. G. Christiansen, *J. Am. Pharm. Assoc.*, *18*, 997–998 (1929).

[422] A. C. Curtis and P. B. Horton, *Am. J. Med. Sci.*, *200*, 102–107 (1940).

[423] O. Andersen, *Acta Paediat.*, *24*, 422–427 (1939).

[424] P. B. Hawk, B. L. Oser, and W. H. Summerson, *Practical Physiological Chemistry*, 12th ed., Blakiston, Philadelphia, 1947, p. 1037.

[425] M. C. Smith and H. S. Spector, *Univ. Arizona, Coll. Agr. Ariz. Agr. Expt. Sta., Tech. Bull. No. 84*, 373–395 (1940).

[426] A. B. McCoord, C. P. Katsampes, C. F. Lavender, F. J. Martin, R. A. Ulstrom, R. H. Tully, III, and A. J. Keenan, *Pediatrics*, *2*, 652–665 (1948).

[427] A. E. Sobel, L. Besman, and B. Kramer, *Am. J. Diseases Children*, *77*, 576–591 (1949).

[428] E. F. Week and F. J. Sevigne, *J. Nutrition*, *39*, 233–250 (1949).

TABLE 8

AVERAGE STORAGE OF VITAMIN A IN LIVERS OF VITAMIN A-DEPLETED CHICKS AFTER FEEDING OF 30,000 UNITS OF VITAMIN A IN THE FORM OF ALCOHOL, ACETATE, OR NATURAL ESTER IN THREE DIVIDED DOSES WITH SEVERAL DILUENT OILS[a]

Diluent oil[b]	Oil fed per dose, ml.	Vitamin A concn., units/ml.	Units vitamin A per liver after			% vitamin A stored after		
			Alcohol	Acetate	Natural ester	Alcohol	Acetate	Natural ester
Experiment A[c]								
Cottonseed	2.0	5,000	7,490	6,830	5,320	25.0	22.8	17.7
Corn	2.0	5,000	7,150	5,970	4,940	23.8	19.9	16.5
Sardine	2.0	5,000	3,930	3,960	1,390	13.1	13.2	4.6
Basking shark	2.0	5,000	4,510	2,390	1,880	15.0	8.0	6.3
Mineral oil	2.0	5,000	4,860	2,160	1,465	16.2	7.2	4.9
Experiment B[d]								
Corn	2.0	5,000	10,670	10,080	7,130	35.6	33.6	22.8
	0.1	100,000	10,380	11,690	8,320	34.6	39.0	27.7
Castor	2.0	5,000	8,210	8,020	5,090	27.4	26.7	17.0
Jojoba seed	2.0	5,000	9,850	4,770	2,440	32.8	15.9	8.1
	0.1	100,000	9,170	10,490	8,400	30.5	35.0	28.0
Ethyl laurate	2.0	5,000	8,830	4,240	3,660	29.4	14.1	12.2

[a] Data from E. F. Week and F. J. Sevigne, *J. Nutrition*, *39*, 233–250 (1949).
[b] Control tests on chicks given each diluent oil without vitamin A showed negative results for vitamin A in liver in all cases.
[c] New Hampshire chicks, 52 days of age, 10 chicks per group.
[d] White leghorn cockerels, 56 days of age, 12 chicks per group.

or to rats[429] in jojoba seed oil, ethyl laurate, basking shark liver oil, cottonseed oil, corn oil, castor oil, or mineral oil. The vitamin A absorption as determined from the vitamin A content of the liver under these several conditions is summarized for chickens in Table 8 and for rats in Table 9.

TABLE 9

AVERAGE STORAGE OF VITAMIN A IN LIVERS OF VITAMIN A-DEFICIENT RATS AFTER FEEDING OF 9000 UNITS OF VITAMIN A IN THE FORM OF ALCOHOL, ACETATE, OR NATURAL ESTER IN SIX DIVIDED DOSES WITH SEVERAL DILUENT OILS[a]

Diluent oil	Corn oil	Castor oil	Jojoba seed oil
Oil fed per dose, ml.	0.4; 0.1	0.4	0.4
Vitamin A concentration, units/ml.	7,500; 30,000	7,500	7,500
Units of vitamin A per liver after:			
Alcohol	2,990; 2,920	2,720	2,660
Acetate	3,250; 3,530	2,740	2,610
Ester	2,630; 3,140	2,690	1,770
Per cent vitamin A stored after:			
Alcohol	33.2; 32.4	30.2	29.5
Acetate	36.1; 39.2	30.4	28.9
Ester	29.2; 34.9	29.9	19.7

10 rats were used per group.
[a] Data from E. F. Week and F. J. Sevigne, *J. Nutrition, 39,* 251–257 (1949).

Vitamin A deposition in the livers of chickens, which is an index of relative absorption, would appear to be dependent upon the form of vitamin A used, as well as upon the diluent oil. In all cases, the vitamin A deposition is lowest when the natural ester is fed. In general, vitamin A acetate elicits a slightly lower response than does the free alcohol, but this condition is revised if the quantity of oil given with the vitamin is reduced to a minimum. The amount of vitamin A deposited in the liver when the vitamin was dissolved in corn, cottonseed, or jojoba oil was highest for the alcohol form. When ethyl laurate or castor oil was the diluent, slightly lower results were obtained for all types of vitamin A. However, when sardine oil, basking shark oil, or mineral oil was employed, the vitamin A deposition was reduced to 50 to 60% of that obtained with cottonseed oil.[428]

On the other hand, the greatest discrepancies caused by the several diluent oils were noted in the experiments in which the natural ester was used. In the case of jojoba oil tests at 2.0 ml. of diluent oil per feeding, the vitamin A was only one-fourth as effective (as regards deposition) when

[429] E. F. Week and F. J. Sevigne, *J. Nutrition, 39,* 251–257 (1949).

given in the form of natural esters as when employed in the form of the alcohol (8.1% *vs.* 32.8% of that administered). When the jojoba oil fed was reduced to one-twentieth of this level (0.1 ml. instead of 2.0 ml. for each feeding), the variations in vitamin A deposition as related to the form of the vitamin A largely disappeared. It is therefore difficult to escape the conclusion that the vitamin A esters must have a markedly lower absorption in chickens than do the alcohols when given in large quantities of oil, particularly when such diluent oils are difficultly absorbed. March and co-workers[430] have also reported that the carrier influences the effectiveness of the form in which vitamin A is administered. When an aqueous carrier was employed, the natural ester and the acetate were found to be better utilized by the chick than was the free alcohol. On the other hand, when cottonseed oil was the carrier, the best utilization obtained with the acetate, followed in order by the alcohol and the natural ester. The low utilization of the palmitate ester of vitamin A in an oil carrier was found to be due not to the destruction of vitamin A but rather to an interference with the absorption of vitamin A through the intestinal mucosa.

The results obtained in the tests on chickens were shown to apply to a somewhat lesser extent to rats.[429] Jojoba seed oil was found to have a definite depressing action on vitamin A absorption only when the vitamin was given as the natural ester. Week and Sevigne[431] suggest that mineral oil and ethyl laurate contain factors which inhibit the hydrolysis of vitamin A esters *in vivo*.

In recent work of Week and Sevigne,[432] it was found that the earlier data obtained in chickens and rats may also apply to man. After a dose of 134,000 μg. of vitamin A in 50 g. of margarine was given to male subjects, it was found that the vitamin A absorption, as judged by the vitamin A tolerance curves in the blood, was best with the alcohol, somewhat less with the vitamin A acetate, and poorest with the mixture of natural ester. In the case of female subjects, the vitamin A alcohol yielded a significantly better result than did the vitamin A acetate, while no significant differences could be noted between the response of the vitamin A in alcohol and in the natural ester form. The results of Popper *et al.*[433] on hospital controls and on patients with liver disease differ somewhat from those of Week and Sevigne.[432] When vitamin A was given in either an aqueous or an oily menstruum, the plasma levels were slightly higher

[430] B. E. March, E. English, and J. Biely, *Arch. Biochem. Biophys.*, *36*, 259–268 (1952).

[431] E. F. Week and F. J. Sevigne, *J. Nutrition*, *42*, 525–537 (1950).

[432] E. F. Week and F. J. Sevigne, *J. Nutrition*, *40*, 563–576 (1950).

[433] H. Popper, F. Steigmann, and H. A. Dyniewicz, *Proc. Soc. Exptl. Biol. Med.*, *73*, 188–190 (1950).

after equal doses of vitamin A esters than after the vitamin A alcohol. In both sets of experiments, the increased vitamin A in the blood serum was composed of the ester, irrespective of the form in which the vitamin A preparation was given.

g′. The Effect of Emulsifying Agents: Lecithin has been shown to enhance the absorption of vitamin A. Thus, Esh and Sutton[241] and Scharf[434] noted that vitamin A is better utilized when given with soybean lecithin than when this phospholipid is absent. Esh and co-workers[435] reported that a similar phenomenon obtains in dairy cattle. The transmission of dietary vitamin A to colostrum was increased by feeding lecithin; the liver storage of vitamin A was also augmented when lecithin was included in the diet. Moreover, the ability of vitamin A to depress the carotene level was increased. In the case of man, Adlersberg *et al.*[436] reported that lecithin promotes the absorption of vitamin A in sprue but not in liver disease.

h′. The Utilization of Vitamin A in Aqueous Dispersion: Several groups of workers[415,427,437–450] have found that vitamin A in aqueous dispersion is more effective than when given in an oil solution. The experimental procedures followed in differentiating between the absorption of

[434] A. Scharf, *Food Materials and Equip.*, *7*, 8–9 (1947); cited by P. L. Harris, *Ann. Rev. Biochem.*, *18*, 391–434 (1949), p. 399.

[435] G. C. Esh, T. S. Sutton, J. W. Hibbs, and W. E. Krauss, *J. Dairy Sci.*, *31*, 461–478 (1948).

[436] D. Adlersberg, S. Kann, A. P. Maurer, K. Newerly, W. Winternitz, and H. Sobotka, *Gastroenterology*, *10*, 822–830 (1948).

[437] B. Kramer, A. E. Sobel, and S. P. Gottfried, *Am. J. Diseases Children*, *73*, 543–553 (1947).

[438] J. M. Lewis, O. Bodansky, J. Birmingham, and S. Q. Cohlan, *J. Pediat.* *31*, 496–508 (1947).

[439] C. D. May and C. U. Lowe, *J. Clin. Invest.*, *27*, 226–230 (1948).

[440] A. E. Sobel, L. Besman, and B. Kramer, *Federation Proc.*, *7*, 189–190 (1948).

[441] A. E. Sobel, A. A. Rosenberg, and B. Kramer, *Abst. 114th meeting, Am. Chem. Soc., Div. Biol. Chem.*, Aug. 30, 1948, 15C–16C.

[442] A. E. Sobel and A. A. Rosenberg, *J. Nutrition*, *42*, 557–563 (1950).

[443] A. E. Sobel, A. A. Rosenberg, R. Geduldig, E. Engel, M. West, and B. Kramer, *Federation Proc.*, *8*, 253–254 (1949).

[444] H. Popper, F. Steigmann, and H. A. Dyniewicz, *Gastroenterology*, *10*, 987–1000 (1948).

[445] C. J. Kern and T. Antoshkiw, *Ind. Eng. Chem*, *42*, 709–713 (1950).

[446] A. E. Sobel, M. Sherman, J. Lichtblau, S. Snow, and B. Kramer, *J. Nutrition*, *35*, 225–238 (1948).

[447] H. Popper and B. W. Volk, *Proc. Soc. Exptl. Biol. Med.*, *68*, 562–564 (1948).

[448] G. R. Halpern, J. Biely, and F. Hardy, *Science*, *106*, 40–41 (1947).

[449] G. R. Halpern and J. Biely, *J. Biol. Chem.*, *174*, 817–826 (1948).

[450] B. C. Barnes, E. E. Wollaeger, and H. L. Mason, *J. Clin. Invest.*, *29*, 982–987 (1950).

the aqueous suspensions and of the oil solutions were not exactly comparable, inasmuch as, in the first case, vitamin A alcohol is generally employed, while in the second instance vitamin A ester is used. However, Kagan *et al.*[451] are of the opinion that this difference in utilization is to be traced to the physical state of the vitamin rather than to the alcohol as opposed to the ester. On the other hand, Popper and Volk[447] ascribe the differences to the passage of increased amounts of vitamin A through the epithelium if given in aqueous dispersion and not to a difference in the physical state of the vitamin A after its passage into the cell. Kagan and associates[451] also demonstrated that the increase in plasma vitamin A was similar when aqueous suspensions of vitamin A alcohol and of vitamin A palmitate, respectively, were given. Sobel *et al.*[414] reported that the decrease in the rate of absorption of vitamin A observed in the newborn when the vitamin is administered in oil is overcome when an aqueous dispersion containing 16% of polyoxyethylene sorbitan monolaurate (Tween 20) is employed. Enhanced absorption of vitamin A has been noted in normal men and in individuals whose utilization of fat is impaired. Thus, Barnes and co-workers[450] reported that normal subjects had higher plasma curves and lower fecal losses of vitamin A after the ingestion of the aqueous dispersion than after taking an oil solution of vitamin A. Although the patients with non-tropical sprue had flat plasma curves and excessive fecal losses with both preparations, the results with the aqueous preparation were superior to those with the oil solution. The vitamin A plasma curve was also restored to normal in a patient with diabetes mellitus and steatorrhea after the ingestion of the aqueous dispersion, although the losses in the feces remained high.

Krantz[452] also reported an improvement in the vitamin A utilization in both normal controls and diseased patients when an aqueous dispersion of vitamin A was used. Thus, when 200,000 I.U. of vitamin A ester were given to normal men alone or with 2 g. of Tween 80 (PSM), the speed at which the maximum blood level was reached was found to be quite different.[452] The peak value, when the vitamin A ester was given alone, was reached at five hours while, in the presence of PSM, the blood vitamin A attained the maximum level in only three hours. This would suggest that absorption is more rapid when PSM is present. Another striking example of improved absorption of vitamin A resulting from the use of aqueous solutions stabilized with PSM was in the case of patients suffering from such conditions as subtotal gastrectomy, sprue, pancreatic fibro-

[451] B. M. Kagan, D. A. Jordan, and D. S. Gerald, *J. Nutrition*, *40*, 275–279 (1950).
[452] J. C. Krantz, Unpublished observations cited in *Nutrition Revs.*, *7*, 205–207 (1949).

sis, or regional enteritis. These subjects showed a low or flat vitamin A tolerance curve when vitamin A was given in oil; on the other hand, when PSM solutions of vitamin A were given, a marked increase in the peak levels of vitamin A resulted,[452] with maximum values 400 to 500% higher than those in the control tests.

Sobel and associates[406] reported that aqueous solutions of vitamin A are non-toxic. After repeated intravenous injections of the aqueous dispersion of vitamin A into rabbits, no injury to tissues could be demonstrated by gross examination, by histopathological survey of vital organ sections, or by chemical analysis. However, when corn oil solutions of vitamin A were administered by several routes to vitamin A-deficient rats, Lemley *et al.*[453] found that the subcutaneous administration of vitamin A was only 35% as effective as it was by the oral route, while intramuscular injection was only 2% as efficient.

i′. The Effect of Thyroxine: Reciprocal relationships exist between the metabolism of vitamin A and that of thyroxine. On the one hand, several investigators have reported that large amounts of vitamin A produce an antithyroid action.[454–456] On the other hand, the storage of vitamin A in the liver of rats on a diet free from the vitamin was found to be highest in thyroidectomized animals, intermediate in those treated with thyroxine, and least in the control animals.[457] Whether or not this is a reflection of the effect of the thyroid on vitamin A absorption has not been established. The low value of vitamin A in the control rats was interpreted as due to the utilization of vitamin A for growth.

j′. Miscellaneous Factors Affecting Absorption: Darby, Kaser, and Jones[289] have reported that the ability to absorb vitamin A as well as carotene improved in sprue patients treated with pteroylglutamic acid. When vitamin A was administered to treated patients, an increase in serum vitamin A occurred, as contrasted with a flat curve during a relapse after withdrawal of the treatment.

X-irradiation has little effect on the absorption of vitamin A. Bennett *et al.*[458] found an increase in the absorption of vitamin A six hours after feeding vitamin A alcohol to young female rats, two to six days after

[453] J. M. Lemley, R. A. Brown, O. D. Bird, and A. D. Emmett, *J. Nutrition*, *33*, 53–64 (1947).

[454] I. J. Belasco and J. R. Murlin, *J. Nutrition*, *20*, 577–588 (1940).

[455] D. P. Sadhu and S. Brody, *Am. J. Physiol.*, *149*, 400–403 (1947).

[456] E. Schulze and G. Hundhausen, *Arch. exptl. Pathol. Pharmakol. (Naunyn-Schmiedeberg's)*, *192*, 43–52 (1939).

[457] C. B. Heimer, H. L. Maslow, and A. E. Sobel, *J. Nutrition*, *38*, 345–352 (1949).

[458] L. R. Bennett, V. C. Bennett, A. Shaver, and T. Grachus, *Proc. Soc. Exptl. Biol. Med.*, *74*, 439–443 (1950).

exposure to 625 *r*. After an interval of ten hours, little difference in absorption could be noted between the irradiated and the control rats. However, the distribution of vitamin A in the tissues was modified by x-irradiation. Smaller amounts of vitamin A were found in the liver and larger quantities in the carcass.

(*c*) *The Stability of Vitamin A in the Intestine.* Although Baumann and associates[459] found that vitamin A may be largely destroyed in the gastrointestinal tract, Reifman and co-workers[418] were unable to demonstrate any appreciable destruction in three-hour tests. Moreover, Richardson[460] has indicated that the putrefaction in fish livers is without a marked effect on their vitamin A content. Geiger[461] also reported that no carotene or vitamin A is destroyed by intestinal bacteria over a twenty-four hour period. Bieri[462] found that vitamin A acetate was stable for twenty-eight days in aqueous solutions either in Tween 40 (polyoxyalkylene derivative of sorbitan monopalmitate), or Tween 80, irrespective of whether it was stored under air or nitrogen. On the other hand, β-carotene remained unchanged only in Tween 40 and under nitrogen.

The tocopherols are now recognized to be the most important stabilizing agents for vitamin A and carotene. Bacharach,[463] Moore,[333] and Davies and Moore[334] were the first to demonstrate the synergistic action of tocopherols on the deposition of vitamin A in the tissues. It was later shown by Hickman and associates[464,465] that the growth-promoting effect of vitamin A alcohol, vitamin A acetate, and U.S.P. vitamin A reference oil was enhanced when natural vitamin E preparations (mixed tocopherols) were present. This "sparing" or "synergistic" action of the tocopherols is termed "co-vitamin E" activity.[465] The co-vitamin E action on vitamin A has been confirmed by a number of workers, including Gridgeman,[466] Guggenheim,[343] Lemley *et al.*,[467] Sanders and co-workers,[468] and most recently by Galeone and San Lorenzo.[469]

[459] C. A. Baumann, B. M. Riising, and H. Steenbock, *J. Biol. Chem.*, *107*, 705–715 (1934).

[460] A. Richardson, Personal communication, 1952.

[461] E. Geiger, Personal communication, 1952.

[462] J. G. Bieri, *J. Nutrition*, *44*, 327–334 (1951).

[463] A. L. Bacharach, *Quart. J. Pharm. Pharmacol.*, *13*, 138–149 (1940).

[464] K. C. D. Hickman, P. L. Harris, and M. R. Woodside, *Nature*, *150*, 91–92 (1942).

[465] K. C. D. Hickman, M. W. Kaley, and P. L. Harris, *J. Biol. Chem.*, *152*, 303–311 (1944).

[466] N. T. Gridgeman, *The Estimation of Vitamin A*, 2nd ed., Lever Bros. & Unilever, Port Sunlight, Cheshire, 1–74 (1945), p. 37.

[467] J. M. Lemley, R. A. Brown, O. D. Bird, and A. D. Emmett, *J. Nutrition*, *34*, 205–218 (1947).

[468] R. Sanders, A. Beaty, and M. Dodd, *Abst. Meetings, Am. Chem. Soc., Div. Biol. Chem.*, Cleveland, April 4, 1944, 17B.

[469] A. Galeone and G. San Lorenzo, *Intern. Z. Vitaminforsch.*, *19*, 257–265 (1948).

The mechanism by which tocopherols potentiate the action of vitamin A is not entirely clear. Davies and Moore[334] concluded that the increased storage of vitamin A accompanying the administration of tocopherol is probably to be ascribed to its antioxidant action. They state: "The tocopherols are higher substituted members of the class of hydroxy-aromatic substances, of which hydroquinone is a lower and less hydroxylated member. . . often used for stabilizing vitamin A in fats. The tocopherols may possibly have a similar action under physiological conditions." Quackenbush and co-workers[339] believe that the protective action of tocopherol toward carotene is due to its action "as an antioxidant in the gastrointestinal tract rather than as a vitamin regulating some phase of metabolism in the tissues." Finally, Hickman *et al.*[465] arrived at the same conclusion by showing that synergism is largely lost when vitamin A and vitamin E are administered on alternate days. Moreover, it was found that the co-vitamin E action was lost when the tocopherol was given parenterally, while vitamin A was being introduced orally. This hypothesis was further supported by the demonstration that antioxidants other than tocopherol increased the growth-promoting action.

One must conclude that the synergism of vitamin E toward vitamin A also has another phase separate from the gastrointestinal effect. Hickman and collaborators[465] demonstrated this by showing that orally administered tocopherols augmented the growth of rats receiving vitamin A parenterally. This effect is ascribed to the protection of the vitamin A circulating in the blood stream, since this "will be in danger of destruction each time it passes through the vascular system connected with the intestinal wall."[342] The results of Lemley and her associates,[467] however, are diametrically opposed to this concept of the intestinal action of the tocopherols. In these latter tests, tocopherol exerted a synergism toward vitamin A when the administration of vitamins A and E occurred on alternate days; moreover, an aqueous solution of tocopherol, when administered parenterally, was found to potentiate vitamin A action. Moreover, the prolongation of the depletion of young rats on a vitamin A-free diet[465] must be a reflection of a vitamin E action taking place elsewhere than in the gastrointestinal tract.

α-Tocopherol, but not α-tocopherol acetate, has been shown to increase the rate of gain-in-weight during the bioassay of vitamin A.[470] However, Miles *et al.*[470] reported that animals which received 1.5 mg. of α-tocopherol daily did not gain as much as those receiving only 0.5 mg.

[470] M. C. Miles, E. M. Erickson, and H. A. Mattill, *Proc. Soc. Exptl. Biol. Med.*, *70*, 162–165 (1949).

Any beneficial effect which α-tocopherol acetate exerts on the utilization of vitamin A was shown to be due to the α-tocopherol available in the intestine during the interval between hydrolysis and absorption. The prolonged effect of the administration of α-tocopherol during the vitamin A assay period is evident from the fact that the tocopherol-supplemented animals survived 30 to 80% longer after the assay than did the unsupplemented controls. Major and Watts[281] noted that, when high levels of tocopherol were fed or injected into rabbits on a *purified* diet, the body fat and meat were protected from rancidity. However, no protection from rancidity was afforded the fat or tissues by the administration of tocopherol when a *natural* diet was employed.

b. The Esterification of Vitamin A in the Intestinal Wall. Some mechanism must function to produce esterification of vitamin A alcohol. This is indicated by the fact that the vitamin A in the chyle of the rat and pig is largely in the form of the ester, irrespective of whether vitamin A alcohol, vitamin A ester, or β-carotene is administered.

Proof that esterification of vitamin A occurs in the intestinal wall is afforded by examination of the vitamin A present in this structure. Thompson *et al.*[259] demonstrated that the proportions of vitamin A alcohol and of ester in the wall were about equal; both forms appeared after vitamin A alcohol, vitamin A ester, or carotene had been given. In later work[471] it was indicated that the esterified vitamin A in the intestinal wall approximates 75% of the total.

c. The Transformation of Retinene Into Vitamin A. Ball, Glover *et al.*[472] and Glover and co-workers[473] found that vitamin A-deficient rats were able to convert retinene$_1$ (vitamin A_1 aldehyde) rapidly into vitamin A_1, either in the mucous membranes of the gut or in the liver. This change could be noted after retinene$_1$ was given orally, as well as following subcutaneous injection. It is believed that the transformation is a quantitative one. This implies the presence of an efficient reductase to reduce the aldehyde to the alcohol. According to Gounelle *et al.*,[474] when retinene originating from the oxidation of vitamin A or from cleavage of carotene is ingested by man, it induces a rise in plasma vitamin A which reaches a maximum in six hours.

Retinene$_1$ appears to be absorbed in the same way as vitamin A_1. Glover

[471] S. Y. Thompson, R. Braude, M. E. Coates, A. T. Cowie, J. Ganguly, and S. K. Kon, *Brit. J. Nutrition*, *4*, 398–421 (1950).

[472] S. Ball, J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *41*, xxiv (1947)

[473] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *43*, 109–114 (1948).

[474] H. Gounelle, G. Marnay, R. Chéroux, and Y. Raoul, *Compt. rend. soc. biol.*, *146*, 523–525 (1952).

et al.[473] reported that it occurs along the whole length of the small intestine. The fact that an accumulation of vitamin A results in the gut wall after the administration of the aldehyde indicates that the entrance of the retinene$_1$ into the mucosa and its transformation into vitamin A_1 are rapid, as compared with the transport of vitamin A away from the gut wall. It was suggested by Glover and associates[473] that the change of β-carotene into vitamin A *in vivo* involves the oxidation of the carotenoid to retinene$_1$, which is then rapidly reduced to vitamin A_1. This mechanism would appear to be more probable than that of hydrolytic fission.

Morton *et al.*[475] found that, when retinene$_2$ was fed to vitamin A-deficient rats, it was quickly reduced to vitamin A_2 in the gut wall; the presence of vitamin A_2 could also be demonstrated in the liver.

(2) Vitamins D

a. The Absorption of the Vitamins D from the Intestine. Much less is known about the absorption of the vitamins D than is the case with carotene and the vitamins A. This is largely because the methods for the quantitative estimation of vitamins D are cumbersome, and require much larger amounts of material for analysis than is the case with the other vitamins. In fact, a bioassay is usually the only procedure by which one is able to determine the correct answer.

The utilization of vitamin D is increased when fat is present in the diet. According to Knudson and Floody,[476] healing was improved in rickets when a known amount of vitamin D was given in conjunction with a diet containing 5% fat, as compared with the results observed when a fat-free diet was employed. Diets containing 10 or 20% of fat were reported to be less efficacious than the 5% fat diet, but better than the fat-free regimen. It is uncertain whether this improvement in vitamin D utilization is a result of improved absorption or of an effect on the vitamin D after it is absorbed. Boer,[477] who reported that diets producing rickets were no longer rachitogenic when 10% of the saponifiable fraction of margarine was added, believed that fat exerts a sparing action on vitamin D. Kon and Booth[478,479] have come to the same conclusion. As a further confirmation of the effect of fats in preventing rickets, McDougall[480]

[475] R. A. Morton, M. K. Salah, and A. L. Stubbs, *Biochem. J.*, *41*, xxiv (1947).
[476] A. Knudson and R. J. Floody, *J. Nutrition*, *20*, 317–325 (1940).
[477] J. Boer, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, *9*, 67–68 (1939).
[478] S. K. Kon and R. G. Booth, *Biochem. J.*, *28*, 111–120 (1934).
[479] S. K. Kon and R. G. Booth, *Biochem. J.*, *28*, 121–130 (1934).
[480] E. J. McDougall, *Biochem. J.*, *32*, 194–202 (1938).

demonstrated that, when 11% of lard or olive oil was added to low-calcium diets having a high proportion of wheat flour or bread, the rats did not develop rickets.

As in the case of carotene and vitamin A, the absorption of vitamin D requires the presence of bile. It had long been recognized that, when bile was excluded from the gastrointestinal tract, osteoporosis developed.[481–489] It was suggested by Düttmann[484] that vitamin D is not absorbed in the absence of bile, and that this, in turn, leads to a negative calcium and phosphorus balance. However, Seifert[490] ascribes this deficiency to an inability to absorb vitamin A. The accuracy of the former explanation is indicated by the fact that this pathological condition is relieved by the parenteral administration of vitamin D.[491] Conclusive proof of this hypothesis was brought forward by Greaves and Schmidt,[492] who studied the absorption of vitamin D in choledochocolostomized rats by the use of calcium and phosphorus balances as a criterion of vitamin D absorption. Bile fistula rats were found to be in negative calcium balance, and little or no irradiated ergosterol was absorbed in the gastrointestinal tract. On the other hand, when desoxycholic acid was given orally, irradiated ergosterol could be carried across the intestinal wall of the bile fistula rat.

Heymann[493] likewise reported that vitamin D is absent from the blood, and that a hyperphosphatemic reaction does not occur in dogs whose bile ducts have been ligated and transected following the administration of viosterol (vitamin D_2 in oil) or drisdol (vitamin D_2 in propylene glycol) by stomach tube. Vitamin D was not absorbed unless bile was present in the chyme. In this case, no difference was noted in the relative effect of the solvents employed. On the other hand, when these preparations were given to bile-fistula dogs by intramuscular injection, a considerable

[481] I. P. Pavlov, *Verh. Ges. russ. Aerzte*, *72*, 314 (1904–1905); cited by J. D. Greaves and C. L. A. Schmidt, *J. Biol. Chem.*, *102*, 101–112 (1933), p. 101, and by G. Düttmann, *Beitr. klin. Chir. (Brun's)*, *139*, 720–729 (1927).
[482] E. Looser, *Verhandl. deut. path. Ges.*, *11*, 291–295 (1907).
[483] F. P. Wiener and G. H. Whipple, *Am. J. Physiol.*, *60*, 119–133 (1922).
[484] G. Düttmann, *Beitr. klin. Chir. (Brun's)*, *139*, 720–729 (1927).
[485] E. Gilbert, *Z. ges. exptl. Med.*, *43*, 539–544 (1924).
[486] W. C. Buchbinder and R. Kern, *Arch. Internal Med.*, *40*, 900–910 (1927).
[487] W. C. Buchbinder and R. Kern, *Am. J. Physiol.*, *80*, 273–277 (1927).
[488] H. Seidel, *Münch. med. Wochschr.*, *57*, 2034–2036 (1910).
[489] D. Rigano-Irrera, *Cultura med. mod.*, *10*, 43–50 (1931).
[490] E. Seifert, *Beitr. klin. Chir. (Brun's)*, *136*, 496–498 (1926).
[491] H. Tammann, *Beitr. klin. Chir. (Brun's)*, *142*, 83–120 (1928).
[492] J. D. Greaves and C. L. A. Schmidt, *J. Biol. Chem.*, *102*, 101–112 (1933).
[493] W. Heymann, *J. Biol. Chem.*, *122*, 249–256 (1937–1938).

amount of vitamin D was excreted in the bile of the drisdol-treated dogs, while practically no vitamin D was found in the bile of the dogs receiving the viosterol injection.

In certain cases, vitamin D has been shown to be excreted in the feces. After obstruction of the common bile duct in dogs, a single dose of vitamin D administered as viosterol or drisdol resulted in its excretion in the feces for only ten to sixteen days.[494] On the other hand, when such dosages of vitamin D were given to normal dogs, vitamin D was found in the feces eight months after viosterol and at least six months after drisdol had been given. Moreover, parenterally administered viosterol or drisdol was shown to be excreted in the feces of dogs in which the bile flow or that of pancreatic juice had been excluded from the intestine. Heymann[494] believes that this indicates that a portion of the vitamin D is normally excreted through the intestinal wall. The largest percentage of the vitamin is eliminated by the first third of the small intestine.

The presence of mineral oil has been shown in most cases to reduce the absorption of vitamin D. Although Dutcher *et al.*[495] and Jackson[496] could not demonstrate that mineral oil exerted any deleterious effect on the absorption of vitamin D, more recent studies of Smith and Spector[425,497,498] indicated that mineral oil interferes markedly with the absorption of vitamin D, both in rats and in dogs.

It must be inferred that vitamin D esters are hydrolyzed in the intestine prior to absorption. Windaus and Rygh[499] have shown that those esters of vitamin D which are incapable of hydrolysis have no antirachitic potency. It has not been established whether the non-utilization of such esters is to be ascribed to their failure to be absorbed or to their biological inactivity after absorption in the conjugated form.

No differences in absorption of the different vitamins D have been noted in normal dogs. Morgan and Shimotori[500] found that equal protection from rickets could be noted in dogs receiving vitamin D_2, vitamin D_3, or a tuna liver oil, after a single dose of 20,000 units. When a single massive dose containing 200,000 units was given, symptoms of prostration were observed with both vitamins D_2 and D_3.

[494] W. Heymann, *J. Biol. Chem.*, *122*, 257–262 (1937–1938).

[495] R. A. Dutcher, J. O. Ely, and H. E. Honeywell, *Proc. Soc. Exptl. Biol. Med.*, *24*, 953–955 (1927).

[496] R. W. Jackson, *J. Nutrition*, *7*, 617–622 (1934).

[497] M. C. Smith and H. Spector, *J. Nutrition*, *20*, 19–30 (1940).

[498] M. C. Smith and H. Spector, *J. Nutrition*, *20*, 197–202 (1940).

[499] A. Windaus and O. Rygh, *Nachr. Ges. Wiss. Göttingen*, *Math.-physik. Klasse*, *III*, 202–216 (1928).

[500] A. F. Morgan, and N. Shimotori, *J. Biol. Chem.*, *147*, 189–200 (1943).

The absorption of vitamins D_2 and D_3 has also been proved by the demonstration that the type of vitamin D in egg yolk of hens corresponds to that in the dietary regimen after the feeding of irradiated ergosterol or cod-liver oil.[501,502] Similar variations have been noted by Bethke *et al.*[503] in the type of vitamin D in milk as related to its presence in the food.

(3) Vitamins E (Tocopherols)

When the tocopherols are given in moderate doses, they are relatively poorly utilized. Although McArthur and Watson[504] were unable to prove the presence of any significant amount of the vitamins E in the urine, more recent investigations have indicated that the absorption of these vitamins is not complete, and that definite quantities are excreted in the feces, particularly after large doses.[505]

After feeding α-tocopherol tagged with C^{14} in oil solution to rats, Shantz[506] found that only 20% was absorbed, and that the remaining 80% was excreted in the feces. These figures are in line with the results of Engel and Heins,[507] who reported that 40% of the vitamin E from wheat germ oil and 87% from dried grass were lost in the feces. On the basis of the finding that the feeding of feces resulted in cure of vitamin E deficiency, Pindborg[508] postulated a synthesis of vitamin E in the intestine. Harris[509] criticized this interpretation; he believes that the fecal vitamin E merely represents unabsorbed vitamin E from the food. Swick and Baumann[282] reported that the maximum concentration of tocopherol occurred in the intestinal wall of the rat five hours after the ingestion of free α-tocopherol, and eight hours after the feeding of α-tocopherol acetate. Maximum values were of the same order of magnitude in both cases.

Considerable differences exist in the ability of the animal to utilize the different tocopherols. This is illustrated in the experiments of Quaife

[501] R. M. Bethke, P. R. Record, C. H. Kick, and D. C. Kennard, *Poultry Sci.*, *15*, 326–335 (1936).

[502] R. M. Bethke, P. R. Record, O. H. M. Wilder, and C. H. Kick, *Poultry Sci.*, *15*, 336–344 (1936).

[503] R. M. Bethke, W. E. Krauss, P. R. Record, and O. H. M. Wilder, *J. Nutrition*, *11*, 21–30 (1936).

[504] C. S. McArthur and E. M. Watson, *Can. Chem. Process Inds.*, *23*, 350–352 (1939).

[505] A. Juhász-Schaffer, *Arch. path. Anat. u. Physiol. (Virchow's)*, *281*, 53–65 (1931)

[506] E. M. Shantz, Unpublished data cited by P. L. Harris, *Ann. Rev. Biochem.*, *18*, 391–394 (1949), p. 410.

[507] C. Engel and J. T. Heins, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, *13*, 37 (1943).

[508] J. J. Pindborg, *Nature*, *164*, 493 (1949).

[509] P. L. Harris, *Nature*, *165*, 572 (1950).

et al.[510] on human subjects. After 500 mg. of *d*-α-tocopherol were fed, the maximum level of the serum tocopherol amounted to 1.75 mg. per 100 ml.; this maximum was reached in about four hours. After the administration of a similarly large dose of *d*-γ-tocopherol, the peak value was obtained in the serum after about the same time interval, but the figure amounted to only 1.35 mg. per 100 ml. of serum.

Cows and hens have likewise been shown to exhibit a preferential absorption of α-tocopherol over γ- and δ-tocopherols. Cow milk is an exceedingly poor source of vitamin E. According to Abderhalden,[511] the average tocopherol content of milk is only 0.061 milligram per cent. Quaife and her co-workers[510] were able to demonstrate a considerable rise in the milk tocopherol value when the diet of the cow was supplemented with α-tocopherol. On the other hand, when the supplement was composed of a mixture containing 90% of γ- and δ-tocopherols, the increase in milk tocopherol was much less pronounced.

In the case of chickens, the most sensitive index of the efficiency of tocopherol absorption is to be found in the quantity of this vitamin in the egg.[512] When α-, γ-, or δ-tocopherols were administered to hens in doses of 100 to 4000 mg. per week,[510,513] the tocopherol content of the eggs increased linearly with the dosage. However, the storage of α-tocopherol in the egg was much greater than was that after the administration of γ- or δ-tocopherols. Thus, when the α-, γ-, or δ-tocopherol was fed at a 400 mg. level weekly, the concentration of tocopherols was 24.2, 5.7, and 2.3 mg. per 100 g. of the fresh egg, respectively. The relative efficiency of transfer was 22.1% for α-tocopherol, compared with figures of 3.6% and 2.0% for γ- and δ-tocopherols, respectively.

Bile is required for the absorption of the tocopherols, just as it is for carotene, vitamin A, vitamin D, and for the fats. Greaves and Schmidt[514] reported that vitamin E was not absorbed when administered to the choledochocolostomized rat. Moreover, dogs with bile fistulas have likewise been shown to be incapable of absorbing this vitamin.[515] It is not known whether the bile acids form coordination compounds with the tocopherols or, if not, how they mediate their absorption.

Information as to the transport of the tocopherols from the gastro-

[510] M. L. Quaife, W. J. Swanson, M. Y. Dju, and P. L. Harris, *Ann. New York Acad. Sci.*, *52*, 300–305 (1949).

[511] R. Abderhalden, *Biochem. Z.*, *318*, 47–53 (1948).

[512] G. L. Barnum, *J. Nutrition*, *9*, 621–635 (1935).

[513] M. Y. Dju, M. L. Quaife, and P. L. Harris, *Am. J. Physiol.*, *160*, 259–263 (1950).

[514] J. D. Greaves and C. L. A. Schmidt, *Proc. Soc. Exptl. Biol. Med.*, *37*, 40–42 (1937).

[515] K. M. Brinkhous and E. D. Warner, *Am. J. Pathol.*, *17*, 81–86 (1941).

intestinal tract is also fragmentary. Presumably they are carried in the lymph in a manner similar to that for carotene and vitamin A, although this has not as yet been proved. The tocopherols are found in blood, in which 75% consist of the α-type and the balance of other types of tocopherols.[510] According to Rosenberg,[516] the total blood tocopherol amounts to 56 γ % in men and 64 γ % in women; the blood level can be increased to 100 γ % by feeding tocopherol. Vitamin E occurs in the blood as the free vitamin, even after esters are administered. Quaife and Dju[517] reported that the total tocopherol contained in the entire blood of a man amounted to 64 mg., and in a woman to 45 mg.

(4) *Vitamins K*

a. The Absorption of the Vitamins K from the Gastrointestinal Tract. Vitamin K is quite unique among the fat-soluble vitamins in that it is synthesized by the intestinal bacteria. This is indeed a fortunate circumstance in view of the fact that this vitamin does not have the widespread distribution in plant and animal tissues which characterizes the other fat-soluble vitamins.

The natural forms of vitamin K (vitamins K_1 and K_2) are soluble only in fats, by virtue of the property conferred on them by their long hydrocarbon side chains. On the other hand, menadione (2-methyl-1,4-naphthoquinone), which has the highest vitamin K biopotency of any natural or synthetic product, is considerably more soluble in water, since it does not contain the long aliphatic side chain. It is therefore obvious that factors which are of importance in the absorption of the natural vitamins K may play a considerably less important role in the utilization of menadione and of similar synthetic products.

Although no enzymes are required in order to prepare the natural or synthetic vitamins K for absorption, the presence of bile is a prerequisite. It was shown early that rats,[518] chicks,[519] and dogs[520,521] exhibited such manifestations of vitamin K deficiency as loss of blood coagulability and a low prothrombin level when the bile ducts were ligated. Greaves and Schmidt[518] noted that the symptoms of vitamin K deficiency in rats could

[516] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York-London, 1945, p. 456.

[517] M. L. Quaife and M. Y. Dju, *J. Biol. Chem.*, *180*, 263–272 (1949).

[518] J. D. Greaves and C. L. A. Schmidt, *Proc. Soc. Exptl. Biol. Med.*, *37*, 43–45 (1937).

[519] H. Dam and J. Glavind, *Acta Med. Scand.*, *96*, 108–128 (1938).

[520] W. B. Hawkins and K. M. Brinkhous, *J. Exptl. Med.*, *63*, 795–801 (1936).

[521] H. P. Smith, E. D. Warner, K. M. Brinkhous, and W. H. Seegers, *J. Exptl. Med.*, *67*, 911–920 (1938)

be corrected by the oral administration of vitamin K. However, this was accomplished efficiently only if bile or bile salts were administered concomitantly. Quick[522] suggested the same relationship between bile salts, prothrombin, and vitamin K.

Desoxycholic acid is believed to be the bile component responsible for bringing about the absorption of vitamin K from the gastrointestinal tract.[523] Almquist and Klose[524] prepared a vitamin K-choleic acid complex which was effective in curing vitamin K deficiency in bile-fistula rats.[525] Dam and co-workers[526] reported that a crude vitamin K-desoxycholic acid complex in aqueous solution was effective in curing vitamin K deficiency in chicks when given subcutaneously, while a water emulsion of the crude vitamin was efficacious when given intramuscularly, but not when injected subcutaneously.

Although bile contains no appreciable quantity of vitamin K,[527,528] the feeding of bile alone has been shown to effect an increased prothrombin level in the case of animals with ligated bile ducts.[518,521,528,529] Presumably this results from facilitation of the absorption of the vitamin K in food and of that synthesized by the bacteria in the intestine.

It has also been recognized for a long time that bile is required for the absorption of vitamin K in man. It was reported by Quick and co-workers[530] that low prothrombin levels obtain in obstructive jaundice. Brinkhous, Smith, and Warner,[531,532] and Snell and collaborators,[533] demonstrated that the hemorrhagic tendency in cases of obstructive jaundice could be overcome if vitamin K was administered with bile or bile salts. However, water-soluble vitamin K substitutes can apparently be absorbed from the intestine without the presence of bile salts.[534] The

[522] A. J. Quick, *Am. J. Physiol., 118*, 260–271 (1937).
[523] C. L. A. Schmidt, *Pacific Coast Med., 5*, 7–10 (1938).
[524] H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc., 61*, 745–746 (1939).
[525] E. T. Cohn and C. L. A. Schmidt, *Proc. Soc. Exptl. Biol. Med., 41*, 443–444 (1939).
[526] H. Dam, J. Glavind, L. Lewis, and E. Tage-Hansen, *Skand. Arch. Physiol., 79*, 121–133 (1938).
[527] H. J. Almquist, *Science, 87*, 538 (1938).
[528] J. D. Greaves, *Am. J. Physiol., 125*, 429–436 (1939).
[529] J. D. Greaves, *Am. J. Physiol., 125*, 423–428 (1939).
[530] A. J. Quick, J. M. Stanley-Brown, and F. W. Bancroft, *Am. J. Med. Sci., 190*, 501–511 (1935).
[531] K. M. Brinkhous, H. P. Smith, and E. D. Warner, *Am. J. Med. Sci., 196*, 50–57 (1938).
[532] E. D. Warner, K. M. Brinkhous, and H. P. Smith, *Proc. Soc. Exptl. Biol. Med., 37*, 628–630 (1938).
[533] A. M. Snell, T. B. Magath, E. W. Boland, A. E. Osterberg, H. R. Butt, J. L. Bollman, and W. Walters, *Proc. Staff Meetings Mayo Clin., 13*, 65–80 (1938).
[534] E. D. Warner and J. D. Flynn, *Proc. Soc. Exptl. Biol. Med., 44*, 607–608 (1940).

relationship of bile to vitamin K absorption has been excellently reviewed by Almquist.[535]

The production of a vitamin K deficiency is accelerated in mice[536] and in rats[537,538] by the feeding of mineral oil. Javert and Macri[539] reported that the prothrombin time is also increased in human subjects after the administration of petroleum oil. The effect of mineral oil is apparently not confined to the removal of the exogenous vitamin K from the gastrointestinal tract; it must also prevent the absorption of the endogenous vitamin K which is constantly being synthesized by the intestinal bacteria.

b. The Synthesis of Vitamin K_2 in the Gastrointestinal Tract. It was proved that the antihemorrhagic tendency of foods depends to a marked extent upon bacterial spoilage,[540,541] and that the droppings of vitamin K-deficient chicks also contain a similar antihemorrhagic substance.[542] It has also been shown that vitamin K is present in many bacteria, including the water microbe, *Bacillus cereus* and the "hay bacillus" from air, water, and soil, *B. subtilis*.[543] The vitamin K synthesized by bacteria differs slightly from that produced in plants; it is 2-methyl-3-difarnesyl-1,4-naphthoquinone, and is referred to as vitamin K_2. Vitamin K_1, which is present in plants, is 2-methyl-3-phytyl-1,4-naphthoquinone. Vitamin K_2 has a biopotency slightly less than that of vitamin K_1; the entire difference can be accounted for by its somewhat higher molecular weight.

Intestinal bacteria of most higher animals can synthesize vitamin K_2 readily. Not only has the presence of vitamin K been demonstrated in chick feces,[542] but also this vitamin has been reported in human feces.[534,544,545] McElroy and Goss[546] likewise proved the presence of vitamin K in the rumen of the cow, even when the animal was receiving a vitamin K-free diet.

[535] H. J. Almquist, *Physiol. Revs*, *21*, 194–216 (1941).

[536] W. A. Barnes, *Proc. Soc. Exptl. Biol. Med.*, *49*, 15–19 (1942).

[537] M. C. Elliott, B. Isaacs, and A. C. Ivy, *Proc. Soc. Exptl. Biol. Med.*, *43*, 240–245 (1940).

[538] E. K. Bacon, S. Lassen, S. M. Greenberg, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *47*, 383–398 (1952).

[539] C. T. Javert and C. Macri, *Am. J. Obstet. Gynecol.*, *42*, 409–414 (1941).

[540] H. J. Almquist and E. L. R. Stokstad, *Nature*, *136*, 31 (1935).

[541] H. J. Almquist and E. L. R. Stokstad, *J. Biol. Chem.*, *111*, 105–113 (1935).

[542] H. J. Almquist and E. L. R. Stokstad, *J. Nutrition*, *12*, 329–335 (1936).

[543] H. J. Almquist, C. F. Pentler, and E. Mecchi, *Proc. Soc. Exptl. Biol. Med.*, *38*, 336–338 (1938).

[544] H. Dam, *Angew. Chem.*, *50*, 807–811 (1937).

[545] E. M. Nelson and C. D. Tolle, *Ann. Rev. Biochem.*, *8*, 415–434 (1939).

[546] L. W. McElroy and H. Goss, *J. Nutrition*, *20*, 527–540 (1940).

Black and co-workers[547,548] were the first to demonstrate that sulfaguanidine reduces the growth rate of young rats, and that this effect is accompanied by a hypoprothrombinemia[548]; this effect could be counteracted by the administration of vitamin K. Succinyl sulfathiazole (sulfasuxidine) has also been found to reduce the growth rate in the rat and to lower the prothrombin level. The effects of this sulfonamide were likewise found to be counteracted by vitamin K, as well as by a liver extract.[548] Kornberg, Daft, and Sebrell[549] reported shortly thereafter that sulfapyrazine, sulfadiazine, and sulfathiazole were more effective in producing vitamin K deficiency than was sulfaguanidine, succinyl sulfathiazole, or sulfanilamide. These workers also proved that neither absorption, utilization, nor alteration in vitamin K requirement could be considered significant elements in the production of vitamin K deficiency by the sulfonamides.

It is known that coliform organisms produce vitamin K *in vitro*,[550] and that sulfaguanidine and succinyl sulfathiazole reduce the coliform count in the feces of rats.[551] On the other hand, *p*-aminobenzoic acid, which Black *et al.*[548] showed was effective in counteracting vitamin K deficiency produced by feeding sulfaguanidine is known to antagonize sulfonamide bacteriostasis.

The action of sulfaguanidine in producing vitamin K deficiency is explained by Black and associates[548] as due in part to the effect of this drug in inhibiting the synthesis of vitamin K by the intestinal flora. This theory is substantiated by Kornberg, Draft, and Sebrell,[552] who demonstrated that the cecal contents and the collected feces of rats with vitamin K deficiency produced by sulfonamides contained only slight vitamin K activity or none at all. On the other hand, these products from control rats had a much greater vitamin K activity. The work of Day *et al.*,[553] who found that cecectomy increased the incidence of vitamin K deficiency in rats fed succinyl sulfathiazole, also supports the above hypothesis.

[547] S. Black, J. M. McKibbin, and C. A. Elvehjem, *Proc. Soc. Exptl. Biol. Med.*, *47*, 308–310 (1941).

[548] S. Black, R. S. Overman, C. A. Elvehjem, and K. P. Link, *J. Biol. Chem.*, *145*, 137–143 (1942).

[549] A. Kornberg, F. S. Daft, and W. H. Sebrell, *Pub. Health Reports*, *U. S. Pub. Health Service*, *59*, 832–844 (1944).

[550] S. Orla-Jensen, A. D. Orla-Jensen, H. Dam, and J. Glavind, *Zentr. Bakteriol.*, *Abt. 2*, *104*, 202–204 (1941); *Chem. Zentr.*, *113*, 1155 (1942).

[551] O. K. Gant, B. Ransone, E. McCoy, and C. A. Elvehjem, *Proc. Soc. Exptl. Biol. Med.*, *52*, 276–279 (1943).

[552] A. Kornberg, F. S. Daft, and W. H. Sebrell, *J. Biol. Chem.*, *155*, 193–200 (1944).

[553] H. G. Day, K. G. Wakim, M. M. Krider, and E. E. O'Banion, *J. Nutrition*, *26*, 585–600 (1943).

The hemorrhagic disease of the newborn is now recognized as a type of alimentary vitamin K deficiency. In this condition, the level of prothrombin, although normal at birth, is very low during the early days of life,[554–558] presumably because of the lack of vitamin K in the diet coupled with a failure of vitamin K to be synthesized in the sterile intestinal contents, the so-called meconium. Recovery from this condition may be spontaneous when food is taken, probably through the establishment of intestinal flora.[559–561]

10. The Transport of Carotenoids and of Fat-Soluble Vitamins in Lymph and Blood

(1) The Transport of Carotenoids in the Lymph and Blood

Drummond, Bell, and Palmer[562] were able to demonstrate, in a patient with chylothorax, that carotene and vitamin A are carried in the chyle. Although administered vitamin A alcohol could be quantitatively recovered in the chyle, only 20% of the carotene given could be accounted for. The failure to obtain a quantitative recovery of carotene in the chyle in these tests may be an indication that carotene is partly split in the intestinal wall in the human subject, and that the portion unaccounted for may have been transported in the chyle in the form of vitamin A.

Proof that carotene is practically completely broken down to vitamin A in the intestine of a number of species of animals has been afforded by the demonstration that no appreciable amount of carotenoid appears in the blood or lymph, even after large doses of β-carotene have been given. Thus, Thompson and associates[231] reported that no carotene could be found in the systemic or portal blood of pigs or of normal or vitamin A-deficient rats after the feeding of carotene, but that a concomitant increase in vitamin A occurred in the lymph. Vitamin A was noted in the mesenteric lymph nodes of pigs shortly after the feeding of carotene, according to Thompson, Ganguly, and Kon.[258] This observation was confirmed by Coates *et al.*[366] in the rat. Woytkiw and Esselbaugh[365]

[554] K. M. Brinkhous, H. P. Smith, and E. D. Warner, *Am. J. Med. Sci.*, *193*, 475–480 (1937).

[555] K. Kato and H. G. Poncher, *J. Am. Med. Assoc.*, *114*, 749–755 (1940).

[556] K. K. Nygaard, *Acta Obstet. Gynecol. Scand.*, *19*, 361–370 (1939).

[557] C. A. Owen, G. R. Hoffman, S. E. Ziffren, and H. P. Smith, *Proc. Soc. Exptl. Biol Med.*, *41*, 181–185 (1939).

[558] A. J. Quick, *Wisconsin Med. J.*, *38*, 746 (1939).

[559] A. J. Quick and A. M. Grossman, *Am. J. Med. Sci.*, *199*, 1–9 (1940).

[560] A. J. Quick and A. M. Grossman, *Proc. Soc. Exptl. Biol. Med.*, *41*, 227–228 (1939).

[561] A. J. Quick and A. M. Grossman, *Proc. Soc. Exptl. Biol. Med.*, *40*, 647–648 (1939).

[562] J. C. Drummond, M. E. Bell, and E. T. Palmer, *Brit. Med. J.*, *1935*, *I*, 1208–1210

demonstrated that dosage of guinea pigs with either carotene or vitamin A caused an increase in the vitamin A content of the mesenteric lymphatics, as well as of the blood of the dorsal aorta. Carotene absorption was reported to be negligible in the guinea pig, as demonstrated by its absence from the lymph. Absorption of carotene or of vitamin A could not be shown to take place *via* the portal vein. McGillivray[371] also reported high levels of vitamin A in the intestinal lymph of sheep and of a bullock after carotene feeding. This is interpreted as evidence for the view that the intestine may be the site of conversion of carotene to vitamin A in cattle as well as in sheep.

The vitamin A found in lymph after the administration of carotene has been shown to consist almost exclusively of vitamin A ester.[231,259,471] The proportion of vitamin A ester to that of vitamin A alcohol in the lymph after carotene administration was similar to that observed when vitamin A was given in either alcohol or ester form.[259,471]

The portal blood has been shown to play a negative role in the transport of carotene or of vitamin A formed from it in the intestinal mucosa. Thus, Goodwin, Dewar, and Gregory[263] reported that carotene was absent not only from the systemic blood of sheep and goats, but also from the portal blood.

Considerable evidence has been presented which indicates that vitamin A from ingested carotene or from ingested vitamin A is transported exclusively by way of the lacteals and lymphatics. In the first place, the quantitative recovery of ingested vitamin A in the lymph indicates that this is the sole pathway.[562] Secondly, Thompson *et al.*[471] proved that no differences could be demonstrated between the level of vitamin A in systemic and in portal blood of pigs after β-carotene had been given in peanut oil, or after the administration of vitamin A ester. Eden and Sellers[563] reported that vitamin A values were higher in the systemic blood than in the portal blood in bullocks which had been given large doses of vitamin A two to twenty-four hours before slaughtering. The best proof, however, was furnished by the experiments of Thompson *et al.*,[471] which indicated that no increase in the vitamin A level of blood or liver occurred in rats after the feeding of carotene when the lymph was diverted and prevented from emptying into the blood stream.

(2) *The Transport of Vitamin A in the Lymph and Blood*

The classical proof that vitamin A is transported from the intestine exclusively in the chyle is that of Drummond, Bell, and Palmer,[562] who were

[563] E. Eden and K. C. Sellers, *Biochem. J.*, *42*, xlix (1948).

TABLE 10

THE VITAMIN A CONTENT OF THE BLOOD AND LYMPH (EXPRESSED IN I.U./100 ML.) OF BULLOCKS THAT HAD RECEIVED 5000 I.U. OF VITAMIN A PER KILOGRAM 2 TO 24 HOURS BEFORE SLAUGHTERING, OR THAT HAD RECEIVED NO SUPPLEMENTARY VITAMIN A[a]

Category	No vitamin A fed		Vitamin A fed	
	Before dosing	After dosing	Before dosing	After dosing
Blood vitamin A	87	—	72	—
Systemic	—	83	—	162
Portal	—	90	—	147
Lymph vitamin A	—	—	—	—
Non-intestinal	—	130	—	175
Intestinal				
duodenal	—	225	—	1500
jejunal	—	185	—	1028
ileal	—	130	—	459
colonic	—	132	—	169

[a] Data adapted from E. Eden and K. C. Sellers, *Biochem. J.*, *42*, xlix (1948).

able to demonstrate a quantitative recovery of administered vitamin A from the chyle of their patient who had a chylothorax. Thompson, Ganguly, and Kon[258] as well as Thompson *et al.*,[471] also proved that vitamin A is transported in the lymph of the rat as well as of the pig. The high content of vitamin A in lymph originating from various portions of the gastrointestinal tract of bullocks after feeding vitamin A, as shown by the experiments of Eden and Sellers,[563] is proof that this route of transport obtains for cattle. As early as 1944, Popper and Volk[411] demonstrated the presence of vitamin A in the lacteals, as revealed by fluorescent microscopy, within twenty-five minutes after the administration of vitamin A. This was confirmed by Radice and Herraiz.[564] It has been repeatedly shown that vitamin A is present as the ester in the lymph and blood of rats and of pigs[258,471] and in the blood serum of man.[432]

A number of investigators[258,264,562,563,565,566] have proved that the portal vein does not transport the vitamin A from the intestine, but merely contains the vitamin A poured into the blood stream *via* the thoracic duct. Thus, Eden and Sellers[565] note that, although the vitamin A content of the portal blood increased after the dosing of bullocks, sheep, and rats with this vitamin, the average figures for vitamin A in the portal blood were, if anything, slightly lower than those of the systemic blood. The only evidence for vitamin A transport *via* the portal system is that of Radice

[564] J. C. Radice and M. L. Herraiz, *Rev. Asoc. med. argentina*, *61*, 287–292 (1947).
[565] E. Eden and K. C. Sellers, *Biochem. J.*, *44*, 264–267 (1949).
[566] L. S. Palmer and C. H. Eckles, *J. Biol. Chem.*, *17*, 223–236 (1914).

and Herraiz,[564] who observed a fluorescence of vitamin A in portal blood similar to that noted by them in lymph. One must conclude that the evidence is overwhelming that vitamin A is not transported in appreciable amounts from the intestine by way of the portal route. Table 10 records some especially convincing experiments which have been cited as proof of the above statement.

According to Eden and Sellers[565] and Bean *et al.*,[567] most of the absorption of vitamin A occurs in the upper part of the intestine.

(3) The Transport of the Vitamins D in the Lymph and Blood

The pathway for transportation of the vitamins D from the intestine to the tissues is not known. There is no reason to suppose that these vitamins do not follow the same pathway as has been reported for fats, carotenoids, vitamins A and K. This would involve the lymphatic system as the principal if not the exclusive route for the transfer of the vitamins D from the epithelial cells of the intestine to the blood stream, after which its further distribution would be mediated through the blood stream. In keeping with this concept, the portal route would be of little or no importance in bringing about the removal of vitamin D from the intestine.

The presence of vitamin D in the blood has been established by a number of investigators. Warkany[568,569] reported that 100 ml. of human serum contained an average of 100 I.U. (50 to 135 I.U.) while, in rabbits, the mean vitamin A content was 50 I.U. per 100 ml. Hess and his collaborators[570] have reported that, in cows, the plasma contains about four times the concentration of vitamin D that is present in the corpuscles. Moreover, higher levels of vitamin D were found after high dosages of irradiated ergosterol had been given.[571] The concentration of vitamin D in whole blood was shown to vary between 40 and 67 I.U. per 100 ml., depending upon the quantity of vitamin D_2 administered.[569]

(4) The Transport of the Vitamins E in the Lymph and Blood

There is no reason to suppose that the method of transport of the vita-

[567] W. B. Bean, M. Franklin, J. F. Embick, and K. Daum, *J. Clin. Invest.*, *30*, 263–273 (1951).

[568] J. Warkany, *Am. J. Diseases Children*, *52*, 831–847 (1936).

[569] J. Warkany, *Biochem. Z.*, *293*, 415–426 (1937).

[570] A. F. Hess, R. F. Light, C. N. Frey, and J. Gross, *J. Biol. Chem.*, *97*, 369–377 (1932).

[571] A. F. Hess, M. Weinstock, and J. Gross, *Proc. Soc. Exptl. Biol. Med.*, *30*, 1357–1358 (1933).

mins E from the intestine to the liver and to other tissues should vary from the route of the other fat-soluble vitamins. However, strangely enough, there does not appear to be any information in the literature in regard to the presence of the vitamins E in the lymph, or any evaluation of the proportion of these vitamins carried *via* the lymph or the portal system. Harris[571a] states that the rates of absorption of orally administered vitamins A and E are sufficiently similar to constitute presumptive evidence that the 2 vitamins travel from the intestine to the blood stream by the same pathway.

(5) *The Transport of the Vitamins K in the Lymph and Blood*

Although little has been known concerning the pathway by which the vitamins K are transported, the fact that the vitamins are present in the liver and other tissues in increased amounts after their administration would indicate that they are distributed by the lymph or by the blood stream or both. Demonstration of the type of vitamin K laid down in the liver under such conditions (K_1 *vs.* K_2) would also be proof of its transport from the gastrointestinal tract.

Evidence that the lacteals and the lymphatics are the main routes for the absorption of the vitamins K has recently been adduced by Mann and co-workers.[572,573] By means of a new operative procedure, devised by Bollman, Cain, and Grindlay[574] for the collection of lymph from the liver, it was demonstrated that vitamin K deficiency can be produced rapidly by external drainage of the intestinal lymph. The vitamin K carried *via* the portal system may be insufficient to prevent the hypoprothrombinemia which is the characteristic symptom of vitamin K deficiency. While the results of Mann *et al.*[572,573] prove that *natural* vitamins K are transported in the lymph, they do not afford information as to the route by which such synthetic forms as menadione are carried.

11. The Excretion of Lipids by Way of the Large Intestine

Although water-soluble derivatives of the lipids are normally excreted in the urine, the large intestine is usually regarded as the chief pathway

[571a] P. L. Harris, *Personal communication* to the author (March, 1954).

[572] J. D. Mann, F. D. Mann, J. L. Bollman, and E. van Hook, *Am. J. Physiol.*, *158*, 311–314 (1949).

[573] F. D. Mann, J. D. Mann, J. L. Bollman, and E. van Hook, *J. Lab. Clin. Med.*, *36*, 234–237 (1950).

[574] J. L. Bollman, J. C. Cain, and J. H. Grindlay, *J. Lab. Clin. Med.*, *33* 1349–1352 (1948).

for the excretion of those lipids which still retain their characteristic solubility patterns. Mammals, and especially birds, excrete considerable portions of lipids by way of the skin. Various samples of human sweat were reported by Pemberton *et al.*[575] to contain from 20 to 216 milligram per cent of the lipids. An increase in blood lipids occurs prior to sweating.[576] In the case of man, 0.1 to 0.2 g. of cholesterol is excreted through the skin daily.[577] Kayser and Balot[578] reported that the normal human subject excretes about 1 mg. of cholesterol daily in the urine. Hypercholesteroluria results when albumin or mucosal casts are present.

In addition to the lipids in the food which are not capable of absorption and those which fail to be absorbed, the feces contain additional fatty-like material which has been added at various levels in the gastrointestinal tract. These include the lipids present in cell debris, those added in the bile, as well as those secreted through the walls of the small and large intestine. Still another source of fecal lipids is the material synthesized by the intestinal bacteria during the passage of food residues through the lower portion of the small intestine and through the large intestine.

(*1*) *The Excretion of Lipids in the Bile*

Bile is the external secretion of the liver. Under normal conditions, it is being formed continuously. In the case of animals having a gall bladder, the bile is stored in this organ until a stimulus is evoked for its ejection into the lumen of the intestine. Food in the intestine provides a suitable stimulus; fat and meat are especially efficacious, as are the bile salts. It is evident that a hormone mechanism is responsible for the emptying of the gall bladder, as has been demonstrated by Doubilet and Ivy,[579] who prepared a substance which, on intravenous injection, caused a contraction of the musculature of the walls of the gall bladder. The hormone has been called *cholecystokinin*. Seager[580] likewise reported the preparation of the purified hormone. It is believed that acid and other substances in the intestine cause the production or liberation of this hormone from the intestinal mucosa.

Bile contains small amounts of cholesterol, phospholipids, fats, soaps,

[575] R. Pemberton, F. A. Cajori, and C. Y. Crouter, *Ann. Internal Med.*, *2*, 1243–1252 (1929).

[576] H. G. Barbour, M. H. Dawson, and I. Neuwirth, *Am. J. Physiol.*, *74*, 204–223 (1925).

[577] W. Hueck, *Verhandl. deut. path. Ges.*, 20th meeting, 18–66 (April, 1925).

[578] F. Kayser and R. Balot, *Compt. rend. soc. biol.*, *146*, 1598–1600 (1952).

[579] H. Doubilet and A. C. Ivy, *Am. J. Physiol.*, *124*, 379–390 (1938).

[580] L. D. Seager, *Proc. Soc. Exptl. Biol. Med.*, *47*, 257–260 (1941).

and bile salts.[581] According to West and Todd,[582] the average composition and range in composition of human fistula bile, in parts per 1000, as reported by various workers since 1900, are as follows: total lipids, 3.4 (2.9–4.2); neutral fat, 1.1 (0.4–3.0); fatty acids (including soaps), 1.1 (0.8–1.4); phosphatides, 0.6 (0.5–0.6); and cholesterol, 1.2 (0.8–1.7). A similar compilation, by these authors, of the composition of human bladder bile gives the following values: total lipids, 22.5 (19–26); neutral fat, 3.7 (1.5–5.6); fatty acids (including soaps), 9.7 (9–10.9); phosphatides, 2.0 (1.8–2.2); and cholesterol, 6.3 (3.5–9.3). Friedman *et al.*[583] reported that the amount of cholesterol in rat bile was 12.7 milligram per cent; the concentration was similar to that in dog bile. Heerschma and Annegers[584] showed that cholecystectomy (removal of the gall bladder), which converts the normal intermittent flow of concentrated bile into a continuous flow of dilute bile into the intestine, did not produce any significant change in the daily excretion of fat in the feces.

Although the bile serves as one pathway for the excretion of cholesterol, Bloor[87] is of the opinion that it is a minor one. Gardner[585] stated that the cholesterol eliminated in the bile is subsequently reabsorbed in the small intestine; if such a reabsorption is quantitative, bile does not serve any useful purpose in removing cholesterol from the organism.

Under certain conditions, the bile is no longer able to hold all of its cholesterol in solution, and the sterol precipitates from it. This phenomenon occurs principally in the gall bladder, where bile undergoes concentration. Any biliary calculi which develop in the bladder may be composed of almost pure cholesterol, although they frequently also contain bile pigments, soaps, $CaCO_3$, and small amounts of other constituents, in addition to cholesterol. It has been mentioned earlier that the term, "cholesterol," was coined to denote the source of this substance in gallstones (solid bile).

a. Factors Altering the Lipid Content of Bile. In the classic experiments of McMaster,[586] it was found that not only was the total cholesterol content of the bile augmented in the dog as a result of the feeding of cholesterol-rich foods, but also higher concentrations of the sterol obtained under such conditions. Some increase in cholesterol output in the bile likewise

[581] G. H. Whipple, *Physiol Revs.*, *2*, 440–459 (1922).

[582] E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951, p. 502.

[583] M. Friedman, S. O. Byers, and F. Michaelis, *Am. J. Physiol.*, *162*, 575–578 (1950).

[584] J. R. Heerschma and J. H. Annegers, *Proc. Soc. Exptl. Biol. Med.*, *69*, 140–141 (1948).

[585] J. A. Gardner, *Biochem. J.*, *18*, 777–784 (1924).

[586] P. D. McMaster, *J. Exptl. Med.*, *40*, 25–42 (1924)

resulted from the ingestion of large amounts of a cholesterol-low diet, although the amounts of cholesterol eliminated were much less than were those resulting from the administration of a cholesterol-rich diet. Actually, the increased cholesterol excretion in dog bile after cholesterol-rich diets comprised only a small proportion of that ingested. After feeding 5 or 6 g. of cholesterol in the form of eggs and brain, McMaster[586] could account for an increased bile cholesterol of only 60 mg.; this amounted to less than 2% of the ingested cholesterol. In fasting, the total excretion of cholesterol in the bile was found to be largely suppressed, although the concentration in this fluid was actually increased.

The level of cholesterol in the bile has also been shown to be a function of the endocrine glands. Thus, Rosenman, Friedman, and Byers[587] reported that the hyperthyroid rat excretes far more cholesterol in its bile than does the normal rat. This result is in contradistinction to the effect of hyperthyroidism on plasma cholesterol, in which case a hypocholesterolemia is observed.

In the case of man, consistent values of 0.045 to 0.055% for cholesterol from bile obtained by gall-bladder drainage have been reported by Nathan.[588] Increased blood cholesterol with a concomitant decrease in bile cholesterol has been reported during pregnancy.[589] Salomon[590] found that the cholesterol content of human bile was increased by butter, and by such high cholesterol-containing foods as eggs and brains.[591,592] This is similar to the response noted earlier in dogs.[586] Thus, in man, bile contained 13 to 21 milligram per cent of cholesterol on a basal diet of protein. This level was increased to 25 to 35 milligram per cent when four eggs were included in the dietary regimen; when 200 g. of butter were added to the basal diet, the bile contained 38 to 76 milligram per cent of cholesterol. It was later shown that the cholesterol level of the duodenal contents was markedly lowered on a cholesterol-low diet consisting of bananas,[591,592] although it is possible that the bile secretion may have been less on such a dietary regimen. More recently, Riegel and coworkers[593] failed to find a correlation between the amount of bile drained externally from their human patients and the cholesterol content of the bile; however, the concentration of bile salts and that of cholesterol were found, in general, to run parallel.

[587] R. H. Rosenman, M. Friedman, and S. O. Byers, *Science, 114*, 210–211 (1951).
[588] M. Nathan, *Arch. path. Anat. u. Physiol.* (*Virchow's*), *228*, 51–67 (1920).
[589] E. E. Pribram, *Arch. Gynäkol., 119*, 57–68 (1923).
[590] H. Salomon, *Arch. Verdauungskrankh., 39*, 46–49 (1926).
[591] H. Salomon, *Arch. Verdauungskrankh., 39*, 325–334 (1926).
[592] H. Salomon and L. L. Silva, *Arch. Verdauungskrankh., 36*, 353–359 (1926).
[593] C. Riegel, I. S. Ravdin, and H. J. Rose, *Am. J. Med. Sci., 193*, 446–447 (1937).

The cholesterol in hepatic bile has been shown to be low when a severe liver injury is present, although the situation is reversed when the liver damage is slight.[593] Bile ordinarily contains only unesterified (free) cholesterol,[67,594,595] although esterified cholesterol to the extent of 29% of the total has been reported from one sample of gall-bladder bile.[594]

After cottonseed oil was given to a patient, a marked increase in bile salts was noted, while the fatty acids and fats were only slightly augmented.[596] The administration of glucose caused no change in bile lipids, whereas peptone had only a slight effect in causing an elevation of the bile lipids.

b. Bile as a Source of Intestinal Lipids. Sperry[597] made an exhaustive study of the role of the bile as the source of fecal lipids. In order to determine this point, he carried out tests on bile-fistula dogs over a period of five weeks. Although considerable variations in the lipid excretion occurred during the course of the tests, no tendency toward a diminution in amount was observed. The lipid excretion continued at levels 1.5 to 4.5 times the normal. The fecal excretion was quite constant. The non-saponifiable fraction comprised 31.4%, and the fatty acids 63.1% (41.9% liquid and 45.5% solid acids). It was concluded that bile is not the source of fecal lipids. The results of Sperry[597] were confirmed by Beumer and Hepner.[598]

(2) The Excretion of Lipids by the Intestinal Wall

It is now recognized that a considerable proportion of the lipid excreted in the feces is secreted into the intestine below the level at which it can be reabsorbed. Sperry[599] reported the presence, in the feces of dogs, of usable free fatty acids not bound to cholesterol; this would indicate that these acids are excreted through the intestinal wall rather than introduced by way of the bile. Since the composition of the fecal fatty acids resembles that of the plasma lipid, it is suggested that the acids represent a leakage which has as its purpose the lubrication of the intestine. This worker also suggests the possibility that the lipid excretion may be concerned with the removal of undesirable excess sterols from the organism. Peretti[600] demonstrated the secretion of fat by the intestinal mucosa in an

[594] C. Riegel, I. S. Ravdin, and H. J. Rose, *Proc. Soc. Exptl. Biol. Med.*, *35*, 94–97 (1936).

[595] W. Wright, *J. Exptl. Med.*, *59*, 407–410 (1934).

[596] C. W. McClure, M. E. Huntsinger, and A. T. Fernald, *Am. J. Physiol.*, *107*, 1–12 (1934).

[597] W. M. Sperry, *J. Biol. Chem.*, *71*, 351–378 (1926–1927).

[598] H. Beumer and F. Hepner, *Z. ges. exptl. Med.*, *64*, 787–797 (1929).

[599] W. M. Sperry, *J. Biol. Chem.*, *68*, 357–383 (1926).

[600] G. Peretti, *Boll. soc. ital. biol. sper.*, *10*, 79–80 (1934).

unequivocal manner. It was shown that, following the feeding of iodized fat to a dog with a Thiry-Vella fistula, the iodized fat was excreted into the isolated intestinal loop.

Beumer and Hepner[598] suggested that cholesterol, also, is secreted through the wall of the intestine of the dog. These workers based their conclusions upon the finding that the cholesterol content of normal dogs was higher in the colon than in the ileum. A similar situation was shown to obtain in a bile-fistula dog after a lipid-free meal; it was found that 0.21% of cholesterol was present in the dried ileum contents, as contrasted with a value of 1.25% in the dried colon contents. According to these investigators, this can only mean that cholesterol has been secreted into the large bowel. Similar results have been reported by Bürger and Oeter[601] for man; the cholesterol content of the wall of the sigmoid of cadavers was found to be higher than that in various sections of the wall of the small intestine. These latter workers have likewise interpreted their data as indicating the passage of cholesterol through the wall of the large intestine.

In addition to cholesterol, the long-chain aliphatic alcohols, which occur in feces, apparently pass through the wall of the intestine into the lumen of the gut. Cetyl or palmityl alcohol, $CH_3(CH_2)_{14}CH_2OH$, was discovered in feces by Gardner,[33] and its presence in the feces of dogs, cats, and man was demonstrated by Schoenheimer and Hilgetag.[34] The latter workers also proved the presence of this alcohol in the walls of human intestine, in meconium, in the feces of bile-fistula dogs maintained on a fat-free diet, and in operatively prepared sterile cysts of the small and large intestine. Schoenheimer and Hilgetag[34] postulate that cetyl alcohol originates in the body, and is secreted through the mucosa into the lumen of the gut in a manner analogous to that of cholesterol secretion. Evidently the origin of the cetyl alcohol occurring in feces must lie in secretion through the walls of the lower gut rather than in the bile or other digestive secretions or in its presence in food. This is indicated by the fact that it is readily absorbed,[36,37,40] and would most certainly not escape such a fate if it were present in the upper part of the intestine where absorption can occur. However, Schoenheimer and Hilgetag[34] suggest that some resorption of cetyl alcohol does take place; under these circumstances, that present in the feces would represent only a fraction of the total alcohol produced. The identification of palmitic acid as a precursor of cetyl alcohol has been discussed elsewhere (see page 257). The occurrence of octadecyl or stearyl alcohol, $CH_3(CH_2)_{16}CH_2OH$, in feces, which has

[601] M. Bürger and H. D. Oeter, *Z. physiol. Chem.*, *182*, 141–147 (1929).

likewise been demonstrated by Schoenheimer and Hilgetag,[34] apparently has an explanation similar to that for cetyl alcohol.

The question which naturally arises is whether the lipid is actually "secreted" by the walls of the large intestine or whether its origin is merely to be traced to the fatty material resulting from desquamated intestinal epithelia. Later experiments of Sperry[602] indicate that the latter explanation is incorrect. In these tests, not only was the lipid content of the mucosa too small to account for the quantity of lipid present in the feces, but the proportion of the lipid occurring in the mucosa varied from that known to originate in the several portions of the gastrointestinal tract. Moreover, a smaller amount of lipids was found in the mucosa of dogs subjected to ileostomy than in that of normal dogs. Finally, it is believed that the metabolic activity of the mucosa rather than its current lipid content is a gauge of the quantity of lipid excretion. These results lead one to question whether any considerable portion of intestinal lipids arises from desquamated cells.

Another possible source of the fecal lipids might be bacterial synthesis in the lower gut. Although some lipids may be newly formed as a result of bacterial action, bacterial lipids probably do not account for more than 40% of the total.[603] Moreover, the ratio of liquid to solid fatty acids is similar to that obtaining in the non-bacterial lipids. This same investigator[604] later reported analogous results as regards the distribution of the lipids in the excretions of bile-fistula dogs, in which the lipid output was two to three times that of normal dogs. It is likewise probable that the fecal cetyl and stearyl alcohols do not owe their formation to bacterial synthesis.

(3) *The Effect of the Food Ingested*

Under ordinary conditions the excretion of lipids in the feces remains quite constant, and is little influenced by the nature of the fat ingested. However, when the dietary fat is incompletely digested, as is the case of high-melting fats such as mutton tallow, deer fat, oleostearine, and completely hydrogenated animal and vegetable fats, sufficient of the dietary fat may remain unabsorbed to alter completely the quantity and the relative composition of fecal fat. For a discussion of the digestibility of fats, the reader is referred to Chapter III.

[602] W. M. Sperry, *J. Biol. Chem.*, *96*, 759–768 (1932).
[603] W. M. Sperry, *J. Biol. Chem.*, *81*, 299–319 (1929).
[604] W. M. Sperry, *J. Biol. Chem.*, *85*, 455–463 (1929–1930).

Although Iwatsura and Nakamura[605] noted that the excreted fat is constant even if various types of fat are added to the diet, Cook,[105] in his excellent review on lipid absorption and excretion, pointed out the fact that lipid excretion is slightly increased on a fat-containing diet in a variety of species, as compared with that on a fat-free regimen. These data are summarized in Table 11.

TABLE 11

LIPID EXCRETION IN THE FECES OF SEVERAL SPECIES ON FAT-LOW AND FAT-CONTAINING DIETS[a]

Species	Total lipid (g./kg.)		Unsaponifiable matter (mg./kg.)	
	Low-fat diet	Fat diet	Low-fat diet	Fat diet
Rat[b]	0.3	0.85	100	200
Guinea pig[b]	—	1.5	—	150
Rabbit[b]	—	0.4	—	80
Cat[c]	0.15	0.2	13	30
Dog[c]	0.05	0.1	12	25
Man[d]	—	0.05	—	20

[a] Adapted from R. P. Cook, "Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, 14–29, Cambridge Univ. Press, 1952, p. 23.
[b] R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol.*, *36*, 61–74 (1951).
[c] W. M. Sperry and W. R. Bloor, *J. Biol. Chem.*, *60*, 261–287 (1924).
[d] D. C. Edwards and R. P. Cook, Unpublished results cited by Cook (footnote *a* above).

According to Wollaeger, Comfort, and Osterberg,[606] the increased lipid excretion in the feces following the ingestion of fat can be calculated from the formula $y = 2.93 + 0.021x$, in which x represents the quantity of ingested fat. Thus, it is calculated that, with an intake of 350 g. of fat, as much as 10 g. of lipid would be excreted in the feces.

Lewis and Partin,[607] reported that the daily fecal excretion of lipids by normal human subjects on a diet practically devoid of fat approximates 5 g. For a man of average weight (70 kg.), this would involve an excretion of 70 mg./kg., which is higher than the value suggested by Cook[105] for human subjects receiving fat diets. Not only does the ingestion of fats cause an increase in fecal lipids, but also, according to Edwards and Cook,[608] the fatty acid excretion in the feces is increased in rats fed cholesterol. The fatty acids excreted are those which are normally present.

[605] R. Iwatsura and T. Nakamura, *J. Biochem.* (*Japan*), *37*, 397–408 (1950).
[606] E. E. Wollaeger, M. W. Comfort, and A. E. Osterberg, *Gastroenterology*, *9*, 272–283 (1947).
[607] G. T. Lewis and H. C. Partin, *Federation Proc.*, *12*, 239 (1953).
[608] D. C. Edwards and R. P. Cook, *Biochem. J.*, *48*, ix (1951).

Under abnormal conditions in the gastrointestinal tract, such as sprue and other related steatorrheas, fat utilization may be greatly impaired, as has already been reported (see Chapter III). Heerschma and Annegers[609] demonstrated this phenomenon as a result of diversion of bile from the gastrointestinal tract in dogs. Thus, while the total fat and fatty acids excreted in the feces of normal dogs remained constant and independent of fat or fiber intake, in the case of bile fistula dogs, the fecal fat increased linearly as dietary fat was increased. It was calculated that the fecal fat under such conditions could be predicted from the sum of the constant amount present on the fat-free diet and 58% of the dietary fat. Moreover, the ratio of free fatty acids to total fecal fat was significantly higher in bile fistula dogs than in normal animals. The fecal fat in bile fistula dogs receiving 15 to 40 g. of triglyceride daily was not altered by the inclusion of 2 to 4 g. of Tween 80 in the diet.[610]

Components of the diet other than lipids may likewise alter the excretion of fecal lipids. Thus, Schulz and Thomas[611] reported that, on a basal diet containing sucrose and 10% soybean oil, the addition of cystine, methionine, vitamin C, bile salts, sodium fluoride, carotene, calcium carbonate, calcium chloride or calcium lactate had no effect upon the percentage of fecal excretion of lipids, or upon the retention of the lipids. However, the inclusion of 20% of charred alfalfa or 10% of agar-agar, as well as the substitution of lactose for sucrose, resulted in an increase in fecal lipids. When starch replaced the sucrose, the addition of carotene to the diet resulted in a significant increase in fecal lipids. Walker[612] suggests that the increased lipid secretion in the intestine brought about by the inclusion of fiber in the diet is due to the stimulating effect of the mechanical action of the fiber itself on the intestinal mucosa. This secretion would then act as a conditioning or lubricating agent for the intestine.

12. The Composition of Fecal Lipids

As early as 1813, Home[613] recognized that fatty materials are present in feces. In 1884, Müller[614] concluded that, since fats are present in the meconium as well as in the feces of the adult during fasting, they must represent excretion products.

[609] J. R. Heerschma and J. H. Annegers, *Am. J. Physiol*, *153*, 143–147 (1953).
[610] J. H. Annegers, *Proc. Soc. Exptl. Biol. Med.*, *81*, 277–278 (1952).
[611] J. A. Schulz and B. H. Thomas, *J. Nutrition*, *42*, 175–187 (1952).
[612] A. R. P. Walker, *Nature*, *164*, 825–827 (1949).
[613] E. Home, *Phil. Trans., Roy Soc. (London)*, *103*, 146–158 (1813).
[614] F. Müller, *Z. Biol.*, *20*, 327–377 (1884).

It was early demonstrated that, under ordinary dietary conditions, fecal fat has a different composition from that of ingested fat, and that the quantity excreted bears little relation to that of the ingested fat. Hill and Bloor[615] showed that fat was invariably present in the feces, irrespective of whether or not this foodstuff was included in the diet. When moderate amounts of fat were present in the diet, no appreciable increase in fecal fat resulted.[615,616] Hill and Bloor[615] concluded that fecal fat is not unabsorbed food fat, but is rather in the nature of an excretion, either directly from the blood or indirectly from intestinal secretions. Holmes and Kerr[617] compared the iodine numbers and saponification values of food fats and fecal fats of human subjects who had ingested the several fatty substances. The values for the iodine number of the food and of fecal fats are respectively as follows: goose fat tests, 68.5 and 28.6; oleo oil tests, 44.3 and 27.9; and corn oil tests, 123.1 and 34.3. The comparative results on saponification values were as follows: goose fat tests, 194.8 and 159.1; oleo oil tests, 207.3 and 146.2; corn oil tests (no value reported for oil fed, but average saponification value is 190), 108.6 (fecal fat).

On the basis of an extensive study, using cats and dogs, Sperry and Bloor[618] concluded that fecal fats do not originate from food fat because: (1) there is almost as much fecal fat on a fat-free diet as on a fat-rich regimen, (2) during fasting there is considerable excretion of lipids with properties resembling those of the lipid excreted when fat is given, and (3) the composition of food fat differs from that of fecal fat. However, ingested fat does to some extent influence fecal fat, since somewhat higher fecal lipids do occur on a fat diet than on a fat-free regimen, and the composition of solid and of liquid fatty acids in the feces bears some relationship to these fractions in the food fats.

Another distinguishing factor which indicates the difference which exists between food fat and fecal fat is the melting point. Fecal fat usually has an appreciably higher melting point than does the corresponding food fat. Thus, the values for food and for fecal fat in the dog were 43° and 50.5°C., respectively, after the feeding of lard, and 52 and 56.0°C. after mutton fat was given.[619] In man, the feces fat melted at 50 to 51.5°C. after milk fat (43°C.) was fed[619]; after the ingestion of lard (35 to 37°C.),

[615] E. Hill and W. R. Bloor, *J. Biol. Chem.*, *53*, 171–178 (1922).
[616] A. Krakower, *Am. J. Physiol.*, *107*, 49–54 (1934).
[617] A. D. Holmes and R. H. Kerr, *J. Biol. Chem.*, *58*, 377–381 (1923).
[618] W. M. Sperry and W. R. Bloor, *J. Biol. Chem.*, *60*, 261–287 (1924).
[619] F. Müller, *Z. klin. Med.*, *12*, 45–113 (1887).

TABLE 12

DISTRIBUTION OF LIPIDS IN NORMAL FECES OF SEVERAL SPECIES OF ANIMALS IN PER CENT TOTAL LIPIDS

Category	Rat[a,b]	Guinea pig[c]	Rabbit[c]	Cat[d]	Dog[d]	Man[e]
Type of solvent extraction	f	g	g	h	h	f
Unsapon. matter	20	10	20	20	30	30
Total sterol	10	2	5	—	15	10
Total acids	80	80	70	60	70	60
Volatile	10	2	3	3	30	10
Non-volatile	—	—	—	—	—	—
Solid	30	—	—	20	10	20
Liquid	40	—	—	30	30	10
Insol. in light petroleum ether	5	Present	10	—	—	10

[a] D. C. Edwards and R. P. Cook, *Biochem. J.*, *48*, ix (1951).
[b] R. O. Thomson, Unpublished observation cited by R. P. Cook," Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams. *Lipid Metabolism*, Biochem. Soc. Symposia No. 9, Cambridge Univ. Press, 1952, p. 24.
[c] R. P. Cook and R. O. Thomson, *Quart. J. Exptl. Physiol.*, *36*, 61–74 (1951).
[d] W. M. Sperry and W. R. Bloor, *J. Biol. Chem.*, *60*, 261–287 (1924).
[e] D. C. Edwards and R. P. Cook, *Biochem. J.*, *49*, xli (1951).
[f] Ether and ethanol extraction.
[g] Ether extraction.
[h] Ether extraction after treatment with light petroleum ether.

fecal fats melted at 43 to 46°C.[620] According to Hecht,[621] fecal fats usually have melting points 4 to 8°C. higher than those for the food fats.

In the fatty acid portion of the lipids from dog feces about 30% were solid acids (palmitic and stearic acids, the latter in a slightly higher prochiportion) and 60% were liquid acids (largely oleic with possibly some arachidonic acid).[599] The balance of the fat fraction (10%) was accounted for as the glycerol moiety. A similar distribution of non-volatile acids is recorded for human feces.[622]

Volatile fatty acids were excreted in a considerable proportion by cats on a coconut oil diet, but only to a slight extent by dogs.[618] However, dogs did excrete volatile fatty acids in definite amounts after a prolonged fat-free diet. The volatile acids were made up of acetic (65%), butyric (23%), and caproic (12%) acids.[599] Cecchini [623] likewise reported that acetic and butyric acids are the main volatile fatty acids in the feces. In the case of human feces, Edwards and Cook[622] reported that the volatile fatty acids comprise 63% of the total lipid.

[620] I. Munk, *Arch. path. Anat. u. Physiol.* (*Virchow's*), *122*, 302–325 (1890).
[621] A. F. Hecht, *Jahrb. Kinderheilk.*, *62*, 613–659 (1905).
[622] D. C. Edwards and R. P. Cook, *Biochem. J.*, *49*, xli (1951).
[623] A. Cecchini, *Arch. pathol. clin. med.*, *2*, 361–392 (1923).

Sperry[599] reported that the non-saponifiable fraction in the fecal lipids of dogs accounts for 35 to 40% of the total, while the fatty acids comprise 55 to 60%. The non-saponifiable fraction contains principally cholesterol, coprosterol, and other sterols. Bürger and Winterseel[103] reported that about 50% of the sterol in human feces consisted of cholesterol, and 50% of coprosterol; 10 to 30% of the sterols were in an esterified form. Edwards and Cook[622] cited a value of 34% for the unsaponifiable content in human feces. The proportion of cholesterol to coprostanol is increased when cholesterol dissolved in oil is added to a mixed diet.

The comparative distribution in normal feces as summarized by Cook[105] is given in Table 12.

Much information as to the nature of fecal fat has been afforded by the use of Thiry-Vella loops and by a number of other types of fistulas. Angevine[624] reported that the lipid secretion from such Thiry-Vella fistulas in dogs is extremely constant when based upon the amount per kilogram body weight per day; no consistent variations were noted when a high-fat or low-fat regimen replaced the normal diet. In an ingenious series of tests, Sperry and Angevine [625] demonstrated a much greater secretion of fat into the ileum than could be recovered from the isolated colon. On the basis of these data, the hypothesis was formulated that considerable amounts of the lipids secreted into the intestine are reabsorbed.

[624] R. W. Angevine, *J. Biol. Chem.*, *82*, 559–565 (1929).
[625] W. M. Sperry and R. W. Angevine, *J. Biol. Chem.*, *96*, 769–786 (1932).

CHAPTER V

BLOOD LIPIDS

1. Introduction

All types of lipids which are present in the tissues of animals occur in greater or lesser amounts in the blood. Although the lymphatics provide the chief initial pathway for the transport of the fats and other lipids from the intestine, the blood is the ultimate avenue for the further distribution of these substances to the liver and to the several fat depots. Moreover, aside from the gastrointestinal phase, the blood serves as the medium of transfer of the lipid components from one organ to another.

The composition of the blood is considerably influenced by the ingestion of food. Although the maximum level of the glucose or amino acids in the blood is reached two to four hours after carbohydrates or proteins are ingested, the highest level of lipids is usually not attained in the blood earlier than six hours after a heavy fat meal. Sometimes as long as nine or ten hours may elapse before the effect of the food fat has disappeared from the blood and the preprandial values are obtained. The lipids remain fairly constant over a fasting period of several days. However, as the glycogen stores are used up, an increased fat metabolism obtains, with the result that a moderate *hyperlipemia* (increased blood fat level) may occur.

Although the blood contains considerable amounts of the several lipids, it is generally accepted that the oxidation of these components occurs only in the tissues. There is no proof that any active metabolic changes can take place in the blood stream. Not only are the lipids transported in the blood stream largely in droplets which are in suspension in the plasma, but the heterogeneity of the whole blood renders impossible any uniform distribution throughout this fluid.

The blood lipids, which are carried from the intestinal tract in the chyle, are mixed with the blood when the thoracic duct empties its contents into the blood stream. The fine droplets, which are composed largely of neutral fat, originally present in the chyle, are retained in suspension in the blood.

These are referred to either as hemoconia, or "blood dust,"[1,2] or, more commonly, as "chylomicrons." The latter connotation was the term coined by Gage and Fish.[3] The chylomicrons are 1 micron or less in diameter, and it is believed that they are stabilized by protein films.[4] This supposition is based upon the fact that an aggregation occurs at a *p*H of 4.7 to 5.3, which represents the approximate isolelectric point of the albumins and globulins of the blood plasma. Coalescence of the fat droplets will occur when the acid is sufficiently strong to precipitate the protein, thus destroying the protective film.

The mechanism by which the lipids other than neutral fats are carried in the blood is not certain. It is possible for the fat-soluble components such as cholesterol and the fat-soluble vitamins to be dissolved in the droplets of fat. On the other hand, there is increasing evidence that cholesterol,[5] carotene,[6] and the vitamins A[6] are carried, not in the fat fraction, but as coordination compounds where they are in combination with the plasma proteins. Lecithin may likewise be transported as a protein complex; it has long been recognized that this phosphatide is not directly extractable from blood or tissues without a preliminary disruption of its combination with protein. The high relative concentration of lecithin in the corpuscles may indicate that not only does this phospholipid play a role in controlling the permeability of the red cells, but it is also possible that the cells may at the same time function as a mechanism for lecithin transport. Bloor,[7] in his comprehensive monograph, has given a most complete discussion of the lipid composition of the blood, together with a consideration of the factors which alter it.

2. The Nature of Blood Lipids

The blood lipids consist of fatty acids, neutral fats, phospholipids, and unsaponifiable components including cholesterol, carotenoids, vitamins A, D, E, and K, and other substances in relatively small amounts.

[1] A. Neumann, *Wien. klin. Wochschr.*, *20*, 851–853 (1907).
[2] E. Neisser and H. Braeuning, *Z. exptl. Pathol. Therap.*, *4*, 747–760 (1907).
[3] S. H. Gage and P. A. Fish, *Am. J. Anat.*, *34*, 1–85 (1924).
[4] S. De W. Ludlum, A. E. Taft, and R. L. Nugent, *Colloid Symposium Annual*, *7*, 233–248 (1929).
[5] M. A. Macheboeuf, *Bull. soc. chim. biol.*, *11*, 268–293 (1929).
[6] J. Ganguly, N. Krinsky, J. W. Mehl, and H. J. Deuel, Jr., *Arch. Biochem. Biophys.*, *38*, 275–282 (1952).
[7] W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

(1) *Fatty Acids*

The fatty acids occur in combination with the phospholipids, and in ester combination with cholesterol, as well as with the carotenols and fat-soluble vitamins. According to Luck,[8] fatty acids may likewise be bound by plasma proteins; this fraction may be identical with that referred to as free fatty acids.

There is evidence that, in addition to the usual C_{16}–C_{18} saturated and unsaturated acids in the blood, several of the short-chain (volatile) acids may also be present, especially in the case of ruminants. Thus, Craine and Hansen[9] proved that the peripheral blood of goats contained acetic, propionic, and butyric acids, as well as other unidentified volatilea cids. It was shown that, concurrently with the development of the rumen, the level of blood glucose decreased, while that of the short-chain fatty acids increased. Although McClymont[10] showed that the concentration of butyrate in the blood of ruminants is low, there are appreciable amounts of β-hydroxybutyrate in blood. On the basis of arteriovenous differences, there would seem to be strong evidence that the bovine udder is able to remove measurable amounts of this hydroxy-acid from the blood.[11] It is now believed that acetate is the chief end-product of the breakdown of celluloses and other polysaccharides in the rumen of the goat and cow. These molecules are carried *via* the blood to the udder, where they are removed to be used in the synthesis of long-chain fatty acids. This is discussed in Volume III. The recent reviews of Popják[12] and Folley[13] furnish considerable information on this phenomenon.

The presence of the volatile fatty acids is not, however, confined to the blood of ruminants. Thus, Fonnesu[14] reported that acetic acid is present in human blood. It was found to be higher in the arterial blood (7.5 milligram per cent) than in the venous blood (5.0 milligram per cent), which would indicate that it was removed in the tissues either for oxidation or for fat synthesis. The level of blood acetate rises rapidly after a meal, and reaches a maximum in one hour. It then gradually decreases to the

[8] J. M. Luck, in *Lipoproteins,* General discussion Faraday Soc., No. 6, 44–52, Aberdeen Univ. Press, Aberdeen, 1949.

[9] E. M. Craine and R. G. Hansen, *J. Dairy Sci., 35,* 631–636 (1952).

[10] G. L. McClymont, *Biochem. J., 45,* i–ii (1949).

[11] J. C. Shaw and C. B. Knodt, *J. Biol. Chem., 138,* 287–292 (1941).

[12] G. Popják, "Fat Synthesis from Small Molecules," in R. T. Williams, *Lipid Metabolism;* Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 1952, pp. 37–51.

[13] S. J. Folley, "Aspects of Fat Metabolism in the Ruminant, With Special Reference to the Biosynthesis of Milk Fat," in R. T. Williams, *Lipid Metabolism,* 1952, pp. 52–65.

[14] A. Fonnesu, *Bull. soc. chim. biol., 33,* 1021–1024 (1951).

original preprandial level, which is reached after four to five hours. Fonnesu[14] suggests that the acetic acid in the blood is derived partly from intestinal absorption and partially from the intermediary metabolism of tissues. Although the presence of butyric acid has not been demonstrated in human blood, large amounts of β-hydroxybutyric acid and acetoacetic (diacetic) acid occur normally during fasting, as well as during the ingestion of a carbohydrate-free regimen. Ketone bodies (β-hydroxybutyrate, acetoacetate, acetone) may likewise occur in large quantities in the blood in such diseases as diabetes mellitus. For a discussion of ketosis and ketonemia, the reader is referred to pages 449–451.

No information is available as to whether the volatile fatty acids occur in the blood as triglycerides, as other esters, as free acids, or as salts. Most of the evidence would seem to indicate that they are present in water-soluble form. This would presumably suggest that they occur as free acids, salts, or in combination with water-soluble proteins.

a. Fatty Acids in Phospholipids. The fatty acids present in the phospholipid fraction of liver are ordinarily composed of 2 to 3 parts of liquid (unsaturated) fatty acids to 1 part of solid (saturated) acids.[15] The composition of blood lipids presumably follows the same pattern. The saturated fatty acids in phospholipids consist chiefly of palmitic and stearic acids, while oleic and linoleic acids largely comprise the unsaturated fatty acid fraction.

The fatty acids of phospholipids are not highly unsaturated. In the case of both lactating and non-lactating cows, Schaible[16] found that the average iodine value was lowest in the phospholipid fatty acids, intermediate in those from neutral fat, and highest in those from the cholesterol esters. The iodine values in lactating cows averaged 68, 103, and 145 for the phospholipid, neutral fat, and cholesterol fatty acids, respectively; in non-lactating cows, the figures were 85, 99, and 130, respectively. On the other hand, Bloor and associates[17] reported that the iodine numbers of the fatty acids from human plasma are lowest for neutral fat (102), intermediate for the phospholipid fraction (125), and highest for the fatty acids combined with cholesterol (158). Bloor[18,19] and Channon and Collinson[20] have shown that the phospholipid fatty acids of species other than man have a considerably lower iodine value than the figures for human

[15] R. H. Snider and W. R. Bloor, *J. Biol. Chem.*, *99*, 555–573 (1932–1933).
[16] P. J. Schaible, *J. Biol. Chem.*, *95*, 79–88 (1932).
[17] W. R. Bloor, A. G. Blake, and S. S. Bullen, *J. Allergy*, *9*, 227–230 (1938).
[18] W. R. Bloor, *J. Biol. Chem.*, *59*, 543–556 (1924).
[19] W. R. Bloor, *J. Biol. Chem.*, *63*, 1–15 (1925).
[20] H. J. Channon and G. A. Collinson, *Biochem. J.*, *23*, 1212–1221 (1929).

plasma. In the case of beef plasma, in harmony with the values of Schaible,[16] the iodine value of phospholipid fatty acids[18,19] is about 71; the average figure reported for pigs[18] was 80, while slightly higher values were obtained for dogs,[18,19] namely 89 and 97.

TABLE 1
COMPARATIVE FATTY ACID COMPOSITION OF VARIOUS FRACTIONS OF ACETONE-SOLUBLE PLASMA LIPIDS OF COW BLOOD[a]

Acids	Free fatty acids	Glyceride fatty acids	Cholesterol ester fatty acids	Total
Total weight, g.	11.64	9.88	26.86	48.38
Per cent	24.1	20.1	55.5	100.0
Fatty acid composition, mole per cent				
Myristic	0.8	0.2	—	0.3
Palmitic	34.4	33.7	11.1	21.4
Stearic	5.2	22.2	3.3	7.6
Arachidic	2.7	0.5	0.3	0.8
Palmitoleic	—	2.6	4.2	2.8
Oleic	40.5	21.3	7.9	18.5
Linoleic	16.4	18.4	61.7	42.1
Linolenic	—	—	9.2	5.0
Arachidonic	—	1.1	2.3	1.5
Total saturated	43.1	56.6	14.7	30.1
Total unsaturated	56.9	43.4	85.3	69.9

[a] Adapted from F. E. Kelsey and H. E. Longenecker, *J. Biol. Chem.*, *139*, 727–740 (1941).

Although the differences in iodine value may be a function of species, this figure may also be related to the nature of the predominant food fat. Sinclair[21] was able to demonstrate the incorporation of definite amounts of elaidic acid in the plasma phospholipids of cats within a few hours after this unnatural fat was fed. After several days of continuous feeding with trielaidin, the elaidic acid content of the fatty acids of plasma phospholipids amounted to as much as 37% of the total; however, since no elaidic acid could be detected in the phospholipids of the red blood cells, it was concluded that the erythrocytes do not participate in phospholipid transport. If a similar behavior were noted in the case of phospholipids containing the fatty acids ordinarily present, this would constitute strong evidence that the cell phospholipid does not participate in phospholipid transport.

b. Fatty Acids in Cholesterol Esters. For some time it has been recognized that the most highly unsaturated fatty acids in the blood are present

[21] R. G. Sinclair, *J. Biol. Chem*, *115*, 211–220 (1936).

in combination with cholesterol, as the cholesterol ester fraction. As has been noted above, the iodine numbers of the fatty acids in the cholesterol fractions were 145 and 130 in cows,[16] and 158 in human plasma.[17] Channon and Collinson[20] demonstrated that arachidonic and linoleic acids occur in the acetone-soluble fraction of beef blood, along with palmitic and stearic acids. Kelsey and Longenecker[22] reported 62% of linoleic acid in cholesterol esters of the acetone-soluble fraction of cow plasma, as contrasted with only 18% of this dienoic acid in the glyceride fatty acids These figures correspond to values of 55 and 25%, respectively, found by Channon and Collinson.[20] In sharp contradistinction to these results, Keegan and Gould[23] isolated cholesteryl oleate from dog and human plasma; it is difficult to explain how cholesteryl oleate could be present in sufficient amounts to be isolated, in view of the prevailing iodine numbers previously reported for the cholesteryl esters. The results of Kelsey and Longenecker[22] are summarized in Table 1 on page 353.

c. Fatty Acids Combined with Protein. It is now believed that the plasma fatty acid fraction, which was formerly classed as free fatty acids, actually represents a fraction combined with protein. The first evidence of a protein-fatty acid combination was given by Macheboeuf and Tayeau.[24] Almost simultaneously, Kendall[25] prepared a crystalline serum albumin from human serum which contained 2% of a fatty acid; this could be freed from the protein only by denaturation of the lipoprotein molecule.

Indirect evidence cited by Luck,[8] which confirms the protein-fatty acid association, includes that obtained from dialysis-equilibrium studies, as demonstrated by effects on absorption spectra, and by binding studies on dyes, on indicators, and on synthetic detergents. Scatchard and Black[26] have shown that serum albumin solutions, which have been rendered isoionic by exhaustive dialysis against water, exhibit a decrease in *p*H of as much as 1.65 *p*H units when various neutral inorganic salts are added. These results are interpreted to mean that an anionic binding of the fatty acids has been disrupted by this procedure. The results of Longsworth and Jacobsen[27] and of Velick[28] afford similar evidence. The dialysis-equilibrium method was first introduced as a tool by von Muralt[29] in 1930;

[22] F. E. Kelsey and H. E. Longenecker, *J. Biol. Chem.*, *129*, 727–740 (1941).
[23] P. Keegan and R. G. Gould, *Federation Proc.*, *12*, 228–229 (1953).
[24] M. A. Macheboeuf and F. Tayeau, *Bull. soc. chim. biol.*, *23*, 49–61 (1941).
[25] F. E. Kendall, *J. Biol. Chem.*, *138*, 97–109 (1941).
[26] G. Scatchard and E. S. Black, *J. Phys. & Colloid Chem.*, *53*, 88–99 (1949).
[27] L. G. Longsworth and C. F. Jacobsen, *J. Phys. & Colloid Chem.*, *53*, 126–135 (1949).
[28] S. F. Velick, *J. Phys. & Colloid Chem.*, *53*, 135–149 (1949).
[29] A. L. von Muralt, *J. Am. Chem. Soc.*, *52*, 3518–3523 (1930).

he developed a mathematical treatment, based upon the law of mass action, for the calculation of the binding of the hydrogen ions, which is probably applicable to the estimation of the multiple binding of other ions where a series of association constants are involved. More recently, Klotz[30] simplified these equations and made possible an evaluation of the role of electrostatic factors in binding. This also affords a means for determining the number of ions bound per protein molecule and for assessing the bond energies. Although the results of Klotz concern the combination of calcium ions with casein, they are apparently applicable to the fatty acid-protein complexes.[8]

Changes in the electrophoretic mobility furnish another criterion of anion-protein combinations. For example, Ballou, Boyer, and Luck[31] showed that the speed of migration of serum albumin, which had previously been equilibrated with solutions of the sodium salts of the fatty acids, was increased as the chain length of the acids became greater. Although this evidence is highly suggestive, Luck[8] postulates that phosphate might also increase the net negative charge of the serum albumin. This would offer a possible explanation for the electrophoretic finding.

The thermal stability of serum albumin solutions, as determined by the so-called cloud-point technic, has been shown to be increased by fatty acid anions.[32-35] The stability of the albumin solutions became greater as the length of the fatty acid chains was increased. These results were supported by the demonstration of a similar protective effect which was also augmented with the increasing chain length of the fatty acids when ultrafiltration was the criterion employed.[32] This also applied when the stabilization of serum albumin against urea denaturation, as followed by viscosimetry, was the property investigated.[36,37]

Other indirect evidence cited by Luck[8] includes the results of Davis and Dubos,[38] who observed that the beneficial effect of serum albumin on the growth of tubercle bacilli was due to fixation of the albumin by oleic acid

[30] I. M. Klotz, *Arch. Biochem.*, *9*, 109–117 (1946).

[31] G. A. Ballou, P. D. Boyer, and J. M. Luck, *J. Biol. Chem.*, *159*, 111–116 (1945).

[32] P. D. Boyer, G. A. Ballou, and J. M. Luck, *J. Biol. Chem.*, *167*, 407–424 (1947).

[33] G. A. Ballou, P. D. Boyer, J. M. Luck, and F. G. Lum, *J. Clin. Invest.*, *23*, 454–457 (1944).

[34] G. A. Ballou, P. D. Boyer, J. M. Luck, and F. G. Lum, *J. Biol. Chem.*, *153*, 589–605 (1944).

[35] P. D. Boyer, F. G. Lum, G. A. Ballou, J. M. Luck, and R. G. Rice, *J. Biol. Chem.*, *162*, 181–198 (1946).

[36] P. D. Boyer, G. A. Ballou, and J. M. Luck, *J. Biol. Chem.*, *162*, 199–208 (1946).

[37] E. L. Duggan and J. M. Luck, *J. Biol. Chem.*, *172*, 205–220 (1948).

[38] B. D. Davis and R. J. Dubos, *J. Exptl. Med.*, *86*, 215–228 (1947).

and possibly by other unsaturated acids. Moreover, the presence of various fatty acids in the medium increased the protective effect of serum albumin against hemolysis, as determined by *in vitro* tests. Boyer *et al.*[32] demonstrated a protective action on the part of the albumin against hemolysis due to sodium caprylate, although its beneficial effect was greater than was predicted from the "combined caprylate" content of the medium.

Boyer *et al.*[32] employed the ultrafiltration technic with considerable success for the study of fatty acid binding by protein. These workers used butyrate, caproate, caprylate, caprate, and acetyltryptophane, and the work was later extended to include mandelate.[39] One can apply a simple mass action expression to these data to obtain a quantitative interpretation of the results. Moreover, this technic does not require as sensitive analytical procedures as do several of the other tests, since higher concentrations of protein and anion are involved.

Serum albumin, especially, appears to have a conspicuous ability to bind anions. According to Teresi and Luck,[40,41] bovine serum albumin has the capacity to bind 25 ions per mole in the case of the more strongly associated anions. In later experiments,[42] the mechanism of the combination of fatty acids and serum albumin was investigated by means of carboxyl-labeled C_{14} fatty acids. It was found that there were two groups of binding sites per protein molecule, each of which was characterized by its own particular binding constants. On the other hand, crystalline β-lactoglobulin[43] was found to be able to bind only 2 ions per mole of fatty acids, while crystalline β-amylase[43] appears to be devoid of any such capacity. These data are in line with the classification of Klotz and Urquhart,[44] in which the relative binding power of protein is analyzed as follows: highest binding power, serum albumin and β-lactoglobulin; slight binding power, ovalbumin and conalbumin; no binding power, pepsin, trypsin, chymotrypsin, ribonuclease, and insulin. Also in line with the above findings, Davis and Dubos[38] reported that the protective action of serum albumin against oleic acid, as observed in the cultivation of tubercle bacilli, was shown to a slight extent by β-lactoglobulin, but was not exhibited by any other proteins tested. Luck,[8] on the basis of the shift in absorption spectrum which occurs when fatty acids are combined with serum albumin, believes that gelatin and γ-globulin do not combine with fatty acids; finally, on the basis of

[39] J. M. Luck, *J. Phys. & Colloid Chem.*, *51*, 229–239 (1947).
[40] J. D. Teresi and J. M. Luck, *J. Biol. Chem.*, *174*, 653–661 (1948).
[41] J. D. Teresi and J. M. Luck, *J. Biol. Chem.*, *177*, 383 (1949).
[42] J. D. Teresi and J. M. Luck, *J. Biol. Chem.*, *194*, 823–834 (1952).
[43] J. D. Teresi; cited by J. M. Luck, in *Lipoproteins*, General discussion Faraday Soc., No. 6, 44–52, Aberdeen Univ. Press, Aberdeen, 1949, p. 48.
[44] I. M. Klotz, and J. M. Urquhart, *J. Am. Chem. Soc.*, *71*, 1597–1603 (1949).

cloud point studies, also, he excludes serum γ-globulin, insulin, diphtheria toxin, diphtheria antitoxin, and papain from the group of proteins which manifest the property of combination with fatty acids.

The best basis for the specificity of serum albumin in binding anions would seem to be that it contains a number of positively charged groups. That this is the case is indicated by the more recent results of Teresi,[45] in which it was demonstrated that, when the free ε-amino groups of lysine are eliminated, the number of ions of *m*-nitrophenolate or *p*-nitrophenolate bound by the modified protein is markedly reduced. In the case of the *o*-nitrophenolates (mono-, di-, or trinitro-), no reduction in binding power followed the treatment of the protein with formaldehyde, or its acetylation. This is interpreted as evidence that the guanidine residues may likewise be concerned in the combinations.

In addition to the electrostatic forces, Luck[8] believes that the experiments of Boyer *et al.*[32] prove the importance of the van der Waals' forces as explaining the binding effects. On the other hand, Klotz and Urquhart[44] proposed the theory that the binding capacity of a protein is a direct function of the number of positively charged groups and an inverse function of the number of carboxyl and hydroxyl groups. As a result of his experiments on methyl orange, Klotz and Urquhart[46] feel that the van der Waals' forces play only a minor role. Luck[8] does not accept the latter deduction, since the dye is believed to be atypical, and conclusions obtained from it are not applicable to the binding of fatty acids by proteins.

(2) *Neutral Fats*

Despite extensive knowledge of the fatty acid makeup of the blood, little is known of the nature of the neutral fat component or of the nature of the fatty acid moiety which is normally combined with glycerol. Since the neutral fats are much less soluble than the other lipid components of plasma, they exist as finely emulsified droplets called chylomicrons,[3] which are generally not more than 1.0 μ in diameter.[47] According to Frazer,[48] blood collected after a twelve-hour starvation period contains only a trace of fatty acids and triglycerides. In hyperlipemic blood, the glyceryl esters are considerably increased, without any appreciable alteration in the free fatty acids. The neutral fat consists chiefly of glycerol esters of C_{12} to C_{18}

[45] J. D. Teresi, *J. Am. Chem. Soc.*, *72*, 3972–3978 (1950).

[46] I. M. Klotz and J. M. Urquhart, *J. Am. Chem. Soc.*, *71*, 847–851 (1949).

[47] E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951.

[48] A. C. Frazer, in *Lipoproteins*, General discussion Faraday Soc., No. 6, 81–97, Aberdeen Univ. Press, Aberdeen, 1949.

fatty acids, with oleic acid as the chief unsaturated acid. The more highly unsaturated acids and the short-chain saturated acids are absent from the neutral fat fraction. Red blood cells are practically devoid of neutral fat.[47]

(3) *Phospholipids*

The phospholipids present in blood consist of all of the three main types, namely, lecithins, cephalins, and sphingomyelins. The phospholipids are higher in the red blood cells than in the plasma, and the distribution of lecithin, cephalin, and sphingomyelin differs for these two fractions of blood.

Artom[49] was the first to call attention to the fact that serine-containing phospholipids occur in human plasma. However, it was recognized that they represented only a minor fraction of the total phospholipids. Taurog, Entenman, and Chaikoff[50] later showed that the phospholipids present in the plasma of dog and man consist almost entirely of the choline-containing phospholipids, lecithin and sphingomyelin. Only 5% of the total phospholipid failed to contain choline, and therefore could be considered as belonging to the cephalin fraction. Hack[51] confirmed the work of Taurog *et al.*,[50] insofar as human blood plasma is concerned, by demonstrating the proportion of lecithin:cephalin:sphingomyelin as 78.8:5.2:16.0. Moreover, Sinclair[52] reported that cephalin makes up only 3 to 8% of the total phospholipids of beef, dog, and pig serum, as well as of human serum.

On the other hand, in the case of the fowl, cephalin apparently plays a more important role in the blood than it does in other animals. Thus, Flock and Bollman[53] found that only 53% of the phospholipid in the plasma of the chicken consisted of the choline-containing fraction. Similar results were noted for the turkey (*Meleagris gallipavo*) by Sinclair,[52] who reported that cephalin comprises 20% of the total plasma phospholipids in this species. Using newer and more reliable methods for cephalin,[54,55] Ranney and associates[56] found that the plasma of the normal, immature male fowl contains, on an average, 21% of cephalins, 65% of lecithins,

[49] C. Artom, *J. Biol. Chem.*, *157*, 595–599 (1945).
[50] A. Taurog, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *156*, 385–391 (1944).
[51] M. H. Hack, *J. Biol. Chem.*, *169*, 137–143 (1947).
[52] R. G. Sinclair, *J. Biol. Chem.*, *174*, 343–353 (1948).
[53] E. V. Flock and J. L. Bollman, *J. Biol. Chem.*, *144*, 571–577 (1942).
[54] A. Taurog, C. Entenman, B. A. Fries, and I. L. Chaikoff, *J. Biol. Chem.*, *155*, 19–25 (1944).
[55] G. Schmidt, J. Benotti, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, *166*, 505–511 (1946).
[56] R. E. Ranney, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *180*, 307–313 (1949).

and 14% of sphingomyelins. The cephalins engage in metabolic activities at a rate proportional to that of the plasma lecithins, as evidenced by the fact that both show a six-fold increase after the administration of stilbestrol.

Albrink[57] reported that 70 to 80% of the serum phospholipids of normal patients or of patients with diseases of the liver or biliary tract were of the choline-containing type. Taylor and McKibbin[58] noted that, although variations in the absolute level of phospholipids were observed in different species, the patterns for the distribution of nitrogenous bases were similar in all nine species examined. Thus, 64 to 79% consisted of bases containing choline, while sphingosine nitrogen made up 10 to 21% of the total lipid nitrogen. There was evidence of the existence of non-choline-containing phospholipids in plasma. In later studies of Sinclair,[59] in which a different criterion was employed for the estimation of cephalin than in the earlier studies by this investigator, no cephalin could be detected in beef or dog serum, although it could be accounted for in pig, turkey, and human serum.

The low values for the proportion of plasma cephalins noted in recent work[50–52,59] are in sharp contrast to those reported in the earlier literature. These vary from maximum figures of 47%[60] and 42%[61] to values[62–66] in the range of 20 to 30%. It has been suggested that these high cephalin determinations are probably the result of unsatisfactory analytical technics.[50] In the new procedure of Taurog *et al.*,[54] a much more reliable analytical procedure has been evolved which consists in separation of the phospholipids on a magnesium oxide column.

Little is known as to the importance of sphingomyelin. The values are in fair agreement, being 16%,[51] 14%,[56] 10%,[61] 17%[66] and 19%[62] of the total plasma phospholipid, while Sinclair[52] reported that sphingomyelin comprises 15% of the total phospholipids in dog serum, and 32% in beef serum.

On the other hand, cephalins occur to the highest extent and leci-

[57] M. J. Albrink, *J. Clin. Invest.*, *29*, 46–51 (1950).

[58] W. E. Taylor and J. M. McKibbin, *J. Biol. Chem.*, *188*, 677–683 (1951).

[59] R. G. Sinclair, *J. Biol. Chem.*, *174*, 355–360 (1948).

[60] E. Kirk, *J. Biol. Chem.*, *123*, 637–640 (1938).

[61] S. J. Thannhauser, J. Benotti, and H. Reinstein, *J. Biol. Chem.*, *129*, 709–716 (1929).

[62] B. N. Erickson, I. Avrin, D. M. Teague, and H. H. Williams, *J. Biol. Chem.*, *135* 671–684 (1940).

[63] C. Artom, *J. Biol. Chem.*, *139*, 65–70 (1941).

[64] G. Brante, *Biochem. Z.*, *305*, 136–144 (1940).

[65] G. Blix, *Biochem. Z.*, *305*, 129–135 (1940).

[66] A. D. Marenzi and C. E. Cardini, *J. Biol. Chem.*, *147*, 371–378 (1943).

thins to the smallest amount in red cells, in contradistinction to their distribution in plasma.[60] In fact, some investigators question whether or not lecithin is a component of the erythrocytes, since neither Bürger and Beumer[67] nor Haurowitz and Sládek[68] were able to detect any choline after saponification of the ether-soluble phosphatide fraction from red blood cells.

a. C. A. Preparation. Macheboeuf[5,69–71] was the first to prepare a lipoprotein from horse serum which contained phospholipids and cholesterol. Although as much as 40% of the lipoprotein consisted of lipids, it was water-soluble in neutral or alkaline media at a *p*H greater than 6.5. This lipoprotein has been referred to as "C.A.," for *cenapses* precipitated by *acid*.

In a more recent publication, Macheboeuf and Rebeyrotte[72] further characterized C.A. In one preparation made before the war, the composition was as follows: protein, 59.3%; lecithin, 22.7% with no cephalin; and cholesterol esters, 17.9% (no free cholesterol). However, in preparations made since the war, in a period when the conditions in France resulting from the war damage were such that the horses were undernourished, it was impossible to prepare any new C.A. samples with lipid concentrations as high as 40%. A more recent sample had a composition as follows: protein, 83%; lecithin, 12.5%; and cholesterol, 4.5%. The latter preparation had a molecular weight of approximately 85,000; it is believed that one protein molecule is associated with 13 or 14 lecithin molecules, and with 5 or 6 moles of cholesterol esters.

b. Other Lipoprotein Preparations. Blix *et al.*[73] reported the presence of phospholipids and cholesterol in all of a series of blood proteins separated from each other electrophoretically. The relative percentages of phospholipid and cholesterol, respectively, in the several preparations from normal serum were as follows: albumin, 2.25 and 1.07%; α-globulin, 7.25 and 4.45%; β-globulin, 10.0 and 8.65%; and γ-globulin, 1.0 and 0.41%.

Adair and Adair[74] prepared a lipoprotein from human serum by precipitation at a *p*H of 7 with 50 to 60% of ammonium sulfate. After purification by electrophoresis, a homogeneous product was obtained containing

[67] M. Bürger and H. Beumer, *Biochem. Z.*, *56*, 446–456 (1913).
[68] F. Haurowitz and J. Sládek, *Z. physiol. Chem.*, *173*, 268–277 (1928).
[69] M. A. Macheboeuf, *Bull. soc. chim. biol.*, *11*, 483–503 (1929).
[70] M. A. Macheboeuf, *Rev. gen. colloïdes*, *7*, 352–367 (1929).
[71] M. A. Macheboeuf, *Rev. gen. colloïdes*, *7*, 393–405 (1929).
[72] M. A. Macheboeuf and P. Rebeyrotte, in *Lipoproteins*, General discussion Faraday Soc., No. 6, 62–74, Aberdeen Univ. Press, Aberdeen, 1949.
[73] G. Blix, A. Tiselius, and H. Svensson, *J. Biol. Chem.*, *137*, 484–494 (1941).
[74] G. S. Adair and M. E. Adair, *J. Physiol.*, *102*, 17 P (1943).

8.5% of phospholipids, 16.4% of cholesterol, and 20.4% of fatty acids. Oncley *et al.*[75] prepared two lipoproteins, by low temperature alcohol fractionation of human plasma, which contained 35 and 75% of lipid; these were shown to be related to α_1- and β_1-globulins.

Oncley, Gurd, and Melin[76] separated a β_1-globulin fraction from human serum which contains 29% of phospholipid, as well as 39% of cholesterol esters and 8% of free cholesterol. The nitrogenous portion of this conjugate protein accounts for only 23% of the total weight.

(4) *Cerebrosides*

The cerebrosides (galacto- or glucolipids) are another group of conjugate lipids present in the blood. Although the latter compounds do not contain phosphate, they may be confused with the phospholipids (particularly sphingomyelin), because of the high concentration of sphingosine in their molecules. Moreover, the phospholipids and the cerebrosides both have a wide distribution in animal tissues.[77]

Erickson and her collaborators[78] reported the presence of 15 milligram per cent of cerebrosides in the serum, while Kirk[60] found an average figure of 42 milligram per cent. However, in the later tests, the individual values were extremely variable. These figures represent 2 and 8% of the total plasma lipids, respectively.

Cerebrosides have likewise been noted in leucocytes and pus cells,[79,80] as well as in fish sperm.[81] Schönheimer[82] demonstrated their presence in atherosclerosis of the aorta. Kirk[60] has given the average cerebroside content of red blood cells as 51 milligram per cent. In addition to cerebrosides, Klenk and Lauenstein[83] identified an additional sugar-containing lipid in human red blood cells which was similar in composition to the gangliosides of the spleen. It differed from the latter compounds, however, by the absence of neuraminic acid. The distribution of cleavage products of this glycolipid was as follows: fatty acids (mainly lignoceric), 29%; sphingo-

[75] J. L. Oncley, G. Scatchard, and A. Brown, *J. Phys. & Colloid Chem.*, *51*, 184–198 (1947).

[76] J. L. Oncley, F. R. N. Gurd, and M. Melin, *J. Am. Chem. Soc.*, *72*, 458–464 (1950).

[77] M. Kaucher, H. Galbraith, V. Button, and H. H. Williams, *Arch. Biochem.*, *3*, 203–215 (1943).

[78] B. N. Erickson, H. J. Souders, M. L. Shepherd, D. M. Teague, and H. H. Williams, *Proc. Soc. Exptl. Biol. Med.*, *45*, 153–156 (1940).

[79] A. Kossel and F. Freytag, *Z. physiol. Chem.*, *17*, 431–456 (1893).

[80] F. Hoppe-Seyler, *Med.-Chem. Untersuch.*, *4*, 486–501 (1871).

[81] M. Sano, *J. Biochem.* (*Japan*), *1*, 1–16, 17–20 (1922).

[82] R. Schönheimer, *Z. physiol. Chem.*, *177*, 143–157 (1928).

[83] E. Klenk and K. Lauenstein, *Z. physiol. Chem.*, *288*, 220–228 (1951).

sine, 20%; sugar, 40 to 41%. The sugar was found to consist of galactose, glucose, and chondrosamine. It is believed to be derived from the stroma of the red blood cells.

(5) *Free Fatty Acids*

Kelsey and Longenecker[22] found that beef plasma contained 28.4 milligram per cent of free fatty acids, which accounted for 10.7% of the total lipids. There is some question as to whether the free fatty acids are actually present in blood, or whether they originate during the analytical procedures. Fairbairn[84] demonstrated that the proportion of free fatty acids in tissues is minimal, but that it increases immediately, on removal of the tissue from the animal, by hydrolysis of the phospholipids.

(6) *Unsaponifiable Components*

The unsaponifiable fraction of the blood is composed largely of cholesterol. Anderson[85] reported that, in beef plasma, the unsaponifiable fraction consisted almost entirely of cholesterol, although the animals had received exclusively phytosterols in their food. In the case of dog plasma, this fraction was found to be more complex. Cholesterol could not be prepared from it, although all but 18% was precipitated by digitonin. About 37% of the total unsaponifiable fraction could not be identified. In addition to the cholesterol, in some species, the unsaponifiable fraction will contain β-carotene or carotenoids, while vitamins A, D, and E will appear in this fraction in all cases.

In the case of human serum, Dimter[86] obtained a total lipid content of 360 milligram per cent, of which 66% consisted of the unsaponifiable fraction. About two-thirds of the non-saponifiable fraction was precipitable with digitonin. In the cholesterol-free portion of the unsaponifiable fraction, evidence was adduced for the presence of a cholesterol precursor as well as of an aliphatic alcohol. However, no hydrocarbons of the squalene type could be identified. Koehler and Hill[87] reported the presence of 7-dehydrocholesterol in appreciable quantities in human serum. The total concentration of 7-dehydrocholesterol in the free form was found to vary between 1.8 and 7.0 milligram per cent, while the range of this sterol in combined form for thirty-five subjects was from 2.4 to 35 milligram per cent.

[84] E. Fairbairn, *J. Biol. Chem.*, *157*, 645–650 (1945).
[85] R. J. Anderson, *J. Biol. Chem.*, *71*, 407–418 (1926).
[86] A. Dimter, *Z. physiol. Chem.*, *272*, 189–200 (1942).
[87] A. E. Koehler and E. Hill, *Federation Proc.*, *12*, 232–233 (1953).

There was no correlation between the amount of cholesterol and of 7-dehydrocholesterol, nor could any interrelationship be noted between the concentration of this sterol and any pathological condition.

a. Cholesterol and Cholesterol Esters. Cholesterol is present in the blood both in the form of the free alcohol (unesterified) and as the fatty acid ester. Cholesterol esters account for about two-thirds of the total cholesterol in the plasma of man. On the other hand, the cholesterol in the red blood cells is almost exclusively unesterified. However, it has been stated that appreciable amounts of cholesterol esters occur in the erythrocytes of children.[47] Pfeiffer[88] also reported that cholesterol esters may occur in the blood cells in the summer when, in fact, the total blood cholesterol is increased. In winter, cholesterol esters and total cholesterol are at a minimum level in the erythrocytes. Both Knudson[89] and Bodansky[90] reported an increase in the cholesterol ester content of the corpuscles during fat absorption.

Cholesterol has been found to be largely in the free form in the leucocytes. In fact, Boyd and Stevenson[91] found that approximately 80% of the total cholesterol was unesterified in the white blood cells of rabbits. In the case of young women, Boyd[92] reported that the free cholesterol accounts for about 60% of the total present in the leucocytes.

About one-third of the stromata of the red blood cells of sheep consists of cholesterol, while the remaining two-thirds are composed of equal amounts of sphingomyelin and of other phospholipids.[67] On the other hand, most of the stroma of human red corpuscles is in the form of sphingomyelin.[67] Erickson *et al.*[93] reported that approximately 22% of the dried stroma of sheep, cow, and horse corpuscles was lipid; in the case of man, the figure was 10 to 15% while, in birds, it was only 3%. This lipid was made up of about 60% phospholipid (75% for avian erythrocytes), 30% free cholesterol, and 10% fat and cholesterol esters. In human corpuscles, the stroma weighed about 2 or 3% of the total of the whole corpuscle.

(*a*) *Fatty Acids Combined with Cholesterol.* As has been discussed earlier, the most highly unsaturated acids in the plasma are in ester combination with cholesterol. For a discussion of this, see pages 353–354.

(*b*) *Cholesterol Combined with Protein.* In the original C.A. preparation

[88] G. Pfeiffer, *Biochem. Z.*, *220*, 210–216 (1930).
[89] A. Knudson, *J. Biol. Chem.*, *32*, 337–346 (1917).
[90] M. Bodansky, *Proc. Soc. Exptl. Biol. Med.*, *28*, 628–630 (1931).
[91] E. M. Boyd and J. W. Stevenson, *J. Biol. Chem.*, *117*, 491–500 (1937).
[92] E. M. Boyd, *J. Biol. Chem.*, *101*, 623–633 (1933).
[93] B. N. Erickson, H. H. Williams, S. S. Bernstein, I. Avrin, R. L. Jones, and I. G. Macy, *J. Biol. Chem.*, *122*, 515–528 (1937–1938).

by Macheboeuf,[5,69,71,72] the purified lipoprotein contained 17.9% of cholesterol esters; however, no free (unesterified) cholesterol was present. On the other hand, in the preparations of C.A. from horse serum made since World War II, only 4.5% of cholesterol esters were present.

Blix and co-workers[73] showed that, although all electrophoretic fractions of human serum contained lipids in at least a one-to-one molecular ratio, the α- and β-globulin fractions had a much higher lipid content; cholesterol made up 8.6% and phospholipids comprised 10% of this protein. The high lipid content of the β-globulin fraction is in line with observations of Longsworth *et al.*[94] that the large fractions of this protein occurring in nephrosis and in jaundice are markedly reduced by ether extraction. McFarlane[95] reported a similar phenomenon in the case of normal human serum, when the ether extraction was accompanied by freezing at low temperatures. The β_1-globulin fraction prepared by Oncley and associates[76] from human serum in a relatively homogeneous form was shown to contain 8% by weight of free cholesterol, and 39% of cholesterol esters. This lipoprotein accounts for 5% of total plasma protein, but it carries 75% of the total serum cholesterol.

Gofman and his collaborators[96,97] reported the separation of a cholesterol-containing lipoprotein from the sera of man and of rabbit; this can be separated by ultracentrifugation, since it possesses a lower density than do other components of the serum. This fraction was shown to vary only slightly with a normal dietary.

The lipoproteins were identified by their flotation rates (expressed as Svedberg units). Gofman *et al.*[96] introduced this term, *flotation rate*, in place of the more cumbersome expression, *negative sedimentation rate.* This index is expressed in negative Svedberg units. A Svedberg unit (S) equals 10^{-13} cm./sec./dyne/g. Thus, the molecules described as S_f 5 to 8 are those which have flotation rates consistent with 5 to 8 S units. The flotation rates are not necessarily an index of molecular size. They depend not only upon density but also upon the molecular shapes. In rabbit serum, the major lipoprotein has a flotation rate (S_f) of 5 to 8 S units; in man, the corresponding lipoprotein is characterized by an S_f value of 3 to 8. When cholesterol is fed to the rabbit, an increase in the S_f 5 to 8 component

[94] L. G. Longsworth, T. Shedlovsky, and D. A. MacInnes, *J. Exptl. Med.*, *70*, 399–413 (1939).

[95] A. S. McFarlane, *Nature*, *149*, 439 (1942).

[96] J. W. Gofman, F. T. Lindgren, H. A. Elliott, W. Mantz, J. Hewitt, B. Strisower, V. Herring, and, T. P. Lyon, *Science*, *111*, 166–171, 186 (1950).

[97] J. W. Gofman, F. T. Lindgren, and H. A. Elliott, *J. Biol. Chem.*, *179*, 973–979 (1949).

occurs. In some instances, a new lipoprotein, having a lower density and containing cholesterol, was obtained; this had an S_f of 10 to 30. There appeared to be some correlation between the appearance of this second lipoprotein and the onset of mild to severe atherosclerosis.[96]

In some samples of human sera, a second component of the lipoprotein occurred, similar to that reported for rabbit blood. The human lipoprotein was also characterized by the S_f value of 10 to 20. It was found that, in a study of 104 patients who had previously experienced myocardial infarctions, the appearance of the new lipoprotein fraction was almost universal. The S_f 10 to 20 fraction occurred less frequently in measurable concentrations, in healthy subjects, than in those who had experienced circulatory disturbances. It is believed that the level of the S_f 10 to 20 fraction may afford an index of the susceptibility of an individual to atherosclerosis. A further discussion of these results has been given by Gofman *et al.*[98]

b. Hydrocarbons. Although hydrocarbons form an extremely minor proportion of the unsaponifiable fraction, a number of paraffins and higher alcohols which have been shown to occur in the body tissues undoubtedly are carried in the blood. However, in the studies of Setälä and Ermala[99] on the carcinogenic hydrocarbon, 3,4-benzpyrene, it was found that this substance or its metabolites exist in the blood in association with chylomicrons after the hydrocarbon, dissolved in fats, has been absorbed from the intestines.

c. Carotenoids. β-Carotene may be present in the blood of animals in which the main site of conversion to vitamin A is the liver rather than the intestinal wall. Thus, the presence of carotene has been noted in human blood serum[100,101] and in that of cattle.[102–105] Mehl[106] first observed that nearly all the carotenoid of human plasma is to be found associated with a β-lipoprotein. This result has been confirmed and extended in a recent communication by Ganguly *et al.*,[6] in which it is proved that the

[98] J. W. Gofman, H. B. Jones, F. T. Lindgren, T. P. Lyon, H. A. Elliott, and B. Strisower, *Circulation*, *2*, 161–178 (1950).

[99] K. Setälä and P. Ermala, *Science*, *114*, 151–152 (1951).

[100] T. Willstaedt, and T. Lindqvist, *Z. physiol Chem.*, *240*, 10–18 (1936).

[101] H. Willstaedt and T. K. With, *Z. physiol. Chem.*, *253*, 40–46 (1938).

[102] B. v. Euler, H. v. Euler, and H. Hellström, *Biochem. Z.*, *203*, 370–384 (1928).

[103] L. S. Palmer and C. H. Eckles, *J. Biol. Chem.*, *17*, 223–236 (1914).

[104] L. S. Palmer, *Carotinoids and Related Pigments*, Chem. Pub. Co., New York, 1922, p. 208.

[105] J. Ganguly, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *50*, 73–84 (1953).

[106] J. W. Mehl, Personal communication, 1944; also cited by F. R. N. Gurd, J. L. Oncley, J. T. Edsall, and E. J. Cohn, in *Lipoproteins*, General discussion Faraday Soc., No. 6, 70–74, Aberdeen Univ. Press, Aberdeen, 1949, p. 73.

fraction containing the β-carotene is distinct from that with which the vitamin A is combined.

In addition to β-carotene, a number of other carotenoids have been demonstrated in the blood of several species of animals, but these have been noted only when they were present in the diet. Their occurrence under such conditions can be considered as adventitious. Cryptoxanthin, which is a provitamin A, was reported in the blood serum of cattle,[107] and in that of chickens after it had been administered.[108] In addition, a number of carotenoids which are not precursors of vitamin A have been noted in the blood. These include lycopene (chicken after feeding it,[108] man[109,110]), $C_{40}H_{56}$, which is an isomer of β-carotene; lutein (cattle,[107] chicken[108]), $C_{40}H_{54}(OH)_2$, which is a dihydroxycarotenoid; and zeaxanthin (chicken after feeding it[108]), $C_{40}H_{54}(OH)_2$ which is an isomer of lutein. Ganguly and co-workers[108] reported that chickens on a farm diet had large quantities of lutein in their blood; however, when they were given a carotenoid-free diet for thirty days, neither lutein nor any other carotenoid was found to be present in the blood.

(a) The Nature of the Carotenoid and Vitamin A Combinations in the Blood. Recent evidence would seem to indicate that most carotenoids and vitamin A are carried in the blood in the form of loose combinations with protein. As early as 1915, Palmer[111] reported the fact that it was impossible to extract more than traces of carotene from cow serum by means of ethanol-free ether. This investigator[103] was able to separate a carotene-protein complex from bovine serum which he called "caroto-albumin." On the basis of an ammonium sulfate fractionation, Dzialoszynski and co-workers[112] concluded that, not only carotene, but also vitamin A is associated with a protein factor, which they believed to be an albumin. On the other hand, Bennhold *et al.*,[113,114] Bendien and Snapper[115] and Mehl[116]

[107] A. E. Gillam and M. S. El Ridi, *Biochem. J.*, *29*, 2465–2468 (1935).

[108] J. Ganguly, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *50*, 59–72 (1953).

[109] E. V. Dániel and G. J. Scheff, *Proc. Soc. Exptl. Biol. Med.*, *33*, 26–30 (1935).

[110] E. V. Dániel and T. Béres, *Z. physiol. Chem.*, *238*, 160–162 (1936).

[111] L. S. Palmer, *J. Biol. Chem.*, *23*, 261–279 (1915); *27*, 27–32 (1916).

[112] L. M. Dzialoszynski, E. M. Mystkowski, and C. P. Stewart, *Biochem. J.*, *39*, 63–69 (1945).

[113] H. Bennhold, E. Kylin, and S. Rusznyák, *Die Eiweisskörper des Blutplasmas*, Steinkopff, Dresden-Leipzig, 1938; Chap. 7, H. Bennhold, "Die Vehikelfunktion der Bluteiweisskörper," pp. 220–303.

[114] H. Bennhold, *Verhandl. deut. Ges. inn. Med.*, *45th Congress*, Wiesbaden, April, 1933, 357–359.

[115] W. M. Bendien and I. Snapper, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, *1*, 69–71 (1931).

[116] J. W. Mehl, Personal communication, 1952.

are of the opinion that the protein associated with the β-carotene is a globulin, and probably a β-globulin. Using the method of fractional precipitation, Pett and LePage[117] also reported that carotene in serum (presumably human) is bound with the globulin fraction. Oncley and associates,[76] and Cohn *et al.*[118] reported the presence of carotenoids in a lipoprotein. Ganguly, Krinsky, Mehl, and Deuel[6] found that carotenoids and vitamin A are carried by different protein fractions in the blood. In the case of beef plasma, which contains β-carotene, the carotenoid was found with the more soluble protein fraction (presumably the albumin), along with lutein and vitamin A alcohol.

However, there is some question as to whether or not the fact that the carotenoid is non-extractable may be taken as proof that the carotene is combined with a protein. Thus, Van den Bergh and Müller[119] reported that simple aqueous colloidal solutions of carotene resembled blood carotene in that they were not readily extracted by organic solvents such as ether, although there was no protein to hold the carotene in combination in this case. These observations were confirmed by Drummond and McWalter.[120] On the basis of this experimental evidence, these workers considered that carotene exists in serum in simple colloidal solution. Although Dzialoszynski *et al.*[112] likewise confirmed the fact that colloidal carotene became unextractable when it was added to normal plasma, it was further shown that it remained readily extractable provided the protein in the plasma had been denatured previous to the addition of the carotene, by shaking with ether.

However, there are additional facts which favor the concept that an actual carotene-protein combination exists in blood. For example, such complexes of protein with carotenoids are widespread in nature. These include the lycopene-protein complex in tomato,[121] rhodopsin in the retina, described by Wald,[122] the vitamin A ester and protein combination found by Lovern and Morton[123] in the *tunica propria* of fish intestines, and the astaxanthin-protein compound in lobsters discovered by Kuhn and Søren-

[117] L. B. Pett and G. A. LePage, *J. Biol. Chem.*, *132*, 585–593 (1940).

[118] E. J. Cohn, F. R. N. Gurd, D. M. Surgenor, B. A. Barnes, R. K. Brown, D. Derouaux, J. M. Gillespie, F. W. Kahnt, W. F. Lever, C. H. Liu, D. Mittelman, R. F. Mouton, K. Schmid, and E. Uroma, *J. Am. Chem. Soc.*, *72*, 465–474 (1950).

[119] H. H. Van den Bergh and P. Müller, *Proc. Acad. Sci. Amsterdam*, *22*, 748–757 (1920).

[120] J. C. Drummond and R. J. McWalter, *J. Physiol.*, *83*, 236–242 (1935).

[121] R. Kuhn and H. J. Bielig, *Ber.*, *73*, 1080–1091 (1940).

[122] G. Wald, *J. Gen. Physiol.*, *21*, 795–832 (1937–1938).

[123] J. A. Lovern and R. A. Morton, *Biochem. J.*, *33*, 330–337 (1939).

sen.[124] Stern and Salomon[125] likewise studied the latter compound, and named it ovoverdin.

Vitamin A-protein conjugation products also exist in plasma.[6,112,117] However, vitamin A ester and vitamin A alcohol are reported to be associated with different proteins in chicken blood.[6] Thus, vitamin A ester was found in the least soluble fraction (globulin), while the free alcohol, together with lutein, was present in the most soluble protein fraction (albumin). In pig plasma, the vitamin A alcohol was also associated with the more soluble protein. Ganguly *et al.*[6] suggest that species differences in the absorption of carotenoids and of vitamins A may be related to the specific plasma and lymph proteins available for their transport.

Vitamin A aldehyde (retinene) has likewise been shown to form a combination with protein. Thus, Rajagopal and Datta[126] found that, when vitamin A aldehyde and a plasma albumin solution were mixed, a new peak for the absorption spectrum resulted; this was interpreted as indicating the formation of a new compound. The complex contained 0.968 g. of protein and 2.985×10^{-5} g. of vitamin A aldehyde per 100 ml., giving an aldehyde content which was 0.003% of that of protein.

d. Fat-soluble Vitamins. In addition to the carotenoids, small quantities of several fat-soluble vitamins occur in plasma. Methods are adequate for the accurate determination of vitamins A and E in the blood, but no satisfactory procedure has been evolved for the quantitative assay of vitamins D and K in the quantity of blood which is ordinarily available.

(*a*) *Vitamins A.* In contrast to the limited occurrence of carotenoids in the blood, vitamin A is distributed almost universally in the blood of all species. This vitamin is present largely as the ester in the blood of rats and pigs.[127,128] Week and Sevigne,[129] using the chromatographic procedure of Glover *et al.*[130] for the separation of vitamin A alcohol and vitamin A ester, demonstrated that the alcohol is the predominant form of the vitamin in the blood of the fasting individual. However, after vitamin A is administered, the ester form alone is increased, irrespective of whether the vitamin A was fed as the ester or as the alcohol.[130,131] Whereas only 10 to 20% of the total vitamin A was esterified in the blood of their fasting sub-

[124] R. Kuhn and N. A. Sørensen, *Ber.*, *71*, 1879–1888 (1938).
[125] K. Stern and K. Salomon, *J. Biol. Chem.*, *122*, 461–475 (1938).
[126] K. Rajagopal and P. K. Datta, *Nature*, *170*, 370–371 (1952).
[127] S. Y. Thompson, J. Ganguly, and S. K. Kon, *Brit. J. Nutrition*, *1*, v (1947).
[128] S. Y. Thompson, J. Ganguly, and S. K. Kon, *Brit. J. Nutrition*, *3*, 50–78 (1949).
[129] E. F. Week and F J. Sevigne, *J. Nutrition*, *40*, 563–576 (1950).
[130] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *41*, 94–96 (1947).
[131] H. Hoch, *Nature*, *158*, 59 (1946).

jects, a maximum of as much as 90% of the total vitamin A appeared in this fraction after large doses of vitamin A had been ingested.

In contradistinction to the increase in ester when vitamin A is fed, a rise in the alcohol fraction was noted by Hoch[131] as a consequence of the mobilization of endogenous vitamin A. Thus, after the ingestion of alcohol, the vitamin A alcohol in the blood was increased.

Glover and associates[132,133] reported that the plasma vitamin A level in rats is proportional to the amount of free vitamin A alcohol in the liver, but not to the total stores in this organ, which consist primarily of vitamin A esters. However, the recent studies of Ganguly *et al.*[134] showed opposite results from those of Glover and co-workers,[133] and no correlation could be drawn between the unesterified vitamin A in the liver and the level of plasma vitamin A. Further investigations are necessary to clarify the reasons for the divergent results in these two groups.

According to the recent report of Ganguly *et al.*,[6] not only is β-carotene associated with a protein in the plasma, but the same is true for the vitamin A alcohol and the vitamin A esters. Furthermore, it was demonstrated that the two vitamin fractions are associated with separate proteins. Rajagopal and Datta[126] reported that, in normal human plasma, 120–200 I.U. of vitamin A is associated with 6 to 8 g. of protein.

Krause and Alberghini[135] demonstrated that a factor capable of hydrolyzing vitamin A esters occurs in the blood of man, rats, and rabbits. The factor was shown to be present in the serum and plasma of blood, but not in the cellular elements.

(*b*) *Vitamins D.* Although it is certain that the vitamins D are present in the blood and that the amount and type must vary with the diet and with the species of animal, any specific information as to these vitamins must await the development of adequate methods for their determination and identification.

(*c*) *Vitamins E (Tocopherols).* There are four tocopherols which occur naturally in the ordinary vegetable fats, namely, α, β, γ, and δ. Quaife *et al.*[136] have demonstrated that d-α-tocopherol is absorbed preferentially by the human subject, as compared with d-γ-tocopherol. The α-isomer also appears to be the compound which possesses the highest vitamin E activity and the lowest antioxidant potency. Under normal conditions,

[132] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *40*, lvii (1946).
[133] J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *41*, 97–100 (1947).
[134] J. Ganguly, N. I. Krinsky, and H. J. Deuel, Jr., *Federation Proc.*, *11*, 218 (1952).
[135] R. F. Krause and C. Alberghini, *Arch. Biochem.*, *25*, 396–400 (1950).
[136] M. L. Quaife, W. J. Swanson, M. Y. Dju, and P. L. Harris, *Ann. New York Acad Sci.*, *52*, 300–305 (1949).

the bulk of the blood tocopherol is the α-isomer[137]; about 75% of the total tocopherol consists of the latter compound, while other isomers comprise the remaining one-quarter of the blood tocopherols. The vitamin occurs as the free vitamin, even after a tocopherol ester has been administered.[138,139]

The tocopherols are only slowly extractable from the plasma by hydrocarbon solvents or by ether, and previous or simultaneous addition of ethanol is required for satisfactory extraction.[140] For this reason, Hickman[141,142] suggested that tocopherol must occur in plasma in combination with a plasma protein. Ames and Risley[140] demonstrated that blood plasma is able to solubilize relatively large amounts of the tocopherols (200–300 milligram per cent). On fractionation of the plasma proteins with ammonium sulfate or ethanol, at low temperatures, α-tocopherol was found in all resulting protein fractions. Amino acids or partially hydrolyzed proteins were not capable of bringing about a solution of tocopherol. Since some of the proteins effective in facilitating the solution of α-tocopherol were lipid-free, it is suggested that the tocopherol-protein complex may be entirely independent of any lipoprotein.

(*d*) *Vitamins K.* Although there is every reason to suppose that vitamin K is present in the blood of most animals, including that of man, data are not at present available as to the amount of this vitamin or the type which is present in blood.

(7) *Lipoproteins in the Blood*

Although it has been recognized for many years that protein-fat combinations exist in nature, there had been but little advance in our knowledge of their structure or function until recently. Most of the earlier information is summarized in the monographs of Guilliermond,[143] Macheboeuf,[144]

[137] M. L. Quaife, N. S. Scrimshaw, and O. H. Lowry, *J. Biol. Chem.*, *180*, 1229–1235 (1949).

[138] A. Emmerie and C. Engel, *Rec. trav. chim.*, *58*, 895–902 (1939).

[139] W. F. J. Cuthbertson, R. R. Ridgeway, and J. C. Drummond, *Biochem. J.*, *34*, 34–39 (1940).

[140] S. R. Ames and H. A. Risley, *Ann. New York Acad. Sci.*, *52*, 149–155 (1949).

[141] K. C. D. Hickman, *Biological Antioxidants*, Trans. First Conference on Biological Antioxidants, New York, Oct. 10–11, 1946; "General Discussion on Tocopherols as Biological Antioxidants," pp. 78–81.

[142] K. C. D. Hickman and P. L. Harris, *Advances in Enzymology*, Vol. VI, Interscience, New York-London, 1946, pp. 469–524.

[143] A. Guilliermond, *Les constituants morphologiques du cytoplasme*, Hermann, Paris, 1934, Chap. VII.

[144] M. A. Macheboeuf, *État des lipides dans la matière vivante, Les cénapses et leur importance biologique*, Hermann, Paris, 1937.

Lepeschkin,[145] and Marrack,[146] as well as in several papers by Chargaff and associates.[147–150] Information on lipoproteins is contained in the *Discussions of the Faraday Society,*[151] which records results of a conference on lipoproteins held in Birmingham, England, in August, 1949. Much of the work reported in the above monograph concerns the lipoprotein components of the blood.

The lipoproteins are widely distributed in living matter, where they occur in cell nuclei, mitochondria, cell membranes, chloroplasts, in egg yolk, in milk, and in blood. Chargaff reported the separation of a thromboplastic protein of this type from the lungs[149]; the nature of lipovitellin from egg yolk and of mitochondria lipoproteins has also been described.[152]

A phase of prime importance in the present discussion is the nature of the lipoproteins in blood. In the previous sections, it was pointed out that neutral fats, fatty acids, lecithins and other phospholipids, cholesterol, carotenoids, and vitamin A are combined with plasma proteins. The nature of the protein moiety of the plasma lipoproteins will be considered in more detail in the present section.

Until the classical studies of the Cohn group at Harvard, which involved a large-scale fractionation of human plasma at low ionic strength, by the use of ethanol-water mixtures at low temperatures,[153] it was believed that the blood lipids were present in solution in the blood. It was therefore expected that the lipids would remain in the residual ethanol-water mixture from which the proteins had been precipitated. However, such was not found to be the case. The blood lipids were identified in two distinctly different types of lipoproteins, which were present in readily separable protein fractions.

Further evidence of the nature of lipoproteins has been obtained by Turner *et al.*[154] through the use of centrifugation. About one-half of the

[145] W. W. Lepeschkin, *Kolloidchemie des Protoplasmas*, Steinkopff, Dresden-Leipzig, 1938, p. 155 ff.

[146] J. R. Marrack, *The Chemistry of Antigens and Antibodies*, Med. Research Council, Special report Ser. No. 194, London, 1934.

[147] E. Chargaff, *J. Biol. Chem.*, *125*, 661–670 (1938).

[148] E. Chargaff and M. Ziff, *J. Biol. Chem.*, *131*, 25–34 (1939).

[149] S. S. Cohen and E. Chargaff, *J. Biol. Chem.*, *136*, 243–256 (1940).

[150] S. S. Cohen and E. Chargaff, *J. Biol. Chem.*, *139*, 741–752 (1941).

[151] General Discussion Faraday Soc., No. 6, *Lipoproteins*, 5–167, Aberdeen Univ. Press, Aberdeen, 1949.

[152] E. Chargaff, *J. Biol. Chem.*, *142*, 491–504 (1942).

[153] J. T. Edsall, "The Plasma Proteins and Their Fractionation," *Advances in Protein Chem.*, *3*, 383–479 (1947).

[154] R. H. Turner, J. R. Snavely, W. H. Goldwater, M. L. Randolph, C. C. Sprague, and W. G. Unglaub, *J. Clin. Invest.*, *30*, 1071–1081 (1951).

total serum neutral fat is present in the zone of minimum density at the top of the column, without appreciable amounts of protein. Cholesterol was present in the fourth zone of high-medium density, but only in the form of the ester; the highest concentrations of proteins (principally globulins) were found in the densest zone (fifth zone), together with neutral fat and phospholipids, but with no cholesterol. By the use of zone electrophoresis, with a filter paper or starch medium containing barbital buffer, Kunkel and Slater[155] were able to separate the α- and β-lipoproteins into two main fractions. The α-fraction had a lower free/total cholesterol ratio than did the β-component. The α-lipoprotein could be separated into either two or three fractions, while the β-lipoprotein consistently yielded at least three fractions. In pathological sera with elevated lipid concentrations, the α-lipoproteins were shown to diminish or entirely disappear concomitantly with an increase in the β-type. In some cases, a new component with an abnormal mobility appeared in large amounts.

According to Sandor *et al.*,[156] euglobulins having a relatively alkaline isoelectric point, and which represent 60 to 80% of these proteins, contain only a small proportion of lipids. The amount of all lipids was found to be higher in the euglobulins having an acid isoelectric point. Lipoproteins were found during the precipitation of α- and β-euglobulins.

a. Types of Lipoprotein. The several plasma lipoproteins differ markedly in their solubility in water and in ethanol-water mixtures, in their molecular size and shape, in their electrostatic reactions, as well as in their lipid content. The electrophoretic properties of the plasma lipoproteins make it appear that one is an α_1-serum globulin and the other a β_1-serum globulin. One of the most striking properties reported for the lipoproteins, in contradistinction to the other plasma proteins, is their ready denaturation when frozen or dried. In fact, the only satisfactory procedure for their preparation in the native state is by the use of the Cohn procedure of plasma fractionation,[153,157-159] which depends upon protein-protein interactions, and in which fractional extraction has been largely replaced by fractional precipitation. The great susceptibility of plasma lipoproteins to practically all types of denaturation explains why they were not prepared pre-

[155] H. G. Kunkel and R. J. Slater, *J. Clin. Invest.*, *31*, 677–684 (1952).

[156] G. Sandor, Y. Sabetay, and R. Vargues, *Bull. soc. chim. biol.*, *35*, 273–284 (1953).

[157] E. J. Cohn, J. A. Luetscher, Jr., J. L. Oncley, S. H. Armstrong, Jr., and B. D. Davis, *J. Am. Chem. Soc.*, *62*, 3396–3400 (1940).

[158] E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin, and H. L. Taylor, *J. Am. Chem. Soc.*, *68*, 459–475 (1946).

[159] E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr., and S. H. Armstrong, Jr., *J. Clin. Invest.*, *23*, 417–432 (1944).

viously in pure form. An excellent discussion of the lipoproteins in human plasma is the article of Gurd, Oncley, Edsall, and Cohn.[160]

(*a*) *β-Lipoproteins in Plasma.* About 75% of the lipid in the plasma of a normal fasting man is present in the β-lipoprotein fraction. This constitutes only approximately 5% by weight of the total plasma proteins.[161] The β-lipoproteins are euglobulins, since they are insoluble in water at the isoelectric point in the absence of salt. Small amounts of salt have a profound influence on their solubility near the isoelectric point (*p*H 5.4). The β-lipoproteins in human plasma interact with other proteins in the plasma, such as γ-globulins, to form insoluble complexes. In a *p*H range at which both the γ-globulins and the β-lipoproteins are soluble (the γ-type as cations, the β-type as anions), a complex is formed on mixing, the solubility of which is reduced a hundred-fold.

The β-lipoproteins are large molecules. On the basis of the demonstration of an intrinsic viscosity[75] of 0.041, and of a marked difference in partial specific volume between the anhydrous protein[75] and the hydrated protein,[162] Oncley *et al.*[75] suggest that this lipoprotein possesses a spherical shape. A molecular weight of 1,300,000 has been postulated for the anhydrous protein. Assuming the hydrated molecule to be a sphere, each molecule would have a diameter of 185 A. The β-lipoprotein has a close relationship to the X-protein isolated by Pedersen.[162]

According to Gurd *et al.*,[160] the average composition of three separate lots of β-lipoprotein gave the following figures: protein, 25%; phospholipid, 30%; and cholesterol (esterified and free), 45%. Nearly all of the carotenoid of the plasma has been located in this β-lipoprotein fraction.[106] The absolute amount was found to be only 0.02 to 0.03%, which would be less than one mole per molecule of protein. According to Roberts and Szego,[163] one of the hormones, the common estrogen, estriol, is combined with the β-lipoprotein fraction and not with the α-lipoprotein.

(*b*) *α-Lipoproteins in Plasma.* The α-lipoproteins constitute only about 3% of the plasma proteins, but they include 35% of the total plasma lipids. Their molecular weight is approximately one-sixth of that of the β-lipoprotein; it has been estimated at 200,000.[75] Oncley, Scatchard, and Brown[75] suggested that the α-lipoprotein molecule has an ellipsoid form, with a length of 300 A. and a 50 A. cross-section.

[160] F. R. N. Gurd, J. L. Oncley, J. T. Edsall, and E. J. Cohn, *Lipoproteins,* General Discussion Faraday Soc., No. 6, 70–74, Aberdeen Univ. Press, Aberdeen, 1949.

[161] E. J. Cohn, *Experientia, 3,* 125–136 (1947).

[162] K. O. Pedersen, *Ultracentrifugal Studies on Serum and Serum Fractions,* Almqvist & Wiksells, Upsala, 1945, p. 167.

[163] S. Roberts and C. M. Szego, *Endocrinology, 39,* 183–187 (1946).

In many respects, α-lipoprotein resembles the lipoprotein isolated by Macheboeuf[5,144] from horse serum. Both are readily soluble in water in the absence of salts; the lipoprotein from horse serum also possesses a high electrophoretic mobility,[164] in line with α-lipoprotein. The lipoprotein originally prepared from horse serum had a lipid content of 50%, as compared with a value of 35% for the α-lipoprotein prepared from human plasma. However, in the preparations of lipoprotein from horse serum made since World War II, only about 20% of lipids were found.

(*c*) *Elinin.* Dandliker *et al.*[165] separated a lipoprotein from human erythrocytes which was called elinin. This lipoprotein was found to consist of large asymmetric particles. Elinin is of especial importance because of its relationship to the Rh factor and to the A and B substances.

(*d*) *Lipovitellin.* In a comprehensive study of the nature of the lipoprotein from egg yolk, lipovitellin, Hawke and Lea[166] noted that it contains the phospholipids, phosphatidylcholine, phosphatidylethanolamine, and unsaturated acids. The fatty acids are much more highly unsaturated than are those of the egg-yolk glycerides. Lipovitellin contains much less cholesterol than do the lipoproteins of human serum; it appears to be considerably more stable. The presence of glucose, lactose, and sucrose during freeze-drying of lipovitellin prevented loss of solubility and denaturation.[167]

b. The Nature of the Lipid-Protein Combinations in Lipoproteins. It is generally assumed that the lipids are adsorbed on the serum proteins and that only weak combinations exist between these two components. The osmotic pressure of the lipoprotein solutions has been brought to bear to explain such a combination. Thus, on the one hand, Popják and McCarthy[168] reported that the extraction of lipids from normal plasma does not alter the colloid osmotic properties of serum proteins. On the other hand, Macheboeuf[69] reported that lipemic sera from patients suffering from lipoid nephrosis exerted a higher osmotic pressure per g. of protein than did normal sera. In addition, Rabinowitch[169] suggested that cholesterol in the serum of diabetics exerts an osmotic pressure. In a later communication, Popják[170] reconciles these divergent opinions by demonstrating that the lipids do not exert an osmotic pressure in lipemic sera, but rather increase

[164] M. A. Macheboeuf, J. L. Delsal, P. Lépine, and J. Giuntini, *Ann. Inst. Pasteur,* *69*, 321–333 (1943).

[165] W. B. Dandliker, M. Moskowitz, B. H. Zimm, and M. Calvin, *J. Am. Chem. Soc*, *72*, 5587–5592 (1950).

[166] J. C. Hawke and C. H. Lea, *Biochem. J.*, *54*, 479–483 (1953).

[167] J. C. Hawke and C. H. Lea, *Biochem. J.*, *54*, 475–479 (1953).

[168] G. Popják and E. F. McCarthy, *Biochem. J.*, *37*, 702–705 (1943).

[169] I. M. Rabinowitch, *Arch. Internal Med.*, *46*, 752–767 (1930).

[170] G. Popják, *Biochem. J.*, *40*, 789–803 (1946).

the pressure normally caused by serum proteins, by reducing the volume of solvent available to the proteins, with the result that the effective concentration of the latter is increased.

Tayeau and Rolland[171] observed that, in aging serum, the amount of biliary salts adsorbed diminishes markedly. It is believed that this reduction of adsorption results because the surface of the protein molecules is being modified by transformation of lipid-protein linkages as a result of the action of serum lecithinase on phospholipids.

The hypothesis that varying types of union occur between the lipids and protein in the lipoproteins is largely based upon extraction studies. Thus, Delsal[172] noted that phosphatides could not be extracted from the blood serum by the McFarlane method,[95] but only by means of an ether-alcohol mixture. When cholesterol was removed, the albumin:globulin ratio remained unchanged, while the elimination of phosphatides resulted in a marked diminution of this ratio. This would indicate that the linkages of protein with phosphatides and with cholesterol were different. In fact, Ardry and Fontaine[173] have postulated the presence of three types of lipoprotein linkages in horse serum, namely, loose association, cenapse, and a very strong bond in β_1-lipoprotein. In the last case, the bond is so strong that the lipid cannot be separated without destruction of the protein. Moreover, it was noted that, on aging, a rupture of the loose associations, and molecular transfers, occur which partially block the extraction of other lipids. The variation in linkages is also shown by the use of cationic soaps.[174] In the presence of ether, these soaps assist in the solubilization of almost all cholesterol, but not of phospholipids, while in the absence of ether these soaps precipitate most of the lipids, including phospholipids. However, there is a fraction of lipids which is not precipitated by any quantity of cationic soaps. According to this method of differentiation, the serum lipids are divided into two distinct groups. Feldman and Weinberg[175] reported that a consistent layering of hyperlipemic and cholesteremic sera occurs on standing, and that this process is accentuated by centrifugation. The cholesterol content of the two layers was shown to vary markedly.

c. Factors Altering the Level of Lipoproteins in Blood. A considerable species variation obtains in the nature of blood lipoproteins. Thus, Lewis,

[171] F. Tayeau and R. Rolland, *Compt. rend. soc. biol.*, *143*, 394–395 (1949).
[172] J. L. Delsal, *Bull. soc. chim. biol.*, *31*, 122–125 (1949).
[173] R. Ardry and M. Fontaine, *Bull. soc. chim. biol.*, *33*, 1497–1503 (1951).
[174] M. Macheboeuf and J. Polonovski, *Bull. soc. chim. biol.*, *31*, 125–128 (1949).
[175] M. Feldman, Jr., and T. Weinberg, *Science*, *113*, 697 (1951).

Green, and Page[176] reported that the lipoproteins in the sera of the normal chicken, rat, rabbit, opossum, monkey, man, cat, and dog had flotation rates from S_f 1 to S_f 15. While no lipoproteins were present in the sera of the cat and dog with a rate faster than 23, lipoproteins with rates as high as 30 occurred in the sera of the rat, the opossum, the sheep, the monkey, and of some human subjects. The lipoproteins of the class having rates of 25 or greater are characteristic in the case of guinea pigs, chickens, rabbits, and of some monkey and human sera. Fasoli and co-workers[177] reported that the serum of the dog is richer in high-mobility lipoproteins migrating with albumin, and in α_1-globulin, than is human serum.

Species variations exist not only in the type of lipoproteins but also in the absolute amounts present. In the S_f 1–15 class, the following amounts (milligram per cent) of lipoproteins were found[176]: cat, dog, opossum, and monkey, > 200; man, 150; rabbit, 100; and sheep, 40. In the case of the S_f 15–40 class, the following values (milligram per cent) were reported[176]: rat, 12; rabbit, sheep, opossum, cat, and dog, 50; and man and monkey, > 200.

The proportion of lipoproteins is altered by certain other physiological and pathological conditions. Thus, age appears to be a factor. Forbes *et al.*[178] found that a large proportion of the cholesterol of lyophilized serum of old hens is "readily extractable," in contradistinction to the low proportion in this fraction of the serum of roosters and young chickens. In the case of the old hens, the proportion of "readily extractable" cholesterol shows a correlation with the neutral fat content. When cholesterol is fed to rabbits over a long period, the hypercholesterolemia is accompanied by a hyperglobulinemia; Fishberg *et al.*[179] have shown that this consists of β-globulin. The high level of lipoprotein obtained thirty hours after irradiation is directly correlated with the subsequent death of the animal.[180] It is suggested that x-irradiation brings about a conversion of low-density lipoprotein to higher density components. The injection of heparin following irradiation hastens the return of lipoprotein levels to normal. Hewitt *et al.*[180] also demonstrated that the injection of toluidine

[176] L. A. Lewis, A. A. Green, and I. H. Page, *Am. J. Physiol.*, *171*, 391–400 (1952).

[177] A. Fasoli, E. B. Magid, M. D. Glassman, and P. P. Foà, *XIX Intern. Physiol. Congress, Montreal Abst.* (Aug.–Sept., 1953), 340–341.

[178] J. C. Forbes, G. H. L. Dillard, W. B. Porter, and O. Petterson, *Proc. Soc. Exptl. Biol. Med.*, *71*, 26–28 (1949).

[179] A. M. Fishberg, L. Friedfeld, I. Hoffman, E. R. Slotter, and E. H. Fishberg, *Proc. Soc. Exptl. Biol. Med.*, *75*, 301–303 (1950).

[180] J. E. Hewitt, T. L., Hayes, J. W. Gofman, H. B. Jones, and F. T. Pierce, *Am. J. Physiol.*, *172*, 579–587 (1953).

blue produces changes in the lipoprotein pattern similar to those after irradiation. Whereas exposure to extreme cold (−12°C. for thirty minutes) resulted in increased cholesterol levels and an augmentation of ultracentrifugally determined lipoproteins in rabbits, a milder exposure (0°C.) was found to produce less severe histopathologic changes, although some increase in lipoproteins in the plasma was observed.[181]

The functioning of the liver has been shown to be closely connected with the maintenance of blood lipoproteins. Thus, Lewis, Page, and Thomas[182] reported that although no change in the lipoprotein pattern obtained in the plasma of dogs for the first six hours after hepatectomy, later all lipoproteins and cholesterol began to decrease in the blood, in spite of a general hemoconcentration. However, the lipoprotein pattern was unchanged under the latter condition, which indicated the importance of the liver in the metabolism of all types of lipoproteins. On the other hand, Kunkel and Ahrens[183] stated that the electrophoretic patterns of the sera of patients suffering from an unexplained biliary cirrhosis do show alteration, with marked increase in the β-globulins; the latter were directly proportional to the total lipid concentration. An abnormal lipoprotein composition was demonstrated. Whereas the normal lipoprotein had a protein content of 28%, that prepared from the serum of patients with primary biliary cirrhosis had only 14% protein and 86% lipid.

The concentration of lipoproteins is regulated by the thyroid gland. Thus, Lewis *et al.*[176] reported that the administration of cholesterol to dogs and rats whose thyroid glands had been inactivated by treatment with I^{131} resulted in a great increase in the concentration of lipoproteins with S_f greater than 23. On the other hand, this condition was corrected by feeding desiccated thyroid powder. Species having large concentrations of lipoproteins with S_f 35 or faster are most susceptible to atheroma. For a discussion of the relationship of lipoproteins to atherosclerosis, the reader is referred to pages 466–481.

The administration of estrogens resulted in no marked or sustained change in any of the serum lipid fractions or in the cholesterol:phospholipid ratio. Analysis of the S_f 12–20 lipoproteins showed no consistent trend.[184] In a later report by Lewis and co-workers,[185] it was demonstrated that the

[181] W. W. Calhoun, L. J. Milch, and R. F. Redmond, *Federation Proc.*, *12*, 22 (1953).

[182] L. A. Lewis, I. H. Page, and C. Thomas. *Am. J. Physiol.*, *172*, 83–85 (1953).

[183] H. K. Kunkel and E. H. Ahrens, Jr., *J. Clin. Invest.*, *28*, 1575–1579 (1949).

[184] S. J. Glass, H. Engelberg, R. Marcus, and J. W. Gofman, *Proc. Soc. Exptl. Biol. Med.*, *80*, 264–265 (1952).

[185] L. A. Lewis, G. M. C. Masson, and I. H. Page, *Proc. Soc. Exptl. Biol. Med.*, *82*, 684–686 (1953).

administration of testosterone to castrated immature rabbits altered the lipoprotein pattern by resulting in the appearance of two abnormal components. One of these was an $S_f > 70$ and the second an S_f 40 to 70. Both disappeared on the cessation of treatment. The administration of diethylstilbestrol did not bring about a change in the pattern of lipoproteins.

Fasoli *et al.*[177] studied depancreatized dogs under insulin control, and reported that, although both cholesterol and phospholipid levels were higher than normal, the relative proportions of the lipoproteins were not significantly changed. When insulin was withdrawn, the serum became opalescent and even milky, due to the increased lipids. There was a sharp increase in concentration of low-mobility lipoproteins migrating with the β-globulins, which is probably to be ascribed to the rapid mobilization of body fat. On the other hand, no consistent change occurred in the high-mobility lipoproteins in untreated depancreatized dogs.

3. Quantitative Relations of Blood Lipids

Although the several lipid components in blood vary with diet and species as well as with various physiological and pathological conditions, the fasting values are quite constant in different animals of the same type.

(*1*) *Normal Values of Blood Lipids in Man*

West and Todd[47] cited the values listed in Table 2 as normal for the blood lipids of man. A similar compilation has been made by Boyd,[186] who also calculated the distribution of fatty acids in the plasma of normal women These values are shown in Table 3.

The distribution of lipids reported by Boyd[186] falls within the average normal range given by West and Todd.[47] The lipid values for man have been shown to be the highest, on an average, of any for the common animals. Bauer and Hirsch[187] reported that the esterified fatty acids in the serum of normal men average 9.2 meq. per liter (7.0 to 12.6).

a. Unsaturated Fatty Acids in Blood. The unsaturated fatty acid content has been shown to consist principally of oleic acid, with smaller amounts of diene (linoleic) and tetraene (arachidonic). Whereas the amounts of oleic and of linoleic acids were shown to depend in a large measure upon alimentation, linolenic and arachidonic acids are constant constituents of the blood under normal conditions.[188] Some normal values for

[186] E. M. Boyd, *J. Biol. Chem.*, *101*, 323–336 (1933).
[187] F. C. Bauer, Jr., and E. F. Hirsch, *Arch. Biochem.*, *23*, 137–140 (1949).
[188] A. Chevallier, S. Manuel, C. Burg, and J. Rouillard, *Compt. rend. soc. biol.*, *144*, 577–578 (1950).

TABLE 2
THE NORMAL RANGE OF LIPIDS IN HUMAN BLOOD[a]

Constituent	Blood fraction	Range[b]
Total lipids	Plasma	360–820
Fats	Serum	150–250
Fatty acids	Serum	200
Phospholipids	Serum	135–170
Lipid P	Serum	8–10
Cholesterol esters	Serum	150–193
Cholesterol		
As ester	Serum	90–114
Free	Serum	60–70
Carotenoids	Serum	6–312 μg./100 ml.
Vitamin A	Serum	35–40 I.U./100 ml.
α-Tocopherol (Vitamin E)	Serum	0.6–1.6

[a] Data adapted from E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan New York, 1951, pp. 544, 545.
[b] Expressed as mg./100 ml. unless otherwise designated.

TABLE 3
THE MEAN VALUES OF THE BLOOD PLASMA LIPIDS OF NORMAL WOMEN UNDER CONTROLLED CONDITIONS OF DIET, EXERCISE, AND REST[a]

Plasma lipid component	Lipid content in mg./100 ml. of blood plasma	
	Analytical values as reported	Analytical values (omitting duplication)
Total lipid	589	—
Neutral fat	154	154
Total fatty acid	353	—
Phospholipid fatty acid	130	—[b]
Cholesterol ester fatty acid	77	77
Neutral fat fatty acid	146	—[c]
Total cholesterol	162	162
Combined cholesterol	115	—
Free cholesterol	47	—
Phospholipid	196	196
Total lipid (excluding duplication)	—	589

[a] Adapted from E. M. Boyd, *J. Biol. Chem.*, *101*, 323–336 (1933).
[b] Included in phospholipid figure.
[c] Included in neutral fat figure.

the unsaturated fatty acid content of the blood serum of normal children and adults are given in Table 4.

TABLE 4
AVERAGE VALUES OF UNSATURATED FATTY ACIDS IN BLOOD SERUM OF NORMAL CHILDREN AND IN BLOOD SERUM AND BLOOD CELLS OF NORMAL ADULTS

Fatty acid component	Children[a]		Adults	
	% of total acids	Mg. %[b]	Plasma,[c] mg. %	Cells,[d] mg. %
Oleic	—	—	700 (250–950)	593 (370–720)
Diene	30.9	62	70 (15–115)	23.5 (9–45)
Triene	2.0	4	12 (5–35)	3
Tetraene	12.6	25	45 (35–65)	74 (54–91)

The figures in parentheses represent the ranges in individual values.
[a] H. F. Wiese, A. E. Hansen, and R. H. Gibbs, *Federation Proc.*, *12*, 433–434 (1953).
[b] Based upon a value for total fatty acids of 200 milligram per cent.
[c] A. Chevallier, S. Manuel, C. Burg, and J. Rouillard, *Compt. rend. soc. biol.*, *144*, 577–578 (1950). The figures in parentheses represent the range.
[d] A. Chevallier, S. Manuel, and J. Rouillard, *Compt. rend. soc. biol.*, *145*, 924–927 (1951).

b. Free Choline in Blood. Choline occurs in the free state in plasma. Although earlier experiments suggested values of 2 to 20 μg./ml.,[189] Bligh,[190] using a new technic involving trichloracetic acid as a protein precipitant, followed by ether extraction, acetylation of the choline, and its assay on the rectus abdominis muscle of an eserinized frog, obtained constant values for human plasma which were much lower (1–2 μg./ml.). The level of choline remained constant over a period of six months; it was unaffected by food or by exercise.

The values for plasma choline in the dog and in the cat were found to be similar to those in man.[190] Somewhat more variable results were exhibited by the rabbit (1.2–5.2 μg./ml.) together with a somewhat higher average level.[190] In the case of the rat,[191] the normal level of free choline varied between 1.3 and 1.9 μg./ml. However, when this species was maintained for four months on a choline-deficient diet, the plasma level fell to between 0.6 and 1.1 μg./ml.

Under normal conditions, when choline is injected, it is rapidly removed from the blood. According to Bligh,[192] the liver and kidneys are the organs primarily concerned with the removal of choline since, after the extirpation of these organs, the rate of disappearance of the base was considerably diminished. However, some choline continued to disappear under these conditions. This is taken to indicate that tissues other than the liver and kidney can dispose of additional choline at a slow rate.

[189] H. D. Appleton, B. B. Levy, J. M. Steele, and B. B. Brodie, *Federation Proc.*, *10*, 157 (1951).
[190] J. Bligh, *J. Physiol.*, *117*, 234–240 (1952).
[191] J. Bligh, *J. Physiol.*, *120*, 440–444 (1953).
[192] J. Bligh, *J. Physiol.*, *120*, 53–62 (1953).

(2) Normal Values of Blood Lipids in Various Animal Species

Wide variations in the relative proportions and in the total lipids have been observed in a number of species of animals examined. Table 5 lists the normal fasting values of the lipids in a series of animals, as determined by Boyd.[193] Abderhalden,[194] also reported the distribution of the lipids between the plasma and the cells in a number of different species. These results are recorded in Table 6.

(3) The Lipid Components in Blood Cells

a. Lipids in Erythrocytes. It is evident from the analyses of Abderhalden[194] that the concentration of several of the lipid components in the cells differs widely from their values in the plasma or serum. No appreciable quantities of neutral fat occur in the red blood corpuscles; in fact, the quantities are so small that Abderhalden[194] was unable to assign a value to them. If one calculates the concentration of total lipids in serum, from the data in Table 6, on the basis that all that appears in whole blood is present in the serum fraction, the calculated and observed values are quite close. In three cases, the calculated figures are lower than the observed values, while in five instances they are higher. The average deviation of the calculated from the observed values is 7.25%.

The calculations for serum fat based upon the known determination in whole blood are made as follows:

1000 g. of whole blood occupies 948 ml. (1000:1.055 (specific gravity)).
1000 g. of serum occupies 973 ml. (1000:1.027 (specific gravity)).
948 ml. of whole blood (1000 g.) contain 568 ml. serum. Plasma is 60% of the volume of whole blood.
Fat in 1000 g. of serum (973 ml.) will be (973:568) × concentration in 1000 g. whole blood. (This assumes that neutral fat is exclusively in the serum.)

Although neutral fat is practically completely absent from the erythrocytes, fatty acids occur in combination with phospholipids, and to some extent with cholesterol. In addition, Erickson and her associates[78] demonstrated that some of the cell fatty acids are combined in the cerebroside molecule; the erythrocytes contain 47 milligrams per cent of the latter.

According to Bloor,[195, 196] blood cells contain 400 to 440 mg. of phospholipid per 100 ml., which is about double the concentration in plasma. The

[193] E. M. Boyd, *J. Biol. Chem.*, *143*, 131–132 (1942).
[194] E. Abderhalden, *Physiological Chemistry*, translated by W. T. Hall and G. Defren, Wiley, New York, 1908.
[195] W. R. Bloor, *J. Biol. Chem.*, *22*, 133–144 (1915).
[196] W. R. Bloor, *J. Biol. Chem.*, *26*, 417–430 (1916).

TABLE 5. AVERAGE VALUES FOR LIPIDS (EXPRESSED IN MILLIGRAMS PER 100 MILLILITERS) IN THE BLOOD PLASMA OF SEVERAL NORMAL FASTING ANIMALS[a]

Category	Guinea pig[b]	Albino rat	Rabbit[c]	Cow	Cat	Cockerel[d]	Man[e]
Number of determinations	10	116	89	3	27	22	118
Total lipid	169 ± 34	230 ± 31	243 ± 89	348 ± 51	376 ± 110	520 ± 85	530 ± 74
Neutral fat	73 ± 33	85 ± 30	105 ± 50	105 ± 39	108 ± 65	225 ± 77	142 ± 60
Total fatty acids	116 ± 29	152 ± 23	169 ± 66	202 ± 55	228 ± 82	361 ± 74	316 ± 85
Total cholesterol	32 ± 5	52 ± 12	45 ± 18	110 ± 32	93 ± 24	100 ± 23	152 ± 24
As ester	21 ± 4	31 ± 10	23 ± 12	73 ± 15	63 ± 23	66 ± 19	106 ± 25
As alcohol	11 ± 2	21 ± 8	22 ± 13	37 ± 15	30 ± 10	34 ± 9	46 ± 8
Phospholipid	51 ± 12	83 ± 24	78 ± 33	84 ± 21	132 ± 53	155 ± 34	165 ± 28

The mean values include Pearson's coefficient.

[a] E. M. Boyd, *J. Biol. Chem.*, *143*, 131–132 (1942).
[b] E. M. Boyd and M. D. Fellows, *Am. J. Physiol.*, *114*, 635–641 (1936).
[c] E. M. Boyd, *Can. J. Research*, *16*, *Sect. D*, 31–37 (1938).
[d] E. M. Boyd and E. L. Clarke, *Can. J. Research*, *18*, *Sect. D*, 49–52 (1940).
[e] E. M. Boyd, *Can. J. Research*, *15*, *Sect. D*, 1–23 (1937).

Table 6. Water and Lipid Content of the Blood of Various Species of Animals (Expressed in Grams per 1000 Grams)[a]

Category	Bull	Cow	Sheep (2)	Goat	Horse (2)	Pig	Rabbit	Dog	Cat
Water									
Blood	814.8	808.9	823.5	803.9	772.0	790.6	816.9	801.0	795.5
Serum	913.4	913.6	917.1	907.7	908.6	917.6	925.6	923.5	926.9
Corpuscles	618.6	591.6	616.3	608.7	613.2	625.6	633.5	635.8	624.2
Fat									
Blood	2.363	0.567	0.900	0.535	0.572	1.095	0.734	0.772	0.373
Serum	3.542	0.926	1.307	0 624	1.067	1.956	1.193	1.346	0.788
Corpuscles	—	—	—	—	—	—	—	—	—
Fatty acids									
Blood	0.495	—	0.489	0.395	0.387	0.775	0.507	0.722	0.280
Serum	0.743	—	0.716	0.611	0.604	0.794	0.809	1.238	0.499
Corpuscles	—	—	—	—	0.060	0.062	—	—	—
Lecithin									
Blood	2.197	2.349	2.318	2.466	2.948	2.309	2.827	2.023	2.325
Serum	1.869	1.675	1.654	1.727	1.733	1.426	1.760	1.727	1.716
Corpuscles	2.850	3.748	3.771	3.856	4.414	3.456	4.627	2.432	3.119
Cholesterol									
Blood	1.209	1.935	1.685	1.299	0.461	0.444	0.611	1.110	0.895
Serum	1.901	1.238	1.094	1.070	0.410	0.409	0.547	0.684	0.600
Corpuscles	1.824	3.379	2.976	1.730	0 524	0.489	0.720	1.705	1.281

[a] E. Abderhalden, *Physiological Chemistry*, translated by W. T. Hall and G. Defren, Wiley, New York, 1911.

same proportionate difference in phospholipid distribution between the plasma and the cells has been observed in children.[197] Moreover, a different composition of the phospholipid fraction obtains in the plasma and in the cells. Williams, Erickson, *et al.*[198] reported that cephalin makes up 50 to 60% of the total phospholipid in the cells, while it comprises less than 10% in the plasma.[50–52] Using a new micromethod for the determination of individual phospholipids, Erickson *et al.*[62] later confirmed the earlier results, and showed the following absolute and percentage distribution of phospholipids in human erythrocytes per 100 g.: total phospholipid, 317 mg.; total choline phospholipids, 127 mg., 40%; lecithin, 77 mg., 24%; cephalin, 190 mg., 60%; and sphingomyelin, 50 mg., 16%. Using the same microprocedure, these workers reported the following figures per 100 ml. in human plasma: total phospholipid, 189 mg.; total choline phospholipids, 134 mg., 71%; lecithin, 99 mg., 52%; cephalin, 55 mg., 29%; and sphingomyelin, 35 mg., 19%.

Cholesterol makes up 125 to 150 milligram per cent per 100 ml. of cells, in normal human subjects, but no appreciable amount of the cholesterol is present in the ester form.[199] Although this quantity is somewhat less than that in the plasma, the concentration per 100 ml. of water is approximately the same in the two fractions. Erickson and co-workers[197] reported that the cholesterol in the red blood cells of children averaged 129 milligram per cent, as much as 32% being in ester combination. In adults esterified cholesterol was found to make up 16% of the total blood cholesterol. The comparative levels of cholesterol and phospholipid in the plasma and cells of forty normal adults are summarized in Table 7. The averages include those for twenty young healthy adults and twenty old patients with no known disorder in lipid metabolism. Since practically no differences obtained in the lipid values of the two age groups, Foldes and Murphy[200] reported the results of the combined groups as comprising the normal mean values.

Hagerman and Gould,[201] employing plasma and cells containing C^{14}-labeled cholesterol obtained by feeding C^{14}-acetate, showed that free chole-

[197] B. N. Erickson, H. H. Williams, F. C. Hummel, and I. G. Macy, *J. Biol. Chem.*, *118*, 15–35 (1937).

[198] H. H. Williams, B. N. Erickson, I. Avrin, S. S. Bernstein, and I. G. Macy, *J. Biol. Chem.*, *123*, 111–118 (1938).

[199] G. C. Brun, *Cholesterol Content of the Red Cells in Man*, H. K. Lewis, London, 1939; *Acta med. Scand., Suppl. 99*, 1–237 (1938).

[200] F. F. Foldes and A. J. Murphy, *Proc. Soc. Exptl. Biol. Med.*, *62*, 215–218, 218–223 (1946).

[201] J. S. Hagerman and R. G. Gould, *Proc. Soc. Exptl. Biol. Med.*, *78*, 329–332 (1951).

sterol reached an equipartition in cells and plasma within four hours; actually a 50% equilibration obtained within one hour. On the other hand, esterified cholesterol did not participate in the interchange. These workers believe that it is unlikely that the entire lipoprotein molecules interchange, inasmuch as plasma lipoproteins were found to contain both free and esterified cholesterol, while the red blood cells contained only the free form.

TABLE 7
DISTRIBUTION OF CHOLESTEROL, CHOLESTEROL ESTERS AND PHOSPHOLIPID PHOSPHORUS IN THE PLASMA AND BLOOD CELLS OF MAN[a]

Category	Plasma, mg. %	Cells, mg. %
Cholesterol		
Total	192.7 ± 35.5	173.0 ± 27.6
Esters	129.3 ± 25.0	15.3 ± 20.3
Phospholipid P	9.0 ± 1.2	14.1 ± 1.4

[a] F. F. Foldes and A. J. Murphy, *Proc. Soc. Exptl. Biol. Med.*, *62*, 215–218 (1946).

Changes in the levels in cell lipids occur much less readily than do variations in the plasma lipids. They are not affected appreciably by diet or by a variety of physiological factors. According to Peters and Van Slyke,[202] one may completely miss important variations of the lipid phosphorus or of free cholesterol in plasma if analyses are made on whole blood. This is attributable to the fact that the cells contain much larger proportions of the aforementioned components, and will thereby mask slight changes in these fractions in the plasma.

Globoside is a glycolipid which has been isolated from the stroma of the red blood cells of human blood, as well as from those of the sheep, goat, and hog, by Yamakawa and Suzuki.[203] Since it contains hexosamine (or chondrosamine) but no hemataminic acid, this type of glycolipid is classified as a globoside rather than as a hematoside. On the other hand, Yamakawa and Suzuki[203] note that the stroma lipid isolated from the dog and the horse have hemataminic acid and very little hexosamine. Bovine stroma possesses both components, while neither type was found in chicken stroma.

Klenk and Lauenstein[204] reported the composition of the erythrocyte stroma of man and ox as follows: fatty acids (mainly lignoceric), 25%; sphingosine, 16%; sugar (galactose, glucose, glucosamine), 56 to 57%.

[202] J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry, Vol. I, Interpretations*, 2nd ed., Williams & Wilkins, Baltimore, 1946.

[203] T. Yamakawa and S. Suzuki, *J. Biochem. (Japan)*, *38*, 199–212 (1951); *39*, 393–402 (1952); *40*, 7–10 (1953).

[204] E. Klenk and K. Lauenstein, *Z. physiol. Chem.*, *291*, 249–258 (1953).

Small amounts of gangliosides were likewise present. Klenk and Wolter[205] confirmed the results of Yamakawa and Suzuki[203] concerning the occurrence of the ganglioside type of glucolipids, the so-called hematosides, in the stroma of the horse. Neuraminic acid (presumably identical with prehemataminic acid) was found to be present, while hexosamine was absent. An unsaturated acid, possibly nervonic acid, was also found to occur.

b. Lipids in Leucocytes. An examination of the lipid composition of the white blood cells of women has been made by Boyd.[92] The average values for the white cells (expressed in milligrams per 100 g.) in the case of the eight women subjects examined, together with the standard deviation, are as follows: total lipid, 1710 ± 734; neutral fat, 536 ± 536; total fatty acids, 1103 ± 614; phospholipid fatty acids, 534 ±170; cholesterol ester fatty acids, 73 ± 65; neutral fat fatty acids, 508 ± 508; total cholesterol, 300 ± 60; free cholesterol, 194 ± 110; combined cholesterol, 110 ± 97; and phospholipids, 802 ± 255. The comparative composition of red cells and of polymorphonuclear leucocytes of rabbits is given in Table 8.

TABLE 8

THE COMPARATIVE LIPID COMPOSITION OF RED BLOOD CELLS AND OF THE POLYMORPHONUCLEAR LEUCOCYTES OF RABBITS[a]

Lipid component	Average content, mg. %		L:E ratio	Per cent total lipid	
	Leucocytes	Erythrocytes		Leucocytes	Erythrocytes
Neutral fat	530 ± 130	41 ± 18	12.9	30.2	8.5
Total fatty acids	1140 ± 100	230 ± 18	4.96	64.6	47.6
Cholesterol, total	243 ± 10	146 ± 3	1.66	13.8	30.2
ester	9 ± 2	0	—	0.5	0
free	234 ± 10	146 ± 3	1.60	13.3	30.2
Phospholipids, total	950 ± 40	264 ± 8	3.60	53.9	54.5
Cerebrosides	41 ± 30	33 ± 8	1.24	2.3	6.8
Distribution of phospholipids					
Monoaminophospholipids	670 ± 30	212 ± 9	3.16	70.6	80.4
Lecithin	300 ± 30	94 ± 5	3.19	31.6	35.6
Cephalin	370 ± 20	118 ± 7	3.14	39.0	44.8
Sphingomyelins	280 ± 10	52 ± 2	5.29	29.4	19.6

[a] N. S. Burt and R. J. Rossiter, *Biochem. J.*, *46*, 569–572 (1950).

(4) *Interrelations between Blood Lipids*

Although considerable variations may obtain, under physiological conditions, in the absolute amounts of the several blood components, there is a

[205] E. Klenk and H. Wolter, *Z. physiol. Chem.*, *291*, 259–265 (1953).

tendency to maintain a fair degree of constancy in the ratios between certain lipids. A number of different interrelationships have been postulated, but only a few of these appear to be of sufficient uniformity to be of any considerable importance.

a. Lipemic Constant. The *lipemic constant* (or coefficient) is the value expressed by the ratio of *blood cholesterol:blood fatty acids.* The uniformity of this ratio for each species was first recognized by Mayer and Schaeffer[206] in 1913. Terroine[207] reported that the relationship between cholesterol and fat in the blood is maintained during the absorption of fat, both values rising simultaneously. In later experiments of this worker,[208] it was shown that the ratio (*blood cholesterol:blood fatty acids*) $\times$ *100* averaged 36 in fourteen dogs, with a range of 23 to 50, and with an average variation of only 17%. According to Bloor,[7] other workers have not always supported Terroine, although it seems to be generally agreed that fatty acids and cholesterol both increase during fat absorption. Among others, Bang[209] has indicated that the fat:cholesterol ratio is relatively constant.

b. Lipemic Index. The *lipemic index* is simply the total lipid content of the blood. Under normal conditions, this value remains quite constant. Terroine[207] postulated that, since the lipemic index and the lipemic constant are relatively uniform for any one animal, the combined values furnish a means for identifying any single specific individual.

c. Cholesterol:Lipid Phosphorus Ratio. The most important interrelationship appears to be that which exists between the cholesterol and the phosphatide fraction in the blood. Bloor[210] was the first to report that the *cholesterol:lecithin* ratio in human blood is constant, both under normal and under pathological conditions. Horiuchi[211] also noted a uniformity of this proportion in rabbits, while Grigaut and Yovanovitch[212] were likewise able to maintain a constant relationship between these components under a wide variety of circumstances. Neutral fat was shown to have widely different values, and to bear no constant relationship to the phospholipids.

Since lecithin is the principal phospholipid in blood, it is understandable that the *cholesterol:lipid phosphorus* ratio would also be uniform. Peters and Man[213] have made an extensive study of this latter proportion in a large

[206] A. Mayer and G. Schaeffer, *J. physiol. path. gén.*, *15*, 984–998 (1913).
[207] E. F. Terroine, *J. physiol. path. gén.*, *16*, 212–222 (1914).
[208] E. F. Terroine, *Ann. Sci. Nat. Zool.* [*10*] *4*, 5–397 (1920).
[209] I. Bang, *Biochem. Z.*, *90*, 383–387 (1918).
[210] W. R. Bloor, *J. Biol. Chem.*, *25*, 577–599 (1916).
[211] Y. Horiuchi, *J. Biol. Chem.*, *44*, 345–361 (1920).
[212] A. Grigaut and R. Yovanovitch, *Compt. rend. soc. biol.*, *91*, 1310–1313 (1924).
[213] J. P. Peters and E. B. Man, *J. Clin. Invest.*, *22*, 707–714 (1943).

number of samples of blood; they concluded that the mean value for the ratio was 21.6 ± 2.5 when the average cholesterol concentration was 204.6 milligram per cent. It was found that the ratio varied somewhat, under

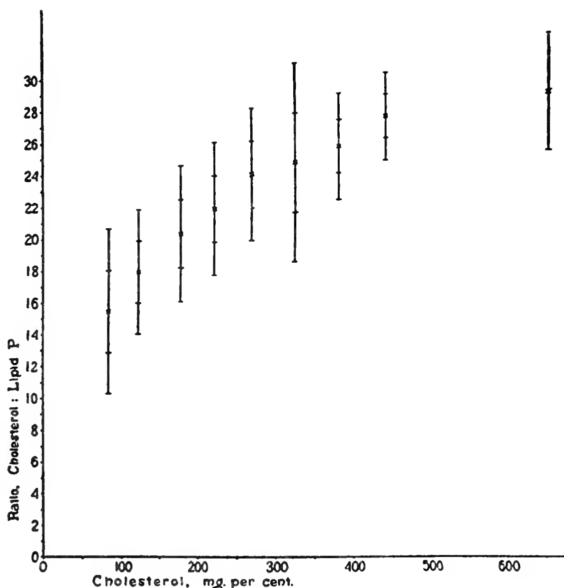


Fig. 1. The relation of blood cholesterol to the ratio, *cholesterol:lipid phosphorus*, in the serum of normal persons, psychiatric persons, and patients with diseases of the thyroid. The crosses on the vertical lines represent the mean values for the ratio in the samples grouped with similar blood-cholesterol values. The cross lines on the vertical lines indicate once or twice the standard deviation, except at the highest cholesterol value, where only the standard deviation is given.[214]

normal and pathological conditions, with changes in blood cholesterol. In general, the values are increased in the presence of higher blood cholesterol figures.[214] These data are represented in Figure 1.

A formula has also been developed to express the relationship between lipid phosphorus and blood cholesterol, which is a straight-line function

[214] J. P. Peters and E. B. Man, *J. Clin. Invest.*, 22, 715–720 (1943).

at cholesterol levels higher than 100 milligram per cent.[215] The formula is as follows:

$$\text{lipid P (mg. \%)} = 0.0294 \text{ cholesterol (mg. \%)} + (3.62 \pm 1.04)$$

d. Cholesterol Ester: Total Cholesterol Ratio. A number of workers have established the fact that the ratio of cholesterol esters to total choles-

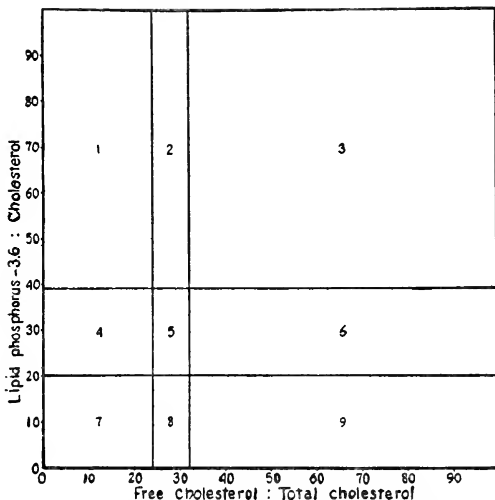


Fig. 2. The interrelations of the serum lipids. The limits of the ratios in normal subjects lie between the two vertical and the two horizontal lines.[215]

terol is a rather precise biological constant. Bloor and Knudson[216] first reported that approximately 60% of the total cholesterol was esterified. However, it now appears that this value is too low, as Boyd[186] obtained a figure of 70% in the case of young women, and Sperry,[217] in an extensive study which included 91 adults, reported an average of 73 ± 1.4%. Sperry states that the constancy of this ratio is much greater than is ordinarily supposed. Peters and Man[213] and Brun[218] confirmed the results of Sperry.

[215] E. B. Man, B. L. Kartin, S. H. Durlacher, and J. P. Peters, *J. Clin. Invest.*, *24*, 623–643 (1945).

[216] W. R. Bloor and A. Knudson, *J. Biol. Chem.*, *27*, 107–112 (1916); *29*, 1–13 (1917).

[217] W. M. Sperry, *J. Biol. Chem.*, *114*, 125–133 (1936).

[218] G. Brun, *Changes in the Lipide Contents of Serum in Patients with Manic-depressive Psychosis*, H. K. Lewis, London, 1940.

Calculated as the ratio of free cholesterol to total cholesterol in blood, the average value is given as 0.28.

Man and collaborators[215] have used a graphical chart to illustrate the relationship between the *lipid phosphorus:cholesterol* ratio and that of *free cholesterol:total cholesterol.* This is illustrated in Figure 2, on page 389.

The values for normal subjects will fall in rectangle 5, in which the ratio of (lipid P − 3.6):cholesterol (*1*) falls between 0.020 and 0.038, while the proportion of free cholesterol : total cholesterol (*2*) varies between 0.24 and 0.32. In abnormal cases, the values may fall in rectangle 2 (Ratio *1* is high and Ratio *2* is normal), rectangle 3 (both ratios high) or rectangle 6 (Ratio *1* is normal and Ratio *2* is high). In some cases, values falling in rectangles 8 or 9 have been recorded. These occur when the blood cholesterol values fall below 100 milligram per cent. The authors state that the chart is not directly applicable under the latter circumstances.

Turner and Pratt[219] noted that incubation of normal human serum and of that of patients with disease not involving the liver results in a decrease by more than 30% of free cholesterol, without change in total cholesterol. Since this reaction does not occur when the serum is heated to 56°C. before incubation, it is assumed that the reaction is probably mediated by an enzyme.

The ratio of cholesterol ester:total cholesterol has been shown by Darraspen *et al.*[220] to vary with species. However, it is fixed for each particular species, with only slight modifications as related to age or sex. In the case of the horse and cow, the value approaches 0.67, while the ratios in the blood serum of dog, pig, and goose approximate 0.75. The ratio for the dog and pig is similar to that found in man.

4. The Constancy in the Level of Blood Lipids

(*1*) *Normal Variations in the Blood Lipids in Animals of the Same Species*

It is, of course, recognized that the mean values for the several blood lipids listed in Tables 2 to 6 are obtained as a result of averaging the figures of a number of determinations, which vary widely, on presumably normal individuals. Animals which are apparently normal may have a value for a lipid constituent deviating widely from the generally accepted "normal" figure.

[219] K. B. Turner and V. Pratt, *Proc. Soc. Exptl. Biol. Med.*, *71*, 633–637 (1949).

[220] E. Darraspen, R. Florio, and P. Emangard, *Compt. rend. soc. biol.*, *143*, 1419–1420 (1949).

Although variations in analytical technic and in other experimental conditions render it difficult to draw any far-reaching conclusions from values obtained by different workers, it is probably worth while to list the values for some blood constituents as reported in the literature.

a. Total Lipids. The values for total lipids (total fatty acids plus cholesterol) in the whole blood of dogs (expressed as milligrams per 100 ml. moist weight), as reported by Terroine,[208] were as follows: sixteen dogs, *Av.* 454 (314–669); six dogs, *Av.* 429 (353–495); and seven dogs, *Av.* 442 (314–564). The Kumagawa-Suto method was used for the estimation of fatty acids, and the Windaus digitonin procedure for cholesterol. Using similar procedures, Lattes[221] reported the mean value for total lipids in the venous blood of thirteen dogs as 382 milligram per cent; the range was from 303 to 428 milligram per cent.

The values for the content of total lipids in dog blood as reported by Bloor are considerably higher than those reported above. This is to be ascribed to the fact that the latter investigator used a nephelometric method for total lipids and fatty acids which yields a considerably higher result than does the Kumagawa-Suto procedure. Moreover, a colorimetric technic was employed for the estimation of cholesterol, which likewise gives a higher value than does the digitonin precipitation method. The results of Bloor (expressed in milligram per cent for whole blood) are as follows: nine dogs, *Av.* 590 (510–660)[222]; five dogs, *Av.* 726 (600–850)[223]; and seven dogs, *Av.* 820 (700–980).[224] When the estimations were made on the blood plasma, the results were as follows: seven tests on two dogs, *Av.* 840 (690–1130)[7,224]; and six dogs, *Av.* 470 (380–620).[7,225] In a series of normal dogs in whose case an extraction procedure resembling the Kumagawa-Suto method was employed, average total lipids were 342 milligram per cent (15 analyses) with a range of 215 to 442 milligram per cent.

Bloor[7] concludes that it is not possible to present satisfactory lipid standards for the blood plasma of dogs. A value of 400 milligram per cent is the usual figure, but determinations showing levels of 300 to 600 milligram per cent may still represent a normal lipid picture.

b. Phospholipids. The averages reported by Bloor and associates for phospholipids are extremely uniform in different series of tests, but here again the individual determinations have a wide range. However, greater

[221] L. Lattes, *Arch. exptl. Pathol. Pharmakol. (Naunyn-Schmiedeberg's)*, *66*, 132–142 (1911).

[222] W. R. Bloor, *J. Biol. Chem.*, *19*, 1–24 (1914).

[223] W. R. Bloor, *J. Biol. Chem.*, *23*, 317–326 (1915).

[224] W. R. Bloor, *J. Biol. Chem.*, *24*, 447–460 (1916).

[225] W. R. Bloor, E. M. Gillette, and M. S. James, *J. Biol. Chem.*, *75*, 61–83 (1927).

uniformity of technic is obtained in a single laboratory than is possible when several laboratories are involved, and this may, in part, explain the greater constancy in these figures. The average phospholipid content in whole blood expressed in milligram per cent was as follows: five dogs, *Av.* 339 (310–370)[223]; and seven dogs, *Av.* 350 (320–400).[224] When blood plasma was used, the phospholipid content was reported as 350 (280–500) for seven dogs,[224] and 400 (250–610) for six dogs.[7,225]

c. Cholesterol. The variations in averages reported for blood cholesterol in dogs are as great as those for total blood lipids. While Terroine,[208] using the Windaus digitonin method, cited an average blood cholesterol value in blood plasma of 122 milligram per cent (90–152) for seven dogs, Bloor and associates, employing a colorimetric procedure, reported mean values of 174 (130–240, five dogs),[223] and 230 (180–300, seven dogs)[7,224] for whole blood, and 220 (150–370, seven dogs)[224] and 100 (80–120, six dogs, modified procedure) for blood plasma. The cholesterol values obtained in the serum of four normal dogs were reported by Mayer and Schaeffer[206] as ranging between 89 and 116 mg. 100 g. moist weight. It is evident that one cannot state a precise value for the level of cholesterol in normal dogs.

(2) *Normal Variations in the Blood Lipids of the Same Individual*

Although one accepts the fact that considerable variations may obtain in the level of a blood constituent in different individuals of a group of normal animals of the same species and of a similar dietary background, it is of considerable interest to inquire as to what degree of constancy in the level of the lipid components may be noted in a single animal over a period of time. It is difficult to establish the experimental conditions in such a manner that changes in diet, environmental conditions, as for instance the season of the year, age, *etc.*, do not play a role.

Terroine[207,226] reported results on the blood lipids in dogs for periods as long as eleven months which show a good degree of uniformity. In experiments on six dogs over periods as long as six months,[208] the maximum variation in total lipids from the mean was 169 mg. per 100 g. In studies on nine dogs over a week, Bloor[222] found that the average variation of each dog from his average was only 6.0%, while the greatest variation noted was 12.0%. The mean value for total lipids in all tests was 590 milligram per cent.

[226] E. F. Terroine, *J. physiol. path. gén.*, *16*, 386–397 (1914).

In a later study which lasted over two years, Bloor[227] followed the blood lipids at frequent intervals in the case of two dogs which were fed a fat-low diet; the food intake was adjusted to maintain a constant body weight. The average value of total lipids found in the first dog was 337 ± 41 milligram per cent (112 samples), with a range of 256 to 412 mg., while the mean in the second animal was 472 ± 40 milligram per cent (80 analyses), with a range of 390 to 572 mg. The average standard deviations amounted to 13 and 9%, respectively. The results on phospholipids and cholesterol are given in the table below.

Test animal	Phospholipid	Cholesterol
Dog 1		
Grand average (mg. %)	169 ± 22	80 ± 14
Range (mg. %)	137–208	60–109
Average standard deviation (%)	13%	17%
Dog 2		
Grand average (mg. %)	234 ± 27	133 ± 14
Range (mg. %)	198–300	110–155
Average standard deviation (%)	11%	11%

5. The Site of Synthesis of Plasma Lipids

It is virtually certain that practically no synthesis of lipids occurs in the blood itself. The cholesterol and phospholipids present in the blood in periods other than during digestion are presumably maintained at a constant level by means of supplies of these components furnished by the liver. It is generally believed that the neutral fat present in the plasma may originate from the gastrointestinal tract, the liver, or from the fat depots, depending upon the state of alimentation.

The experimental data on the origin of plasma phospholipids leave little doubt that they are largely if not entirely synthesized in the liver. Although Fishler and co-workers[228] demonstrated substantially the same amount of phospholipid synthesis in the kidney and small intestine of hepatectomized dogs as of normal animals, after the injection of P^{32}, yet only negligible amounts of newly synthesized phospholipid appeared in the plasma when the liver was absent. Thus, it was concluded that, although the small intestine and kidney can synthesize phospholipids, only the liver can serve as the source of the plasma phospholipids. Ramney *et al.*[229]

[227] W. R. Bloor, *J. Biol. Chem.*, *103*, 699–705 (1933).

[228] M. C. Fishler, C. Entenman, M. L. Montgomery, and I. L. Chaikoff, *J. Biol. Chem.*, *150*, 47–55 (1943).

[229] R. E. Ramney, I. L. Chaikoff, and C. Entenman, *Am. J. Physiol.*, *165*, 596–599 (1951).

also observed that the liver is the site of formation of the plasma phospholipids in the case of the domestic fowl, although extrahepatic synthesis of phospholipids was likewise demonstrated.

In a further study of the role of the liver in phospholipid synthesis, Goldman *et al.*[230] found that 2% of injected palmitic acid-1-C^{14} was present in the phospholipid molecule in normal dogs after seven hours, while only 0.1% was recovered in the liverless dogs. On the other hand, isotopic fatty acids were found in the phospholipids of heart, skeletal muscle, kidney, small intestine and its mucosa, and lung of hepatectomized dogs. The authors concluded that the phospholipid synthesized in these tissues is not normally concerned with the transport of fatty acids from one organ to another.

Not only is the liver concerned with the synthesis of plasma phospholipids, but also this organ is the primary site of their removal from the blood.[231] Labeled plasma phospholipids disappeared at a normal rate in dogs after the removal of the gastrointestinal tract, but not after hepatectomy. When labeled phospholipids were introduced into the blood, 40 to 50% were rapidly lost.[232] About one-third of this was deposited in the liver, while considerable amounts were transferred to other tissues. Approximately 9 to 20% of the radiophospholipids which left the plasma reappeared in the thoracic duct lymph, indicating that some phospholipids can leave the blood stream and undergo circulation through the lymph.

6. The Turnover Rate of Plasma Lipids

The rate of turnover registers the speed at which a tissue component is replaced with new molecules of the same compound. Thus, it is related both to the speed of synthesis and to the rate at which the substance is excreted, decomposed, or converted to a new product.

The chief interest in turnover rates in the plasma has been focussed on the phospholipids. Although phospholipids can be synthesized in the small intestine, kidney, muscle, and brain, the main organ in which this reaction occurs is the liver. The turnover rate of phospholipids is greatest in this organ. Entenman *et al.*[231] found that the turnover rate for plasma phospholipids was 6 to 10 hours in normal dogs weighing 7 to 18 kg., while it

[230] D. S. Goldman, I. L. Chaikoff, W. O. Reinhardt, C. Entenman, and W. G. Dauben, *J. Biol. Chem.*, *184*, 727–733 (1950).

[231] C. Entenman, I. L. Chaikoff, and D. B. Zilversmit, *J. Biol. Chem.*, *166*, 15–23 (1946).

[232] W. O. Reinhardt, M. C. Fishler, and I. L. Chaikoff, *J. Biol. Chem.*, *152*, 79–82 (1944).

was prolonged to 33 to 160 hours in liverless animals. Friedlander and associates[233] noted that the rate of turnover of plasma phospholipids in dogs was increased by the administration of choline. Zilversmit, Entenman, and Chaikoff[234] confirmed the earlier results with choline which proved that the specific activities of choline-containing phospholipids of liver and plasma were markedly increased after a single dose of choline. Nearly all of the increase was found in the case of lecithin; that of liver sphingomyelin was slight, due to its low concentration. Although the animals which responded to choline showed an increased calculated turnover of liver lecithins, there was no rise of that fraction in either plasma lecithin or plasma sphingomyelin. The authors conclude that the increases in specific activities of plasma phospholipid must be interpreted as a reflection of similar increases of their precursors in the liver. In the case of man, Cornatzer and Cayer[235] found that the rate of phospholipid turnover in the plasma of normal persons varies between individuals, but is fairly constant in the same individual. However, neither choline nor methionine produced any stimulatory effect on phospholipid turnover under normal conditions.

In completely depancreatized dogs with diabetes, in which condition considerable amounts of fat migrate from fat depots to liver, the rate of turnover of plasma phospholipids is not increased.[236] These results also fail to support the concept that the phospholipids serve in the transport of fatty acids from one tissue to another. In the case of cholesterol, London and Rittenberg[237] reported that the half-time of serum cholesterol is eight days, while the turnover time is twelve days.

7. Factors Altering the Concentration of Blood Lipids

Although the average values for blood constituents listed earlier are reasonably accurate for the prediction of the several blood lipids in normal individuals under postabsorptive conditions, a number of physiological factors may cause marked variations from the usual values. Such conditions as age, race, sex, species, and environment may have considerable importance, as do also the ingestion of fatty meals, or prolonged fasting. The most pronounced alterations from the normal are to be observed in such

[233] H. D. Friedlander, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *158*, 231–238 (1945).

[234] D. B. Zilversmit, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *176*, 193–208 (1948).

[235] W. E. Cornatzer and D. Cayer, *J. Clin. Invest.*, *29*, 534–541 (1950).

[236] I. L. Chaikoff, D. B. Zilversmit, and C. Entenman, *Proc. Soc. Exptl. Biol. Med.*, *68*, 6–9 (1948).

[237] I. M. London and D. Rittenberg, *J. Biol. Chem.*, *184*, 687–691 (1950).

pathological conditions as diabetes mellitus, and in diseases of the thyroid gland. These several factors will be summarized first for the blood components in which a close interrelation exists (neutral fat, phospholipid, cholesterol) and then for each of the other components separately.

(1) *Factors Altering the Concentration of Neutral Fat, Phospholipids, and Cholesterol*

Since the levels of the three main lipids in the blood, namely, neutral fat, phospholipids, and cholesterol, are interrelated, it seems best to consider them together. The concentration of phospholipids and of cholesterol in the blood is related to that of neutral fat; when fats are ingested, there is a marked rise of the neutral fat level in the blood, together with concomitant changes in the phospholipid and cholesterol fractions. The ingestion of phospholipid affects the concentration of neutral fat and cholesterol.

a. The Effect of Age. (*a*) *Total Blood Lipids in the Newborn and in Young Children.* Although the fatty acids are able to pass into the fetus, the composition of the blood lipids in the newborn substantiates the fact that the maternal-fetal transfer does not bring about an equilibrium in the composition of the blood of mother and child. There is considerable evidence that not only fatty acids, but also phosphatides and cholesterol, are lower in infancy than in the adult.[197,238,239] Boyd[238] found that the total plasma lipid in newborn infants amounted to only 198 ± 80 milligram per cent and 3.3 ± 1.9 meq. of neutral fat-fatty acids per 100 ml.[202] György,[240] Plass and Tompkins,[241] and Hellmuth[242] have all reported low values for the blood lipids of the newborn as contrasted with those of the mother. Total lipids in children three to eleven years old approximate the levels in adults.[243] Newborn rats also have a low blood lipid level.[244]

(*b*) *Blood Phospholipids in the Newborn and in Young Children.* According to Boyd,[238] the lipid phosphorus of the newborn is at the low level of 2.5 ± 1.3 milligram per cent. György[240] reported that the phospholipid level rises more rapidly than does that of cholesterol during the first two weeks following birth, after which cholesterol increases more rapidly. For comparative levels of phospholipid and cholesterol, see Table 9.

[238] E. M. Boyd, *Am. J. Diseases Children, 52*, 1319–1324 (1936).
[239] A. D. Kaiser and M. S. Gray, *Am. J. Diseases Children, 47*, 9–24 (1934).
[240] P. György, *Jahrb. Kinderheilk., 112*, 283–297 (1926).
[241] E. D. Plass and E. H. Tompkins, *J. Biol. Chem., 56*, 309–317 (1923).
[242] K. Hellmuth, *Arch. Gynäkol., 127*, 293–361 (1926).
[243] D. M. Cowie and L. A. Hoag, *J. Am. Med. Assoc., 77*, 1493–1494 (1921).
[244] A. Mayer and G. Schaeffer, *Compt. rend., 159*, 102–105 (1914).

TABLE 9
THE BLOOD LIPIDS IN YOUNG CHILDREN AT BIRTH AND AT SEVERAL PERIODS UP TO THE SIXTH YEAR[a]

Age	Cholesterol, mg. %	Phospholipids,[b] mg. %
Birth[c]	89	102.5
First week	87	105
5–12 months	136	152.5
5–6 years	169	177.5

[a] M. B. Gordon and D. J. Cohn, *Am. J. Diseases Children*, *35*, 193–200 (1928).
[b] Lipid P × 25.
[c] Cord blood.

On the other hand, Green and Macaskill[245] reported that the lipid phosphorus was as high (or higher) in the blood of newborn calves as in that of their mothers; moreover, a further increase in the level of phospholipid took place shortly after birth.

(*c*) *Blood Cholesterol in the Newborn and in Young Children.* A number of workers have noted low blood cholesterol values in early infancy.[238,246–250] Boyd[238] reported the blood cholesterol values of newborn infants as only 34 ±15 milligram per cent; 40% of this was free. Whitelaw[251] observed that the serum cholesterol values of premature infants were the same as those of full-term infants (70.6 miligram per cent). The levels did not vary with the degree of prematurity. It was suggested that the low cholesterol value in the newborn may be due to the depressant action of the estrogenic hormones.

In babies four to twenty-five days of age, Sperry[248] found blood cholesterol values of 71 to 190 milligram per cent, with an average of 135 ± 25 milligram per cent.

Offenkrantz and Karshan[247] noted a gradual rise in the cholesterol level in the plasma between the ages of two months and seven years, by which time the sterol had reached the adult level in the blood. Likewise, Erickson and her associates[197] reported an average cholesterol value of 143 milligram per cent of which 24% was present as the free alcohol, in children from five to nine years of age. Eck and Desbordes,[252] showed that the blood cholesterol of children six to fifteen years of age was somewhat lower

[245] H. H. Green and E. H. Macaskill, *J. Agr. Sci.*, *18*, 384–390 (1928).
[246] Banu, Negresco, and Heresco, *Compt. rend. soc. biol.*, *91*, 730–732 (1924).
[247] F. M. Offenkrantz and M. Karshan, *Am. J. Diseases Children*, *52*, 784–795 (1936).
[248] W. M. Sperry, *Am. J. Diseases Children*, *51*, 84–90 (1936).
[249] J. M. Slemons and H. J. Stander, *Bull. Johns Hopkins Hosp.*, *34*, 7–10 (1923).
[250] D. Rosenbloom, *Proc. Soc. Exptl. Biol. Med.*, *32*, 908–910 (1935).
[251] M. J. Whitelaw, *J. Clin. Invest.*, *27*, 260–262 (1948).
[252] M. Eck and J. Desbordes, *Compt. rend. soc. biol.*, *118*, 494–501 (1935).

TABLE 10

MEAN VALUES (INCLUDING STANDARD ERROR OF THE MEAN) OF VARIOUS LIPID FRACTIONS IN BLOOD OF NORMAL MEN OF WIDELY VARYING AGES[a]

The figures represent milligrams of lipid per 100 ml. of plasma

Age group	Number	Total lipid C	Cholesterol			Lipid P	Lipid NH_2-N	Total lipid N
			Total	Free	Esterified			
20–34	10–7	537 ± 146	214 ± 82	85 ± 16	129 ± 68	6.6 ± 2.9	3.3 ± 1.4	10.2 ± 5.7
40–44	4	519 ± 100	220 ± 26	83 ± 18	137 ± 14	7.9 ± 2.7	2.9 ± 2.1	10.7 ± 3.1
45–49	5	472 ± 75	253 ± 42	74 ± 13	179 ± 31	6.2 ± 3.0	2.4 ± 1.6	10.8 ± 3.1
50–54	5	546 ± 140	220 ± 67	73 ± 17	146 ± 52	7.6 ± 2.3	3.1 ± 1.3	8.7 ± 3.6
55–59	8	681 ± 271	246 ± 68	78 ± 15	163 ± 53	6.4 ± 3.0	2.3 ± 1.4	10.1 ± 3.8
60–64	6	535 ± 170	246 ± 71	90 ± 12	157 ± 70	8.8 ± 3.6	3.8 ± 2.6	10.2 ± 5.7
65–69	4	524 ± 84	222 ± 39	79 ± 32	143 ± 52	9.0 ± 1.6	4.4 ± 1.0	17.6 ± 4.7
70–74	6	575 ± 186	257 ± 44	94 ± 18	163 ± 41	7.6 ± 3.4	3.4 ± 1.1	10.2 ± 4.8
75–79	4	548 ± 125	260 ± 64	77 ± 22	167 ± 46	8.5 ± 4.2	4.0 ± 1.5	12.4 ± 5.7
80–84	7	630 ± 173	199 ± 49	86 ± 25	113 ± 40	8.8 ± 3.1	3.1 ± 1.8	12.1 ± 5.1
85–89	3	592 ± 59	240 ± 24	75 ± 11	165 ± 13	12.0 ± 4.0	4.2 ± 0.7	13.3 ± 3.9
90–91	2	560 ± 164	228 ± 36	83 ± 1	144 ± 35	7.4 ± 1.1	4.4 ± 0.7	12.2 ± 5.7
Grand average		*566 ± 166*[b]	*232 ± 62*	*82 ± 17*	*146 ± 56*	*7.7 ± 3.0*	*3.2 ± 1.6*	*11.1 ± 4.7*

[a] Adapted from I. H. Page, E. Kirk, W. H. Lewis, Jr., W. R. Thompson, and D. D. Van Slyke, *J. Biol. Chem.*, *111*, 613–639 (1935), p. 620.

[b] Expressed as total lipids, value is 735 ± 216 milligram per cent.

than was that of adults. Ward,[253] Parhon and Parhon,[254] and Kaiser and Gray[239] reported that cholesterol increases with age, although Blix[255] does not subscribe to this hypothesis. In any event, the level of blood cholesterol appears to have become stabilized by the advent of puberty. In fact, the work of Hodges, Sperry, and Andersen[256] has failed to demonstrate any significant differences in the cholesterol level in the sera of children, varying in age from two months to thirteen years, from that of adults. According to these workers, the ratio of total to free cholesterol has also become stabilized at as early an age as two months. Sperry[257] stated that the serum cholesterol is maintained in each healthy person at a constant level.

Another variation between the composition of the blood of the mother and that of the infant is in the relative lack of the cholesterol ester fraction in the latter instance. If delivery occurred without the use of anesthetics, lower values for the cholesterol esters were found in the blood of the infant than following the use of anesthetics.[249] The calf, also, has been shown to have a blood devoid of the esterified cholesterol at birth.[258]

(*d*) *Blood Lipids in the Young and in the Aged.* It has been fairly well established that the blood lipid picture remains essentially uniform as life advances, except as it may be influenced by pathological conditions. In order to obtain data on these points, Page and his collaborators[259] made an extensive investigation on a large group of men varying in age from 20 to 101 years. The results of these workers are summarized in Table 10. The average figures of Page *et al.*[259] for 66 male subjects (a) compare with those of Boyd[260] for eight subjects (b), and of Man and Peters[261] for twelve subjects (c) as follows: total lipids, (a) 735 ± 216, (b) 582; neutral fats, (a) 225 ± 137, (b) 137; phosphatides, (a) 181 ± 71, (b) 185 ± 21, (c) 222 ± 29; total cholesterol, (a) 232 ± 62, (b) 177, (c) 207 ± 29; and free cholesterol, (a) 82 ± 17 and (b) 53 ± 8. Thus, there is considerable evidence that advancing age is without effect on the blood lipids levels in human subjects.

[253] K. M. Ward, *Arch. Diseases Childhood, 6*, 329–342 (1931).
[254] C. J. Parhon and M. Parhon, *Compt. rend. soc. biol., 88*, 231–233 (1923).
[255] G. Blix, *Acta Med. Scand., 64*, 142–174, 175–233, 234–259 (1926).
[256] R. G. Hodges, W. M. Sperry, and D. H. Andersen, *Am. J. Diseases Children, 65*, 858–867 (1943).
[257] W. M. Sperry, *J. Biol. Chem., 117*, 391–395 (1937).
[258] R. E. Shope, *J. Biol. Chem., 80*, 141–148 (1928).
[259] I. H. Page, E. Kirk, W. H. Lewis, Jr., W. R. Thompson, and D. D. Van Slyke, *J. Biol. Chem., 111*, 613–639 (1935).
[260] E. M. Boyd, *J. Biol. Chem., 110*, 61–70 (1935).
[261] E. B. Man and J. P. Peters, *J. Biol. Chem., 101*, 685–695 (1933).

However, several workers have maintained that blood cholesterol increases with advancing years. Keys[262] reported that the average serum cholesterol of healthy men increased from 173 milligram per cent at nineteen years to 252 milligram per cent at fifty-two years. For the age range from seventeen to seventy-eight years, Keys *et al.*[263] reported a pronounced curvilinear relationship between age and serum cholesterol in men, a maximum concentration being obtained during the sixth decade. During the period of increase, the average rise in serum cholesterol was found to be 2.2 mg. of total cholesterol/100 ml. serum/year. On the other hand, Sperry and Webb[264] concluded as a result of studies of blood cholesterol on the same fourteen men and eight women over a period of thirteen to fifteen years, that the serum cholesterol concentration may increase with age in some persons, but that the increase is not an obligatory concomitant of aging.

(*e*) *Chylomicrons and Lipemia.* According to Becker, Meyer, and Necheles,[265] there is a definite delay in the rate of absorption of fat, and an increase in the total absorption of corpuscular fat in the aged. These workers base their conclusions upon chylomicron curves, which are believed to serve as an index of postabsorptive lipemia. It was found that the oral administration of Tween 80 or of lipase with the fat meal reduced the hyperchylomicronemia in the aged, but that it was without effect on the chylomicron curves of young persons after a fat meal. The delayed absorption in older persons may also be reflected in the decreased levels of pancreatic lipase as compared with those of young subjects.[266] Becker *et al.*[265] suggest that the mechanism of fat digestion and/or that of fat absorption may change with aging.

b. The Effect of Sex. No definite variations in concentrations or in the partition of blood lipids are ascribable to sex.[210,213,247,267] Gildea and coworkers[268] did report that cholesterol and total fatty acids tended to be high in pyknic (stocky) men, while in leptosomes (slender individuals) the corresponding values were low. On the other hand, no correlation with body build could be demonstrated in females. Moreover, Sperry[257] was

[262] A. Keys, *Federation Proc.*, *8*, 523–529 (1949).

[263] A. Keys, O. Mickelsen, E. O. Miller, E. R. Hayes, and R. L. Todd, *J. Clin. Invest.*, *29*, 1347–1353 (1950).

[264] W. M. Sperry and M. Webb, *J. Biol. Chem.*, *187*, 107–110 (1950).

[265] G. H. Becker, J. Meyer, and H. Necheles, *Science*, *110*, 529–530 (1949).

[266] H. Necheles, F. Plotke, and J. Meyer, *Am. J. Digestive Diseases*, *9*, 157–159 (1942).

[267] J. A. Gardner and H. Gainsborough, *Biochem. J.*, *21*, 130–140, 141–147 (1927).

[268] E. F. Gildea, E. Kahn, and E. B. Man, *Am. J. Psychiat.*, *92*, 1247–1260 (1936).

unable to confirm the observation of Gildea *et al.*[268]; he concluded that the cholesterol level in the blood was a value characteristic of the individual.

On the other hand, the level of the ketone bodies in the blood during fasting or following the ingestion of a protein-fat diet is closely linked with sex. Although the ketone-bodies (acetoacetic acid, β-hydroxybutyric acid, and acetone) do not represent lipids in the true sense of the word, since they are water-soluble, they are the result of lipid metabolism. See pages 449–451 for a discussion of blood ketones.

c. The Effect of Race. There is no evidence that race affects the level or nature of blood lipids to an appreciable degree. The blood cholesterol value reported by Bose and De[269] for Indians, namely, 140 milligram per cent (120–160), is lower than that of occidentals. Boyd and Roy[270] also reported levels for blood cholesterol which were slightly lower than those considered normal for Europeans and Americans. There is no adequate explanation for the low values reported by Radsma[271] for tropical races, although Radsma suggests that infection with intestinal parasites (*Ascaris* spp.) or with the hookworm organism (*Ankylostoma* spp.) may be responsible.

In studies on Eskimos, Corcoran and Rabinowitch[272] detected no specific differences in the lipid patterns of the serum, in spite of the fact that the diet consisted largely of meat; however, the total lipid, cholesterol, and phospholipid values were somewhat lower than the normal figures, and the phospholipid : cholesterol ratios appeared to be slightly higher than the usual normal ratios.

d. Diurnal Variations in Blood Lipids. The bulk of the experimental evidence is against any pronounced diurnal variation in blood lipids which cannot be traced to fat ingestion. A diurnal variation in liver glycogen has been postulated.[273,274] If this change could be demonstrated with carbohydrate, it is possible that it might be correlated with a rhythmic change in fat storage in the liver, and the latter would certainly be associated with variations in blood lipids. In fact, Ohlsson and Blix[275] reported experiments on rats which appeared to demonstrate these cyclic changes in the composition of liver lipids.

However, although more recent work has confirmed the variations in

[269] J. P. Bose and U. N. De, *Indian J. Med. Research*, *24*, 489–508 (1936).
[270] T. C. Boyd and A. C. Roy, *Indian J. Med. Research*, *15*, 643–651 (1928).
[271] W. Radsma, *Geneesk. Tijdschr. Nederland.-Indië*, *69*, 793–805 (1929).
[272] A. C. Corcoran and I. M. Rabinowitch, *Biochem. J.*, *31*, 343–348 (1937).
[273] E. Forsgren, *Skand. Arch. Physiol.*, *53*, 137–151 (1928).
[274] G. Ågren, O. Wilander, and E. Jorpes, *Biochem. J.*, *25*, 777–785 (1931).
[275] B. Ohlsson and G. Blix, *Skand. Arch. Physiol.*, *69*, 182–188 (1934).

liver glycogen during the twenty-four hour period,[276–278] it has been shown that these variations were related to the feeding habits of the animals.[276–278] When rats were fed constant amounts of carbohydrate at spaced intervals throughout the day and night, and the animals were sacrificed twelve hours later, no evidence of a diurnal cycle in carbohydrate storage obtained.[278]

Although there is considerable evidence of marked variations in blood lipids after the ingestion of heavy doses of fats, Boyd[260] could find no significant variations of total lipid in human subjects given normal diets with moderate amounts of fat. Samples were collected at intervals of three or four hours throughout the twenty-four-hour period. The variations between the different subjects were two or three times as great as were any diurnal changes.

Cholesterol appears to remain quite constant in the blood throughout the twenty-four-hour period. Although McEachern and Gilmour[279] noted wide variations in the blood cholesterol values of twenty-eight subjects over five-hour intervals, more recent results have supported the viewpoint that alterations in this lipid fraction are insignificant and inconstant during the course of the day.[260,280] The blood level of cholesterol remains largely unaffected by ordinary meals and by the usual activities.[281,282]

e. The Effect of Menstruation. With the development in our knowledge concerning the hormones which control the female sex cycle, more exact information has become available on the relationship of this function to lipid metabolism. Two hormones are now known to be secreted by the ovaries, namely, estrone, which occurs in the follicles, and progesterone, which is a product of the corpus luteum.

Okey and Boyden[283] reported that a decrease in blood cholesterol occurred in women during or within a few days of the onset of the menstrual period; this was followed by an increased level in blood cholesterol. No similar changes could be consistently demonstrated in the level of fatty acids and phospholipids in the serum. Even more marked changes in the cholesterol level were noted by Kaufmann and Mühlbock,[284] who found a decrease in serum cholesterol as great as 40% at the menstrual period. The fact that

[276] G. M. Higgins, J. Berkson, and E. Flock, *Am. J. Physiol.*, *102*, 673–682 (1932).

[277] G. M. Higgins, J. Berkson, and E. Flock, *Am. J. Physiol.*, *105*, 177–186 (1933).

[278] H. J. Deuel, Jr., J. S. Butts, L. F. Hallman, S. Murray, and H. Blunden, *J. Biol. Chem.*, *123*, 257–265 (1938).

[279] J. M. McEachern and C. R. Gilmour, *Can. Med. Assoc. J.*, *26*, 30–33 (1932).

[280] C. W. McClure and M. E. Huntsinger, *J. Biol. Chem.*, *76*, 1–18 (1928).

[281] M. Bruger and I. Somach, *J. Biol. Chem.*, *97*, 23–30 (1932).

[282] K. B. Turner and A. Steiner, *J. Clin. Invest.*, *18*, 45–49 (1939).

[283] R. Okey and R. E. Boyden, *J. Biol. Chem.*, *72*, 261–281 (1927).

[284] C. Kaufmann and O. Mühlbock, *Arch. Gynäkol.*, *136*, 478–502 (1929).

the rhythm in blood lipids disappeared in lues or after the menopause served as an excellent control test. However, Peters and Van Slyke[202] do not accept these changes as being convincing in magnitude or in consistency.

Several workers have observed definite effects on blood lipids as a result of the administration of sex hormones. Thus, Loeb[285] was able to demonstrate a moderate increase in serum lipids when estradiol benzoate was given to rats receiving a high fat diet which was devoid of essential fatty acids. He later demonstrated that a storage of body fat followed the administration of this hormone.[286] According to Bogdanovitch and Man,[287] the blood fatty acids of guinea pigs were increased not only by estrone but also by antuitrin-S, which is an anterior pituitary gonadotrophic hormone. These hormones produced no effect either on the blood cholesterol or on the blood phospholipids.

Schlegel[288] called attention to the interesting relationship between the menstrual cycle and the serum choline concentration. Thus, the choline level was found to reach the maximum value on the fourteenth day and the lowest level on about the twenty-sixth day. It was deduced that a coincidence obtains between the maxima and minima of the blood choline and of the blood estrogen curves. It was suggested that the known effect of estrogen upon the serum cholinesterase[289] in rats, rabbits, and guinea pigs may provide an explanation for this relationship. In addition to the lunar variation of blood choline, a seasonal one was also noted; the choline content of the serum was found to be five times higher in February–March than in June–July.[288]

f. The Effect of Ovulation. Since the ovulation process is intimately connected with the sex cycle, it would seem probable that this might likewise be associated with changes in the level of some of the blood lipids. Studies in this field have been largely confined to fowls.

Lawrence and Riddle[290] reported that the blood plasma of laying hens contained more alcohol-soluble substances and phosphorus than did that of non-laying hens or of males. The relative values of phosphorus were as follows: males, 100, non-laying females, 115, and laying hens, 205. The ratios of alcohol-soluble substances were 100, 116, and 181, respectively. In the collared ring dove (*Streptopelia risoria*), an increase of 35% in the

[285] H. G. Loeb, *Proc. Soc. Exptl. Biol. Med.*, *49*, 340–342 (1942).
[286] H. G. Loeb, *Proc. Soc. Exptl. Biol. Med.*, *51*, 330–332 (1942).
[287] S. B. Bogdanovitch and E. B. Man, *Am. J. Physiol.*, *122*, 73–80 (1938).
[288] J. U. Schlegel, *Am. J. Physiol.*, *158*, 345–350 (1949).
[289] C. H. Sawyer and J. W. Everett, *Am. J. Physiol.*, *148*, 675–683 (1947).
[290] J. V. Lawrence and O. Riddle, *Am. J. Physiol.*, *41*, 430–437 (1916).

TABLE 11

THE MEAN VALUES OF THE BLOOD LIPIDS (INCLUDING STANDARD ERRORS OF THE MEAN) IN MALE CHICKENS, AND IN FEMALE CHICKENS BEFORE AND DURING THE LAYING PERIOD[a]

The values are expressed in milligram per cent of whole blood

Category	Low-fat diet			High-fat diet		
	Males	Immature females	Laying females	Males	Immature females	Laying females
No. of tests	12	7	36	12	6	32
Total lipid	428 ± 13	446 ± 25	1689 ± 149	461 ± 22	480 ± 41	1345 ± 115
Phospholipid	299 ± 8	282 ± 11	642 ± 33	304 ± 10	288 ± 12	572 ± 30
Total fatty acids	314 ± 13	329 ± 19	1564 ± 146	331 ± 18	361 ± 33	1209 ± 110
Residual fatty acids	99 ± 13	115 ± 16	1122 ± 133	97 ± 13	140 ± 23	795 ± 94
Total cholesterol	114 ± 4	117 ± 8	125 ± 5	131 ± 7	119 ± 8	136 ± 6
Free cholesterol	92 ± 4	83 ± 4	109 ± 1	88 ± 3	80 ± 4	108 ± 5
Ester cholesterol	22 ± 3	35 ± 6	16 ± 3	42 ± 6	39 ± 7	29 ± 4

[a] Adapted from F. W. Lorenz, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *122*, 619–633 (1938).

ether-soluble substances of the blood and of 50% in the phosphorus level during the ovulation cycle were reported.[291] Higher blood cholesterol values have likewise been noted in hens during the laying period.[292,293] Because of the high concentration of cholesterol in the yolk of the egg, it is logical to assume that a mobilization of this substance in the blood for the transport of the sterol to the yolk must precede its deposition in the egg.

Abrupt changes in the level of blood lipids occur at the onset of laying. Such changes are strikingly demonstrated in the blood lipid pattern of young hens just prior to their first egg-laying period. Lorenz, Entenman, and Chaikoff[294] reported a sudden rise in blood fat to a value of over 300 milligram per cent, with a concomitant increase of phospholipids to approximately 1000 milligram per cent. Although the level of blood cholesterol also increased, the rise was much less spectacular. Marked variations in all of the blood lipid constituents occurred during the course of the egg-laying period. It was later shown[295] that the hormone, estrone, produced a 100% increase in the blood lipids in immature females within twelve hours; a marked increase in the blood lipids of males was likewise provoked by this hormone. The variations in the blood lipids of hens as affected by egg-laying are shown in Table 11.

Results substantially similar to those of Lorenz *et al.*[294,295] have been obtained by Walker, Taylor, and Russell.[296] These workers reported that laying hens on normal rations had average plasma lipids amounting to 1476 milligram per cent. Phospholipid made up 30% of the total, neutral fat, 62%, and cholesterol, 7%. The blood plasma may contain as much as 13% of lipids. Although a slight decrease in blood lipids occurred when the birds were placed on a low-fat diet, no change was noted when a high-fat regimen was used.

Diethylstilbestrol, which is widely used for stimulating the growth of chickens, has been shown to have a profound effect on lipid metabolism. Stamler and associates[297] reported a hyperlipemia, sustained over fifteen weeks, in chicks into which 25 mg. pellets of diethylstilbestrol had been implanted at the age of five weeks. A simultaneous increase in cholesterol,

[291] O. Riddle and F. H. Burns, *Am. J. Physiol.*, *81*, 711–724 (1927).
[292] C. J. Parhon and M. Parhon, *Compt. rend. soc. biol.*, *89*, 349–353 (1923).
[293] C. J. Parhon and M. Parhon, *Compt. rend. soc. biol.*, *90*, 150–152 (1924).
[294] F. W. Lorenz, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *122*, 619–633 (1938).
[295] F. W. Lorenz, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *126*, 763–769 (1938).
[296] H. A. Walker, M. W. Taylor, and W. C. Russell, *Poultry Sci.*, *30*, 525 (1951).
[297] J. Stamler, C. Bolene, M. Dudley, and E. Levinson, *Endocrinology*, *46*, 375–381 (1950).

phospholipid, and neutral fat, in individual organs and in the carcass, occurred concomitantly with the hyperlipemia.

According to Chaikoff and Entenman[298] the values of blood cholesterol, phospholipids, and fatty acids in the painted terrapin (*Chrysemys picta bellii*) and the fresh-water scribe turtle (*Pseudemys scripta*) are related to ovarian activity, as is the case with the fowl. These fractions were augmented during ovarian function.

g. The Effect of Pregnancy. Many studies have been made of the effect of pregnancy on the level of blood lipids in human subjects. Hermann and Neumann[299] reported an average increase in the total lipids from 590 to 780 milligram per cent; the increases were largely in neutral fat and cholesterol esters. Some workers have noted slight increases in fatty acids,[300-302] and also in phospholipids,[300,301,303] as a result of pregnancy.

Blood cholesterol exhibits the most pronounced changes of any of the lipids during pregnancy. There is general agreement that the cholesterol fraction of the blood increases markedly in this condition.[299-301,304-307] The increase in cholesterol begins after the second month and continues up to the thirtieth week, after which it again decreases until delivery.[304] The maximum values are 50 to 100% higher than the normal values for non-pregnant women.

The increase in cholesterol concentration during pregnancy may be associated with a change in the ratio of free to esterified cholesterol. According to Bloor and Knudson,[216] the ester fraction, chiefly, is increased; Boyd[301] found a normal partition of cholesterol between the free and esterified forms. On the other hand, Gardner and Gainsborough[304] reported that free cholesterol alone is increased, while the ester fraction may even show a decrease. In these tests, the ratio of free to total cholesterol reached a value as high as 0.90. The discrepancies reported by the several investigators might be due to their failure to recognize that the cholesterol may be bound with plasma protein. Eufinger[308] reported that the fraction of cholesterol in this combination is considerably increased toward the end of

[298] I. L. Chaikoff and C. Entenman, *J. Biol. Chem.*, *166*, 683–689 (1946).
[299] E. Hermann and J. Neumann, *Biochem. Z.*, *43*, 47–55 (1912).
[300] C. Fahrig and L. Wacker, *Klin. Wochschr.*, *11*, 886–891 (1932).
[301] E. M. Boyd, *J. Clin. Invest.*, *13*, 347–363 (1934).
[302] H. Knauer, *Jahrb. Kinderheilk.*, *Abhandl.*, *22*, 1–164 (1928).
[303] B. L. Oser and W. G. Karr, *Arch. Internal Med.*, *36*, 507–515 (1925).
[304] J. A. Gardner and H. Gainsborough, *Lancet*, *1929*, *I*, 603–606.
[305] E. E. Pribram, *Arch. Gynäkol.*, *119*, 57–68 (1923).
[306] W. Stepp, *Münch. med. Wochschr.*, *65*, 781–785 (1918).
[307] M. Tyler and F. P. Underhill, *J. Biol. Chem.*, *66*, 1–14 (1925).
[308] H. Eufinger, *Arch. Gynäkol.*, *133*, 475–489 (1928).

pregnancy. After delivery, the concentration of cholesterol and, in fact, of all blood lipids, gradually returns to normal.[304,309] The rate of decline is to some extent dependent upon whether or not the mother nurses her infant.[309] Peters and associates[310] confirmed the progressive rise of total and free cholesterol, phospholipids, and neutral fat from the twelfth week of pregnancy to delivery, after which the value returned to normal. Neutral fat was found to increase proportionally far more before delivery and to decline more rapidly after parturition than did the other lipid fractions.

Little information is available as to the changes in blood lipids in the lower animals during pregnancy. Blood fat has been reported to increase as term approaches, in the guinea pig, following fat feeding,[311] while a high liver fat has been noted in pregnant rabbits.[312] However, Kaufmann and Erdmann[313] detected no increase in blood cholesterol in pregnant rats, although increased amounts of this sterol were reported in the adrenals of non-pregnant female rabbits, as compared with the values for the bucks. This difference largely disappeared in pregnant rabbits.[314]

h. The Maternal-Fetal Transfer of Lipids. The question as to whether or not a transfer of lipids from the maternal blood to the fetus occurs has been answered both negatively and affirmatively. The experimental procedures employed to investigate this question have usually involved the feeding of fats having a fatty acid composition differing from that normally found in the animal. In the case of dogs, Thiemich[315] was unable to demonstrate that feeding cocoa butter or a highly unsaturated fat such as linseed oil to the mothers exerted any influence on the iodine number of the fats in the pups. However, this worker[316] later observed that unsaturation had some effect on the composition of fetal fat; therefore, he concluded that fetal fat may, in part, be derived from maternal fat. Hofbauer[317] also reported the presence of lauric acid in the fetal fat after feeding coconut oil to pregnant guinea pigs. However, other workers have questioned the methods of analysis employed.[318,319]

[309] E. M. Boyd, *Am. J. Obstet. Gynecol.*, *29*, 797–805 (1935).

[310] J. P. Peters, M. Heinemann, and E. B. Man, *J. Clin. Invest.*, *30*, 388–394 (1951).

[311] T. Oshima, *Zentr. Physiol.*, *21*, 297–301 (1907).

[312] R. Coope and V. H. Mottram, *J. Physiol.*, *49*, 23–33 (1914).

[313] C. Kaufmann and R. Erdmann, *Biochem. Z.*, *249*, 438–442 (1932).

[314] E. N. Chamberlain, *J. Physiol.*, *68*, 259–264 (1929).

[315] M. Thiemich, *Zentr. Physiol.*, *12*, 850–852 (1898).

[316] M. Thiemich, *Jahrb. Kinderheilk.*, *61*, 174–177 (1905).

[317] J. Hofbauer, *Biologie der menschlichen Plazenta*, W. Braumüller, Vienna-Leipzig, 1905, p. 87, 88.

[318] L. G. Wesson, *Bull. Johns Hopkins Hosp.*, *38*, 237–241 (1926).

[319] J. Needham, *Chemical Embryology*, Cambridge Univ. Press, Vol. 2, 1931, p. 1192.

It has also been claimed that fat-soluble dyes, when fed to pregnant animals, appear in the fetus.[317] However, this conclusion has been denied by at least three groups of workers,[320–322] who were unable to demonstrate the appearance of dye in the fetal fat after it had been fed to the mother. However, these negative results do not necessarily prove that fat cannot be transmitted across the placenta, but only that a non-physiological dye is unable to penetrate this barrier.

A number of recent studies have given strong positive evidence that the lipid in the maternal blood is transferred to the fetus. Bickenbach and Rupp[323,324] demonstrated that the composition of the maternal and that of the fetal fat of rabbits were identical after the amyl or methyl ester of oleic or palmitic acid had been injected into pregnant rabbits. Miura[325] also found that the iodine number of fetal fat, as well as that of the maternal fat, was increased when linseed oil was given to the mother, while this index was lowered in the fat of both mother and fetus when coconut oil was fed. Moreover, Chaikoff and Robinson[326] showed a correspondence between the iodine numbers and the saturation of the fat fed; however, the maternal fat was far more sensitive to the dietary lipid than was the fetal fat. Sinclair[327] confirmed these observations, while McConnell and Sinclair[328] demonstrated the passage of the unnatural fat, elaidic acid, into the fetuses of rats. Popják[329] reported that a marked increase in the fat content in the decidual cells of the maternal portion of the placenta occurs in rabbits fed diets high in cholesterol during the last three weeks of pregnancy. This lipid storage in the placenta apparently interfered with the nutrition of the fetuses, as they were one-third lighter than usual. Neutral fat was actually lower in these fetuses than in the controls. It is suggested that this may have resulted from a partial blockage of the placenta to the transfer of fat precursors. The consensus of the experimental evidence is that, under certain conditions, fetal fat may be derived from that of the mother, although part may be synthesized within the fetus.

According to Boyd and Wilson,[330] phospholipids and cholesterol (both

[320] S. H. Gage and S. P. Gage, *Anat. Record*, *3*, 203–204 (1909).
[321] L. B. Mendel and A. L. Daniels, *J. Biol. Chem.*, *13*, 71–95 (1912–1913).
[322] E. J. Baumann and O. M. Holly, *Am. J. Physiol.*, *75*, 618–632 (1926).
[323] W. Bickenbach and H. Rupp, *Klin. Wochschr.*, *10*, 63–64 (1931).
[324] W. Bickenbach and H. Rupp, *Z. Geburtshülfe u. Gynäkol.*, *100*, 1–16 (1931).
[325] K. Miura, *J. Biochem. (Japan)*, *25*, 579–593 (1937).
[326] I. L. Chaikoff and A. Robinson, *J. Biol. Chem.*, *100*, 13–26 (1933).
[327] R. G. Sinclair, *Am. J. Physiol.*, *103*, 73–74 (1933).
[328] K. P. McConnell and R. G. Sinclair, *J. Biol. Chem.*, *118*, 123–129 (1937).
[329] G. Popják, *J. Physiol.*, *105*, 236–254 (1946).
[330] E. M. Boyd and K. M. Wilson, *J. Clin. Invest.*, *14*, 7–15 (1935).

free and esterified) can pass into the fetus. However, Popják[329] recorded a unilateral permeability of the rabbit placenta to cholesterol; according to this concept, the placenta may take up large amounts of cholesterol from the maternal circulation, but it does not pass it to the fetus. Popják and Beeckmans,[331] using phospholipids tagged with P^{32}, proved that, although the fetal placenta took up appreciable amounts of phospholipid from the maternal circulation, it did not transmit the whole phospholipid molecule either to the fetal liver or to the rest of the fetus. Moreover, these investigators[332] later demonstrated that glycerophosphate, which is the first decomposition product of lecithin in the tissues, does not pass unhydrolyzed through the placenta. It is therefore believed that a unilateral permeability for phospholipids exists in the placenta of rabbits similar to that for cholesterol to some extent, but that the degree of transfer of these substances through the placenta is definitely limited. A comparison of the composition of the lipids in maternal and in fetal blood at birth and during the lactation period is found in the following section.

i. The Effect of Lactation. At the start of the lactation cycle, the level of lipids in the maternal blood is high, while that in the fetal blood has a low value. Slemons and Stander[249] reported that, at the time of delivery, the blood of the mother had total cholesterol values varying between 195 and 330 milligram per cent, as contrasted with figures of 120 and 230 milligram per cent in the fetal blood. These figures correspond closely to those reported by Rosenbloom,[250] which were 223 and 120 milligram per cent, respectively.

As lactation proceeds, there is a rapid change in the levels of blood lipid in the mother and child. This results from the lowering of the high values in the mother's blood, as well as from an augmentation in the low figures of the infant's blood. The colostrum of women contains considerably more cholesterol than does the milk secreted later.[333–335]

During lactation, the blood lipids remain elevated over much longer periods in the cow than in the human subject. Maynard and associates[336] found that the total fatty acids, phospholipids, and cholesterol in cows rose rapidly following parturition in an approximately parallel manner; this was followed by a gradual drop to the prelactation figure as the dry

[331] G. Popják and M. L. Beeckmans, *Biochem. J.*, *45*, x (1949).
[332] G. Popják and M. L. Beeckmans, *Biochem. J.*, *46*, 99–103 (1950).
[333] F. W. Fox and J. A. Gardner, *Biochem. J.*, *18*, 127–135 (1924).
[334] L. Wacker and K. F. Beck, *Berl. klin. Wochschr.*, *58*, 452–457 (1921).
[335] L. Wacker and K. F. Beck, *Z. Kinderheilk.*, *27*, 288–292 (1921).
[336] L. A. Maynard, E. S. Harrison, and C. M. McCay, *J. Biol. Chem.*, *92*, 263–272 (1931).

period approached. The variations in blood lipids were shown to be independent of fat ingestion and of total food intake. Lactation therefore must exert an effect on blood lipids which is not connected with the food intake. In later work from the same laboratory, Schaible[16] determined blood lipids in individual cows over the entire lactation cycle. Whereas the various figures for the different animals in the lactating or non-lactating states showed considerable variation, blood lipids always increased during lactation, irrespective of how high the prelactation figure had been. Despite the marked increase in blood lipid which resulted on lactation, the character of the fatty acids, as determined by the iodine number, remained unchanged. Typical results of these experiments are given in Table 12.

TABLE 12

THE AVERAGE PLASMA LIPIDS OF LACTATING AND NON-LACTATING COWS, AND OF STEERS[a]

		Total fatty acids		
Category	No. of tests	Amt., mg./100 ml.	Iodine number	Lipid P, mg./100 ml.
Non-lactating cows	5	166	101	4.70
Lactating cows	6	374	111	7.38
Steers	2	160	103	4.60

[a] P. J. Schaible, *J. Biol. Chem.*, *95*, 79–88 (1932).

j. The Effect of Immunization. Chino[337] reported that the immunization of rabbits with egg albumen or with sheep corpuscles results in an increase in the immune sera of both free and esterified cholesterol, phospholipids, neutral fat and fatty acids. The increase in the lipid content of the serum is apparently not essential to the formation of the antibodies or to the antigen-antibody reaction. The rise in serum lipid may rather be due to the stimulation resulting from the injection of an antigenic substance.

(2) *The Effect of Diet on the Level of Blood Lipids*

a. The Effect of the Ingestion of Fats. Whenever fatty substances are ingested which are capable of being absorbed, they are carried to the blood stream by way of the chyle. Under such conditions, there is a rapid increase in the microscopic fat droplets (chylomicrons). They consist largely of neutral fat, with practically no admixture of phospholipids or cholesterol. The chylomicrons have a diameter of one micron or less. Ludlum and co-workers[4] have suggested that the particles are stabilized by protein films; these workers were able to demonstrate an aggregation of

[337] H. Chino, *J. Biochem. (Japan)*, *39*, 4P (1952).

particles when the pH of the solution approached the isoelectric point of the protein. Thus, a coalescence of the particles was observed when the acidity was sufficient to precipitate the protein films. For a further discussion, see pages 426–450.

(*a*) *Immediate Effects of the Ingestion of Fat.* a′. The Effect of the Ingestion of Fat on Blood Fats and Fatty Acids: As early as 1877, Ahlfeld[338] demonstrated that the blood serum of a dog became milky, due to its increased fat content, after a heavy fat meal was given. Subsequently, it was shown that a hyperlipemia could readily be produced in carnivora,[223,224,339] but not in herbivora. In most cases, attempts to produce an alimentary lipemia in man have produced only slight effects, or have even resulted negatively.[281,340–344] The marked variation between the susceptibility of carnivora and of man to alimentary lipemia, after fat ingestion, is believed to be partially explained by differences in the dosages of fat employed. Recognizing this fact, Man and Gildea[345] carried out tests, with normal adults, in which the fat dosage was fixed at 3.5 g. per kilogram body weight. By means of this experimental procedure, these workers were able to demonstrate a marked alimentary lipemia in man. On the other hand, when the dosage was reduced to 0.5 g. per kilogram body weight, only minor changes in the levels of blood lipids were noted, and the results were quite inconstant.

After the ingestion of a large dose of fats, there is a gradual increase in blood lipids; the serum lipids may remain elevated for six hours or longer, depending upon the quantity of fat given. The maximum level may not be reached until as late as the sixth hour. The fat tolerance curve obtained after a test meal of fat stands in sharp contrast to the glucose tolerance curve, not only in respect to the slow rate of development, and the prolonged period required to reach the maximum, but also as regards the prolonged decay curve.

On the basis of experiments on dogs, Bang[346] reported differences in the hyperlipemia produced by various fats. Thus, lard did not cause any increase in blood lipids, but butter and olive oil elicited a considerable

[338] F. Ahlfeld, *Centr. Gynäkol.*, *1*, 265–267 (1877).
[339] K. Reicher, *Verhandl. deut. Kongr. inn. Med.*, *28*, 327–330 (1911).
[340] A. Hiller, G. C. Linder, C. Lundsgaard, and D. D. Van Slyke, *J. Exptl. Med.*, *39*, 931–955 (1924).
[341] I. L. Chaikoff, T. H. McGavack, and A. Kaplan, *J. Clin. Invest.*, *13*, 1–13 (1934).
[342] J. A. Gardner and H. Gainsborough, *Biochem. J.*, *22*, 1048–1056 (1928).
[343] I. H. Page, L. Pasternak, and M. L. Burt, *Biochem. Z.*, *223*, 445–456 (1930).
[344] H. R. Rony and A. J. Lévy, *J. Lab. Clin. Med.*, *15*, 221–228 (1929–1930).
[345] E. B. Man and E. F. Gildea, *J. Biol. Chem.*, *99*, 61–69 (1932–1933).
[346] I. Bang, *Biochem. Z.*, *91*, 111–121 (1918).

response. Bloor[222] likewise followed the blood-fat picture in normal dogs after the feeding of olive oil. He found that the maximum values for blood lipids were reached in four to six hours after the ingestion of the fat; the blood lipids were still elevated seven to nine hours after the fat meal. When the thoracic duct was ligated, no variation in these components was

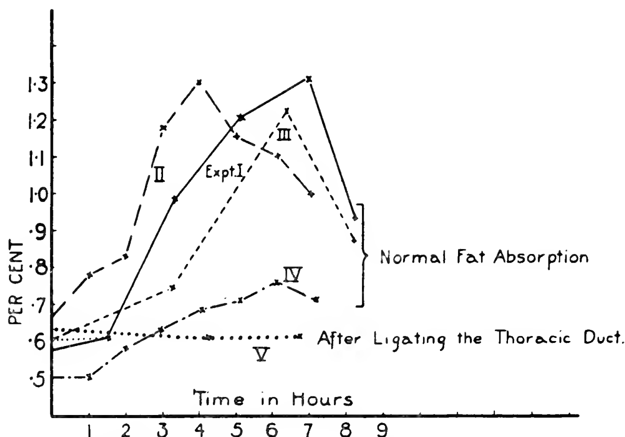


Fig. 3. Changes in the fat content of the blood of dogs, following the feeding of olive oil.[222]

noted. These results are illustrated in Figure 3. Jacobson *et al.*[347] reported that the greatest effect on blood lipids of calves was obtained by feeding diets containing whole milk or crude soybean oil; the lowest degree of lipemia was noted when diets containing hydrogenated soybean oil were fed, while butter oil or lard produced an intermediate effect.

Man and Gildea[345] reported an average increase of 62% (34–133%) in the serum fatty acids of man following the ingestion of meals containing 3.4 g. of fat per kilogram body weight. However, the rise in phospholipid fatty acids was much less pronounced; the mean increase was 18%, with a range between 5 and 28%. The values were at the maximum after six hours, when the tests were terminated. Figure 4 is a graphic representation of these data as presented by Peters and Van Slyke.[202]

b′. The Effect of the Ingestion of Fat on Blood Phospholipids: The

[347] N. L. Jacobson, J. H. Zaletel, and R. S. Allen, *J. Dairy Sci.*, *36*, 832–842 (1953).

evidence which was accumulated during the past forty years has consistently shown that the ingestion of fat causes a moderate but definite concomitant rise in phospholipids. Reicher[339] reported an increase of 32% in blood lecithin after fat feeding; this was greater than the rise in the neutral fat fraction. Other workers likewise obtained higher phospholipid values in blood serum after fat feeding, although these increases were less marked.[89, 90, 345, 348–350] Jacobson and co-workers[347] reported that the ingestion of fat by newborn calves is followed by an increase in blood phospholipids. Bloor alone,[223] and in collaboration with Gillette and James,[225]

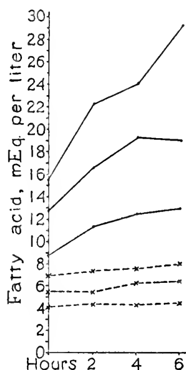


Fig. 4. Serum fatty acids after the ingestion of a meal containing 3.4 g. to 4.0 g. of fat per kilogram body weight, in the form of cream and butter. The 3 top curves represent the maximum, average, and minimum responses, respectively, of total fatty acids, while the lower 3 curves show the corresponding data for the phospholipid fatty acids.[202, 345]

reported that an increase in blood lecithin occurred in the plasma after fat feeding. An increase in the lecithin in the blood corpuscles was observed by Knudson[89] and by Bodansky.[90] Wendt[351] found that the increase in phospholipids occurred only in the plasma, and that the red blood cells took no part in the response to the feeding of neutral fat. Artom and Ped-

[348] I. Bang, *Biochem. Z.*, *91*, 104–110 (1918).
[349] W. Hueck and L. Wacker, *Biochem. Z.*, *100*, 84–99 (1919).
[350] T. F. Zucker, *Proc. Soc. Exptl. Biol. Med.*, *17*, 89–91 (1920).
[351] H. Wendt, *Biochem. Z.*, *250*, 212–219 (1932).

retti[352] repeated these studies, by the use of iodized fats; they observed a rise in the levels of total fat, fatty acid, and phospholipid in the corpuscles and plasma, although the increase was slight. On the other hand, Vahlquist[353] was unable to observe any increase in cell phospholipid after fat feeding, and this result was also obtained by Bloor in his later work.[7] One must conclude, as a result of these findings, that the ingestion of neutral fat is normally followed by a rise in the blood phospholipids, which may or may not be reflected in the erythrocytes.

c'. The Effect of the Ingestion of Fat on Blood Cholesterol: The results of various workers have given quite variable answers to the question as to whether or not the administration of fat causes a concomitant rise in the blood cholesterol level. Terroine,[226] Reicher,[339] Widal *et al.*,[354] Bloor, Gillette, and James,[225] and Li and Freeman[355] reported positive results on dogs, and Milbradt[356] confirmed the findings on rabbits. However, Bloor,[223] in his earlier studies, was unable to demonstrate any change in blood cholesterol following the feeding of fat. Blix[255] and Bang[348] likewise obtained negative results on dogs. In those instances in which the findings were positive, it was observed that the cholesterol:fatty acid ratio remained approximately constant.

Most of the earlier data on human subjects failed to produce convincing evidence that hypercholesterolemia is a necessary concomitant of a fat meal. Thus Hiller *et al.*[340] noted only irregular increases in blood cholesterol after fat feeding, and no uniformity in the cholesterol:fatty acid ratio obtained. Brun[218] failed to demonstrate alterations in blood cholesterol, while Turner and Steiner[282] reported that the blood cholesterol in man is remarkably independent of food intake. On the other hand, Wendt[351] found definite evidence that hypercholesterolemia followed the ingestion of olive oil; however, the increase in cholesterol took place only in the plasma. Several reports indicate that a higher blood cholesterol may occur when the fat intake is high. For example, Schmidt-Thomé and associates[357] noted that the serum cholesterol declined in normal subjects in Germany over the years 1942 to 1947, which coincided with the period of increasing fat shortages. Over this interval, total cholesterol dropped from 191 to 160 milligram per cent, free cholesterol decreased from 51

[352] C. Artom and G. Pedretti, *Boll. soc. ital. biol. sper.*, *7*, 980–984 (1932).
[353] B. Vahlquist, *Biochem. J.*, *25*, 1628–1633 (1931).
[354] F. Widal, A. Weill, and M. Laudat, *Semaine méd.*, *32*, 529–531 (1912).
[355] T. W. Li and S. Freeman, *Am. J. Physiol.*, *145*, 660–666 (1946).
[356] W. Milbradt, *Biochem. Z.*, *223*, 278–322 (1930).
[357] H. Schmidt-Thomé, G. Schettler, and H. Goebel, *Z. physiol. Chem.*, *283*, 63–68 (1948).

to 44 milligram per cent, and esterified cholesterol changed from 141 to 116 milligram per cent. The authors ascribe these changes to the deficient diet. Moreover, Anderson and Keys[358] reported that the serum cholesterol of patients decreased 21 milligram per cent over a four-week period when the fat was decreased from 140 to 70 g. daily in an equicaloric diet containing 0.7 g. of cholesterol. After the preliminary decrease during the first four weeks, no further adjustment in blood cholesterol was noted over the following thirty-two weeks. Tanner[359] suggested that a relationship exists between the serum cholesterol level and physique; a correlation was also noted between the cholesterol level and the thickness of various fat folds.

Various explanations have been offered for the increased cholesterol in cases in which a hypercholesterolemia follows fat feeding. Some workers ascribed the increased blood cholesterol to synthesis, although Terroine[226] states that there is no evidence for such an hypothesis. Moreover, Alfin-Slater and co-workers[360] have reported that no differences in the rate of cholesterol synthesis obtain in rats receiving diets containing 30% of fat, and regimens with only 2% of this foodstuff, respectively. Moreover, there are no known reserves of cholesterol sufficient to account for the increase in cholesterol which has been described by some investigators. Milbradt,[356] who observed an increase in plasma cholesterol in rabbits following the feeding of triolein, explained the rise as resulting from a washing out of cholesterol from the tissues, since he noted a simultaneous decrease in adrenal cholesterol.

Bloor[7] suggested the possibility that a reabsorption of bile cholesterol may well be the source of this sterol in the blood. On the basis of the calculations of Bürger,[361] an adequate supply of the sterol would be available if a recirculation of bile cholesterol followed the administration of fat. Another suggestion as to a possible relationship between cholesterol and dietary fat is that of Schramm and Wolff.[362] These workers suggest that cholesterol aids in the absorption of fat by combining with fatty acids and facilitating their transport from the intestine. When the total fat intake is reduced, less cholesterol is required, and a decrease in blood cholesterol occurs.

It has recently been observed by Alfin-Slater *et al.*[363] that, although a

[358] J. T. Anderson and A. Keys, *Federation Proc.*, *12*, 169 (1953).

[359] J. M. Tanner, *J. Physiol.*, *109*, 13 P (1949).

[360] R. B. Alfin-Slater, M. C. Schotz, F. Shimoda, and H. J. Deuel, Jr., *J. Biol. Chem.*, *195*, 311–315 (1952).

[361] M. Bürger, *Ergeb. inn. Med. u. Kinderheilk.*, *34*, 583–701 (1928).

[362] G. Schramm and A. Wolff, *Z. physiol. Chem.*, *263*, 61–72 (1940).

[363] R. B. Alfin-Slater, L. Aftergood, A. F. Wells, and H. J. Deuel, Jr., *Arch. Biochem. Biophys.*, in press, 1954.

decrease in plasma cholesterol occurs in rats fed over a period of weeks on a fat-free diet, the cholesterol level in the liver and adrenal glands is increased markedly above the normal value. In contradistinction to this, rats fed on a 12.5% fat diet presented normal levels of plasma cholesterol, together with a normal cholesterol content in the liver and adrenals. Thus, while the cholesterol level in the livers of animals on the high-fat diet was approximately 2 milligram per cent, that found for the rats on the fat-free diet varied from 3 to over 6 milligram per cent. It is thus evident that plasma cholesterol may not mirror the storage and the concomitant deposition of this lipid occurring in the liver.

(*b*) *Postabsorptive Effects of the Ingestion of Fat.* In addition to the immediate effects of fat ingestion on the level of various components of the serum lipids, there may likewise be a prolonged effect, which persists over several days, on the levels of their constituents. Bloor[364] found that the feeding of moderately high fat diets to dogs over considerable periods of time resulted in increased amounts of serum phospholipids, but that the neutral fat and cholesterol in the serum were less affected. Bloor[227] later reported that the phospholipid level of dogs was elevated over several days following a single large feeding of fat or carbohydrate (making up more than 50% of the calories); protein, however, did not provoke this augmentation in blood phospholipid. Hansen and his collaborators[365] likewise found that the feeding of fatty diets increased the blood lipids of dogs; in their experiments, all fractions of the blood were affected. Entenman and Chaikoff[366] were able to demonstrate that, after one feeding, an increase in blood cholesterol occurred, while the fatty acid and the phospholipid in the serum were only slightly increased. On the other hand, Flock *et al.*[367] reported that the postprandial level of blood lipids was increased in their dogs only when phospholipids were fed concomitantly with neutral fat; the higher percentages of neutral fat resulted in a greater response. Bloor[364] noted that the rabbit is much more sensitive to changes in dietary fat, as reflected in the level of blood lipids, than is the dog or man.

According to a number of reports of tests on human subjects, a relationship appears to obtain between the dietary fat and the level of serum lipids. Thus, McQuarrie and associates[368] reported a marked increase in

[364] W. R. Bloor, *J. Biol. Chem.*, *95*, 633–644 (1932).
[365] A. E. Hansen, W. R. Wilson, and H. H. Williams, *J. Biol. Chem.*, *114*, 209–222 (1936).
[366] C. Entenman and I. L. Chaikoff, *J. Biol. Chem.*, *142*, 129–139 (1942).
[367] E. V. Flock, W. C. Corwin, and J. L. Bollman, *Am. J. Physiol.*, *123*, 558–565 (1938).
[368] I. McQuarrie, C. Husted, and W. R. Bloor, *J. Clin. Invest.*, *12*, 255–265 (1933).

serum lipids in epileptic children receiving ketogenic diets composed almost exclusively of protein and fat. Tolstoi[369] likewise reported that a hyperlipemia and a hypercholesterolemia occurred in two human subjects who lived on an exclusive meat and fat diet for over a year. As soon as the diet was discontinued, the levels of these components returned to normal. In experiments designed for the study of the effect of dietary fat on the iodine value of the blood fatty acids, Nhavi and Patwardhan[370] also demonstrated that a correlation exists between the fat intake and the level of lipid components in fasting blood. The results of these tests are summarized in Table 13.

TABLE 13
AVERAGE VALUES OF SERUM LIPIDS AS RELATED TO FAT INTAKE[a]

Group No.	Range of daily fat intake, g.	No. of tests	Values, mg./100 ml. serum			Av. iodine value of fatty acids[b]
			Total lipids	Total fatty acids	Total cholesterol	
I	0– 30	12	543	385	158	108 ± 29.5
II	31– 60	23	539	384	155	115 ± 25.7
III	61– 90	22	555	392	163	130 ± 22.4
IV	91–130	17	616	445	171	135 ± 18.1
Av. I + II	*0– 60*	*35*	*540*	*384*	*156*	*112 ± 25.9*
Av. III + IV	*61–130*	*39*	*581*	*415*	*166*	*132 ± 20.5*

[a] Adapted from N. G. Nhavi and V. N. Patwardhan, *Indian J. Med. Research, 34,* 257–262 (1946).
[b] Including standard deviation.

b. The Effect of the Ingestion of Phospholipid on the Level of the Blood Lipids. Although there is no doubt that phospholipids can originate from fats in the intestinal wall and liver, there is some evidence that the blood picture can be considerably modified when they are included in the diet. Thus, in the tests of Flock, Corwin, and Bollman,[367] although the composition of the blood lipids in the dogs was not altered when fat was administered, either alone or with sodium choleate, a marked lipemia developed within a week after the daily addition of 1 g. of crude lecithin from adrenal glands to the regimen. This lipemia continued for the six-week period during which the supplementation was continued. When the administration of phospholipid was discontinued, the blood lipids promptly returned to normal. Neutral fat was the first blood constituent to be affected.

[369] E. Tolstoi, *J. Biol. Chem., 83,* 753–758 (1929).
[370] N. G. Nhavi and V. N. Patwardhan, *Indian J. Med. Research, 34,* 257–262 (1946).

c. The Effect of the Ingestion of Cholesterol on the Level of Blood Lipids. (*a*) *Hypercholesterolemia in Man.* There are conflicting reports as to the effect of ingested cholesterol on the level of blood cholesterol in man. A number of workers have been unable to demonstrate any direct relationship between these two values. Thus, Gardner and Gainsborough[342] reported that no hypercholesterolemia occurred after cholesterol was fed. Likewise, Turner and Steiner[282] could detect no increase in serum cholesterol after the administration of daily doses as high as 20 g. Moreover, Keys[371] reported that single doses of cholesterol, even when large, produced only trivial and transient changes of serum cholesterol in man. It was also stated that the serum cholesterol levels in the blood of "*normal*" men was not significantly related to differences in the habitual cholesterol intake over a range of 250 to 800 mg. daily. On the other hand, Keys[371] did note that the ingestion of a fat-free, cholesterol-free, calorically deficient diet produced a marked decline in the serum of *hypertensive* men. The critical level below which a decrease in serum cholesterol occurs is probably between 0 and 200 mg./day. It is suggested that this critical level may vary from individual to individual. It should be recalled here that although the plasma level of normal rats fed a fat-free diet may be lowered, there is a concomitant increase in the cholesterol deposited in the liver and adrenal glands. Whether a similar situation obtains in man has not been ascertained.

Okey and Stewart[372] are among the investigators who proved that a noticeable increase in the serum cholesterol obtained in young women when cholesterol was fed in the form of egg yolk; this result was confirmed by Steiner and Domanski[373]; however, they attributed the cholesterolemia to the lecithin in the egg yolk, inasmuch as comparable results were not obtained by feeding diets rich in fat and cholesterol, even when the daily amount of dietary cholesterol was increased. Apparently cholesterol is effectively absorbed only when adequate amounts of lecithin are present. Egg yolk affords one of the best sources of this phospholipid. It should be noted, however, that earlier workers failed to obtain hypercholesterolemia when eggs were fed.[374,375] Friedman *et al.*,[376] working with rats, reported that, when normal rabbit serum was substituted for their own plasma,

[371] A. Keys, *Science*, *112*, 79–81 (1950).
[372] R. Okey and D. Stewart, *J. Biol. Chem.*, *99*, 717–727 (1933).
[373] A. Steiner and B. Domanski, *Am. J. Med. Sci.*, *201*, 820–824 (1941).
[374] A. L. Mjassnikow, *Z. klin Med.*, *103*, 767–778 (1926).
[375] H. M. Hunt, *New England J. Med.*, *201*, 659–667 (1929).
[376] M. Friedman, R. H. Rosenman, and S. O. Byers, *Proc. Soc. Exptl. Biol. Med.*, *81*, 393–395 (1952).

hypercholesterolemia rapidly developed in these animals when they were placed on a high cholesterol diet.

(*b*) *Hypercholesterolemia in Animals Other Than Man.* Rats and rabbits differ from man in their reaction to dietary cholesterol. The feeding of an excess of cholesterol in the diet brings about a hypercholesterolemia in rats,[202] as well as fatty livers.[377–380] When cholesterol-containing diets are fed to rabbits with bile salts, not only hypercholesterolemia,[380] but also atherosclerosis occurs.[381,382] In the case of guinea pigs, hypercholesterolemia follows the administration of the sterol, and a fatty infiltration also occurs in the liver.[379,383] Knudson[384] reported an increase in free cholesterol in both the blood plasma and the corpuscles, when either free or esterified cholesterol was fed to dogs. However, no change in cholesterol esters resulted. Although Bloor[7] believes that cholesterol is not appreciably absorbed from the intestine in the absence of fat, Dömösi and Egyed[385] showed that cholesterol was readily absorbed from the alimentary tract if administered in the form of an amorphous suspension in water. Blood cholesterol was shown to rise in four to five days from about 80 to 300–400 mg./100 ml., and in three to four weeks to 1300–1800 mg./100 ml., concomitant with the development of atheroma in the aorta and pulmonary arteries. These results have been confirmed by Popják[386]; the latter investigator notes that amorphous cholesterol is much more effective in increasing blood cholesterol than is cholesterol dissolved in fat.

(*c*) *The Effect of Ingested Cholesterol on Other Blood Lipids.* There is considerable evidence that lipid components other than cholesterol in the blood may be altered by the ingestion of cholesterol-containing diets. Page and Bernhard[387] noted that, when cholesterol in olive oil was fed to rabbits, the phospholipids were increased in the plasma. Weinhouse and Hirsch[388] confirmed this finding and noted that the natural fat and cholesterol fractions of the blood were likewise increased when cholesterol-

[377] C. H. Best, H. J. Channon, and J. H. Ridout, *J. Physiol.*, *81*, 409–421 (1934).
[378] C. H. Best and J. H. Ridout, *J. Physiol.*, *78*, 415–418 (1933).
[379] R. P. Cook, *Biochem. J.*, *30*, 1630–1636 (1936).
[380] L. Swell and D. F. Flick, *Am. J. Physiol.*, *174*, 51–53 (1953).
[381] K. B. Turner, *J. Exptl. Med.*, *58*, 115–125 (1933).
[382] K. B. Turner and E. H. Bidwell, *J. Exptl. Med.*, *62*, 721–732 (1935).
[383] R. Okey, *Proc. Soc. Exptl. Biol. Med.*, *51*, 349–350 (1942).
[384] A. Knudson, *J. Biol. Chem.*, *45*, 255–262 (1921).
[385] P. Dömösi and M. Egyed, *Magyar Orvosi Arch.*, *40*, 242 (1939); cited by G. Popják, *Biochem. J.*, *40*, 608–621 (1946), p. 608.
[386] G. Popják, *Biochem. J.*, *40*, 608–621 (1946).
[387] I. H. Page and W. G. Bernhard, *Arch. Pathol.*, *19*, 530–536 (1935).
[388] S. Weinhouse and E. F. Hirsch, *Arch. Pathol.*, *30*, 856–867 (1940).

containing diets were fed to rabbits. Vermeulen and co-workers[389] also reported a considerable rise of the "non-cholesterol fraction" of serum lipids of rabbits fed cholesterol dissolved in sunflower-seed oil. Dubach and Hill[390] observed that the hypercholesterolemia produced in adult white rabbits by feeding either lanolin or cholesterol was invariably accompanied by a lipemia, and by an increase in all blood lipids. A decrease in the albumin: globulin ratio also obtained after two or three months of cholesterol feeding.

(*d*) *The Effect of Cholesterol Injected Intravenously.* Byers and Friedman[391] prepared a cholesterol suspension which could be injected into rats without producing gross toxicity. Under these conditions, free cholesterol rose to a maximum in the plasma, and decreased gradually for the succeeding forty-eight hours, concomitantly with a rise in the ester fraction. Within thirty-six hours after the injection, however, the cholesterol ester content had returned approximately to the preinjection level. Horlick *et al.*[392] described an intravenous tolerance test for cholesterol in the chicken.

(3) *The Effect of Inanition on the Level of Blood Lipids*

a. The Effect of Complete Starvation. Fat provides the main source for the storage of calories in the animal body. The carbohydrate reserves are relatively small, and are exhausted within two or three days after the initiation of a fast. When this occurs, the body must fall back on its reserve fat depots as a source of calories. Under this condition, the route of the fat is the opposite of that noted in normal alimentation. Instead of following the usual pathway from the intestinal lumen to the lacteals, to the lymphatics, to the blood stream, to the liver, and then to the fat depots, the course is reversed. The fat in the depots is believed to be taken up by the blood stream, then to be carried to the liver and to the extrahepatic tissues for oxidation. As soon as this mechanism becomes established, the rate of transfer of lipids from the fat stores to the blood stream is adjusted to keep pace with the rate of utilization of this foodstuff by the tissues; a state of equilibrium then is reached. The limiting factor in the continuation of this replenishment of the blood lipids from the tissue stores will

[389] C. Vermeulen, L. R. Dragstedt, D. E. Clark, O. C. Julian, and J. G. Allen, *Arch. Surg.*, *44*, 260–267 (1942).

[390] R. Dubach and R. M. Hill, *J. Biol. Chem.*, *165*, 521–531 (1946).

[391] S. O. Byers and M. Friedman, *J. Biol. Chem.*, *177*, 841–846 (1949).

[392] L. Horlick, M. Feldman, Jr., and L. N. Katz, *Proc. Soc. Exptl. Biol. Med.*, *168*, 243–245 (1948).

obviously be the quantity of storage fat available. When these storage depots are exhausted, the protein metabolism is suddenly increased, and death ensues shortly after this so-called "premortal rise."

The experimental data as to the effect of fasting on the composition of blood lipids have been quite variable. Probably one of the most important factors involved in bringing about this lack of uniformity has been the variation associated with the species of the animal.

In experiments on dogs, it was reported[221,222,393] that either an increase or no change in serum lipids occurred on fasting. Terroine[226] obtained variations in total lipid from +45 to −54% in seven dogs fasted from twenty-two to thirty-five days. Although Greene and Summers[394] demonstrated an increase of 200% in the blood fat of fasted puppies, they were unable to find any significant rise in the blood lipids of adult dogs even after prolonged fasting. Underhill and Baumann[395] reported a decrease in blood lipids during the early period of the fast, followed by a rise in blood lipids to normal as the period of the fast progressed. In line with these results, Ling[396] reported a decrease in blood lipids of dogs fasted seven days, while Entenman, Changus, Gibbs, and Chaikoff[397] found no lipemia in dogs subjected to acute fasting for as long as thirty days, or to prolonged undernutrition; however, when the undernutrition resulted in a severe loss in weight, a decrease in the cholesterol, fatty acids, and phospholipid of the blood ensued. Kartin and associates[398] likewise showed that the blood lipids were not increased in the dog on fasting, although their results on the monkey and on man were somewhat different.

According to the results of Sure and collaborators,[399] the rat is also a species in which a decrease in the fatty acids and phospholipids of the blood occurs on fasting; blood cholesterol was found to remain constant. However, Kohn[400] reported that the response of blood cholesterol to one week of fasting varied with the strain of the rats. In the case of the Sprague-Dawley and the Osborne-Mendel strains, cholesterol levels reached twice those of the Holtzman and the Tumblebrook hooded strains. This variation in response had a genetic basis which involved a number of genes.

[393] E. Freudenberg, *Biochem. Z.*, *45*, 467–487 (1912).

[394] C. W. Greene and W. S. Summers, *Am. J. Physiol.*, *40*, 146–147 (1916).

[395] F. P. Underhill and E. J. Baumann, *J. Biol. Chem.*, *27*, 169–172 (1916).

[396] S. M. Ling, *Chinese J. Physiol.*, *5*, 381–397 (1931).

[397] C. Entenman, G. W. Changus, G. E. Gibbs, and I. L. Chaikoff, *J. Biol. Chem.*, *134*, 59–69 (1940).

[398] B. L. Kartin, E. B. Man, A. W. Winkler, and J. P. Peters, *J. Clin. Invest.*, *23*, 824–835 (1944).

[399] B. Sure, M. C. Kik, and A. E. Church, *J. Biol. Chem.*, *103*, 417–424 (1933).

[400] H. I. Kohn, *Am. J. Physiol.*, *163*, 410–417 (1950).

Following hypophysectomy, the blood cholesterol rose to a new level, which was the same in both the "high" and the "low" genetic types.

Strangely enough, the results on mice are diametrically opposed to those for rats. It was proved by MacLachlan[401] that a statistically significant rise in total blood lipids and acetone-soluble lipids occurred when three-month old male albino mice were fasted. Blood phospholipid was apparently related to the prefasting level. No change in the unsaturation of the several fat fractions was noted during fasting, which would indicate that no preferential or selective utilization of storage fats occurred. In a later study[402] it was found that lipemia occurred in the early part of the period, followed by a drop in concentration on the fifth day of fasting.

In the rabbit, the blood cholesterol is elevated on fasting.[403,404] Shope[403] reported earlier that hypocholesterolemia accompanies fasting in the cat, guinea pig, swine, and man. Mann and White[405] found that the hypocholesterolemia observed in dogs subjected to inanition resulted in a disproportionate reduction of the esterified fraction. This plasma change was found to be associated with parallel alterations in the size and cholesterol content of the adrenal glands. It is possible to produce a similar plasma cholesterol change in normal dogs with ACTH, but not with cortisone.

It is generally believed that man, in contradistinction to other species, usually develops a hyperlipemia during inanition. Fahrig and Wacker[300] found that all components of the blood lipids of men were increased during fasting. These findings were confirmed in the extensive experiments of Kartin *et al.*[398] on man. It was found that, after two days of complete fasting, the cholesterol and lipid phosphorus had increased perceptibly in only a few of their subjects. When the fast was continued for six days, both of these fractions increased in all cases. The changes were roughly parallel to those of the ketone bodies, which had appeared in appreciable amounts in the blood in all cases after this interval.

Petersen[405a] noted that the increase in serum phospholipids which occurred during fasting in human subjects was confined to the sphingomyelin fraction, while no changes were observed in the level of serum lecithins or cephalins. It was found that the level of serum sphingomyelin was re-

[401] P. L. MacLachlan, *J. Biol. Chem.*, *152*, 391–394 (1944).

[402] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. A. Welch, S. L. Kornberg, and M. Falkenheim, *J. Biol. Chem.*, *169*, 707–711 (1947).

[403] R. E. Shope, *J. Biol. Chem.*, *75*, 101–113 (1927).

[404] G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, *B85*, 385–393 (1912).

[405] C. V. Mann and H. S. White, *Metabolism*, *2*, 47–58 (1953).

[405a] V. P. Petersen, *Acta Med. Scand.*, *143*, 249–259 (1952).

stored to normal within three hours after the fast was broken by the ingestion of glucose.

On the other hand, Man and Gildea[406] noted a decrease of blood lipids associated with undernutrition. Blix,[407] using lipid glycerine as an index of triglyceride fat, found that the triglyceride content of the blood of fasting individuals was in the range of 30 to 70 milligram per cent, instead of the earlier values of 150–250 milligram per cent. It has been shown that a hyperlipemia of 2 meq. per liter in plasma fatty acids occurs in children after only twenty-four hours of fasting, and that this elevation still persists after forty-eight hours. Peters and Van Slyke[202] attribute this earlier rise of plasma lipids in children, as compared with adults, to the fact that the former are more susceptible to ketosis than are the latter. It is also known that the monkey, which is like man in developing a fasting ketosis, also develops a rapid hyperlipemia during fasting.[398] These data all fit in with the hypothesis that the hyperlipemia of fasting is related to the attendant ketosis. Cholesterol and phospholipids are the main lipids which bring about the increase in total blood lipids. If the above theory is correct, then the rise in blood lipids should appear more promptly and reach a higher level in women than in men. This would be expected, in view of the fact that ketonuria appears sooner after the start of a fast, and is much more severe during the course of this period, in women than in men.[408]

The serum lipoproteins have been found to be increased in man during fasting. Thus, Rubin and Aladjem[408a] reported a significant increase in the concentration of low-density serum lipoproteins in five of six subjects studied within a fast period of four or five days. However, the high-density lipoproteins were not increased under these conditions. The level of blood lipoproteins was restored to normal within twenty-four hours after the resumption of the usual diet, although it was not significantly altered three hours after the ingestion of sucrose. In contradistinction to the results on man, Aladjem and Rubin[408b] found that all fractions of lipoprotein (S_f 0–12, 12–20, 20–100, 100–400) were significantly increased by fasting for three days, in the case of rabbits. After seven days of fasting, the level of S_f 0–12 was further increased, in the blood of the animals, that of the S_f 12–20 and 20–100 remained constant, while a drop in the level of the S_f 100–400 fraction was noted.

[406] E. B. Man and E. F. Gildea, *J. Clin. Invest.*, *15*, 203–214 (1936).
[407] G. Blix, *Biochem. Z* , *305*, 145–149 (1940).
[408] H. J. Deuel, Jr., and M. Gulick, *J. Biol. Chem.*, *96*, 25–34 (1932).
[408a] L. Rubin and F. Aladjem, *Am. J. Physiol.*, in press (1954).
[408b] F. Aladjem and L. Rubin, *Am. J. Physiol.*, in press (1954).

b. The Effect of Carbohydrate Deficiency. In an earlier section, it was explained that serum lipids in man are increased on a carbohydrate-low diet (see page 417). The interpretation of the reason for such a change was that it was due to the high fat levels ordinarily found in these carbohydrate-low diets. However, this dietary regimen will likewise produce ketosis, and, in fact, the diet is frequently referred to as a "ketogenic" diet. In experiments on epileptic children, McQuarrie *et al.*[368] found serum cholesterol values as high as 555 milligram per cent. Lipid phosphorus also increased, as evidenced by the cholesterol:lipid phosphorus level, which varied greatly in the patients studied. The ratio of lecithin to cholesterol was always higher at or near the time of the convulsions. Likewise, in the experiments reported by Tolstoi,[369] a serum cholesterol level as high as 800 milligram per cent was noted, while values of 400 and 600 milligram per cent were found on other occasions. In the case of two subjects observed by Tolstoi, who partook of an exclusive meat-fat diet for a year, ketonuria was constantly present. It is therefore evident that the hyperlipemia observed on the protein-fat diets may as readily be attributed to the attendant ketonuria as to the high fat intake. However, it is possible that both factors are involved, and that one condition may reinforce the other.

(4) *The Effect of Overnutrition on the Level of Blood Lipids*

Overnutrition, as evidenced by obesity, does not appear to produce any special characteristics of the blood lipid patterns,[213,255,409,410] or results in only slight hyperlipemia.[411] Arnoldi and Collazo[412] reported that the blood lipids of obese individuals were lower than the normal. A similar finding was reported somewhat later in hogs, by Knauer,[413] who found that low blood lipid values were the rule during fattening. Hetényi[411] likewise noted a greater depression in blood fatty acids in obese persons maintained for eight days on highly inadequate diets than in normal persons who had received similar diets.

However, some of the more recent results have not demonstrated a depressed fat level in the blood of overweight individuals, but rather normal values. Thus, Gildea *et al.*[268] found that the serum lipids were the same in obese children and adults, of both sexes, as in corresponding individuals of

[409] M. Bruger and C. A. Poindexter, *Arch. Internal Med.*, *53*, 423–434 (1934).
[410] W. Denis, *J. Biol. Chem.*, *29*, 93–110 (1917).
[411] G. Hetényi, *Deut. Arch. klin. Med.*, *179*, 134–141 (1936).
[412] W. Arnoldi and I. A. Collazo, *Z. ges. exptl. Med.*, *40*, 323–340 (1924).
[413] H. Knauer, *Z. physiol. Chem.*, *176*, 151–172 (1928).

normal weight. Similar conclusions can be reached on the basis of the work of Bruger and Poindexter,[409] as well as of that of Rony and Lévy.[344] Moreover, no correlation was found between weight changes in obese subjects and the concentration of their blood lipids.[213,414] It is believed that reductions of blood fat occurring during the period when a loss of body weight is observed[213,411] are referable to the manner in which reduction was effected, rather than to the loss of fat in itself.[202] Nissen[415] reported that a greater increase in blood fat occurs in obese than in normal persons after fat is fed.

The inability to demonstrate a correlation between blood lipids and obesity is to be expected. No greater rate of utilization of fat occurs in obesity than in normal conditions; in all probability the opposite situation obtains. Since fat is not being oxidized in unusual amounts in obese individuals, no increased transport of the lipids from the fat depots to the liver is required; hence, a hyperlipemia does not occur. The fact that the metabolism of fat is slower in the obese male during fasting is indicated by the experiments of Deuel and Gulick[408] in which the single subject who was overweight exhibited the lowest level of ketonuria.

(5) *The Effect of Work on the Level of Blood Lipids*

A number of investigators have shown that an increase in blood lipids accompanies hard work. Gage and Fish[3] first reported that an increase in the chylomicron count in the blood occurred concomitantly with exercise. This would indicate that a rise in the neutral fat level had taken place. Stewart *et al.*[416] likewise reported an increase in blood lipid after strenuous exercise; they believe that only the triglyceride fraction (neutral fat) is involved. Several other workers[300,417] have also reported an increased lipemia as a result of heavy energy output. On the other hand, Hiramatsu[418] found no change in the blood fat of rats following exercise, if they had been on a rice diet. However, if the previous dietary regimen had been largely protein, a marked decrease in blood lipids resulted after exertion.

Reports on the effect of hard work on blood cholesterol levels are conflicting. Robinson and co-workers[419] reported a marked drop in chole-

[414] C. A. Poindexter and M. Bruger, *Arch. Internal Med.*, *56*, 884–890 (1935).
[415] N. I. Nissen, *Acta Med. Scand.*, *73*, 99–124 (1930).
[416] C. P. Stewart, R. Gaddie, and D. M. Dunlop, *Biochem. J.*, *25*, 733–748 (1931).
[417] J. R. Murlin and J. A. Riche, *Am. J. Physiol.*, *40*, 146 (1916).
[418] T. Hiramatsu, *Biochem. Z.*, *255*, 304–305 (1932).
[419] S. H. G. Robinson, W. R. Brain, and H. D. Kay, *Lancet*, *1927*, *II*, 325–326.

sterol in both the plasma and the corpuscles following exertion. On the other hand, Patterson[420] observed increases in blood fat of as much as 40% after exercise, although this increase could be prevented if sugar was given. However, Patterson,[420] Rakestraw,[421] and Cattoretti[422] reported that the blood cholesterol remains unchanged after strenuous exercise. Tanner[423] found a mean value for serum cholesterol in forty-six young men of 190.5 ± 28.5 milligram per cent. A statistically significant correlation was found to exist between serum cholesterol and subscapular subcutaneous tissue, as well as between the cholesterol and the endomorphic component of physique. The effect of exercise on ketosis is discussed later (see Volume III).

(6) Specific Compounds that Affect Alimentary Lipemia

a. The Effect of Heparin. (*a*) *Heparin as an Anticoagulant.* Heparin is a substance originally isolated from liver, in 1918, by Howell and Holt,[424] who recognized that it possesses an anticoagulating action in blood. It was subsequently further purified by Howell,[425] and by Charles and Scott,[426] who also found that beef lung was superior to beef liver as a source of the product.[427] It is now known that the heparin molecule contains a hexuronic acid (presumably glucuronic acid), glucosamine, esterified sulfuric acid groups, and acetylated amino groups.[428–432] This composition would suggest that heparin should be classified as a chondroitin sulfuric acid. The anticoagulant properties are presumably referable to the presence of sulfuric acid in the molecule. According to Charles and Scott,[427] heparin is widely distributed in tissues; these workers cite the following concentration on the substance (expressed in Howell units per kilogram of tissue): dog liver, 4400; beef liver, 1900; hog liver, 1700; beef lung, 2200; beef muscle, 1900; beef heart, 380; and beef blood, 60.

(*b*) *Heparin as a Clearing Agent.* The ability of heparin to cause rapid

[420] J. W. T. Patterson, *Biochem. J.*, *21*, 958–966 (1927).
[421] N. W. Rakestraw, *J. Biol. Chem.*, *47*, 565–591 (1921).
[422] F. Cattoretti, *Arch. ital. biol.*, *63*, 113–121 (1915).
[423] J. M. Tanner, *J. Physiol.*, *115*, 371–390 (1951).
[424] W. H. Howell and E. Holt, *Am. J. Physiol.*, *47*, 328–341 (1918).
[425] W. H. Howell, *Am. J. Physiol.*, *63*, 434–435 (1923).
[426] A. F. Charles and D. A. Scott, *J. Biol. Chem.*, *102*, 425–429 (1933).
[427] A. F. Charles and D. A. Scott, *J. Biol. Chem.*, *102*, 431–435 (1933).
[428] W. H. Howell, *Bull. Johns Hopkins Hosp.*, *42*, 199–206 (1928).
[429] A. F. Charles and D. A. Scott, *Biochem. J.*, *30*, 1927–1933 (1936).
[430] A. F. Charles and A. R. Todd, *Biochem. J.*, *34*, 112–118 (1940).
[431] E. Jorpes, *Biochem. J.*, *29*, 1817–1830 (1935).
[432] E. Jorpes, *Acta Med. Scand.*, *88*, 427–433 (1936).

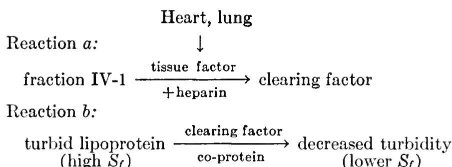
clearing of the cloudiness in the plasma obtained during an alimentary lipemia was first recognized, in 1943, by Hahn.[433] Meng and Youmans[434] found that when young unanesthetized dogs were injected intravenously with 10% fat emulsion in doses of 1 g./kg. body weight, the administration of heparin reduced the total maximum fatty acid level. The total cholesterol in the plasma decreased in the heparin-injected group, in contrast to an increase in the control group. No significant differences in the plasma lipid P in the two groups, respectively, were observed. Block and collaborators[435] reported that the injection of heparin caused a greater decrease in the cloudiness of the lipemic plasma of men than in that of women.

Although turbidity could be decreased *in vivo* when heparin was injected, the substance does not produce this effect when added to the lipemic serum outside of the body.[433,436–438] However, when the heparin reaction has been initiated within the body, it continues when the blood is removed from the body.[439,440] This heparinized plasma has been shown to be active in the *in vitro* clearing of a synthetic fat emulsion as well.[441] Anderson and Fawcett[439] interpreted these results as indicating that the injection of heparin activates or stimulates the production of a substance which appears in the plasma, and which possesses "antichylomicronemic" properties. The action of clearing plasma is shared by other heparin-like substances,[442] as well as by a cholesterol-free brain fraction.[443]

The experiments of Anfinsen *et al.*[444] confirmed the property of heparin in clearing up lipemic plasma. However, these latter workers demonstrated that heparin alone is inactive, as is a tissue extract prepared from heart. However, when the tissue extract and heparin are combined, the turbidity of lipemic plasma can be reduced. The scheme on the following page has been suggested by these investigators.

Brown *et al.*[445] reported that the rate of clearing is proportional to the concentration of co-protein, if the clearing factor is kept at a constant level.

[433] P. F. Hahn, *Science, 98*, 19–20 (1943).
[434] H. C. Meng and J. B. Youmans, *Federation Proc., 12*, 424 (1953).
[435] W. J. Block, F. D. Mann, and N. W. Barker, *Circulation, 4*, 464–465 (1951).
[436] C. B. Weld, *Can. Med. Assoc. J., 51*, 578 (1944).
[437] J. J. Spitzer, *Am. J. Physiol., 174*, 43–45 (1953).
[438] A. Comfort, *Biochem. J.*, xxiii (1953).
[439] N. G. Anderson and B. Fawcett, *Proc. Soc. Exptl. Biol. Med., 74*, 768–771 (1950).
[440] J. J. Spitzer, *Am. J. Physiol., 171*, 492–498 (1952).
[441] C. Hollett, W. Cole, and H. C. Meng, *Federation Proc., 12*, 70 (1953).
[442] J. M. Waldron and M. H. F. Friedman, *Federation Proc., 7*, 130 (1948).
[443] R. J. Jones, S. C. Kraft, E. L. Balter, and S. Huffman, *Federation Proc., 12*, 227 (1953).
[444] C. B. Anfinsen, E. Boyle, and R. K. Brown, *Science, 115*, 583–586 (1952).
[445] R. K. Brown, E. Boyle, and C. B. Anfinsen, *J. Biol. Chem., 204*, 423–434 (1953).



In a later report, Brown and Kauffman[446] reported that the *in vivo* administration of heparin to a variety of animals causes the production of a plasma enzyme, termed the "clearing factor." This protein, in the presence of a second plasma protein, or "co-protein," brings about a decrease in the turbidity of lipemic plasmas, a decrease in the ultracentrifugation flotation rates of low density lipoproteins, and a concomitant production of α-lipoprotein molecules. The lipemia clearing factor can be produced *in vitro* when heparin is incubated with rat pylorus; the activity is stable for three months, in contrast to the instability of preparations obtained when rat heart or lung extracts are employed.

Only injected heparin will initiate the production of the clearing factor. Thus, Levy and Swank[447] reported that conditions such as shock, due to anaphylaxis or to the injection of certain histamine liberators, which is known to bring about the appearance of native heparin in the blood, does not necessarily coincide with the clearing of a lipemia. Heparin does not inhibit cortisone-induced lipemia or hypercholesterolemia in the rabbit; in fact, Constantinides *et al.*[448] reported that it even tended to increase it.

The most important effect of heparin is on the formed fat particles. Fidlar and Jaques[449] suggested that heparin reduces the number of blood-platelets, in a manner similar to its effect upon the dispersion of the chylomicrons. It presumably combines with the lipoprotein complex or splits off the lipid portion and becomes itself bound with the protein moiety.[450] Snellman *et al.*[451] isolated a compound present in tissue mast cell cytoplasm containing heparin associated with a polypeptide and a lipid residue. All three components must be present to produce the antithrombin effect. It is not known whether or not this is similar to the clearing factor. Swank and Levy[452] also postulate that the mechanism by which heparin lowers

[446] R. K. Brown and D. L. Kauffman, *Federation Proc.*, *12*, 183–184 (1953).

[447] S. W. Levy and R. L. Swank, *Proc. Soc. Exptl. Biol. Med.*, *82*, 553–556 (1953).

[448] P. Constantinides, G. Szasz, and M. Darrach, *XIX Intern. Physiol. Congress*, Montreal (Aug.–Sept., 1953), *Abst.* 277–278.

[449] E. Fidlar and L. B. Jaques, *J. Lab. Clin. Med.*, *33*, 1410–1423 (1948).

[450] E. Chargaff, *Advances in Protein Chem.*, *1*, 1–24 (1944).

[451] O. Snellman, B. Sylven, and C. Julen, *Biochim. et Biophys. Acta*, *7*, 98–109 (1951).

[452] R. L. Swank and S. W. Levy, *Am. J. Physiol.*, *171*, 208–217 (1952).

hyperlipemia is by dissolution of the chylomicrons. The injection of heparin greatly accelerates the passage of the neutral fat in the chylomicrons through the capillary walls to the tissues, where its metabolism is facilitated. This transport mechanism is absent from the brain; this organ uses only insignificant amounts of fat in its metabolism. According to Swank,[453] the tendency of the chylomicrons to form clusters is increased after a fat meal; this tendency is slight in whole blood without heparin, as well as in oxalated or citrated blood, but it is marked in heparinized blood. The tendency toward clustering also varies with different individuals[453,454]; it is less after the feeding of unsaturated fats than after the administration of saturated fats. Pregnancy and high-fat diets increase this property, while it is reduced when a low-fat diet is taken. Zinn and co-workers[455] reported that heparin and a related product, "treburon," were both equally effective in reducing lipomicronemia within thirty to sixty minutes. In addition to its effect on neutral fat in the chylomicrons, Basu and Stewart[456] reported that the injection of heparin reduces both free and esterified cholesterol in the plasma.

Grahm and co-workers[457] were the first to demonstrate that heparin may have an important part in the regulation of the size of the lipoprotein molecule elaborated in the plasma. When heparin was given to rabbits or to human subjects, a considerable reorientation of the low density S_f 3–8 lipoproteins occurred. However, the most important reaction was the retardation in the formation of the S_f 10–50 lipoproteins which would normally occur during cholesterol feeding. Boyle, Bragdon, and Brown,[458] by the use of ultracentrifugation and by chemical means, also demonstrated that heparin causes an increase in the high-density α_1-lipoproteins at the expense of certain low-density lipoproteins. Such changes were shown to occur *in vitro* in plasma obtained after the injection of heparin. They suggest that an enzymatic conversion of one lipoprotein class to another results from the injection of heparin. Milch alone[459] and with co-workers[460]

[453] R. L. Swank, *Am. J. Physiol.*, *164*, 798–811 (1951).
[454] W. J. Messinger and Y. Porosowska, *Proc. Soc. Exptl. Biol. Med.*, *82*, 164–167 (1953).
[455] W. J. Zinn, J. B. Field, and G. C. Griffith, *Proc. Soc. Exptl. Biol. Med.*, *80*, 276–278 (1952).
[456] D. P. Basu and C. P. Stewart, *Edinburgh Med. J.*, *57*, 596–599 (1950).
[457] D. M. Graham, T. P. Lyon, J. W. Gofman, H. B. Jones, A. Yankley, and J. Simonton, *Circulation*, *4*, 465 (1951).
[458] E. Boyle, J. H. Bragdon, and R. K. Brown, *Proc. Soc. Exptl. Biol. Med.*, *81*, 475–477 (1952).
[459] L. J. Milch, *Federation Proc.*, *12*, 351 (1953).
[460] L. J. Milch, R. F. Redmond, W. W. Calhoun, and H. I. Chinn, *Am. J. Physiol.*, *170*, 346–350 (1952).

confirmed the fact that heparin induced a reorientation of blood lipoproteins, so that the concentrations of cholesterol-bearing S_f 12–20 and S_f 20–100 are markedly reduced. However, plasma cholesterol levels were unaffected by the exogenous heparin, *in vivo*. On the other hand, adenosine-5-monophosphate caused a reduction of the concentrations in the plasma and aorta of chickens, without producing significant changes in the levels of the several lipoproteins. The effects of heparin and AMP were shown to be additive, in normal and in hyperlipoproteinemic rabbits.[459]

In addition to the clearing of turbidity and the redistribution of lipoproteins, heparin causes a lipolysis. Shore *et al.*[461] noted that when plasma, from human subjects who had received intravenous heparin a short time before the sample was withdrawn, was incubated with certain lipoproteins for four to eight hours at 37°C., a partial hydrolysis of the glyceride component occurred, with a concomitant release of fatty acids. Brown and co-workers[445] also noted the appearance of fatty acids after clearing, which produced turbidity due to the formation of calcium soaps. These workers presented evidence that the plasma esterases are responsible for the formation of the fatty acids.

The clearing factor produced by the injection of heparin was found by Spitzer[440] to be dialyzable. It occurs in the albumin fraction. It is inactivated irreversibly at 49°C. but its action is not affected by thrombin or by thromboplastin. The smallest concentration of heparin which was found to be active was 0.5 µg./ml. The effect of a dosage of 2 mg. lasted forty-five to sixty minutes in a rabbit. Levy and Swank[447] reported that the injection of dextran (molecular weight = 75,000) into dogs produced a marked clearing of a heavy alimentary lipemia in the absence of heparin activity. The explanation for this effect is not immediately apparent.

b. The Effect of Protamine. Heparin and protamine have been shown to have antagonistic effects. Brown[462,463] was the first to observe that the effect of heparin in abolishing lipemia can be counteracted by the administration of protamine. He also proved that the total amount of serum fatty acids, as well as the visible alimentary lipemia, could be increased by the administration of protamine, even when heparin had not previously been injected. However, it failed to influence the endogenous lipemia of pregnancy. Spitzer[437,464] reported that protamine was effective only *in vivo*. Moreover, it failed to influence the fat content in control, fasting rats.[464]

[461] B. Shore, A. V. Nichols, and N. K. Freeman, *Proc. Soc. Exptl. Biol. Med.*, *83*, 216–220 (1953).

[462] W. D. Brown, *Proc. XVIII Intern. Physiol. Congr.*, Copenhagen, 1950.

[463] W. D. Brown, *Quart. J. Exptl. Physiol.*, *37*, 75–84, 119–129, 215–219 (1952).

[464] J. J. Spitzer, *Federation Proc.*, *12*, 137 (1953).

According to Spitzer,[437] the hyperlipemia sets in very slowly, requiring about forty minutes. It occurs only when visible lipemia is already present. The effect can be repeated. Since two other antiheparin substances, namely, toluidine blue and neutral red, failed to influience vsible lipemia, Spitzer [437] suggests that the effect of protamine is a *sui generis* action rather than a consequence of its antiheparin property.

c. The Effect of Bile Acids. Cholic acid, when injected as the sodium salt, causes a hypercholesterolemia in fasting rats.[465] Friedman and Byers[465] suggest that this result may indicate that various hypercholesterolemic states, heretofore considered as primary or idiopathic, may be secondary to an initial derangement of cholate metabolism. In later work, Byers and Friedman[466] reported that, when cholic acid was fed to rats with obstructed bile ducts, the hypercholesterolemia associated with the obstruction was further increased. However, this effect was specific for cholic acid, since desoxycholic, glycocholic, and dehydrocholic acids did not produce this effect.

Siperstein and colleagues[467] demonstrated an interesting effect exerted by ferric chloride, which is related to the bile acids. It was found that the feeding of $FeCl_3$ prevented the hypercholesterolemia and the associated atheromata produced by cholesterol feeding in the cockerel. These findings suggest that the binding of bile salts in the intestinal tract, and consequent suppression of cholesterol absorption, may offer a method for controlling the development of atherosclerosis. It has long been recognized that ferric chloride is able to precipitate bile acids *in vitro*.

d. The Effect of Miscellaneous Substances. Insulin-free pancreatic extracts were found to decrease blood lipids in the case of rabbits.[468] The maximum effect was reached in six hours, and this was followed by a rise above normal during the following eighteen hours. The action of choline was similar but less marked. On the other hand, the intravenous administration of hypercholesterolemic rabbit plasma to normal rabbits resulted in a rapid increase in total serum cholesterol, followed by a gradual decrease.[469] Chung and Shaw[470] noted that the intravenous injection of a surface-active agent, triton, into a goat and two cows resulted in a marked increase in all plasma lipids except phospholipid. In the case of one cow,

[465] M. Friedman and S. O. Byers, *Proc. Soc. Exptl. Biol. Med.*, *78*, 528–529 (1951).

[466] S. O. Byers and M. Friedman, *Am. J. Physiol.*, *168*, 138–139 (1952).

[467] M. D. Siperstein, C. W. Nichols, Jr., and I. L. Chaikoff, *Science*, *117*, 386–389 (1953).

[468] J. Schneider and F. Stutinsky, *Compt. rend. soc. biol.*, *143*, 475–477 (1949).

[469] E. Oppenheim and M. Bruger, *Proc. Soc. Exptl. Biol. Med.*, *75*, 636–638 (1950).

[470] A. C. Chung and J. C. Shaw, *J. Dairy Sci.*, *34*, 1180–1185 (1951).

plasma lipids were found to increase as much as five-fold, while neutral fat was augmented to seventeen times the normal value. Cholesterols (both free and ester forms) were increased four times. A single injection of the surface-active agent resulted in a maximum plasma lipid content of 2.05 milligram per cent. Blood lipids remained above 1 milligram per cent for nine days following the injection. Herrmann[471] reported that the administration of methionine causes a reduction in cholesterol and cholesterol esters in the blood of old hens, concomitantly with an increase of blood phospholipids. Peterson and co-workers[472] likewise noted that the administration of soy sterols to chickens receiving cholesterol prevented the increase in serum cholesterol which would otherwise occur. Streptomycin causes hyperlipemia, presumably as the result of the inositol present in the molecule.[473]

X-radiation has been shown to cause an increase in plasma phospholipid in the case of the rabbit, dog, mouse, guinea pig, and rat, but the time of onset of the changes varied in different species. The alteration of phospholipid can be correlated with the time of death of the animal.[474]

(7) *The Effect of Abnormal Conditions on the Level of Blood Lipids*

a. Idiopathic Familial Hyperlipemia. This is a congenital disorder in which hyperlipemia is a constant accompaniment. It is an exceeding rare condition which has been reported in both children and adults. The classical symptoms are hepatosplenomegaly (enlargement of liver and spleen), xanthomatosis, lipemia retinalis, and a high level of neutral fat in the blood, with only slightly elevated phospholipids and cholesterol, in the absence of other conditions producing hyperlipemia. The blood fat is decreased on a low-fat diet.

Holt and co-workers [475] were unable to improve the extreme lipemia in an eleven-year-old girl with idiopathic familial lipemia by means of lecithin, choline, thyroxine, insulin, lipocaic or by treatment with antuitrin; however, the hyperlipemia could be controlled to some extent by a low-fat diet. In this condition, a large amount of fat can be demonstrated in the liver, which is enlarged.[475,476] It is believed that this condition is due to

[471] G. R. Herrmann, *Proc. Soc. Exptl. Biol. Med.*, *64*, 284–285 (1947).

[472] D. W. Peterson, E. A. Shneour, N. F. Peek, and H. W. Gaffey, *J. Nutrition*, *50*, 191–201 (1953).

[473] L. Mosonyi, L. Pollák, J. Juthász, and R. Zulik, *Lancet*, *1951*, *II*, 81.

[474] C. Entenman, R. A. Neve, and C. A. Olmstead, *Federation Proc.*, *12*, 40 (1953).

[475] L. E. Holt, Jr., F. X. Aylward, and H. G. Timbres, *Bull. Johns Hopkins Hosp.*, *64*, 279–314 (1939).

[476] O. C. Bruton and A. J. Kanter, *Am. J. Diseases Children*, *82*, 153–159 (1951).

a defective mechanism for the removal of blood fat by the liver, a mechanism in which a humoral factor may be involved.[475]

b. The Blood Lipids Following Tissue Injury. Man and co-workers[477] observed that, after surgery, small but unequivocal decrements occurred in serum lipid fractions, total and free cholesterol, lipid phosphorus, and fatty acids. The lipid components were found to be proportionally affected, without distortion such as occurs in liver disease.

c. The Blood Lipids Following the Administration of Various Anesthetics. The several anesthetics have widely varying effects on the blood lipids. Some of them, such as ether and chloroform,[422] produce a marked hyperlipemia, while others are apparently without effect on the blood lipid pattern.

One of the early conceptions of the mechanism of ether anesthesia as described by Reicher[478] was to the effect that the ether dissolved a portion of the fatty material in the brain, and that the fat was transported by the blood to the liver. This theory would offer an explanation for the hyperlipemia observed during anesthesia. However, since increased blood lipids are not an invariable concomitant of anesthesia, one is led to question the above hypothesis as an explanation of the cause of anesthesia. In fact, this assumption was abandoned after the Meyer-Overton theory of narcosis was proposed and had been generally accepted.

The increase of blood lipids is quite marked when ether or chloroform is administered. Reicher[478] was able to demonstrate that blood lipids were increased as much as 300% by various narcotics, and that the increase affected equally the neutral fat, cholesterol, and phospholipid fractions. Bloor[222] and Hospers[479] confirmed the hyperlipemia which resulted from ether anesthesia, and also noted that all lipid constituents were involved in this augmentation. It was later reported by Mahler[480] that this hyperlipemia could be abolished by insulin; this is in line with the recognized impairment in carbohydrate metabolism which is produced by ether anesthesia. The formation of ketone bodies as a delayed result of ether anesthesia[481,482] is likewise in accordance with this physiological behavior.

On the other hand, there is little evidence that chloroform anesthesia elevates the blood lipids. Lattes[221] was unable to confirm the positive

[477] E. B. Man, P. G. Bettcher, C. M. Cameron, and J. P. Peters, *J. Clin. Invest.*, *25*, 701–708 (1946).
[478] K. Reicher, *Z. klin. Med.*, *65*, 235–268 (1908).
[479] C. A. Hospers, *Arch. Surg.*, *26*, 909–922 (1933).
[480] A. Mahler, *J. Biol. Chem.*, *69*, 653–659 (1926).
[481] J. J. Short, *J. Biol. Chem.*, *41*, 503–513 (1920).
[482] W. Thalhimer, *J. Am. Med. Assoc.*, *81*, 383–385 (1923).

results of Reicher,[478] and Bloor[222] could not demonstrate an increased lipemia as a result of subjecting dogs on a low-fat diet to chloroform for three hours. In the case of one dog on a high-fat diet, Bloor[222] did report a hyperlipemia as a result of chloroform anesthesia. Gray[483] demonstrated an increase in blood cholesterol in rabbits three weeks after they had been subjected to several daily doses of chloroform. On the other hand, Lehnherr[484] found that all blood lipids, including cholesterol, were depressed during carbon tetrachloride poisoning. Both of the latter results are probably correct, but they merely reflect the effect of differences in dosage.

When large amounts of alcohol are given to human subjects and to animals, a hyperlipemia develops.[485-488] Feigl[488] was able to separate the alcohol effect into three distinct steps. In the first place, a hyperlecithinemia occurred in three to eight hours. This was followed by a hypercholesterolemia, which was largely due to the increase in the free cholesterol fraction. Finally, after fifteen to eighteen hours, a marked increase in the neutral fat level occurred in the blood. In chronic alcoholism, hyperlipemia was also frequently observed. A visible lipemia was noted in about 20% of the cases, while a moderate rise in the level of blood lipids was observed in 66% of the subjects. About 28% of the group had a normal amount of blood lipids.

Morphine was shown by Bloor[222] to produce no immediate effect on blood lipids; an increase in this fraction did not result until twenty-four hours after the drug was given. This delayed effect is probably referable to the damage to the liver. The injury to the liver may also account for the delayed hyperlipemia occurring after chloroform intoxication. According to Gray,[483] paraldehyde and urethane are innocuous as far as effects on blood lipids are concerned. Hospers[479] reported that neither ethylene nor nitrous oxide appears to affect the blood cholesterol.

d. The Blood Lipids as Affected by Vitamin Deficiencies. Following early studies based upon diets completely free from vitamins, several investigators reported the occurrence of a hyperlipemia of considerable magnitude.[356,489-491] Asada[491] suggested that, in avitaminosis, the body

[483] S. H. Gray, *J. Biol. Chem.*, *87*, 591–596 (1930).
[484] E. R. Lehnherr, *Arch. Internal Med.*, *56*, 98–104 (1935).
[485] V. Ducceschi, *Arch. ital. biol.*, *70*, 93–114 (1920).
[486] V. Ducceschi, *Arch. Fisiol.*, *13*, 147–153 (1915); *16*, 117–124 (1918).
[487] V. Duccheschi and V. Barilari, *Arch. Fisiol.*, *14*, 21–27 (1916).
[488] J. Feigl, *Biochem. Z.*, *92*, 282–317 (1918).
[489] J. A. Collazo and G. Bosch, *Biochem. Z.*, *141*, 370–378 (1923).
[490] K. Onohara, *Biochem. Z.*, *163*, 67–74 (1925).
[491] K. Asada, *Biochem. Z.*, *142*, 44–52 (1923).

cells lose the power to take up fat, and that a resultant hyperlipemia develops.

More recently, when the deficiency was limited to vitamin B (chiefly a deficiency in thiamine), increased plasma lipids were recorded.[492] Cholesterol and fatty acids increased to about three times their normal value. Lecoq[493] is of the opinion that the assimilation of lipids requires the presence of vitamin B.

Although low blood cholesterol values have been recorded in human scurvy,[494] increased fatty acids and phospholipids have been noted in the blood of scorbutic guinea pigs, the level of blood cholesterol remaining unchanged, except in the terminal period, during which a decrease occurs.[495]

In spite of the few positive correlations between blood lipids and avitaminosis, there is little evidence that such changes are directly due to the lack of the vitamins. It would seem that other factors, such as the partial starvation resulting from anorexia developed during avitaminosis, might more properly be considered as the causes responsible for the alterations in the blood picture.

e. The Blood Lipids as Affected by Essential Fatty Acid Deficiency. It has long been recognized that the fatty acids combined with cholesterol

TABLE 14

EFFECT OF A FAT-DEFICIENT DIET ON THE CHOLESTEROL LEVEL OF THE LIVER AND ADRENAL GLANDS, AND OF THE BLOOD PLASMA, OF THE RAT[a]

Diet	Group No.	No. of rats	Plasma cholesterol, mg. %	Cholesterol in moist tissue, mg./g.	
				Liver	Adrenal
Control, 12.5% cottonseed oil....	1	10	65.6	2.04	35.4
	2	9	63.2	2.08	35.3
Fat-free diets					
Vitamin-test casein............	3	8	38.4	3.15	48.9
	4	7	50.4	4.06	50.4
Commercial casein............	5	10	41.1	4.72	49.6
	6	9	44.9	4.24	46.2

[a] R. B. Alfin-Slater, L. Aftergood, A. F. Wells, and H. J. Deuel, Jr., *Arch. Biochem. Biophys.*, in press, 1954.

in the plasma are more unsaturated than are those in neutral fat or in phospholipids (see page 353). In fact, it has usually been assumed that cholesterol functions in the transport of the highly unsaturated acids. It

[492] H. Lawaczeck, *Z. physiol. Chem.*, *125*, 229–247 (1923).
[493] R. Lecoq, *Bull. soc. chim. biol.*, *15*, 1498–1507 (1933).
[494] N. A. Ssokoloff, *Deut. Arch. klin. Med.*, *145*, 236–239 (1924).
[495] S. Ohata, *J. Biochem. (Japan)*, *16*, 191–206 (1932).

is now known that the metabolism of cholesterol and that of the essential fatty acids are closely interrelated.

It was first demonstrated by Alfin-Slater and her collaborators[363] that the presence of essential fatty acids is required to aid in the regulation of the cholesterol in the blood and tissues. The variations in the plasma cholesterol level as affected by diet are shown in Table 14 (page 435).

It would thus appear that, although diets low in fats cause a decrease in the level of plasma cholesterol, they also result in a concomitant increase in the cholesterol deposited in the liver, adrenal glands, and possibly other organs. It is not known whether or not this results from the inability of cholesterol to be transported from the liver when the supply of linoleate is deficient, or whether the essential fatty acids are required in the catabolism of cholesterol. Bromer and Day[496] have reported that the increase in liver cholesterol was greater after cholesterol was fed to rats on an essential fatty acid-free diet than when it was given to animals on a normal diet; moreover, a greater hypercholesterolemia obtained, under these conditions, in the rats on the fat-free regimen than in the normal controls. Methyl linoleate was shown to lower the cholesterol level in both the blood and the liver.

f. The Blood Lipids as Affected by Disturbances of the Endocrine Glands. The most potent factors which influence the metabolism of the lipids are the secretions of the endocrine glands. Of these hormones, those produced by the thyroid gland and by the islet tissue of the pancreas share the limelight as the most important in relation to the blood lipids. However, the pituitary gland (both the anterior and the posterior portions), the adrenal cortex, and the sex glands also have an influence on the utilization of several of the lipids. Although one finds alterations in lipid distribution when abnormalities of one gland become prominent, the resultant effects must, in most cases, be due to simultaneous variations in function of several of the endocrine glands. Workers in the field of endocrinology have come to realize that the activity of any one endocrine gland cannot be segregated, but practically always results in a variation in behavior of one or more of the other members of the group.

(*a*) *Variations Primarily Related to the Thyroid Gland.* Practically all experimental and clinical evidence has indicated the very marked control exerted by the thyroid gland on the several blood lipids. When the secretion of the thyroid hormone is excessive, as occurs in exophthalmic goiter or other types of hyperthyroidism, a marked decrease in all blood lipids

[496] W. W. Bromer and H. G. Day, *Proc. Am. Chem. Soc.*, Paper No. *140*, Chicago, Sept., 1953.

takes place. On the other hand, when the thyroid gland is extirpated, or when its rate of secretion is at a low level, all blood lipids tend to be increased considerably above their normal values. Although all of the several lipid fractions of blood are affected by variations in the thyroid secretion, the most striking effects are usually to be noted in the cholesterol fraction.

a′. Serum Cholesterol and Thyroid Secretion: A number of workers reported high cholesterol levels following removal of the thyroid gland. Fleischmann *et al.*[497] found an increase in blood cholesterol after thyroidectomy in rabbits; Handler[498] confirmed this finding in rats, while Thompson and Long,[499] and Schmidt and Hughes,[500] noted a similar result in the dog. In the latter instance, the hypercholesterolemia was cleared up by the administration of thyroxine,[500] or by hypophysectomy.[499] High blood cholesterol values have also been reported in thyroidectomized horses,[501] and after subtotal thyroidectomy in man.[501] Boyd and Connell[502] reported that cholesterol and, in fact, all blood lipids, increased following the operation.

High blood cholesterol values have also been noted in cases in which the gland is non-functional, as well as after its surgical removal. Thus, hypercholesterolemia occurs in rats treated with thiouracil,[498] but the increased level was found to be somewhat reduced in choline-deficient animals. Westra and Kunde[503] observed a high blood cholesterol in cretin rabbits; the figure was somewhat reduced when thyroid tissue was fed. Heckscher[504] also reported a hypercholesterolemia in human cretins. In addition to these findings, a number of investigators[505–508] reported high blood cholesterol values in myxedema and in other forms of hypothyroidism in man. The serum phosphatides and fatty acids are also increased in these conditions.[508]

Foldes and Murphy[200] noted that the ratios of cell cholesterol/plasma cholesterol and of cell P/plasma P were significantly decreased in hypo-

[497] W. Fleischmann, H. B. Shumacker, Jr., and L. Wilkins, *Am. J. Physiol.*, *131*, 317–324 (1940).
[498] P. Handler, *J. Biol. Chem.*, *173*, 295–303 (1948).
[499] K. W. Thompson and C. N. H. Long, *Endocrinology*, *28*, 715–722 (1941).
[500] L. H. Schmidt and H. B. Hughes, *Endocrinology*, *22*, 475–482 (1938).
[501] H. Heckscher, *Biochem. Z.*, *158*, 417–421 (1925).
[502] E. M. Boyd and W. F. Connell, *Quart. J. Med.*, *n.s. 5*, 455–460 (1936).
[503] J. J. Westra and M. M. Kunde, *Am. J. Physiol.*, *103*, 1–4 (1933).
[504] H. Heckscher, *Biochem. Z.*, *158*, 422–427 (1925).
[505] L. M. Hurxthal, *Arch. Internal Med.*, *51*, 22–32 (1933).
[506] L. M. Hurxthal, *Arch. Internal Med.*, *53*, 762–781 (1934).
[507] L. M. Hurxthal and H. N. Simpson, *J. Clin. Endocrinol.*, *1*, 450–452 (1941).
[508] E. F. Gildea, E. B. Man, and J. P. Peters, *J. Clin. Invest.*, *18*, 739–755 (1939).

thyroidism. Only the ratio of cell cholesterol/cell P remained unchanged. These variations occur because the cell lipid values remain relatively constant in both hypothyroidism and hyperthyroidism.

In addition to the interrelationship between the level of serum cholesterol and the activity of the thyroid, this gland also exerts some control on the ratio of free to esterified cholesterol. Schwarz and Topper[509] found that the ratio of free to total cholesterol was especially high in cases of hypothyroidism (cretinism) in children. However, in adults with myxedema and other thyroid disturbances, a normal cholesterol partition has been observed.[214] Foldes and Murphy[200] likewise reported that the ratios of cholesterol ester: total cholesterol and of total cholesterol: P were significantly increased in the plasma in hypofunction of this organ. The latter effect is to be traced to the fact that, in the case of thyroid hypofunction, the increase in plasma cholesterol greatly exceeds that in the plasma phospholipids.

On the other hand, an increased activity of the thyroid gland results in a reduction of the lipid components of the blood. Again, cholesterol is the lipid component which shows the greatest regularity in response to the increased amount of thyroid hormone. Low blood cholesterol and phosphatide values have been reported in patients having exophthalmic (or toxic) goiter (also called Graves' or Basedow's disease) or other forms of hyperthyroidism.[510–513] Moreover, an increased ratio of free cholesterol: total cholesterol occurs in hyperthyroidism, in which case the esters are decreased to a greater extent than is the free fraction.[502] However, Foldes and Murphy[200] state that the changes in plasma lipids are less consistent in hyperthyroidism than they are in hypothyroidism.

"Antu," α-naphthylthiourea, which is an antithyroid drug, has been shown to produce a reversible increase in serum cholesterol in thyroidectomized dogs maintained on a dose of thyroid adequate to prevent thyroid deficiency.[514] Fleischmann *et al.*[514] suggest that this effect is at least partly independent of its antithyroid action.

b'. Serum Cholesterol and Basal Metabolism: Although the basal metabolism has usually been considered to be the most satisfactory index

[509] H. Schwarz and A. Topper, *J. Pediat.*, *3*, 242–246 (1933).
[510] H. J. Bing and H. Heckscher, *Biochem. Z.*, *158*, 403–416 (1923).
[511] L. M. Hurxthal, *Arch. Internal Med.*, *52*, 86–95 (1933).
[512] E. B. Man, E. F. Gildea, and J. P. Peters, *J. Clin. Invest.*, *19*, 43–59 (1940).
[513] E. G. Nicholls and W. A. Perlzweig, *J. Clin. Invest.*, *5*, 195–204 (1928).
[514] W. Fleischmann, J. L. Stubbs, and W. P. McShane, *Proc. Soc. Exptl. Biol. Med.* *70*, 246–248 (1949).

of the functioning of the thyroid gland, Hurxthal,[505,511] McElroy *et al.*,[515] Mason, Hunt, and Hurxthal,[516] and Gilligan *et al.*[517] suggested that the serum cholesterol level affords a more reliable index in thyroid disorders than does the basal metabolism. Thus, in hyperthyroidism, serum cholesterol levels averaged 130 milligram per cent[516]; in myxedema, the mean value for serum cholesterol was 321 milligram per cent. The variations from the normal are more frequently noted in cases of hypothyroidism than in hyperthyroidism.[508,512]

However, differences in basal metabolism and in serum cholesterol may not necessarily be associated. Thus, in undernutrition, both basal metabolism and serum cholesterol decrease. Moreover, when dinitrophenol is administered, an increased basal metabolism obtains, without a concomitant decrease in the value of serum cholesterol.[518] Most workers, therefore, do not agree that serum cholesterol values are as satisfactory an index of thyroid activity as has been claimed.[500,508,512,516,519] For a further discussion of the topic, see Epstein and Lande.[520]

c′. Blood Phospholipids and Fats, and Thyroid Secretion: Most blood lipids run parallel to cholesterol in that they are increased or decreased from normal by hypothyroidism or hyperthyroidism, respectively. Blood phospholipid seems best to mirror the change in blood cholesterol, while the neutral fat fraction shows a somewhat less satisfactory correlation with blood cholesterol. Low values for phospholipids and fats have been reported in hyperthyroidism,[502,510–513] while high values for these components have been noted in the blood of thyroidectomized horses[501] and of cretins,[504] as well as in several other types of hypothyroidism.[502,508] Boyd and Connell[502] state that the decrease in phospholipid is less than that of cholesterol in hyperthyroidism, although Man *et al.*[512] found a parallelism between blood cholesterol and blood phospholipid.

Although Boyd and Connell[502] reported that neutral fat decreases more than does cholesterol in hyperthyroidism, Peters and Man[214] have not verified this conclusion. In fact, these workers state that neutral fat is largely uninfluenced by thyroid activity. High levels of neutral fat were obtained

[515] J. S. McElroy, E. B. Schuman and J. O. Ritchey, *Ann. Internal Med.*, *12*, 106–114 (1938).

[516] R. L. Mason, H. M. Hunt, and L. M. Hurxthal, *New England J. Med.*, *203*, 1273–1278 (1930).

[517] D. R. Gilligan, M. C. Volk, D. Davis, and M. L. Blumgart, *Arch. Internal Med.*, *54*, 746–757 (1934).

[518] W. C. Cutting, D. A. Rytand, and M. L. Tainter, *J. Clin. Invest.*, *13*, 547–552 (1934).

[519] J. P. Peters and E. B. Man, *J. Clin. Invest.*, *29*, 1–11 (1950).

[520] A. A. Epstein and H. Lande, *Arch. Internal Med.*, *30*, 563–577 (1922).

in patients with hyperthyroidism and a concomitant hypocholesterolemia. Moreover, no correlation was found between the response to therapy and the blood neutral fat, either in myxedema or in thyrotoxicosis. One is led to the conclusion that hyperthyroidism is associated with low values for serum cholesterol, serum phospholipid and to a lesser extent for the serum fatty acids; in hypothyroidism, the values for these components are generally high. When the thyroid secretion is brought back to normal (by partial thyroidectomy in the case of Graves' disease or by the administration of thyroxine or thyroid in cases of hypofunction of the thyroid gland), then the levels of the several blood lipids tend to assume their normal values.

(b) *Variations Primarily Related to the Anterior Lobe of the Pituitary Gland.* It is possible to prepare, from the anterior lobe of the pituitary gland, an extract which will produce a ketonuria in fasted rats and in man.[521–528] It has been called a fat metabolic, a ketogenic or a diabetogenic hormone. Anselmino and Hoffman,[521,522] who were the first to discover the activity of the anterior pituitary extracts in producing ketosis, stated that these extracts resulted in fatty infiltration of the liver, and regulated the level of the lipids in the blood. Thyroidectomy does not alter the ketogenic response to anterior pituitary extract or prevent the ketosis resulting from phlorhizin.[526]

Foglia and Mazzocco[525] have shown that hyperlipemia accompanies the ketonuria occurring after the injection of the anterior pituitary preparation. Ketonuria does not occur in fed rats,[529] and it can be abolished by carbohydrate.[530,531] The hyperlipemia is not eliminated by pancreatectomy.[525] For a further discussion on this type of ketonuria, see Volume III.

Chaikoff and associates[532] found that hyperlipemia occurred occasionally following hypophysectomy. It was reported that the development of fatty livers following removal of the pancreas was not prevented by com-

[521] F. Hoffman and K. J. Anselmino, *Klin. Wochschr.*, *10*, 2383–2386 (1931).
[522] K. J. Anselmino, F. Hoffman, and E. Rhoden, *Arch. ges. Physiol. (Pflüger's)*, *237*, 515–516 (1936).
[523] C. H. Best and J. Campbell, *J. Physiol.*, *86*, 190–203 (1936).
[524] P. T. Black, J. B. Collip, and D. L. Thomson, *J. Physiol.*, *82*, 385–391 (1934).
[525] V. G. Foglia and P. Mazzocco, *Compt. rend. soc. biol.*, *127*, 150–152 (1938).
[526] E. G. Fry, *Endocrinology*, *21*, 283–291 (1937).
[527] E. M. MacKay and R. H. Barnes, *Am. J. Physiol.*, *118*, 525–527 (1937).
[528] A. H. Neufeld and J. B. Collip, *Endocrinology*, *25*, 768–774 (1939).
[529] R. A. Shipley and C. N. H. Long, *Biochem. J.*, *32*, 2242–2256 (1938).
[530] J. S. Butts, C. H. Cutler, and H. J. Deuel, Jr., *J. Biol. Chem.*, *105*, 45–58 (1934).
[531] N. Nelson, I. Grayman, and I. A. Mirsky, *J. Biol. Chem.*, *132*, 711–715 (1940).
[532] I. L. Chaikoff, G. E. Gibbs, G. F. Holton, and F. L. Reichert, *Am. J. Physiol.*, *116*, 543–550 (1936).

plete removal of the hypophysis. It is believed that the hyperlipemia and the hypercholesterolemia which are frequently found in acromegaly (overproduction of the growth hormone of the anterior pituitary gland) are not related to the pituitary hormone, but rather to the diabetes or other disorders usually associated with this disease.[533] Normal values for serum lipids obtain in pituitary basophilism.[533]

(c) *Variations Primarily Related to the Adrenal Glands.* Since the adrenal cortex plays such an important role in the manufacture of steroid hormones, it is natural to suppose that it would exert some control over the cholesterol content of the blood. In fact, in the report of Ingle,[534] a direct or secondary control of fat metabolism by the adrenal cortex is implied.

A number of investigators[535–537] failed to demonstrate any change in the serum cholesterol level after adrenalectomy. However, Medvei[538] observed that blood cholesterol is somewhat elevated in Addison's disease, which primarily affects the adrenal cortex.

Hoffmeyer[539] reported an increase in the blood cholesterol of rabbits following the transplantation of adrenals or the injection of adrenal cortical extract. Adrenalectomy did not result in any definite alterations in the serum cholesterol. According to other sources, some decreases in serum cholesterol may occur after the injection of cortical extracts.[538,540,541] However, the action of desoxycorticosterone acetate (DCA) and that of corticosterone are markedly different. Thus Zilversmit *et al.*[542] reported a marked decrease in plasma phospholipid concentration, as well as in total circulating plasma phospholipids, in bilaterally adrenalectomized dogs maintained on DCA. In a later report,[543] it was shown that the plasma cholesterol was likewise reduced in DCA-maintained adrenalectomized dogs. After four weeks of DCA therapy, the concentration of these lipids was lowered 30 to 50% below the original value, without alteration of the cholesterol:phospholipid ratio. The decrease in phospholipid concentration cannot be ascribed to dilution or to increased utilization of phospho-

[533] E. B. Man, Unpublished observation; cited by J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, 2nd ed., Vol. I, Williams & Wilkins, Baltimore, 1946.

[534] D. J. Ingle, *J. Clin. Endocrinol.*, *3*, 603–612 (1943).

[535] E. J. Baumann and O. M. Holly, *J. Biol. Chem.*, *55*, 457–475 (1923).

[536] F. S. Randles and A. Knudson, *J. Biol. Chem.* *76*, 89–93 (1928).

[537] C. Reid, *J. Physiol.*, *75*, 34P–35P (1932).

[538] C. V. Medvei, *Z. klin. Med.*, *128*, 58–68 (1935).

[539] J. Hoffmeyer, *Acta Physiol. Scand.*, *10*, 31–41 (1945).

[540] J. Bauer and A. Buttu, *Z. klin. Med.*, *122*, 601–606 (1932).

[541] S. Thaddea and W. Fasshauer, *Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's*, *182*, 477–498 (1936).

[542] D. B. Zilversmit, T. N. Stern, and R. R. Overman, *Am. J. Physiol.*, *164*, 31–34 (1951).

[543] N. R. Di Luzio, M. L. Shore, and D. B. Zilversmit, *Federation Proc.*, *12*, 197 (1953).

lipids[542]; it may or may not be traced to a decreased rate of synthesis.

Di Luzio *et al.*[543] reported that, when cortisone replaced DCA, the abnormally low plasma phospholipid and cholesterol concentrations in adrenalectomized dogs were restored to their preoperative levels within a period of two to three days. During a subsequent period of cortisone treatment, further increases in these plasma lipids were observed. In the case of human patients, Adlersberg and collaborators[544] demonstrated a marked increase in serum phospholipids and cholesterol, coincident with a decrease in the neutral fat in the serum, following the administration of cortisone in various diseased conditions. Following ACTH, a moderate decrease in total and esterified cholesterol in the serum was noted, and a slight increase in serum phospholipids. Adlersberg *et al.*[545] noted average increases of 15% in blood cholesterol and 26% in phospholipids simultaneously with a decrease of 51% in neutral fat in the blood of patients receiving cortisone; ACTH produced much less pronounced changes. The lipid picture in Cushing's pituitary basophilism and in the adrenocortical syndrome involving cortical stimulation does not show any regular decrease.[533,546–549]

Since the advent of cortisone, considerable new information has been amassed concerning its effect on the general lipid constituents of the blood. Thus, in man, cortisone therapy results in an increased concentration of cholesterol and of phospholipids in the serum, and in a decrease in the serum neutral fat.[550,551] In the case of rats, total and esterified cholesterol, as well as lipid phosphorus, are increased in the serum following treatment with cortisone.[552,553] The effect of cortisone was not manifest in adrenalectomized rats maintained with desoxycorticosterone acetate.[552]

In rabbits, also, the administration of adrenal extract and cortisone has been shown to cause an increase in the cholesterol, lipid phosphorus, and total fatty acids of the serum.[539,554,555] Moreover, adrenocorticotropic hormone (ACTH) produces fatty infiltration of the liver both in the case of the rabbit[555] and in the rat.[556] However, adrenalectomy was shown to prevent fat mobilization in the liver of rats given ACTH,[526,527] while cortisone was unable to effect an infiltration of fat in the liver in intact or in adrenalectomized mice.[557] Lipid granules did occur, however, in the hepatic cells of intact rabbits treated with cortisone.[555]

The adrenal cortex is of importance in the regulation of ketonuria. The

[544] D. Adlersberg, L. E. Schaefer, and R. Dritch, *J. Clin. Endocrinol.*, *10*, 814–815 (1950).

[545] D. Adlersberg, L. E. Schaefer, and R. Dritch, *Proc. Soc. Exptl. Biol. Med.*, *74*, 877–879 (1950).

[546] G. Giraud, J. Margarot, and P. Rimbaud, *Presse méd.*, *43*, 841–843 (1935).

[547] K. H. Hildebrand, *Klin. Wochschr.*, *14*, 951–957 (1935).

removal of this gland suppresses the ketosis and the fat infiltration into the liver produced by the injection of the anterior pituitary extracts,[526,527] although demedullation of the adrenals did not prevent the accumulation of liver fat in response to the anterior pituitary extract.[526] Moreover, adrenalectomy was shown to decrease starvation ketosis,[558] as well as to abolish sex differences in ketonuria.[559] On the other hand, the injection of cortical extracts does not result in the production of ketonuria.[560] For a further discussion of the subject of ketosis, see Volume III.

Epinephrine (or adrenalin), the chief hormone present in the adrenal medulla, is now believed to be without an appreciable effect on the level of serum lipids.[561–563] Himwich and Spiers[564] observed an increase in serum lipids after the injection of moderate doses of epinephrine into dogs; similar results were reported in cats following emotional excitement and fright, due to exposure to barking dogs,[565] to nocuous stimulation of the adrenals by the application of electric stimuli to the pads of the feet,[566] as well as after the faradic stimulation of the proximal end of the sciatic nerve.[567] However, Long and Venning[563] believe that these results are erroneous; they ascribe the positive findings of the above investigators to errors in the analytical technic. Page, Pasternak, and Burt[568] reported that the injection of epinephrine was followed by a decrease in the level of phospholipid, cholesterol,

[548] C. Hunter, J. C. McMillan, W. Boyd, and A. T. Cameron, *Can. Med. Assoc. J.*, *25*, 188–193 (1931).
[549] C. A. Wright, *Med. Record*, *141*, 191–196 (1935).
[550] D. Adlersberg, L. E. Schaefer, and S. R. Drachman, *J. Clin. Endocrinol.*, *11*, 67–83 (1951).
[551] J. W. Conn, W. C. Vogel, L. H. Louis, and S. S. Fajans, *J. Lab. Clin. Med.*, *35*, 504–517 (1950).
[552] C. J. Migeon, *Proc. Soc. Exptl. Biol. Med.*, *80*, 571–574 (1952).
[553] C. A. Winter, R. H. Silber, and H. C. Stoerk, *Endocrinology*, *47*, 60–72 (1950).
[554] S. D. Kobernick and R. H. More, *Proc. Soc. Exptl. Biol. Med.*, *74*, 602–605 (1950).
[555] A. R. Rich, T. H. Cochran, and D. C. McGoon, *Bull. Johns Hopkins Hosp.*, *88*, 101–109 (1951).
[556] G. Sala, A. Amira, M. Borasi, and C. Cavallero, *Lancet*, *1951*, *I*, 641–642.
[557] L. Levin and R. K. Farber, *Proc. Soc. Exptl. Biol. Med.*, *74*, 758–763 (1950).
[558] E. M. MacKay and R. H. Barnes, *Am. J. Physiol.*, *118*, 184–189 (1937).
[559] E. M. MacKay and R. H. Barnes, *Endocrinology*, *22*, 351–353 (1938).
[560] R. A. Shipley and E. G. Fry, *Am. J. Physiol.*, *135*, 460–463 (1942).
[561] M. Bruger and H. O. Mosenthal, *J. Clin. Invest.*, *13*, 399–409 (1934).
[562] E. F. Gildea and E. B. Man, *J. Clin. Invest.*, *15*, 295–300 (1936).
[563] C. N. H. Long and E. M. Venning, *J. Biol. Chem.*, *96*, 397–404 (1932).
[564] H. E. Himwich and M. A. Spiers, *Am. J. Physiol.*, *97*, 648–653 (1931).
[565] H. E. Himwich and J. F. Fulton, *Am. J. Physiol.*, *97*, 533–534 (1931).
[566] J. F. Fazikas, M. A. Spiers, and H. E. Himwich, *Proc. Soc. Exptl. Biol. Med.*, *29*, 236 (1931–1932).
[567] Y. D. Koskoff and J. G. Dusser de Barenne, *Science*, *74*, 550 (1931).
[568] I. H. Page, L. Pasternak, and M. L. Burt, *Biochem. Z.*, *232*, 295–309 (1931).

and total fat in the serum and kidneys; on the other hand, there was an increase in fatty acids, cholesterol, and total fat in the liver, and a decrease in brain phosphatides, with a simultaneous increase in cholesterol. According to these results, adrenalin has an insulin-like action.

The injection of epinephrine has been shown to provoke a temporary ketonemia, as well as a ketonuria[569]; this effect is presumably due to the physiological action of this hormone in inhibiting the combustion of carbohydrates.

(*d*) *Variations Primarily Related to the Pancreas.* Hyperlipemia and hypercholesterolemia are well known symptoms of diabetes mellitus in man. However, since the involvement in diabetes may be extensive and not limited to the pancreas alone, much more definite information can be obtained by the study of the blood picture following experimental pancreatectomy than in the diabetic syndrome in man.

a′. Hyperlipemia in Experimental Pancreatectomy: The most convincing information which is available as to the role played by the pancreas in the control of the level of blood lipids is that obtained by study of depancreatized animals. In this case, the data are largely based upon experiments with dogs, because of the relative ease of the surgical procedure and the ability of this species to survive the operation without untoward effects. A large number of investigators[225,570–576] have demonstrated that all lipid components of the plasma increase markedly following the extirpation of the pancreas. Gibbs and Chaikoff reported that the hyperlipemia, hypercholesterolemia, and hyperphosphatidemia occur in depancreatized dogs deprived of insulin only when they are fed,[572] or when they are given raw pancreas prior to and following the operation.[573] Allen[570] reported that the blood of depancreatized dogs receiving a high-fat diet may contain as much as 15% of fat. This investigator suggested that, in addition to the effect of a high fat diet, the presence of a hyperlipemia in these dogs is dependent upon a clinically severe diabetes, resulting from the operation, as evidenced by the glycosuria and hyperglycemia. Thus, Allen believes that the high lipid levels in the blood reflect a secondary

[569] R. S. Hubbard and F. R. Wright, *J. Biol. Chem.*, *49*, 385–388 (1921).
[570] F. M. Allen, *J. Metabolic Research*, *2*, 219–298 (1922).
[571] H. E. Himwich, W. H. Chambers, A. L. Hunter, and M. A. Spiers, *Am. J. Physiol.*, *99*, 619–625 (1932).
[572] G. E. Gibbs and I. L. Chaikoff, *Endocrinology*, *29*, 877–884 (1941).
[573] G. E. Gibbs and I. L. Chaikoff, *Endocrinology*, *29*, 885–899 (1941).
[574] A. L. Lichtman, *J. Biol. Chem.*, *120*, 35–40 (1937).
[575] I. I. Nitzescu, C. Popescu-Inotesti, and I. Cadariu, *Compt. rend. soc. biol.*, *90*, 538–539 (1924).
[576] Y. Seo, *Arch. exptl. Pathol. Pharmakol. Naunyn Schmiedeberg's*, *61*, 1–6 (1909).

breakdown in fat metabolism which is not directly connected with the loss of the endocrine function of the pancreas, with the excess of fat, or with the loss of the sugar in the urine. Wide differences in susceptibility to hyperlipemia obtain, both in dogs and in man. On the other hand, Bloor *et al.*[225] link the hyperlipemia with the lack of the internal secretion of the pancreas.

In addition to precipitating hyperlipemia, the removal of the pancreas produces ketosis and a resultant ketonuria. The ketonemia and ketonuria which result in dogs following pancreatectomy, or after the administration of phlorhizin, disappear when glucose is given intravenously. However, it was not possible to abolish the ketonuria in depancreatized dogs by giving glucose by mouth, presumably because it was not absorbed rapidly enough to build up the prerequisite glycogen in the liver and tissues.

(a′) The Effect of Insulin on the Hyperlipemia.—When depancreatized dogs were maintained over long periods by treatment with insulin, Chaikoff and Kaplan[577] demonstrated a marked fall in hyperlipemia to values which

TABLE 15

SUMMARY OF THE AVERAGE VALUES (WITH RANGES) FOR BLOOD LIPIDS IN NORMAL AND IN DEPANCREATIZED DOGS MAINTAINED WITH INSULIN[a]

The values are expressed in milligram per cent

Category	Normal dogs	Depancreatized dogs
Number of dogs	10	10
Cholesterol		
Total	170 (137–205)	109 (96–124)
Free	129 (104–144)	109 (95–127)
As ester	40 (29–66)	1 (0–7)
As ester (% of total)	23 (19–33)	1 (0–7)
Total fatty acids	394 (309–439)	266 (216–347)
Phospholipid	330 (286–391)	204 (146–263)
Residual fatty acid	142 (85–203)	128 (91–171)
Total lipid	563 (465–644)	374 (315–471)
Ratios		
Total fatty acids:total lipid	0.70 (0.67–0.73)	0.71 (0.67–0.74)
Total cholesterol:total lipid	0.30 (0.27–0.33)	0.29 (0.26–0.33)
Phospholipid:total cholesterol	1.9 (1.5–2.5)	1.9 (1.5–2.2)

[a] Adapted from I. L. Chaikoff and A. Kaplan, *J. Biol. Chem.*, *106*, 267–279 (1934).

were lower than the preoperation levels. A summary of their results is given in Table 15.

The decrease in concentration of the total cholesterol, total fatty acids,

[577] I. L. Chaikoff and A. Kaplan, *J. Biol. Chem.*, *106*, 267–279 (1934).

phospholipid, and total lipid, respectively, is proportional in the insulin-treated depancreatized dogs. The averages of the ratios of these constituents to each other are remarkably uniform in the normal and depancreatized dogs. However, there is one marked difference which is quite apparent. The cholesterol fraction present as the ester has completely disappeared in the pancreatectomized insulin-treated dogs. Whereas the combined cholesterol makes up 23% of the total cholesterol in the blood of normal dogs, it accounts for only 1% in the experimental animals. However, the esterified cholesterol still comprises approximately 75% of the total cholesterol in the liver of the insulin-treated operated animals.[578]

The effect of insulin in preventing the hyperlipemia of depancreatized dogs is completely reversed when the animals are fed raw pancreas by mouth.[579] Under such conditions, the values for the several blood lipids were found to be markedly increased above the preoperative level. The ratio of cholesterol ester to total cholesterol was increased from a preoperative level of 23% to one of 45%. The response was likewise similar if raw pancreas was added to the diet of the operated dog after the blood lipids had been depressed by previous treatment with insulin alone. When the feeding of the raw pancreas was suspended and the insulin injections were continued, the level of blood lipids rapidly dropped. There is no explanation for this phenomenon; it seems possible that the relatively large intake of cholesterol, phospholipid, and total lipids present in the 250 g. of raw pancreas fed daily may have been partially responsible for the increased blood lipid values. Insulin does not alter the concentration of the blood lipids in normal men with adequate glycogen stores.[533,580] However, Rony and Ching[581] reported that, although insulin is without effect on the blood lipids of fasting dogs, it is able to inhibit alimentary hyperlipemia in these animals.

In addition to the depressing effect which insulin exerts on the blood lipids, it will also decrease or completely abolish any existing ketonemia and ketonuria. However, the reaction within the body depends somewhat upon the accompanying nutritional conditions. When carbohydrate reserves are available, the injection of insulin results in an immediate decrease in blood ketones, which occurs concomitantly with the oxidation of carbohydrate.[582,583] On the other hand, when the carbohydrate reserves

[578] A. Kaplan and I. L. Chaikoff, *J. Biol. Chem.*, *108*, 201–216 (1935).
[579] I. L. Chaikoff and A. Kaplan, *J. Biol. Chem.*, *112*, 155–165 (1935).
[580] H. U. Hartmann, *Biochem. Z.*, *146*, 307–317 (1924).
[581] H. R. Rony and T. T. Ching, *Endocrinology*, *14*, 355–363 (1930).
[582] L. Salomonsen, *Am. J. Diseases Children*, *40*, 718–724 (1930).
[583] M. Somogyi, *J. Biol. Chem.*, *141*, 219–227 (1941).

are completely exhausted, and the body is forced to rely upon protein and fat as a source of calories, the injection of insulin will cause an increased ketonemia.[582,584]

b′. The Blood Lipids in Diabetes Mellitus: (a′) The Presence of Hyperlipemia.—Very high values for blood lipids in men have been reported in cases of diabetes mellitus, as compared with other pathological conditions.[585–597] The maximum value for total lipids in diabetes reported in the literature is one of 48% cited by Chase.[594] The ordinary Babcock centrifugation method for butterfat was employed in the determination of this value. Other high values reported are 22%,[596] 19.7%,[588] and 15%.[593] In the case of children suffering from diabetes, the figures are much lower. Boyd[595] reported that the values for total lipid were higher in the case of diabetic children than for normal subjects; in acidosis, the total blood lipids approximated 1% while, in coma, figures as high as 2% were found.

Although it was recognized for a long period that a large proportion of fatty material occurred in the blood of diabetics, Fischer[587] and Klemperer and Umber[589,590] were the first to emphasize the fact that constituents other than the neutral fats were likewise present in increased quantities. Thus, cholesterol has been reported in amounts of 1.5% (14.1% total lipid),[591] and 1.41% (22% total lipid).[596] Feigl[592] found a maximum of 0.92% of lecithin in diabetic blood containing a total of 15% of total lipid,[593] while Herbert[596] reported a value of 0.95% for phospholipids (total lipid, 22%). However, in spite of the marked percentage increase which may occur in the total cholesterol and phospholipid, it is evident that the bulk of the fatty material present, in the above cases of extreme hyperlipemia, must consist of neutral fat.

According to Blix,[255] the instances of the high degree of lipemia noted above are rare, and a moderate lipemia obtains in the largest proportion of

[584] A. Gottschalk and A. Springborn, *Klin. Wochschr.*, *8*, 1660–1661 (1929).
[585] M. Bönninger, *Z. klin. Med.*, *42*, 65–71 (1901).
[586] L. Schwarz, *Deut. Arch. klin. Med.*, *76*, 233–289 (1903).
[587] B. Fischer, *Arch. path. Anat. Physiol.* (*Virchow's*), *172*, 30–71 (1903).
[588] E. Neisser and L. Derlin, *Z. klin. Med.*, *51*, 428–438 (1904).
[589] G. Klemperer and H. Umber, *Z. klin. Med.*, *61*, 145–152 (1907).
[590] G. Klemperer and H. Umber, *Z. klin. Med.*, *65*, 340–351 (1908).
[591] C. G. Imrie, *J. Biol. Chem.*, *20*, 87–90 (1915).
[592] J. Feigl, *Biochem. Z.*, *92*, 1–83 (1918).
[593] J. Feigl, *Biochem. Z.*, *90*, 173–214 (1918).
[594] L. A. Chase, *Can. Med. Assoc. J.*, *17*, 197–204 (1927).
[595] G. L. Boyd, *Am. J. Diseases Children*, *36*, 298–309 (1928).
[596] F. K. Herbert, *Biochem. J.*, *29*, 1887–1893 (1935).
[597] S. H. Rubin, *J. Biol. Chem.*, *131*, 691–702 (1939).

cases. He believes that hyperglycemia is a more sensitive index of the severity of diabetes than is the hyperlipemia.

(*b′*) The Cause of the Hyperlipemia.—A number of suggestions have been advanced as to the cause of the hyperlipemia which occurs in diabetes. Such leaders in the field of diabetes research as Allen *et al.*,[598] Joslin and associates,[599] and Bloor[196] concluded that some defect in transportation, disposal, or metabolism of fat accounts for the hyperlipemia in diabetes. For this reason, the use of appreciable amounts of fat in diabetic diets has been discouraged.

However, Blix,[255] Blatherwick,[600] Marsh and Waller,[601] and Freyberg *et al.*[602] found that hyperlipemia in diabetes bears little relation to the level of dietary fat. In fact, Bloor, Gillette, and James[225] noted that a fall in the level of blood lipids frequently obtained in their diabetic dogs when fat was fed. In the pre-insulin days, Newburgh and Marsh[603-605] employed diets containing as much as 200 g. of fat daily, with beneficial effects, in cases of diabetes. When the patients improved on these diets, blood fats decreased.[600,601] According to Peters and Van Slyke,[202] when a diabetic is enabled by diet or by insulin to utilize an adequate amount of carbohydrate, the metabolism of the lipids is normal, and the pattern and concentration of the serum lipids are also normal.[606,607]

In spite of the several reports that dietary fat is of little importance in the causation of diabetic hyperlipemia, Cochrane, Michaels, and Kinsell[608] found that the substitution of vegetable fat for "animal fat" in the high-protein, high-fat diabetic diets resulted in a major fall in the levels of plasma cholesterol and phospholipids. It is suggested that this reduction in hyperlipemia is not to be explained merely by the absence of dietary cholesterol. Felch and Dotti[609] reported that inositol is an effective agent in

[598] F. M. Allen, E. Stillman, and R. Fitz, *Total Dietary Regulation in the Treatment of Diabetes*, Monograph, Rockefeller Inst. Med. Research, New York, No. 11, October, 1919.

[599] E. P. Joslin, W. R. Bloor, and H. Gray, *J. Am. Med. Assoc.*, *69*, 375–378 (1917).

[600] N. R. Blatherwick, *J. Biol. Chem.*, *49*, 193–199 (1921).

[601] P. L. Marsh and H. G. Waller, *Arch. Internal Med.*, *31*, 63–75 (1923).

[602] R. H. Freyberg, L. H. Newburgh, and W. A. Murrill, *Arch. Internal Med.*, *58*, 589–597 (1936).

[603] L. H. Newburgh and P. L. Marsh, *Arch. Internal Med.*, *26*, 647–662 (1920).

[604] L. H. Newburgh and P. L. Marsh, *Arch. Internal Med.*, *27*, 699–705 (1921).

[605] L. H. Newburgh and P. L. Marsh, *Arch. Internal Med.*, *31*, 455–490 (1923).

[606] I. L. Chaikoff, F. S. Smyth, and G. E. Gibbs, *J. Clin. Invest.*, *15*, 627–631 (1936).

[607] E. B. Man and J. P. Peters, *J. Clin. Invest.*, *14*, 579–594 (1935).

[608] G. C. Cochrane, G. D. Michaels, and L. W. Kinsell, *J. Clin. Nutrition*, *1*, 295–298 (1953).

[609] W. C. Felch and L. B. Dotti, *Proc. Soc. Exptl. Biol. Med.*, *72*, 376–378 (1949).

reducing both cholesterol and lipid P in hypercholesteremic diabetics.

Another suggestion as to the cause of the hyperlipemia in diabetes is to the effect that a greater than normal lipemic reaction may occur on the ingestion of fatty meals. Abnormal responses were reported by Bing and Heckscher,[610] and by Hatakeyama *et al.*,[611] but they were not obtained by Blix[255] or by Man.[533] Hatakeyama and collaborators[611] also assume that hyperlipemia in diabetes may be traced to diets high in fat and low in carbohydrate. Freyberg *et al.*[602] deny that, in modern methods of treatment, the diets used cause a consistent and a constant hyperlipemia.

Although hyperlipemia is a constant concomitant of acidosis, many causes of hyperlipemia have been reported in which no acidosis could be demonstrated. In the cases of diabetic patients not suffering from acidosis, reported by Man and Peters,[607] 24% of the 79 patients presented hyperlipemia, evidenced by hypercholesterolemia. After an examination of the patients, these authors suggest that lesions of the hypothalamus may account for the instability of the autonomic system, and for the hyperlipemia.

(c′) Diabetic Acidosis and Hyperlipemia.—When carbohydrate cannot be metabolized, as occurs in diabetes, or when the supply of carbohydrate is exhausted, as a result of fasting, the body must fall back on fat and protein as sources of its energy. In the absence of carbohydrate oxidation, the fatty acids derived from the neutral fat and from certain amino acids fail to be utilized completely, leaving intermediate acids in the blood stream. These acids result in the production of the so-called diabetic acidosis.

The condition of diabetic acidosis is brought about when the rate of production of these intermediate acids is sufficient to result in a reduction of the alkali reserve of the blood. The ketone body acids are acetoacetic (or diacetic) acid, CH_3COCH_2COOH, and β-hydroxybutyric acid, $CH_3CHOHCH_2COOH$. Acetone, CH_3COCH_3, is formed in this series of reactions. For this reason, these three components (the two acids and acetone) are referred to collectively as the "acetone bodies" or "ketone bodies." The appearance of these products in the blood is described as a "ketosis," while their appearance in larger than normal amounts in the blood is known as a "ketonemia." When the ketone bodies are excreted in the urine, the condition is called a "ketonuria" or an "acetonuria."

The state of acidosis develops because the diacetic and β-hydroxybutyric acids remove the fixed bases (sodium and potassium) from the blood. The

[610] H. I. Bing and H. Heckscher, *Biochem. Z.*, *149*, 83–99 (1924).

[611] T. Hatakeyama, K. Takahashi, Y. Tutumi, and K. Yamazaki, *Biochem. Z.*, *300*, 392–402, 403–413 (1938–1939).

reason for this is that these acids react with sodium bicarbonate, with the consequent production of carbonic acid and of the alkali salts of the ketone body acids. The latter are excreted in the urine, with a resultant loss of fixed base from the blood. The carbonic acid formed is largely broken down to water and carbon dioxide; the latter is excreted in the expired air.

During the constant and steady production of the ketone body acids inherent in the diabetic syndrome, additional amounts of sodium bicarbonate continue to be used for neutralization; a progressive decrease in the CO_2-combining power of the blood occurs. As the supply of bicarbonate is depleted, a larger proportion of the acid is neutralized by ammonia. The ammonium hydroxide, required for this reaction, is made available at the expense of the urea. Moreover, as the acidosis becomes more severe, the body tends to secrete a more acid urine, in which a greater proportion of the ketone body acids is eliminated in the form of free acids, rather than as their sodium, potassium, or ammonium salts.

The requirement for fat is augmented both in fasting and in diabetes mellitus. However, the requirement may be somewhat greater in the latter case, since no glucose can be used. Even the carbohydrate formed from the amino acids fails to be oxidized in severe diabetes, and this loss in calories must be compensated by increased fat oxidation. Moreover, because the ketonuria is frequently extremely severe in diabetes, additional energy must be rendered available by the partial oxidation of more fat, to compensate for the loss of energy occurring as the result of the loss of the potential calories arising from the excretion of the unoxidized ketone bodies. However, the intensity of the ketonuria which occurs, particularly in women during fasting uncomplicated by diabetes, should not be underestimated. It may be extremely severe, and in the same range as that which occurs in diabetic acidosis.[408]

It is therefore understandable that, under conditions of diabetic acidosis, a high requirement for fat exists in the tissues. Thus, the serum lipids have invariably been found to be high when a diabetic acidosis is present.[596,612,613] In addition to the increased requirement caused by the failure of carbohydrate to be utilized and also by the inefficient utilization of the calories of the fat which is broken down, another phenomenon occurs which tends to exaggerate the increase in blood lipid. This factor is the hemoconcentration which is usually present. There is a considerable loss of fluid from the blood, due to the glycosuria and ketonuria which are occurring concomitantly, with the result that the substances remaining in the

[612] E. B. Man and J. P. Peters, *J. Clin. Invest.*, *13*, 237–261 (1934).
[613] E. Sorkin and M. Batuschanskaja, *Z. ges. exptl. Med.*, *74*, 138–147 (1930).

blood stream are present in higher percentages in the concentrated blood.

The neutral fat fraction is increased to a greater degree than are the other blood lipid fractions, in diabetic acidosis. Man and Peters[612] reported that, following recovery from diabetic acidosis, lipid phosphorus, cholesterol, and serum proteins showed a parallel decrease. The fatty acids increased out of proportion to the phospholipids and cholesterol during the active phase of the acidosis. The degree of increase of phospholipids was somewhat greater than was that of cholesterol, so that the cholesterol:lipid phosphorus ratio tended to fall below normal during the diabetic acidosis. Harris *et al.*[614] found that hemoconcentration plays an important role in the production of hyperlipemia as a whole. However, there is also an accession to the plasma of other lipids. This increment is composed chiefly of neutral fat with lesser quantities of phospholipids, free cholesterol, and cholesterol esters in descending order of magnitude. These are all believed to be in a free state rather than in a protein combination.

(d′) Ketosis in Diabetes Mellitus.—As has been discussed in the previous section, one of the outstanding features of diabetes mellitus is the occurrence of the ketone bodies in the blood and urine. The intensity of this ketosis varies with several factors. The most important is the occurrence of concomitant infections, which markedly increase the level of blood ketone bodies. Another condition upon which the degree of ketosis depends is the adequacy of the insulin therapy. When insulin injections are omitted, or insufficient doses are administered, increased ketosis may be anticipated.

Allen *et al.*[598] cited a case of diabetic acidosis in which 368 milligram per cent of ketones (calculated as acetone) were found in the blood. Magnus-Levy[615] reported a case in which a boy eliminated 97.5 g. of ketone bodies daily in the urine, over a period of three days. In several other instances a ketonuria of 40 g. per day has been recorded.[615–617] The CO_2-combining power of the blood, which is an excellent index of the severity of the acidosis, may reach as low a value as 10 volumes per cent, compared with a normal level of 55 to 60 volumes.

The severity of the ketosis and ketonuria during fasting is much less, for male subjects, than in severe diabetes. However, in the case of five normal females who fasted, the maximum ketonurias noted on the third

[614] L. V. D. Harris, M. J. Albrink, W. F. Van Eck, E. B. Man, and J. P. Peters, *Metabolism*, *2*, 120–132 (1953).

[615] A. Magnus-Levy, *Ergeb. inn. Med.*, *1*, 352–419 (1908).

[616] A. Magnus-Levy, *Johns Hopkins Hosp. Bull.*, *22*, 46–53 (1911).

[617] W. M. Marriott, *J. Biol. Chem.*, *18*, 507–517 (1914).

fast day amounted to 20.65, 19.15, 13.88, 9.96, and 6.68 g., respectively.[408] In one case the CO_2-combining power of the blood was decreased to 26.9 volume per cent on the fourth fast day.[408]

There is no entirely satisfactory explanation to account for the precipitation of an acidosis. Peters and Van Slyke[202] do not attribute the appearance of ketosis to overindulgence in carbohydrate. Some factor which reduces the ability of the individual to burn sugar is the most important underlying cause. The recent report of Mirsky *et al.*[618] demonstrates that both carbohydrate and insulin are necessary to counteract the incidence of ketosis. When smaller doses of insulin were given to patients than were required to control their glycosuria, together with large doses of carbohydrate, ketosis disappeared, although an increased glycosuria obtained. On the other hand, when the insulin was given without the carbohydrate, ketosis increased. Finally, when insulin was omitted and carbohydrate was given, the ketosis could not be controlled, except in a few cases in which the carbohydrate intake was very high. These results prove that sugar is required to alleviate the ketosis, together with insulin. Either by itself is ineffective. Presumably the ingested sugar functions in the presence of insulin, not only to provide carbohydrate for combustion, but also to serve as a precursor for replenishing the stores of glycogen in the liver.

(e′) Lipidoses in Diabetes Mellitus.—Several types of abnormal lipid deposition are frequently associated with diabetes. The two commonest types of lipidoses are called *xanthomatosis* and *lipemia retinalis*. They usually occur when a marked hyperlipemia is present.

a″. Xanthomatosis: In this condition, fatty nodules appear on the skin, chiefly on the eyelids. The condition is frequently known as *cutaneous xanthomatosis*. In the cases of this disease studied by Yamakawa and Kashiwabara,[619] the cholesterol and neutral fat in the serum were three times the normal value, and the phospholipid was slightly elevated. Rosen and Krasnow[620] likewise reported a hypercholesterolemia, as well as a hyperphospholipidemia, in this condition. In xanthomatosis, large amounts of free and esterified cholesterol were present in the tumors, but practically no phospholipids.[619]

Xanthomas occur in diabetes associated with hyperlipemia. However, the hyperlipemia *per se* is not the sole cause of the condition, since

[618] I. A. Mirsky, A. N. Franzblau, N. Nelson, and W. E. Nelson, *J. Clin. Endocrinol.*, *1*, 307–313 (1941).

[619] S. Yamakawa and M. Kashiwabara, *Tôhoku J. Exptl. Med.*, *3*, 317–332 (1922).

[620] I. Rosen and F. Krasnow, *Arch. Dermatol. Syphilol.*, *26*, 48–55 (1932).

the disease is usually absent in pathological states other than diabetes in which hyperlipemia exists. However, Ahrens and Kunkel[621] reported the occurrence of xanthomas in eighteen cases of primary biliary cirrhosis. The presence of xanthomas coincided with an elevation of total serum lipids; they tended to disappear when the total serum lipid decreased. Xanthomatosis may, however, occur without concomitant hypercholesterolemia. One such case reported by Rosenthal and Braunisch[622] was improved by treatment with adrenalin. Diabetic xanthomatosis responds to treatment with insulin.[622–625] According to one report, the xanthomatosis regressed with the improvement of the diabetes, and was intensified when the diabetes became more severe.[626] A similar relationship has been reported as existing between diabetes on the one hand and both xanthomatosis and lipemia retinalis on the other hand.[627]

Another allied condition called *essential xanthomatosis*, or *essential cholesterolemia*, which occurs independently of diabetes, involves the formation of nodules in various locations in the body. These are composed largely of cholesterol. Blood cholesterol has been found to be at a level of 400 to 500% of that of normal subjects, but the other blood lipids are not proportionately increased. It has been suggested that this condition is related to a congenital inability to excrete cholesterol. Although some cases may respond to a cholesterol-free diet, Sperry and Schick[628] were unable to note any improvement as a result of this dietary measure. According to Chanutin and Ludewig,[629] the free cholesterol, phospholipids, and total lipids in the blood were increased in xanthomatosis, while cholesterol ester and neutral fats were reduced. Schick and Sperry[630] reported that a patient with essential xanthomatosis whom they had observed over a fifteen-year period, during thirteen years of which he had lived on a diet containing negligible amounts of cholesterol, exhibited hypercholesterolemia throughout the interval. These workers emphasize the familial nature of essential xanthomatosis, and support the concept that inherited hypercholesterolemia is the primary disturbance in this disease.

[621] E. H. Ahrens, Jr., and H. G. Kunkel, *J. Clin. Invest.*, *28*, 1565–1574 (1949).

[622] F. Rosenthal and R. Braunisch, *Z. klin. Med.*, *92*, 429–441 (1921).

[623] A. Chauffard, P. Brodin, and R. Yavanovitch, *Bull. mém. soc. méd. hôp. Paris* [*3*], *48*, 1573–1582 (1924).

[624] W. H. Gordon and M. S. Feldman, *J. Michigan State Med. Soc.*, *23*, 231–233 (1924).

[625] T. H. McGavack and H. C. Shepardson, *Ann. Internal Med.*, *7*, 582–604 (1933).

[626] J. A. Buchanan and J. C. Indelicato, *Am. J. Med. Sci.*, *195*, 50–53 (1938).

[627] A. C. Curtis, J. M. Sheldon, and H. C. Eckstein, *Am. J. Med. Sci.*, *186*, 548–556 (1933).

[628] W. M. Sperry and B. Schick, *Am. J. Diseases Children*, *51*, 1372–1384 (1936).

[629] A. Chanutin and S. Ludewig, *J. Lab. Clin. Med.*, *22*, 903–911 (1937).

[630] B. Schick and W. M. Sperry, *Am. J. Diseases Children*, *77*, 164–174 (1949).

b″. Lipemia Retinalis: The diffuse deposition of lipids in the retina frequently occurs in diabetic patients who have a high level of lipemia. This type of lipidosis seems to be definitely related to the diabetic syndrome, since it occurs only in connection with this disease, and is not found in nephrosis, in which a comparable degree of lipemia exists.[631] Moreover, it disappears when the diabetic symptoms are alleviated and the hyperlipemia is relieved.[631,632]

c′. The Blood Lipids in Pancreatitis: Pancreatitis may also be associated with a hyperlipemia. Although hyperlipemia has not ordinarily been reported in this disease in children, Kennedy and Collett[633] observed chronic relapsing pancreatitis associated with a decided hyperlipemia in the case of a boy eight years of age.

d′. The Blood Lipids in Alloxan Diabetes: Kendall and associates[634] noted that a transient period of hyperlipemia and hypercholesterolemia occurred in rabbits following the injection of alloxan. When a cholesterol-rich diet was given to alloxan diabetic rabbits,[635] blood cholesterol levels reached much higher levels than was the case of uninjected controls. Atheromata appeared consistently in the controls but in only one diabetic rabbit.[635] According to Payne and Duff,[636] a transitory hyperlipemia may or may not occur during the early stages of alloxan diabetes. In the hyperlipemic serum, the lipid constituents are, to a great extent, "readily extractable," suggesting that they are not closely associated with the serum proteins, as is the case in normal rabbits.

(*e*) *Variations in the Blood Lipids Primarily Related to the Sex Hormones.* Although, under normal conditions, other than during fasting, sex has little influence on the blood lipids, some of the natural and synthetic sex hormones have a marked effect upon these compounds. Glass and coworkers[637] reported that estradiol did not cause any change in the serum lipids or in the low-density lipoproteins of male and female patients. On the other hand, Eilert[638] found that, when ethinyl estradiol was given to women in the ordinary doses employed during menopause, a slight increase in total serum lipids was noted; serum phospholipids were markedly

[631] S. H. McKee and I. M. Rabinowitch, *Can. Med. Assoc. J.*, *25*, 530–534 (1931).

[632] L. A. Chase, *J. Am. Med. Assoc.*, *97*, 171–172 (1931).

[633] R. L. J. Kennedy and R. W. Collett, *Am. J. Diseases Children*, *78*, 80–87 (1949).

[634] F. E. Kendall, W. Meyer, L. Lewis, and J. Victor, *Proc. Soc. Exptl. Biol. Med.*, *60*, 190–195 (1945).

[635] H. C. McGill, Jr., and R. L. Holman, *Proc. Soc. Exptl. Biol. Med.*, *72*, 72–75 (1949).

[636] T. P. B. Payne and G. L. Duff, *Proc. Soc. Exptl. Biol. Med.*, *73*, 332–337 (1950).

[637] S. J. Glass, H. Engelberg, R. Marcus, H. B. Jones, and J. W. Gofman, *Metabolism*, *2*, 133–136 (1953).

[638] M. L. Eilert, *Metabolism*, *2*, 137–145 (1953).

increased, while a significant depression of the total serum cholesterol and of the total cholesterol:lipid P ratio occurred in female patients. The results obtained with diethylstilbestrol were similar to those obtained with ethinyl estradiol.[638]

a′. The Effect of Natural Estrogenic Hormones and of Stilbestrol on Chicks: Entenman and co-workers[639] were the first to demonstrate that the injection of pregnant mare serum into immature female birds produced an increase in blood lipids similar to that which had been observed earlier in the domestic fowl, coincident with the laying of eggs.[294,640] Lorenz, Chaikoff, and Entenman[295] subsequently showed that the injection of estrone in a dosage of 3000 rat units caused a doubling of the blood lipids of the immature female birds within twelve hours. Moreover, after the injection of 2000 rat units of estrone into male birds, blood lipid values well over 1000 milligram per cent were obtained. In contradistinction to the results on women, all lipid constituents shared in the increase in the fowl, although the most pronounced effect was exerted upon the neutral fat. In a later study, Entenman *et al.*[641] reported that the following crystalline estrogenic substances increased the concentration of total fatty acids, phospholipids, and cholesterol in the blood: estrone, estradiol, estradiol benzoate, and ethinyl estradiol. The synthetic estrogenic compound, stilbestrol, was also active and, in fact, increased the blood lipids to the greatest extent. Estradiol benzoate resulted in a more marked increase than did any of the other natural estrogenic substances investigated. The male hormone, testosterone, as well as progesterone and DCA, yielded negative results when administered in amounts as high as 60 mg. Flock and Bollman[53] confirmed the earlier results of Entenman and collaborators.

In a study of the phospholipid response to estrogenic hormones and stilbestrol, it was found that the increase in plasma phospholipid coincided with a decrease of this component in the liver.[53] Since the liver is presumably the site of synthesis of the plasma phospholipids, Flock and Bollman[642] suggest that an increase in phospholipid synthesis may occur in the liver. Burr and Barnes[643] suggest as an alternate explanation that the estrogens may inhibit the destruction of phospholipids. As a result of experiments with P^{32} (injected intraperitoneally into cells), Flock and Bollman[642] came to the conclusion that the administration of diethylstilbestrol

[639] C. Entenman, F. W. Lorenz, and I. L. Chaikoff, *J. Biol. Chem.*, *126*, 133–139 (1938).

[640] F. W. Lorenz, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *123*, 577–585 (1938).

[641] C. Entenman, F. W. Lorenz, and I. L. Chaikoff, *J. Biol. Chem.*, *134*, 495–504 (1940).

[642] E. V. Flock and J. L. Bollman, *J. Biol. Chem.*, *156*, 151–160 (1944).

[643] G. O. Burr and R. H. Barnes, *Ann. Rev. Biochem.*, *12*, 157–182 (1943).

increased both the rate of formation and the rate of turnover of the phospholipids.

Stamler *et al.*[644] reported that, when chicks were given a subcutaneous implantation of 25 mg. of stilbestrol, they developed hyperlipemia followed by atherosclerosis. When desiccated thyroid powder was also given to the chicks, the hyperlipemia was temporarily suppressed, but after several weeks both groups of chicks exhibited similar symptoms. Forbes and Petterson[645] reported that the subcutaneous administration of diethylstilbestrol to cockerels caused a marked increase in the concentration of the "readily extractable" cholesterol of the plasma, as well as of total cholesterol and neutral fat. However, the level of both total and "readily extractable" cholesterol was decreased below normal when the diet contained 10% of soybean lecithin.

g. The Blood Lipids in Anemia. (*a*) *The Blood Lipids in Experimental Anemias.* When a chronic anemia is produced in rabbits by the daily removal of a portion of the animal's blood, a marked lipemia results. According to Boggs and Morris,[646] the increase in lipid consists largely of the lecithin fraction. When rabbits were fed high-fat diets, the increase in total fatty acids in the plasma amounted to twenty-five times the preanemia level; in one case, on a low-fat diet, the increase was about eight times the normal.[647] The fats were shown to be those mobilized from the tissues of the body; the largest proportion was derived from the fat in the skin, while the spleen, brain, kidney, lungs, and adrenal glands contributed in that order.[346] Schmitz and Koch,[648] likewise, reported an increase in lecithin in hemorrhagic anemia in rabbits. The cephalin fraction was also increased to a level which was the optimum for clotting. Several workers have reported that cholesterol increases concomitantly with lecithin, or with general lipemia, in the chronic anemia of rabbits.[647,649–651] In fact, Horiuchi[647] noted an average maximum increase of seven times the original value for lecithin, and 7.2 times the original value for cholesterol, in rabbits with acute anemia and fed a high-fat diet. The comparative values for rabbits having acute anemia but receiving a fat-free diet were 2.5 and

[644] J. Stamler, A. J. Miller, L. Akman, C. Bolene, and L. N. Katz, *Circulation, 2,* 523–529 (1950).

[645] J. C. Forbes and O. Petterson, *Proc. Soc. Exptl. Biol. Med., 78,* 883–885 (1951).

[646] T. R. Boggs and R. S. Morris, *J. Exptl. Med., 11,* 553–560 (1909).

[647] Y. Horiuchi, *J. Biol. Chem., 44,* 363–379 (1920).

[648] E. Schmitz and F. Koch, *Biochem. Z., 223,* 257–277 (1930).

[649] E. H. Fishberg, *J. Biol. Chem., 81,* 205–214 (1929).

[650] E. N. Chamberlain and R. L. Corlett, *Brit. J. Exptl. Pathol., 18,* 299–310 (1932).

[651] W. R. Bloor, *J. Biol. Chem., 49,* 201–227 (1921).

2.0 for the ratio of the maximum levels of lecithin and of cholesterol, respectively, during the acute phase of the disease, as compared with the values for the pre-anemic period.

When dogs were employed as experimental animals, the marked change in anemia was the increased unsaturation of the lipid fraction. In Bloor's experiments,[19] the lecithin fraction, which was increased by 30%, also exhibited the greatest increase in unsaturation (+24%). Terroine,[208] however, found little change in any serum lipids after removal of a large proportion of blood over several days. Some increase was observed in serum cholesterol. Bloor and MacPherson[652] found that the concentration of lipids in the blood cells was less affected by hemorrhage than was that of the lipids in the plasma.

Plasma lipids were found to respond markedly to hemorrhagic anemia, in the case of the guinea pig. Feigl[653] reported that total fatty acids were increased ten times, lecithin four times, and cholesterol seven times by this condition. High lipid values were also reported for a human subject who had undergone a severe loss of blood.[653]

(*b*) *The Blood Lipids in Anemias of Man.* In contradistinction to the results on rabbits, lipid phosphorus and cholesterol are depressed in the plasma of man in several types of anemia.[210,302,652,654–658] In most of these instances, a concomitant rise in the plasma fatty acids was noted, which would indicate an increase in neutral fat.[210,652,654,658] The alterations in blood lipid do not usually occur until the erythrocyte count is less than 50% of the normal[659]; however, in idiopathic anemia, no constant proportionality exists between the severity of the anemia and the change in blood lipids.[656,657] When the anemia is cleared up, the serum lipids return to normal.[656–658]

The reason why a disturbance in the lipid concentration of the blood occurs is obscure. One suggestion as to the cause of the increase in blood lipids in anemia is that they result from the diminished oxygen-carrying power of the blood.[7] Although anoxemia induces hyperlipemia in rabbits,[660]

[652] W. R. Bloor and D. J. MacPherson, *J. Biol. Chem.*, *31*, 79–95 (1917).

[653] J. Feigl, *Biochem. Z.*, *115*, 63–70 (1921).

[654] B. N. Erickson, H. H. Williams, F. C. Hummel, P. Lee, and I. G. Macy, *J. Biol. Chem.*, *118*, 569–598 (1937).

[655] J. Feigl, *Biochem. Z.*, *93*, 257–288 (1919).

[656] G. L. Muller, *Am. J. Med. Sci.*, *179*, 316–337 (1930).

[657] G. L. Muller and C. W. Heath, *Arch. Internal Med.*, *52*, 288–305 (1933).

[658] H. H. Williams, B. N. Erickson, S. Bernstein, F. C. Hummel, and I. G. Macy, *J. Biol. Chem.*, *118*, 599–618 (1937).

[659] W. MacAdam and C. Shiskin, *Quart. J. Med.*, *16*, 193–203 (1923).

[660] U. Starup, *Biochem. Z.*, *270*, 74–92 (1934).

MacLachlan[661] was unable to change the serum lipid in cats or dogs by exposure to low atmospheric pressures for periods of three to six hours. No consistent changes could be demonstrated in the plasma of human beings who had remained for several days at the reduced pressures encountered at elevations of 10,000 to 14,000 feet.[662] Another suggestion as to the reason for the increase in lipid in anemia is that advanced by Fishberg,[649] who suggested that the lipid can compensate for the loss of blood proteins by aiding in the adjustment of the colloid osmotic pressure in the blood.

Variations in the pattern of blood lipids occur in different types of anemia. Thus, in the erythroblastic anemia of children, the blood cells were shown to contain increased amounts of neutral fats.[654] A greater proportion of esterified cholesterol was observed in sickle-cell anemia,[654] whereas, in pernicious anemia, the erythrocytes contain more than the normal amount of cholesterol esters, and an abnormally low proportion of phospholipid.[658] The ether-insoluble phospholipids are increased when pernicious anemia is treated with liver extracts.[663]

h. The Blood Lipids in Diseases of the Gastrointestinal Tract. Any condition which retards the absorption of fats from the gastrointestinal tract will alter the normal curve for alimentary lipemia. This will occur when fat absorption is depressed, due to faulty secretion of pancreatic juice or of bile, and to a lesser extent of gastric juice.

Any obstruction of the gastrointestinal tract will cause vomiting, with a ketosis followed by a hyperlipemia. However, as this condition is prolonged, the level of blood lipids falls to subnormal values.[533] When the foodstuffs are delayed in the stomach for a prolonged period, due to the failure of this organ to empty, the alimentary lipemia is delayed.[664]

In cases of pancreatic steatorrhea, in which pancreatic juice is excluded from the lumen of the intestine, considerable amounts of lipid escape absorption, and are excreted in the feces. This circumstance explains why the alimentary lipemic reaction is decreased or completely abolished in pancreatic steatorrhea.[665] Furthermore, in other types of steatorrhea and allied conditions (non-pancreatic steatorrhea, celiac disease, tropical and non-tropical sprue), there is a decrease in fat absorption. In all these conditions, the hyperlipemia following the ingestion of fat is reduced or entirely eliminated.[666–670] It has been suggested[202] that the postabsorptive concentrations of lipid phosphorus and cholesterol are usually reduced in patients suffering from steatorrhea.

[661] P. L. MacLachlan, *J. Biol. Chem.*, *129*, 465–469 (1939).
[662] G. L. Muller and J. H. Talbott, *Arch. Internal Med.*, *47*, 855–860 (1931).
[663] E. Kirk, *Am. J. Med. Sci.*, *196*, 648–654 (1938).
[664] B. Hejda, *Am. J. Med. Sci.*, *180*, 84–90 (1930).

i. The Blood Lipids in Hepatic Disease. (*a*) *In Cholelithiasis.* When gallstones are formed which do not become lodged in the bile ducts, no abnormalities in lipid metabolism or in the level of blood lipids can be noted. Since the gallstones are frequently composed of cholesterol, it has been postulated that they result from an abnormally high amount of cholesterol in the bile. However, it has been demonstrated that non-obstructive cholelithiasis is not associated with hypercholesterolemia.[671–673] Turner *et al.*[674] reported that the activity of the cholesterol-esterifying enzyme was normal in cholelithiasis and stricture of the common bile duct, as well as in cholecystitis.

(*b*) *In Obstruction of the Common Bile Duct.* It is obvious that, when the common bile duct is occluded by calculi or by a tumor, the absorption of lipids will be retarded, due to the lack of bile acids in the intestine. The effect of such a condition would be akin to that described for steatorrhea, in which there is a considerable excretion of fat in the stools. One would expect that, in the event of bile stoppage, the alimentary lipemic response would be largely abolished.

However, the retention of cholesterol caused by the inability to excrete it in the bile causes a pronounced increase in serum cholesterol,[215,671,673,675,676] which may reach as high as 1000 milligram per cent.[672] Heinlein[676] suggests that the hypercholesterolemia occurring in obstructive jaundice is due not to a defect in excretion but to a disturbance of the storage function of the liver. The higher levels of cholesterolemia are generally found in cases of long-standing obstruction. Albrink *et al.*[677] reported that an increase in the ratio of free to esterified cholesterol obtained in this condition. However, if the obstruction is not relieved, the blood cholesterol falls prior to death.[672] Byers and co-workers[678] reported that the rapid and marked increase in the cholesterol content of the plasma of rats after

[665] N. Poczka and W. Fischel, *Deut. Arch. klin. Med.*, *177*, 14–28 (1934).
[666] D. Adlersberg and H. Sobotka, *J. Nutrition*, *25*, 255–263 (1943).
[667] W. H. Barker and C. P. Rhoads, *Am. J. Med. Sci.*, *194*, 804–810 (1937).
[668] C. E. Kellett, *Lancet*, *1932*, *II*, 1270–1272.
[669] T. E. H. Thaysen, *Quart. J. Med.*, *n.s. 4*, 359–395 (1935).
[670] P. Vogt-Møller and B. Lawaetz, *Acta Med. Scand.*, *92*, 105–134 (1937).
[671] E. Z. Epstein, *Arch. Internal Med.*, *50*, 203–222 (1932).
[672] E. Z. Epstein and E. B. Greenspan, *Arch. Internal Med.*, *58*, 860–890 (1936).
[673] J. A. Gardner and H. Gainsborough, *Quart. J. Med.*, *23*, 465–483 (1930).
[674] K. B. Turner, G. H. McCormack, Jr., and A. Richards, *J. Clin. Invest.*, *32*, 801–806 (1953).
[675] W. B. Hawkins and A. Wright, *J. Exptl. Med.*, *59*, 427–439 (1934).
[676] H. Heinlein, *Z. ges. exptl. Med.*, *91*, 638 (1933).
[677] M. J. Albrink, E. B. Man, and J. P. Peters, *J. Clin. Invest.*, *29*, 781–788 (1950).
[678] S. O. Byers, M. Friedman, and F. Michaelis, *J. Biol. Chem.*, *188*, 637–641 (1951).

obstructive ligation of the common bile duct could not be prevented by prior castration, adrenalectomy, viscerectomy, or ligation of the thoracic duct. However, total or partial hepatectomy prevented the development of hypercholesterolemia. Moreover, no increase in cholesterol could be produced in the partially hepatectomized animal by intravenous infusion of choline, inositol, lecithin, or unchanged bile. It was concluded that the liver itself discharged the excess cholesterol into the blood stream following ligation of the bile duct, although it was not assumed that the liver is necessarily concerned with the formation of the cholesterol in the plasma of normal intact rats.

Lipid phosphorus increases somewhat more than does cholesterol, in biliary obstruction, with a resultant increase in the lipid phosphorus: cholesterol ratio.[215] On the other hand, the neutral fat fraction is somewhat increased at the height of the obstruction, although this increase is considerably less than that of the other fractions.

Friedman and Byers[679] also found an increase of lecithin as well as of bile acids in the plasma, following biliary obstruction. These investigators[680] were of the opinion that the hypercholesterolemia which occurs after the occlusion of the bile duct is due to the retention of some substance other than cholesterol, which, in turn, induces an increased level of blood cholesterol. Since the administration of lecithin was without an effect on hypercholesterolemia, it is suggested that the bile acids are responsible for this condition.[679]

Turner *et al.*[674] reported that, in 16 of 21 cases of obstructive jaundice due to neoplasm, the activity of the cholesterol-esterifying enzyme in human serum was decreased. This is in line with the report of Albrink and coworkers[677] that the ratio of free to total cholesterol rises markedly in obstructive jaundice. The total cholesterol was lowest where the element of parenchymal damage was greatest, and highest when the element of obstruction was highest.[677] The level of phospholipids was found to bear a close relationship to that of the free cholesterol, even when the ratio of free cholesterol:total cholesterol was considerably distorted.[677] It is suggested that this constancy may reflect a basic process in the liver whereby fatty acids are apportioned to the phospholipids and to cholesterol. Byers and collaborators[681] likewise reported that, after simple ligation of the bile duct in rats, the free cholesterol of the plasma was rapidly increased, while a much slower and less rapid rise in the esterified cholesterol level

[679] M. Friedman and S. O. Byers, *Am. J. Physiol.*, *168*, 292–296 (1952).
[680] M. Friedman and S. O. Byers, *J. Exptl. Med.*, *95*, 19–24 (1952).
[681] S. O. Byers, M. Friedman, and F. Michaelis, *J. Biol. Chem.*, *184*, 71–75 (1950).

occurred. When cholesterol was injected into such rats, an increase in free cholesterol was noted in the plasma; this augmentation was superimposed upon that attributable to the biliary obstruction, and did not affect the rate of the development of the latter. Byers *et al.*[681] suggest that the regulation of the free cholesterol level in the plasma of the rat may be dependent upon its destruction or excretion by the hepatobiliary system.

In spite of the rather clear-cut changes of free cholesterol which occur in the blood of rats after ligation of the bile duct, the blood changes occurring in obstructive jaundice in man are less decisive. Epstein and Greenspan[672] observed an increase in the cholesterol ester in the blood of a majority of their patients suffering from obstructive jaundice. On the other hand, Man *et al.*[215] actually noted a reduction of cholesterol ester in the blood of such patients.

(*c*) *In Acute Hepatitis.* In acute diseases of the liver, the serum cholesterol may rise,[306,610,682–686] or there may be no change.[306,410,610] The explanation for the differences is obscure, but the cholesterol level may be associated with the nature of the infection, with its duration, or with the nutritional state of the patient. In the condition referred to as catarrhal jaundice or infectious hepatitis, higher levels of serum cholesterol are usually found,[215,361,687] together with an increase in the ratio of free to esterified cholesterol,[677] and also an increase in the level of lipid phosphorus. Neutral fat increased in this condition to a greater extent than in obstructive jaundice. Oser and Karr[303] made the interesting observation that, whereas the cell cholesterol increases in the jaundice of infectious diseases, the plasma cholesterol is usually diminished. Thus, an analysis of whole blood might indicate that no change in cholesterol content had occurred in this condition.

In the so-called "yellow atrophy of the liver," which is a particularly toxic form of hepatitis, cholesterol may increase[687] or decrease,[672] while the proportion of esters falls. In a number of other cases of liver disease, serum cholesterol values are actually lower than normal for some of the patients suffering from hepatitis.[215,675,676,688] It is not known what particular condition is responsible for the hypocholesterolemia, although it is suspected that it is related to the severity of the disease.

[682] A. Adler and H. Lemmel, *Deut. Arch. klin. Med.*, *158*, 173–213 (1927–1928).
[683] J. Feigl, *Biochem. Z.*, *86*, 1–47 (1918).
[684] J. M. H. Campbell, *Quart. J. Med.*, *18*, 123–31, 393–422 (1924).
[685] A. Grigaut and A. L'Huillier, *Compt. rend. soc. biol.*, *73*, 202–203 (1912).
[686] A. Chauffard, G. Laroche, and A. Grigaut, *Compt. rend. soc. biol.*, *73*, 23–25 (1912).
[687] R. Mancke, *Deut. Arch. klin. Med.*, *170*, 358–368 (1931).
[688] E. M. Boyd and W. F. Connell, *Arch. Internal Med.*, *61*, 755–761 (1938).

Irrespective of whether the serum cholesterol is high or low, an elevation in the ratio of free cholesterol:total cholesterol is the rule. In cases in which the total cholesterol has reached abnormally low levels, the proportion of esterified cholesterol may approach zero. One of the widely accepted criteria of liver function over a number of years has been the ability of this organ to form cholesterol esters. The lowering of the combined fraction of this sterol in severe hepatitis is therefore an expression of the weakening of that function. On the other hand, Turner *et al.*[674] reported a low activity on the part of the cholesterol-esterifying enzyme in human serum in hepatitis. These workers state that the decreasing enzyme activity is an ominous prognostic sign.

(*d*) *In Cirrhosis of the Liver.* No consistent results have been reported in regard to the pattern of serum lipids in this condition. In the terminal stages coincident with a decreased amount of functioning liver tissue, serum cholesterol reaches subnormal levels,[215,533,672] lipid phosphorus is reduced, but the lipid phosphorus:cholesterol ratio remains practically unchanged. Man *et al.*[215] reported relatively low values for neutral fat in the serum under these conditions. A larger proportion of the cholesterol is also free.[215,256] This is in line with the report of Turner *et al.*,[674] who observed a lower concentration of the cholesterol-esterifying enzyme in the serum of such patients.

(*e*) *Following Partial or Functional Hepatectomy.* Friedman and associates[689] reported that when the plasma cholesterol levels of rats were drastically reduced by plasmapheresis, the restoration of this sterol was inhibited or prevented for twenty-four hours following partial hepatectomy. These workers concluded that the liver is responsible for the maintenance of the normal plasma cholesterol content. However, Chanutin and Gjessing[690] did not report a decrease in cholesterol, total lipid or cholesterol in rats after partial hepatectomy, laparotomy, thermal injury, or after the injection of tris(β-chloroethyl)amine. However, plasmapheresis was not employed, and the degree of functional activity of the liver may have varied from that in the experiments of Friedman *et al.*[689]

Ranney and Chaikoff[691] observed that the hyperlipemia which is ordinarily observed in birds following the injection of diethylstilbestrol does not occur in fowl subjected to functional hepatectomy. This indicates that the augmentation of the blood lipids following this estrogenic agent is dependent upon the presence of the liver.

[689] M. Friedman, S. O. Byers, and F. Michaelis, *Am. J. Physiol.*, *164*, 789–791 (1951).
[690] A. Chanutin and E. C. Gjessing, *J. Biol. Chem.*, *178*, 1–5 (1949).
[691] R. E. Ranney and I. L. Chaikoff, *Am. J. Physiol.*, *165*, 600–603 (1951).

j. The Blood Lipids in Diseases of the Kidney. (*a*) *In Nephroses.* Marked lipemia occurs in nephrosis coincident with edema, when the latter is of non-cardiac origin.[210,340,585,613,692,693] The concentration of lipids is so high that the serum frequently exhibits a lactescence (milky appearance). Serum cholesterol values as high as 1% have been noted. Although Lichtenstein and Epstein[694] reported that the esters were present in an unusually high proportion, most other workers have reported a normal free cholesterol: total cholesterol ratio.[695–698] The lipid phosphorus also increases, but to a lesser degree than does the cholesterol. Thomas *et al.*[698] have reported that the increase in neutral fat is disproportionally great in children with nephrotic lipemia.

Although the most pronounced degree of hyperlipemia is usually found in the patients with the lowest serum albumin and the greatest albuminuria (and hence the greatest degree of edema), it has not been possible to correlate the lipid concentration with the decrease in serum albumin.[694,696–700] However, Thomas *et al.*[698] suggest that there is some evidence of a correlation between lipid levels in the blood and those of albumin and γ-globulin.

In some cases, edema and lipemia exhibit an inverse relationship.[697] However, when the edema disappears, due either to a regression or to a recovery from the disease, a concomitant decrease in hyperlipemia usually occurs. In some cases, hyperlipemia may continue for some time after all evidence of edema has vanished.[701] In the terminal or uremic phases of the nephrotic syndrome, the blood lipids drop to subnormal levels.[696,697,702–704] These lower values may obtain during the terminal stages of the disease, irrespective of whether or not the edema has subsided.[696,697] The decrease in cholesterol following the initial maximal rise is prevented by the oral administration of sodium cholate.

In addition to the hyperlipemia associated with nephroses, Rosenman

[692] F. Erben, *Z. klin. Med.*, *50*, 441–463 (1903).
[693] F. Erben, *Z. klin. Med.*, *57*, 39–69 (1905).
[694] L. Lichtenstein and E. Z. Epstein, *Arch. Internal Med.*, *47*, 122–127 (1931).
[695] H. Gainsborough, *Quart. J. Med.*, *23*, 101–127 (1929).
[696] I. H. Page, E. Kirk, and D. D. Van Slyke, *J. Clin. Invest.*, *15*, 101–107 (1936).
[697] J. P. Peters and E. B. Man, *J. Clin. Invest.*, *22*, 721–726 (1943).
[698] E. M. Thomas, A. H. Rosenblum, H. B. Lander, and R. Fisher, *Am. J. Diseases Children*, *81*, 207–214 (1951).
[699] I. H. Page and L. E. Farr, *J. Clin. Invest.*, *15*, 181–191 (1936).
[700] J. Bing and U. Starup, *Acta Med. Scand.*, *86*, 12–21 (1935).
[701] J. K. Calvin and A. H. Goldberg, *Am. J. Diseases Children*, *41*, 1066–1080 (1931).
[702] B. I. Ashe and M. Bruger, *Am. J. Med. Sci.*, *186*, 670–678 (1933).
[703] A. A. Epstein and M. A. Rothschild, *J. Biol. Chem.*, *29*, iv–v (1917).
[704] J. Maxwell, *Quart. J. Med.*, *3*, 79–83 (1934).

and co-workers[705] pointed out that a *hypercholatemia* (increase in bile acids in the blood) occurs in human patients with nephrosis. This condition was also observed in rats in whose case an experimental nephrosis had been induced by the injection of anti-rat kidney serum,[705a] whenever hypercholesterolemia was also present. Moreover, the nephrotic rat was found to exhibit a diminished ability to rid the blood of injected cholate.

In later work by Byers, Friedman, and Rosenman[705b] in which this method for producing experimental nephrosis in the rat was confirmed, it was found that the hypercholesterolemia produced in rats by the injection of anti-rat kidney serum was not associated with any change in the rate of cholesterol synthesis. The conclusion is reached that the hypercholesterolemia in the nephrotic is neither initiated nor maintained by an accelerated rate of cholesterol production in the liver. It has also been established that the hypercholesterolemia in the nephrotic rat cannot be ascribed to an increased absorption of dietary cholesterol from the gastrointestinal tract.[705c]

(*b*) *In Nephritides.* In those forms of kidney disease in which edema is not a prominent symptom, hyperlipemia may be absent. Thus, in acute nephritis, blood lipids remain at a normal level in the early stages, but a hyperlipemia develops in the later stages, coincident with the edema.[704] In forms of nephritis in which little or no edema exists, the blood lipids usually remain at a normal level.[696,697] When lipemia develops in nephritis, neutral fat plays an important role.[340] The amounts vary greatly, and do not exhibit the regular relationships shown by phosphorus and cholesterol. The differences in response may be related to the effects of a single large fatty meal[196,706]; however, the fat intake apparently does not affect the postabsorptive level of serum lipids.[361,699,706,707]

The basic reason for the hyperlipemia in kidney disorders is not clear. According to the data of Hiller *et al.*,[340] there would not appear to be any inability to burn fat. According to Peters and Van Slyke,[202] an impairment in the processes concerned with the mobilization of lipids would seem to be the main cause of the abnormality. Epstein[707] suggested the use of thyroid preparations in the treatment of nephrosis, since he believed that the disorder was caused by hypothyroidism. However, Page and Farr[699] could not alter the characteristic hypercholesterolemia of nephritis by the

[705] R. H. Rosenman, M. Friedman, and S. O. Byers, *J. Clin. Invest.*, *32*, 121–124 (1953).

[705a] W. Heymann and H. Z. Lund, *Pediatrics*, *7*, 691–706 (1951).

[705b] S. O. Byers, M. Friedman, and R. H. Rosenman, *Am. J. Physiol.*, in press (1954).

[705c] R. H. Rosenman, M. Friedman, and S. O. Byers, *Am. J. Physiol.*, in press (1954).

[706] A. I. Odinow and S. N. Guschtschina, *Z. klin. Med.*, *128*, 358–364 (1935).

[707] A. A. Epstein, *Am. J. Med. Sci.*, *163*, 167–186 (1922).

use of thyroid preparations. Moreover, the nephritic type of hyperlipemia differs from that in myxedema by virtue of the high neutral fat content in the blood.

No increase in blood lipids could be demonstrated in nephrolithiasis, pyelitis, or cystitis, although a moderate hyperlipemia was observed in pyelonephritis.[610]

(c) *In Experimental Nephrectomy.* Heymann and Clark[708] showed that the blood lipids are increased in rats and dogs following nephrectomy or after the parenteral administration of nephrotic agents. After the complete ablation of the kidneys, a progressive increase in blood cholesterol has been shown to occur, until death ensues,[709] in cats as well as in dogs; in the latter case, both bilateral nephrectomy and also destruction of the kidneys by means of mercuric chloride were employed.[710] When carbon tetrachloride was administered orally, prior to the subcutaneous administration of mercuric chloride, the hyperlipemia could usually be prevented in dogs.[711] In experiments with dogs and monkeys, in which the kidneys were extirpated or the ureters were tied, Winkler and associates[712] noted that not only was cholesterol increased in the blood, but an elevation of the phospholipid and of the neutral fat level also occurred. The development of the hyperlipemia could not be traced to the operative procedures nor to fasting. When the bile duct was ligated in dogs simultaneously with a bilateral nephrectomy, Diaz and Mendoza[713] observed an increase of the fat deposits in the liver, a decrease in the phospholipids, and an increase in the cholesterol esters. The cholesterol esterase activity is decreased in the plasma. The authors conclude that the kidney plays an important role in fat utilization.

In contradistinction to the lipemia in experimental nephrectomy in the lower animals, opposite results have been observed in human subjects. Thus, Heymann[711] observed that no hyperlipemia or hypercholesterolemia occurred in the case of three patients subjected to unilateral nephrectomy. Atlas and co-workers[714] likewise reported no alterations in total lipids, phospholipids or cholesterol in the serum of patients following nephrectomy

[708] W. Heymann and E. C. Clark, *Am. J. Diseases Children*, *70*, 74–82 (1945).
[709] W. Nekludow, *Z. ges. exptl. Med.*, *47*, 70–76 (1925).
[710] W. Heymann, *Science*, *96*, 163–164 (1942).
[711] W. Heymann, *Proc. Soc. Exptl. Biol. Med.*, *66*, 82–86 (1947).
[712] A. W. Winkler, S. H. Durlacher, H. E. Hoff, and E. B. Man, *J. Exptl. Med.*, *77*, 473–486 (1943).
[713] C. Jiménez Díaz and H. Castro-Mendoza, *Rev. clin. españ.*, *37*, 232–235 (1950); *Chem. Abst.*, *44*, 10856 (1950).
[714] D. M. Atlas, M. M. Cash, and M. M. Kirschen, *Am. J. Clin. Pathol.*, *19*, 962–965 (1949).

or during fatal mercuric chloride poisoning. Heymann[711] also observed normal or low values for total lipids, cholesterol, and phospholipids in the serum of six patients with mercuric chloride poisoning. The lipid content is also depressed in human beings during the terminal stages of nephritis.

k. The Blood Lipids in Arteriosclerosis. In the condition of *arteriosclerosis*, there is a loss in the elasticity of the arteries, associated with a thickening and a hardening of their walls. This condition usually occurs in hypertension, although increased blood pressure may originate from a variety of other causes, as well. In the type of arteriosclerosis produced experimentally in animals, which is referred to as *atherosclerosis*, only the intima (the innermost of the three layers of the arterial wall) is involved. The atheromatous lesions (or "patches") present in this layer contain small droplets of lipid material.

(*a*) *Experimental Studies on Atherosclerosis.* a'. The Relationship of Cholesterol to the Formation of the Atheromatous Lesions: The atheromas have been shown, both by microscopical examination and by chemical analysis, to contain considerable proportions of lipids. Cholesterol can readily be detected microscopically in these tissues, because of its characteristic property of birefringence. Morrison and Johnson[715] observed that the average cholesterol content of the coronary arteries of patients who had died of acute coronary thrombosis was four times the average for a group of control patients. A number of investigators have demonstrated that cholesterol accumulates in these atheromatous patches by preference.[716–720] The amount of cholesterol deposited in the aorta of presumably normal persons has been shown by Bürger[361] and by Rosenthal[718] to increase with advancing age. In fact, Bragdon[721] reported that a spontaneous atherosclerosis may be present in aging rabbits. In the later stages of atherosclerosis, calcium is deposited in the atheromas. Because of the predominance of cholesterol in the atheromatous masses, the belief has become widespread that the deposition of cholesterol in the arterial wall is probably attributable to a hypercholesterolemia.

Bjorksten[722] demonstrated that the intima of fresh hog aortas can be made to adsorb and retain cholesterol from a suspension, provided that

[715] L. M. Morrison and K. D. Johnson, *Amer. Heart J.*, *39*, 31–34 (1950).
[716] T. Leary, *Arch. Pathol.*, *32*, 507–555 (1941).
[717] C. S. McArthur, *Biochem. J.*, *36*, 559–570 (1942).
[718] S. R. Rosenthal, *Arch. Pathol.*, *18*, 473–506, 660–698, 827–842 (1934).
[719] R. Schönheimer, *Arch. path. Anat. Physiol.* (*Virchow's*), *249*, 1–42 (1924).
[720] R. Schönheimer, *Arch. path. Anat. Physiol.* (*Virchow's*), *251*, 732–738 (1924).
[721] J. H. Bragdon, *Circulation*, *5*, 641–646 (1952).
[722] J. Bjorksten, *Proc. Soc. Exptl. Biol. Med.*, *81*, 350–353 (1952).

the intima was first treated with certain cross-linking agents, of which lead acetate is one of the most efficacious. On the basis of these results, the hypothesis is advanced that the primary cause of cholesterol deposition and the subsequent pathologic involvement of the arteries may be a chemical process affecting the protein material in the lining of the arteries. These are changed from cholesterophobic to cholesterophilic substances, presumably due to a gradual cumulative action on the part of certain protein cross-linking agents.

Injury may cause the formation of atheromas. Thus, Schlichter, Katz, and Meyer[723] obtained some evidence that atheromas developed in dogs when cholesterol was given following cauterization of the aortas, in contradistinction to the negative results in the control animals which had not been cauterized prior to the oral or intraperitoneal administration of cholesterol. Moreover, Duff[724] demonstrated that injury to the intima precedes the deposition of cholesterol in the blood vessels. The primary cause of the atheroma may not be a hypercholesterolemia or a disturbance in cholesterol metabolism, but rather a chemical disturbance in the tissue which favors the deposition of cholesterol in a particular area. This disturbance may be exaggerated or increased by high plasma levels of cholesterol, and thus act as a contributory cause of atherosclerosis, although Duff[724] failed to find direct evidence of any such effect.

Another suggestion as to the relationship of blood cholesterol to atherosclerosis, by Alvarez and Neuschlosz,[725] is that the sterol is present in the blood in this disease in a less readily soluble form; it is thought that, under such conditions, cholesterol will precipitate even if present at normal levels. However, neither Medvei[726] nor Holden[727] was able to substantiate this theory.

The formation of atheromas is believed to be related to the physical state of the atherogenic agents. Thus, the large particles of fatty materials in the blood during hyperlipemia are removed by the reticuloendothelial cells in the arterial intima, and are retained due to the barrier action of the internal elastic membrane. Moreton[728] demonstrated that this action was not confined to lipid particles in the blood, but also occurred when other coarsely suspended foreign substances, such as methyl cellulose, pectin, and polyvinyl alcohol, were present in this fluid. Cholesterol deposition

[723] J. G. Schlichter, L. N. Katz, and J. Meyer, *Am. J. Med. Sci.*, *218*, 603–609 (1949).
[724] G. L. Duff, *Arch. Pathol.*, *20*, 81–123, 259–304 (1935).
[725] C. Alvarez and S. M. Neuschlosz, *Klin. Wochschr.*, *10*, 244–247 (1931).
[726] C. V. Medvei, *Klin. Wochschr.*, *11*, 414–416 (1932).
[727] R. F. Holden, Jr., *J. Clin. Invest.*, *16*, 763–765 (1937).
[728] J. R. Moreton, *Science*, *107*, 371–373 (1948).

in arterial walls was likewise demonstrated in *in vitro* tests of Wilens,[729] who reported that a visible lipid retention occurred if normal human blood serum was filtered through the walls at normal arterial pressures for twenty-four hours or longer. It was estimated that 2 to 38% of the cholesterol was retained intramurally. It was suggested that atherosclerosis results from filtration of serum through the walls of the artery; the major portion of the cholesterol fails to enter the arterial intima, due to its linkage with large protein molecules. It is postulated that only free cholesterol can pass into the arterial walls.

Although there is considerable indication that cholesterol deposition is the primary cause for the development of atheromas, Peters and Van Slyke[202] do not subscribe to this hypothesis. The latter investigators state that the presence of cholesterol in the atheromatous patches does not prove that a hypercholesterolemia or any disturbance in cholesterol metabolism has been the cause of the deposition. In fact, Chernick, Srere, and Chaikoff,[730] as well as Werthessen *et al.*[731] have shown that cholesterol can be synthesized in the arterial wall.

b′. Dietary and Other Factors Related to the Formation of Atheromas: A large number of workers have shown that a diet high in cholesterol causes the development of atherosclerosis in rabbits,[732–734] in chickens,[735,736] and in geese.[737] Wissler and co-workers[738] reported that it was possible to produce lipomatous lesions in the coronary arteries of rats fed for a year on a diet containing a high proportion of lard and a high level of choline, with or without added cholesterol. On the other hand, no significant lesions were noted in rats receiving similar diets, for the same period, when corn oil was substituted for the lard, or in rats on a commercial stock diet. The incidence of the lesions was correlated with the progressive changes in the lipid components of the blood. Although Anitschkow[739] was unable

[729] S. L. Wilens, *Science, 114*, 389–393 (1951).

[730] S. Chernick, P. A. Srere, and I. L. Chaikoff, *J. Biol. Chem., 179*, 113–118 (1949).

[731] N. T. Werthessen, L. J. Milch, R. F. Redmond, L. L. Smith, and E. C. Smith, *Am. J. Physiol., 178*, 23–29 (1954).

[732] N. Anitschkow and S. S. Chalatow, *Zentr. allgem. Pathol. u. path. Anat., 24*, 1–9 (1913).

[733] O. J. Pollak, *Am. Heart J., 38*, 459–460 (1949).

[734] A. Kuntz and N. M. Sulkin, *Arch. Pathol., 47*, 248–260 (1949).

[735] L. Horlick, and L. N. Katz, *Am. Heart J., 38*, 336–349 (1949).

[736] J. E. Peterson and A. E. Hirst, *Circulation, 3*, 116–119 (1951).

[737] J. B. Wolffe, A. S. Hyman, M. B. Plungian, A. D. Dale, G. E. McGinnis, and M. B. Walkow, *J. Gerontol., 7*, 13–23 (1952).

[738] R. W. Wissler, J. L. Collins, M. Schroeder, and K. Soules, *Federation Proc., 12*, 407 (1953).

[739] N. Anitschkow, *Verhandl. deut. pathol. Ges., 20*, 149–154 (1925).

to produce atherosclerosis in cats or dogs by cholesterol feeding, Steiner *et al.*[740] obtained positive results when thiouracil was also given. These results would indicate that atherogenesis may occur more readily when hypofunction of the thyroid gland obtains. This is in line with the report of Katz and co-workers[741] that the thyroid hormone is able to suppress both hypercholesterolemia and atherogenesis. This is accomplished by a direct effect on lipid metabolism, since it does not follow the administration of the thyroid-stimulating hormone of the anterior pituitary gland.

Hormones other than those associated with the thyroid are also active in relation to atherogenesis. Thus Compound F (17-hydroxycorticosterone) markedly intensifies hypercholesterolemia and hyperlipemia in cholesterol-fed chicks, without influencing blood pressure or atherogenesis.[741] ACTH in large doses duplicates these effects, in contrast to the opposite behavior of cortisone and desoxycorticosterone. The estrogenic hormones, when given with a high cholesterol diet, completely prevent the development of atherosclerosis. In chicks with developed atherosclerosis resulting from cholesterol ingestion, the coronary lesions were completely reversed, when estrogens were given, despite the continued feeding of atherogenic material. These effects could be maintained when androgenic hormones were given simultaneously. The estrogen effect was shown to be associated with significant alterations of the plasma lipid-lipoprotein patterns. It was found that the total cholesterol:lipid P ratio was reduced toward normal levels.[741]

Vitamin B_6 has also been considered to be associated with the development of atherosclerosis. Thus, in the case of the monkey, Rinehart and Greenberg[742,743] succeeded in producing an atherosclerosis by means of a pyridoxine-deficient diet, while prolonged feeding with cholesterol failed to produce significant lesions. In a later study,[744] it was shown that a greater degree of cholesterolemia was present in the pyridoxine-deficient monkey receiving cholesterol than in the normal animal which received considerably higher quantities of cholesterol. Mice and rats are resistant to atherosclerosis, as are also hamsters.[745–747] Although Altschul[746] was

[740] A. Steiner, F. E. Kendall, and M. Bevans, *Am. Heart J.*, *38*, 34–42 (1949).

[741] L. N. Katz, R. Pick, and J. Stamler, *XIXth Intern. Physiol. Congress*, Montreal (Aug.–Sept., 1953), *Abst.*, 505–506.

[742] J. F. Rinehart and L. D. Greenberg, *Am. J. Pathol.*, *25*, 481–491 (1949).

[743] J. F. Rinehart and L. D. Greenberg, *Arch. Pathol.*, *51*, 12–18 (1951).

[744] L. D. Greenberg and J. F. Rinehart, *Proc. Soc. Exptl. Biol. Med.*, *76*, 580–583 (1951).

[745] J. Goldman, *Arch. Pathol.*, *49*, 169–172 (1950).

[746] R. Altschul, *Am. Heart J.*, *40*, 401–409 (1950).

[747] W. Marx, L. Marx, and H. J. Deuel, Jr., *Am. Heart J.*, *42*, 124–128 (1951).

able to produce typical vascular lesions by the use of cholesterol in the diet, the reactions were less generalized. The effect of cholesterol feeding on the production of atherosclerosis is discussed in the monograph of Cowdry,[748] as well as in a number of more recent reviews.[749–755]

Some investigators, for example Gould,[756] believe that an equilibrium exists between plasma cholesterol and liver cholesterol. This is not invariably the case, however, as is shown by the results of Marx *et al.*[747]; these workers were unable to demonstrate any constant relationship, in several species of animals, between the level of liver and of plasma cholesterol and their relative susceptibility to atherosclerosis. These data are summarized in Table 16.

TABLE 16

CHOLESTEROL CONTENT OF THE BLOOD PLASMA AND LIVERS OF SEVERAL SPECIES OF ANIMALS BEFORE AND AFTER ADMINISTRATION OF A DIET CONTAINING CHOLESTEROL[a]

Species	Diet supplement		Length of feeding, weeks	No. of animals	Av. cholesterol content	
	Cholesterol	Bile salts			Plasma, mg. %	Liver, %
Rat	0	0	—	17	60 ± 3	0.21 ± 0.02
	+	+	26–38	9	133 ± 12	2.03 ± 0.11
Hamster	0	0	—	9	57 ± 5	0.22 ± 0.04
	+	0	25	6	160 ± 14	5.11 ± 0.17
	+	+	16–25	13	282 ± 34	8.68 ± 0.88
Chicken	0	0	—	5	100 ± 3	0.41 ± 0.07
	+	+	10	4	685 ± 164	1.67 ± 0.21
Rabbit	0	0	—	5	35 ± 2	0.17 ± 0.02
	+	0	15–21	7	455 ± 36	1.33 ± 0.10

[a] W. Marx, L. Marx, and H. J. Deuel, Jr., *Am. Heart J.*, *42*, 124–128 (1951).

The data summarized in Table 16 leave no doubt that the feeding of cholesterol along with bile salts markedly increases the level of this sterol in the plasma over that obtained on the cholesterol-free basal diet. In the

[748] E. V. Cowdry, *Arteriosclerosis*, MacMillan, New York, 1933, pp. 281–294.

[749] L. N. Katz, *Circulation*, *5*, 101–114 (1952).

[750] A. Keys, *Circulation*, *5*, 115–118 (1952).

[751] J. W. Gofman, H. B. Jones, T. P. Lyon, F. T. Lindgren, B. Strisower, D. Colman, and V. Herring, *Circulation*, *5*, 119–134 (1952).

[752] I. H. Page, *Biol. Symposia*, *11*, 43–73 (1945).

[753] L. N. Katz and D. V. Dauber, *J. Mt. Sinai Hosp.*, *12*, 382–410 (1945–1946).

[754] R. Gubner and H. E. Ungerleider, *Am. J. Med.*, *6*, 60–83 (1949).

[755] W. B. Kountz, A. Sonnenberg, L. Hofstatter and G. Wolff, *Biol. Symposia*, *11*, 79–86 (1945).

[756] R. G. Gould, D. J. Campbell, F. B. Kelly, Jr., C. B. Taylor, J. A. Hagerman, and I. Warner, *Circulation*, *4*, 479 (1951).

case of the hamster, the mean value of plasma cholesterol, when cholesterol is administered with bile salts, is practically double that observed when cholesterol is given alone. The highest levels of plasma cholesterol were found in chickens and rabbits, which are two of the species susceptible to

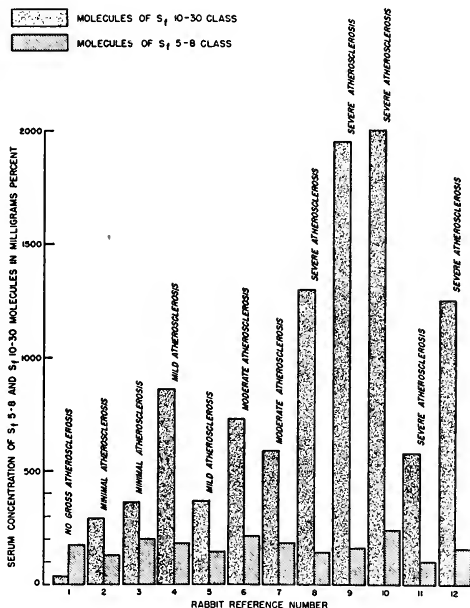


Fig. 5. Plot showing the relationship of the severity of atherosclerosis (as determined by autopsy) to the concentration of S_f 5–8 and S_f 10–30 classes of molecules in the serum of rabbits which had received cholesterol for 15 weeks prior to examination.[96] See p. 472.

atherosclerosis. On the other hand, the picture is reversed when one compares the figures for liver cholesterol in the species which are resistant to atherosclerosis and in those which are susceptible to the condition, respectively. The highest averages were reported in the livers of the hamsters and rats; these animals are not subject to atheromas. Possibly the fact that hamsters and rats have the ability to store the cholesterol more effi-

ciently in the liver than do the chicken or the rabbit accounts for the variation in blood cholesterol noted in the several species.

The relationship between dietary cholesterol and blood cholesterol in the rabbit has recently been investigated from an entirely new viewpoint by Gofman and his co-workers.[96,98] These investigators found that the plasma of normal rabbits contains a lipoprotein, having a hydrated density of 1.03, which contains about 30% of cholesterol by weight. When serum from normal rabbits was subjected to ultracentrifugation, the lipoprotein appeared with a flotation value of 5–8 (S_f = 5–8).

When cholesterol was fed to rabbits in dosages of 3 g. per week, an interesting sequence of events ensued. At first there was an increased concentration of the S_f 5–8 fraction amounting to as much as four times the previous values. However, after a longer period (thirty to forty days) another series of molecules appeared which had flotation rates varying between S_f 10 and 30. The S_f 10–30 class components, also, do not usually appear until the serum cholesterol exceeds 200–250 milligram per cent.[96] In some instances, components having a higher S_f value are likewise formed. Subsequent to the appearance of the S_f 10–30 class, the S_f 5–8 molecules are maintained at approximately the same level, while the S_f 10–30 class continues to increase. Variations obtain between different rabbits in the amount of these components, which range from none at all to any number of the higher S_f components. A comparison of the proportionate amounts of S_f 5–8 and S_f 10–30 in the blood of rabbits, after fifteen weeks of cholesterol feeding, with the extent of atherosclerosis, is shown in Figure 5. A further discussion of the flotation rates of cholesterol compounds is given in the next section, dealing with investigations on man.

(*b*) *Arteriosclerosis in Man.* a′. Comparison of Atherosclerosis in Rabbits and Arteriosclerosis in Man: Although a hypercholesterolemia following cholesterol feeding is associated with the production of an atherosclerosis in rabbits, there is some question as to whether or not these experimental data apply to arteriosclerosis in man. Although Leary[757] reported that the lesions obtained from the blood vessels of rabbits having an experimentally produced atherosclerosis and those obtained from the coronary arteries of atherosclerotic human patients on autopsy were similar, their distribution is not confined to such a limited area in the rabbit as in man[718]; in fact, atheromatous lesions have been demonstrated not only in the aortic and systemic vessels of rabbits but also in the pulmonary system[719] and even in the veins.[720] According to Peters and Van Slyke,[202] the experimental atherosclerosis of rabbits differs from the disease

[757] T. Leary, *Arch. Pathol.*, *17*, 453–492 (1934).

condition in man by the speed of development and also by the unusual susceptibility of the former to iodine and to the activity of the thyroid gland.

b′. Gross Blood Lipid Changes in Arteriosclerosis: There is also considerable disagreement as to whether or not hypercholesterolemia is an invariable concomitant of hypertension in man, and inversely whether hypertension invariably follows the exposure of the body to a hypercholesterolemia over an extended period. A number of investigators, including Fahrig and Wacker,[300] Koch and Westphal,[758] Medvei,[726] Wacker and Fahrig,[759] and Harris[760] reported that cholesterol is increased in hypertension. Some workers have indicated that all blood lipids, as well, are increased.[300,759] Alford[761] found that coronary heart disease and xanthoma tuberosum are associated with hereditary hyperlipemia. Increases either in the total serum lipids or in the serum cholesterol, or in both, were found. Gertler and Garn[762] likewise observed that serum cholesterol was considerably higher in males who had experienced myocardial infarction than it was in healthy, active males. In the coronary disease group, both cholesterol and phospholipids were increased in the serum, but the cholesterol : phospholipid ratio was likewise increased, indicating that the rise in phospholipid had not kept pace with that of cholesterol. It is suggested that the factors favoring the deposition of cholesterol in the intima are enhanced because of the lack of sufficient phospholipid to act as a colloid stabilizer.

On the other hand, one must recognize that hypercholesterolemia exists in a variety of diseases other than hypertension. Several investigators are of the opinion that a normal cholesterol level may occur in hypertension unless a concomitant disorder such as nephritis is likewise present.[210,697,725,763,764] The occurrence of hypertension is not considered to be adequate proof of the existence of atherosclerosis.[202]

c′. The Relationship of the S_f 10–20 Fraction to Atherosclerosis: Gofman and his associates[96,98] have advanced an attractive theory to explain the relationship of blood cholesterol to atherosclerosis. These workers point out that, while the total serum cholesterol level may exhibit no uniform relationship to hypertension, the concentration of the

[758] K. Koch and K. Westphal, *Deut. Arch. klin. Med.*, *181*, 413–484 (1937–1938).
[759] L. Wacker and C. Fahrig, *Klin. Wochschr.*, *11*, 762–766 (1932).
[760] I. Harris, *Lancet*, *257*, 283–285 (1949).
[761] R. M. Alford, *Arch. Internal Med.*, *84*, 1002–1019 (1949).
[762] M. M. Gertler and S. M. Garn, *Science*, *112*, 14–16 (1950).
[763] I. Harris and I. J. Lipkin, *Brit. Med. J.*, *1930*, *1*, 587–588.
[764] I. H. Page, E. Kirk, and D. D. Van Slyke, *J. Clin. Invest.*, *15*, 109–113 (1936).

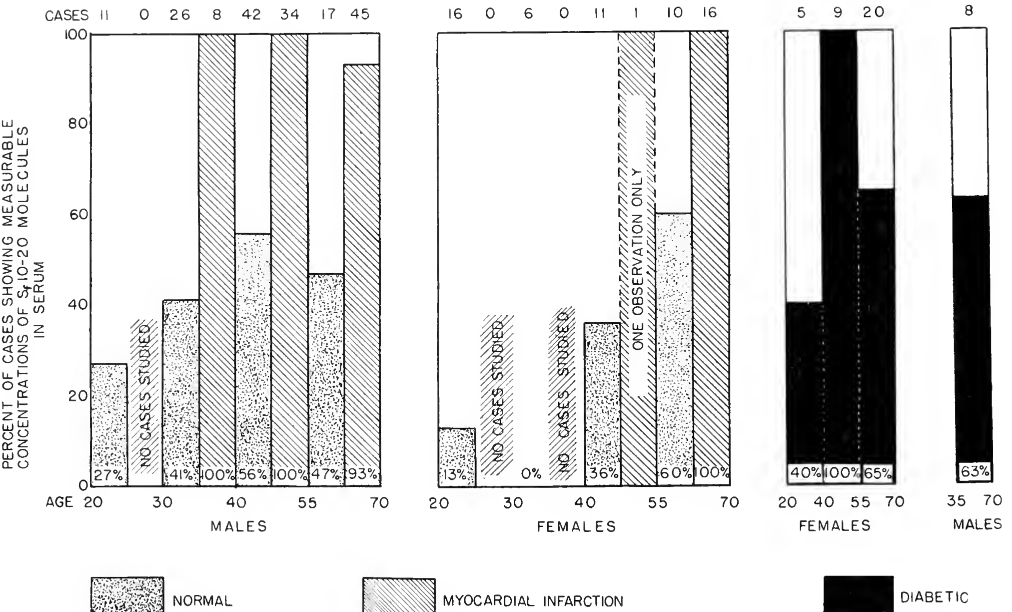


Fig. 6. The concentration of S_f 10–20 molecules in the serum of normal males and females, of those who had previously experienced myocardial infarcts, and of those who had diabetes. The blank areas are for "normal" individuals, the lined areas for those with a previous history of myocardial infarcts, while the solid areas are for diabetic patients.[96] See p. 476.

blood lipoprotein which is combined with cholesterol may offer a fairly constant relationship. Thus, it was shown that, in the human subject, in addition to S_f 3–8 molecules, varying quantities of a fraction described as

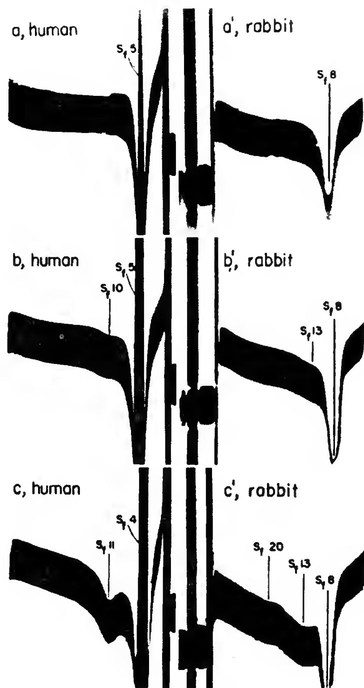


Fig. 7. Ultracentrifugal flotation diagrams of lipids and liproproteins in human and rabbit sera:[96] (a) normal human serum showing only S_f 3–8 class; (b) human serum showing moderate amount of S_f 10–20 class; (c) human serum showing high concentration of S_f 10–20 class; (a′) normal rabbit serum showing only a lipoprotein of S_f 5–8 class; (b′) rabbit serum from animal shortly after start of cholesterol feeding, showing small amount of S_f 10–30 class with S_f 5–8 fraction; (c′) rabbit serum from animal after extended period of cholesterol feeding (this has a large amount of S_f 10–30 fraction and also lipoproteins of S_f 5–8 class). The analytical runs were made at a rotor speed of 52,640 r.p.m. in a cell of 0.8 ml. capacity.

S_f 10–20 occur. Molecules in the S_f 3–8 class contain about 25% of protein, while the S_f 10–20 class has little protein but 30% of cholesterol by weight.

In a study of a large number of normal men and women of widely different age groups, Gofman *et al.*[96] found that the incidence of increased concentration of S_f 10–20 molecules was much more frequent in the "normal" males twenty to forty years of age than in the same female "normal" age group. In the series of normals over forty, both males and females had a definite increase in the S_f 10–20 fraction over the levels in the corresponding younger groups. These facts are in line with the incidence of atherosclerosis as related to age in the two sexes. All but 3 of 104 patients who had previously experienced a myocardial infarction had concentrations of S_f 10–20 molecules above normal. It is known that more than 95% of the myocardial infarctions occur in individuals who have hypertension. The high incidence of exaggerated levels of the S_f 10–20 fraction in 97% of the cases examined offers an interesting correlation between hypertension and this specific cholesterol fraction. Observations made on diabetic subjects also indicated that the S_f 10–20 fraction was markedly elevated above normal in a number of cases. A graphic representation of the relationship between the concentration of the S_f 10–20 fraction in the serum of normal men as contrasted with the values for this fraction in the serum of patients who had previously suffered from myocardial infarction or who had diabetes, is given in Figure 6 on p. 474.

Figure 7 gives examples of flotation diagrams obtained by Gofman *et al.*[96] from samples of rabbit and human blood serum after ultracentrifugation. See p. 475.

d′. The Relationship of Fractions Higher Than S_f 10–20 to Atherosclerosis: In more recent studies of the Gofman group,[98,765–768] it has been disclosed that the group of serum lipoproteins containing cholesterol represents a large number of different varieties having densities from 1.12 g./ml. to less than 1.0 g./ml. Lindgren *et al.*[766] and Jones *et al.*[767] have been able to demonstrate several discrete entities as follows: S_f 2, S_f 4, S_f 6, S_f 8, S_f 10, S_f 13, and S_f 17. These differ from each other in physical properties as well as in chemical structure. There are likewise additional

[765] J. W. Gofman, F. T. Lindgren, H. B. Jones, T. P. Lyon, and B. Strisower, *J. Gerontol.*, *6*, 105–119 (1951).

[766] F. T. Lindgren, H. A. Elliott, and J. W. Gofman, *J. Phys. & Colloid Chem.*, *55*, 80–93 (1951).

[767] H. B. Jones, J. W. Gofman, F. T. Lindgren, T. P. Lyon, D. M. Graham, B. Strisower, and A. V. Nichols, *Am. J. Med.*, *11*, 358–380 (1951).

[768] J. W. Gofman, *Bull. New York Acad. Med.*, *28*, 279–293 (1952).

fractions designated as S_f 17–20, S_f 20–40, and S_f 40–40,000. The S_f 40,000 class appears to consist of the chylomicrons.

Gofman[768] described the chemical nature of the several flotation areas, from unpublished work of Lindgren, Freeman, and Nichols. All S_f samples contain some cholesterol. The total content of cholesterol decreases steadily from 30% in the S_f 4–6 fraction to 5% in the S_f 40–40,000 molecule class, while the proportion of esterified cholesterol changes from 75 to

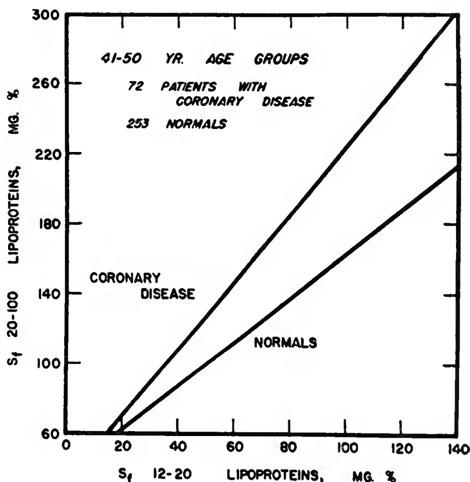


Fig. 8. The average S_f 12–20 lipoproteins plotted against the S_f 20–100 lipoproteins in serum of normal individuals and of patients with coronary disease.[768]

0% within these limits. Phospholipid and protein both decrease gradually from 25% levels in the S_f 4–6 fraction to approximately 5% in the largest molecules. On the other hand, glyceryl esters are absent, or are present only in very low percentages in the S_f 4–6 fraction; however, they *increase* steadily until they comprise 75 to 85% in the S_f 40–40,000 fraction within which the chylomicrons fall.

The comparative amounts of S_f 12–20 and S_f 20–100 lipoproteins in a group of normal persons and of some coronary patients are given in Figure 8.

The seriousness of atherosclerosis is believed to be related to the total

lipoprotein concentration. The following chart prepared by Gofman[768] lists the defects in progressive order.

"Normal" Pattern

(1) Lipoproteins of S_f 4 and/or S_f 6 present at low or moderate concentrations. Minimal levels of higher S_f components except for transient elevations in S_f 30–40,000 following fatty meals.

"Minimal" Defect

(2) Lipoproteins of S_f 4 and/or S_f 6 at increased concentrations but without any increase in higher S_f components as compared with (1).

"Minor" Defect

(3) Lipoproteins of S_f 4 and/or S_f 6 plus S_f 8 in increasing concentration.

Progressively "More Severe" Defect

(4) S_f 4 + S_f 6 + S_f 8 + S_f 10
(5) S_f 4 + S_f 6 + S_f 8 + S_f 10 + S_f 13
(6) S_f 4 + S_f 6 + S_f 8 + S_f 10 + S_f 13 + S_f 17
(7) S_f 4 + S_f 6 + S_f 8 + S_f 10 + S_f 13 + S_f 17 + S_f 17–20
(8) S_f 4 + S_f 6 + S_f 8 + S_f 10 + S_f 13 + S_f 17 + S_f 17–20 + S_f 20–40
(9) S_f 4 + S_f 6 + S_f 8 + S_f 10 + S_f 13 + S_f 17 + S_f 17–20 + S_f 20–40 + S_f 40–40,000

(In this group the S_f 40–40,000 can be transient in existence, following meals, or may be sustained even postabsorptively.)

(10) "Most Severe" Defect

As in (9) except that the S_f 4 and S_f 6 may be depressed to quite low concentrations. (This may be regarded as a general shift toward higher S_f lipoproteins, and is comparable to that which appears in the rabbits in the later phases of cholesterol-Wesson oil feeding.)

Lipoprotein transport as a measure of lipid metabolic defect in man.[768]

e′. The Relationship of Total Serum Cholesterol to the Concentration of the S_f 10–20 Fraction: Gofman and co-workers[96] stated that analytically determined values for blood cholesterol were highly unsatisfactory in reflecting the occurrence or progress of atherosclerosis. No correlation was found between the occurrence of the "giant" cholesterol molecules and the serum cholesterol values. Although there was a general trend toward higher concentrations of the S_f 10–20 molecules when the plasma cholesterol exceeded 200 milligram per cent, sera having 120 to 140 milligram per cent showed appreciable concentrations of the large cholesterol molecules in some cases. However, after a thorough statistical evaluation of Gofman's data, Keys[769] concluded: "At the present time, it is entirely unjustified

[769] A. Keys, *Bull. Johns Hopkins Hosp.*, *88*, 473–483 (1951).

to attribute to G measurements any special virtues beyond that for simple cholesterol measurements for the prediction of atherosclerosis or the estimation of the activity of the atherosclerotic process." On the other hand, Koehler and Hill[770] posed the question as to whether or not cholesterol forms definite lipoprotein compounds. These workers proposed that cholesterol becomes adsorbed on the protein molecules without forming a

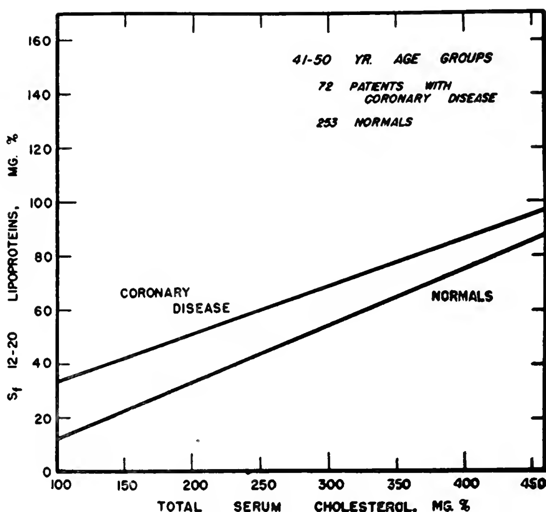


Fig. 9. The average S_f 12–20 lipoproteins of patients with coronary disease, and of normal persons of the 41- to 50-year age group plotted against the total serum cholesterol.[768]

definite compound. On the basis of electrophoretic mobility studies, these workers[771] postulate that no large serum lipoprotein complexes exist in arteriosclerosis.

In more recent studies, Jones, Gofman *et al.*[767] reported marked differences between the S_f 12–20 lipoproteins in the sera of blood of normal patients forty-one to fifty years of age, as contrasted with patients with coronary disease, of the same age group. These results plotted in Figure 9.

[770] A. E. Koehler and E. Hill, *Federation Proc.*, *11*, 241 (1952).
[771] A. E. Koehler and E. Hill, *Federation Proc.*, *12*, 232 (1953).

Barr and collaborators[772] demonstrated that patients who have survived coronary occlusion, or who have other evidence of atherosclerosis, exhibit an abnormal distribution of proteins and lipids in the plasma. These variations from normal include a reduction in albumin and α-lipoprotein, along with an absolute increase in the β-lipoprotein fraction. Increases also take place in other components of Cohn's Fractions I and III. These changes can occur without the development of a hypercholesterolemia or any significant elevation in the cholesterol:phospholipid ratio. This ratio does not deviate from normal in the atherosclerotic group, namely about 0.50 for the α-lipoprotein-containing fraction and approximately 1.40 for the fraction which contains the β-lipoprotein. Such changes in protein and cholesterol distribution are known to occur in conditions which predispose to an early and extensive atherosclerosis. In many cases of diabetes, these changes may be recognized before any vascular complications are demonstrable. Such data would seem in general to support the Gofman hypothesis that it is not cholesterol *per se* which may be the offender in atherosclerosis, but rather the nature of the cholesterol-protein lipoprotein in the blood. Although Gertler *et al.*[773] are of the opinion that blood cholesterol is increased in coronary disease, and that the level of phospholipids is augmented, they suggest that the important consideration is the ratio of these components rather than their absolute amounts.

f′. The Effect of Lipotropic Factors on Atherosclerosis: A number of reagents which are known to cause the reduction of liver lipids under certain fixed conditions have also been shown to have definite effects on atherosclerosis.

(a′) Choline.—Although Duff and Meissner[774] were unable to demonstrate that choline exerted any effect on the atherosclerosis, produced by feeding cholesterol, in the rabbit, several investigators noted beneficial results when choline therapy was employed.[774–777] Moreover, Morrison *et al.*[778] reported that choline appears to have a beneficial effect in counteracting atherosclerosis in the human patient. Hermann[779] found a reduction of 20% in the blood cholesterol in 111 patients suffering from atheromatous

[772] D. P. Barr, E. M. Russ, and H. A. Eder, *Am. J. Med.*, *11*, 480–493 (1951).
[773] M. M. Gertler, S. M. Garn, and E. F. Bland, *Circulation*, *2*, 517–522 (1950).
[774] G. L. Duff and G. F. Meissner, *Circulation*, *4*, 468–469 (1951).
[775] A. Steiner, *Arch. Pathol.*, *45*, 327–332 (1948).
[776] L. M. Morrison and A. Rossi, *Proc. Soc. Exptl. Biol. Med.*, *69*, 283–284 (1948).
[777] L. M. Morrison, *Geriatrics*, *4*, 236–238 (1949).
[778] L. M. Morrison and W. F. Gonzalez, *Proc. Soc. Exptl. Biol. Med.*, *73*, 37–38 (1950).
[779] G. R. Herrmann, *Exptl. Med. and Surg.*, *5*, 149–159 (1947).

disease, after choline was given. This author [780] also noted a decrease in cholesterol deposits in old hens after choline feeding.

(b′) Inositol.—Inositol, $C_6H_{12}O_6$, is another lipotropic agent which has proved effective as a decholesterolizing agent. Herrmann[781] reported a decrease in the cholesterol content of the arteries of hens after inositol feeding while, in some later work,[779] he was able to demonstrate that this substance decreased the blood cholesterol of patients with atheromas. The reduction of cholesterol was accompanied by an increased phospholipid content. Dotti and associates[782] reported that inositol also prevents the expected rise in cholesterol and phospholipids in the serum of rabbits on high-cholesterol diets.

(c′) Lecithin.—This phospholipid has been given both favorable and unfavorable criticism insofar as its effect on atherosclerosis is concerned. Although large doses of lecithin were found to be harmful, Downs[783] reported that this phospholipid, in small doses, protected the rabbit from atherosclerosis when it was fed with cholesterol in varying proportions over a four-month period. Similar findings were noted by Kesten and Silbowitz[784] when crude soya lecithin was used in a lecithin:cholesterol ratio of 6:1. However, Pollak[785] was unable to demonstrate any beneficial effect on the part of lecithin when it was injected intravascularly along with colloidal cholesterol. In fact, lecithin was shown to damage the large blood vessels, in contradistinction to cholesterol, which acts more frequently on the smaller arteries.

(d′) Heparin.—Although heparin can hardly be classed as a lipotropic agent, it has recently been shown to have a pronounced effect in increasing the solubility of lipoproteins and, by virtue of these properties, it must be considered as playing an important role in atherosclerosis. Block and collaborators[435] reported that the action of heparin was less in atherosclerotic male patients than it was in normal male and female subjects. For a discussion of the action of heparin in relation to lowering lipemia, the reader is referred to pages 426–430.

1. The Blood Lipids in Diseases of the Central Nervous System. There has been a more or less general belief that the central nervous system controls fat metabolism in some manner. It has been known that obesity

[780] G. R. Herrmann, *Proc. Soc. Exptl. Biol. Med.*, *61*, 302–304 (1946).

[781] G. R. Herrmann, *Proc. Soc. Exptl. Biol. Med.*, *63*, 436–438 (1946).

[782] L. B. Dotti, W. C. Felch, and S. J. Ilka, *Proc. Soc. Exptl. Biol. Med.*, *78*, 165–167 (1951).

[783] W. G. Downs, Jr., *Am. Med.*, *41*, 460 (1935).

[784] H. D. Kesten and R. Silbowitz, *Proc. Soc. Exptl. Biol. Med.*, *49*, 71–73 (1942).

[785] O. J. Pollak, *Geriatrics*, *6*, 73–80 (1951).

may result as a consequence of tumors or other lesions of the hypothalamus. In fact, Brobeck and his co-workers[786,787] produced adiposity in rats by experimental injury to this area of the brain. However, no abnormality in fat metabolism is associated with this obesity, as the animals utilized the foodstuff normally; moreover, no significant increase in blood lipids was noted. Obesity occurred simply as a result of continued overeating.[786] Man and Peters[607] have suggested that hyperlipemia may result from lesions of the hypothalamus in man, and that this may account for the autonomic instability which is sometimes associated with clinical diabetes. De Langen[788] states that, because hyperlipemia occurs in sulfonal narcosis, the blood lipids must be controlled by the central nervous system. However, the weight of evidence indicates that, if any control of the level of blood lipids is mediated by the central nervous system, it is an indirect effect.

Some workers have attributed to the higher nerve centers a function related to serum lipids. However, there is little decisive information as to changes in blood lipids in psychoses. Fatty acids and cholesterol have been reported to be low in schizophrenia.[789] On the other hand, in manic depressive psychoses, these blood lipids tended to be high. Schube[790] reported wide variations of blood cholesterol in manic depressive psychoses, the low values being found in the manic phase and the high ones being attributed to the depressive phase of the disease. These and other reports,[218,791] appear to agree that the fasting, as well as the postprandial values of the blood lipids are higher than normal.

Some workers[792] have been unable to demonstrate any relation between the serum cholesterol level and the development of convulsive seizures in epilepsy, or between the cholesterol and lecithin content of the blood and the clinical findings, in cerebrospinal syphilis and dementia paralytica.[793] However, Pezzali[794] claimed that serum cholesterol values decrease during epileptic seizures, while Robinson *et al.*[419] state that the

[786] J. R. Brobeck, J. Tepperman, and C. N. H. Long, *Yale J. Biol. Med.*, *15*, 831–853 (1943).

[787] J. Tepperman, J. R. Brobeck, and C. N. H. Long, *Yale J. Biol. Med.*, *15*, 854–874 (1943).

[788] C. D. De Langen, *Acta Med. Scand.*, *97*, 427–439 (1938).

[789] E. F. Gildea, E. B. Man, and R. W. Biach, *Arch. Neurol. Psychiat.*, *43*, 932–947 (1940).

[790] P. G. Schube, *J. Lab. Clin. Med.*, *22*, 240–245 (1936–1937).

[791] D. Slight, C. N. H. Long, and R. W. Salter, *Am. J. Psychiat.*, *13*, 141–152 (1933).

[792] I. McQuarrie, W. R. Bloor, C. Husted, and H. A. Patterson, *J. Clin. Invest.*, *12*, 247–254; 255–265 (1933).

[793] I. Rosen, F. Krasnow, and J. Notkin, *Arch. Neurol. Psychiat.*, *28*, 399–404 (1932).

[794] G. Pezzali, *Riforma méd.* (*Napoli*), *39*, 433–437 (1923).

cholesterol level drops prior to the attack. Cholesterol levels are slightly higher in epileptics than in normal subjects, and the ratios of cholesterol to lipid phosphorus are slightly elevated in the epileptic patients. It is believed that the latter ratio may be correlated with the frequency of epileptic seizures.

m. The Blood Lipids in Infectious Diseases. Most lipids decrease in the serum during the acute stages of febrile infectious diseases.[410,795–798] Cholesterol is the chief lipid component affected in infectious fevers, while the fatty acids constitute the component which is the least influenced. The hypocholesterolemia resulting during the acute stages of the infection occurs at the onset of the infection and disappears on convalescence[306,410,795,798,799]; in fact, as the patient recovers, a temporary hypercholesterolemia may develop, due to the fact that the normal level is exceeded.[302,796,800] Chauffard *et al.*[801] report that the decrease in cholesterol is proportional to the intensity of the infection.

In the group of acute infections, hypocholesterolemia has been found in scarlet fever,[802] in hog cholera, alternating with periods of hypercholesterolemia,[803] in colds and similar infections,[283] in typhoid fever,[796] in rats infected with the paratyphoid organism (*Salmonella enteritides* (*danyszi*))[804] and in pneumonia.[800,805] No variation in blood lipids can be effected by artificial fever produced by diathermy or by the administration of phenylethylhydantoin or typhoid vaccine.[798] D'Alessandro[806] found that the blood cholesterol was slightly increased in the tertiary benign and febrile quaternary forms of malaria.

A number of investigators have reported that a hypocholesterolemia obtains in a variety of chronic infections.[270,807–809] Thus, lower than normal cholesterol values have been reported in blood serum in the early

[795] H. A. Kipp, *J. Biol. Chem.*, *44*, 215–237 (1920).

[796] S. Marino, *Arch. farmacol. sper.*, *55*, 1–27 (1933).

[797] I. Rosen, F. Krasnow, and M. A. Lyons, *Arch. Dermatol. Syphilol.*, *27*, 383–391 (1933).

[798] A. V. Stoesser and I. McQuarrie, *Am. J. Diseases Children*, *49*, 658–671 (1935).

[799] C. Achard, A. Grigaut, A. Le Blanc, and M. David. *J. physiol. et path. gén.*, *26*, 415–425 (1928).

[800] A. Steiner and K. B. Turner, *J. Clin. Invest.*, *19*, 373–377 (1940).

[801] A. Chauffard, G. Laroche, and A. Grigaut, *Semaine méd.*, *31*, 577–581 (1911).

[802] G. Stern, *Z. Kinderheilk.*, *25*, 129–133 (1920).

[803] R. E. Shope, *J. Exptl. Med.*, *51*, 179–187 (1930).

[804] W. M. Sperry and V. A. Stoyanoff, *J. Biol. Chem.*, *105*, lxxxii (1934).

[805] A. V. Stoesser, *Proc. Soc. Exptl. Biol. Med.*, *32*, 1324–1325 (1935).

[806] R. D'Alessandro, *Arch. farmacol. sper.*, *52*, 258–268 (1931).

[807] L. Eichelberger and K. L. McCluskey, *Arch. Internal Med.*, *40*, 831–839 (1927).

[808] B. H. Henning, *J. Biol. Chem.*, *53*, 167–170 (1922).

[809] S. E. King and M. Bruger, *Ann. Internal Med.*, *8*, 1427–1435 (1935).

acute phases of syphilis,[797,810] leprosy,[270] in the terminal stages of tuberculosis,[809] in the active stages of rheumatic fever,[811] and also in rheumatic heart disease associated with cardiac failure.[811,812] The blood carotene is reduced in various forms of leprosy, and the serum vitamin level is low in lepra nervorum. It is possible that slightly higher than normal cholesterol values obtain in filariasis[813]; apparently this also occurs in cerebrospinal syphilis.[797]

The decrease in blood cholesterol in infections occurs at the expense of the cholesterol ester, with the result that the ratio, free cholesterol:total cholesterol rises.[800,805,814] The feeding of cholesterol to animals has been shown to increase their resistance to infection.[815] Tunnicliff[816] also reported that the addition of small doses of cholesterol doubled the opsonic and cytophagic indices of blood as determined by both *in vitro* and *in vivo* tests, while stronger concentrations had the opposite effect.

Lipids other than cholesterol have been shown to remain constant or to decrease in fever.[817–819] Phospholipids are reduced on the onset of fever, and increase during convalescence.[796,799,820] Bing and Heckscher[610] and Bönniger[585] reported that fatty acids are increased in pneumonia, although the opposite results were noted in six cases reported by Stoesser and McQuarrie.[798]

n. The Blood Lipids in Cancer. The reports on the effect of cancer on the blood lipids of patients with cancer and on experimental animals suffering from cancer are quite conflicting. Thus, Mattick and Buchwald[821,822] found that the cholesterol was higher in plasma than in whole blood in most cases of cancer, while the opposite situation usually obtained in normal subjects. A plasma hypercholesterolemia was usually found. On the other hand, the results of Downes and Pack[823] and of Denis[410]

[810] F. Feraru and F. M. Offenkrantz, *Am. J. Syphilis, Gonorrhea, Venereal Diseases, 21*, 267–281 (1937).

[811] F. M. Offenkrantz, *Am. J. Diseases Children, 56*, 67–82 (1938).

[812] C. A. Poindexter and M. Bruger, *Arch. Internal Med., 61*, 714–719 (1938).

[813] T. C. Boyd and A. C. Roy, *Indian J. Med. Research, 17*, 949–953 (1930).

[814] A. V. Stoesser, *Proc. Soc. Exptl. Biol. Med., 43*, 168–170 (1940).

[815] E. Leupold and L. Bogendörfer, *Deut. Arch. klin. Med., 140*, 28–38 (1922).

[816] R. Tunnicliff, *J. Infectious Diseases, 33*, 285–288 (1923).

[817] H. Hamano, *Proc. Imp. Acad. (Tokyo), 7*, 80–81 (1931).

[818] E. M. Boyd, J. H. Orr, and G. B. Reed, *Proc. Soc. Exptl. Biol. Med., 35*, 479–482 (1936).

[819] W. Raab, *Z. ges. exptl. Med., 89*, 616–621 (1933).

[820] I. McQuarrie and A. V. Stoesser, *Proc. Soc. Exptl. Biol. Med., 29*, 1281–1283 (1932).

[821] W. L. Mattick and K. W. Buchwald, *J. Cancer Research, 12*, 236–245 (1928).

[822] W. L. Mattick and K. W. Buchwald, *J. Cancer Research, 13*, 157–166 (1929).

[823] H. R. Downes and G. T. Pack, *Am. J. Cancer, 16*, 290–296 (1932).

show no abnormalities of blood cholesterol in patients with malignancies. In fact, Guthmann,[824] De Voss[825] and Drevon[826] all noted that serum cholesterol was decreased in cancer. No constant differences in blood cholesterol were found in normal and in tumor-bearing rats, respectively.[313,827]

o. The Effect of Drugs on Blood Lipids. (*a*) *Phlorhizin.* Phlorhizin is a glucoside obtained from the bark and root of the peach and pear tree which, on hydrolysis, breaks down to glucose and phloretin. This substance is of interest in relation to blood lipids, since it acts on the kidneys to produce glycosuria, with a subsequent ketosis and ketonuria. The course of metabolism following daily injections of phlorhizin parallels that which occurs in experimental pancreatic diabetes in the dog, or in diabetes mellitus in man. In fact, the condition which obtains after an animal has been "phlorhizinized" is referred to as "phlorhizin glycosuria" or "phlorhizin diabetes." This technic has been widely employed by the Lusk school to determine what compounds are glucose formers. The results of Deuel, Wilson, and Milhorat[828] and of Deuel alone[829] indicate that the diabetic symptoms observed during phlorhizinization are to be traced to carbohydrate depletion following the primary action of the drug on the tubules of the kidneys. For a review of phlorhizin and glycosuria prior to 1912, the reader is referred to Lusk[830]; Nash[831] contributed an excellent review of the more recent work. See also Chapter II of this volume.

Since the typical symptoms of phlorhizinized animals, especially when fasted, resemble those of diabetes, one would also expect a hyperlipemia to occur. This is actually the case, as has been proved by Lattes[221] as well as by Terroine.[208] The latter worker reported that the level of both fatty acids and cholesterol in the blood was increased after the drug was given; moreover, the changes in each of these two lipid components proceeded independently. Allen[570] was unable to demonstrate a lipemia after phlorhizin had been given; however, these discrepancies may well be due to nutritional differences in the experimental animals, since the ketosis and ketonuria disappear when the phlorhizinized dog receives more sugar in the diet than it is physically able to eliminate. One would not expect the animal to exhibit the diabetic symptoms of ketosis, ketonuria, or hyper-

[824] H. Guthmann, *Arch. Gynäkol.*, *140*, 202–225 (1930).
[825] G. De Voss, *Z. physiol. Chem.*, *205*, 20–24 (1932).
[826] B. Drevon, *Compt. rend. soc. biol.*, *142*, 974–977 (1948).
[827] F. Dannenberg, *Biochem. Z.*, *244*, 128–132 (1932).
[828] H. J. Deuel, Jr., H. E. C. Wilson, and A. T. Milhorat, *J. Biol. Chem.*, *74*, 265–298 (1927).
[829] H. J. Deuel, Jr., *J. Biol. Chem.*, *89*, 77–91 (1930).
[830] G. Lusk, *Ergeb. Physiol.*, *12*, 315–392 (1912).
[831] T. P. Nash, Jr., *Physiol. Revs.*, *7*, 385–430 (1927).

lipemia, although a severe glycosuria would occur. It has been shown that fat is removed from the blood by the muscles and liver of phlorhizinized dogs.[571]

(*b*) *Phosphorus.* Phosphorus is a substance which, when injected into animals, causes a migration of fat to the liver (the so-called fatty infiltration of the liver). During the initial phases of this reaction, a hyperlipemia occurs; after the fat has been transferred to the liver, blood fat decreases, and a hypolipemia supervenes, if the animal survives for a sufficient period.[832] Cholesterol and neutral fat are high in both stages, the phospholipids being considerably reduced.

(*c*) *Chloroform and Carbon Tetrachloride.* Both chloroform, $CHCl_3$, and carbon tetrachloride, CCl_4, are liver poisons; fatty infiltration of the liver results after either substance is administered. The reaction of the body is similar to that obtained in phosphorus poisoning, namely an initial hyperlipemia followed by a subsequent hypolipemia.[484,832]

8. Factors Altering the Concentration of the Carotenoids and of the Fat-Soluble Vitamins in Blood

(1) Carotenoids and Vitamins A

In view of the close association of β-carotene, the principal carotenoid found in blood and the most important of the provitamins A, with vitamin A, it is logical that these compounds should be considered simultaneously.

a. Normal Values for Serum Carotene and Vitamin A. The blood serum of normal adults contains β-carotene in amounts varying from 50 to 500 microgram per cent, with an average of approximately 200 microgram per cent. Kimble[833] reported mean values of 166 microgram per cent for β-carotene in the case of thirty healthy men (range 50–300). The average figure for thirty-four healthy women was given as 187 microgram per cent (range 90–340). Murrill *et al.*[834] reported a mean value of 213 ± 72 microgram per cent, while Sobotka[835] recorded figures between 100 and 250 microgram per cent. The value cited by Ralli and her associates[836] was

[832] H. Heinlein and M. Angermann, *Centr. allgem. Pathol. u. path. Anat.*, *58*, *Suppl.*, 81–95 (1933); *Chem. Abst.*, *27*, 5423 (1933).

[833] M. S. Kimble, *J. Lab. Clin. Med.*, *24*, 1055–1065 (1939).

[834] W. A. Murrill, P. B. Horton, E. Leiberman, and L. H. Newburgh, *J. Clin. Invest.*, *20*, 395–400 (1941).

[835] H. H. Sobotka, Personal communication; cited by M. E. Yarbrough and W. J. Dann, *J. Nutrition*, *22*, 597–607 (1941), p. 601.

[836] E. P. Ralli, E. Bauman, and L. B. Roberts, *J. Clin. Invest.*, *20*, 709–713 (1941).

123 microgram per cent, while Yarbrough and Dann[837] reported values of 183 ± 18.4 microgram per cent and Caveness *et al.*[838] cited an average figure of 138 ± 62 microgram per cent. Hsu[839] gave 140 microgram per cent as an average for the blood carotene of thirty-three young healthy Chinese adults. The results of Abels and co-workers[840] on sixty-two normal men and sixty-two normal women do not support those of Kimble,[833] who stated that women have a higher average value than men do. In fact, Abels *et al.*[840] found higher values for men than for women, the averages being 210 microgram per cent (range 40–500) and 180 microgram per cent (range 80–400), respectively. Later, Harris, Hickman, Jensen, and Spies[841] reported a mean of 210 ± 31 microgram per cent (range 80–370) for seventy "normal" subjects. According to the arbitrary standards of normality used in the Oxford Nutrition Survey as reported by Sinclair,[842] a value of 100 microgram per cent represents the lower limit of normal blood, while a value below 50 microgram per cent indicates extreme abnormality.

The plasma vitamin A values more or less coincide with those of carotene. The range for the vitamin A in normal individuals is from 20 to 60 microgram per cent (67 to 200 I.U.).[843] Kimble [833] reported averages of 38 and 27 microgram per cent for men and women (127 and 91 I.U., respectively). Other average figures are as follows: Murrill *et al.*,[834] 27.9 microgram per cent (93 ± 15 I.U.); Sobotka,[835] 18 to 24 microgram per cent; Ralli and associates,[836] 47.4 microgram per cent; Yarbrough and Dann,[837] 21.3 microgram per cent (71 ± 3.3 I.U.); Caveness *et al.*,[838] 78.9 microgram per cent (263 ± 96 I.U.); Abels and co-workers,[840] 51 microgram per cent (170 I.U. with a range of 132 to 208 I.U.) for men, and 44.7 microgram per cent (149 I.U. with a range of 103 to 195 I.U.) for women; and Harris, Hickman, Jensen, and Spies,[841] 61 ± 13 microgram per cent (range 36 to 89 μg.). Hsu[839] cites a figure of 16.8 μg. (56 I.U. with a range of 36 to 99 I.U.) for healthy young Chinese adults, while Harris and Moore[844] recorded a value of 35.4 μg. (118 I.U.) as the average

[837] M. E. Yarbrough and W. J. Dann, *J. Nutrition*, *22*, 597–607 (1941).

[838] H. L. Caveness, G. H. Satterfield, and W. J. Dann, *Arch. Ophthalmol.*, *25*, 827–832 (1941).

[839] H.-C. Hsu, *Chinese Med. J.*, *61*, 238–243 (1943).

[840] J. C. Abels, A. T. Gorham, G. T. Pack, and C. P. Rhoads, *J. Clin. Invest.*, *20*, 749–764 (1941).

[841] P. L. Harris, K. C. D. Hickman, J. L. Jensen, and T. D. Spies, *Am. J. Pub. Health*, *36*, 155–160 (1946).

[842] H. M. Sinclair, *Vitamins and Hormones*, *6*, 101–162 (1948).

[843] One International Unit is equivalent to 0.3 μg.

[844] A. D. Harris and T. Moore, *Brit. Med. J.*, *1947*, *1*, 553–559.

for forty-one normal individuals in Great Britain, based upon the findings of Leitner and Moore.[845] Van Bruggen and Straumfjord[846] reported a vitamin A value for thirty-six control subjects on a hospital diet as 44.7 microgram per cent (149 I.U.), while Youmans *et al.*[847] consider any figure for plasma vitamin A in excess of 21 microgram per cent (70 I.U.) as normal. Sinclair[842] reported a value of 21 microgram per cent (70 I.U.) as the lowest normal value for serum vitamin A for adults. Any figure below 9 microgram per cent (30 I.U.) is considered to be abnormally low. Popper and Steigmann[848] cited quite high figures for hospital patients. Average values for 2673 determinations on 454 patients were as follows: vitamin A, men, 58 microgram per cent and women, 47 microgram per cent; carotenoids, men, 74 microgram per cent and women, 85 microgram per cent. According to these investigators, considerable significance can be attached to the ratio of vitamin A:carotene. Apparently, a real sex difference exists in this proportion, which was 0.78 for males and 0.57 for females.

Serum vitamin A is normally quite high in the dog.[849–851] Maddock *et al.*[851] reported values varying between 173 and 355 microgram per cent for the vitamin A value in the serum of normal dogs.

Wide variations occur in the carotenoid content of the bloods of animals which contain these pigments. In the case of the cow, values as high as 1520 microgram per cent have been recorded,[852] when large amounts of carotene are available in the diet, although figures as low as 140 microgram per cent are also recorded during periods when there are no green feeds. In addition to β-carotene, considerable amounts of the carotenols, zeaxanthin, lutein, and cryptoxanthin are also present.[107] Rasmussen *et al.*[853] reported that the serum carotene level in the horse is 97 ± 78 microgram per cent, while the plasma vitamin A content is only 12.5 ± 3.5 microgram per cent. It is believed that the horse converts carotene into vitamin A quite inefficiently.

b. General Physiological Factors Related to the Level of Serum Carotene

[845] Z. A. Leitner and T. Moore, *Lancet, 1946, I,* 262–265.

[846] J. T. Van Bruggen and J. V. Straumfjord, *J. Lab. Clin. Med., 33,* 67–74 (1948).

[847] J. B. Youmans, E. W. Patton, W. R. Sutton, R. Kern, and R. Steinkamp, *Am. J. Pub. Health, 34, 368–378* (1944).

[848] H. Popper and F. Steigmann, *J. Am. Med. Assoc., 123,* 1108–1114 (1943).

[849] S. W. Clausen, W. S. Baum, A. B. McCoord, J. O. Rydeen, and B. B. Breese, *Science, 91,* 318–319 (1940).

[850] G. E. Wakerlin and W. G. Moss, *Proc. Soc. Exptl. Biol. Med., 53,* 149–152 (1943).

[851] C. L. Maddock, S. B. Wolbach, and S. Maddock, *J. Nutrition, 39,* 117–137 (1949).

[852] W. Braun, *J. Nutrition, 29,* 61–71 (1945).

[853] R. A. Rasmussen, C. L. Cole, and M. J. Miller, *J. Animal Sci., 3,* 346–350 (1944).

[854] W. J. Dann, *Biochem. J., 26,* 1072–1080 (1932).

and Vitamin A. (*a*) *Maternal-Fetal Transfer of Carotene and Vitamin A.* Dann[854] is of the opinion that the placenta is impervious to the carotene in the maternal blood. On the other hand, Clausen and McCoord[855] indicated that the carotene value in the blood plasma of the umbilical vein (blood flowing to the fetus) was greater than that in the umbilical arteries (blood flowing from the fetus), although it is possible that the missing carotene may have been retained in the fetus as vitamin A. However, the more recent results of Lund and Kimble[856] and of Byrn and Eastman[857] are particularly illuminating in this connection. Carotene values of cord blood in 149 normal infants at term were exceedingly low, averaging 23 μg. (range 9 to 75), as contrasted with a figure of approximately 220 μg. in the mothers (range 50 to 550).[856] Thus, the carotene ratio in the maternal and fetal bloods was 10:1. In the studies of Byrn and Eastman,[857] the values of carotene in fetal blood averaged only 2.01 microgram per cent, as contrasted with a mean in the maternal blood of 106.3 microgram per cent. This gives a ratio for carotene in maternal and in fetal blood of 50:1. These data all support the fact that the placenta largely bars the entrance of carotene into the fetus, with the result that the carotene level of the blood of the newborn is exceedingly low. Hoch[858] reported that, whereas β-carotene represents 53 to 72% of the total carotenoids in maternal blood, it accounted for only 37 and 40% of the total in two cases of newborn infants.

There is a much closer correspondence in the values of vitamin A in the maternal and in the fetal bloods, respectively. According to the studies of Lund and Kimble,[856] 90% of the infants had plasma vitamin A values between 9 and 21 microgram per cent (30 to 70 I.U.), while the values in the maternal bloods ranged from 22.5 to 36 microgram per cent (75 to 120 I.U.). Ordinarily, the concentration of vitamin A in the fetal blood was about one-half of that in the mother's blood. The vitamin A levels in the maternal blood were much more subject to change than were those in the fetal blood, which remained quite constant, irrespective of whether increased or decreased values for vitamin A obtained in the mother's blood. The average value for vitamin A in fetal blood, as reported by Byrn and Eastman,[857] was also definitely lower than that of maternal blood, but the variations were not as great as those reported earlier. The average plasma vitamin A level for non-pregnant women was given as 40.2 microgram per cent, while

[855] S. W. Clausen and A. B. McCoord, *J. Pediat.*, *13*, 635–650 (1938).
[856] C. J. Lund and M. S. Kimble, *Am. J. Obstet. Gynecol.*, *46*, 207–221 (1943).
[857] J. N. Byrn and N. J. Eastman, *Bull. Johns Hopkins Hosp.*, *73*, 132–137 (1943).
[858] H. Hoch, *Biochem. J.*, *38*, 304–308 (1944).

that for women at term was reported as 35.4 microgram per cent. On the other hand, the average value for vitamin A in fifty samples of cord blood was found to be 27.4 microgram per cent.

(b) *Plasma Carotene and Vitamin A in Children.* The most comprehensive studies on the effect of age on the carotene and vitamin A content of the blood of children were made by Szymanski and Longwell.[859] These workers determined the plasma carotene and vitamin A in ninety-five healthy children from two days to sixteen years of age. The median value (in italics) of plasma carotene in microgram per cent (including minimum and maximum) were as follows: 2 days, *34* (18–61); 1 month, *37* (14–111); 2 months, *42* (7–135); 3 months, *45* (18–157); 6 months, *174* (49–337), 9 months, *268* (166–592); 12 months, *328* (183–672); 15 months, *281* (123–581); 18 months, *253* (109–452); 21 months, *233* (96–377); 24–27 months, *201* (52–391); 30 to 33 months, *171* (64–285); 3 to 4 years, *155* (84–281); 4.5 to 5.5 years, *150* (56–304); 6 to 7 years, *149* (40–299); 7.5 to 8.5 years, *147* (61–297); 9 to 10 years, *130* (54–286); 10.5 to 11.5 years, *117* (51–283); 12 to 13 years, *114* (63–272); and 13 to 16 years, *126* (61–258). These values are in line with the ranges reported by May and collaborators,[860] for children two to twelve years of age, *i.e.*, *140* (47 to 233 microgram per cent) (based upon a conversion key given by Henley *et al.*[861]), and of Krause and Pierce[862] for 179 presumably normal school children, who gave values of 116 ± 45.7 microgram per cent. They also agree with the averages of Robinson *et al.*[863] for normal and underprivileged children in Michigan from six to sixteen years old, which varied between 100 and 168 microgram per cent. It would thus appear that the carotene levels in the blood plasma start at minimum values, which are maintained for the first three months of life, after which they rapidly rise to a maximum at twelve months. Following this, the blood carotene values gradually decrease until, after the age of two years, they reach a level which corresponds to that noted in some of the surveys on adults. The blood carotene values for the school children would seem to be lower than those recorded in most tests on adults.

Szymanski and Longwell[859] recorded the following median values (in

[859] B. B. Szymanski and B. B. Longwell, *J. Nutrition, 45,* 431–442 (1951).

[860] C. D. May, K. D. Blackfan, J. F. McCreary, and F. H. Allen, *Am. J. Diseases Children, 59,* 1167–1184 (1940).

[861] T. H. Henley, M. Dann, and W. R. C. Golden, *Am. J. Diseases Children, 68,* 257–264 (1944).

[862] R. F. Krause and H. B. Pierce, *J. Nutrition, 33,* 633–640 (1947).

[863] A. Robinson, M. Lesher, A. P. Harrison, E. Z. Moyer, M. C. Gresock, and C. Saunders, *J. Am. Dietet. Assoc., 24,* 410–416 (1948).

italics) for plasma vitamin A in microgram per cent (including minimum and maximum): 2 days, *23* (5–46); 1 month, *29* (11–72); 2 months, *36* (18–213); 3 months, *42* (21–120); 6 months, *47* (17–100); 9 months, *56* (23–125); 12 months, *52* (32–113); 15 to 18 months, *47* (19–143); 21 to 24 months, *44* (17–113); 27 to 36 months, *41* (27–102); 3.2 to 4.8 years, *39* (14–62); 5 to 6 years, *36* (18–96); 6.5 to 16 years, *35* (14–110). These results are in line with those of Henley *et al.*[861] who found abnormally low values for vitamin A in three-week-old premature infants; mean values were only 20.4 microgram per cent (68 I.U.). These figures likewise agree with those reported by Lewis and associates[864] for the fourth day of life, after recovery from a precipitous drop to 11.1 microgram per cent (37 I.U.) within forty-eight hours after birth. It is suggested that both failure of the liver to mobilize adequate quantities of vitamin A and the low intake of the foodstuff are the responsible factors in the case of the newborn. Lewis and co-workers[865] found that the serum vitamin A in somewhat older infants varied between 13.5 and 42.3 microgram per cent (45 and 141 I.U.); the results were practically identical for infants who had received a daily supplement of 17,000 I.U. of vitamin A and for those who had not been given the extra vitamin A. The values for the older children were in the range reported by Robinson and co-workers[863] for children six to sixteen years of age; they reported averages of 30 to 36 microgram per cent for the normal group and 31 to 35 microgram per cent for the underprivileged group. As is the case with carotene, minimum values of plasma vitamin A are noted in the newborn; a maximum level is reached at the age of nine months, after which a gradual decrease obtains. These data lead one to conclude that, although the blood levels of carotene are greatly suppressed and those of vitamin A are depressed to a lesser extent in the newborn, the deficit is quickly overcome, and the levels soon reach those considered normal for adults.

(*c*) *Plasma Carotene and Vitamin A in the Aged.* The values for carotene and vitamin A in the plasma of aged individuals would appear to be somewhat lower than those reported for normal middle-aged adults. Thus, Rafsky, Newman, and Jolliffe[866] reported the average plasma carotene as 80 microgram per cent (males, 74.7 and females, 85 microgram per cent), while plasma vitamin A averaged 27.1 microgram per cent (males, 29.3 and females, 25 microgram per cent) in the case of fourteen males and fif-

[864] J. M. Lewis, O. Bodansky, and L. M. Shapiro, *Am. J. Diseases Children*, *66*, 503–510 (1943).

[865] J. M. Lewis, O. Bodansky, and C. Haig, *Am. J. Diseases Children*, *62*, 1129–1148 (1941).

[866] H. A. Rafsky, B. Newman, and N. Jolliffe, *Gastroenterology*, *8*, 612–615 (1947).

teen females varying in age from sixty-nine to eighty-three years. According to the studies of Kirk and Chieffi,[867] there would appear to be a definite decrease in carotene and vitamin A with advancing age. The latter data are summarized in Table 17.

TABLE 17
TOTAL CAROTENE, α- AND β-CAROTENES AND VITAMIN A IN BLOOD PLASMA OF INDIVIDUALS OF VARIOUS AGES[a]

Age group, years	No. of individuals	Micrograms per 100 ml.			
		Total carotenes[b]	α + β-Carotenes[b]	Vitamin A[b,c]	Vitamin A[b,d]
16–39	47	320 ± 91 (120–540)	190 ± 77 (70–380)	23 ± 73.4 (1–57)	30 ± 14.7 (3–64)
40–59	34	220 ± 87 (100–380)	120 ± 61 (30–270)	20 ± 8.9 (1–35)	25 ± 9.4 (6–41)
60–69	25	200 ± 67 (70–320)	110 ± 53 (20–210)	22 ± 12.8 (1–56)	27 ± 13.6 (3–61)
70–79	45	210 ± 91 (40–440)	110 ± 53 (20–260)	17 ± 10.5 (1–46)	23 ± 11.8 (4–58)
>80	51	210 ± 87 (80–410)	110 ± 55 (20–240)	18 ± 9.5 (1–45)	23 ± 9.5 (5–49)

[a] Data adapted from E. Kirk and M. Chieffi, *J. Nutrition, 36*, 315–322 (1948).
[b] Including the standard deviation. Figures in parentheses are the range of values.
[c] Correction applied for total carotenes.
[d] Correction applied for α- and β-carotenes.

(*d*) *Plasma Carotene and Vitamin A as Influenced by Sex.* Although sex must play only a minor role in determining the normal levels of carotene and vitamin A in the blood, there is some indication that it may have importance. Getz and Koerner[868] reported sex differences in blood vitamin A. According to the results of Kimble[833] and of Popper and Steigmann,[848] the blood carotene values are higher in women than in men, while the plasma vitamin A in males exceeds the value in females. Moreover, in an extensive experiment involving repeated tests on eighteen male and seven female subjects, Week and Sevigne[129] demonstrated that the plasma vitamin A levels were consistently lower for female than for male subjects, not only during fasting but at all periods up to twenty-four hours after the administration of 134,000 μg. of vitamin A. For a summary of these experiments, see page 500. Additional data indicate variations in the sex patterns. Thus, Szymanski and Longwell,[859] showed that, in infants and young children, both plasma vitamin A and plasma carotene are higher

[867] E. Kirk and M. Chieffi, *J. Nutrition, 36*, 315–322 (1948).
[868] H. R. Getz and T. A. Koerner, *Am. J. Med. Sci., 202*, 831–847 (1941).

in the girls than in the boys. However, just before the start of adolescence, there was a decrease in carotene in the case of girls, with the result that a lower average was obtained than in boys of the same age. It was suggested that this variation at puberty is associated with differences in rate of growth in the two sexes at that time. Abels *et al.*[840] reported that both carotene and vitamin A in the plasma are higher in males than in females.

(*e*) *Seasonal Changes in Plasma Carotene and Vitamin A.* A marked seasonal variation in the serum content of carotene and vitamin A occurs in the blood of cattle. Braun[852] reported that these variations coincided with changes in the quantity of carotenoids available in the diet. The age and breed of the cows had only a minor effect. It was found that the vitamin A : carotenoid level in the plasma decreased with increasing carotenoid levels in the blood, but tended to reach a constant value when the carotenoid levels were high. The average maximum level of carotenoids in the Holsteins was 1.35 milligram per cent (1350 microgram per cent) in March when on green pasturage, while the minimum mean value which was noted in October was 0.28 milligram per cent. The maximum levels for vitamin A also approximated 43.5 microgram per cent (145 I.U.) in the March samples, while the minimum average obtained in the September and October samples was 10 microgram per cent (35 I.U.). The values for the Holsteins were somewhat lower than those for other breeds during the period when the blood carotene and vitamin A were at a high level.

Lord[869] reported a similar seasonal variation for Ayrshire cows, with a corresponding difference in the carotene and vitamin A level in the butter. The averages for serum carotene and vitamin A in the summer during pasture feeding were 1350 and 45 microgram per cent (150 I.U.), respectively, as compared with values during stall feeding, in the winter, of 230 and 9.3 μg. (31 I.U.), respectively.

Since, in most areas, the intake of green foods does not vary to the same extent in man as it does in cattle, such variations in blood level do not exist ordinarily; however, it is known that the maintenance of blood carotene is dependent upon a continued supply of this carotenoid in the food. On the other hand, Szymanski and Longwell[859] reported a significant seasonal variation in plasma carotene levels in children; the average figures (microgram per cent) were as follows: June–November (166 determinations) 152; December–May (176 determinations) 132. No seasonal variations in plasma vitamin A were evident.

[869] J. W. Lord, *Biochem. J.*, *39*, 372–374 (1945).

(*f*) *Plasma Carotene and Vitamin A as Influenced by Parturition.* Sutton and co-workers[870] reported that, at or immediately after parturition, the plasma carotenoid and vitamin A levels of cows drop sharply, to recover slowly during the following fourteen to twenty-one days. Goodwin and Wilson[871] confirmed these observations; however, these later workers do not consider these changes significant for vitamin A and β-carotene *per se*, but state that they are probably only a reflection of the changes in the concentration of blood constituents during this period.

(*g*) *The Effect of Hormones on Plasma Carotene and Vitamin A.* Williamson[872] reported that the injection of large doses of estradiol benzoate over a nineteen-day period into thyroidectomized rabbits was followed by a significant decrease in plasma vitamin A. No change was noted when this hormone was administered to normal rabbits. On the other hand, when estrogen was given to immature pullets, it evoked an increase in serum vitamin A. The increase in vitamin A was confined to the ester fraction.[873] The estrogen effect was not counteracted by progesterone, although the latter hormone reduced the hypertrophy of the oviduct caused by estrogen.[873] Gardiner *et al.*[873] reported that testosterone did not affect serum vitamin A, although it might significantly increase the level of β-carotene in the blood. However, on the other hand, Danish and Klopp[874] reported that the concentration of vitamin A in the plasma of man increases as a result of the administration of testosterone.

Young and Wald[875] reported that the intravenous injection of adrenalin into rabbits caused a mobilization of vitamin A in the blood. However, Goodwin and Wilson[876] were unable to confirm this finding, in the case of either the rabbit or the rat, so that there is some doubt as to whether or not the effect actually occurs.

On the other hand, 3-β-acetoxy-17α-hydroxyallopregnan-20-one, the so-called "Compound L," which, according to Bodansky and Markardt,[877] was isolated from the adrenal gland by Reichstein,[878] has been found to decrease the plasma vitamin A of male rats considerably.[877] Thus, Bodansky and Markardt[877] observed that, when 15 to 30 mg. daily doses of Compound L were injected into male rats four months of age, over a period of

[870] T. S. Sutton, H. E. Kaeser, and P. A. Soldner, *J. Dairy Sci.*, *28*, 933–939 (1945).

[871] T. W. Goodwin and A. A. Wilson, *Biochem. J.*, *49*, 499–503 (1951).

[872] M. B. Williamson, *Proc. Soc. Exptl. Biol. Med.*, *66*, 621–623 (1947).

[873] V. E. Gardiner, W. E. Phillips, W. A. Maw, and R. H. Common, *Nature*, *170*, 80–81 (1952).

[874] A. Danish and C. T. Klopp, *Cancer Research*, *10*, 211 (1950).

[875] G. Young and G. Wald, *Am. J. Physiol.*, *131*, 210–215 (1940).

[876] T. W. Goodwin and A. A. Wilson, *Biochem. J.*, *45*, 370–372 (1949).

[877] O. Bodansky and B. Markardt, *J. Biol. Chem.*, *190*, 83–93 (1951).

ten days, plasma and kidney vitamin A was decreased, concomitantly with an increase in the level of liver vitamin A. It is suggested that Compound L may function by exerting a regulatory effect on the equilibrium between liver and plasma vitamin A.

c. The Effect of Diet on the Level of Carotene and Vitamin A in the Blood. (*a*) *The Effect of Ingested Carotene on the Level of Plasma Carotene and Vitamin A.* The amount of carotenoids present in ordinary meals has little or no effect on the carotene or vitamin A content of the serum.[833] When large quantities of carotene are ingested, either in oil solution or by the ingestion of foods rich in carotene, both the carotene and the vitamin A content of the plasma are augmented. Getz[879] was able to increase the plasma carotene of human subjects from 900 to 1100 microgram per cent after feeding 120,000 μg. (120 mg.) of carotene. Vitamin A in the plasma reached a maximum of only 51 microgram per cent (170 I.U.). It was believed that this result indicated a relatively poor utilization of a carotene in man; the conversion of carotene to vitamin A was estimated at 15 to 20%. At low levels, the increase in blood carotene is proportional to the dose. However, with gradually increasing doses, the rise in blood carotene does not occur in proportion to the dosage; this is probably to be ascribed to increasing losses in the feces. Vitamin A in the serum increases in proportion to that of blood carotene after the administration of carotene.[833,834]

When carotene is administered in a moderate dose, the maximum level in the serum may not be reached for seven to ten hours after its administration,[880] in contradistinction to vitamin A, following which the peak of serum vitamin A occurs within three to five hours.[881] Murrill *et al.*[834] found that, when somewhat larger doses (80 mg.) were given, the maximum blood carotene was noted in six hours, although a second increase in this value occurred which reached a maximum figure twenty-four to forty-eight hours after the original dosage. In the case of serum vitamin A, only a single rise was experienced, and this was completed within nine hours. On the other hand, when massive doses of carotene in oil (600 mg.) were given human subjects,[880] the hypercarotenemia was prolonged for sixty to seventy-two hours before the maximum value was obtained. In the experi-

[878] T. Reichstein, *Helv. Chim. Acta, 19,* 29–63, 402–412 (1936).

[879] H. R. Getz, "Induction of Vitamin A Deficiency in Man," presented at Vitamin Conference, A. A. A. S., Gibson Island, July, 1944; cited by J. C. Fritz, *Ann. Rev. Biochem., 14,* 525–560 (1945), p. 533.

[880] E. P. Ralli, H. Brandaleone, and T. Mandelbaum, *J. Lab. Clin. Med., 20,* 1266–1275 (1935).

[881] S. W. Clausen, *J. Am. Med. Assoc., 101,* 1384–1388 (1933).

ments of Ralli and her co-workers,[880] a second dose of carotene, given during the interval when hypercarotenemia was still present, was shown to exert a smaller effect on the blood plasma than did the original dose. Wendt[882] carried out feeding tests on human subjects with even higher doses of carotene than those employed by Ralli *et al.*[880] After the continued administration of massive doses of carotene, a serum value as high as 1500 µg. was noted during the early part of the test; however, this fell to an average maximum value of 490 microgram per cent after four weeks, and no further increase in carotene followed the continued administration of the carotenoid.[882]

Szymanski and Longwell[859] proposed the following prediction formula for plasma carotene, based upon the dietary sources of this provitamin:

$$X_0 = 0.46I_0 + 0.06\, I_3 + 86$$

in which X_0 is the expected plasma carotene in microgram per cent, I_0 is the average daily intake of vitamin A (carotene) from plant sources in I.U. per kilogram of body weight during the preceding three months, and I_3 is the average daily intake of vitamin A (carotene) from plant sources in I.U. per kilogram of body weight during the period three to six months prior to the date of the prediction.

Most of the data available on the effect of ingested carotene on blood carotene are obtained from human subjects, who absorb carotene as such. However, well-controlled tests have been made, by Davis and Madsen,[883] on dairy cattle fed carotene-free and vitamin A-free diets and diets containing various amounts of carotene. The data are summarized in Table 18.

In the rat, chick, and guinea pig, which do not absorb carotene as such,

TABLE 18

LEVELS OF CAROTENE AND VITAMIN A IN THE BLOOD PLASMA OF COWS RECEIVING VARIOUS AMOUNTS OF CAROTENE IN THEIR DIET[a]

Carotene intake, µg./kg.	No. of tests	Plasma carotene (av. and range), µg./100 ml.	Plasma vitamin A (av. and range), µg./100 ml.
Low	14	14 (6–24)	10 (3–16)
30	8	46 (34–58)	18 (14–21)
45	4	41 (36–43)	19 (16–23)
60	5	81 (62–96)	26 (20–31)
120	2	103 (96–110)	35 (33–36)
High (pasture)	11	813 (560–1362)	49 (40–65)

[a] Adapted from R. E. Davis and L. L. Madsen, *J. Nutrition*, *21*, 135–146 (1941).

[882] H. Wendt, *Klin. Wochschr.*, *14*, 9–14 (1935).
[883] R. E. Davis and L. L. Madsen, *J. Nutrition*, *21*, 135–146 (1941).

the administration of carotene is followed only by a rise in vitamin A in the blood.

(*b*) *The Effect of Ingested Vitamin A on the Level of Plasma Vitamin A.* When vitamin A is administered as the ester or alcohol, a prompt increase is noted in the vitamin A ester in the blood, which usually reaches the maximum after three hours. The concentration cannot be increased to any marked extent by the continued administration of this vitamin. However, there did appear to be a slight effect on the postabsorptive blood levels when single large doses were given to individuals previously "saturated" with vitamin A as the result of an ample feeding of this vitamin. Ralli and her colleagues[836] found higher maximum values for serum vitamin A in men after the administration of 30,000 μg. (100,000 I.U.) of vitamin A than after 6000 μg. (20,000 I.U.) had been given. However, in both series of tests, normal values for serum vitamin A were found within twenty-four hours.

Lewis *et al.*[884] demonstrated that, in the case of rats, a direct relationship obtains between the intake of vitamin A and the plasma level of this component. These results are summarized in Table 19.

TABLE 19

RELATION OF VITAMIN A INTAKE IN THE RAT TO GAIN IN WEIGHT AND TO VITAMIN A CONTENT OF BLOOD PLASMA, LIVER, AND RETINA[a]

Vitamin A dosage per day, μg.	No. of rats	Av. body weight, g.		Av. vitamin A (μg.) after 6 weeks		
		Start	After 6 weeks	In plasma, μg./100 ml.	In liver, μg./g.	In retina, μg./g.
0	13	45	98	0	0	4.2
0.3	11	44	119	2.1	0	—
0.6	31	40	140	4.2	0	6.0
3.0	31	40	159	10.5	0	7.5
7.5	16	41	172	20.7	0.9	6.0
15	15	41	172	30.0	10.2	—
30	21	42	173	33.6	33.9	7.8
300	26	43	177	33.0	381.0	7.5
30,000[b]	20[c]	—	—	143.4	5760	22.5

[a] Data adapted from J. M. Lewis, O. Bodansky, K. G. Falk, and G. McGuire, *J. Nutrition, 23*, 351–363 (1942).
[b] Experiments continued 4 weeks only. Rats sacrificed 48 hours after last vitamin A dosage.
[c] Averages for 14 only; 6 animals died during experiment.

It will be noted that the optimum effect on weight gain of rats over a six-weeks period after weaning occurs when 7.5 μg. of vitamin A are ad-

[884] J. M. Lewis, O. Bodansky, K. G. Falk, and G. McGuire, *J. Nutrition, 23*, 351–363 (1942).

ministered daily. This is the smallest dose which results in any detectable deposition of vitamin A in the liver. However, the apparently normal vitamin A level in the plasma is evidently not reached until 30 μg. has been given; it remains constant when ten times the dose is administered. The retina has an almost constant vitamin A content throughout, while the liver vitamin A increases progressively after the dosage is high enough to permit storage in that organ. When a massive dose of vitamin A was given (30,000 μg. daily), the rats suffered from hypervitaminosis A, and 30% died. However, the serum, liver, and retina all contained the highest vitamin A levels recorded in any of the tests.

a′. Vitamin A Tolerance Curves: A number of workers have assessed the vitamin A status of man by the effect of fixed doses of vitamin A on the blood picture. When this procedure was employed by Krause and Pierce[862] in the case of school children who received either 7500 or 15,000 μg. (25,000 or 50,000 I.U.) of vitamin A in a single dose, the data recorded in Table 20 were obtained. No differences are evident, for the children with and without folliculosis, respectively, in the fasting levels of vitamin A in the plasma or in those resulting after the administration of fixed doses of this vitamin.

TABLE 20
VITAMIN A CONTENT IN SERA OF SCHOOL CHILDREN WITH AND WITHOUT FOLLICULOSIS, FOLLOWING THE INGESTION OF SINGLE DOSES OF VITAMIN A[a]

Category	Group I		Group II	
	Control	Folliculosis	Control	Folliculosis
Number of children	5	7	12	12
Vitamin A administered, μg.	7500	7500	15000	15000
Plasma vitamin A, μg.%[b]				
0 hours (basal)	26 ± 7.6	24 ± 5.4	27.6 ± 5.4	28.0 ± 6.9
4 hours after	68 ± 26.2	41 ± 24.0	67.2 ± 31.2	50.8 ± 10.9
5 " "	68 ± 23.4	68 ± 20.8	123.8 ± 49.0	123.9 ± 20.8
6 " "	68 ± 27.8	65 ± 39.2	87.0 ± 26.9	95.5 ± 13.1
8 " "	45 ± 17.9	38 ± 9.8	52.9 ± 26.9	67.3 ± 6.7
24 " "	48 ± 7.0	25 ± 5.8	35.5 ± 6.9	40.6 ± 4.4

[a] Adapted from R. F. Krause and H. B. Pierce, *J. Nutrition*, *33*, 633–640 (1947).
[b] Including Standard Deviation of the Mean.

Although no appreciable differences were observed in the curves for serum vitamin A between the "normal" children and those having folliculosis, the maximum levels obtained on the two dosages are quite different. Thus, after 15,000 μg. of vitamin A had been given, the maximum blood

level was 124 microgram per cent; on the other hand, when the dosage was only 7500 μg. of vitamin A, the highest value for vitamin A in the serum was 68 microgram per cent. In both cases, the peak values were obtained five hours after the ingestion of the vitamin. The basal values for vitamin A were reached twenty-four hours after the lower dose of vitamin A; the vitamin A levels were still slightly elevated in the twenty-four hour samples following the higher vitamin A intake (15,000 μg.).

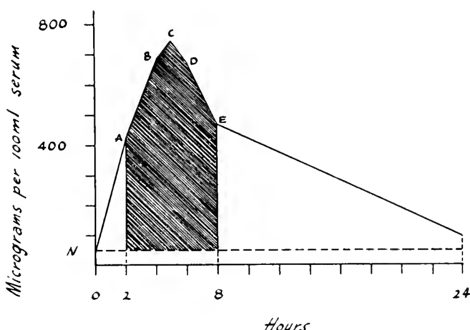


Fig. 10. A typical vitamin A tolerance curve. "N" represents the basal level. Shaded area represents the increase in vitamin A level for the two- to eight-hour period, expressed in microgram hours. The calculated area $= (A + C + E) + 1.5(B + D) - 6N$.[129]

Week and Sevigne[129] carried out an extensive series of tests with human subjects, to whom they gave 134,000 μg. of vitamin A in the form of vitamin A alcohol, acetate, or palmitate, along with 50 g. of a commercial brand of margarine. A typical curve for assessing the increase in blood vitamin A is shown in Figure 10. A comparison of the amount of vitamin A in the plasma as the ester or as the alcohol is given in Table 21.

The administration of vitamin A as the free alcohol, acetate, or natural ester in the amount of 134,000 μg. gives rise to a curve for plasma vitamin A which reaches the maximum in five hours, for male subjects. In most cases of women, the peak is reached under comparable conditions in four hours. The figures for plasma vitamin A remain somewhat above the basal value, even after twenty-four hours. There is practically no change in the value for vitamin A alcohol during the entire experimental period. On the other hand, there is a hundred-fold increase in the concentration of vitamin A ester. Although vitamin A alcohol constitutes 80 to 90% of the

TABLE 21. FREE AND ESTERIFIED VITAMIN A IN SERUM OF TWO SUBJECTS AT VARIOUS TIME INTERVALS AFTER ADMINISTRATION OF 134,000 μG. OF VITAMIN A IN THE FORM OF ALCOHOL ACETATE OR NATURAL ESTER[a]

Data expressed in microgram per cent

Supplement administered	Time after administration of vitamin A (hours)							
	0		5		6		24	
	Alcohol	Ester	Alcohol	Ester	Alcohol	Ester	Alcohol	Ester
Male subject:								
Vitamin A alcohol	54.6	7.5	60.6	697	52.6	473	54.8	57.2
Vitamin A acetate	51.8	5.9	50.0	580	—	—	49.8	21.4
Vitamin A ester	56.0	12.5	59.4	480	—	—	51.0	33.8
Female subject:								
Vitamin A alcohol	34.0	5.1	66.8	540	51.2	315	42.3	92.7
Vitamin A acetate	31.3	7.8	36.6	370	—	—	39.0	22.8
Vitamin A ester	34.6	5.4	26.7	335	—	—	33.0	52.4

[a] Adapted from E. F. Week and F. Sevigne, *J. Nutrition*, *40*, 563–576 (1950).

TABLE 22. AVERAGE TOTAL VITAMIN A IN PLASMA OF MEN AND WOMEN AFTER ADMINISTRATION OF 134,000 μG. OF VITAMIN A AS ALCOHOL, ACETATE, OR NATURAL ESTER[a]

Supplement given	Sex	No. of subjects	Plasma vitamin A in μg. % in hours after vitamin A						
			(Basal) 0	2	4[b]	5[b]	6	8	24
Vitamin A alcohol	Male	18	53.3	425	692	*750*	680	478	102.5
	Female	7	40.8	285	*458*	437	342	283	71.6
Vitamin A acetate	Male	18	54.2	329	565	*658*	628	482	96.3
	Female	7	40.0	248	*345*	328	270	188	59.1
Vitamin A ester, No. 1 (natural)	Male	18	51.4	269	513	*596*	549	408	91.0
	Female	7	43.5	214	278	*295*	261	237	64.5
Vitamin A ester, No. 2	Male	18	53.7	274	482	*539*	512	405	95.0
	Female	7	40.3	221	*419*	406	322	238	71.8

[a] Data assembled from E. F. Week and F. Sevigne, *J. Nutrition*, *40*, 563–576 (1950), pp. 566, 567.
[b] The maximum values are italicized.

total vitamin A in the fasting samples, it accounts for only about 10% of the total in the samples at approximately the apex of the plasma vitamin A curve.

There is a marked sex difference, not only in the basal levels for vitamin A in the plasma, but also during the absorption period, coincident with high vitamin A values. The differences in plasma vitamin A levels between the sexes can be seen from the data summarized in Table 22.

b′. The Relationship between Plasma and Liver Levels of Vitamin A Alcohol: Lewis and co-workers[884] were of the opinion that plasma tends to resist any changes in vitamin A concentration, despite the wide variations in the amount of vitamin A stored in the liver. This viewpoint has been supported to some extent by the work of several investigators.[836,885–891] In fact, in the case of rachitic calves, the plasma vitamin A level is not considered to be a reliable index of the vitamin A intake,[890] of the carotene intake,[892] or of the liver storage of vitamin A.[891] According to Horton *et al.*,[887] on the other hand, the concentration of vitamin A in the blood is an index of the vitamin A nutrition. Furthermore, Almquist[893] noted that, in chicks and turkeys, plasma vitamin A shows an essentially linear relation to the log of the concentration of vitamin A in the liver.

Glover and co-workers[132,133] and Horton *et al.*[887] suggested that a direct relationship exists between the concentrations of vitamin A alcohol in the liver and in the plasma of rats. Ganguly and Krinsky[894] refuted the conclusion proposed by Glover *et al.*[132,133] On the basis of experiments in which wide variations were observed in the concentrations of the vitamin A alcohol in the liver, the plasma level for vitamin A alcohol was shown to remain relatively constant. Ganguly *et al.*[134] postulated that the plasma vitamin A alcohol concentration is determined by the amount of a specific protein carrier in the plasma.

Aron[895] believes that the liver plays an important role in the regulatory processes controlling the amount of vitamin A circulating in the blood in

[885] H. W. Josephs, *Bull. Johns Hopkins Hosp.*, *71*, 253–264 (1942).
[886] S. Brenner, M. C. H., Brookes, and L. J. Roberts, *J. Nutrition*, *23*, 459–471 (1942).
[887] P. B. Horton, W. A. Murrill, and A. C. Curtis, *J. Clin. Invest.*, *20*, 387–393 (1941).
[888] A. G. Van Veen, *Mededeel. Dienst Volksgesondheid Ned. Indië*, *26*, 300–307 (1937); cited by P. C. Leong, *Biochem. J.*, *35*, 806–812 (1941), p. 806.
[889] R. F. Krause, *J. Nutrition*, *38*, 535–542 (1949).
[890] J. W. Thomas, W. C. Jacobson, and L. A. Moore, *J. Dairy Sci.*, *35*, 679–686 (1952).
[891] P. C. Leong, *Biochem. J.*, *35*, 806–812 (1941).
[892] J. W. Thomas and L. A. Moore, *J. Diary Sci.*, *35*, 687–692 (1952).
[893] H. J. Almquist, *Arch. Biochem.*, *39*, 243–244 (1952).
[894] J. Ganguly and N. I. Krinsky, *Biochem. J.*, *54*, 177–181 (1953).
[895] H. C. S. Aron, *Am. J. Diseases Children*, *77*, 763–773 (1949).

man. When this regulatory mechanism is not functioning, clinical disorders such as hepatic disease, fever, and cutaneous diseases occur. The regulatory mechanism functions abnormally in pregnancy.[895]

(c) *The Effect of Massive Doses of Carotene on Plasma Carotene and Vitamin A Levels.* When excessive quantities of carotene are ingested by individuals who have severe hepatic disease, diabetes mellitus, or myxedema which prevents a normal utilization of carotene, or even by completely healthy individuals, excessively high values of blood carotene obtain, as indicated by the name of this abnormal condition, *carotenemia.* It is also sometimes referred to as "*carotinosis.*"

Carotenemia should be distinguished from hypervitaminosis A, since the causative agent in one case is carotene and in the other instance, vitamin A. Whereas carotenemia is a harmless metabolic condition, hypervitaminosis A may result fatally. Both abnormalities can be cleared up by reduction of the intake of the offending substance to a normal level. The amount of carotene is increased not only in the serum, but likewise in the tissues, giving the individual the yellow appearance also characteristic of icterus or jaundice. Normal individuals, especially children, have been known to develop this coloration after an excessive intake of carrots,[896] Hubbard squash (*Cucurbita maxima*), "mikan," or Japanese orange (*Citrus nobilis*, Lour., var. *microcarpa*),[897] or other food sources rich in carotene.[898]

(d) *The Effect of Massive Doses of Vitamin A on Plasma Carotene and Vitamin A Levels.* When massive doses of vitamin A were given to cows (70,000 to 1,690,000 μg. per day), a marked suppression of the carotene content of butter fat occurred.[899,900] Coincident with the marked reduction in milk carotene which occurred after the cows had received the high vitamin A supplements for four to six weeks, there was a decrease in blood carotene. This feached as low a level as 30 microgram per cent after eleven weeks of feeding with massive doses of vitamin A. Since a proportionality was shown to exist between the vitamin A content of plasma and of milk,[900] the low levels of the latter in many butter samples would indicate that the blood carotenes are concomitantly low. Fountaine and Bolin[901] and Jensen *et al.*[902] confirmed the depressing action of massive doses of vitamin A on the milk carotene in cows.

[896] A. F. Hess and V. C. Myers, *J. Am. Med. Assoc.*, *73*, 1743–1745 (1919).

[897] H. Hashimoto, *J. Am. Med. Assoc.*, *78*, 1111–1112 (1922).

[898] H. Koch, *Biochem. J.*, *37*, 430–433 (1943).

[899] H. J. Deuel, Jr., N. Halliday, L. Hallman, C. Johnston, and A. J. Miller, *J. Nutrition*, *22*, 303–313 (1941).

[900] H. J. Deuel, Jr., L. F. Hallman, C. Johnston, and F. Mattson, *J. Nutrition*, *23*, 567–579 (1942).

[901] F. C. Fountaine and D. W. Bolin, *J. Dairy Sci.*, *27*, 155–158 (1944).

It has likewise been shown that the administration of massive doses of vitamin A to chickens results in a pronounced decrease in the carotene level of the plasma and of the egg yolk,[903] while vitamin A is being markedly increased in these locations. These data are related to the observation of Hammond and Harshaw[904] that there is a material in fortified cod-liver oil which interferes with the deposition of xanthophyll in the shanks and skin of chicks. The pigmentation-suppressing factor was later shown by Rubin and Bird[905] to be vitamin A. Table 23 furnishes a summary of these data.

TABLE 23
EFFECT OF SEVERAL LEVELS OF VITAMIN A INTAKE ON CAROTENOID AND VITAMIN A CONTENT OF PLASMA, LIVER, FAT, AND EGG YOLK OF CHICKENS[a]

Vitamin A supplement per lb. of food, μg.	Serum		Yolks		Liver		Body fat	
	Car., μg. %	Vit. A, μg. %	Car., μg./g.	Vit. A, μg./g.	Car., μg./g.	Vit. A, μg./g.	Car., μg./g.	Vit. A, μg./g.
0	143.2	139	32.3 ± 2.0	14.0	7.8	111	9.5	9.7
333	218.0	166	29.4 ± 1.5	14.0	11.7	200	4.5	7.0
666	—	—	33.0 ± 1.7	13.9	—	—	—	—
4,500	—	—	25.3 ± 0.7	14.5	—	—	—	—
9,000	111.4	134	21.7 ± 1.1	16.4	6.7	1378	5.9	12.7
18,000	105.8	174	12.9 ± 0.8	18.7	6.3	2082	6.1	17.8
30,000	62.6	133	9.7 ± 0.6	29.8	4.0	1396	2.3	27.8
60,000	37.0	207	8.4 ± 0.5	36.2	2.7	1388	8.0	66.8

[a] Data from H. J. Deuel, Jr., M. C. Hrubetz, F. H. Mattson, M. G. Morehouse, and A. Richardson, *J. Nutrition, 26,* 673–685 (1943).

When massive doses of vitamin A are administered over prolonged periods, symptoms of hypervitaminosis A develop. According to Josephs,[906] a hypervitaminemia A occurs, together with a lowered level of blood carotene; in the one child studied, the serum vitamin A equaled 273 microgram per cent, while the serum carotene amounted to 110 microgram per cent. In other reports of hypervitaminosis A in man, even higher serum vitamin A levels were reported, namely 400 microgram per cent, by Toomey and Morissette,[907] and 624 microgram per cent, by Roth-

[902] C. Jensen, P. D. Boyer, P. H. Phillips, I. W. Rupel, and N. S. Lundquist, *J. Dairy Sci., 25,* 931–937 (1942).
[903] H. J. Deuel, Jr., M. C. Hrubetz, F. H. Mattson, M. G. Morehouse, and A. Richardson, *J. Nutrition, 26,* 673–685 (1943).
[904] J. C. Hammond and H. M. Harshaw, *Poultry Sci., 20,* 437–444 (1941).
[905] M. Rubin and H. R. Bird, *Science, 103,* 584–586 (1946).
[906] H. W. Josephs, *Am. J. Diseases Children, 67,* 33–43 (1944).
[907] J. A. Toomey and R. A. Morissette, *Am. J. Diseases Children, 73,* 473–480 (1947).

man and Leon.[908] Most animals, including man, respond to either large or toxic doses of vitamin A by increases in serum cholesterol,[882,909] although Muller and Suzman[910] were unable to note any correlation between the amounts of vitamin A and of cholesterol in the livers of patients at autopsy. Fasold[911] reported that large dosages of vitamin A did not produce changes in the cholesterol in the bodies of rats. However, neither of these findings directly refutes the positive results on blood lipids, since variations in blood lipids may occur without influencing the quantity in the liver or total body. Peters and Van Slyke[202] suggested that the hypothesis of a hyperlipemia following excessive vitamin A intake must be discounted, because the vitamin was administered in oil. However, one must question this objection in view of the relatively large quantity of oil required to produce hyperlipemia.

In the case of the dog, the hypervitaminosis A is also distinguished by hypervitaminemia A.[851] Serum vitamin A values, which are remarkably high in the normal dog,[849–851] are increased 8- to 40-fold in hypervitaminosis A. A value as high as 6012 microgram per cent has been recorded by Maddock *et al.*[851] following the administration of vitamin A in the dosage of 90,000 μg./kg. body weight to a puppy for forty-five days. However, the dog is unusual in its failure to respond to hypervitaminosis A by the development of hypercholesterolemia[851,882] or by an increase in serum phospholipids.[851]

The toxicity of hypervitaminosis A may be due to a hypoprothrombinemia,[912–914] a condition which can be corrected by the administration of vitamin K.[912] Moore and Wang[915] call attention to the prevalence of uterine hemorrhages in pregnant rats given an excess of cod-liver oil. This may also be related to the hypoprothrombinemia. No similar toxicities can be produced by the feeding of high dosages of carotene, although a high blood carotene and pigmentation of the skin result. The failure of carotene to effect a hypervitaminosis A similar to that caused by excessive doses of vitamin A *per se* may be due to the limited absorbability of the

[908] P. E. Rothman and E. E. Leon, *Radiology*, *51*, 368–374 (1948).
[909] F. Lasch, *Klin. Wochschr.*, *13*, 1534–1536 (1934).
[910] G. L. Muller and M. M. Suzman, *Arch. Internal Med.*, *54*, 405–411 (1934).
[911] H. Fasold, *Z. ges. exptl. Med.*, *94*, 35–37 (1934).
[912] R. F. Light, R. P. Alscher, and C. N. Frey, *Science*, *100*, 225–226 (1944).
[913] S. E. Walker, E. Eylenburg, and T. Moore, *Biochem. J.*, *41*, 575–580 (1947).
[914] C. L. Maddock, S. B. Wolbach, and D. Jensen, *Federation Proc.*, *7*, 275 (1948).
[915] T. Moore and Y. L. Wang, *Biochem. J.*, *39*, 222–228 (1945).
[916] K. Rodahl, *The Toxic Effect of Polar Bear Liver*, Skrifter No. 92, Norsk Polarinstitut, Kommisjon Has Jacob Dybwad, Oslo, 1949, 99 pp.; cited by T. Moore, *Ann. Rev. Biochem.*, *19*, 319–338 (1950), p. 325.

pigment. The symptoms of hypervitaminosis A have been reviewed by Rodahl[916] and by Maddock *et al.*[851] Other characteristic lesions, which occurred in young animals, are skeletal fractures and external hemorrhages.[915,917]

(*e*) *The Effect of Carotene-free and Vitamin A-free Diets on Plasma Carotene and Vitamin A.* When vitamin A-free diets were given to rats, the vitamin disappeared from the plasma within twenty days,[885] and definite signs of avitaminosis A appeared in twenty-six days. On the other hand, the depletion of the human subjects is much slower. Murrill and coworkers[834] were unable to detect deficiency symptoms, other than a reduction of carotene and vitamin A in the blood, in either of two men who had received a vitamin A-free diet for forty days. Even when the patients were maintained on a diet low in vitamin A for six months, following a month of high vitamin A intake, Wald *et al.*[918] were unable to detect vitamin A-deficiency symptoms in any of five men.

On the other hand, Hsu[839] reported an average blood carotene figure of 23.8 microgram per cent (range 0–128), and a mean vitamin A value of 1.7 microgram per cent (range 0–8.1) in the case of eighty-five individuals classed as vitamin A-deficient. Hartzler[919] was able to prove that the level of serum carotene depends upon the presence of this substance in the diet, and that of vitamin A upon the ingestion of carotene or vitamin A. She demonstrated a progressive decrease both in carotene and in vitamin A in a subject receiving a completely vitamin A-free diet. The blood plasma, which contained 149 microgram per cent of carotene and 39.9 microgram per cent of vitamin A at the start, had only 15 microgram per cent of carotene and 22.8 microgram per cent of vitamin A at the end of the 140-day depletion period. It is likewise possible to produce experimentally, and maintain over an indefinite period, by dietary means, a carotene-free plasma without any symptoms of vitamin A deficiency. This can be done by the administration of a carotene-free diet which contains an adequate amount of preformed vitamin A. The alternative condition, namely one in which carotene is present in the blood plasma without vitamin A, obviously cannot occur, since a portion of the carotene will always be changed to vitamin A in order to maintain a normal value of the latter compound in the blood and tissues.

(*f*) *The Effect of Miscellaneous Substances on Plasma Carotene and Vitamin A.* The administration of mineral oil concomitantly with carotene

[917] K. Rodahl and T. Moore, *Biochem. J.*, *37*, 166–168 (1943).
[918] G. Wald, L. Brouha, and R. E. Johnson, *Am. J. Physiol.*, *137*, 551–556 (1942).
[919] E. Hartzler, *J. Nutrition*, *36*, 381–390 (1948).

has been found to reduce its absorption, as demonstrated by the decrease in plasma carotene levels.[920–922] On the other hand, under similar conditions, the absorption of vitamin A was not appreciably decreased, as judged by the fact that there was no reduction in plasma vitamin A.[921]

According to Ronning and Knodt,[923] sulfonamide therapy is without detrimental effects on the normal vitamin A and carotene utilization in calves, as judged from the constancy of the values of the latter components in the blood plasma. On the other hand, the total plasma carotenoids and the concentration of vitamin A in the liver were significantly elevated in the chick by the administration of dietary penicillin.[924] Crowley and Allen[925] reported a 14 to 18 microgram per cent increase in blood plasma vitamin A in calves and goats after feeding ethyl alcohol. The extent of increase in vitamin A in the plasma was found to be proportional to that stored in the liver; no increase occurred in vitamin A-depleted animals. Shaw[926] observed that the addition of 30% ground raw soybeans to the diet of calves decreased both plasma and liver vitamin A markedly, and plasma carotene to a somewhat lesser extent.

d. Plasma Carotene and Vitamin A as Indices of Nutritional Status. According to Sinclair,[842] levels of serum carotene below 100 microgram per cent and of vitamin A lower than 21 microgram per cent are considered to be indicative of vitamin A deficiency. However, on a carotene-free diet containing adequate amounts of vitamin A, there is no reason why a value of zero for plasma carotene should indicate any degree of avitaminosis A, provided, of course, that the plasma vitamin A value is normal. Youmans *et al.*,[847] for example, reported that 33% of the white and 15% of the colored subjects examined in Tennessee had serum vitamin A values lower than 21 microgram per cent. In the North Carolina mill village studied by Yarbrough and Dann,[837] blood carotene was found to be 131 microgram per cent, as contrast with the level of 183 microgram per cent presented by the medical staff, while vitamin A was only 15.3 microgram per cent as contrasted with 21.3 μg. for the medical group. Aykroyd *et al.*[927] pointed

[920] A. C. Curtis and E. M. Kline, *Arch. Internal Med.*, *63*, 54–63 (1939).

[921] B. Alexander, E. Lorenzen, R. Hoffmann, and A. Garfinkel, *Proc. Soc. Exptl. Biol. Med.*, *65*, 275–278 (1947).

[922] A. E. Mahle and H. M. Patton, *Gastroenterology*, *9*, 44–53 (1947).

[923] M. Ronning and C. B. Knodt, *J. Dairy Sci.*, *33*, 424–429 (1950).

[924] R. C. Burgess, M. Gluck, G. Brisson, and D. H. Laughland, *Arch. Biochem.*, *33*, 339–340 (1951).

[925] J. W. Crowley and N. N. Allen, *J. Dairy Sci.*, *36*, 156–160 (1953).

[926] J. C. Shaw, *J. Dairy Sci.*, *34*, 176–180 (1951).

[927] W. R. Aykroyd, N. Jolliffe, O. H. Lowry, P. E. Moore, W. H. Sebrell, R. E. Shank, F. F. Tisdall, R. M. Wilder, and P. C. Zamecnik, *Can. Med. Assoc. J.*, *60*, 329–352 (1949).

out that the plasma vitamin A of residents of Newfoundland increased from 20.0 ± 2.0 microgram per cent in 1944, to 45.0 ± 2.0 microgram per cent in 1948. They attribute this result to the dissemination of information on the importance of vitamin A from a nutritional standpoint. Hospital patients may, in some instances, have lower average blood carotene and vitamin A levels than do non-hospital patients[839,841,848]; this reflects the lower quality of the diets in these cases as contrasted with those of the controls.

e. Plasma Carotene and Vitamin A Levels in Abnormal Conditions. Some abnormalities such as folliculosis, xerophthalmia, keratosis, and nyctalopia develop because of vitamin A deficiency. Conversely, a vitamin A deficiency may develop as a secondary condition following a diseased condition.

(*a*) *Plasma Carotene and Vitamin A Levels in Diabetes.* One of the main conditions associated with diabetes mellitus is the faulty metabolism of carotene and vitamin A. As a result of the inability of the liver to metabolize carotene effectively, a carotenemia obtains which is frequently associated with low values for the plasma vitamin A.[928–933] On the other hand, Kimble *et al.*,[934] studying deviations in blood levels, were unable to demonstrate any tendency toward carotenemia or to a low level of plasma vitamin A in 116 unselected cases of diabetes, prior to hospital treatment.

However, in a number of instances, not only was a fasting carotenemia demonstrated, but also the blood carotene tolerance curve reached higher than normal levels, and remained elevated over a longer period than in normal patients.[880,928] Ralli and her collaborators[880] also reported that the carotene levels were higher in the liver of diabetics than in this organ of patients with other diseases. Although there is no physiological evidence that the destruction of carotene is associated with the utilization of carbohydrate, it is open to question as to whether or not carotenemia is directly concerned with the diabetic disorder.[202] Rabinowitch[931] is of the opinion that carotenemia is directly correlated with the severity of the diabetes. Since it has been shown that acetonemia in cows is associated with a low

[928] E. P. Ralli, A. C. Pariente, H. Brandaleone, and S. Davidson, *J. Am. Med. Assoc.*, *106*, 1975–1978 (1936).

[929] G. H. Stueck, G. Flaum, and E. P. Ralli, *J. Am. Med. Assoc.*, *109*, 343–344 (1937).

[930] W. Heymann, *J. Am. Med. Assoc.*, *106*, 2050–2052 (1936).

[931] I. M. Rabinowitch, *Arch. Internal Med.*, *45*, 586–592 (1930).

[932] H. Brandaleone and E. P. Ralli, *Proc. Soc. Exptl. Biol. Med.*, *32*, 200–201 (1934).

[933] J. G. Brazer and A. C. Curtis, *Arch. Internal Med.*, *65*, 90–105 (1940).

[934] M. S. Kimble, O. A. Germek, and E. L. Sevringhaus, *Am. J. Med. Sci.*, *212*, 574–585 (1946).

vitamin A level in the blood, it is possible that this association may account for the failure of carotene to be metabolized in diabetes.

(*b*) *The Relationship of Acetonemia to Plasma Vitamin A.* Patton[935] was the first to demonstrate that a high serum carotene (620 microgram per cent) obtains in acetonemia of cattle, while the vitamin A in the plasma is extremely low (1.2 microgram per cent). This would seem to indicate that the high carotene and the low vitamin A value in the plasma result from an inability of the cow to transform the provitamin A to vitamin A. Mackay,[936] Patton,[935,937] and Burt[938] have been able to treat acetonemia of cattle successfully by the administration of vitamin A. This resulted in increased levels of blood vitamin A and in a decrease in blood carotene. The question arises as to whether or not vitamin A deficiency *per se* or the inability to convert carotene to vitamin A is the cause of acetonemia.

(*c*) *The Effect of Liver Disease on Plasma Carotene and Vitamin A.* Not only is the carotenemia in diabetes mellitus ascribable to an upset in liver function, but also conditions in which liver damage is the primary lesion may exhibit abnormalities in plasma carotene and vitamin A. Harris and Moore[844] reported a level of 16.4 microgram per cent of plasma vitamin A in hepatitis, which increased to 35.4 microgram per cent when a cure was effected. Fiessinger *et al.*,[939,940] Popper and associates,[941] and Haig and Patek[942] reported low vitamin A levels in cases of liver damage in man. Furthermore, the response is depressed when vitamin A is administered.[836,941,942]

(*d*) *The Effect of Miscellaneous Diseases on Plasma Carotene and Vitamin A.* Somewhat lower than normal carotene and vitamin A levels have been reported by Abels and co-workers[840] in the plasma of fifty-one patients who had cancer of the gastrointestinal tract. Ikegaki[943] noted that the blood carotene was reduced in all forms of leprosy; on the other hand, the serum vitamin A levels were reduced only in *lepra nervorum* and in *lepra tuberosa,* while they remained normal in *lepra maculosa.*

Krause and Pierce[862] were unable to demonstrate any appreciable differences in the fasting level of plasma carotene or of plasma vitamin A, or in the blood levels following the administration of 7500 or 15,000 μg. of vita-

[935] J. W. Patton, *Vet. Med., 39,* 271–278 (1944).
[936] J. Mackay, *Vet. Record, 55,* 455 (1943).
[937] J. W. Patton, *Vet. Med., 39,* 150–153 (1944).
[938] A. C. Burt, *Can. J. Comp. Med. Vet. Sci., 8,* 187–188 (1944).
[939] N. Fiessinger and H. Torres, *Compt. rend. soc. biol., 135,* 636–637 (1941).
[940] N. Fiessinger, H. Torres, and A. Gasnier, *Compt. rend. soc. biol., 135,* 697–698 (1941).
[941] H. Popper, F. Steigmann, and S. Zevin, *J. Clin. Invest., 22,* 775–783 (1943).
[942] C. Haig and A. J. Patek, Jr., *J. Clin. Invest., 21,* 309–317 (1942).
[943] I. Ikegaki, *Z. Vitaminforsch., 6,* 206–209 (1937).

min A, in the case of normal children or of those with folliculosis, respectively. During the active phase of pneumonia, both carotene and vitamin A are reduced in the plasma[944]; they return to normal values on convalescence. Reduced values for serum vitamin A are found in rheumatic patients.[945] On the other hand, no changes in the provitamin A levels in the blood occur in nephrosis.[946] However, after the feeding of vitamin A alcohol, higher values were obtained, indicating that, in the nephrotic syndrome, the liver does not store vitamin A as rapidly as in normal individuals. In the case of patients with severe renal failure, Johns *et al.*[947] found little change in serum vitamin A, in spite of the fact that the patients were depleting the liver vitamin A by excreting as much as 1000 I.U. daily in the urine. Huszäk and Geréb[948] listed the vitamin A and carotene content of blood serum in various diseases of the nervous system.

(*2*) *Tocopherols* (*Vitamins E*)

a. Normal Values for Plasma Tocopherols. According to Ames and Harris,[949] the normal concentration of tocopherol in blood plasma ranges from 0.9 to 1.2 milligram per cent, although extreme variations of from 0.1 to 3.0 milligram per cent have been noted in some cases. Lemley and co-workers[950] reported a mean serum tocopherol level of 1.09 ± 0.17 milligram per cent in twenty-one young healthy adults, while Klatskin and Krehl[951] cited an average of 1.23 milligram per cent for their twenty-three normal adults. The value recorded by Darby *et al.*[952] is 1.06 ± 0.06 milligram per cent. Engel[953] found that tocopherol levels in normal adults receiving 15 mg. of vitamin E daily approximated 0.8 milligram per cent. Levels as high as 1 2 milligram per cent were seldom observed, and did not occur in the case of patients receiving 120 mg. of vitamin E daily. The earlier figures of Varangot *et al.*[954] for plasma vitamin E, *i.e.*, 0.19 milligram

[944] H. W. Josephs, *Am. J. Diseases Children*, *65*, 712–727 (1943).

[945] R. E. Shank, A. F. Coburn, L. V. Moore, and C. L. Hoagland, *J. Clin. Invest.*, *23*, 289–295 (1944).

[946] B. M. Kagan, E. M. Thomas, D. A. Jordan, and A. F. Abt, *J. Clin. Invest.*, *29*, 141–145 (1950).

[947] R. Johns, H. Hoch, and J. R. Marrack, *Biochem. J.*, *41*, liii (1947).

[948] S. Huszäk and T. Geréb, *Z. Vitaminforsch.*, *19*, 330–335 (1947).

[949] S. R. Ames and P. L. Harris, *Intern. Rev. Vitamin Research*, *22*, 26–34 (1950).

[950] J. M. Lemley, R. G. Gale, R. H. Furman, M. E. Cherrington, W. J. Darby, and G. R. Meneely, *Am. Heart J.*, *37*, 1029–1034 (1949).

[951] G. Klatskin and W. A. Krehl, *J. Clin. Invest.*, *29*, 1528–1541 (1930).

[952] W. J. Darby, M. E. Cherrington, and J. M. Ruffin, *Proc. Soc. Exptl. Biol. Med.*, *63*, 310–312 (1946).

[953] C. Engel, *Ann. New York Acad. Sci.*, *52*, 292–299 (1949).

[954] J. Varangot, H. Chailly, and N. Rieux, *Compt. rend. soc. biol.*, *137*, 210–211 (1943).

per cent for men and 0.22 milligram per cent for women, are quite out of line with the later results. Couperus[955] reported a somewhat higher value for serum tocopherol, namely 0.7 milligram per cent.

In cows, the tocopherol content is somewhat lower than in man. Thus, van der Kaay and associates[956] reported an average of 800 microgram

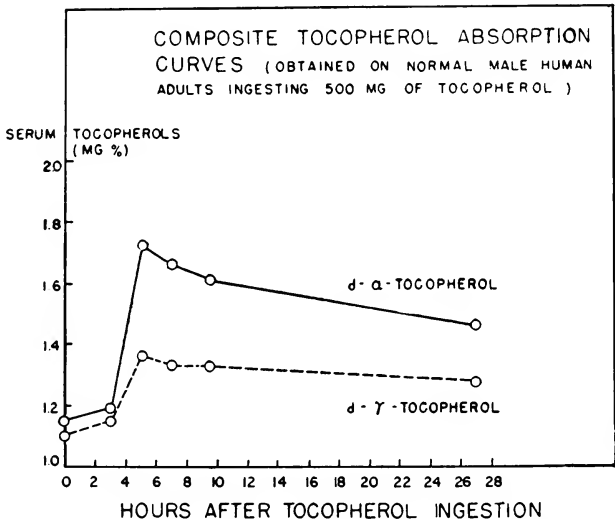


Fig. 11. The comparative absorption of *d*-α- and *d*-γ-tocopherols by normal human adults as determined by the resulting tolerance curves for serum tocopherols.[136]

per cent (0.80 milligram per cent) in cows on pasture, while the serum tocopherol was reduced to 0.1 to 0.2 milligram per cent in winter, presumably when green pasturage was not available. Meunier *et al.*[957] cited a range of 200 to 300 microgram per cent as the normal plasma tocopherol level in sheep. Quaife *et al.*,[136] recorded a value of 0.70 milligram per cent for male rats receiving a vitamin E-deficient diet supplemented with 1

[955] J. Couperus, *Z. Vitaminforsch.*, *13*, 193–207 (1943).

[956] F. C. van der Kaay, G. H. B. Teunissen, A. Emmerie, and M. van Eekelen, *Ann. New York Acad. Sci.*, *52*, 276–283 (1949).

[957] P. Meunier, R. Ferrando, and P. Chenavier, *Compt. rend. soc. biol.*, *142*, 525–527 (1948).

mg. of α-tocopherol daily. On the other hand, Quaife and Harris[958] reported serum tocopherol values of 1.72 milligram per cent and 1.68 milligram per cent for rats whose ration was supplemented with vitamin E at a level of 12 mg./week, and which were subjected to stress or were free from stress, respectively. On the other hand, rats which were vitamin E-deficient and were subjected to similar experimental conditions showed a zero value for plasma tocopherol.

b. General Physiologic Factors Related to the Level of Plasma Tocopherols. (*a*) *Relation to Ingested Tocopherol.* There can be no doubt that the administration of tocopherol to animals and to man in sufficiently large doses results in augmented levels of this component in the blood plasma. Quaife *et al.*[136] studied the effect of 500 mg. doses of *d*-α- and *d*-γ-tocopherols on the levels of serum tocopherols of normal human adults. It was shown that the peak values were not reached until a period of six hours had elapsed; the tocopherol levels in the blood were still elevated twenty-four hours after the supplements were given. These results are charted in Figure 11.

Meunier and co-workers[957] found a significant decrease in plasma tocopherol when cod-liver oil was administered to sheep. Ferrando and associates[959] also observed that the administration of cod-liver oil to lactating cows results in a reduction of blood tocopherol, as well as in a decrease of the fat in the milk. It is suggested that this phenomenon is not the result of an antagonistic action between the unsaturated fatty acids of cod-liver oil and tocopherol, but rather that a critical ratio of tocopherol:unsaturated fatty acids exists. In later studies, Brion, Ferrando, and collaborators[960] observed that cod-liver oil in normal doses did not affect the plasma tocopherol level of the pig.

(*b*) *Maternal-Fetal Transfer of Tocopherols.* Mason and Bryan[961] reported, in 1938, that the placental transfer of vitamin E was negligible in the rat, and that the mammary transfer was likewise decidedly limited. In later studies by these workers,[962] it was shown that the maximum transfer of vitamin E to newborn female rats attributable to supplementation of the maternal diet during pregnancy and during lactation is insufficient to insure fertility of these young on reaching maturity. Moreover, the store

[958] M. L. Quaife and P. L. Harris, *Proc. Soc. Exptl. Biol. Med.*, *78*, 188–191 (1951).

[959] R. Ferrando, P. Chenavier, and M. Cormier, *Bull. soc. chim. biol.*, *31*, 810–816 (1949).

[960] A. Brion, R. Ferrando, P. Chenavier, and J. Portal, *Bull. soc. chim. biol.*, *33*, 151–154 (1951).

[961] K. E. Mason and W. L. Bryan, *Biochem. J.*, *32*, 1785–1791 (1938).

[962] K. E. Mason and W. L. Bryan, *J. Nutrition*, *20*, 501–517 (1940).

of vitamin E in the newborn rat cannot be appreciably altered by the intake of vitamin E by the mother, although the length of time required to produce vitamin E-deficiency symptoms was prolonged when the mother's diet included 2000 to 3000 times the minimum daily requirement of vitamin E throughout pregnancy and during the first two weeks of lactation. Evidence that some maternal-fetal transfer of tocopherol occurs in rats and mice is to be found in the fact that the young of vitamin E-deficient mothers develop symptoms of vitamin E deficiency during the first two weeks of life,[963–965] while the young of normally fed females show no such symptoms.

In the case of ewes, Willman *et al.*[966,967] also showed that a vitamin E deficiency, the so-called "stiff-lamb disease" occurred in a high percentage of cases when the mothers were fed alfalfa hay and cull beans during gestation and lactation. A diet of this type is extremely low in vitamin E. Whiting and Loosli[968] demonstrated a marked increase in the tocopherol content of the blood plasma of lambs and kids whose parent had been supplemented *prepartum* with mixed tocopherols, as compared with those from mothers who had been given alfalfa and clover hay and red kidney beans, and who had received no added tocopherol during the gestation period. However, the tocopherol feeding of the mothers was without effect on the tocopherol level of newborn pigs. On the other hand, tocopherol feeding produced a two-fold increase in the colostrum content in all species. It was also shown by Whiting and Loosli[968] that colostrum normally contains as much as four times the vitamin E which is present in the normal milk secreted as early as four days after parturition. A summary of these data is included in Table 24.

In the case of dairy cows, wide discrepancies exist in the tocopherol level of the dams and of their calves. The effect of the administration of relatively large doses of mixed tocopherols on maternal-placental transfer was shown to be minimal, by Parrish *et al.*,[969] whose results are summarized in Table 25. When the serum tocopherol of the dam was increased fourfold by the administration of large doses of tocopherols, that of the newborn calf was only slightly augmented, with the result that the ratio of

[963] H. M. Evans and G. O. Burr, *J. Biol. Chem.*, *76*, 273–297 (1928).

[964] H. S. Olcott, *J. Nutrition*, *15*, 221–225 (1938).

[965] A. M. Pappenheimer, *Am. J. Pathol.*, *18*, 169–175 (1942).

[966] J. P. Willman, J. K. Loosli, S. A. Asdell, F. B. Morrison, and P. Olafson, *J. Animal Sci.*, *4*, 128–132 (1945).

[967] J. P. Willman, J. K. Loosli, S. A. Asdell, F. B. Morrison, and P. Olafson, *Cornell Vet.*, *36*, 200–204 (1946).

[968] F. Whiting and J. K. Loosli, *J. Nutrition*, *36*, 721–726 (1948).

[969] D. B. Parrish, G. H. Wise, C. E. Latschar, and J. S. Hughes, *J. Nutrition*, *40*, 193–202 (1950).

TABLE 24

TOCOPHEROL CONTENT OF PLASMA AND LIVER OF NEWBORN LAMBS, KIDS, AND PIGS WHOSE MOTHERS HAD RECEIVED NO TOCOPHEROL, OR A SUPPLEMENT OF 80 MG. OF MIXED TOCOPHEROL PER 100 LBS. OF BODY WEIGHT[a]

Species	Tocopherol in plasma,[b] μg. %		Tocopherol in liver,[b] μg./g.	
	Controls	Supplemented	Controls	Supplemented
Lambs	20 ± 10 (6)	94 ± 62 (6)	25.3 ± 2.7 (6)	30.0 ± 4.3 (6)
Kids	16 ± 8 (5)	65 ± 40 (5)	10.4 ± 1.5 (5)	13.1 ± 2.0 (6)
Pigs	120 ± 28 (6)	101 ± 33 (7)	24.7 ± 3.6 (8)	26.4 ± 4.7 (10)

[a] F. Whiting and J. K. Loosli, *J. Nutrition*, *36*, 721–726 (1948).
[b] Including the Standard Error of the Mean. The figures in parentheses are the number of samples analyzed.

serum tocopherol of the dam to the serum tocopherol of the calf was greatly increased.

Placental transfer of vitamin E is likewise decidedly limited in the human subject, as is evident from the data of Wright, Filer, and Mason.[970] Whereas the mothers who had nursed their infants had a level of serum tocopherol of 1.82 milligram per cent and those who had not nursed the babies had an average serum tocopherol of 2.05 milligram per cent on the second day *postpartum*, the average tocopherol content of the newborn was only 0.38 ± 0.18 milligram per cent.

(*c*) *Plasma Tocopherols in the Newborn and Young Animal.* As is evident from the data presented in the previous section, the level of tocopherol in the blood serum of the newborn is much lower than in the adult. There is a rapid increase in the tocopherol levels as soon as the young animal partakes of colostrum. This occurs irrespective of whether or not the dam was supplemented with tocopherol prior to parturition. In the experiments reported by Parrish *et al.*,[969] the average tocopherol content listed in Table 26 was noted in the blood of calves after parturition (p. 515).

In the case of human subjects, Abderhalden[971] reported a figure of 96 microgram per cent for the tocopherol content of the newborn infant, while Straumfjord and Quaife[972] recorded a figure of 340 microgram per cent. More recently, Moyer[973] has found a mean value of 0.22 ± 0.13 milligram per cent (220 μg.) for the cord blood of thirty-three full-term infants, and of 0.24 milligram per cent for the venous blood of these babies. The mixed blood of fifty-three full-term infants, whose plasma tocopherol

[970] S. W. Wright, L. J. Filer, Jr., and K. E. Mason, *Pediatrics*, *7*, 386–393 (1951).
[971] R. Abderhalden, *Schweiz. med. Wochschr.*, *75*, 281–283 (1945).
[972] J. V. Straumfjord and M. L. Quaife, *Proc. Soc. Exptl. Biol. Med.*, *61*, 369–371 (1946).
[973] W. T. Moyer, *Pediatrics*, *6*, 893–896 (1950).

TABLE 25
AVERAGE CONCENTRATIONS IN THE BLOOD SERUM OF DAIRY COWS AFTER PARTURITION, AND OF NEWBORN CALVES PRIOR TO COLOSTRUM INGESTION[a]

Prepartal supplement of dam						
	Daily amount, g.			Tocopherol levels in blood serum,[b] μg. %		
Vitamins	28–14 days	13–0 days	No. of animals	Dam	Calf	Ratio of tocopherol, dam/calf
None	0	0	7[c]	276 ± 27	44 ± 31	6.3
None	0	0	1[d]	347	25	13.9
Vitamin A	0.15	0.30	14	255 ± 86	53 ± 31	4.8
Vitamin A	0.15	0.30				
α,γ-Tocopherols	0.5	1.0	6	350 ± 31	74 ± 36	4.7
α,γ-Tocopherols	0.5	1.0	1	396	43	9.2
α,γ-Tocopherols	5	5	1	817	43	19.0
Mixed tocopherols	4	4	1	1003	68	14.7
Mixed tocopherols	10	10	3	1101 ± 235	79 ± 20	13.9

[a] Adapted from D. B. Parrish, G. H. Wise, C. E. Latschar, and J. S. Hughes, *J. Nutrition*, *40*, 193–202 (1950).
[b] Including standard deviation.
[c] Results from 5 cows and 7 calves.
[d] Cow milked throughout gestation.

TABLE 26

TOCOPHEROL CONCENTRATIONS IN BLOOD PLASMA OF CALVES FROM DAMS RECEIVING UNSUPPLEMENTED RATIONS OR DIETS SUPPLEMENTED WITH TOCOPHEROLS DURING THE LATER STAGES OF GESTATION[a]

Prepartal supplement of dam		Tocopherol in blood serum in μg. % on following days after birth							
Vitamin fed	Amt. of tocopherols, g.	0[c]	1	2	3	7	14	21	28
None	0	47(6)	159(6)	175(6)	202(6)	237(4)	307(4)	225(4)	180(4)
None[b]	0	25(1)	68(1)	62(1)	80(1)	—	—	—	—
Vitamin A alcohol or ester	0	40(7)	131(7)	178(7)	196(7)	221(3)	221(3)	190(3)	141(3)
Vitamin A alcohol plus tocopherols	0.5–1.0	105(2)	223(2)	335(2)	304(2)	—	—	—	—
Tocopherols	0.5–1.0	43(1)	334(1)	260(1)	229(1)	192(1)	297(1)	291(1)	192(1)
Tocopherols (mixed)	4	68(1)	520(1)	588(1)	520(1)	198(1)	260(1)	186(1)	105(1)
Tocopherols (mixed)	10	90(2)	371(2)	597(2)	450(2)	706(1)	415(1)	210(1)	155(1)

[a] D. B. Parrish, G. H. Wise, C. E. Latschar, and J. S. Hughes, *J. Nutrition, 40*, 193–202 (1950).
[b] Dam milked throughout gestation.
[c] The figures in parentheses represent the number of samples analyzed.

TABLE 27

EFFECT OF TOCOPHEROL SUPPLEMENTS DURING TERMINAL STAGES OF GESTATION ON PLASMA TOCOPHEROLS OF DAMS BEFORE AND AFTER PARTURITION[a]

Days before or after parturition	Days of supplement	Plasma tocopherols of dams in $\mu g.$ % following daily tocopherol supplements						
		None[b]	None[c]	0.5–1.0 g.[b,d]	0.5–1.0 g.	4 g.	5 g.	(Mixed)[b] 10 g.
Prepartum:								
35	—	511 (8)	—	690 (5)	384	724	619	351 (3)
28	—	499 (14)	283	535 (6)	396	588	378	479 (3)
14	+	381 (17)	341	466 (6)	569	1187	919	1268 (3)
7	+	345 (18)	353	414 (6)	559	1278	861	1333 (3)
3	+	296 (19)	353	391 (6)	557	1271	805	1201 (3)
1	+	275 (19)	365	388 (6)	378	1136	813	1164 (3)
Parturition	—	260 (19)	347	350 (6)	396	1003	817	1011 (3)
Postpartum:								
1	—	244 (19)	396	292 (6)	279	891	650	1044 (3)
2	—	226 (19)	359	255 (6)	347	848	582	1007 (3)
3	—	240 (19)	316	279 (6)	285	823	557	852 (3)
7	—	306 (19)	241	323 (6)	369	836	396	593 (3)
14	—	423 (7)	379	—	501	799	594	582 (3)
21	—	451 (7)	—	—	513	799	680	643 (3)
28	—	575 (5)	—	—	616	842	799	659 (3)

[a] Data adapted from C. E. Latschar, G. H. Wise, D. B. Parrish, and J. S. Hughes, *J. Nutrition*, *38*, 503–516 (1949).
[b] Figures in parentheses represent the number of samples. Where no parenthetical value is included, the value is for a single sample.
[c] Milked throughout entire gestation.
[d] Also received vitamin A supplement.

amounted to 0.24 milligram per cent, agrees well with these values. After two to five days, plasma tocopherol had increased to 0.36 milligram per cent. Wright and collaborators[970] found a somewhat higher value for plasma tocopherol in newborn infants than Moyer[973] reported; the average plasma tocopherol in the case of seventy-seven infants on the first or second day of life was found to be 0.38 ± 0.18 milligram per cent.

The tocopherol levels in the blood of premature infants do not show any striking variations from those of full-term infants. Thus, a value of 0.43 milligram per cent (thirty infants) has been cited for premature babies during the first ten days of extrauterine life,[970] and of 0.22 milligram per cent (eleven infants) shortly after birth.[973] This average figure had decreased to only 0.09 milligram per cent after thirty-one to forty days.[970] Low values for serum tocopherol were also shown to be characteristic of infants having poor fat utilization; this condition of decreased fat digestibility exists in premature infants.

Wright, Filer, and Mason[970] demonstrated the rapid increase in serum tocopherols occurring during the early days of life. In nursing infants, blood tocopherol had reached 1.46 milligram per cent by the sixth day; it continued to remain above 1.25 milligram per cent thereafter. In the case of artificially fed babies, plasma tocopherol was found to be 0.50 milligram per cent by the sixth day. At the age of five to eight months, the levels were approximately 0.75 milligram per cent. Minot and Frank[974] reported that normal children eight to nineteen years of age had plasma tocopherol concentrations of 0.64 to 1.12 milligram per cent.

(*d*) *Plasma Tocopherols in Pregnancy.* In the case of cows, Latschar *et al.*[975] showed that a progressive decrease in plasma tocopherols occurs as term approaches. The minimum value is reached seven to fourteen days following delivery, after which the serum tocopherol begins to increase. When tocopherol is given during the last four weeks prior to parturition, the level of the blood tocopherol is promptly raised; a decrease occurs before and following parturition, but the maximum and minimum levels are somewhat higher. These data are recorded in Table 27 (p. 516).

In the case of women, the changes in plasma tocopherols during pregnancy do not follow as definite a pattern as in cattle.[972,976,977] Although

[974] A. S. Minot and H. E. Frank, *Am. J. Diseases Children*, *67*, 371–375 (1944).

[975] C. E. Latschar, G. H. Wise, D. B. Parrish, and J. S. Hughes, *J. Nutrition*, *38*, 503–516 (1949).

[976] J. Varangot, *Compt. rend.*, *214*, 691–692 (1942).

[977] J. Varangot, H. Chailley, and N. Rieux, *Compt. rend. soc. biol.*, *137*, 393–394 (1943).

Athanassiu[978,979] reported that, in a large proportion of women who aborted, low vitamin E levels were observed in the serum (0.2–0.3 milligram per cent), a number of other workers have found no differences in plasma tocopherol levels in non-pregnant, pregnant, or aborting women.[980–982] Scrimshaw and co-workers[982] report that plasma tocopherols in women increase progressively during the course of normal pregnancy, from 1.05 ± 27 milligram per cent during the first eight weeks of gestation to 1.51 ± 0.44 milligram per cent by the thirty-third to fortieth week. However, Latschar *et al.*[975] do not consider that the parturient decreases in the concentrations of vitamin E in the blood of cattle are necessarily unique for that species.

It is of considerable interest in this connection that the placenta has been shown to be a tissue high in vitamin E. Thus, Athanassiu[983] reported an average value of 0.75 milligram per cent (0.56 to 1.07 milligram per cent) for placenta obtained from women who had had normal deliveries.

c. The Plasma Tocopherol Levels in Abnormal Conditions. Although the deficiency of vitamin E in the diet produces characteristic symptoms in a large variety of the smaller animals, little is known about the blood picture under such conditions. However, van der Kaay and associates[956] noted that the serum tocopherol is normal in cows aborting due to *Brucella abortus* Bang infection, as well as in sterile nymphomaniac and anaphroditic cows.

(*a*) *Plasma Tocopherols in Sprue.* Darby and co-workers[952] demonstrated a marked lowering of plasma tocopherol levels in sprue. The vitamin E values in these patients ranged from 0.1 to 0.3 milligram per cent, as contrasted with 1.06 ± 0.06 milligram per cent for the normal adult controls. When the patients suffering from sprue recovered after treatment with liver extract, the blood tocopherol returned to normal (0.97 ± 0.08 milligram per cent). Low tocopherol values in the plasma in premature infants have been ascribed to poor lipid absorption; this condition also obtains in sprue.

(*b*) *Plasma Tocopherols in Diabetes.* An examination of the results of 270 tests on the serum tocopherols of sixty-three diabetic patients showed that the values were essentially normal[984] in all but seven instances. Bens-

[978] G. Athanassiu, *Z. Geburtshülfe u. Gynäkol.*, *127*, 169–195 (1946).

[979] G. Athanassiu, *Med. Monatsschr.*, *2*, 186–189 (1948).

[980] K. Faaborg-Andersen, *Nord. Med.*, *32*, 2401–2404 (1946).

[981] O. Käser, *Schweiz, med. Wochschr.*, *78*, 535–536 (1948).

[982] N. S. Scrimshaw, R. B. Greer, and R. L. Goodland, *Ann. New York Acad. Sci.*, *52*, 312–321 (1949).

[983] G. Athanassiu, *Klin. Wochschr.*, *24–25*, 170–171 (1946).

ley *et al.*[984] consider that two factors are related to the plasma level of tocopherol in diabetics, namely, the tocopherol intake and the level of plasma cholesterol. The apparent lack of relationship between plasma tocopherol and the intake of vitamin E in diabetics not receiving supplements may be due to the concomitant effect of cholesterol. It is believed that the tocopherol levels in diabetic patients are related to the cholesterol levels.[984,985]

(*c*) *Plasma Tocopherols in Muscular Dystrophies.* Although Morgulis and Spencer[986] found an increase in the lipid phosphorus and cholesterol in rabbits having muscular dystrophy, no alterations in serum tocopherol have been established. For example, Minot and Frank[974] reported plasma levels of tocopherol ranging from 0.73 to 1.28 milligram per cent in the case of eight boys five to nineteen years of age who were afflicted with pseudohypertrophic muscular dystrophy, while the range of plasma tocopherol in normal subjects of the same age was from 0.64 to 1.12 milligram per cent. When tocopherol was administered to the dystrophic patients, a prompt rise of 20 to 50% occurred in the levels of blood tocopherol; however, no improvement in creatinuria was noted.

(*d*) *Plasma Tocopherols in Miscellaneous Abnormalities.* Lemley and co-workers[950] were unable to show that heart disease exerted any effect on blood tocopherol. Thus, the mean value of tocopherol in the plasma of sixty-two cardiac patients was 0.94 ± 0.35 milligram per cent, as contrasted with an average of 0.92 ± 0.29 milligram per cent in a comparable hospital group of forty-two patients. These workers reported 1.09 ± 0.17 milligram per cent as the average figure for plasma tocopherol of young healthy adults.

Liver disease, also, probably fails to cause any decrease in serum tocopherol. Although the average figure of 0.95 milligram per cent for the blood vitamin E in forty-three patients with hepatic disease is considerably lower than the figure for normal healthy adults obtained by this investigator, namely, 1.23 milligram per cent, it does, however, closely approximate the value obtained in the case of fifty-seven hospitalized patients free from liver disease who had a mean plasma tocopherol level of 1.02 milligram per cent.

[984] E. H. Bensley, A. F. Fowler, M. V. Creaghan, B. A. Moore, and E. K. McDonald, *J. Nutrition*, *40*, 323–327 (1950).

[985] W. J. Darby, M. E. Ferguson, R. H. Furman, J. M. Lemley, C. T. Ball, and G. R. Meneely, *International Conference on Vitamin E*, *New York Acad. Sci.*, Apr. 15–16, 1949; cited by E. H. Bensley, A. F. Fowler, M. V. Creaghan, B. A. Moore, and E. K. McDonald, *J. Nutrition*, *40*, 323–327 (1950), p. 326.

[986] S. Morgulis and H. C. Spencer, *J. Nutrition*, *12*, 173–190 (1936).

Reduced values for tocopherols have been reported in fibrositis.[987] Vitamin E therapy was followed by a rise in plasma tocopherol coincident with clinical improvement.

Owens and Owens[988] noted low values for blood tocopherol in infants suffering from retrolental fibroplasia. This is a disease which develops primarily in premature infants shortly after birth. The greater the prematurity, the more likely the disease is to develop. However, the figure given for such affected babies, namely 0.25 milligram per cent (forty-six cases), does not seem to be much lower than that reported for premature babies not suffering from this condition, which has been given as 0.22 milligram per cent by one group of workers[973] and as 0.43 milligram per cent by another group.[970] After the administration of 150 mg. of vitamin E daily, over a relatively short period, to the babies with fibroplasia, the average figure for plasma tocopherol was 4.12 milligram per cent (103 cases).

[987] M. Ant and H. D. Appleton, *Med. Record*, *162*, No. 12, 12–14 (Dec., 1949).
[988] W. C. Owens and E. U. Owens, *Am. J. Ophthalmol.*, *32*, 1631–1637 (1949).

CHAPTER VI

THE OCCURRENCE OF LIPIDS IN THE ANIMAL AS A WHOLE

1. Introduction

Since the levels of the several lipids in the blood mirror the conditions which obtain in the tissues, any abnormality which results in variations in the blood picture is usually to be ascribed to an alteration of the metabolic breakdown in the tissues themselves. Blood is generally regarded as an indifferent fluid insofar as the lipids are concerned; it functions chiefly in providing a means for transporting the latter from the gastrointestinal tract to the tissues for storage, or from one tissue to another to supply material for oxidation. The blood also helps to regulate the intermediary metabolism of lipids by rendering available, at the site of transformation of the fats or other lipids, any hormones necessary for the chemical transformations.

The intermediary metabolism of each of the several lipids is quite distinct, and involves its own characteristic metabolic transformations. However, the fate of neutral fat and that of phospholipids are sufficiently closely related to render a simultaneous consideration desirable. On the other hand, the metabolism of the steroids and of the vitamins is each of a sufficiently specific nature to warrant separate treatment.

In the study of the intermediary metabolism of the fats and phospholipids, one is first concerned with the nature of the changes occurring in the liver, with their storage in the fat depots, with their conversion to other substances, and finally with their complete oxidation to carbon dioxide and water. The partial breakdown of fatty acids to the ketone bodies, which occurs in diabetes and in other conditions, helps to explain the pathways of transformation involved when a complete oxidation obtains.

The lipids occurring in different tissues vary markedly in nature. In some cases, the lipid component represents a metabolically active storage depot for fat. On the other hand, the lipid may actually comprise the structural tissue. This applies to the cerebrosides in the brain and, to

some extent, to the sterols in the spinal cord. In still other situations, the lipids may represent foodstuffs undergoing oxidation to serve as a source of energy for the animal as a whole. The liver is the best example of this type of organ.

The tissue lipids exercise a number of varied functions. One usually considers that the primary function of tissue fat, particularly in the so-called fat depots, is to serve as a reserve source of calories. Another most important role played by fats is that of an insulator, to prevent too rapid loss of heat from the surface of the body or too great an absorption of heat from the surrounding environment. Fatty tissues likewise serve as cushions for the bony projections, and in this way pad the bones; they act to protect the nerves and organs from shock. In addition, they serve as anchoring tissue to fill in and occupy any space otherwise vacant. They protect and hold the blood vessels in position. Finally, they have a most important function in providing the necessary essential material for the production of the semipermeable cell membranes of the blood cells, as well as of the cells of most fixed tissues and organs. A very complete and satisfactory treatise on the tissue lipids is included in the monograph of Bloor.[1]

2. Theoretical Considerations in the Deposition of Lipids in Different Species

In 1936, Hilditch and Lovern[2] proposed a theory to explain the divergence in the depot fats laid down by various species of animals. This theory was based upon a simplification in the structure of fats which conformed to the increasing complexity of the animal. Thus, the greatest variety of fatty acids are found in fats from the simplest marine forms; these contain not only the saturated C_{14}, C_{16}, and C_{18} acids, but also unsaturated acids varying in chain length from C_{14} to C_{22} and even, in some cases, to C_{24}. On the other hand, the higher land mammals contain as the major fatty acids in their depot fat only three representatives, namely palmitic, stearic, and oleic acids. In the case of animals whose evolutionary development is intermediate between the most primitive and the most complex form, the fatty acid makeup represents a distribution somewhat simpler than that of the marine animals, but with more acids than occur in the higher land mammals. Hilditch and Lovern[2] were of the opinion that these differences were not adventitious, but were the result of

[1] W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

[2] T. P. Hilditch and J. A. Lovern, *Nature, 137*, 478–481 (1936).

an altered metabolism related to the increasing complexity of the animal organism. Each animal presumably lays down its own typical fat. For a further discussion, see Volume I, pages 184–194.

Shorland[3] has since proposed a new theory to account for species differences in fat composition. This ascribes more importance to the ingested fat and less to the ability of the animal to lay down its own typical fat. Thus, in the early stages of evolutionary development, an organism such as the fish absorbs the dietary fat, and deposits it, with only minor changes in its composition, such as hydrogenation, dehydrogenation, or, in some cases, shortening of the carbon chains. At this stage of development, the animals are unable to synthesize fat from proteins and carbohydrates, with the result that the fat more or less resembles that ingested.

In the case of amphibians, which represent an intermediate stage in development, the animal can still deposit the dietary fat in an unchanged form. However, at this stage, the animals have developed the capacity to synthesize fats. These fats have, as their main components, palmitic, stearic, and oleic acids. The resultant depot fat of any species in this category will depend upon the relative proportion of dietary and synthetic fat which the animals contain.

The final stage is represented by ruminants, which have, to a large extent, lost their ability to deposit any of the dietary fat[4] in their tissues; most of the fatty acids of such fat are broken down by bacteria in the rumen before assimilation by the animal.[3] Under such conditions the depot fat largely mirrors the composition of the fat synthesized in the body from carbohydrates and proteins.

Proof that the fat in the lower forms is related to diet has been adduced in several ways. For example, Green and Hilditch[5] showed that, when carp are fed on grass, they deposit diene and triene fatty acids characteristic of the grass lipids[3]; these differ in composition from those of other marine forms. Lovern[6] likewise demonstrated that the composition of the original body fat of eels may be modified if fats of a different fatty acid makeup are fed to this species in relatively large amounts. Shorland[3] has indicated that the following effects on the body fat of different species would be noted on feeding linseed oil: *first stage animals* (fishes): depot fat resembling linseed oil; *second stage animals* (amphibians): depot fat consisting of a mixture of synthetic fat and linseed oil, the proportion de-

[3] F. B. Shorland, *Nature, 170,* 924–925 (1952).
[4] F. B. Shorland, *Nature, 165,* 766 (1950).
[5] T. G. Green and T. P. Hilditch, *J. Soc. Chem. Ind., 55,* 4–8T (1936).
[6] J. A. Lovern, *Biochem. J., 32,* 1214–1224 (1938).

pending upon the amount of fat in the diet, and the rate of growth; and *third stage animals* (ruminants such as the cow): depot fat unaffected by linseed oil and consisting essentially of synthetic fat. Hilditch,[7] in commenting on the Shorland article, advances the fact that the composition of pig fat, from an animal fed on whale oil, may resemble the latter fat to a great extent.[8] Moreover, reference is made to the fact that linolenic acid appears in the fat of grass-fed horses; this apparently originates from the grass glycerides.[9,10] Although these examples are entirely correct, they do not invalidate the Shorland hypothesis, since neither the pig nor the horse can be classed as a ruminant.

3. Sources of Fat in the Animal Body

(1) *Dietary Fat as a Source of Lipids*

The primary substances from which one might expect the tissue lipids to originate are the fats which occur in the diet. This assumption is of only extremely limited application, in the case of the higher animals, and a correspondence between the ingested fat and that deposited in the fat depots can ordinarily not be demonstrated. However, when large amounts of unusual fats are given to animals, evidence of the deposition of the unchanged food fat in the fat depots of the animal may sometimes be demonstrated. As has already been stated, a much closer correspondence exists between the dietary fat and the body fat in the lower animals than in those higher in the evolutionary scale.

Under ordinary conditions in which a mixed diet is employed, animals lay down a fat characteristic of the species. The nature of this fat is influenced by sex hormones as well as by other hormones, and probably also by environmental conditions. These usually assist in establishing the fatty acid composition which is characteristic of the particular species.

In some cases, dietary fat plays no role in the composition of deposit fat. Shorland[4] has pointed out that the composition of beef and mutton tallow is substantially unaffected by the nature of the dietary fat. It is proposed to designate this type of fat as *heterolipids*, to indicate its lack of resemblance to dietary fat. On the other hand, most animal fats are subject to a modification in composition as a result of the dietary fat. These fats are referred to as *homolipids*, which term connotes that they readily incorporate

[7] T. P. Hilditch, note to a letter of F. B. Shorland, *Nature, 170,* 925–926 (1952).
[8] G. A. Garton, T. P. Hilditch, and M. L. Meara, *Biochem. J., 50,* 517–524 (1952).
[9] E. G. Brooker and F. B. Shorland, *Biochem. J., 46,* 80–85 (1950).
[10] S. S. Gupta and T. P. Hilditch, *Biochem. J., 48,* 137–146 (1951).

dietary fatty acids into their structure. While most animal fats belong to this latter group, some, such as cow's milk fat, are of an intermediate type; the composition of this type of fat is not readily altered by the nature of the dietary fat. However, by feeding certain oils, such as rapeseed oil, it is possible to incorporate small proportions of erucic acid into the milk fat.

There are many instances in which the effect of diet on homolipid fats has been followed. When an unusually large proportion of fat is given in the diet, it will not all be metabolized, but will be laid down in the tissues, largely in its original form. If this resembles the normal body fat of the particular species involved, then the only result may be that the total proportion of fat in the tissues is increased. On the other hand, if some of the ingested fatty acids are foreign to the body, they may be absorbed and deposited in the fat depots in sufficient amount to reveal the effect of the food fat on the body fat. The higher the concentration of the fat in the diet, the shorter the period which will be required for the storage fat to be altered.

The fats in the several tissues have different rates of turnover; this explains why a substitution of a new fat may occur in one tissue before it occurs in another tissue. Thus, the blood and liver lipids are the most susceptible to alteration resulting from the fats consumed, since they are primarily concerned with fat transport and fat oxidation. In contradistinction to the rapid interchange of food fats with those of the blood and liver, the storage fats are much more slowly altered by dietary means. However, the nature of the fats in the fat depots may also be greatly changed before any appreciable alteration obtains in brain lipids.[11,12] The sluggishness of the brain in altering its fat composition may be related to the inability of this tissue to oxidize fat or ketone bodies. For a more complete exposition of the relationship of diet to fat deposition, the reader is referred to the monograph of Leathes and Raper,[13] as well as to the more recent reviews of Anderson and Williams,[14] and of Burr and Barnes.[15]

The classical experiments on the interplay of dietary and body fats are those of Lebedeff.[16] This investigator fasted two dogs until each had lost

[11] R. G. Sinclair, *J. Biol. Chem.*, *86*, 579–586 (1930).

[12] K. P. McConnell and R. G. Sinclair, *J. Biol. Chem.*, *118*, 131–136 (1937).

[13] J. B. Leathes and H. S. Raper, *The Fats*, 2nd ed., Longmans, Green, London-New York, 1925.

[14] W. E. Anderson and H. H. Williams, *Physiol. Revs.*, *17*, 335–372 (1937).

[15] G. O. Burr and R. H. Barnes, *Physiol. Revs.*, *23*, 256–278 (1943).

[16] A. Lebedeff, *Centr. Med. Wissensch.*, *20*, 129–130 (1882); *Arch. ges. Physiol. (Pflüger's)*, *31*, 11–59 (1883).

30 to 40% of its original body weight. The animals were then fed rations high in specific fats until they had regained their original body weight. In the case of one dog which was fed linseed oil, the resulting body fat would not solidify at 0°C., although dog fat normally melts at between 30 and 42°C. In the case of the second dog, which received mutton fat, the deposited fat melted above 50°C. and yielded a fat "which was almost identical with mutton tallow." Munk[17] performed a similar experiment in which the dietary fat consisted of a high proportion of rapeseed oil. This investigator was able to isolate from the dog fat considerable quantities of erucic acid, which is the principal fatty acid in rapeseed oil, and which is ordinarily absent from animal fat; he correctly postulated that the glyceride of the food fat had been deposited in the tissues.

The above experiments, which may be classed as the extreme type, illustrate the profound effect which dietary fat may exert upon the composition of storage fat. However, more recent work proves that a similar replacement of normal body fat by specific food fats may occur without the drastic procedure of first removing most of the animal fat by a prolonged fast period. The more recent data show that a gradual substitution of the new fat occurs if a specific type of diet is continued over a rather extended period.

a. The Effect of Dietary Fats Containing Short-Chain Acids on the Composition of Storage Fat. A number of workers[18–22] have reported that when such fats as butter and coconut oil, which have a considerable content of relatively short-chain acids, are fed to animals, the iodine value of the depot fat is somewhat reduced. Although this change might indicate that a deposition of saturated short-chain acids was taking place in the storage fat, neither Leube[23] nor Zuntz,[24] who fed butter fat, nor Lebedeff,[25] who fed tributyrin, could detect any increase in the volatile fatty acids in depot fat. Eckstein,[26] using rats, and Davis,[27] experimenting with chickens, have reinforced our evidence that the butyryl radical (C_4) cannot be de-

[17] I. Munk, *Arch. pathol. Anat. u. Physiol. (Virchow's)*, *95*, 407–467 (1884).
[18] V. Henriques and C. Hansen, *Skand. Arch. Physiol.*, *11*, 151–165 (1901).
[19] V. Henriques and C. Hansen, *Oversigt kgl. Danske Videnskab. Selskabs Forh.*, *1899*, *No. 4*, 333–372.
[20] W. Lummert, *Arch. ges. Physiol. (Pflüger's)*, *71*, 176–208 (1898).
[21] J. König and J. Schluckebier, *Z. Untersuch. Nahr. u. Genussm.*, *15*, 641–661 (1908).
[22] H. D. Gibbs and A. Agcaoili, *Philippine J. Sci.*, *5*, Sect. A, 33–43 (1910).
[23] W. Leube, *Verhandl. Kongr. inn. Med.*, *13*, 418–432 (1895).
[24] N. Zuntz, *Z. Untersuch. Nahr. u. Genussm.*, *4*, 126–127 (1901).
[25] A. Lebedeff, *Z. physiol. Chem.*, *6*, 139–154 (1882).
[26] H. C. Eckstein, *J. Biol. Chem.*, *81*, 613–628 (1929).
[27] R. E. Davis, *J. Biol. Chem.*, *88*, 67–75 (1930).

posited in the tissues. The same situation also obtains in the case of the caproyl radical (C_6),[28] and likewise of the caprylyl group (C_8).[29]

Powell reported, however, that capric acid (C_{10}) was laid down to the extent of 15% in the storage fat after the administration of tricaprin,[30] while trilaurin (C_{12}) is likewise capable of being stored. In her tests, Powell[29] proved that the saponification number of the deposited fat was increased from 199 to 218 by the administration of trilaurin, which would indicate that approximately 25% of the short-chain acids were present in the storage depots. Channon *et al.*[31] were able to produce somewhat similar results in rats by the administration of a diet containing 40% of coconut oil. After twenty-one days, the depot fats contained 1.2% of capric acid (C_{10}), 17.2% of lauric acid (C_{12}), and 13.8% of myristic acid (C_{14}). Longenecker[32] confirmed these results in rats fed high coconut oil rations for fourteen days. The depot fat was found to contain as much as 31.8 mole per cent of lauric acid (C_{12}) and 18.1 mole per cent of myristic acid (C_{14}), as contrasted with a palmitic acid (C_{16}) content of 18.6 mole per cent. Although myristic acid ordinarily comprises only 1% or less of the total fatty acids in the rat,[32] the results obtained by Channon *et al.*[31] and by Longenecker[32] confirm the earlier results of Eckstein,[26] which indicated that a large amount of myristic acid was laid down in the tissue lipids after its administration. Lovern[33] also reported that the administration of ethyl myristate to eels was followed by an increased tetradecenoic acid content, in the eel fat, but that no increase in myristic acid obtained. However, an increase in both palmitic and hexadecenoic acids occurred in the eel body fat after the administration of ethyl palmitate.

b. The Effect of Fats Containing Fatty Acids with an Odd Number of Carbon Atoms on the Composition of Storage Fat. Natural vegetable and animal fats invariably contain only fatty acids having an even number of carbon atoms. With the exception of the formic acid residue and that of propionic acid (C_3), the odd-carbon fatty acids can largely be considered as unphysiological compounds. Whereas the even-chain fatty acids give rise to ketone bodies,[34] and no glycogen deposition follows their administration,[35]

[28] H. C. Eckstein, *J. Biol. Chem.*, *84*, 353–357 (1929).
[29] M. Powell, *J. Biol. Chem.*, *89*, 547–552 (1930).
[30] M. Powell, *J. Biol. Chem.*, *95*, 43–45 (1932).
[31] H. J. Channon, G. N. Jenkins, and J. A. B. Smith, *Biochem. J.*, *31*, 41–53 (1937).
[32] H. E. Longenecker, *J. Biol. Chem.*, *130*, 167–177 (1939).
[33] J. A. Lovern, *Biochem. J.*, *34*, 704–708 (1940).
[34] H. J. Deuel, Jr., L. F. Hallman, J. S. Butts, and S. Murray, *J. Biol. Chem.*, *116* 621–639 (1936).
[35] H. J. Deuel, Jr., J. S. Butts, L. F. Hallman, and C. H. Cutler, *J. Biol. Chem.*, *112*, 15–23 (1935).

the odd-carbon fatty acids form no ketone bodies, or limited amounts of the latter.[34,36,37] Moreover, the acids can be transformed to glycogen to the extent that they are converted to propionic acid.[35]

It is therefore quite suprising to find that triundecylin (C_{11}) can be readily deposited in the fat storage depots of the rat. Visscher[38] reported that as much as 24% of the fatty acids present in the tissues consisted of undecylic acid, after triundecylin had been fed to the rats over a six-week period.

Appel, Böhm and associates, also,[39] have reported that the saturated odd-carbon acids from C_{11} to C_{19}, which occur in the fat synthesized from paraffin, are stored in depot fat as the corresponding C_9 to C_{10} unsaturated acids. Only minimum amounts of the branched-chain acids were found to be deposited in animal fats, the greater amount being excreted in the urine as C_6 to C_{10} dicarboxylic acids.

c. The Effect of Unsaturated Fats in the Diet on the Composition of Storage Fat. (*a*) *Oleic Acid.* Oleic acid, $CH_3(CH_2)_7CH{:}CH(CH_2)_7$-COOH, is the most important unsaturated fatty acid found in animal as well as in vegetable fats, both from a qualitative and from a quantitative standpoint. The concentration of this fatty acid in the tissue fats is less influenced by diet than is that of the other unsaturated acids. This is probably to be ascribed to the fact that tissue oleic acid possesses a dual origin. In the first place, along with many other fatty acids, oleic acid in the fat depots may represent that originating from the diet. Secondly, oleic acid may be synthesized from saturated fatty acids which, in turn, may be derived from carbohydrate and protein.

Schoenheimer and Rittenberg[40-42] have shown that the body is able to desaturate stearic acid and other saturated acids to produce unsaturated acids such as oleic. In fact, the reverse reaction may also take place in the tissues; this may indicate that an equilibrium exists which is able to control the proportion of oleic acid laid down in body fat. On the other hand, the animal body is unable to produce a dienoic acid either from a monoethenoid acid or from a saturated acid,[43] although Reiser[44] has shown that such acids may originate from more highly unsaturated ones. In

[36] J. S. Butts, C. H. Cutler, L. F. Hallman, and H. J. Deuel, Jr., *J. Biol. Chem.*, *109*, 597–613 (1935).

[37] E. M. MacKay, A. N. Wick, and C. P. Barnum, *J. Biol. Chem.*, *136*, 503–507 (1940).

[38] F. E. Visscher, *J. Biol. Chem.*, *162*, 129–132 (1946).

[39] H. Appel, H. Böhm, W. Keil, and G. Schiller, *Z. physiol. Chem.*, *282*, 220–244 (1947).

[40] R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, *113*, 505–510 (1936).

[41] R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, *114*, 381–396 (1936).

[42] D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, *121*, 235–253 (1937).

[43] K. Bernhard and R. Schoenheimer, *J. Biol. Chem.*, *133*, 707–712 (1940).

[44] R. Reiser, *Arch. Biochem. Biophys.*, *32*, 113–120 (1951).

view of the fact that stearic acid can readily be synthesized *in vivo* from carbohydrate (see page 538), it is obvious that the animal body is not dependent upon the dietary oleic acid for supplying the needs of this monoethenoid acid in tissue lipids.

(*b*) *Elaidic Acid.* Elaidic acid, *trans*-9,10-octadecenoic acid, is the geometric isomer of oleic acid. Unlike the latter compound, elaidic acid is never found normally in animal or vegetable fats. In contradistinction to oleic acid and trioleins, which are liquids at ordinary temperatures, elaidic acid and its triglyceride, trielaidin, are solids at room temperature, although they melt at temperatures only slightly above that of the body, *i.e.*, at 43.7°C. and 42°C., respectively. The elaidinization reaction, which is a reversible one in the laboratory, can readily be brought about by the use of mercurous nitrate, nitrous acid, nitrous oxide, sulfurous acid and selenium. For a further discussion of this reaction, see Volume I, pages 85 and 86.

In the case of the animal body, neither the process of elaidinization nor the reverse reaction can be effected. Barbour[45] reported that "isoöleic acid," which is present in hydrogenated cottonseed oil, is deposited in the adipose tissues of the rat, and is eventually utilized as fuel for the animal body in a similar manner, and to the same extent, as are other dietary fatty acids. Although the exact composition of the so-called isooleic acid is uncertain, Barbour suggests that it consists largely of elaidic acid, also of some $\Delta^{12,13}$-acid, resulting from the partial hydrogenation of linoleic acid, as well as still other isomers of oleic acid.

Sinclair[46] was the first to use pure elaidic acid and trielaidin for following the intermediary fat metabolism. These compounds behaved in exactly the same manner as normal fatty components. The course of the intermediary metabolism of elaidic acid could be followed, since its properties allowed it to be readily differentiated from the other fatty acids. It was found that, when elaidic acid was fed to normal growing rats, it made up one-third of the total fatty acids in the phospholipids of the liver and skeletal muscle. On the other hand, when it was administered to adult rats on ordinary diets, elaidic acid was shown to replace 25 to 30% of the natural fatty acids. The saturated acids were the ones largely replaced. In later studies, McConnell and Sinclair[12] were able to demonstrate that elaidic acid could be introduced to the extent of 30% into the lecithin and cephalin fractions of the rat. Kohl[47] confirmed the fact that elaidic acid is

[45] A. D. Barbour, *J. Biol. Chem.*, *101*, 63–72 (1933).
[46] R. G. Sinclair, *J. Biol. Chem.*, *111*, 515–526 (1935).
[47] M. F. F. Kohl, *J. Biol. Chem.*, *126*, 709–719 (1938).

laid down in the adipose tissues, as well as in the phospholipids. He has also shown that the elaidic acid is apparently utilized, as it gradually disappears from the fat stores.

(c) *Linoleic Acid.* Linoleic acid, $CH_3(CH_2)_4CH:CHCH_2CH:CH(CH_2)_7COOH$, is one of the essential fatty acids. It cannot be synthesized in the body,[43] and that present in the fat stores must have been obtained from the diet. When linoleic acid is fed to the pig, large amounts may be retained.[48,49] Anderson and Mendel[49] reported that, when 60% of the calories of the diet came from unsaturated fats, the iodine number of the storage fat of rats approximated that of the dietary fat. Even when more saturated fats such as lard or Crisco were fed, there was a selective absorption of the unsaturated acids, so that the body fat was more unsaturated than the dietary fat.

Although no linoleic acid could be demonstrated in the adipose tissue of the rat on a low-fat diet, Spadola and Ellis[50] were able to detect it in the tissues when it was present in the diet to the extent of only 0.2%. When cottonseed oil was present to the extent of 8% in the diet, linoleic acid made up 27.3% of the component acids. However, under these circumstances, the amount of palmitoleic acid in the fat was markedly suppressed. When a low-fat diet was employed, palmitoleic acid made up 14.0% of the total fatty acids of the adipose tissue, while linoleic acid was absent. In the test cited above, in which linoleic acid comprised 27.3% of the total fatty acids, the proportion of palmitoleic acid was reduced to 2.0%.

More recently, Longenecker demonstrated that the storage fat of rats contained 2.2 mole per cent of linoleic acid when a low-fat diet[51] was given, but that this was increased to 18.6 mole per cent on a 5% oil diet,[51] and to 32.3 mole per cent on a diet containing a high proportion of corn oil.[52] When coconut oil was the dietary fat,[32] the molar percentage of linoleic acid was reduced to 1.2. Ellis and collaborators[53] have shown that, whereas the back fat of the pig contained 8.6% of linoleic acid when a low-fat diet was given, the corresponding amount of linoleate was increased to 14.6%, after the ingestion of 4% of cottonseed oil, to 18.6% when 8% of cottonseed oil was included in the diet, and to 26.8% when the diet contained 12% of cottonseed oil.

(d) *Linolenic Acid.* Linolenic acid is 9,12,15-octadecatrienoic acid.

[48] N. R. Ellis and O. G. Hankins, *J. Biol. Chem.*, *66*, 101–122 (1925).
[49] W. E. Anderson and L. B. Mendel, *J. Biol. Chem.*, *76*, 729–747 (1928).
[50] J. M. Spadola and N. R. Ellis, *J. Biol. Chem.*, *113*, 205–218 (1936).
[51] H. E. Longenecker, *J. Biol. Chem.*, *128*, 645–658 (1939).
[52] H. E. Longenecker, *J. Biol. Chem.*, *129*, 13–22 (1939).
[53] N. R. Ellis, C. S. Rothwell, and W. O. Pool, Jr., *J. Biol. Chem.*, *92*, 385–398 (1931).

This fatty acid has been reported to be widely distributed in the fat of various species of animals. Cruickshank[54] alone, and with associates,[55] proved that linolenic acid is readily deposited in egg yolk. Reiser[56] also noted the presence of this trienoic acid in the neutral fat of the carcass of growing chicks after isomerized linolenate and fats containing linolenic acid had been fed. However, Chu and Kummerow[57] reported that linolenic acid was present only in skin lipids, and not in liver lipids, after 6, 12, or 25% of linseed oil had been included in the diets. Skin lipids contained 25–29% of linolenic acid, irrespective of the level of linseed oil fed. These findings are not in accordance with those of Matsubara,[58] who observed that both linoleic and linolenic acids were present in liver and also in muscle lipids of pigeons after the feeding of linseed oil. They were largely present in the phospholipid fraction.

Linolenate may occur in the depot fat of the mammals, as well as in the fowl. Beadle and associates[59] demonstrated that, after a diet of highly unsaturated fats has been fed, as much as 27.6% of the fatty acids present in rats, and 11.4% of those composing pig fat, may consist of trienoic acids, of which linolenic acid is the principal acid. This compound has likewise been reported in the abdominal fat[60] of the horse to the extent of 4.48%, as well as in the body fat of this animal,[61] in a 1.69% concentration. Gupta and Hilditch[10] confirmed the positive findings of Brooker and Shorland,[9,62] and of Crowell,[63] who found linolenic acid present in the mesenteric fat of the horse. On the other hand, Holmberg and Rosenqvist[64] reported contradictory results so far as linolenate is concerned; they noted a higher proportion of linoleic acid. These differences are probably to be ascribed to the diet of the horses previous to slaughtering. Eckstein[65] found that linolenic acid is a normal constituent of human fat; on the other hand,

[54] E. M. Cruickshank, *Biochem. J.*, *28*, 965–977 (1934).

[55] E. M. Cruickshank, J. Houston, and T. Moore, *Biochem. J.*, *33*, 1630–1634 (1939).

[56] R. Reiser, *J. Nutrition*, *42*, 325–336 (1950).

[57] T. K. Chu and F. A. Kummerow, *Poultry Sci.*, *29*, 846–851 (1950).

[58] K. Matsubara, *J. Biochem. (Japan)*, *36*, 17–41 (1944).

[59] B. W. Beadle, O. H. M. Wilder, and H. R. Kraybill, *J. Biol. Chem.*, *175*, 221–229 (1948).

[60] H. A. Schuette, T. M. Garvin, and E. J. Schwoegler, *J. Biol. Chem.*, *107*, 635–639 (1934).

[61] A. Heiduschka and A. Steinruck, *J. prakt. Chem.*, *102*, 241–266 (1921).

[62] F. B. Shorland, *J. New Zealand Inst. Chem.*, *13*, 5–20 (1949); cited by E. G. Brooker and F. B. Shorland, *Biochem. J.*, *46*, 80–85 (1950).

[63] G. K. Crowell, *J. Assoc. Offic. Agr. Chemists*, *27*, 448–451 (1944).

[64] J. Holmberg and U. Rosenqvist, *Svensk. Kem. Tid.*, *61*, 89–97 (1949); *Chem. Abst.*, *43*, 6839 (1949).

[65] H. C. Eckstein, *J. Biol. Chem.*, *64*, 797–806 (1925).

Wagner[66] failed to confirm this fact. It is altogether possible that this discrepancy may have been related to the diet of the two individuals before death.

(*e*) *Elaeostearic Acid.* Elaeostearic acid, 9,11,13-octadecatrienoic acid, is a conjugated trienoic acid, present in high concentration in tung oil and in other tropical plants. According to Burr *et al.*,[67] purified α-elaeostearic acid is ineffective as a source of essential fatty acids. Therefore it cannot be converted to the corresponding non-conjugated trienoic acid, linolenic acid, *in vivo*. If this were the case, it would cure essential fatty acid deficiency, just as linolenic acid does.

Miller and Burr[68] reported that α-elaeostearic acid may be deposited in the tissues of rats. However, the acid rapidly undergoes a transformation to dienoic acids. Cruickshank *et al.*[55] likewise demonstrated that elaeostearic acid may be deposited in the tissue lipids of hens. Reiser[44] proved that trienoic acids continue to be excreted in the egg yolks for as long as twelve days after conjugated linolenic acid has been administered to hens in 18 g. doses divided over a three-day period. Although the unsaturated acids were present in both the neutral fat and the phospholipid fraction, they were in a higher concentration in the latter. Moreover, when α-elaeostearic acid in the form of tung oil was fed to laying hens in amounts as high as 6 g., a slow rise was noted in the trienoic acid content of the egg-yolk fat, which reached the maximum in five days. In these tests also, the greater concentration of the unsaturated acids was noted in the phospholipid fraction.

(*f*) *Arachidonic Acid.* Arachidonic acid, 5,8,11,14-eicosatetraenoic acid, is widely distributed in animal fats, but it does not occur in the plant kingdom. It is present in the liver,[69,70] where it is the only highly unsaturated acid,[71] and in the brain, in which case it has been shown to occur in both the lecithin[72] and the cephalin[72,73] fractions. The wide distribution of this acid in tissues has been demonstrated by Wesson,[74] who demonstrated its occurrence in the liver of rats and in the liver, pancreas, kidney, lung, spleen, lymph glands, and muscle fat of dogs. Brown[75] reported the

[66] O. Wagner, *Biochem. Z.*, *174*, 412–419 (1926).
[67] G. O. Burr, M. M. Burr, and E. S. Miller, *J. Biol. Chem.*, *97*, 1–9 (1932).
[68] E. S. Miller and G. O. Burr, *Proc. Soc. Exptl. Biol. Med.*, *36*, 726–729 (1937).
[69] R. H. Snider and W. R. Bloor, *J. Biol. Chem.*, *99*, 555–573 (1933).
[70] P. A. Levene and H. S. Simms, *J. Biol. Chem.*, *48*, 185–196 (1921); *51*, 285–294 (1922).
[71] J. B. Brown, *J. Biol. Chem.*, *80*, 455–460 (1928).
[72] P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *54*, 91–98, 99–100 (1922).
[73] L. G. Wesson, *J. Biol. Chem.*, *60*, 183–187 (1924).
[74] L. G. Wesson, *J. Biol. Chem.*, *65*, 235–250 (1925).
[75] J. B. Brown, *J. Biol. Chem.*, *83*, 777–782 (1929).

presence of arachidonic acid in thyroid, spleen, and suprarenal lipids, in which it comprised 0.4, 4.0, and 5.5% of the total fatty acids. However, the highest concentration of this C_{20}-unsaturated acid is in the suprarenal glands, in which it makes up 11.2% of the total fatty acids[76] and as much as 22% of the suprarenal phospholipids.[77,78] According to Holman,[79] the best practical source of arachidonic acid in animal tissue is bull testicle.

Arachidonic acid is presumably a component of most animal fats. The acid has been shown to be present to the extent of 2.2% in the rat,[51] and 2.0% in pig depot fat.[80,81] It has likewise been found to occur in the fat of fowl (duck, goose, chicken),[82] of the dog (as cited earlier),[74] and of man.[65,66,83] Small amounts of arachidonic acid have been reported in egg lecithin[84] and in butterfat.[85]

When weanling rats were kept on a fat-free diet, the arachidonate content was found by Longenecker[51] to be extremely low (0.2–0.3%). On the other hand, when the diet contained a source of arachidonate (dog chow), considerable amounts of this acid were observed (2.2%). When these animals were fasted, the arachidonate was spared to a considerable extent. Smedley-MacLean and Hume[86] reported very little change in the arachidonate content of the fat-free dry weight of rats kept on a fat-free diet for from six to eleven months. It was very low in the subcutaneous tissue, and only after ten weeks of dosing with methyl arachidonate was a definite increase in the arachidonate content noted in this tissue. However, an immediate increase occurred in the liver when curative doses of this acid were given. When large Walker tumors were implanted in rats, and the animals were maintained on a fat-free diet over a period of 97 days, a marked decrease was noted in the highly unsaturated acid in the subcutaneous fat, but not in the carcass fat.[87] It has been suggested that the arachidonate is reduced when new tissue is being formed; however, its constant value when new tissue is not being laid down indicates that it is not required for the normal maintenance of the cell.[86]

[76] J. B. Brown, unpublished data, cited by W. C. Ault and J. B. Brown, *J. Biol. Chem.*, *107*, 607–614 (1934), p. 607.
[77] W. C. Ault and J. B. Brown, *J. Biol. Chem.*, *107*, 607–614 (1934).
[78] G. Y. Shinowara and J. B. Brown, *J. Biol. Chem.*, *134*, 331–340 (1940).
[79] R. T. Holman, personal communication to the author, 1953.
[80] H. K. Dean and T. P. Hilditch, *Biochem. J.*, *27*, 1950–1956 (1933).
[81] A. Banks and T. P. Hilditch, *Biochem. J.*, *26*, 298–308 (1932).
[82] J. B. Brown and C. C. Sheldon, *J. Am. Chem. Soc.*, *56*, 2149–2151 (1934).
[83] D. L. Cramer and J. B. Brown, *J. Biol. Chem.*, *151*, 427–438 (1943).
[84] P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *51*, 507–513 (1922).
[85] A. W. Bosworth and E. W. Sisson, *J. Biol. Chem.*, *107*, 489–496 (1934).
[86] I. Smedley-MacLean and E. M. Hume, *Biochem. J.*, *35*, 990–995 (1941).
[87] I. Smedley-MacLean and E. M. Hume, *Biochem. J.*, *35*, 996–1002 (1941).

(*g*) *Miscellaneous Fatty Acids.* Winternitz[88] demonstrated, more than fifty years ago, that iodized fats can be deposited in fat storage depots. Artom and Peretti[89] reported that fatty livers resulted after the feeding of iodized natural fats. Zummo[90] obtained a similar response with brominated ethyl oleate, while Artom and Swanson[91] reported comparable results with several brominated esters. It has also been found that erucic acid, 13,14-docosenoic acid, is incorporated in the adipose tissue of the dog when rape-seed oil is fed in large proportion to the previously fasted dog.[4] Garton, Hilditch, and Meara[8] showed that the characteristic unsaturated fatty acids of whale oil are laid down in the fat depots when a diet rich in whale oil is fed to pigs. They observed that the whale oil glycerides were deposited principally in the outer back fat, and also, to the extent of 26%, in the perinephric fat. When the diet included 50% of the whale oil, it appeared that the glycerides of this fat had been absorbed and deposited in the body fat without any chemical alteration.

Arachidic acid, $CH_3(CH_2)_{18}COOH$, which is not a normal component of animal fats, has been reported by Ellis and Isbell[92] in hog fat obtained from animals which had previously been fed on large amounts of peanuts. This fatty acid obviously had its origin in the peanut oil fed, since this oil contains a high percentage of the acid. On the other hand, arachidonic acid, a tetraenoic C_{20} acid, is found only in animal tissues; thus it must be synthesized there. However, it is probable that the dienoic or trienoic acids serve as building stones for this compound.[44]

d. The "Soft Pork Problem" in Relation to the Ingestion of Unsaturated Fats. Considerable interest has been evinced as to the laws which govern the laying down of adipose tissue, because of the widespread occurrence of the so-called "soft pork." For some time during the twenties, a considerable proportion of hogs which were slaughtered contained a fat graded as "oily" or "soft," as contrasted with the usual grade classed as "hard." In some carcasses, the fat exhibited such a lack of firmness that the products presented a typical soft, flaccid, shapeless condition; this rendered them inconvenient to handle, as well as unattractive to customers. In extreme cases, the meat cuts were practically unsalable. The whole problem has been one of great economic importance. An understanding of the causes of this phenomenon has been obtained from the research of

[88] H. Winternitz, *Z. physiol. Chem.*, *24*, 425–448 (1898).
[89] C. Artom and G. Peretti, *Arch. intern. physiol.*, *36*, 351–370 (1933).
[90] C. Zummo, *Arch. sci. biol.* (*Italy*), *24*, 162–168 (1938).
[91] C. Artom and M. Swanson, *Federation Proc.*, *1*, 99 (1942).
[92] N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, *69*, 219–238, 239–248 (1926).

Ellis and his group[48,50,53,92–95] at the U. S. Department of Agriculture, as well as from the investigations of Anderson and Mendel.[49]

(*a*) *The Relationship of the Linoleate Content to the Type of Fat Ingested.* Ellis and Isbell[92] reported that the lards from hogs fed on a corn + skim milk ration (4.5% fat) or on a brewer's yeast + tankage ration (1% fat) were "hard," and had iodine numbers of 59 and 53, respectively. In contradistinction to these results, the fat from hogs on a peanut diet was "soft," while that from hogs receiving soybeans was graded as "oily" and, in one case, had an iodine value[92] as high as 101. The "peanut lard" appeared to resemble peanut oil much more than the "soybean lard" resembled the soybean oil.[92]

The most marked variation in composition between the "hard" and "soft" lards was in their linoleic acid content. Whereas this dienoic acid accounted for only 1.9% of the total fatty acids in the first case, the amounts were greatly increased in the "soft" types, reaching a maximum of 30.6% in one sample from a hog which had received the soybean regimen. To compensate for this change in composition, there was a marked decrease in the proportion of saturated acids; these averaged 38% of the total in the "hard" samples and only 26% in the "soft" lards. In all cases, a similar palmitic acid:stearic acid ratio of 2:1 obtained. It was concluded that the primary cause of the softening was the presence of the increased proportion of linoleate.

Ellis, Rothwell, and Pool[53] demonstrated that the deposition of linoleic acid in the tissues was governed by different laws when cottonseed oil was the food fat than when corn or soybean oils were ingested. In the first case, the increase in the amount of linoleate which appeared in the hog fat, as the proportion of cottonseed oil in the diet was augmented, was at the expense of the oleic acid fraction; the saturated fats remained practically constant. When increasing linoleate deposition occurred with higher concentrations of corn oil, the oleic acid remained constant but the saturated acids dropped proportionally. Spadola and Ellis[50] found that the increased linoleate laid down in rats when cottonseed oil was fed was at the expense not only of the oleic acid but also of the palmitoleic acid, which comprises an important fraction of the unsaturated acid in this species. Practically no difference in total saturated acids obtained between rats which received a fat-low diet (saturated acids, 37.4%; linoleate, 0.0%)

[93] O. G. Hankins and N. R. Ellis, *U. S. Dept. Agric., Dept. Bull. No. 1407*, 1–68 (April, 1926).

[94] O. G. Hankins, N. R. Ellis, and J. H. Zeller, *U. S. Dept. Agric., Dept. Bull. No. 1492*, 1–50 (Feb., 1928).

[95] N. R. Ellis, *U. S. Dept. Agric. Tech. Bull. No. 368*, 1–14 (July, 1933).

and those on 8% of cottonseed oil (saturated acids, 39.6%; linoleate, 27.3%).

(*b*) *The Hardening Effect of Cottonseed Oil.* It has been beautifully demonstrated by Ellis and Isbell[92] and by Ellis, Rothwell, and Pool[53] that cottonseed oil exerts a marked hardening effect on the storage fat of the pig when fed at a 4% level in the diet. This is in contradistinction to the behavior of corn, soybean, and peanut oils, which produce a definite softening of the lard when administered at this level. Other workers[96,97] had also previously shown that the lard produced by hogs on a ration of cottonseed meal was firm and had a high melting point, while a decreased proportion of the volatile fatty acids was noted in butterfat after a cottonseed meal, or when cottonseed oil was present in the diet.[98] Table 1 illustrates the effects of the several types of dietary fat on the content of unsaturated acids in the lard, while Table 2 shows the effect of progressively increasing amounts of cottonseed oil on the composition of the lard, in both saturated and unsaturated acids.

TABLE 1

THE EFFECT OF THE VEGETABLE OIL IN THE DIET ON THE FIRMNESS AND COMPOSITION OF LARD[a]

Supplement added to corn and tankage diet	Firmness of lard	Iodine number of lard	% unsatd. acids in lard: Total satd. acids	Oleic acid	Linoleic acid
Peanut oil, 4.1%	Medium soft	72.4	32.5	47.9	13.8
Soybean oil, 4.1%	Medium soft	75.7	33.8	43.3	18.6
Cottonseed oil, 4.1%	Hard	64.4	43.0	35.9	15.7
Corn oil, 4.1%	Medium soft	76.3	33.0	45.0	16.8
Corn oil, 11.5%	Oily	97.2	23.1	41.4	31.4

[a] Adapted from N. R. Ellis, C. S. Rothwell, and W. O. Pool, *J. Biol. Chem.*, *92*, 385–398 (1931).

(*c*) *Factors Affecting the Ratio of Palmitic to Stearic Acid.* In addition to the variations in linoleic and oleic acids in hog fat as related to diet, the relationship of palmitic to stearic acid is of considerable interest. Bhattacharya and Hilditch[99] have reported the analyses of a number of lards in which the palmitic acid:stearic acid ratio varied from 3:1 to 1.5:1, as

[96] H. H. Harrington and D. Adriance, *Texas Agric. Expt. Sta. (College Sta.), Bull. No. 29*, 347–355 (1893).

[97] C. L. Hare, *J. Ind. Eng. Chem.*, *5*, 410–414 (1913).

[98] C. H. Eckles and L. S. Palmer, *Missouri Agric. Expt. Sta., Research Bull. No. 27*, 3–44 (1916).

[99] R. Bhattacharya and T. P. Hilditch, *Biochem. J.*, *25*, 1954–1964 (1931).

TABLE 2
THE EFFECT OF THE AMOUNT OF COTTONSEED OIL IN THE DIET ON THE BACK FATS OF HOGS RECEIVING A BASAL DIET OF HOMINY[a]

Category	Group basal diet	Groups receiving basal diet plus 4% oil	8% oil	12% oil
Average refractive index (40°C.)	1.4591	1.4595	1.4599	1.4609
Iodine number	60.6	60.5	64.4	77.4
Saponification number	196.2	197.9	197.6	195.2
Melting point, °C.	41.7	45.7	46.3	40.9
Saturated acids:				
Total, %	39.0	45.5	44.0	39.6
Myristic, %	1.6	1.1	0.8	1.0
Palmitic, %	24.3	24.1	20.9	13.2
Stearic, %	13.0	20.3	22.3	25.3
Unsaturated acids:				
Total, %	56.4	50.9	53.6	55.8
Oleic, %	47.9	38.2	34.2	30.4
Linoleic, %	8.5	12.7	17.4	25.4

[a] Adapted from N. R. Ellis, C. S. Rothwell, and W. O. Pool, *J. Biol. Chem.*, *92*, 385–398 (1931), pp. 392–393.

compared to a value of approximately 2:1 in the hogs on the several diets other than cottonseed oil. Hilditch[100] likewise reported that palmitic acid is a more important saturated acid in beef tallow than is stearic acid, although in mutton tallow a reverse relationship obtains. The importance of palmitic acid in the fat of rats has also been noted by Banks *et al.*[101]

(*d*) *The Hardening Effect of Carbohydrates.* It has long been known that much of the body fat deposited under normal conditions is derived from carbohydrate (see page 538). As the results of Ellis and Isbell[92] demonstrated, fat from hogs on a low-fat diet tends to be hard and to have an iodine value approximating 60. Ellis[95] has shown that, when a low-fat diet (corn) was substituted for a peanut ration, a marked alteration in the type of adipose tissue ensued. The fat changed from the "soft" type to the "hard" type, coincident with a reduction of the linoleic acid content from 23 to 9%. This was reflected in the change of the iodine number, which decreased from 93 to 63 during the corn diet. Saturated acids increased from 19 to 33% during the hardening period. Anderson and Mendel[49] reported a similar result in the rat. After the deposition of the "soft" fat in this species, the fat in the diet was replaced equicalorically by corn starch. A progressive "hardening" of the fat resulted over the period

[100] T. P. Hilditch, *J. Soc. Chem. Ind.*, *54*, 139–145, 163–167, 184–189 (1935).
[101] A. Banks, T. P. Hilditch, and E. C. Jones, *Biochem. J.*, *27*, 1375–1382 (1933).

of weeks during which the replacement diet continued. The replacement of the "soft" fat by "hard" fat was accelerated if the animals were first subjected to a fasting period before the new regimen was instituted. It was reasoned that the unsaturated fatty acids were oxidized preferentially by such a procedure, which left a more saturated fat in the tissues when the corn diet was started. However, Longenecker[51] failed to demonstrate any preferential utilization of the unsaturated fatty acids in rats having a normal body fat, when these rats were fasted until they had lost 15 to 25% of their body weight.

(2) *Carbohydrates as a Source of Body Fat*

It has already been shown that the nature of the storage fat laid down in the rat or in the pig is profoundly influenced by the presence of carbohydrate in the diet. It has long been recognized that foodstuffs other than lipids can be a source of body fat. The widespread use of corn as a fattening agent for hogs is certainly not based upon its fat content, which is minimal, but rather upon its starch content, which makes up over 70% of the grain. For a discussion of the interconversion of foodstuffs, the reader is referred to the excellent review of Rapport.[102] Deuel and Morehouse[103] have also discussed the interplay between carbohydrate and fat.

a. Experimental Proof of the Conversion of Carbohydrate to Fat. The first experimental evidence of the transformation of carbohydrate to fat was obtained by Lawes and Gilbert,[104] in 1866, in England, and by Meissl and Strohmer,[105] in 1883, in Germany. By the use of balance experiments, these workers found that a considerable portion of the ingested carbon could not be accounted for in the excreta (urine, feces, and expired carbon dioxide), after large amounts of food had been given. After subtracting the carbon, which could have been retained in the stored protein (as determined from the nitrogen retained), a large balance of carbon was still unaccounted for. Since this residual carbon exceeded the carbon present in all stored carbohydrate (as determined by the analysis of the carcass), the conclusion is inescapable that this carbon moiety must have been converted to fat and stored as such. The conversion of carbohydrate to fat

[102] D. Rapport, *Physiol. Revs.*, *10*, 349–472 (1930).

[103] H. J. Deuel, Jr., and M. G. Morehouse, *Advances in Carbohydrate Chem.*, *2*, 119–160 (1946).

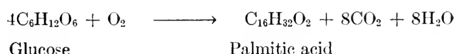
[104] J. B. Lawes and J. H. Gilbert, *Phil. Mag.* [*4*], *32*, 439–451 (London, Edinburgh and Dublin, 1866).

[105] E. Meissl and F. Strohmer, *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, 88, Abt. III*, 205–218 (1883).

as determined by balance experiments has been confirmed in the goose[106] and in the dog.[107]

A second method, also indirect, by which this thesis has been proved, is based upon the fact that an elevation of the Respiratory Quotient (R.Q., *i.e.*, volume of respiratory CO_2/volume of respiratory O_2) occurs over a considerable period following the ingestion of large amounts of carbohydrate. This phenomenon is to be observed during the interval when a conversion of carbohydrate to fat is presumably taking place. For a discussion of the R.Q. and its implications, see the review of Richardson.[108]

When carbohydrate is completely oxidized to carbon dioxide and water, the R.Q. is 1.00. However, when an oxygen-rich foodstuff such as carbohydrate is converted to an oxygen-poor foodstuff such as fat, extra oxygen is available for metabolic purposes; accordingly, less oxygen need be absorbed, and the R.Q. for this reaction will exceed 1.00. In fact, Bleibtreu,[109] as cited by Lusk[110] calculated that the theoretical R.Q. for the conversion of carbohydrate to fat is 8.00. The postulated reaction for this change is:



$$\underset{\text{Glucose}}{4C_6H_{12}O_6} + O_2 \longrightarrow \underset{\text{Palmitic acid}}{C_{16}H_{32}O_2} + 8CO_2 + 8H_2O$$

However, since a certain amount of carbohydrate continues to be completely oxidized to furnish energy, with a resultant R.Q. of 1.00, and protein must likewise be metabolized to supply the body needs (R.Q. = 0.801), the observed R.Q. of the animal during the process of fat synthesis never approaches the theoretical value of 8.00. The resultant R.Q. will be the sum of the R.Q.'s of the myriad of reactions taking place concomitantly.

According to a number of reports in the literature, the respiratory quotient under these conditions actually does exceed 1.00. Bleibtreu[109] observed a ratio of 1.33 in a goose which was stuffed with carbohydrate, although when the animal was fasted the R.Q. was only 0.73. Under the latter circumstances, fat was largely oxidized; this foodstuff has an R.Q. of 0.707. A ratio of respiratory carbon dioxide to respiratory oxygen of 1.39 has been re-

[106] K. B. Lehmann and E. Voit, *Z. Biol.*, *42* [*n.s. 24*], 619–671 (1901).

[107] M. Rubner, cited by G. Lusk, *The Elements of the Science of Nutrition*, 4th ed., Saunders, Philadelphia-London, 1928, p. 395.

[108] H. B. Richardson, *Physiol. Revs.*, *9*, 61–125 (1949).

[109] M. Bleibtreu, *Arch. ges. Physiol.* (*Pflüger's*), *85*, 345–400 (1901).

[110] G. Lusk, *The Elements of the Science of Nutrition*, 4th ed., Saunders, Philadelphia-London, 1928, pp. 206, 275, 396–398.

ported by Pembrey[111] for the hibernating marmot; Grafe[112] observed a non-protein R.Q. (total R.Q. corrected for that due to the protein moiety) of 1.31 for dogs given 300% of their daily requirement as carbohydrate. Finally, Wierzuchowski and Ling,[113] working in Lusk's laboratory, obtained an average non-protein R.Q. of 1.40 in a pig, for a period of twenty hours after the ingestion of 700 g. of starch; the maximum value noted for a single period was 1.58. It was calculated that fat was synthesized at a maximum rate of 7.1 g. per hour. During the twenty-four hour period, a total of 125 g. of fat was produced, with an average hourly production of 5.2 g. Under these unusual conditions, 27% of the metabolized starch was required to satisfy the basal metabolism, and 13% was expended for the specific dynamic action. The balance of 56% of the polysaccharide was estimated to be converted to fat.

Although it was formerly considered by some investigators that the conversion of carbohydrate to fat occurs only when the R.Q. exceeds 1.00, this concept is not held at the present time. It is certain that the formation of fat continues to take place even when the R.Q. is considerably below 1.00. Although the level of the R.Q. is without question an index of the intensity of the carbohydrate → fat reaction, this transformation will still proceed, at a slower rate, when the oxidation of carbohydrate and protein becomes the predominant reaction and thus results in a masking of the higher respiratory quotient. This dynamic concept of fat synthesis is in line with the results of Schoenheimer and Rittenberg,[41] which demonstrate that a constant and rapid exchange of fat occurs in the fat depots. Moreover, the adipose tissues offer a site for the temporary storage of the caloric reserves after heavy carbohydrate meals, when the capacity of the storage facilities for glycogen may be inadequate to store the unoxidized reserves as glycogen. In fact, making use of injected deuterium oxide, Stetten and Boxer[114] demonstrated that the major part of administered glucose which is not burned is converted directly to fat by the rat, and does not undergo a preliminary transformation to glycogen. On the other hand, in the fasted rat, which possesses considerable additional storage capacity for glycogen over that afforded in the well-fed animal, a much larger proportion of administered glucose is converted to glycogen.[115]

b. The Site of Conversion of Carbohydrate to Fat. Although one is naturally led to ascribe to the liver the function of mediating the carbo-

[111] M. S. Pembrey, *J. Physiol.*, *27*, 407–417 (1901–1902).
[112] E. Grafe, *Deut. Arch. klin. Med.*, *113*, 1–91 (1914).
[113] M. Wierzuchowski and S. M. Ling, *J. Biol. Chem.*, *64*, 697–707 (1925).
[114] De W. Stetten, Jr., and G. E. Boxer, *J. Biol. Chem.*, *155*, 231–236 (1944).
[115] G. E. Boxer and De W. Stetten, Jr., *J. Biol. Chem.*, *155*, 237–242 (1944).

hydrate → fat transformation,[116-118] Tepperman *et al.*[119] proved that it can likewise take place in extrahepatic tissues. In the case of rats, trained to ingest large amounts of a high-carbohydrate diet within a short period, the R.Q. rose to 1.2% shortly following the feeding; when evisceration was carried out immediately after the food was ingested, the resultant R.Q. was shown to be 0.95. However, when insulin was also injected, an R.Q. of 1.16 was observed. These results are interpreted to indicate that the peripheral tissues are able to convert carbohydrate to fat when a sufficient supply of carbohydrate is available.[119]

The results of the Yale group support the earlier data of Tuerkischer and Wertheimer[120] indicating that the synthesis of fat may occur in the adipose tissue itself. When fasted rats were placed on a diet rich in carbohydrate, glycogen in concentrations of 1% was found to accumulate in the adipose tissues after realimentation for periods up to four days. It is suggested by these workers not only that the adipose tissues can synthesize glycogen, but also that they can effect the synthesis of fat. These observations were further supported by the results of Henle and Szpingier,[121] who demonstrated that, in the isolated adipose tissue of the rat, the R.Q. rose above unity simultaneously with the disappearance of glucose. In an extension of this work, Mirski[122] was able to demonstrate that the adipose tissue of rats could phosphorylate glycogen to form glucose-1-phosphate, as well as to synthesize the polysaccharide from this ester. Moreover, when adipose tissue obtained from such rats was suspended in a glucose-serum medium, an R.Q. of 1.27 was found; similar adipose tissue obtained from fasted rats was also observed to exhibit an R.Q. of 1.15. These data indicate that the adipose deposit should probably not be considered as an indifferent and passive tissue but as one which can synthesize glycogen and oxidize carbohydrate, and which likewise can convert carbohydrate to fat.

c. The Mechanism of the Transformation of Carbohydrate to Fat. Up to the present time, we have no exact information as to the intermediary changes required to effect the transformation of carbohydrate to fat.

[116] T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947.

[117] W. R. Bloor, *Physiol. Revs.*, *19*, 557–577 (1939).

[118] R. Schoenheimer, *The Dynamic State of Body Constituents*, Harvard Univ. Press, Cambridge, 1942.

[119] J. Tepperman, J. R. Brobeck, and C. N. H. Long, *Yale J. Biol. Med.*, *15*, 855–874 (1943).

[120] E. Tuerkischer and E. Wertheimer, *J. Physiol.*, *100*, 385–409 (1941–1942).

[121] Henle and W. G. Szpingier, *Arch. exptl. Path. Pharmakol.* (*Naunyn-Schmiedberg's*) *180*, 672–689 (1936).

[122] E. Mirski, *Biochem. J.*, *36*, 232–241 (1942).

Lusk[110] has calculated that, even when this change is proceeding at a maximum rate, a hog weighing 13.5 kg. can synthesize a maximum of only 2 mg. of fat per second. If such a reaction is a generalized one which is taking place simultaneously throughout the organism, as most certainly must be the case, the maximum concentration of fat synthesized per second would amount to 0.15 μg. per gram of tissue. In all probability, the intermediates would not be present in any greater amount than the end-product; thus the identification of such intermediates poses an extremely difficult analytical problem.

Since the chief fatty acids which are synthesized are the C_{16} and C_{18} acids,[51,123] it would seem that the building stones should consist of a compound in which the number of carbon atoms is a common denominator of these values, namely 2. One might also postulate that three glucose molecules are converted to a C_{18} acid, which can then be degraded to a C_{16} acid. Such an *in vivo* conversion of stearic (C_{18}) to palmitic (C_{16}) acid has been demonstrated by Schoenheimer and Rittenberg[124]; the reverse change, namely the synthesis of the C_{18} acid from a C_{16} acid, has likewise been proved by these workers. One can also assume that fatty acids having a shorter chain length than C_{16} can be produced in an analogous manner. Thus, myristic acid (C_{14}) might readily be synthesized *in vivo* from palmitic acid. There is no experimental evidence to support the hypothesis that a condensation of three glucose molecules is involved in fat synthesis.

Of the possible initial building stones, the fragments with 2 carbons would seem to be the logical units. Moreover, the fact that even-numbered carbon acids shorter than C_{16} are found, especially in such sources as the milk fats, renders the process of synthesis by steps of two carbons at a time a most attractive one. In any event, a three-carbon intermediate is almost certainly ruled out, inasmuch as this would result in the production of odd-carbon atom acids in the intermediate stages. Since, practically without exception, all animal fats are composed of fatty acids with an even number of carbon atoms, the three-carbon intermediate would appear to be highly improbable.

Magnus-Levy[125] has postulated that acetaldehyde is the two-carbon intermediate in the biosynthesis of fat from carbohydrate. He has proposed that two such molecules condense in a reaction similar to the aldol

[123] H. E. Longenecker, G. Gavin, and E. W. McHenry, *J. Biol. Chem.*, *134*, 693–699 (1940).

[124] R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, *120*, 155–165 (1937).

[125] A. Magnus-Levy, cited by G. Lusk, *The Elements of the Science of Nutrition*, 4th ed., Saunders, Philadelphia-London, 1928, p. 350.

condensation to form hydroxybutyral. This condensation product is reduced to butyraldehyde; successive condensations of acetaldehyde with the new fatty aldehyde occur, followed by a reduction of the subsequent β-hydroxyl groups. When the chain has reached the desired length, oxidation of the terminal aldehyde group to an acid group occurs, yielding the typical fatty acids present in animal fats. These fatty acids can combine with glycerol (which is readily produced from glucose) to form the neutral fat.

Although there is little direct evidence that acetaldehyde may serve as the building stone for fatty acids, it is known to be a physiological compound, and has been reported to be a component of urine.[126] An especially compelling argument for acetaldehyde as an intermediate is the important role of pyruvic acid in fat synthesis. Considerable amounts of pyruvic acid are present in the organism whenever carbohydrate metabolism is proceeding at a high rate. Neuberg and Karczag[127] have proved that pyruvic acid is normally converted to acetaldehyde as a result of the action of the enzyme, carboxylase. It is now known that a coenzyme is also required, which is referred to as co-carboxylase. The coenzyme has been identified as thiamine pyrophosphate. A similar reaction occurs *in vivo*, as is indicated by the fact that, when the breakdown of pyruvic acid is prevented by the absence of the coenzyme, as is the case in thiamine deficiency, an accumulation of pyruvic acid in the blood can be demonstrated, both directly[128] and indirectly, as implied from the urinary pyruvate, especially when carbohydrate is ingested.[129,130] Since it has also been found that thiamine is necessary for fat synthesis,[123,131-133] one may well assume that thiamine acts in this capacity by allowing the reaction, pyruvic acid → acetaldehyde to proceed in a normal manner. By *a priori* reasoning, one may therefore conclude that acetaldehyde is the essential intermediate in the synthesis of fat. On the other hand, Quackenbush and associates[134] reported that other B vitamins, in addition to thiamine, are equally concerned with the fat synthesis reaction.

Additional proof that thiamine is related to fat synthesis is to be found

[126] W. Stepp and R. Feulgen, *Z. physiol. Chem.*, *119*, 72–75 (1922).
[127] C. Neuberg and L. Karczag, *Biochem. Z.*, *36*, 68–75 (1911).
[128] R. H. S. Thompson and R. E. Johnson, *Biochem. J.*, *29*, 694–700 (1935).
[129] H. A. Harper and H. J. Deuel, Jr., *J. Biol. Chem.*, *137*, 233-238 (1941).
[130] M. E. Shils, H. G. Day, and E. V. McCollum, *J. Biol. Chem.*, *139*, 145–161 (1941).
[131] D. V. Whipple and C. F. Church, *J. Biol. Chem.*, *114*, cvii–cviii (1936).
[132] E. W. McHenry, *Science*, *86*, 200 (1937).
[133] E. W. McHenry and G. Gavin, *J. Biol. Chem.*, *125*, 653–660 (1938).
[134] F. W. Quackenbush, H. Steenbock, and B. R. Platz, *J. Biol. Chem.*, *145*, 163–167 (1942).

in the experiments of Ring.[135] This investigator reported that, when glucose and thiamine were administered together, the specific dynamic action was twice that resulting from the administration of a similar dose of glucose without the vitamin. Inasmuch as no effect was noted when a like amount of thiamine was given alone or with fat, the hypothesis is suggested that the additional heat set free when thiamine and glucose are given together, as contrasted with the effect when the vitamin is omitted, is to be ascribed to the waste energy produced when carbohydrate is converted to fat.

The possibility that aldehydes may act as intermediates in the synthetic production of fats from carbohydrates is strengthened by the demonstration that the aldehydes of the higher fatty acids occur normally in the tissues. Thus, palmityl and stearyl aldehydes have been proved to be natural components of tissues.[136,137] Moreover, Möckel[138] reported that such lipoaldehydes are present in increased amounts whenever fat synthesis or degradation occurs. Smedley[139,140] postulated an aldol condensation of pyruvic acid in the synthesis of fatty acids. However, in view of our more recent knowledge of the function of carboxylase and co-carboxylase, this hypothesis would conform to the earlier one, in considering acetaldehyde as an intermediate product.

Acetic acid is another two-carbon compound which has been suggested as a possible intermediate in fat synthesis. Rittenberg and Bloch[141,142] demonstrated that C^{13} appears in the body fat when it is administered to mice in either the methyl or the carboxyl group of acetic acid. It is now a well-recognized fact that acetic acid can be formed in tissues from carbohydrates. Bloch and Rittenberg[143] have shown, by the aid of deuterium, that acetic acid can be derived from pyruvic acid. It is possible that a condensation of acetic acid molecules takes place directly, or after reduction to acetaldehyde by the mechanism originally suggested by Magnus-Levy.[125] The role of acetic acid in the synthesis of body fat, and especially of milk fat, has been reviewed by Popják[144] and by Folley.[145]

[135] G. C. Ring, *Am. J. Physiol.*, *138*, 488–490 (1942–1943).
[136] R. Feulgen, K. Imhäuser, and M. Behrens, *Z. physiol. Chem.*, *180*, 161–179 (1929).
[137] R. Feulgen and M. Behrens, *Z. physiol. Chem.*, *256*, 15–20 (1938).
[138] G. Möckel, *Z. physiol. Chem.*, *277*, 135–146 (1943).
[139] I. Smedley, *J. Physiol.*, *45*, xxv–xxvii (1912-1913).
[140] I. Smedley and E. Lubrzyńska, *Biochem. J.*, *7*, 364–374 (1913).
[141] D. Rittenberg and K. Bloch, *J. Biol. Chem.*, *154*, 311–312 (1944).
[142] D. Rittenberg and K. Bloch, *J. Biol. Chem.*, *160*, 417–424 (1945).
[143] K. Bloch and D. Rittenberg, *J. Biol. Chem.*, *155*, 243–254 (1944).
[144] G. Popják, "Fat Synthesis from Small Molecules," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Univ. Press, Cambridge, 1952, pp. 37–51.
[145] S. J. Folley, "Aspects of Fat Metabolism in the Ruminant with Special Reference to the Biosynthesis of Milk Fat," in R. T. Williams, *Lipid Metabolism*, pp. 52–65.

On the other hand, fatty acids with more than two carbons apparently cannot be incorporated into the long-chain fatty acids. Rittenberg, Schoenheimer, and Evans[146] were unable to demonstrate the presence of any deuterium in the long-chain fatty acids of rats after deuteriobutyric acid or deuteriocaproic acid had been fed. Morehouse[147] likewise proved that all the deuterium present in the rat after the feeding of deuteriotributyrin was present in the unmetabolized tributyrin temporarily present. No deuterium was demonstrable in the storage fat.

Although these two sets of observations may appear to refute each other, it is possible that the acids themselves cannot be used for further synthesis of the long-chain acids, but only their aldehydes. Although butyric acid can be converted to acetic acid,[143] the amount of such conversion may be too small to be demonstrated when the total fatty tissues are examined. For the most recent hypothesis on the chemical mechanism of fat synthesis, the reader is referred to Volume III, Chapter II.

d. The Nature of the Fat Synthesized from Carbohydrate. As has been discussed earlier, the fats laid down after a high-carbohydrate diet are hard fats composed largely of palmitic, stearic, palmitoleic, and oleic acids. Although we now know that it is possible to synthesize the monoethenoid acids from the saturated acids, there is conclusive proof that diethenoid or polyethenoid acids cannot be produced *de novo* in the animal body.

Longenecker[51] reported that the storage fat of rats contained only approximately 5% of hexadecenoic (palmitoleic) acid when they received a normal diet. On a high-carbohydrate regimen, as well as on a diet composed almost exclusively of protein, the concentration of palmitoleic acid was increased from 4 to 16%.

Schoenheimer and Rittenberg[40] demonstrated that monoethenoid acids can originate directly from the corresponding saturated acids *in vivo*. Presumably stearic acid is the parent substance for oleic acid.

From both a qualitative and a quantitative standpoint, carbohydrate is a less satisfactory source of body lipids than is fat itself. The experiments of Williams and co-workers[148] demonstrated that the amount of fat laid down, as well as the essential lipids in this fat, were distinctly inferior in rats which were sacrificed after receiving 3000 Calories contained in the particular diet under investigation, when a high-carbohydrate diet was fed as compared with a high-fat regimen. The animals were twenty-two days old when the

[146] D. Rittenberg, R. Schoenheimer, and E. A. Evans, Jr., *J. Biol. Chem.*, *120*, 503–510 (1937).

[147] M. G. Morehouse, *J. Biol. Chem.*, *155*, 33–38 (1944).

[148] H. H. Williams, H. Galbraith, M. Kaucher, and I. G. Macy, *J. Biol. Chem.*, *161*, 463–474 (1945).

feeding tests were started, and the period over which the diet was consumed averaged sixty-six days. The data are summarized in Table 3.

TABLE 3
THE EFFECT OF DIET ON THE COMPOSITION OF MALE RATS SACRIFICED AFTER RECEIVING 3000 CALORIES OF THE DIET UNDER STUDY[a]

Lipid analyzed	Control[b]	Adequate diet[c]	High-fat diet[c]	High-carbohydrate diet[c]
Calculated in % dry weight				
Total lipid	16.58	44.80	49.10	36.01
Neutral fat	8.71	40.56	45.16	32.28
Essential lipid	7.87	4.24	3.94	3.73
Calculation in % on water-free, neutral fat-free basis				
Essential lipid	8.62	7.13	7.19	5.51
Cerebrosides	1.35	2.67	2.55	1.20
Free cholesterol	0.47	0.25	0.26	0.22
Cholesterol esters	0.82	0.24	0.26	0.37
Phospholipid	5.98	3.97	4.12	3.72
Cephalin	2.73	1.41	1.77	1.49
Lecithin	2.52	2.04	1.69	1.67
Sphingomyelin	0.73	0.52	0.66	0.56

[a] Adapted from H. H. Williams, H. Galbraith, M. Kaucher, and I. G. Macy, *J. Biol. Chem.*, *161*, 463–474 (1945).
[b] Rats sacrificed at 22 days of age.
[c] Rats averaged 88 days of age.

(3) *Protein as a Source of Body Fat*

It has long been known that the nitrogen-free moiety of protein could be converted to carbohydrate. If one admits the thesis that carbohydrate can be converted to fat, one must likewise accept the hypothesis that protein can be changed to fat, carbohydrate acting as an intermediary product. There is no difference qualitatively between the glucose or glycogen arising in the animal body from protein and that which is formed after sugars or starches are ingested. The experimental proof of the transformation of protein to fat is more difficult than is that for carbohydrate. It has been investigated by the use of balance experiments, by interpretation of the data obtained from experiments on respiratory metabolism, and indirectly by the proof of the conversion of proteins and amino acids to carbohydrate.

a. Balance Experiments as Proof of the Conversion of Protein to Fat. As early as 1862, Pettenkofer and Voit,[149,150] and later Lawes and Gilbert[104]

[149] M. Pettenkofer and C. Voit, *Ann. Chem. u. Pharm.*, *2nd Suppl.*, 52–70, 361–377 (1862).
[150] M. Pettenkofer and C. Voit, *Z. Biol.*, *7*, 433–497 (1871).

and Meissl and Strohmer,[105] reported that, although all the nitrogen of the protein fed was excreted in the urine, an appreciable amount of the carbon ingested as protein failed to be accounted for in the urine or expired air, and must therefore have been converted to fat and stored in that form. The quantity of carbon retained was of such magnitude as to preclude its storage exclusively as carbohydrate.

In calculating the carbon content of the protein, in their original studies, Pettenkofer and Voit[149,150] had used the factor 3.68 for the C:N ratio. However, Rubner[151] showed that the above factor was too high when it was determined on meat completely extracted with ether; according to the latter investigator, the figure for the C:N ratio in meat protein should be 3.28:1. When the new factor was used for the calculation of carbon retention in the original Pettenkofer-Voit experiments, the carbon retained was very small, or amounted to zero. On this basis, Pflüger[152] wrote his polemic refutation of this proof of transformation of protein to fat.

TABLE 4

COMPARISON OF THE POTENTIAL GLYCOGEN FORMATION FROM PROTEIN CARBON RETAINED BY A CAT RECEIVING LEAN MEAT OVER AN 8-DAY PERIOD WITH THAT ACTUALLY FOUND ON ANALYSIS[a]

Category	Total per day		Total for 8 days	
	N	C	N	C
1. Protein metabolized (from urinary N)	13.0	41.6[b]	104.0	332.8[b]
2. Carbon excreted				
a. Urine	—	7.5	—	60.0
b. Feces	—	1.4	—	11.2
c. Respiration	—	25.4	—	203.2
d. Total	—	34.3	—	274.4
3. Carbon retained (*1* − *2d*)	—	7.3	—	58.4
4. Glycogen equivalent (*3*/0.444)[c]	—	16.4	—	131.5
5. Glycogen found	—	—	—	35.0

[a] Data adapted from M. Cremer, *Münch. med. Wochschr.*, *44*, 811 (1897); *Z. Biol.*, *38*, 309–314 (1899).
[b] Calculated on a C:N ratio of 3.2:1.
[c] Based on a value of 44.4% carbon in glycogen.

However, the subsequent results of Cremer[153] can be interpreted as offering proof of the interconversion of protein and fat. After removal of much of the storage fat from a cat by a fast continuing over a number of

[151] M. Rubner, *Z. Biol.*, *21* [*n.s. 3*], 250–334 (1885).
[152] E. Pflüger, *Arch. ges. Physiol.* (*Pflüger's*), *52*, 239–322 (1892).
[153] M. Cremer, *Münch. med. Wochschr.*, *44*, 811 (1897); *Z. Biol.*, *38* [*n.s. 20*], 309–314 (1899).

days, the animal was fed all the lean meat it would eat over an eight-day period. During this interval, the cat was kept in a respiration chamber, and the total excreta were collected. The carbon present in meat protein was calculated from the urinary nitrogen, using the low figure of 3.2, which is even lower than the ratio (3.28) obtained by Rubner.[151] At the conclusion of the test, the animal was sacrificed and the total glycogen in the tissues was determined. The essential data for the interpretation of the experiment are given in Table 4 (page 547).

Since Cremer (see Table 4) was able to account for only 35 g. of glycogen in the tissues of the cat, sacrificed after receiving 3600 g. of lean meat over an eight-day period, and since sufficient carbon was retained from the protein to give rise to 131.5 g. of glycogen, one must conclude that most of the carbon moiety of protein was stored in some form other than carbohydrate. The only possible non-protein component in the tissues other than carbohydrate which could account for this amount of carbon is fat. The conclusion is inescapable, therefore, that protein can be converted to fat in the cat. Since there is nothing particularly different in the metabolic picture of the cat from that of other mammals, one can readily accept this experiment as proving a general principle.

b. Respiration Experiments as Proof of the Conversion of Protein to Fat. Atkinson, Rapport, and Lusk[154] designed an interesting experimental procedure based upon data obtained from respiratory metabolism experiments for testing the validity of the hypothesis that protein is convertible to fat. By maintaining the glycogen reserves of an animal at a maximum as a result of a previous carbohydrate meal, these workers provided a condition which would be most favorable for determining whether the carbon moiety retained at the height of protein metabolism following a protein meal was in the form of carbohydrate or of fat. If the protein had been completely oxidized, the resultant R.Q. would have been 0.801. On the other hand, if the protein carbon retained had been in the form of carbohydrate, the stored material removed from the metabolic interchange would have had a potential R.Q. of 1.00; the actual R.Q. observed, therefore, would have been reduced below that for protein (0.801), in proportion to the quantity stored as carbohydrate.

However, if the carbon retained from protein had been stored as fat, this portion of the protein would have been removed from the metabolic interplay as a material having a potential R.Q. of 0.707. The R.Q. of the remaining portion of protein which would then be metabolized would have been increased over and above 0.801 in proportion to the quantity of re-

[154] H. V. Atkinson, D. Rapport, and G. Lusk, *J. Biol. Chem.*, *53*, 155–166 (1922).

tained material stored as fat. The results of Atkinson, Rapport, and Lusk[154] indicate that, under the conditions of their experiments, the latter situation existed. Table 5 gives a summary of the data upon which their conclusions are based.

TABLE 5

THE RESPIRATORY METABOLISM OF DOG 18 FIVE HOURS AFTER THE FEEDING OF 1000 GRAMS OF MEAT[a]

Category	CO_2, g.	O_2, g.	R.Q.	Cal.
1. Respiratory gases equivalent to 1.44 g. nitrogen (urine)[b]	13.46	12.23	0.802	38.17
2. Found in respiration	10.10	8.72	0.842	—
3. Respiratory gases equivalent in pabulum retained (*1* − *2*)	3.36[c]	3.51	0.700	11.32[d]
4. Calories produced (calculated) indirect (*1* − *3*)	—	—	—	26.85
5. Calories found (direct)	—	—	—	27.52

[a] Adapted from H. V. Atkinson, D. Rapport, and G. Lusk, *J. Biol. Chem.*, *53*, 155–166 (1922); cited by G. Lusk, *Science of Nutrition*, 4th ed., Saunders, Philadelphia, 1928.
[b] 1 g. urinary nitrogen is equivalent to 9.35 g. CO_2 and 8.49 g. oxygen.
[c] Equivalent to 0.92 g. carbon ($3.36 \times {}^{12}/_{44}$) or 1.2 g. fat (0.92/0.765).
[d] Obtained by multiplying fat carbon (0.92) by 12.32 Calories.

Atkinson *et al.*[154] calculated that, had the retained carbon been stored as carbohydrate, 2.3 g. of glucose would have been formed, with a caloric equivalent of 8.63 Calories. Under these circumstances, the calories calculated would have been 29.54 instead of 26.85 (based upon retention as fat) compared with a value of 27.54 actually determined. These calculations further support the data from the respiratory quotient in indicating a conversion of protein to fat.

Lusk[110] refers to his findings published in 1906, in the first edition of his book, in which he stated that 40% of the protein carbon which was capable of conversion into glucose could be retained either as glycogen or as fat. According to Williams, Riche, and Lusk,[155] this figure was indirectly shown to be 33% while, according to the investigations of Atkinson, Rapport, and Lusk,[154] 50% of the potential glucose was deposited as fat.

c. Proof of the Conversion of Proteins and Amino Acids to Fat, Based upon the Formation of Carbohydrate. Although the direct demonstration of the synthesis of fat arising from protein is a rather difficult one, the proof of the conversion of protein to carbohydrate is relatively easy to obtain. Since the transformation of carbohydrate to fat is likewise a readily demonstrable change and one which is generally accepted, proof of the formation

[155] H. B. Williams, J. A. Riche, and G. Lusk, *J. Biol. Chem.*, *12*, 349–376 (1912).

of carbohydrate from protein can be considered as constituting equally valid evidence for fat formation and for carbohydrate synthesis.

A number of methods can be employed for demonstrating the production of carbohydrate from protein. The most effective include the use of diabetic subjects (diabetes mellitus, pancreatic, phlorhizin, or alloxan diabetes) for quantitative determinations, estimation of the glycogen deposition (particularly in the liver), and investigation of the effect of the substance in preventing or reducing ketonuria.

(*a*) *The Formation of Glycogen or Glucose.* a′. Experiments with Protein: According to Lusk[110] Claude Bernard was of the opinion that glycogen could originate from protein. Voit[156] had a similar viewpoint, except that he believed that the protein molecule was ruptured into two main fragments without the liberation of any considerable amount of energy. One of these fragments, which was the nitrogenous portion, was rapidly oxidized, as indicated by the prompt elimination of the nitrogenous end-products in the urine. The nitrogen-free fragment, which contained the major part of the potential energy, could be temporarily stored either as carbohydrate or as fat until it was used for energy.

The first experimental demonstration of carbohydrate formation from protein was that of Wolffberg,[157] who gave carbohydrate-free meat powder to fowls previously fasted for several days to remove the glycogen. The appearance of considerable quantities of glycogen in the liver and muscles was taken as evidence of the conversion of protein to carbohydrate. Külz[158] reached a similar conclusion. In 1910, Pflüger and Junkersdorf[159] reported convincing evidence of the transformation. After having denied the validity of the proof for this change for many years, Pflüger became convinced when it was demonstrated that dogs, previously depleted of liver and muscle glycogen by a ten-day fast followed by the injection of phlorhizin, stored considerable quantities of glycogen after large amounts of codfish had been given. The liver glycogen increased from a control value of 0.1% to an average of 6.5% (maximum, 9.9%) after the ingestion of codfish, while the muscle glycogen rose from 0.2 to 1.0%. Since the codfish contained only inappreciable amounts of carbohydrate (0.03%), and since fat was without effect on the glycogen store, the only interpretation possible is that the carbohydrate originated from protein.

[156] C. Voit, *Z. Biol.*, *28* [*n.s. 10*], 245–292 (1891).

[157] S. Wolffberg, *Z. Biol.*, *12*, 266–314 (1876).

[158] E. Külz, in *Festschrift für Carl Ludwig*, Marburg, 1891, p. 83; cited by G. Lusk, *The Elements of the Science of Nutrition*, 4th ed., Saunders, Philadelphia-London, 1928, p. 206.

[159] E. Pflüger and P. Junkersdorf, *Arch. ges. Physiol.* (*Pflüger's*), *131*, 201–301 (1910).

Although Kossel[160] suggested that hexone bases and leucine might be sugar precursors, inasmuch as they contained six carbon atoms, and Müller and Seemann[161] strongly supported this hypothesis, Stiles and Lusk[162] presented the first experimental evidence by demonstrating the conversion of a protein hydrolysate to "extra sugar" when it was given to a phlorhizinized dog. Reilly, Nolan, and Lusk,[163] as well as Janney,[164] confirmed these results. It is generally agreed that a D:N ratio of approximately 3.6:1 obtains in a phlorhizinized dog after the ingestion of meat (or when fasting); this would account for a conversion of approximately 58% of protein to sugar. Many workers have confirmed these early classic demonstrations of the conversion of protein to glycogen or to glucose.

b′. Experiments with Amino Acids: The larger proportion of the amino acids is known to be convertible to carbohydrate. Of the twenty-two common amino acids, only leucine, lysine, methionine, and tryptophane have been proved to be non-glycogenic, while the evidence for the end-product is not clear in the case of hydroxylysine and phenylalanine.

Friedberg and Marshall[165] summarized the experimental work carried out on glycogenicity and ketogenicity of the amino acids. These workers indicated that, since the same amino acid may give rise to pyruvate and to acetate (which is convertible to acetoacetate), the categorization of these substances into "ketogenic" and "glycogenic" lacks meaning in the light of present knowledge of intermediary metabolism. It is suggested that an amino acid might yield both acetoacetate and glycogen but that, since these are mutually antagonistic, there would be no evidence of the formation of either metabolite in *in vivo* tests. Although this is an attractive hypothesis, the fact remains that most amino acids are primarily glycogen formers, or are convertible to acetoacetate. A list of the amino acids which are definitely glycogenic is included in Table 6 (page 552).

(*b*) *Respiration Experiments as Proof of the Conversion of Protein to Carbohydrate.* The experiments employed by Williams, Riche, and Lusk[155] to demonstrate the change of protein to carbohydrate corresponded to those previously employed in proving the formation of fat following the ingestion of protein.[154] The chief difference in the technic was that, in the first case, the glycogen storehouses were not previously charged before the administration of the test dose of protein, while in the latter case a high

[160] A. Kossel, *Deut. med. Wochschr.*, *24*, 581–582 (1898).
[161] F. Müller and J. Seemann, *Deut. med. Wochschr.*, *25*, 209–211 (1899).
[162] P. G. Stiles and G. Lusk, *Am. J. Physiol.*, *9*, 380–385 (1903).
[163] F. H. Reilly, F. W. Nolan, and G. Lusk, *Am. J. Physiol.*, *1*, 395–410 (1898).
[164] N. W. Janney, *J. Biol Chem.*, *20*, 321–350 (1915).
[165] F. Friedberg and L. M. Marshall, personal communication to the author, 1954.

carbohydrate meal was used to fill the carbohydrate depots before the test dose of protein was administered. Thus, Williams, Riche, and Lusk[155] observed an oxygen uptake in a dog, over the fourteen hours after receiving

Table 6
Glucose Formation from Various Amino Acids as Determined by Several Experimental Procedures

Amino acid	Test employed for detection of glucose formation		
	"Extra sugar" in phlorhizin dogs	Deposition of liver glycogen	Decrease in ketonuria
Alanine	a	b-d	e
Arginine	a	f	f
Aspartic acid	a	g	g
Glutamic acid	a	g	g
Glycine	a	e,h,i	e
Histidine	—	j	j
Hydroxyproline	—	k	—
Isoleucine	—	l-n	—
Norleucine	—	l	l
Proline	a	—	—
Serine	a	c,o	—
Threonine	—	o,p	p
Tryptophane	—	—	q
Tyrosine	—	r,s	s
Valine	t	—	—

a. H. D. Dakin, *J. Biol. Chem.*, *14*, 321–333 (1913).
b. C. Reid, *Biochem. J.*, *33*, 723–725 (1939).
c. F. A. Schofield and H. B. Lewis, *J. Biol. Chem.*, *169*, 373–378 (1947).
d. R. H. Wilson and H. B. Lewis, *J. Biol. Chem.*, *85*, 559–569 (1930).
e. J. S. Butts, M. S. Dunn, and L. F. Hallman, *J. Biol. Chem.*, *112*, 263–274 (1935).
f. J. S. Butts and R. O. Sinnhuber, *J. Biol. Chem.*, *140*, 597–602 (1941).
g. J. S. Butts, H. Blunden, and M. S. Dunn, *J. Biol. Chem.*, *119*, 247–255 (1937).
h. E. M. MacKay, A. N. Wick, and H. O. Carne, *J. Biol. Chem.*, *132*, 613–617 (1940).
i. W. Sakami, *J. Biol. Chem.*, *176*, 995–996 (1948).
j. L. F. Remmert and J. S. Butts, *J. Biol. Chem.*, *144*, 41–46 (1942).
k. W. C. Hess and I. P. Shaffran, *J. Am. Chem. Soc.*, *73*, 474 (1951).
l. J. S. Butts, H. Blunden, and M. S. Dunn, *J. Biol. Chem.*, *120*, 289–295 (1937).
m. L. C. Terriere and J. S. Butts, *J. Biol. Chem.*, *190*, 1-5 (1951).
n. J. J. Wirth, *Biochem. Z.*, *27*, 20–26 (1910).
o. W. C. Hess, *J. Am. Chem. Soc.*, *72*, 1407 (1950).
p. W. K. Hall, J. R. Doty, and A. G. Eaton, *Am. J. Physiol.*, *131*, 252–255 (1940).
q. R. Borchers, C. P. Berg, and N. E. Whitman, *J. Biol. Chem.*, *145*, 657–666 (1952).
r. J. S. Butts, M. S. Dunn, and L. F. Hallman, *J. Biol. Chem.*, *123*, 711–718 (1938).
s. J. S. Butts, R. O. Sinnhuber, and M. S. Dunn, *Proc. Soc. Exptl. Biol. Med.*, *46*, 671–673 (1941); *Ibid.*, *J. Biol. Chem.*, *140*, xxii–xxiii (1941).
t. W. C. Rose, J. F. Johnson, and W. F. Haines, *J. Biol. Chem.*, *145*, 679–684 (1942).

1200 g. of meat, of 186.2 g., as compared with a calculated value of 184.6 g. if the carbon retained was assumed to be present as carbohydrate. Had the carbon retained been stored as fat, only 169.7 g. of oxygen would have

been required over the test period. These data justify the conclusion of the authors that protein can change to carbohydrate.

4. The Lipid Content and Composition of the Animal as a Whole

(1) Methods for Determining the Lipid Content of Animals, Including Man

Two general types of methods are available for the assessment of the fat content of animals, namely by direct analysis of the animal tissues, or by tests in which the fat content may be calculated indirectly. The latter procedure has the advantage that it can be carried out more rapidly, and that it can be employed not only once, but repeatedly with live animals without injury to them. A comprehensive review of the methods which are available for the estimation of body fat in man and in animals was compiled by Keys and Brožek.[166]

a. Direct Analyses. The determination of the lipid content of the body as a whole, or of specific tissues, is a slow and laborious process. Although considerable data have been obtained with laboratory animals by this process, the results available for human subjects are negligible. The procedure involved consists in analyzing the entire animal or an aliquot part of the homogenized tissue for the several components, namely water, protein, minerals, and fat. The partition of water into extracellular and intracellular fractions is recommended, and will provide important additional information. A further refinement of the technic involves the separation of the total fat into the structural portion (as in the nervous system) and the depot or storage fat.

b. Indirect Methods for the Estimation of Body Fat. Although the direct method for estimating the composition of animal tissues is an accurate one, it is unsatisfactory for application to living individuals. Several indirect methods are available, however, which include determination of body weight, estimation of the extent of fat deposits in the subcutaneous fat layer, determination of body water following the administration of water-soluble compounds, or the use of fat-soluble dyes. Each of these procedures has its advantages and disadvantages.

(*a*) *The Use of "Standard" Weight Tables.* In addition to the subjective impressionistic appraisal of the relative fatness or leanness of a patient by the clinician, the most widely employed criterion for establishing the nutritive state is by comparison of the actual weight with the so-called

[166] A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953).

standard or ideal weight. Normal values have been established for different ages, height, and sex. The per cent deviation from the standard can be expressed by the following equation[166]:

$$\Delta\ \% = 100\ \frac{M - M_s}{M_s}$$

in which M is actual weight, M_s is the standard weight, and $\Delta\ \%$ gives the extent of the deviation; this latter value is positive in the case of overweight individuals and negative in those instances in which the body weight is less than the predicted normal. The percentage deviation from normal as expressed by relative weight ($M_R\ \%$), is obtained by the following expression:

$$M_R\ \% = 100\ \frac{M}{M_s}$$

However, neither of the above formulas takes into consideration the fact that variations in weight may result from changes in composition of tissue components other than fat. Even when evaluated with reference to the size of the skeleton, body weight does not afford an accurate index of fatness. This fact constitutes the fundamental limitation in the use of this type of data for assessing the leanness or fatness of an individual. Keys and Brožek[166] summarized in a comprehensive manner the limitations of the standard weight method, as well as the results obtained by the employment of this technic.

The best criteria for the indirect measurement of body composition would appear to be anthropometric data, which include the determination of subcutaneous fat deposits by making use of the skinfold technic or of roentgengrams, the determination of body density, the estimation of total water content, or the employment of the so-called fat-soluble indicators to determine the fat content by dilution. Each of these procedures will be described below.

(*b*) *The Use of Subcutaneous Fat Estimates.* Since the subcutaneous layer of adipose tissue constitutes an important proportion of the storage fat, a physical measurement of this deposit can be used as an indirect method for the estimation of the total depot fat. According to Wilmer,[167] the skin together with the subcutaneous fat layer comprises 17% of the body weight of the adult man. This total can be divided into 6% for the proportionate skin weight and 11.5% for that of the subcutaneous fatty tissue. In the case of women, the weight of the skin is given as 6%, that of the subcutaneous tissues as 23.7% of body weight.

[167] H. A. Wilmer, *Proc. Soc. Exptl. Biol. Med.*, *43*, 386–388 (1940).

a'. Skinfold Measurements: Although the assessment of the thickness of the skin and subcutaneous tissue has long been a standard procedure for the grading of live turkeys,[168] it has not generally been applied for the determination of the nutritive condition in other animals or in man. In the case of the turkey, fatness is judged on the basis of skinfolds on the side of the breast, and is rated from *1* (skin equaling or exceeding 0.18 in. in thickness) to *9* (skin paper-thin, 0.03 in. in thickness); in the latter case the skin appears devoid of fat.

Keys and Brožek[166] cite the time-honored custom of assessing the nutritional status of human subjects by pinching the skin between the thumb and forefinger. This procedure lacks precision, and can be considered to be at best only a very rough approximation. Meredith and Stuart[169] proposed a quantitative procedure involving skinfold measurements below the scapula and above the crest of the ilium. The results are assessed on a scale of 1 to 5. Different standards are employed for the sexes, as well as for each year from the ages of four to eighteen.

Special calipers were employed by Franzen[170] for measuring the thickness of skinfolds; systematic studies of the different types of calipers were made by Sandler.[171] Although variations in results are entailed by the use of different types of calipers, quite consistent data have been obtained in the case of a single observer using a particular procedure, as reported by Tanner and Weiner[172] and by Newman and White.[173]

For uniform results, it is necessary that the determination of skinfold fat be measured at comparable sites. Several different sites were employed by Franzen,[170] while Matiegka[174] tested seven specified locations. In choosing the area for comparison in different subjects, it is essential that the fold must be capable of being lifted, and accurately located in different subjects.[175] According to Keys and Brožek,[166] the minimum number of areas include one on the upper arm (midway on the posterior line), below the scapula, and above the iliac crest (in the mid-axillary line). Although

[168] S. J. Marsden and J. H. Martin, *Turkey Management*, 5th ed., Interstate, Danville, Ill., 1949.

[169] H. V. Meredith and H. C. Stuart, *Am. J. Public Health*, *37*, 1435–1438 (1947).

[170] R. Franzen, *Physical Measures of Growth and Nutrition*, School Health Research Monogr. *No. 2*, Am. Child Health Assoc., New York, 1929, p. 108.

[171] B. Sandler, unpublished work; cited by A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 258.

[172] J. M. Tanner and J. S. Weiner, *Am. J. Phys. Anthropol.*, *7* (*n.s.*), 145–186 (1949).

[173] R. W. Newman and R. M. White, personal communication, 1951; cited by A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 261.

[174] J. Matiegka, *Am. J. Phys. Anthropol.*, *4*, 223–230 (1921).

[175] D. A. W. Edwards, *Clin. Sci.*, *9*, 259–270 (1950).

these values are not the highest predicted levels of total fat, they do correlate well with specific gravity, and the locations are readily accessible for measurement in both sexes. The best indicator of total fat can be obtained in men by measurement of the chest skinfold above the nipple.

Ordinarily, the actual values of the skinfolds can be used as a criterion of fatness. One may employ the thickness of one skinfold (one-half the mean skinfold minus the average skin thickness) or a mean of several skinfolds, treated in the same way, or a combination of several skinfold measurements in the form of a multiple regression equation, body fat being considered the "dependent" variable.

Keys and Brožek[166] postulated that a relative value for the skinfolds in which the body size was included might be a better index of the degree of fatness than the absolute measurement. This value could be calculated from the following equation:

$$I \text{ (index of fatness)} = \frac{S \text{ (skinfold measurement)} \times A \text{ (surface area)}}{M \text{ (body weight)}}$$

If the true average value of the thickness of subcutaneous fat is available, SA would represent the volume of subcutaneous adipose tissue; by multiplying this by the density value one could obtain the total weight of the subcutaneous fat.

b′. Fat-Skeleton-Muscle Analyses: Matiegka[174] based his calculations of skeleton, skin plus subcutaneous fat, and muscles upon stature and upon the anthropometric characteristics of the extremities. The skinfold measurements were employed in these calculations, which, however, were not validated by determinations of these values on corpses. Edwards[175] presented mean "fatfold" thicknesses (skinfolds less the average values for double skin thickness) at each of fifty-three sites in female subjects with an average weight of 148 lb. Neither height nor average age was specified.

c′. Roentgenograms: Apparently Stuart *et al.*[176] were among the first to measure the thickness of the subcutaneous fat layer by the application of roentgenography. The technic was developed further by Stuart and Sobel,[177] and by Reynolds,[178] who used children as subjects. This technic was also employed in the tissue analysis of children in Madrid,[179] as well as

[176] H. C. Stuart, P. Hill, and C. Shaw, *The Growth of Bone, Muscle, and Overlying Tissues as Revealed by Studies of Roentgenograms. Monogr. Soc. Research Child Develop., 5, Ser. 26, No. 3,* Child Develop. Publ., Evanston, Ill., 1940, pp. 1–189.

[177] H. C. Stuart and E. H. Sobel, *J. Pediat., 28,* 637–647 (1946).

[178] E. Reynolds., *Child Develop., 15,* 181–205 (1944).

[179] W. D. Robinson, J. H. Janney, and F. (Covian) Grande, *J. Pediat., 20,* 723–739 (1942).

in Marseilles.[180,181] It has been used for the investigation of body composition by Reynolds[182] at the Fels Research Institute for the Study of Human Development, at Antioch College. In order to obtain consistent results, it was necessary to take the roentgenograms at precise locations. In the trochanteric region, relatively slight changes in the level at which the width of the fat layer was measured caused variations of as much as 50% in the values obtained.

d′. Direct Measurement of The Thickness of the Subcutaneous Layer: Another procedure, employed mainly in the case of hogs, by McKeekan,[183] involves the determination of the thickness of the fat layer along the back line. Hazel and Kline[184] employed this method by first making small skin incisions into the back at certain points, and then measuring the depth to which a narrow metal ruler could be inserted before it was stopped by a well-defined muscle layer.

(c) *The Use of Density Measurements.* Since the density of body fat is considerably less than that of the fat-free constituents of the tissues, the figure for the density of the body as a whole will vary according to the relative proportion of fat. The higher the content of fat of the body, the lower will be the value recorded for its density. The density of the entire body is simply the weight divided by the volume which it occupies. The latter value can be readily obtained by determining the weight of the object completely submerged in water as compared with that when it is surrounded by air. Keys and Brožek[166] presented the following formula for the estimation of specific gravity:

$$\text{Sp. gr.} = \frac{M_A}{M_A - M'_W - D_W V_R}$$

where M_A is the weight in air, M'_W is apparent weight under water, D_W is density of water in tank, and V_R is weight of water displaced by residual air (lungs and respiratory passages).

According to Keys and Brožek,[166] the specific gravity of the fat-free eviscerated body of the guinea pig was estimated by Pace and Rathbun[185] at 1.0939; Keys and Brožek calculated it as 1.0934. In the case of man, Rathbun and Pace[186] cited a value of 1.10 for the density of the fat-free

[180] H. C. Stuart and D. Kuhlman, *J. Pediat.*, *20*, 424–453 (1942).

[181] H. C. Stuart, *J. Pediat.*, *25*, 257–264 (1944).

[182] E. L. Reynolds, *Distribution of Subcutaneous Fat in Childhood and Adolescence. Monogr. Soc. Research Child Develop.*, *15*, *Ser. 50*, *No. 2*, 1950. Child Develop. Publ., Evanston, Ill., 1951.

[183] C. P. McKeekan, *J. Agr. Sci.* (*England*), *31*, *Part I*, 1–49 (1941).

[184] L. N. Hazel and E. A. Kline, *J. Animal Sci.*, *11*, 313–318 (1952).

[185] N. Pace and E. N. Rathbun, *J. Biol. Chem.*, *158*, 685–691 (1945).

[186] E. N. Rathbun and N. Pace, *J. Biol. Chem.*, *158*, 667–676 (1945).

human body, on the basis of data of Behnke and associates.[187] Keys and Brožek state that the value should have been 1.098, and should refer to a human body containing 10% of its weight as essential lipids.

The specific gravity of human subcutaneous fat, as given by Ulzer and Klimont,[188] was 0.9179 at 15°C. Fidanza, Keys, and Anderson,[189] on the basis of determinations of the densities of twenty samples of fat from adult men and women, obtained values ranging from 0.8982 to 0.9009, with an average value of 0.9000/g. at 37°C. The mean change in density of human fat from 15° to 37°C. per 1°C. was found to be 0.00074; it was quite uniform over this temperature range. At 15°/15°C., the specific gravity was 0.9171, which approximates a value (0.9179) cited earlier by Jaeckle,[190] as well as that of Ulzer and Klimont.[188] The density of dog body fat was found to be lower than that of man, but in other species the values for the density of the body fat are higher than in the case of man.[189] Tester[191] showed that a linear relationship exists between the oil content of the Pacific herring (*Clupea pallasii*) and a factor based upon specific gravity. Rathbun and Pace[186] proved that a similar phenomenon occurs in the guinea pig. On the basis of studies of fifty guinea pigs the specific gravity of whose eviscerated bodies ranged from 1.021 to 1.096, and whose fat content varied from 1.5 to 35.8%, the following formula was derived to calculate the body fat of these animals from the specific gravity:

$$\%\text{ fat (guinea pig)} = 100\left(\frac{5.362}{\text{sp. gr.}} - 4.880\right)$$

In order to apply the same formula to man, Rathbun and Pace modified the preceding formula as follows:

$$\%\text{ fat (man)} = 100\left(\frac{5.548}{\text{sp. gr.}} - 5.044\right)$$

Kraybill *et al.*[192] showed that, in the case of swine, the fat content can be accurately calculated, by the following formula, from the specific gravity of the eviscerated animal:

[187] A. R. Behnke, B. G. Feen, and W. C. Welham, *J. Am. Med. Assoc.*, *118*, 495–498 (1952).

[188] F. Ulzer and J. Klimont, *Allgemeine und physiologische Chemie der Fette, für Chemiker, Mediziner, und Industrielle*, Springer, Berlin, 1906; cited by A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 271.

[189] F. Fidanza, A. Keys, and J. T. Anderson, *The Density of Human Body Fat*, in press; cited by A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 271.

[190] H. Jaeckle, *Z. physiol. Chem.*, *36*, 53–84 (1902).

[191] A. L. Tester, *J. Fisheries Research Board, Can.*, *4, No. 5*, 461–471 (1940).

[192] H. F. Kraybill, E. R. Goode, R. S. B. Robertson, and H. S. Sloane, *J. Applied Physiol.*, *6*, 27–32 (1953).

$$\% \text{ fat (swine)} = 100 \left(\frac{5.405}{\text{sp. gr.}} - 4.914\right)$$

Messinger and Steele[193] likewise showed that body fat and body water can be calculated from specific gravity with considerable accuracy.

(*d*) *The Use of Body Water for the Determination of Body Fat.* Since an inverse relationship is known to obtain between the proportion of body water and that of body fat,[185,193,194] the determination of the former has been employed in the estimation of the total content of the latter. The proportion of body water is highly variable unless calculated on a fat-free basis.[193] According to Pace and Rathbun,[185] water constitutes 72.42 ± 2.11% of the fat-free mass of the guinea pig. Pace and Rathbun[185] summarized figures from the literature for the water content of fat-free tissues of various species, as follows: rat, 72.7%; guinea pig, 73.3%; rabbit, 74.9%; cat, 72.4%; dog, 72.2%; and monkey, 73.3%. The grand average for all species was 73.2%. In the case of man, McCance and Widdowson[195] concluded that the fat-free tissues contain 71% of water. Osserman and co-workers,[196] making use of the antipyrine method, estimated this figure to be 71.8 ± 2.99 for healthy men eighteen to forty-six years of age. Keys and Brožek are of the opinion that the water content of fat-free human tissues should be 72 ± 3; moreover, it is suggested that this value is not independent of the fatness of the individual, and that greater deviations are to be expected in emaciated and in obese subjects.

a′. Test Solutes for the Determination of Water: In order to estimate the proportion of water in the tissues of an individual, the usual procedure is to determine the concentration of a test solute in the blood some time after its administration, and to calculate total water on the dilution principle. In order that the solute may prove satisfactory as a test substance, it must (*a*) rapidly penetrate and dissolve in all the water of the body, (*b*) not be adsorbed on, be combined with, or be destroyed by other constituents of the body (or if such is the case, the extent of destruction must be predictable), (*c*) be eliminated from the organism at a measurable rate, (*d*) be nontoxic when given in the required dosage, and (*e*) be readily and accurately measurable in the blood serum.[166] Several such substances have been used successfully.

(*a*′) Urea.—It is generally believed that urea is distributed in the body

[193] W. J. Messinger and J. M. Steele, *Proc. Soc. Exptl. Biol. Med., 70*, 316–318 (1949).
[194] E. Da Costa and R. Clayton, *J. Nutrition, 41*, 597–606 (1950).
[195] R. A. McCance and E. M. Widdowson, *Proc. Roy. Soc. (London), 138 B*, 115–130 (1951).
[196] E. F. Osserman, G. C. Pitts, W. C. Welham, and A. R. Behnke, *J. Applied Physiol., 2*, 635–639 (1950).

tissues in proportion to their water content.[197–199] A number of workers have employed this compound as a solute for such estimations,[195,200,201] although an equal number[202–204] refused to use it on the basis of an assumption,[205] and a report[206] that the distribution of urea between blood cell and plasma water is not exactly equal in the postabsorptive state.[207] However, Keys and Brožek[166] express the opinion that, in all probability, urea may serve satisfactorily as a solute, inasmuch as: (*a*) although the distribution of urea between cells and plasma is not exactly unity in the postabsorptive state, it is highly constant, and is the same in men and in women, (*b*) there is no proof that *added* urea might not have a distribution ratio of unity in the blood, (*c*) there is no evidence that urea is unequally distributed in the water of tissues other than blood, and (*d*) no critical tests have actually been made on the validity of water estimates based upon the results of the administration of urea. In more recent tests with ordinary urea,[207] and with N^{15}-labeled urea,[208] this substance was shown to yield reasonable values when applied to man; the results agree with the data obtained by the use of heavy water. It is reported that the urea space is slightly less than that obtained with deuterium water, but this is to be expected in view of the deuterium (hydrogen) exchange in the body.

(*b'*) Deuterium Oxide (Heavy Water).—The use of deuterium oxide as an ideal test substance for determining the total body water was first proposed by Hevesy and Hofer.[209] A number of workers[202,210–214] have em-

[197] E. K. Marshall, Jr., and D. M. Davis, *J. Biol. Chem.*, *18*, 53–80 (1914).

[198] K. L. Gad-Andresen, *Biochem. Z.*, *116*, 266–302 (1921).

[199] E. E. Painter, *Am. J. Physiol.*, *129*, 744–755 (1940).

[200] E. M. Widdowson and R. A. McCance, *Effect of Undernutrition and of Posture on the Volume and Composition of Body Fluids. Med. Research Council (Brit.), Spec. Rept. Ser. No. 275*, 165–174 (1951).

[201] H. L. Kornberg and R. E. Davies, *Nature*, *169*, 502–503 (1952).

[202] N. Pace, L. Kline, H. K. Schachman, and M. Harfenist, *J. Biol. Chem.*, *168*, 459–469 (1947).

[203] M. F. Levitt and M. Gaudino, *Am. J. Med.*, *9*, 208–215 (1950).

[204] B. B. Brodie, *Methods in Medical Research*, Vol. 4, Year Book Pub., Chicago, 1951, pp. 31–38.

[205] J. P. Peters, *Harvey Lectures*, *33*, 112–142 (1937–1938).

[206] J. O. Ralls, *J. Biol. Chem.*, *151*, 529–541 (1943).

[207] M. G. Eggleton, *J. Physiol.*, *115*, 482–487 (1951).

[208] A. San Pietro and D. Rittenberg, *J. Biol. Chem.*, *201*, 445–455 (1953).

[209] G. Hevesy and E. Hofer, *Nature*, *134*, 879 (1934).

[210] E. J. McDougall, F. Verzár, H. Erlenmeyer, and H. Gaertner, *Nature*, *134*, 1006–1007 (1934).

[211] E. Swift, Jr., *J. Am. Chem. Soc.*, *61*, 198–200 (1939).

[212] F. D. Moore, *Science*, *104*, 157–160 (1946).

[213] G. Hevesy, *Radioactive Indicators. Their Application in Biochemistry, Animal Physiology, and Pathology*, Interscience, New York-London, 1948.

[214] J. D. Hardy and D. L. Drabkin, *J. Am. Med. Assoc.*, *149*, 1113–1116 (1952).

phasized the value of deuterium and of tritium[213] from a theoretical standpoint. Deuterium oxide has been shown experimentally to give results which appear reasonable on the basis of other data.[210,212,215–220] It has been shown that labile hydrogen in organic compounds in the body exchanges with deuterium.[213,221,222] However, Hevesy and Jacobsen[223] estimated this labile hydrogen to be equivalent to only 0.5 to 2.0% of the body weight as water. Schloerb and co-workers[219] showed that the heavy water space in the body exceeds the total body water by about 1% of the body weight; this probably accounts for the discrepancy due to labile hydrogen exchange.

(*c′*) Antipyrine.—The dilution of non-physiologic compounds in the body has likewise been employed for the calculation of body water, and indirectly for the estimation of body fat. One of the most satisfactory substances for this purpose appears to be antipyrine (1,5-dimethyl-2-phenyl-3-pyrazolone), which has been used as an antipyretic since 1883. Brodie and associates[204,224] reported that antipyrine is uniformly distributed in the water of various tissues. It is relatively non-toxic,[166,204] readily determined, and only slowly metabolized by man.[166] The total body water in normal men, as estimated by the use of antipyrine, agreed well with that calculated by the use of heavy water, although less satisfactory results were obtained in the case of edematous patients. The values obtained for the fat content as calculated from the water content (estimated by the antipyrine method) corresponded well with those based upon measurements of density.[194] Antipyrine has given good results in the hands of many investigators[194,196,225–228]

[215] L. B. Flexner, A. Gellhorn, and M. Merrell, *J. Biol. Chem.*, *144*, 35–40 (1942).

[216] L. B. Flexner, W. S. Wilde, N. K. Proctor, D. B. Cowie, G. J. Vosburgh, and L. M. Hellman, *J. Pediat.*, *30*, 413–415 (1947).

[217] F. D. Moore, *Surg. Gynecol. Obstet.*, *86*, 129–147 (1948).

[218] V. Hollander, P. Chang, and F. W. Co Tui, *J. Lab. Clin. Med.*, *34*, 680–687 (1949).

[219] P. R. Schloerb, B. J. Friis-Hansen, I. S. Edelman, A. K. Solomon, and F. D. Moore, *J. Clin. Invest.*, *29*, 1296–1310 (1950).

[220] J. D. Hardy, P. K. Sen, and D. L. Drabkin, *Surg. Gynecol. Obstet.*, *93*, 103–106 (1951).

[221] A. Krogh and H. H. Ussing, *Skand. Arch. Physiol.*, *75*, 90–104 (1936).

[222] H. H. Ussing, *Skand. Arch. Physiol.*, *78*, 225–241 (1938).

[223] G. Hevesy and C. F. Jacobsen, *Acta Physiol. Scand.*, *1*, 11–18 (1940).

[224] B. B. Brodie, J. Axelrod, R. Soberman, and B. B. Levy, *J. Biol. Chem.*, *179*, 25–29 (1949).

[225] R. Soberman, B. B. Brodie, B. B. Levy, J. Axelrod, V. Hollander, and J. M. Steele, *J. Biol. Chem.*, *179*, 31–42 (1949). R. J. Soberman, *Proc. Soc. Exptl. Biol. Med.*, *71*, 172–173 (1949).

[226] M. Herrold and L. A. Sapirstein, *Proc. Soc. Exptl. Biol. Med.*, *79*, 419–421 (1952).

[227] J. M. Steele, E. Y. Berger, M. F. Dunning, and B. B. Brodie, *Am. J. Physiol.*, *162*, 313–317 (1950).

[228] G. W. Dupertuis, G. C. Pitts, E. F. Osserman, W. C. Welham, and A. R. Behnke, *J. Applied Physiol.*, *4*, 364–367 (1951).

for the indirect estimation of fat in man and other mammals. Kraybill *et al.*[229] reported that the antipyrine method is satisfactory for the estimation of fat in cattle.

In spite of the relatively poor results obtained by Soberman and coworkers[225] by the use of antipyrine in the case of edematous patients, other workers found that this drug may yield accurate data when a rapid change in body water obtains. Thus, Steele and associates[227] reported that the antipyrine method is accurate to 0.5 liter for measurement of body water before and after paracentesis (tapping of the abdominal cavity) in ascitic patients. Antipyrine and heavy water have both been employed successfully in the determination of body water in edematous patients, both before and after the cessation of the edema.[230] The antipyrine method yields somewhat lower results than does the heavy water method in edema; in the case of infants and in growing children, the results obtained with antipyrine have been found by Friis-Hansen *et al.*[231] to be about 1.8% lower than those obtained by the heavy water method. The discrepancy has been attributed to a systematic error in the latter procedure, attributable to the hydrogen exchange. Greenberg[232] has compiled a critical review on antipyrine.

(*d′*) Miscellaneous Compounds.—A number of substances do not meet the qualifications as test solutes, or there are insufficient data at the present time to prove their usefulness. Potassium is not satisfactory.[233] Alcohol[234] and glycerol,[235] which do mix readily with body water, are metabolized at such high and possibly variable rates as to render them unreliable as test solutes. Thiourea, which was proposed as a rough measure of change in body water, by Danowski,[236] has been criticized as a test solute.[202–204] Sulfanilamide has been suggested by Painter[199] for this purpose, because of its ready penetration into all tissues; however, it has been rejected, largely on the basis that it progressively disappears from the tissues.[237,238] Keys and

[229] H. F. Kraybill, O. G. Hankins, and H. L. Bitter, *J. Applied Physiol.*, *3*, 681–689 (1951).

[230] W. W. Hurst, F. R. Schemm, and W. C. Vogel, *J. Lab. Clin. Med.*, *39*, 36–40 (1952).

[231] B. J. Friis-Hansen, M. Holiday, T. Stapleton, and W. M. Wallace, *Pediatrics*, *7*, 321–327 (1951).

[232] L. A. Greenberg, *Antipyrine. A Critical Bibliographic Review*, Hillhouse Press, New Haven, 1950.

[233] A. W. Winkler and P. K. Smith, *J. Biol. Chem.*, *124*, 589–598 (1938).

[234] E. M. Widmark, *Die theoretische Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung*, Urban, Berlin-Vienna, 1932. *Fortschr. naturwiss.-Forsch.*, *No. 11*, 1932; Review, *J. Am. Med. Assoc.*, *98*, 1834 (1932).

[235] E. J. Holst, *Acta Physiol. Scand.*, *7*, 69–79 (1944).

[236] T. S. Danowski, *J. Biol. Chem.*, *152*, 207–212 (1944).

[237] H. S. Sise, *Proc. Soc. Exptl. Biol. Med.*, *40*, 451–454 (1939).

[238] A. Waterhouse and J. A. Shannon, *Proc. Soc. Exptl. Biol. Med.*, *50*, 188–192 (1942).

Brožek[166] suggest that sulfanilamide might be a satisfactory test solute for the estimation of body water if an empirical correction factor were applied.

Tritium-labeled water has been recommended as having some advantages over deuterium oxide as a test substance.[202,239] Prentice *et al.*[240] found that the exchange of tritium with labile organic hydrogen is small or undetectable in fat, somewhat greater in muscle and gut, and considerable in liver, kidney, and plasma solids. Tritium appears to measure the same water volume as deuterium, averaging 2 to 4% higher than with antipyrine, and undergoes a similar degree of exchange with organic molecules as does deuterium. The exchange error can be eliminated by a constant correction factor. Other disadvantages of tritium-labeled water include its expense, the complication of its analysis, and the fact that some question arises as to the health hazard to man entailed by its long half-life (twelve years).[214] Presumably no danger factor is involved by its use in a single test.[240] Moreover, no extensive data are as yet available as to the reliability and uniformity of the results obtained by the employment of this radioisotope.

Brodie and co-workers[241] proposed the use of *N*-acetyl-4-aminopyrine (NAAP) instead of antipyrine for the estimation of body water. In contradistinction to antipyrine, NAAP does not combine with proteins to an appreciable extent.

b′. Formula for the Calculation of Fat Content from Body Water Data: The general equation for the calculation of total fat from the calculation of body water is as follows[166]:

$$F = 1 - kA$$

in which F is body fat as a proportion of body weight, A is total body water as a proportion of body weight, and k is a constant. The value of k has been assumed to be 1.393, which is the ratio of 1.000/0.718; the denominator is the assumed percentage of water in fat-free tissue.[196] The figures obtained by the use of this equation gave a standard error of $\pm 3.8\%$ of the body weight as fat, when compared with the results obtained from densiometry. Keys and Brožek[166] proposed a value of 1.4 for k based upon a water content of 71% in fat-free tissue.

However, according to Keys and Brožek,[166] data are still unavailable to support a final conclusion as to the absolute validity of the estimation of

[239] E. A. Pinson and E. C. Anderson, *Am. J. Physiol.*, *163*, 741 (1950).

[240] T. C. Prentice, W. Siri, N. I. Berlin, G. M. Hyde, R. J. Parsons, E. E. Joiner, and J. H. Lawrence, *J. Clin. Invest.*, *31*, 412–418 (1952).

[241] B. B. Brodie, E. Y. Berger, J. Axelrod, M. F. Dunning, Y. Porosowska, and J. M. Steele, *Proc. Soc. Exptl. Biol. Med.*, *77*, 794–798 (1951).

TABLE 7

BODY COMPOSITION BASED UPON DIRECT ANALYSIS OF CADAVERS[a]

Subject No.	Age	Height, cm.	Weight, kg.	% total weight				% Fat-free weight			Ash/ Protein
				Water	Fat	Protein	Ash	Water	Protein	Ash	
				Female subject							
1[b]	42	169	45.1	56.0	23.6	14.4	7.6[a]	73.2	18.8	9.9[a]	52.7[a]
				Male subjects							
2[c]	46	168.5	53.8	55.1	19.4	18.6	5.4	68.4	23.1	6.7	29.0
3[a,d]	35	183	70.6	67.9	12.5	14.4	4.8	77.6	16.5	5.5	33.3
4[a]	25	179	71.8	61.8	14.9	16.6	7.5	72.6	17.5	8.8	50.3
5[a]	48	—	63.8	81.5	1.1	12.8	4.9	82.4	12.9	5.0	38.8

[a] Adapted from A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 249.
[b] E. M. Widdowson, R. A. McCance, and C. M. Spray, *Clin. Sci.*, *10*, 113–125 (1951).
[c] R. M. Forbes, A. R. Cooper, and H. H. Mitchell, unpublished manuscript, cited by A. Keys and J. Brožek, *Physiol. Revs.*, *33*, 245–325 (1953), p. 249.
[d] H. H. Mitchell, T. S. Hamilton, F. R. Steggerda, and H. W. Bean, *J. Biol. Chem.*, *158*, 625–637 (1945).

total fat in man by the determination of total body water. The results of Rathbun and Pace[186] on eviscerated guinea pigs and of Da Costa and Clayton[194] on rat carcasses show a close but not perfect inverse relationship between fat and water content when these components are measured by classical analytical methods.

(*e*) *The Use of Fat-Soluble Indicators.* The dilution method would be an ideal procedure for the estimation of body fat if test solutes could be employed which were soluble only in fat. However, from a practical standpoint, since such compounds must be distributed by the blood, it has been necessary to employ compounds preferentially soluble in fat but which are also somewhat soluble in water and fat-free cells.

Nitrogen and helium gases have been employed as such test substances. According to Shaw and co-workers,[242] nitrogen gas is about five times as soluble in fat as in water; the amount of nitrogen gas eliminated by subjects breathing oxygen over a period of six to twelve hours has been shown by Behnke, Thomson, and Shaw,[243] and by Behnke,[244–246] to yield values for body fat of the correct general order of magnitude.

Cyclopropane constitutes a more practical compound as a fat diluent than does nitrogen or helium. Thus, Lesser and associates[247] reported that good agreement could be obtained between fat calculated from the quantity of cyclopropane disappearing in a closed system respirometer and from ether extraction of the tissues. Equilibrium required 1.5 to 2.5 hours.[166]

(*2*) *The Normal Fat Content of Man and Animals*

The results of body composition which have been determined on man are summarized in Table 7.

The analyses of the composition of a man thirty-three years old, based upon data of Bischoff as corrected by Voit and cited by Vierordt,[248] and recalculated by Keys and Brožek[166] are as follows for total solids and fat, respectively, in percentage of wet weight: skeleton, 12.6, 3.8; voluntary

[242] L. A. Shaw, A. R. Behnke, A. C. Messer, R. M. Thomson, and E. P. Motley, *Am. J. Physiol.*, *112*, 545–553 (1935).

[243] A. R. Behnke, R. M. Thomson, and L. A. Shaw, *Am. J. Physiol.*, *114*, 137–146 (1935).

[244] A. R. Behnke, *U. S. Naval Med. Bull.*, *35*, 219–240 (1937).

[245] A. R. Behnke, *Harvey Lectures*, *37*, 198–226 (1941-1942).

[246] A. R. Behnke, *Medicine*, *24*, 359–379 (1945).

[247] G. Lesser, A. G. Blumberg, and J. M. Steele, *Am. J. Physiol.*, *169*, 545–553 (1952).

[248] H. Vierordt, *Daten und Tabellen für Mediziner und Ärzte*, 1st ed., Fischer, Jena, 1888.

muscles, 10.3, 0.93; brain and nerves, 0.67, 0.33; "Fett," 12.8, 12.8; all other tissues, 4.9, 1.4; and total, 41.3, 19.3.

The body composition of a normal and of a fat man, and the composition

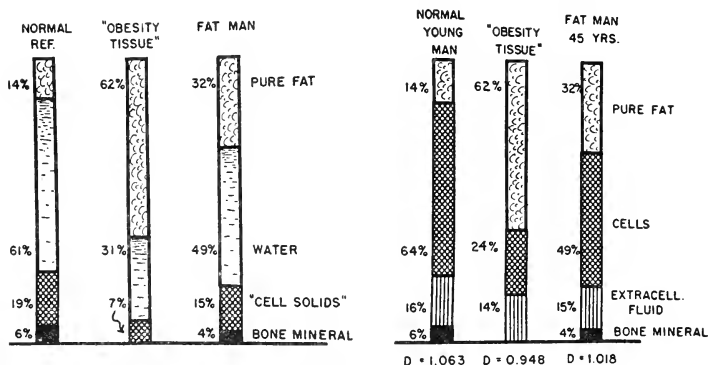


Fig. 1. Body composition of a normal and of a fat man and of "obesity" tissue, as calculated by densitometric analysis (left) and by water analysis (right).[166]

TABLE 8

THE AVERAGE COMPOSITION OF EVISCERATED MALE AND FEMALE RATS 15 WEEKS OF AGE THAT HAD PREVIOUSLY RECEIVED A MINERALIZED SKIMMED MILK DIET CONTAINING SEVERAL FATS FOR 12 WEEKS FOLLOWING WEANING[a]

Category	Male rats *(1)*	Female rats *(2)*	M.D.: S.E.M.D. of *(1)* *vs.* *(2)*[b]
Number of rats	51	51	—
Body weight, total g	254.8	171.2	—
" " , after evisceration, g	237.0	158.3	—
Composition:[c]			
Water, %	58.2 ± 0.4	56.8 ± 0.4	2.45
Protein, %	17.3 ± 0.2	16.4 ± 0.2	3.10
Lipid, %	18.1 ± 0.5	20.1 ± 0.6	2.70
Carbohydrate (by difference), %	3.38 ± 0.37	3.53 ± 0.12	0.40
Ash, %	2.95 ± 0.03	3.26 ± 0.04	6.20
Ca, mg. %	911 ± 11	1028 ± 16	6.03

[a] H. J. Deuel, Jr., L. F. Hallman, E. Movitt, F. H. Mattson, and E. Wu, *J. Nutrition*, *27*, 335–338 (1944).

[b] Ratio of Mean Difference to Standard Error of the Mean Difference. A figure greater than 3.0 is considered highly significant.

[c] Including Standard Error of the Mean.

of "obesity" tissue, as determined by densitometric analysis, as well as by water analysis, is given in Figure 1.

Although analyses of the composition of the tissues of a number of animal species have been reported, only those for normal adult rats will be given here. These are recorded in Table 8.

Although wide differences in fat content may occur in conjunction with variations in nutritional condition, the values for normal rats (18 to 20%) correspond roughly with the figures in man, namely 1–23%.

(3) The Normal Composition of Animal Fats

The fats which are laid down in the fat depots, as well as in supporting tissues, are characteristic of the species of animal from which they are obtained. Although the composition of these fat depots may be influenced to some extent by extreme changes in the diet of the animal, animals of different species will, on the whole, synthesize entirely different types of body fat when they are given the same basal diet. Moreover, the animal possesses an ability to produce, from the same basic foodstuffs, specific fats for deposition in different organs. It has been known for a long time that the fat laid down in the internal organs has a higher melting point and a lower iodine value than has that deposited in the subcutaneous tissues of the same animal. Thus, the animal must be able to manufacture simultaneously, from the same basic components, a number of types of fats in the several organs. The nature of the body fat and the site at which it is deposited are likewise controlled by certain hormones; of these agents, the sex hormones are probably the most important, since sex plays a most important role in the metabolism of fats.

Many investigations on the subject of animal fats have been concerned with determining the composition of the mixed fat from the animal as a whole, rather than from a specific tissue. This is obviously the only practical solution for investigations of small organisms, when it is impossible to effect an adequate separation of tissues and to obtain a sufficient supply of material for an analysis. However, even this difficulty has been overcome by the application of new technics for the mechanical separation of the structural elements of the cell; these will be discussed in a later section (see Section 6, page 603).

A number of studies on the qualitative and quantitative aspects of fat storage of animals as large as the rat have been made, employing a mixed fat from the entire animal. These samples have been obtained either by a rendering technic applied to the macerated tissues of the animal as a whole, as employed by Anderson and Mendel,[49] or by the ether extraction of an

aliquot of the macerated, dried tissues.[249] In spite of the fact that considerable variations in the composition of the lipids obtain in the various tissues, the values noted for the mixed animal fats are characteristic of the different species.

The animal fats differ from the vegetable fats in containing a larger variety of fatty acids. For a further discussion of the composition of animal and vegetable fats, the reader is referred to Hilditch,[116] and to Volume I, pages 184–234.

(4) The Distribution and Composition of the Lipids in the Storage Depots and in the Several Organs

a. The Normal Distribution of Lipids. Fat is stored in definite locations in the animal; these differ somewhat in importance according to age and sex, but not according to diet. These areas include (1) intermuscular, (2) genital, (3) subcutaneous, (4) perirenal, (5) mesenteric, and (6) omental. These have been exactly defined and described by Reed and co-workers.[250]

According to Reed *et al.*,[250] normal male rats weighing 250 g. after having completed their period of most active growth were shown to have the following distribution in per cent of total fat in the several fat depots: intermuscular, 9; genital, 13; subcutaneous, 55; perirenal, 16.4; mesenteric, 6.0; and omental, 2.6. On the other hand, the comparative values for female rats weighing 250 g., which were somewhat older than the male rats, were as follows: intermuscular, 5; genital, 18; subcutaneous, 61; perirenal, 9.6; mesenteric, 5.0, and omental, 2.4. It would thus appear that male rats have a higher proportion of storage fat in the intermuscular and perirenal depots, while the female has a larger proportion of subcutaneous and genital fat.

b. The Comparative Lipid Composition of Different Tissues. According to Bloor,[251] those tissues and organs which have the greatest variety of physiologic activities contain the highest concentration of lipids. In tissues as, for instance, muscle, in which physiologic activity such as oxidation is predominant, the cells contain ten to twenty times the concentration of protein as of lipid.[252] On the other hand, in brain, liver, and other organs

[249] H. J. Deuel, Jr., L. F. Hallman, E. Movitt, F. H. Mattson, and E. Wu, *J. Nutrition*, *27*, 335–338 (1944).

[250] L. L. Reed, F. Yamaguchi, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *87*, 147–174 (1930).

[251] W. R. Bloor, *J. Biol. Chem.*, *132*, 77–82 (1940).

[252] M. Kaucher, H. Galbraith, V. Button, and H. H. Williams, *Arch. Biochem.*, *3*, 203–215 (1943).

in which widely varying reactions occur other than oxidation, the lipids are much more prominent, and occur in amounts varying from one-sixth to a quantity equivalent to that of protein.

The nature of the lipids which are present as components of the various organs varies markedly from that of the depot fats. The lipids in the organs contain practically no neutral fat, but only phospholipids, cerebrosides, and cholesterol. On the other hand, the depot fats are almost entirely composed of neutral fats. The depot fats are present in different amounts, according to the diet and nutritional condition.

Phospholipids have consistently been shown to comprise the largest proportion of the essential lipids. According to Kaucher and her collaborators,[252] cerebrosides are second in importance in skeletal and cardiac muscle, followed by cholesterol, which is a minor constituent. In smooth muscle such as that of the stomach and intestine, the cholesterol fraction is more important than are the cerebrosides. The above investigators likewise reported that sphingomyelin was of greater importance in the soft tissues of the body than it was in the skeletal musculature.

The composition of the lipids present in the various organs of rats fifteen and seventy days of age is summarized in Table 9, while a further analysis of the phospholipid components is included in Table 10 (pages 570, 571).

The proportion of the several tissues composed of essential lipids increases with age. The highest concentration of lipid occurs in the brain, where the essential lipids increase from 29.82% at fifteen days to 42.68% at seventy days. The testes show the next greatest percentage change; in these organs the lipid increases from 13.73% at fifteen days to 20.41% at seventy days. The phospholipids make up the major portion of the essential lipids. The increase in proportion of this component is consistent, in all tissues, with increasing age. This accounts for most of the rise in total essential lipids. The cerebrosides are of paramount importance, especially in the brain, where they increase from 3.77 to 8.42% of the total lipids during the fifty-five day increment in age between the two series of tests.

There are considerable variations, from a qualitative standpoint, in the composition of the phospholipids in the several tissues. In most instances, the cephalins increase markedly between the ages of fifteen days and seventy days, so that they equal or exceed the proportion of choline-containing phospholipids at the later age period. In a majority of cases, the choline phospholipids decline with increasing age, a change which is largely to be ascribed to a decrease in the lecithin fraction.

TABLE 9

LIPID COMPOSITION OF VARIOUS TISSUES OF MALE RATS AT 15 AND 70 DAYS OF AGE[a]

The values are in % dry weight

Rat tissue	Phospholipids		Cerebrosides		Free cholesterol		Cholesterol ester		Essential lipid		Neutral fat		Total lipid	
	15[b] days	70[c] days	15 days	70 days	15 days	70 days	15 days	70 days	15 days	70 days	15 days	70 days	15 days	70 days
Brain	21.34	27.19	3.77	8.42	4.44	7.05	0.27	0.02	29.82	42.68	2.80	1.95	32.62	44.63
Heart	12.85	15.38	2.32	1.37	0.77	0.45	0.46	0.21	16.40	17.41	2.90	—	19.30	—
Kidney	11.99	15.19	1.20	1.30	1.16	1.00	0.84	0.94	15.19	18.43	4.43	3.16	19.62	21.59
Lung	10.75	13.75	0.86	0.91	1.00	1.43	1.44	1.02	14.05	17.11	6.92	4.75	20.97	21.86
Testes	10.32	14.97	1.86	3.96	1.31	0.82	0.24	0.66	13.73	20.41	5.96	2.77	19.69	23.18
Liver	11.67	13.90	0.12	0.13	0.27	0.29	1.44	0.66	13.50	14.98	6.35	5.22	19.85	20.20
Thymus	9.72	10.75	1.34	1.14	0.57	0.24	0.46	0.61	12.09	12.74	6.89	—	18.98	—
Spleen	6.93	10.76	0.60	0.84	1.34	1.08	0.08	0.51	8.95	13.19	2.74	—	11.69	—
Skeletal muscle	5.95	8.57	1.45	3.57	0.20	0.12	0.61	0.14	8.21	12.40	19.58	3.44	27.79	15.84

[a] Data adapted from H. H. Williams, H. Galbraith, M. Kaucher, E. Z. Moyer, A. J. Richards, and I. G. Macy, *J. Biol. Chem.*, *161*, 475–484 (1945).

[b] 88 to 116 animals used in 15-day tests.

[c] 6 rats used for 70-day tests.

TABLE 10

PHOSPHOLIPID DISTRIBUTION IN THE TISSUES OF RATS 15 AND 70 DAYS OF AGE[a]

The values are in % dry weight

Rat tissue	Total phospholipids		Cephalin		Total choline phospholipids		Lecithin		Sphingomyelin	
	15[b] days	70[c] days	15 days	70 days	15 days	70 days	15 days	70 days	15 days	70 days
Brain	21.34	27.19	10.37	18.05	10.97	9.14	7.04	4.87	3.93	4.27
Heart	12.85	15.38	5.53	9.01	7.32	6.37	6.30	5.89	1.02	0.48
Kidney	11.99	15.19	5.83	7.40	6.16	7.79	5.21	5.96	0.95	1.83
Lung	10.75	13.75	3.73	4.63	7.02	9.12	5.04	6.53	1.98	2.59
Testes	10.32	14.97	3.19	7.74	7.13	7.23	6.03	6.19	1.10	1.04
Liver	11.67	13.90	5.18	7.70	6.49	6.20	6.05	5.85	0.44	0.35
Thymus	9.72	10.75	5.23	6.75	4.49	4.00	3.63	3.28	0.86	0.72
Spleen	6.93	10.76	1.38	6.17	5.55	4.59	5.03	3.48	0.52	1.11
Skeletal muscle	5.95	8.57	1.42	4.84	4.53	3.73	4.05	3.56	0.48	0.17

[a] Adapted from H. H. Williams, H. Galbraith, M. Kaucher, E. Z. Moyer, A. J. Richards, and I. G. Macy, *J. Biol. Chem.*, *161*, 475–484 (1945).

[b] 88 to 116 rats in 15-day groups.

[c] 6 rats in 70-day groups.

TABLE 11

DISTRIBUTION OF STORAGE FAT IN MALE AND FEMALE RATS ON A LOW-FAT DIET (CORN) AND ON A HIGH-FAT DIET (CRISCO)[a]

No. of rats	Body wt., g.	Age, days	Sex	Total fatty acids: Wt., g.	Total fatty acids: % body wt.	Proportion of depot fat (fatty acids) to total depot fat (fatty acids), %: Intermuscular	Genital	Subcutaneous	Perirenal	Mesenteric	Omental
					Rats on corn starch diet						
3	50	25	M	2.55	5.09	3.5	3.9	85.0	3.7	2.6	1.2
2	150	48		7.68	5.10	9.0	10.2	64.2	7.8	6.0	2.6
2	250	90		28.44	11.08	7.6	12.0	56.8	14.0	7.2	2.2
3	50	25	F	2.81	5.61	3.3	3.0	88.8	2.3	1.8	0.7
2	150	51		7.70	5.14	8.5	11.6	65.1	6.3	5.9	2.4
2	250	145		26.74	10.90	2.9	18.2	63.6	7.9	5.2	2.2
					Rats on Crisco diet						
3	50	25	M	2.61	5.22	3.1	5.5	84.1	3.3	3.0	1.0
2	150	49		14.13	9.40	6.1	11.1	65.4	8.4	6.0	2.9
2	250	96		34.40	13.76	5.6	13.6	53.9	18.8	5.0	3.0
3	50	25	F	3.04	6.09	2.8	3.6	87.0	3.2	2.4	0.8
2	150	46		13.62	9.08	6.9	12.9	63.8	8.2	5.7	2.4
2	250	141		31.05	12.53	5.8	17.4	58.5	11.3	4.7	2.6

[a] Adapted from L. L. Reed, F. Yamaguchi, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *87*, 147–174 (1930).

(5) *Factors Altering the Distribution, Content, and Composition of Tissue Lipids*

a. The Effect of Age. (*a*) *Distribution of Tissue Lipids as Affected by Age.* In young animals of both sexes, the subcutaneous fat comprises 85 to 90% of the total storage fat. The quantities of intramuscular, genital, and perirenal fat are especially low in young rats when compared with the values in the adult animals. The proportion of subcutaneous fat was reduced to 65% in all tests when the animals were at the 150 g. level; this decrease was accompanied by an increase in the proportion of storage fat in the other fat depots. A still further readjustment occurs in the 250 g. rat. The same relationship exists, irrespective of whether or not the rats have been receiving a low fat diet (corn) or a high-fat diet (Crisco). These data are summarized in Table 11.

(*b*) *Content of Tissue Lipids as Affected by Age.* No reliable data are available on the relative fat content of the tissues of children during infancy or childhood. Reynolds[253] noted that the thickness of the subcutaneous fat, as determined by x-ray pictures of the calf of the leg, is increased during infancy; a decrease occurs during childhood, and this is followed by another rise during adolescence, and a postadolescent leveling off.

However, considerable data demonstrate in an unequivocal manner that the fat content of the adult increases progressively with advancing years. Making use of tritium as the test solute, for the indirect determination of the fat content, Prentice *et al.*[240] found that the water content of four young men averaging 22.2 years of age was 60.4%, as contrasted with a value of 52.1% for fifteen men who had a mean age of 44.5 years. Similar variations were obtained by Edelman *et al.*[254] over a wider age span when deuterium oxide was employed as the test solute. Thus, the following values were obtained for the water content of human tissues: 25.0 yr. (34 men), 61.1%; 40.9 yr. (10 men), 55.4%; and 66.0 yr. (6 men), 54.3%. In the case of women, the same trend was observed but there was a consistently lower water content (and presumably a higher fat content) throughout, *viz.*, 28.8 yr. (18 women), 51.2%; 47.3 yr. (6 women), 48.2%; and 73.0 yr. (5 women), 46.2%.

The results obtained by the use of specific gravity measurements, by Brožek,[255] for five age groups have given results in conformity with those listed above; the fat was computed by the formula of Rathbun and Pace.[186] These data are as follows: 20.3 yr., 9.9% fat; 25.2 yr., 14.4% fat; 46.0

[253] E. L. Reynolds, *Anat. Record, 100*, 621–630 (1948).

[254] I. S. Edelman, H. B. Haley, P. R. Schloerb, D. B. Sheldon, B. J. Friis-Hansen, G. Stoll, and F. D. Moore, *Surg. Gynecol. Obstet., 95*, 1–12 (1952).

[255] J. Brožek, *Federation Proc., 11*, 784–793 (1952).

TABLE 12

TOTAL LIPIDS AND ESSENTIAL LIPID CONTENT OF NEWBORN RATS AND OF RATS 15, 45, AND 70 DAYS OF AGE[a]

Lipid constituent	Lipid content (%) on dry basis				Essential lipids (%) on water-free, neutral fat-free basis			
	Newborn	15 days old	45 days old	70 days old	Newborn	15 days old	45 days old	70 days old
Number of rats	28	6	4	2	28	6	4	2
Total lipid	21.26	26.68	38.23	40.72	—	—	—	—
Neutral fat	11.72	19.60	32.34	35.50	—	—	—	—
Essential lipids	9.54	7.08	5.89	5.22	10.81	8.80	8.70	8.09
Cerebrosides	1.15	1.17	1.07	1.04	1.30	1.46	1.58	1.61
Free cholesterol	0.95	0.49	0.34	0.25	1.08	0.61	0.50	0.39
Cholesterol esters	0.42	0.22	0.22	0.30	0.48	0 27	0.33	0.47
Phospholipid	7.02	5.20	4.26	3.63	7.95	6.46	6.29	5.62
Cephalin	1.37	2.13	2.50	1.82	1.55	2.65	3.69	2.82
Lecithin	5.22	2.60	1.42	1.35	5.91	3.23	2.10	2.09
Sphingomyelin	0.43	0.47	0.34	0.46	0.49	0.58	0.50	0.71

[a] Adapted from H. H. Williams, H. Galbraith, M. Kaucher, and I. G. Macy, *J. Biol. Chem.*, *161*, 463–474 (1945).

yr., 22.2% fat; 50.0 yr., 24.0% fat; and 54.6 yr., 25.2% fat. In a later study made by Brožek and associates[256] on sixty-two women, who were in age groups 24.2, 39.1, and 56.0 years, the specific gravities were 1.0459, 1.0336, and 1.0218, which correspond to values of 26.1, 32.4, and 38.8% of the body weight accounted for by fat, as calculated by the Rathbun-Pace formula.[186] When a linear prediction equation was employed, the mean values for fat in the case of women twenty-five, thirty-five, forty-five, and fifty-five years of age were reported to be 26.5, 30.5, 34.5, and 38.5%, respectively.

The average skinfold measurements made at ten sites showed mean values of 19.5, 23.3 and 24.9 mm., respectively, with increasing age.[166]

(c) *The Composition of Tissue Lipids as Affected by Age.* Marked alterations also occur in the chemical composition, as well as in the total amount of the body lipid during growth and aging. Such changes as those which occur during the early days of life are, without question, of a physiologic nature. On the other hand, it is considerably more difficult to evaluate the alterations in tissue composition in the aged, inasmuch as many of the changes are the result of disease, and hence are unphysiologic and unpredictable. Moulton,[257] who has carried out extensive studies in the field, concluded that mammals "show a rapid decrease in relative water content, and an increase in protein (nitrogen) and ash content from earliest life until the time of chemical maturity is reached." However, the interpretations of Moulton may be open to some question, since they are all based upon data calculated on the fat-free basis. This same investigator likewise notes that the most striking change in the chemical composition of the body of mammals associated with growth and development is an increase in the fat content. Although it is probably justifiable to exclude adipose tissue from the discussion, it is questionable whether one should exclude the so-called essential lipids which represent the *élément constant* (see page 597). Williams and associates[148] showed that, although the proportion of total lipids and likewise of essential lipids does decrease with age, cerebrosides and cholesterol esters actually increase coincident with a decrease in phospholipids and free cholesterol. McCay[258] has written an excellent review on the biological and medical aspects of the subject of aging.

Williams *et al.*[148] determined the content of various lipids in the whole

[256] J. Brožek, K. P. Chen, W. Carlson, and F. Bronczyk, *Federation Proc.*, *12*, 21–22 (1953).

[257] C. R. Moulton, *J. Biol. Chem.*, *57*, 79–97 (1923).

[258] C. M. McCay, in E. V. Cowdry, *Problems of Aging, Biological and Medical Aspects*, 2nd ed., Williams & Wilkins, Baltimore, 1942.

rat at birth, and at fifteen, forty-five, and seventy days of age. Their data are summarized in Table 12 (page 574).

The most striking change in the composition of rats during the first seventy days of life is the tremendous increase in the fat content. Thus, the percentage of total lipids practically doubles over this interval, increasing from 21.3% at birth to 40.7% at seventy days of age. When one takes into consideration the great increase in body weight which occurs during this period, one finds that the actual amount of lipid material accumulated is much greater than is indicated by the increased percentage.

The neutral fat continues to be the principal lipid constituent. In this category, there is more than a three-fold increase, namely from 11.7% at birth to 35.5% at seventy days. The neutral fat fraction is deposited in adipose tissues, in contradistinction to the essential lipids, which are concerned with cell structure. In this latter group of lipids, the cholesterol esters are highest at birth, and lowest at fifteen days, after which they return to their initial level at seventy days. In the case of phospholipids and free cholesterol, a synthesis must occur, since the total content of the body is greatly increased after seventy days. However, since the rate of increase is somewhat less than for protein and other structural elements, the percentage of the above lipids is somewhat reduced as the animal becomes older.

On the other hand, cerebrosides continue to increase in amount during the entire period of the test, while the proportion of cephalin rises up to the forty-fifth day. The increased concentration of both of these components can be explained largely by the fact that the muscle tissue, which contains a relatively high amount, represents a higher percentage of body weight in the seventy-day-old rat than in the newborn animal. Thus, according to Donaldson,[259] the musculature at birth accounts for 24% of the body weight while, at seventy-eight days of age, it comprises 41% of the total body substance.

Although considerable variations obtained in the amount of fat laid down by rats from the twenty-second to the eighty-eighth day of life when they were fed a high-fat *vs.* a high-carbohydrate diet, the distribution of lipids was quite similar. Whereas the total lipids amounted to 16.58% at twenty-two days, the figures were 44.80% for those on an adequate diet, 49.10% for those on the high-fat diet, and 36.01% for those on the high-carbohydrate diet, after each had been fed 3000 Calories of their respective diets. On the other hand, the proportion of essential lipid components on

[259] H. H. Donaldson, *The Rat*, Memoirs Wistar Inst. Anat. Biol., 2nd ed., Philadelphia, 1924.

the dry, neutral fat-free basis was 8.62% (control, twenty-two days), 7.13% (adequate diet), 7.19 (high-fat diet), and 5.51% (high-carbohydrate diet). In confirmation of the above results, Dvorak[260] reported that total lipids (especially glycerides) increase most rapidly in very young rats (before eyes are open), while dry matter increases more rapidly in somewhat older rats (after eyes are open). He believes that this variation is related to the state of development of the hypophysis.

In another report of Williams *et al.*[261] in which the lipid concentration of the several individual organs in rats was correlated with increasing age, it

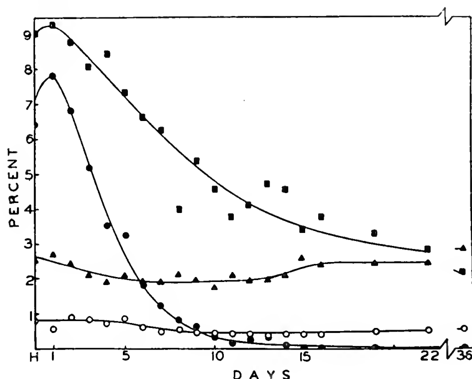


Fig. 2. Liver lipids of the chick from the day of hatching (Day H) to 36 days of age: (■) total fatty acids; (●) esterified cholesterol; (○) free cholesterol; (▲) phospholipids. The values are expressed in per cent of the wet weight of the tissue. Each point represents the average values obtained in 2 to 5 pools, each of which contained 2 to 4 livers. More pools were taken from younger than from older chicks.[262]

was shown that the fundamental change, common to all tissues, was the increase in cephalin content. An increase in cerebrosides occurred in testes and skeletal muscle, while a decrease in this component obtained in cardiac muscle. There was an increased amount of sphingomyelin in kidney, lung,

[260] Z. Dvorak, *Nature*, *171*, 432–433 (1953).

[261] H. H. Williams, H. Galbraith, M. Kaucher, E. Z. Moyer, A. J. Richards, and I. G. Macy, *J. Biol. Chem.*, *161*, 475–484 (1945).

[262] C. Entenman, F. W. Lorenz, and I. L. Chaikoff, *J. Biol. Chem.*, *133*, 231–241 (1940).

and spleen, whereas this phospholipid, together with free cholesterol, decreased in both skeletal and cardiac muscle. All essential lipid components except lecithin and cholesterol esters were found to increase in the brain during growth.

Fundamental differences distinguish the lipid metabolism of birds from that of mammals. Entenman, Lorenz, and Chaikoff[262] reported that, in contradistinction to the rat, the blood and liver lipids of chickens are exceedingly high at the time of hatching. The total liver lipids of the newly hatched chicks averaged 13 to 14.7%, based upon wet weight, while values as high as 23% were reported immediately after hatching. The high lipid

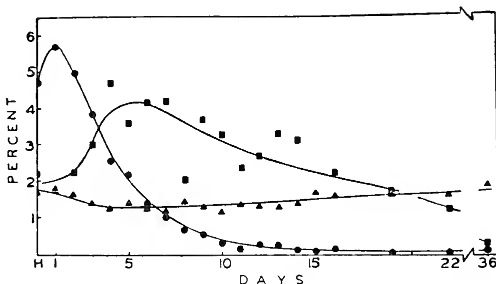


Fig. 3. Liver fatty acids present as triglycerides, cholesterol esters, and phospholipid: (●) cholesterol ester fatty acids in per cent; (▲) phospholipid fatty acids; (■) neutral fat fatty acids. Day H is day of hatching.[262]

content was maintained for only a short period after hatching; in most cases a decline had already begun by the fourth day, and in all cases the decrease was quite definite by the sixth day. The values for liver lipids in the eight-day chick were 5.0 to 5.6%, while those on the twenty-second and thirty-sixth day after hatching were between 1.6 and 3.2%. The changes in the composition of the livers are indicated in Figures 2 and 3.

In examining the composition of the lipids of the livers of the newly hatched chicks, one is impressed by the enormous concentration of cholesterol. During the first three days after hatching, values of 5.9 to 9.7% of total cholesterol were recorded.[262] At this time, cholesterol comprised as much as 48% of the total liver lipids. After three days, the decrease in cholesterol content was rapid, with values of 1.0% recorded for the nine-day-old and 0.5% for the eleven-day-old chick. Cholesterol levels in the

livers of the twenty-two-day and thirty-six-day-old chicks varied from 0.3 to 0.4%, which approximates the value in mature birds.

Another variation in the lipid pattern of the chicken immediately after hatching is the fact that the major portion of the cholesterol is in the esterified form. The values of esterified cholesterol were not decreased to that of adult animals until the birds were fourteen days of age. The decrease in cholesterol ester was associated with an increase in the triglyceride content of the liver.

b. The Effect of Embryonic Development. The lipid composition of the early embryo varies markedly from that of the newborn animal. The phospholipid content has been shown to be highest, on the dry weight basis, in the very young pig fetus[263]; the proportion of this component drops to 50% of the original level at term. The fatty acids in this fraction were shown to have an iodine number of 82.

Although the non-phospholipid fatty acids have usually been considered to be neutral fat, Gortner[263] reported that they are largely free fatty acids which are present in unesterified form. The fetal glycerides do not begin to assume importance until the middle of gestation, after which they gradually increase until term. However, even in the newborn pig, the glycerides account for only a minor proportion of the total lipids.

On the other hand, the unsaponifiable lipids in the pig fetus gradually decrease proportionately as gestation proceeds. There is a parallel drop in the amount of free and total cholesterol. The total lipid and the lipid: protein ratio remain constant for a large part of the embryonic growth period.

c. The Effect of Sex. (*a*) *The Effect on Total Fat.* The fat content of the female tends to be higher than that of the male. Thus, in the experiments of Deuel *et al.*,[249] the total tissue lipids of female rats on several fat diets exceeded those of males on similar regimens, while the males showed a somewhat higher water and protein content than did the females. Making use of a specific gravity method for the calculation of body fat, Rathbun and Pace[186] found that female guinea pigs average 4.7% more body fat than do the males. Moreover, Jamin and Müller[264] stated that the specific gravity of the partial body volume (not including the head) was higher in men (1.095) than in women (1.081). Presumably, the fat content varies inversely with the specific gravity.

Although the relationship between sex and lipid content seems to be a general one in different species, there is some evidence that the variation

[263] W. A. Gortner, *J. Biol. Chem.*, *159*, 135–143 (1945).
[264] F. Jamin and E. Müller, *Münch. med. Wochschr.*, *50*, 1454–1457, 1511–1515 (1903).

may be greater in some laboratory animals, such as the rabbit and rat,[249,265] than in other species. Kraybill and associates[229] likewise noted a sex difference in the fat content of cattle. Thus, the average total fat content of six Hereford heifers was found to be 30.5% as contrasted with a value of 25.9% for seven steers of the same species.

(*b*) *The Effect on Subcutaneous Fat.* The higher content of fat in the female than in the male is especially evident from the thickness of the subcutaneous fat layer. In the case of human subjects, Wilmer[167] reported that the subcutaneous layer of fat accounted for only 11.5% of the total body weight in man and 23.7% of the total body weight in women. Similar differences have been noted in the results based upon roentgenographic determination of subcutaneous fat layers. In a group of one-hundred men and one-hundred women whose average age was approximately thirty-eight years, Reynolds and Asakawa[266] demonstrated a greater fat thickness in the broadest portion of the calf in women (20.5 mm.) as compared with men (10.8 mm.), while an opposite relationship obtained as regards the breadth of the muscle (67.4 *vs.* 60.3 mm.) and that of bone (39.4 *vs.* 32.5 mm.). The index of fat thickness to bone breadth was 27.6 for men and 62.2 for women. Reynolds[267] reported that this sex variation appears early. Thus, the ratio of fat thickness to bone index was found to be the following for males and females, respectively: 7.5 yr., 44 and 51; 10.5 yr., 42 and 52; 13.5 yr., 38 and 51; 16.5 yr., 30 and 59; and young adults, 28 and 61. An examination of Figure 4 will convince one that the sex variation in thickness of the subcutaneous fat layer is generalized, and occurs at as early an age as 7.5 years. Keys and Brožek[166] state that the thickness of the subcutaneous tissues in the Dutch population immediately after the liberation of Leiden and of the Hague, as investigated by the Oxford Survey Team in World War II was, on an average, 3.7 mm. for men and 5.4 mm. for women.

Edwards[268] reported that skinfold measurements made at fifty-three sites averaged 7.8 mm. for young men and 12.1 mm. for women. The latter value for women is 155% higher than that for men. Edwards[268] showed that men had relatively larger fat deposits on the trunk while, in the case of women, the extremities and especially the legs, had the greater fat deposits.

In a personal communication to Keys and Brožek,[166] from the Division of Chronic Diseases and Tuberculosis of the U. S. Public Health Service, the following average values were noted for skin-fold thickness in 103 negro

[265] C. M. Spray and E. M. Widdowson, *Brit. J. Nutrition, 4,* 332–353 (1950).
[266] E. L. Reynolds and T. Asakawa, *Am. J. Phys. Anthropol., 8 (n.s.),* 343–365 (1950).
[267] E. L. Reynolds, *Human Biol., 21,* 199–204 (1949).
[268] D. A. W. Edwards, *Clin. Sci., 10,* 305–315 (1951).

adult males and 200 adult females, respectively: dorsal part of the upper arm, 9.8 and 14.9 mm.; above the iliac crest along the midaxillary line,

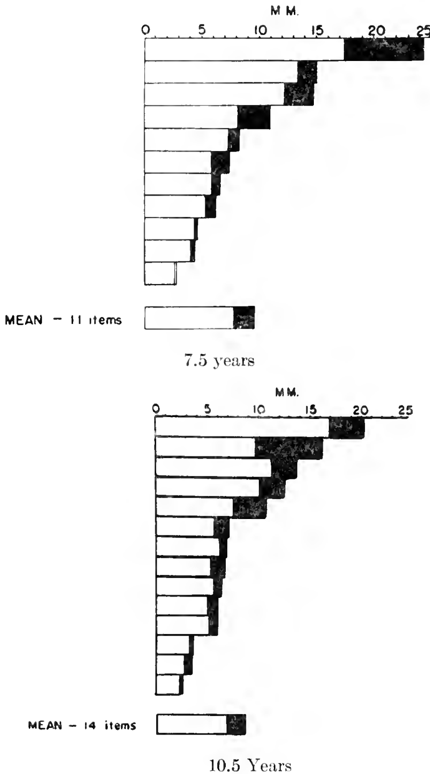


Fig. 4. Means for the breadth of subcutaneous fat in 11 and 14 body areas of boys and girls 7.5 years of age or 10.5 years of age. The dark areas represent the excess of the average values for girls over that for boys.[182]

13.7 and 23.3 mm.; and below the scapula, 12.9 and 20.3 mm. The relative thicknesses of the subcutaneous fat layer in women at these three sites were 152, 170, and 157% respectively, of the values for men.

The studies on the oldest group of men (54.6 yr.) and women (56.0 yr.) investigated by Brožek[255] also show that the greater thickness of the subcutaneous layer in women is a generalized phenomenon. The following figures are reported for the breadth of the fat layer in these several areas in men and in women respectively[166]: above the patella, 9.4 and 17.2 mm.; dorsal part of upper arm, 15.4 and 27.0 mm.; and abdomen, 26.0 and 34.8 mm. The upper trunk showed lesser variations. Additional data of Brožek *et al.*[256] have been cited earlier (see page 575).

De Smet[269] observed that, following castration, male rats present an increase in the fat content under the skin and around the kidneys, while there is a concomitant decrease in abdominal fat. When female rats are ovariectomized, an increase in the fat storage in the abdominal and kidney areas occurs, while the carcass fat shows a simultaneous decrease. In a later report, De Smet[270] noted that, following castration in adult male rats, an initial decrease in total lipids occurred; this was followed by an increase. In the case of female rats, an immediate rise in total body lipids was noted, following the removal of the gonads.

(*c*) *The Effect on the Fat Content of Specific Tissues.* Greisheimer[271] was the first to point out that male rats fed on various diets had a higher level of liver glycogen and a lower figure for liver fat than was noted in the female animals. An analysis of the data of Ponsford and Smedley-MacLean[272] on the liver glycogen and liver fat in rats after the administration of the salts of several organic acids is especially convincing, although these investigators do not comment on this fact. The liver fats (expressed in per cent) for the male and female rats, respectively, in the several groups were as follows: control group, 7.43, 8.30; acetate group, 4.63, 6.24; fumarate group, 3.10, 4.97; malate group, 4.04, 5.09; succinate group, 3.90, 4.81; and glucose group, 4.34, 5.73. Although Deuel and co-workers[273] were unable to demonstrate a sex difference in the level of liver lipids of unfasted rats, they found increasingly higher values for liver lipids in the females, as compared with the males, during a subsequent fasting period. In the experiments of Gulick *et al.*[274] on ovariectomized rats, the values for liver glycogen (which behaves in a manner opposite to that of liver lipids) of fasted operated animals were similar to those for males, thus indicating that

[269] J. De Smet, *Compt. rend. soc. biol.*, *147*, 542–544 (1953).
[270] J. De Smet, *Compt. rend. soc. biol.*, *147*, 726–729 (1953).
[271] E. Greisheimer, *J. Nutrition*, *4*, 411–418 (1931).
[272] A. P. Ponsford and I. Smedley-MacLean, *Biochem. J.*, *26*, 1340–1344 (1932).
[273] H. J. Deuel, Jr., M. Gulick, C. F. Grunewald, and C. H. Cutler, *J. Biol. Chem.*, *104*, 519–530 (1934).
[274] M. Gulick, L. T. Samuels, and H. J. Deuel, Jr., *J. Biol. Chem.*, *105*, 29–34 (1934).

a control is exerted by the female sex hormones over carbohydrate metabolism. The sex difference in liver glycogen in normal rats is shown by markedly higher values for males than for females, except in immature and old rats, in which cases the figures are the same.[275] Blatherwick and associates[276] likewise confirmed the sex differences in liver glycogen values in this species.

Further evidence of sex variation in the composition of the liver lipids is to be found in later work of Deuel *et al.*[277]; in these experiments, fatty livers were produced by dietary means. A sex difference in glycogen content was consistently observed in the livers of *unfasted* rats fed choline-free diets containing different fats, in that higher levels were observed in the male rats as compared with the females. Furthermore, the liver lipids were usually found to be considerably higher in the female rats than in the males.[277] Similar variations in liver lipids have been recorded by Best *et al.*[278] Shorland[279] recorded higher values for lipids in the livers of the female New Zealand grouper (*Polyprion oxygeneios*) than in the male fish, although the differences were not statistically significant.

Several workers have noted that different lipids have separate sex patterns. Thus, Okey and co-workers[280] reported that, in spite of the fact that the total lipid in the livers of female rats was consistently higher than in male animals, total cholesterol was higher in the males. This occurred both on a normal diet in which the cholesterol levels were 1.2 and 1.0 milligram per cent respectively, for the livers of males and of females, and on a regimen containing 1% of cholesterol, in which the liver cholesterol figures were 15.2 and 11.7 milligram per cent, respectively. Likewise, the lecithin values were slightly higher in the livers of male rats than in females. Obviously, the neutral fat fraction must be considerably higher in the female than in the male to account for the higher total lipid content in the former case. The results obtained by Okey *et al.*[280] have been confirmed by Barnes, Miller, and Burr,[281] who noted that the higher lipid value in the females as compared with the males was confined to the acetone-soluble fraction (neutral fat), while it was not observed in the phospholipid fraction.

[275] H. J. Deuel, Jr., J. S. Butts, L. F. Hallman, S. Murray, and H. Blunden, *J. Biol. Chem.*, *119*, 617–620 (1937).

[276] N. R. Blatherwick, P. J. Bradshaw, O. S. Cullimore, M. E. Ewing, H. W. Larson, and S. D. Sawyer, *J. Biol. Chem.*, *113*, 405–410 (1936).

[277] H. J. Deuel, Jr., L. F. Hallman, and S. Murray, *J. Biol. Chem.*, *119*, 257–268 (1937).

[278] C. H. Best, J. H. Ridout, J. M. Patterson, and C. C. Lucas, *Biochem. J.*, *48*, 448–452 (1951).

[279] F. B. Shorland, unpublished observations, 1953.

[280] R. Okey, H. L. Gillum, and E. Yokela, *J. Biol. Chem.*, *107*, 207–212 (1934).

[281] R. H. Barnes, E. S. Miller, and G. O. Burr, *J. Biol. Chem.*, *140*, 247–253 (1941).

Holt[282] stated that the extent of fat storage in the liver of a female with congenital idiopathic hyperlipemia was considerably greater than in the case of two brothers who also suffered from this abnormality.

Another expression of sex difference in lipid storage is to be found in the studies of Lorenz, Chaikoff, and Entenman.[283] These workers reported that the level of neutral fat was much higher in the livers of laying hens than in non-laying hens or in male birds. Although no similar variation in phospholipid and cholesterol obtained in the liver under these conditions, a significant increase in the values of these lipid components, as well as of neutral fat in the blood, was noted.

Loeb and Burr[284] reported that the fatty acids in the neutral fats of the rat carcass are selectively affected by sex. Thus, although no sex differences could be established in the degree of unsaturation of fatty acids obtained from the body fat of rats raised on diets of 20% lard, hydrogenated coconut oil, or on fat-free diets, it was reported that, on a high-fat diet deficient in essential fatty acids, females stored more fat than did males. It was suggested that the males are more sensitive to a deficiency in essential fatty acids than are females, when both derive the bulk of their calories from fat.

(*d*) *The Effect of Ovariectomy on the Distribution of Storage Fat.* It is generally agreed that removal of the ovaries results in obesity. For example, Stotsenburg[285] reported that ovariectomized rats presented more fat at autopsy than did the non-spayed control rats. Slonaker[286] likewise noted that castrated female rats had a greater increment in body weight than did normal animals, but he suggested that this could be attributed to a greater increase in body length as well as to fatness. On the other hand, Marshall[287] did not note any marked tendency to fattening in ovariectomized rats. Finally, Reed, Anderson, and Mendel[288] reported a slight increase in body fat, together with a very considerable increase in body weight, following the removal of the ovaries. However, a marked decrease in genital fat was observed in the operated animals, which was compensated for by a proportionate augmentation of the subcutaneous fat. These experiments are summarized in Table 13.

[282] L. E. Holt, Jr., personal communication to the author, 1953.
[283] F. W. Lorenz, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *123*, 577–585 (1938).
[284] H. G. Loeb and G. O. Burr, *J. Nutrition*, *33*, 541–551 (1947).
[285] J. M. Stotsenburg, *Anat. Record*, *7*, 183–194 (1913).
[286] J. R. Slonaker, *Am. J. Physiol.*, *93*, 307–317 (1930).
[287] F. H. A. Marshall, *The Physiology of Reproduction*, 2nd ed., Longmans, Green, London-New York, 1922, pp. 390, 391.
[288] L. L. Reed, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *96*, 313–323 (1932).

TABLE 13
THE EFFECT OF OVARIECTOMY AND OF THYROXINE FEEDING ON THE DISTRIBUTION AND QUANTITY OF FAT IN FEMALE RATS[a]

Category	Series I		Series II		
				Thyroxine-fed rats	
	Normal female rats	Ovari-ectomized rats	Normal female rats	Started at 100 g.	Started at 200 g.
Number of rats	5	7	4	4	4
Final body weight, g.	216	252	222	188	235
Proportion of total depot fat, %					
Intermuscular	8.8	8.7	8.7	6.6	8.9
Genital	22.4	12.9	22.0	23.2	21.5
Subcutaneous	44.3	56.0	44.9	43.7	42.2
Perirenal	13.5	13.0	14.8	16.0	15.9
Mesenteric	7.9	6.6	5.6	7.7	8.8
Omental	3.2	2.5	2.5	3.1	2.7
Depot fat, % body wt.	12.6	14.2	13.8	6.3	6.4

[a] Adapted from L. L. Reed, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *96*, 313–323 (1932).

Although the effect of ovariectomy in increasing the total body fat is not very marked in the rat, there can be no doubt that the operation results in a marked redistribution of fat. Reed *et al.*[288] found no change in the character of the fat following ovariectomy, since the iodine numbers of the fat of the experimental and of the control animals were practically identical. Apparently obesity results from gonadectomy to a greater extent in fowls than in the rat. According to Korenchevsky[289] "birds develop obesity so regularly after the removal of the sexual glands that this operation is employed commercially for fattening animals for food."

(*e*) *Sex Differences in Fat Metabolism.* In addition to variations in the total amount and the distribution of lipids, as related to sex, there is considerable evidence that a sex difference obtains in the metabolism of this foodstuff. The metabolic difference may be related to the variations in fat storage. A more intense fat oxidation can be demonstrated in the female than in the male, during fasting. This accelerated rate of fat breakdown is indicated by the markedly higher degree of ketonuria which occurs in fasting women as contrasted with fasting men. Deuel and Gulick[290] reported that the average ketone body excretion during fasting, based upon surface area, is approximately five times as great in the case of women as in men. A similar sex difference in ketonuria has likewise been demonstrated in the exogenous ketonuria produced when acetoacetic (diacetic) acid, bu-

[289] V. Korenchevsky, *Brit. J. Exptl. Pathol.*, *6*, 21–35 (1925).
[290] H. J. Deuel, Jr., and M. Gulick, *J. Biol. Chem.*, *96*, 25–34 (1932).

tyric acid, or other ketogenic acids are given as their sodium salts to fasting rats and guinea pigs.[291] Beach and co-workers[292] recently confirmed this sex difference in ketonuria occurring in alloxan diabetes. Moreover, the high ketonuria noted in the normal female rat was reduced following ovariectomy to the value found in the normal male animals.[293] Finally, a similar sex difference was also noted in the endogenous ketonuria occurring in rats with fatty livers produced by fasting without the administration of supplementary ketogenic acids.[277] Although these data do not prove that a sex difference obtains in the lipid composition of rats, they do indicate that a quantitative variation must exist in the intermediary metabolism of lipids as related to sex.

Finally, the relationship of the higher lipid values in the liver to the increased level of fat metabolism in the female as contrasted with the male animal is evident from later studies by the Deuel group.[294] After the production of fatty livers in male and female rats by the administration of a choline-free, high-fat diet, a greater decline in the value of liver lipid obtained in the females than in the males during a subsequent fasting period. This increased rate of decrease in liver lipid in the female occurs coincidentally with the higher level of endogenous ketonuria produced during this period, as compared with that in the males.

d. The Effect of Environmental Temperature. (*a*) *The Effect on Animal Fats.* There is some evidence that environment may also play a role in determining the type of fat laid down in the animal. Thus, the fat of fishes living in cold water and that of aquatic animals which inhabit the Arctic regions have a much lower melting point than do those from animals from tropical regions. Lovern[295] has shown that the fat from the tuna or tunny (*Thunnus thynnus*), which spends a considerable portion of time in relatively warm water, and which likewise has a body temperature 3°C. higher than the water, differs from that of other marine fats in several respects. This fat contains less myristic acid, more palmitic acid, and an unusual proportion of stearic acid. In the unsaturated acids, the average unsaturation is less than that in other marine animals. All of these differences in composition tend to produce a fat having a higher melting point.

[291] J. S. Butts and H. J. Deuel, Jr., *J. Biol. Chem.*, *100*, 415–428 (1933).
[292] E. F. Beach, P. J. Bradshaw, and N. R. Blatherwick, *Am. J. Physiol.*, *166*, 364–373 (1951).
[293] C. F. Grunewald, C. H. Cutler, and H. J. Deuel, Jr., *J. Biol. Chem.*, *105*, 35–43 (1934).
[294] H. J. Deuel, Jr., S. Murray, L. F. Hallman, and D. B. Tyler, *J. Biol. Chem.*, *120*, 277–288 (1937).
[295] J. A. Lovern, *Biochem. J.*, *20*, 2023–2026 (1936).

Henriques and Hansen[18] reported that fat from the back of the pig has a melting point related to the environmental temperature. In one pig, kept in a room at 30 to 35°C. for two months, the iodine number of the fat was 69.4. In a litter-mate kept on the same diet at an environmental temperature of 0°C., the iodine value of the back fat was 72.3 while, in a third pig from the same litter, which was also maintained at 0°C. but which was protected by a fur coat, the iodine value of the fat was only 67.0. Thus, it was suggested that a low environmental temperature tends to result in the production of a low-melting fat (high iodine number), while more elevated outside temperatures cause the production of higher-melting fats having lower degrees of unsaturation.

Variations in the melting point of a fat may be related to the site at which it is deposited in the body. In birds, where the skin is well protected from the effect of external temperature, the depot fats have a fairly uniform composition, irrespective of whether they represent samples from internal organs or from superficial tissues. On the other hand, in the pig, which does not possess an external covering, corresponding to feathers, for protection from cold, the perinephric fat has the highest melting point, while that from the outermost back fat has the lowest melting point. The differences in melting point and iodine value for fats obtained from different sites in the pig are shown in Table 14.

TABLE 14
A COMPARISON OF THE SOLIDIFYING POINT AND IODINE VALUES OF FAT WITH THE TEMPERATURE OF THE TISSUES OF THE PIG[a]

Source of body fat	Solidifying pt., °C.	Iodine value	Site for detn. of body temp.	Body temp., °C.
Outer layer of back fat			Back tissue	
1st layer	—	60.0	1 cm. deep	33.7
2nd layer (inner)	26.4	57.1	2 cm. deep	34.8
Inner layer of back fat				
3rd layer	28.0	51.8	3 cm. deep	37.0
4th layer	27.7	50.6	4 cm. deep	39.0
Perinephric fat	29.6	47.7	Rectum	39.9

[a] V. Henriques and C. Hansen, *Skand. Arch. Physiol., 11,* 151–165 (1901), pp. 155, 161.

The results of Henriques and Hansen[18] are in agreement with those of Lummert,[20] and have been confirmed by Schirmer[296] and by Dean and Hilditch.[80] However, the latter workers reported that the unsaturation

[296] O. Schirmer, *Arch. exptl. Pathol. u. Pharmakol. (Naunyn-Schmiedeberg's), 89,* 263–279 (1921).

was confined to the outermost layer of the outer back fat, and was not noted in the inner layer. Thus, the iodine number of five samples of sow back fat representing layers progressively deeper below the surface were 70.4, 67.4, 62.9, 63.0, and 62.8. In the deeper samples, some increase was noted in the proportion of the saturated C_{16} and C_{18} acids, while a definite decrease obtained in the oleic and linoleic acid content, as well as in the unsaturated C_{20}–C_{22} acids.

On the other hand, the fats laid down in the several fat depots of the rat were shown by Reed *et al.*[250] to have similar iodine numbers. The differences found between the samples from the diverse fat depots were less than the variations between the fats from any one depot obtained from different rats within the same group. The similarity in deposit fat in different locations likewise applies for the dog and for the duck.

Although the temperature of the tissues may to some extent be associated with the hardness of the fat deposited, this is not invariably the case. Such warm-blooded marine animals as the Indian dugong or sea-cow (*Halicore dugong*), which has a body temperature of 102° to 104°F., and the porpoise (*Phocaena* spp.),[297] with a body temperature of 96° to 98.6°F., have fats similar to those of the cold-blooded fishes; the same series of highly unsaturated acids are present in both types of marine life. Although it is evident that body temperature may play some part in determining the nature of deposit fat, it is obviously a less important factor than is species or the type of food fat ingested.

(*b*) *The Effect on Vegetable Fats.* It is well known that the composition of vegetable oils may, to some extent, be related to the environmental conditions under which they are produced. The most important factor in determining the composition of such fats is temperature. Ivanov[297–302] and Juschkevitsch[303] are of the opinion that fats having a larger proportion

[297] S. Ivanov, *Bull. Appl. Bot. and Plant Breed. (Leningrad), 13, No. 2,* 483–491 (1922–1923); *Chem. Abst., 20,* 2349 (1926).

[298] S. Ivanov, *Fortschr. Naturwiss.-Forsch. (Abderhalden's) n.s., 5,* 1–39 (1929).

[299] S. Ivanov, *Ber. deut. Botan. Ges., 44,* 31–39 (1926); also cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats,* 2nd ed., Wiley, New York, 1947, p. 162.

[300] S. Ivanov, *Z. angew. Chem., 42,* 292 (1929).

[301] S. Ivanov, *Chem. Umschau Gebiete Fette, Öle, Wachse, u. Harze, 38,* 96–100 (1931); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats,* 2nd ed., Wiley, New York, 1947, p. 162.

[302] S. Ivanov, *Allgem. Oel-Fett-Ztg., 29,* 149–150 (1942); *Chem. Abst., 27,* 2053–2054 (1933); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats,* 2nd ed., p. 162.

[303] S. Juschkevitsch, *Fettchem. Umschau, 40,* 197–200 (1933); *Chem. Abst., 28,* 355 (1934); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats,* 2nd ed., p. 162.

of unsaturated acids (and hence a lower melting point) are produced in cold environments, while those produced in tropical or semitropical districts tend to have a higher proportion of saturated acids (and hence higher melting points). However, Hilditch[116] points out that some of the most highly unsaturated fats come from plants indigenous to the tropical or subtropical areas. Although it is improbable that fats solid at the prevailing temperatures would be produced by plants in the cooler districts, Hilditch does not consider that the evidence proves that high environmental temperatures *per se* cause or favor the production of more saturated fats. It is true that the seed oils from plants grown in either cold or hot climates have a greater proportion of characteristic unsaturated acids than do those produced in plants raised in areas where the environmental temperatures are intermediate between these extremes.

There are numerous instances in which oils from the seeds of species indigenous to cool climates have been shown to be more unsaturated than are those from warmer districts. This is true in the case of seeds from the several varieties of pine (*Pinus*).[304–312] The same relationship has been shown to exist in the seed oils of the *Moraceae* (mulberry family); the oil from hempseed (*Cannabis sativa*), which grows in temperate zones, is more highly unsaturated than is the seed oil from the African variety of breadfruit (*Treculia africana*),[313–315] which is native to the more tropical Sierra Leone.[316] Another example of a similar variation occurs in the seed oils from the bittersweet; the variety indigenous to North America (*Celastrus scandens*)[317] contains more linoleic and linolenic acids and less oleic and

[304] S. L. Ivanov and S. B. Resnikova, *Schriften zentral. biochem. Forsch.-Inst. Nahr. u. Genussmittelind.* (*U. S. S. R.*), *3*, 239–245 (1933); *Chem. Abst.*, *28*, 2557 (1934); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., p. 156.

[305] A. Eibner and F. Reitter, *Chem. Umschau Gebiete Fette, Öle, Wachse, u. Harze*, *33*, 114–124, 125–129 (1926); *Chem. Abst.*, *20*, 3243 (1926); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., p. 156.

[306] O. von Friedrichs, *J. Soc. Chem. Ind.*, *39*, 304 A (1920).

[307] O. von Friedrichs, *Svensk Farm. Tidskr.*, *23*, 445–451, 461–463, 500–505 (1919); *Chem. Abst.*, *14*, 205–206 (1920).

[308] G. V. Pigulevskiĭ and M. A. Ivanova, *J. Applied Chem.* (*U. S. S. R.*), *7*, 569–571 (1934); *Chem. Abst.*, *29*, 2007 (1935).

[309] M. Adams and A. Holmes, *Ind. Eng. Chem.*, *5*, 285–287 (1913).

[310] J. Semb, *J. Am. Pharm. Assoc.*, *24*, 609–613 (1935).

[311] A. H. Gill, *Oil & Soap*, *10*, 7–8 (1933); *Chem. Abst.*, *27*, 1223 (1933).

[312] H. Matthes and W. Rossié, *Arch. Pharm.*, *256*, 289–302 (1918).

[313] H. N. Griffiths and T. P. Hilditch, *J. Soc. Chem. Ind.*, *53*, 75–81 T (1934).

[314] H. P. Kaufmann and S. Juschkevitsch, *Z. angew. Chem.*, *43*, 90–92 (1930).

[315] P. Shestakov and P. Kupchinsky, *Z. deut. Öl.-Fett-Ind.*, *42*, 741–743, 774–776 (1922); *Chem. Abst.*, *17*, 889 (1923).

[316] M. B. Ichaporia, *Dissertation*, Liverpool, 1937; cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 162.

[317] C. Barkenbus and C. F. Krewson, *J. Am. Chem. Soc.*, *54*, 3993–3997 (1932).

saturated acids than does the variety native to India and the East Indies (*Celastrus paniculata*).[318] According to Hilditch,[116] a similar but less striking variation in unsaturation of seed oils as related to environmental temperatures can be noted for the several species of beech (*Fagaceae*), of argemone and poppy (*Papaveraceae*), of sesame (*Pedaliaceae*), of safflower (*Compositae*), of grape (*Vitaceae*), and even of the pea, or pulse (*Leguminosae*). Painter and associates[319–321] have ascribed the wide variations in the iodine number of linseed oils partly to the temperatures occurring during ripening, as well as to the conditions of moisture.

On the other hand, there is ample evidence that the most highly unsaturated fats are to be found in seed oils from tropical plants. In a plant family such as the *Rosaceae*, the oils from the rose-hip, or wildbriar (*Rosa canina*)[322] and blackberry seed (*Rubus caesius*),[323] which are from temperate zones, are much more saturated than are those from such tropical species as oiticica (*Licania rigida*),[324] "fungu" or "behurada" (*Parinarium campestre*) from Surinam, and "po-yoak" (*Parinarium sherbroense*) from Sierra Leone.[325,326] In the latter cases, triethenoid and tetraethenoid acids are to be found. A similar pattern obtains in the different members of the *Euphorbiaceae*. Thus, seed oil from the caper spurge (*Euphorbia lathyrus*),[327] which is a plant from the temperate zone, does not contain the highly unsaturated fatty acids (such as linolenic) present in the tropical Brazilian (Para) and Nigerian rubberseed (*Hevea brasiliensis*),[313,328,329] or those, such as elaeostearic, in the tung oils, *Aleurites cordata* (Chinese varnish tree),[330] *A. fordi*, and *A. montana* ("mu oil" or chinawood),[331,332] and in the West African gingerbread plum, or "neou" (*Parinarium macrophyllum*) which is used as a substitute for tung oil.[332]

[318] B. G. Gunde and T. P. Hilditch, *J. Chem. Soc., 1938*, 1980–1985.
[319] E. P. Painter, *Oil & Soap, 21*, 343–346 (1944).
[320] E. P. Painter and L. L. Nesbitt, *Ind. Eng. Chem., Anal. Ed., 15*, 123–128 (1943).
[321] E. P. Painter and L. L. Nesbitt, *Oil & Soap, 20*, 208–211 (1943).
[322] P. Vasterling, *Arch. Pharm., 260*, 27–44 (1922).
[323] R. Krzizan, *Chem. Rev. Fett- u. Harz.-Ind., 15*, 7–9, 29–30 (1908); *Chem. Abst., 2*, 1352 (1908); cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 166.
[324] J. Van Loon and A. Steger, *Chem. Umschau Gebiete Fette, Öle, Wachse, u. Harze, 37*, 337–340 (1930).
[325] A. Steger and J. Van Loon, *Rec. trav. chim., 57*, 620–628 (1938).
[326] A. Steger and J. Van Loon, *Fette u. Seifen, 49*, 769–770 (1942); *Chem. Abst., 38*, 1655 (1944).
[327] B. Tyutyunnikov, A. Sobel, and V. Erschova, *Masloboĭno-Zhirove Delo., 11*, 132–133 (1935); *Chem. Abst., 29*, 8375–8376 (1935).
[328] F. D. Gunstone and T. P. Hilditch, *J. Soc. Chem. Ind., 65*, 8–13 (1946).
[329] G. S. Jamieson and W. F. Baughman, *Oil & Fat Ind., 7*, 419–421, 437 (1930).
[330] R. S. McKinney and G. S. Jamieson, *Oil & Soap, 14*, 2–3 (1937).
[331] R. S. McKinney and G. S. Jamieson, *Oil & Soap, 12*, 92–93 (1935); *15*, 30–32 (1938).
[332] T. P. Hilditch and J. P. Riley, *J. Soc, Chem. Ind., 65*, 74–81 (1946).

A number of high-melting fats or "butters" contain a large percentage of stearic acid, or of palmitic and stearic acids. Without exception, all these fats are obtained from tropical plants. The fats with the highest proportion of stearic acid belong to the genera *Guttiferae* (garcinia family), and *Sapotaceae* (sapodilla family). Thus, *Allanblackia sacleuxii* Kagné ("hua," a bouandja-like plant)[333] and *Allanblackia klainei* Pierre (garcinia, bouandja from the Belgian Congo),[334] which have 82 and 62% of stearic acid, respectively, are both African tropical plants belonging to the genus *Guttiferae*.

Thus, it would appear that, as a result of climatic conditions, only liquid oils with a large content of unsaturated acids are to be found in plants from cold regions. On the other hand, the distribution of high-melting fats or butters having a large proportion of saturated acids is confined to the tropics. Highly unsaturated acids may likewise occur in the tropical plants.

e. The Effect of Exercise on the Distribution of Storage Fat. According to the results of Reed and associates,[250] no change in the distribution of fat was noted, whether rats were inactive or active, or whether or not they were subjected to a limited diet. However, forced activity or voluntary activity at night did cause an increase in the proportion of intermuscular fat over that in the control groups. In the case of the rats on the low-fat diet, forced activity and undernutrition resulted in a decreased storage of genital fat. These results were later confirmed by Bloor,[251] who reported that an artificial enhancement of activity by exercise through second and third generations results in higher concentrations of cholesterol and phospholipid in the muscles of rats. A summary of the experiments of Reed *et al.*[250] is included in Table 15 (page 592).

f. The Effect of Diet on the Distribution of Storage Fat. Although the total fat stored in the fat depots is markedly higher on a high-fat diet than on a high-carbohydrate ration, as is evident from Table 11, the distribution of the fats in the several fat depots is not appreciably altered. The same conclusion can be reached by an examination of Table 15, which records the data on the distribution of storage fat in male rats receiving a low-fat diet (corn), or high-fat diets consisting of soybean oil or coconut oil.

Although the distribution of fats between the several fat depots does not appear to be greatly altered in the rat by variations in fat intake, Pitts[335] observed that, in the guinea pig, the fat content of the liver, spleen, and central nervous system was independent of changes in total body fat, which

[333] H. Jumelle, *Les huiles végétales*, Paris, 1921, p. 228; cited by T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 194.
[334] L. Adriaens, *Mat. grasses*, *25*, 9931–9932, 9961–9962 (1933).
[335] G. C. Pitts, *Federation Proc.*, *12*, 109 (1953).

TABLE 15

EFFECT OF VARIOUS DEGREES OF ACTIVITY AND OF DIFFERENT DIETS ON THE DEPOSITION OF STORAGE FAT IN SEVERAL FAT DEPOTS IN THE RAT[a]

No. of rats	Calories	Activity	Forced or voluntary activity	Proportion of fat to body wt., %	Proportion of depot fat to total depot fat, %					
					Intermuscular	Genital	Subcutaneous	Perirenal	Mesenteric	Omental
				Rats on starch diet						
5	Unlimited	Inactive	—	9.6	5.3	20.2	47.3	13.2	14.1	
3	Limited	Inactive	—	7.8	5.3	12.3	58.1	12.0	8.1	3.6
3	Fasting	Inactive	—	5.5	4.9	22.9	51.4	9.2	7.0	4.4
4	Unlimited	Active	Voluntary	6.2	5.4	22.0	47.2	10.2	15.4	
5	Limited	Active	Voluntary	7.8	5.6	17.9	53.5	8.6	14.5	
5	Unlimited	Active	Forced	5.2	9.7	12.5	53.3	12.0	10.0	3.2
				Rats on soybean oil diet						
11	Unlimited	Inactive	—	11.6	5.5	21.1	46.2	14.3	9.7	3.1
3	Limited	Inactive	—	13.1	5.6	21.3	51.5	11.8	7.0	2.4
3	Fasting	Inactive	—	5.3	5.2	20.4	52.2	12.0	5.3	4.3
4	Unlimited	Active	Voluntary	8.8	6.3	24.2	45.2	11.8	8.9	3.3
5	Limited	Active	Voluntary	7.8	5.6	19.8	48.1	12.6	11.6	3.2
5	Unlimited[b]	Active	Voluntary	3.7	8.0	21.0	46.0	12.4	7.4	5.1
6	Unlimited	Active	Forced	7.8	8.8	18.5	49.6	11.3	8.4	3.4
				Rats on coconut oil diet						
6	Unlimited	Inactive	—	14.1	4.6	19.8	53.2	11.6	7.8	3.1
3	Limited	Inactive	—	14.1	4.6	21.2	54.6	10.6	6.2	2.6
2	Fasting	Inactive	—	2.9	9.4	27.5	37.5	13.4	6.2	6.5
4	Unlimited	Active	Voluntary	7.2	7.4	22.6	45.3	13.1	7.6	3.7
4	Limited	Active	Voluntary	8.9	5.9	21.1	51.4	10.8	7.6	3.2
5	Unlimited	Active	Forced	9.5	6.6	22.0	48.5	12.5	7.5	3.2

[a] Adapted from L. L. Reed, F. Yamaguchi, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *87*, 147–174 (1930).
[b] Night. Other similar tests during day.

ranged from 5 to 50% of the lean body mass. On the other hand, the fat content of heart, kidney, gut, muscle, bone, skin, and adipose tissue was directly proportional to total body fat. The skin was found to be the most sensitive to changes in total body fat.

Moreover, the type of fat laid down in the different fat depots is usually quite uniform, irrespective of diet. Only the omental fat consistently shows some variation, in that it is slightly more saturated than the other samples. The average iodine numbers of the fats from different storage sites are given in Table 16.

TABLE 16

THE IODINE NUMBERS OF STORAGE FATS FROM VARIOUS SITES AS AFFECTED BY DIET[a]

Fat source	Starch diet	Soybean oil diet	Coconut oil diet
Intermuscular	55.8	109.3	31.3
Genital	54.3	113.4	29.2
Subcutaneous	56.3	114.1	31.4
Perirenal	53.7	113.9	29.9
Mesenteric	51.4	110.5	29.1
Omental	51.6	105.2	27.8

[a] L. L. Reed, F. Yamaguchi, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *87*, 147–174 (1930).

g. The Effect of Inanition on the Content and Composition of Body Fat. When animals are fasted, a sparing action appears to be exerted on the fatty acids which are specialized in their behavior, and above all on those which are essential. On the other hand, short-chain fatty acids, which have been deposited in the fat depots by feeding a coconut oil diet, are most rapidly utilized. All of these phenomena may result in a considerable modification of the fatty acid distribution, as compared with the normal, as inanition progresses.

In the experiments of Hilditch and Pedelty,[336] which were carried out on pigs after a previously normal diet, little variation was to be found in the composition of the fat, even after 135 days of fasting, except for a decrease in the oleic acid, with a small concomitant increase in stearic acid. The linoleic acid, however, remained practically constant. The data for this experiment are summarized in Table 17.

Hilditch and Pedelty,[337] in a later communication, showed that, when fat ewes were fasted for periods up to 209 days, a reduction of the palmitic acid occurred in the storage depot, together with a slight increase in stearic acid.

[336] T. P. Hilditch and W. H. Pedelty, *Biochem. J.*, *34*, 40–47 (1940).
[337] T. P. Hilditch and W. H. Pedelty, *Biochem. J.*, *35*, 932–939 (1941).

TABLE 17

EFFECT OF A PROLONGED FAST ON THE DISTRIBUTION OF THE FATTY ACIDS (IN WEIGHT PER CENT) IN THE FAT OF PIGS[a]

Category	Perinephric fat obtained			Inner back fat obtained			Outer back fat obtained		
	At start	After 51 days	After 135 days	At start	After 51 days	After 135 days	At start	After 51 days	After 135 days
Iodine number[b]	56.4	56.6	54.8	58.9	59.9	54.7	63.9	65.0	60.0
Saturated acids									
C_{12}	—	0.1	—	0.1	—	0.1	0.1	—	—
C_{14}	0.9	0.8	0.9	0.8	0.6	0.9	0.9	0.9	0.9
C_{16}	29.3	31.3	30.3	27.5	29.4	30.7	26.5	26.0	30.1
C_{18}	17.4	17.6	21.5	15.1	15.0	18.8	12.8	13.0	15.1
Unsaturated acids									
C_{14}	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C_{16}	1.8	1.0	2.2	1.7	2.4	1.7	1.9	1.7	2.6
C_{18} (oleic)	40.3	38.8	34.1	44.2	40.0	37.2	46.8	45.6	39.5
C_{18} (linoleic)	8.1	8.3	7.3	7.3	9.6	7.1	7.9	9.1	8.2
C_{20-22}	1.9	2.0	3.5	3.1	2.8	3.3	2.9	3.5	3.4

[a] Adapted from T. P. Hilditch and W. H. Pedelty, *Biochem. J.*, *34*, 40–47 (1940).
[b] T. P. Hilditch, *The Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947.

However, in the ewes as well as in the pigs, the variations brought about by fasting were minor.

There is some question as to whether or not a definite sparing action is exerted upon the essential fatty acids during fasting. Chevallier *et al.*[338] found that the amount of arachidonic acid in the organism of the rat remained remarkably constant during fasting, despite a decrease in total lipids. The constancy with which the concentration of tetraenoic acid is maintained in the tissues during fasting does not appear to be shared by the dienoic and trienoic acids. Thus, it was noted by Manuel and Chevallier[339] that the injection of thyroxine, which causes a decline in total lipids in the organism, similar to that brought about by fasting, is followed by a decrease of the dienoic acids in the rat and guinea pig, and of trienoic acids in the guinea pig. Little variation in the quantity of tetraenoic acid obtains in these two species as a result of the injection of the thyroid hormone. On the other hand, Evans[340] found that the absolute decrease in the fat content of the bone-marrow of starved rabbits affected equally the polyunsaturated fatty acids, oleic acid, and the saturated acids.

When short-chain fatty acids are present in the depot fats at the start of the fast, these are first oxidized. Channon *et al.*[31] were the first to demonstrate that, after a diet containing 40% of coconut oil was fed for fourteen and twenty-one days, respectively, the depot fat contained 0.9 and 1.2% of capric acid, 7.8 and 17.2% of lauric acid, and 13.4 and 13.8% of myristic acid. In the studies of Reed *et al.*[250] in the Yale laboratories, it was found that the depot fat of rats, previously on a coconut oil diet, became more unsaturated when the animals were subjected to fasting. It was suggested that a relative increase in phospholipids during fasting might account for the increased unsaturation which they observed. When the depot fats consisted primarily of palmitic, oleic, and linoleic acids, no comparable changes were observed during inanition.[51,52] Longenecker[32] confirmed these earlier results by an analytical demonstration of the rapid loss of the saturated acids during fasting on the part of rats previously on a coconut oil diet. The author considers that these results lend unqualified support to the hypothesis that acids of lower molecular weight are preferentially utilized. However, the increased rate of utilization of the C_{12} to C_{16} acids may be attributable to the fact that they are present in the glycerides in maximum concentration (two molecules per triglyceride). Under these

[338] A. Chevallier, C. Burg, and R. Wagner, *Compt. rend. soc. biol.*, *144*, 1396–1397 (1950).

[339] S. Manuel and A. Chevallier, *Compt. rend. soc. biol.*, *145*, 1369–1371 (1951).

[340] J. D. Evans, *Federation Proc.*, *12*, 41 (1953).

circumstances, if one molecule of the fat were used at a time, a greater proportion of these shorter-chain acids would be utilized, while only one-half the amount of the C_{18} acids was being oxidized. The data of Longenecker are summarized in Table 18.

TABLE 18
THE EFFECT OF FASTING ON THE CONTENT OF PRINCIPAL FATTY ACIDS OF RATS PREVIOUSLY FED A COCONUT OIL DIET[a]

Acids	Non-fasted group, g.	Fasted groups: 15% loss in body weight, Grams	Fasted groups: 15% loss in body weight, % utilized	Fasted groups: 30% loss in body weight, Grams	Fasted groups: 30% loss in body weight, % utilized
Total acids	13.50	8.46	37.3	4.95	63.7
Lauric (C_{12})	3.60	1.69	53.1	0.92	74.4
Myristic (C_{14})	2.34	1.56	33.3	0.70	69.9
Palmitic (C_{16})	2.69	1.73	35.8	0.80	70.1
Oleic (C_{18})	3.21	2.63	19.1	1.73	46.2

[a] H. E. Longenecker, *J. Biol. Chem.*, *130*, 167–177 (1939).

Black and her co-workers[341] recorded an interesting phenomenon related to the caloric intake in the Cape John dory (*Zeus capensis* C. and V.). The composition of the fat differed, depending upon whether it was obtained from the thin or from the fat fish. Whenever an increased fat content was present in the fish, the proportion of highly unsaturated C_{20}, C_{22}, and C_{24} acids was increased, not only in the body fat but also in that in the liver.

h. The Effect of Thyroxine on the Distribution of Storage Fat. When thyroxine was administered to rats, there was a marked reduction in the total fat laid down.[288] This result is not unexpected, in view of the fact that the thyroid hormone may increase heat production to such an extent that it is no longer possible for the animal to have a sufficient surplus of calories to provide for the deposition of fat. One interesting feature brought out by the experiments of Reed *et al.*[288] is the finding that, despite the fact that the total fat laid down was decreased more than 50% when thyroxine was fed, no change in distribution could be observed. Apparently all fat depots were equally affected. In contradistinction to the results on ovariectomized rats, the degree of saturation of the fat of the thyroxine-fed rats was found to be changed. Whereas the average iodine number of the body fat stored in the several fat depots was 33.3 in the normal rats, it amounted to 41.2 and 45.2 in the rats fed the identical diet but

[341] M. M. Black, W. S. Rapson, H. M. Schwartz, and N. J. Van Rensburg, *J. Soc. Chem. Ind.*, *65*, 13–15 (1946).

started on thyroxine when they had reached a body weight of 100 or 200 g., respectively.

According to Reed and co-workers,[288] "of all the factors, including food, undernutrition, fasting, muscular activity, ovariectomy, and the administration of thyroxine, which we have studied thus far, the character of the diet and the thyroid hormone represent the only influences that have appreciably altered the quality of depot fat."

5. Variable and Constant Components of Lipids

In 1914, Terroine[342,343] first clearly differentiated between the two categories of lipids in vertebrates; these he called the *élément variable* (which consists of the reserve fat) and the *élément constant* (which represents the essential components of the protoplasm). These will be referred to here as the variable and the constant components of fat. Since it was not possible to attain complete inanition in the invertebrates, Terroine[342] could not separate these two groups in the lower forms.

The concept that two types of lipids occur in the tissues is based upon the fact that animals which have died as a result of fasting still contain an appreciable amount of fat, which appears to be fairly constant for each species. When the animal is forced to use this constant component as a source of calories, death results shortly. On the other hand, the quantity of the variable element may have a wide range; changes in this fraction, either up or down, appear to have little effect upon the well-being of the animal. The theory of the individuality of the function of the variable and constant components of fat is based upon intensive experiments of Mayer and Schaeffer[344–347] and of Terroine.[348–350] A more recent exposition is to be found in a review by Terroine.[351]

The variable and constant components of fats differ chemically and physiologically from each other. The variable component consists almost

[342] E. F. Terroine, *Compt. rend.*, *159*, 105–108 (1914).
[343] E. F. Terroine, *Contribution à la connaissance de la physiologie des substances grasses et lipoidiques*, Masson, Paris, 1919; *Ann. sci. nat. Zool.* [*10*], *4*, 5–397 (1920).
[344] A. Mayer and G. Schaeffer, *J. physiol. et path. gén.*, *15*, 510–524 (1913).
[345] A. Mayer and G. Schaeffer, *J. physiol. et path. gén.*, *15*, 535–548 (1913).
[346] A. Mayer and G. Schaeffer, *J. physiol. et path. gén.*, *15*, 773–788, 984–998 (1913).
[347] A. Mayer and G. Schaeffer, *J. physiol. et path. gén.*, *16*, 1–16, 23–38 (1914).
[348] E. F. Terroine, *J. physiol. et path. gén.*, *16*, 386–397 (1914).
[349] E. F. Terroine, *J. physiol. et path. gén.*, *16*, 408–418 (1914).
[350] E. F. Terroine and H. Barthélémy, *Arch. intern. physiol.*, *19*, 88–102 (1922); *Chem. Abst.*, *16*, 2531 (1922); E. F. Terroine and J. Weill, *J. physiol. et path. gén.*, *15*, 549–563 (1913).
[351] E. F. Terroine, *Ann. Rev. Biochem.*, *5*, 227–246 (1936).

exclusively of neutral fats, the composition of which depends to a considerable extent upon the nature and proportion of dietary fat. It may also be related to the level of carbohydrate in the diet, since this foodstuff may serve as a source of much of the storage fat. The quantity of the variable component will naturally depend upon the level of alimentation.

On the other hand, the constant component of the fat present in an animal, which constitutes as much as 23% of the dry matter in the mouse and 25% in the chicken, consists of phospholipids, cerebrosides, and other essential fatty components such as cholesterol, as well as of some neutral fat. It is believed that these lipids serve as essential elements of the protoplasm.

(1) The Nature of the Constant Component

Terroine and Belin[352] were of the opinion that the constant component was related both qualitatively and quantitatively to the phospholipids. It was relatively constant, in spite of all physiologic variations. These authors have shown that the ratio, total fatty acids: lipid phosphorus, approximates the value for lecithin. They believe that the lecithin type of phospholipid is the only one present to any considerable degree in this fraction. According to Terroine and Belin,[352] the nature of the food is without influence on the fatty acid composition of the constant component of a wide variety of tissues and organisms. It was also indicated that the iodine number of the phospholipid fatty acids in the lung, kidney, liver, and muscle of the same species was within a relatively narrow range. The unsaturation of the fatty acids was therefore believed to constitute a second index of the constant component of the fatty tissues which was characteristic of the species. Thus, the constant component for any species was characterized not only by the uniformity in total quantity but also by the similarity in qualitative composition as expressed in the degree of unsaturation of the component fatty acids.

However, the studies by MacLachlan and co-workers[353] force one to revise the concept that phospholipids are all equally important as components of the constant element of fat. Although the total quantity of phospholipid in the liver of the mouse is decreased by about 50% after four days of fasting, the size of the liver is likewise reduced to about one-half of what it was previous to fasting. Thus, the *concentration* of phospholipids remains fairly constant in the liver during inanition. This led Hodge, MacLachlan

[352] E. F. Terroine and H. Belin, *Bull. soc. chim. biol.*, *9*, 12–48 (1927).

[353] P. L. MacLachlan, H. C. Hodge, W. R. Bloor, E. A. Welch, F. L. Truax, and J. D. Taylor, *J. Biol. Chem.*, *143*, 473–490 (1942).

et al.,[354] in their original work, to state that "In its lipid distribution, the liver of the fasted mouse is like the normal liver."

In their later investigations, MacLachlan and his collaborators[353] demonstrated that a remarkable change in the composition of phospholipids takes place during this interval of fasting. Whereas the proportion of α-lecithin and β-cephalin was decreased by as much as 80%, that of β-lecithin and α-cephalin remained constant, or was actually slightly increased over this period. This results in a seemingly constant lecithin:cephalin ratio, in spite of the marked qualitative variations. These changes are summarized in Table 19.

TABLE 19
THE DISTRIBUTION OF PHOSPHOLIPIDS IN THE LIVERS OF UNFASTED MICE, AND OF MICE FASTED FOR 1 TO 4 DAYS[a]

Category	Number of days of fasting				
	0	1	2	3	4
Initial wt., g.	20 4	20.7	21.1	21.0	20.8
Carcass wt., g.[b]	18.30	16.50	15.08	13.65	12.70
Liver wt., g.	1.265	1.076	0.957	0.803	0.648
Liver/g. mouse, mg.	65	62	60	56	49
Total lipids, mg.	66	107	110	42	20
Total phospholipid, mg.	41	33	30	25	18
Phospholipid fractionation[c]					
α-Lecithin, *mg.* and (%)	12.2 (34)	8.0 (26)	7.3 (25)	5.4 (21)	3.6 (16)
β-Lecithin, *mg.* and (%)	7.2 (20)	10.0 (32)	9.2 (31)	8.8 (34)	8.1 (35)
α-Cephalin, *mg.* and (%)	7.0 (19)	7.8 (25)	7.2 (24)	8.5 (32)	8.8 (39)
β-Cephalin, *mg.* and (%)	9.7 (27)	5.4 (17)	5.9 (20)	3.4 (13)	2.4 (10)
Total lecithin, % phospholipid	58	57	57	56	55
Phospholipid, % liver wt.[d]	3.24	3.07	3.14	3.12	2.79
Total lecithin: total cephalin[d]	1.16	1.36	1.26	1.19	1.04

[a] Adapted from P. L. MacLachlan, H. C. Hodge, W. R. Bloor, E. A. Welch, F. L. Truax, and J. D. Taylor, *J. Biol. Chem.*, *143*, 473–490 (1942).
[b] Carcass, not including liver weight.
[c] Including per cent of total phospholipid (in parentheses).
[d] Calculations by present author.

Thus, it has been suggested that α-lecithin and β-cephalin might be described as "metabolic" phospholipids which presumably owe their normal existence to the ordinary lipid metabolism of the liver. Such compounds may likewise be considered as "labile" phospholipids, which can be utilized during an emergency such as inanition. This concept, that two types of

[354] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, C. A. Stoneburg, M. C. Oleson, and R. Whitehead, *J. Biol. Chem.*, *139*, 897–915 (1941).

phospholipid exist in the liver, is not a new one, but was suggested earlier by Artom *et al.*[355] and by Sinclair.[46] The latter investigator demonstrated that the fatty acids in one portion of the liver phospholipids are readily replaced by elaidic acid, while the second portion is resistant to such a change. MacLachlan *et al.*[353] do not believe that the interchange of α- and β-phospholipids takes place physiologically, although they do not completely discount this as a possibility.

In contradistinction to the role of the α-lecithins and β-cephalins as metabolic phospholipids, the β-lecithin, α-cephalin fraction must be regarded as the true *élément constant.* The hypothesis of Terroine and Belin[352] that the phospholipid portion of the constant component consists only of the lecithin type of phospholipids must be revised in the light of this more recent work.

Moreover, the question as to whether or not the quantity of the components in the *élément constant* is uniform is a much debated problem. In his later review, Terroine,[351] cites the work of Alf Klem[356] on the uniformity of the constant component in rats (fatty acids equaled 1.54 and 1.58% of body weight), and of Valla[357] for the values in mice (2% of body weight). The French investigator suggested that a more accurate index, from a biometric point of view, for the constant component would be the ratio, at the time of death, of the fatty acids in the entire organism to the total nitrogen.

On the other hand, there is considerable evidence which is contrary to the thesis that the level of phospholipids is a constant value. Thus, Boyd[358] has shown that the phospholipids in the leucocytes vary with the physiologic activity of the organism as a whole, or of a certain tissue. After an operation, the phospholipids increase 200% if recovery ensues, or fall if postoperative complications occur[358]; a similar phenomenon obtains following fever. The phospholipids in the white corpuscles of women increase for three weeks following parturition.[359] An increase in phospholipid content occurs in the rabbit ovary during pregnancy,[360] which reaches a maximum of 300% of the normal. However, this also returns to normal before parturition.[360] It has been shown that, in such closely related organs as the mammary glands, uterine mucosa, and the corpus luteum, the

[355] C. Artom, G. Sarzana, and E. Segré, *Arch. intern. physiol.*, *47*, 245–276 (1938).

[356] A. Klem, *Norske Videnskaps-Akad., Oslo, Hvalrådets Skrifter, No. 11,* 49–108 (1935).

[357] S. Valla, *Bull. soc. chim. biol.*, *17*, 1715–1740 (1935).

[358] E. M. Boyd, *Can. Med. Assoc. J.*, *31*, 626–633 (1934).

[359] E. M. Boyd, *Surg. Gynecol. Obstet.*, *59*, 744–751 (1934).

[360] E. M. Boyd, *J. Biol. Chem.*, *108*, 607–617 (1935).

phospholipid content increases to a maximum concomitantly with an increase of activity to the highest pitch.[361–363] On the other hand, no similar variation occurs in the cholesterol fraction. When the corpus luteum relapses into inactivity, the phospholipid content decreases, while that of cholesterol increases.

A lack of uniformity in the phospholipid and cholesterol content of muscles has also been demonstrated. In fact, Bloor[364,365] alone and with Snider[366] reported that the phospholipid and cholesterol content of muscles cannot be assigned a fixed value. Differences in the quantities of these components were shown to exist between voluntary, involuntary, and cardiac muscle in the same animal, between different muscles of the same type, according to the extent of the activity, and finally between identical muscles in different species of animals. Both phospholipids and cholesterol are higher in malignant than in benign tumors.[367,368] Sinclair,[369] in a review of the physiology of phospholipids, cited a number of other instances in which the concentration of phospholipids was altered by the physiologic activity of the tissues. Bloor[1] believes that the constant factor in tissue lipids is a valuable feature in characterizing a tissue or organ, especially in species having settled habits of activity.

In further support of the theory that lipids in the constant component exhibit not only a quantitative constancy but also a qualitative constancy, Terroine and Belin[352] suggest that the phospholipids are not constant in composition in the static sense, but rather in the dynamic sense. The constancy is a consequence of two types of processes operative in opposite directions; one of these tends to modify the *élément constant* and the other, which depends upon the specific properties of the tissues, attempts to reconstitute it.

However, the present balance of opinion is contrary to the concept that the composition of the phospholipids is constant. In the first place, Aylward and co-workers[370] believe that the above hypothesis is in contradiction to that of the activity of the phospholipids in fat metabolism. Moreover, Sinclair has consistently denied that phospholipids are uniform in composi-

[361] W. R. Bloor, R. Okey, and G. W. Corner, *J. Biol. Chem., 86*, 291–306 (1930).
[362] R. Okey, W. R. Bloor, and G. W. Corner, *J. Biol. Chem., 86*, 307–314 (1930).
[363] C. Kaufmann and K. Raeth, *Arch. Gynäkol., 130*, 128–151 (1927).
[364] W. R. Bloor, *J. Biol. Chem., 72*, 327–343 (1927).
[365] W. R. Bloor, *J. Biol. Chem., 114*, 639–648 (1936).
[366] W. R. Bloor and R. H. Snider, *J. Biol. Chem., 107*, 459–470 (1934).
[367] M. Yasuda and W. R. Bloor, *J. Clin. Invest., 11*, 677–682 (1932).
[368] R. Bierich and A. Lang, *Z. physiol. Chem., 216*, 217–223 (1933).
[369] R. G. Sinclair, *Physiol. Revs., 14*, 351–403 (1934).
[370] F. X. Aylward, H. J. Channon, and H. Wilkinson, *Biochem. J., 29*, 169–178 (1935).

tion. It was found that their degree of saturation could readily be changed by food fats, rapidly when greater unsaturation was involved, and more slowly when the opposite change was brought about.[371] Thus, Sinclair noted[372] that the iodine values for the fatty acids of phospholipids extracted from the whole carcass of rats varied from 104 to 165, depending upon the nature of the dietary fat. However, the ratio of solid to liquid fatty acids in the phospholipids was constant. The differences in unsaturation of the acids are rather to be ascribed to differences in the relative amounts of the several types of unsaturated acids.

On the other hand, the feeding of a fat containing saturated acids, such as would occur when hydrogenated coconut oil was given, does not alter the unsaturation of the phospholipid fatty acids.[373] Moreover, such saturated fats were unable to represent the effect of small amounts of highly unsaturated fats in increasing the iodine number of the tissue phospholipid. Once established, these highly unsaturated fatty acids were shown to be maintained over long periods, despite prolonged fasting or the feeding of a diet rich in saturated fat. In later work, Sinclair[46] showed that elaidic acid may be substituted in the liver phospholipids in the proportion of one-third of the fatty acids. The entrance of this unphysiologic acid into the phospholipids of the liver was rapid, while it occurred only slowly in the muscle phospholipid. On the basis of these results, Sinclair was led to postulate that two types of phospholipids exist in cells, insofar as functional activity is concerned. One class of these compounds, which consists of the more highly unsaturated phospholipids, functions in the essential make-up of the cell. The other type, which is made up of the less unsaturated phospholipids, acts as an intermediary product in the metabolism of fat. Terroine[351] suggests that this eclectic point of view may offer the promise of a compromise between the diametrically opposed opinions of Terroine and of Sinclair concerning both the constancy and the qualitative uniformity of the phospholipids.

(2) *The Nature of the Variable Component*

The variable component of fat present in the animal body increases or decreases with an augmentation or decline in the extra calories ingested, as compared with those used. The composition of this deposit fat varies with the nature of the diet, with the sex of the animal, with environment, with the activity of the tissue, and with the site at which the fat is deposited.

[371] R. G. Sinclair, *J. Biol. Chem.*, *95*, 393–408 (1932).
[372] R. G. Sinclair, *J. Biol. Chem.*, *111*, 261–273 (1935).
[373] R. G. Sinclair, *J. Biol. Chem.*, *111*, 275–284 (1935).

The total fat in the depots may be reduced by 95% as a result of prolonged starvation, compared to a loss of 70% in the spleen, to one of 50% in the liver, and to one of only 5% in the brain, spinal cord, eyes, and heart.[374] On the other hand, the tissues may contain an extremely high level of fat. Smith[375] cited a case of hypothalamic injury in which the fat made up 74% of the total carcass weight. Hetherington and Ranson[376] reported that the depots in the subcutaneous tissue, in the omentum, and in the mesenteries of the viscera were filled with fat, as were also those in the perirenal tissues, the pericardium and, in fact, in every location where adipose tissue normally exists. Moreover, large amounts of fat were stored in the liver. This organ was yellow, and was twice its normal size. The presence of twice the normal content of fat in these livers is quite common. This would account for about four times the normal amount of fat. However, instances have been reported in which, when choline-low diets were given, the enlarged livers contained as much as twenty times the amount of lipid normally present (see page 635).

Extreme obesity can be produced experimentally by injury to the hypothalamus.[376] A number of investigators[376–379] have described an experimental obesity which may begin to develop within a few hours after an operation in which the hypothalamus is injured. An adult female rat may increase in weight by 15% within the first twenty-four hours after operation,[380] and the rate of gain will continue at ten times the normal for several weeks. The gain is not maintained indefinitely, as the body weights of the animals eventually reach a plateau. Brobeck[380] reviewed the subject of obesity in animals with hypothalamic lesions. For a further discussion of the deposition of excessive amounts of fat, see page 623.

6. The Lipid Distribution in the Cell

Lipids are present in the cell membrane, where they serve structural rather than metabolic purposes. Investigations of a number of the cell components have been rendered possible by the development of new methods for the separation of various anatomical structures within the cell.

[374] M. R. Everett, *Medical Biochemistry*, 2nd ed., Hoeber, New York, 1946, p. 249.
[375] P. E. Smith, *J. Am. Med. Assoc.*, *88*, 158–161 (1927).
[376] A. W. Hetherington and S. W. Ranson, *Anat. Record*, *78*, 149–172 (1940).
[377] A. D. Keller and W. Noble, *Am. J. Physiol.*, *113*, 79–80 (1935); *116*, 90–91 (1936).
[378] P. E. Smith, *Am. J. Anat.*, *45*, 205–256 (1930).
[379] J. R. Brobeck, J. Tepperman, and C. N. H. Long, *Yale J. Biol. Med.*, *15*, 831–853 (1943).
[380] J. R. Brobeck, *Physiol. Revs.*, *26*, 541–559 (1946).

Stoneburg[381] investigated the lipid composition of cell nuclei. He employed a new technic of Crossmon,[382] in which the nuclei are ejected from the cells when small pieces of tissue are placed in 5% citric acid solution. Since the lipids are not water-soluble, the application of this procedure should not result in a loss of any of the lipids from the freed nuclei. The data of Stoneburg[381] are summarized in Table 20.

TABLE 20
AVERAGE LIPID ANALYSIS OF CELL NUCLEI ON A DRY BASIS[a]

	Source of nuclei			
Category	Beef heart muscle	Rabbit thigh muscle	Tumor cells	Pus cells
Number of tests	6	8	6	1
Acetone-sol. lipids				
Neutral fat and fatty acids	—	—	—	—
Amount, %	6.5	1.8	18.0	26.0
Iodine number	126	80	—	—
Cholesterol, %	3.6	3.6	4.6	2.5
Acetone-insol. lipids				
Phospholipid, %	15.7	4.0	11.5	—
Phospholipid fatty acids	—	—	—	—
Amount, %	9.8	3.0	7.5	12.0
Iodine number	70	70	30	27
Phospholipid:cholesterol ratio	3.9	1.3	2.3	5.0

[a] Adapted from C. A. Stoneburg, *J. Biol. Chem.*, *129*, 189–196 (1939).

The nuclei were shown to contain considerable amounts of lipids; these were predominantly phospholipids and cholesterol. The lipid concentrations of the nuclei tend to conform to those of the tissue from which they were obtained, but at a higher level.

Unfortunately, the Crossmon technic for removing the nuclei could not be applied to liver cells. However, by the application of a different procedure for the separation of the nuclei, Williams and his co-workers[383] were able to study the lipid composition of the nuclei of the liver cells of normal rats and dogs, and of rats previously treated with *p*-dimethylaminoazobenzene (butter yellow). It was found that the lipid pattern of the cell nuclei differed from that of the whole liver. The structural or essential lipids were shown to comprise 12 to 14% of the dry weight of the nuclei;

[381] C. A. Stoneburg, *J. Biol. Chem.*, *129*, 189–196 (1939).

[382] G. Crossmon, *Science*, *85*, 250 (1937).

[383] H. H. Williams, M. Kaucher, A. J. Richards, E. Z. Moyer, and G. R. Sharpless, *J. Biol. Chem.*, *160*, 227–232 (1945).

they made up about three-fourths of the total lipid. The essential lipid was composed of phospholipid to the extent of over 90%, sphingomyelin making up less than 5% of the total. Butter yellow was shown to effect a change in the structure of the liver cell, as indicated by the alterations in the lipid pattern. Nuclei isolated from tumorous liver cells exhibited a greatly reduced phospholipid concentration and an increased amount of cholesterol ester. These authors suggest that the pathological derangement of phospholipid occurs primarily in the nucleus.

In a further examination of the nature of cell nuclei, Wang *et al.*[384] demonstrated that the lipid components were present in combination with protein. These lipoproteins of the nuclei of rat liver, calf liver, ox spleen, and chicken erythrocytes were found to contain approximately 10% of lipids. In the case of those prepared from rat and calf liver, 20 to 30% of the dry weight was found to consist of the lipoprotein.

The lipid composition of mitochondria has also been studied. Mayer and Schaeffer,[385,386] employing relatively crude methods, reported that mitochondria consist largely of phospholipids. Using the modern technic of gravitational fractionation, Bensley[387,388] reported that 35% of the guinea pig mitochondria is lipid. Phospholipid (as lecithin) was shown to account for 45 to 58% of the total lipid.[389] On the other hand, Chargaff[390] noted that the phosphatides in rabbit liver made up only a small part of the extracted lipids of the mitochondrial fraction.

Chargaff[390] listed the following composition for these mitochondria, expressed in per cent of dry matter: phosphatides, 4.0; glycerides (as triolein), 5.4; cholesterol, 1.2; cerebrosides, 3.3; lysophosphatides (as palmityl lysolecithin), 4.3; extraction residue, 72.0. However, Swanson and Artom[391] have called attention to the fact that the lipids in mitochondria are present in unusually firm combinations with protein, rendering their complete extraction very difficult. Phospholipids were found to be present to the extent of 79% in the lipids extracted from the trichloroacetic acid precipitate of mitochondria. Neutral fat and other lipids made up 17%, and cholesterol comprised the remaining 4%. The phospholipid fraction was composed of 45% lecithin, 8% sphingomyelin, and 47% non-

[384] I. Y. Wang, M. J. Carver, R. H. Ramsey, A. J. Funckes, and L. E. Thomas, *Federation Proc.*, *11*, 306 (1952).

[385] A. Mayer and G. Schaeffer, *J. physiol. et path. gén.*, *16*, 325–336 (1914).

[386] A. Mayer and G. Schaeffer, *J. physiol. et. path gén.*, *16*, 344–359 (1914).

[387] R. R. Bensley, *Anat. Record*, *69*, 341–352 (1937).

[388] R. R. Bensley and N. L. Hoerr, *Anat. Record*, *60*, 449–455 (1934).

[389] R. R. Bensley, *Science*, *96*, 389–393 (1942).

[390] E. Chargaff, *J. Biol. Chem.*, *142*, 491–504 (1942).

[391] M. A. Swanson and C. Artom, *J. Biol. Chem.*, *187*, 281–287 (1950).

choline phospholipids (mostly phosphatidylethanolamine). Except for the high phospholipid content, the composition of the lipid mixture extracted from the mitochondria was similar to that of the whole unfractionated liver.

In addition to occurring in the nuclei and mitochondria, the lipids also appear to be concentrated in the other particulate matter of the cytoplasm. Kretchmer and Barnum[392] reported that, although the large granules and microsomes contain only 38% of the solids of the cytoplasm of mouse liver cells, they account for 62% of the total lipids, and 85% of the phospholipids. Lecithin and cephalin are the phospholipids concentrated in the particulate matter, while the supernatant also contains sphingomyelin. This finding was based upon the demonstration of a N:P ratio of 1:1 in the particulate phospholipids, as compared with a 1.3:1 ratio of N to P in the phospholipids present in the supernatant fraction. A marked variation in fatty acids between the particulate and the supernatant fractions also obtains. In the former case, the acids are highly unsaturated, and contain 20% of tetraenoic acids. Although unsaturated acids are likewise present in the supernatant fraction, a much larger proportion of monoethenoid acids and a lower level of tetraenoic acids have been reported. Ada[393] found a difference in composition between the small granules (microsomes) and the large granules in rabbit liver. The microsomes contained 43.4% of lipid, while the large granules had 29.6% of lipid material. The total solids in both of these fractions were accounted for as lipid, protein, and nucleic acid. According to Ada, 26.4% of the liver cytoplasmic phospholipids occurred in the large granules, 64.6% was in the microsomes, and 9% was accounted for in the supernatant fraction.[393]

On the basis of studies with isotopic P, it was found that at least three phospholipid fractions occur in the cytoplasm of the liver cells; each of these fractions has specific activities. The rate of synthesis of phospholipid is slower in the nuclei than in the cytoplasm.[393,394] Apparently phospholipids are synthesized separately and metabolized independently of each other in different morphological structures of the liver cell.

In an overall study of the comparative lipid composition in various structures of liver cells, Chauveau and associates[395] found that there was a progressive increase in the unsaturation of the fatty acids of phospholipids from mitochondria, through the microsomes to the supernatant phase.

[392] N. Kretchmer and C. P. Barnum, *Arch. Biochem. Biophys.*, *31*, 141–147 (1951).

[393] G. L. Ada, *Biochem. J.*, *45*, 422–428 (1949).

[394] G. Hevesy, *Nature*, *158*, 268 (1946).

[395] J. Chauveau, G. Clément, J. Clément, and E. Le Breton, *Compt. rend.*, *232*, 2261–2263 (1951).

On the other hand, the unsaturation of the triglyceride fatty acids changed in the opposite direction, being highest in the mitochondria and lowest in the homogenic phase.

The distribution of cholesterol as the ester and free alcohol was investigated by Alfin-Slater and co-workers,[396] by Rice *et al.*,[397] and by Schotz,[398] who used a modification of the Schneider-Hogeboom centrifugation technic.[399] It was found that the esterified cholesterol was largely present in the "cream" layer, which represents unorganized material from the cytoplasm, while the free cholesterol was concentrated in the microsomal fractions. Using a similar technic, Krinsky and Ganguly[400] demonstrated that vitamin A occurs in different fractions of the liver, depending upon whether it is in the form of the free alcohol or of the ester. The vitamin A ester was found in the fat of the cream layer, whereas the alcohol occurred in the particulate matter as well as in the cream.

7. The Physiology of Adipose Tissue

Adipose tissue has long been considered to be an accumulation of inert fatty material which possesses little or no metabolic activity. However, this viewpoint has been challenged by Wertheimer and Shapiro,[401] in a review on adipose tissue. These workers maintain that fatty tissue constitutes an important link in the metabolic processes of the animal. Wells[402] likewise reviewed this topic from the anatomical and pathological viewpoint, and arrived at essentially the same conclusion. In this section, evidence will be summarized which indicates that adipose tissue has an independent origin, reacts to stimuli other than those activating the adjoining connective tissues, has its specific blood supply, and responds in its own characteristic way to factors which alter its metabolism.

(1) The Specific Nature of Adipose Tissue

The view is still quite widespread that adipose tissue is nothing more than connective tissue in which the fatty cells have been laid down. However, adipose tissue has been shown to develop from specific primitive

[396] R. B. Alfin-Slater, L. I. Rice, and M. C. Schotz, *Federation Proc.*, *12*, 167 (1953).
[397] L. I. Rice, M. C. Schotz, R. B. Alfin-Slater, and H. J. Deuel, Jr., *J. Biol. Chem.*, *201*, 867–871 (1953).
[398] M. C. Schotz, L. I. Rice, and R. B. Alfin-Slater, *J. Biol. Chem.*, *204*, 19–26 (1953).
[399] W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, *183*, 123–128 (1950).
[400] N. I. Krinsky and J. Ganguly, *J. Biol. Chem.*, *202*, 227–232 (1953).
[401] E. Wertheimer and B. Shapiro, *Physiol. Revs*, *28*, 451–464 (1948).
[402] H. G. Wells, *J. Am. Med. Assoc.*, *114*, 2177–2183, 2284–2289 (1940).

cells which possess a characteristic structure entirely distinct from the fibroblasts of the connective tissue. Moreover, the specific gland-like structure of the brown adipose tissue has been demonstrated histologically by Maximow and Bloom.[403]

Further proof of the characteristic nature of deposit fat is afforded by experiments with embryonic tissue. Hausberger[404] demonstrated that, when primitive "fat organs" from the embryonic tissue of rats were transplanted into adult animals, typical fatty tissues developed from them. On the other hand, negative results were obtained when embryonic connective tissue was transplanted in a similar manner. The embryonic adipose and connective tissues were indistinguishable morphologically, and could be recognized only by their location and development.

There is also evidence that adipose cells belong to the reticuloendothelial system which, of course, is not the case with cells in the connective tissue. Thus, adipose tissue cells in the unfatted fat organ have a gland-like structure similar to that of the reticuloendothelial cells, and fat tissue has an affinity for such cells.[405] The two tissues have similar functions. Moreover, the ability of omental cells to form antibodies is apparently correlated with the occurrence of aggregates of reticuloendothelial cells.[406] In addition, Wasserman[405] has shown that, under certain conditions, adipose tissue cells may reestablish their function of blood formation. This is in line with the behavior of bone-marrow, the cells of which may exhibit both fat storage and blood-forming functions. Vital dyes such as trypan blue are stored equally well by the cells of the two types of tissue.[407-409] Adipose tissue cells are able to take up these dyes especially well when they are depleted of fat. These data lead one to the conclusion that adipose and connective tissue differ, and also that the adipose tissue cells are capable of functions other than that of fat storage.

(2) *The Specific Innervation and Blood Supply of Adipose Tissue*

Although early investigators such as Hofmeister[410] and Rasmussen[411] were of the opinion that fat mobilization in adipose tissue is unrelated to

[403] A. A. Maximow and W. Bloom, *Histology*, 6th ed., Saunders, Philadelphia, 1952, p. 73.

[404] F. X. Hausberger, *Verhandl. Ges. f. Verdauungs- u. Stoffwechselkrankheiten*, 14th meeting, Stuttgart, Sept. 22–24, 1938, pp. 450–454 (1939).

[405] F. Wasserman, *Z. Zellforsch. u. mikroskop. Anat.*, *3*, 235–328 (1926).

[406] B. Portis, *J. Infectious Diseases*, *34*, 159–185 (1924).

[407] G. C. Dogliotti, *Z. Zellforsch. u. mikroskop. Anat.*, *8*, 222–248 (1929).

[408] J. L. Bremer, *Anat. Record*, *70*, 263–281 (1938).

[409] A. W. McCullough, *J. Morphol.*, *75*, 193–201 (1944).

[410] F. Hofmeister; cited by E. Wertheimer and B. Shapiro, *Physiol. Revs.*, *28*, 451–464 (1948), p. 452.

[411] A. T. Rasmussen, *J. Morphol.*, *38*, 147–205 (1923).

nervous stimuli and, in fact, that nerves are not present in these tissues, later investigators have reached opposite conclusions.[412–414] Nordmann[415] believed that the nerve supply to adipose tissue was dependent upon that to the blood vessels supplying this tissue. However, considerable proof that a nerve supply exists not only to the blood-vessels but also to the cells has been furnished by Hausberger[414] and by Boecke.[416] Goering[412] suggests the existence of a nerve center in the floor of the third ventricle which regulates the deposition and the disappearance of fat.

The blood-vessels in adipose tissue are likewise characteristic. Gersh and Still,[417] who made a quantitative examination of the capillary supply of adipose tissue, found that, in rats, the capillaries in the fat depots showed no orientation such as occurs in muscle tissue, but formed loose meshes running in all directions in the tissues enclosing the fat cells. Considerable variation was noted in the number of capillaries in different parts of the tissue, but all fat cells were in contact with at least one vessel.

When a comparison of the circulation in the adipose tissue and muscle, respectively, is based upon the relation of the surface of the capillary bed to the volume of tissue supplied, the ratio is 52 in fat-rich tissue and about 222 in fat-poor tissue. This latter figure corresponds to the value in poorly supplied muscle. On the other hand, when the comparison is made between the surface area of the capillaries and the volume of the protoplasm—which is a better standard of comparison for metabolic purposes—the ratio for fat-rich tissue based upon surface of open capillaries is 978, while that based upon total capillaries is 2160. This means that, for metabolic purposes, the capillary bed of adipose tissue is richer than that of muscle.

(3) Changes in Fat Composition Occurring in Fat Depots

The fat deposited in adipose tissue is characteristic of the animal as well as of the site of deposition. The composition of the depot fat can also be influenced by diet,[372] as has been demonstrated by the feeding of trielaidin,[46] or by the administration of deuterium-labeled fat.[418] Moreover, the proportion of unsaturated acids present in this adipose tissue can be greatly increased, particularly when the diet is low in protein and carbohydrate, and

[412] D. Goering, *Z. ges. Anat., Abt. II, Z. Konstitutionslehre, 8,* 312–335, 458 (1922).
[413] E. Wertheimer, *Arch. ges. Physiol. (Pflüger's), 213,* 262–279, 287–297 (1926).
[414] F. X. Hausberger, *Z. mikroskop.-anat. Forsch., 36,* 231–266 (1934).
[415] M. Nordmann, *Z. ges. exptl. Med., 48,* 84–110 (1925).
[416] J. Boecke, *Z. mikroskop.-anat. Forsch., 33,* 233–275 (1933).
[417] I. Gersh and M. A. Still, *J. Exptl. Med., 81,* 219–232 (1945).
[418] De W. Stetten, Jr. and R. Schoenheimer, *J. Biol. Chem., 133,* 329–345 (1940).

no hardening agent is present in the diet. For a further discussion of the effect of diet on the nature of the fat deposited, see page 524.

In spite of these variations in depot fat which can be ascribed to diet, adipose tissue itself is able to bring about a certain amount of modification of the fat supplied to it before new adipose tissue is laid down. While Schoenheimer and Rittenberg[40] demonstrated that the body could desaturate deuteriostearic acid to deuterium-containing unsaturated acids, the site of the transformation was not determined. However, a number of workers[419–422] proved that desaturation can be carried out in adipose tissue. This assumption also affords a convenient explanation for the finding of Henriques and Hansen[18] that the fats deposited in deeper layers of the body are more saturated, as determined by their iodine number, than are those laid down in the more superficial layers of storage fat. Wertheimer and Shapiro[401] suggest that this relationship between tissue temperature and the saturation of the deposited fat may result from the effect of temperature on the equilibrium in the interchange between saturated and unsaturated acids. Hilditch[116] proposed, as an alternative explanation, that the several fat depots have the ability to select the correct proportion of saturated and unsaturated fatty acids from the fatty acids furnished them. The desaturation of the fatty acids would occur in other tissues, such as the liver. Although this latter hypothesis has no definite experimental support, its acceptance also predicates that the adipose tissue is not a passive storage depot, but a site in which active work can be done to make the proper selection of fats for such deposition.

a. The Mobile Nature of Fat in Fat Depots. The earlier and widely accepted explanation of the addition or removal of the fat from adipose tissue was that these changes occurred only in the presence of a caloric excess or a caloric deficit, respectively. However, the results of Schoenheimer and Rittenberg[423] with labeled fats demonstrated that ingested fats become incorporated in the body fats even though no change in the total depot fats obtains. After deuterio-fats were fed over a period of four days on a diet which supported a caloric equilibrium, as much as 50% of the ingested fats appeared in the storage depots. Moreover, Bernhard and Steinhauser[424] demonstrated a similar interchange of fat in fasted mice. These data indi-

[419] G. Quagliariello, *Rend. accad. nazl. Lincei*, [*6*], *16*, *Classe sci. fis. mat. nat.*, 552–554 (1932).

[420] S. Yosii, *J. Biochem.* (*Japan*), *26*, 397–424 (1937).

[421] B. Shapiro and E. Wertheimer, *Biochem. J.*, *37*, 102–104 (1943).

[422] J. Champougny and E. Le Breton, *Compt. rend. soc. biol.*, *141*, 43–45, 45–48 (1947).

[423] R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, *114*, lxxxvii (1936).

[424] K. Bernhard and H. Steinhauser, *Helv. Chim. Acta*, *27*, 207–210 (1944).

cate that mobilization and replenishment of body fat proceeds continuously, irrespective of the nutritional status of the animal. When an excess of calories is ingested, the total fat stores are increased. It is well known that, under the opposite condition, the total fat reserves become depleted. However, even in this latter situation, new fat replaces some of the old fat present in the fat depots.

Schoenheimer[118] noted an apparent constancy in the fat stores in normal adult animals. This is not to be traced to an inertia of the fat depots, but rather to the fact that a very delicate balance exists between the processes leading to the deposition of depot fat and the processes tending to cause a mobilization and utilization of adipose tissue. A decrease in the magnitude of the fat depots occurs not only in states of fasting or undernutrition in which deposition of new fat is retarded, but in such abnormal conditions as thiamine deficiency[425] and diabetes.[426] The extent of fat deposition may also decrease as a result of an increased mobilization of depot fat such as may occur in a variety of conditions described in Section **c** below.

b. Fat Synthesis in Adipose Tissue. It has already been demonstrated that fat is normally synthesized continuously from carbohydrate and protein in the animal organism (see pages 538–552). In addition to the earlier demonstrations based upon balance experiments or upon changes in R.Q., the subsequent demonstration of the incorporation of deuterium in the tissue fat of animals receiving a carbohydrate diet clearly proves this synthesis.[41] It has been generally believed that the newly synthesized fat, consisting of C_{16} and C_{18} acids,[427] is manufactured in the liver.

However, several sets of experimental data are difficult to correlate with the assumption that the liver is the sole site of fat synthesis. Although the half-life of fatty acids in the liver is 2.6 to 2.8 days, the rate of synthesis of fat from carbohydrate would require a complete turnover of fats in the liver four times daily.[428] Secondly, Tepperman and associates[119] have postulated that the conversion of carbohydrates to fats may occur not only in the liver but also in the extrahepatic tissues. This conclusion is based upon a high R.Q. following the administration of glucose, not only in control rats which had been trained to consume their food rapidly, but also in rats in which the liver was functionally absent.

Since it was shown that glycogen can be deposited in adipose tissue, Tuerkischer and Wertheimer[120] suggested that the adipose tissue is also a

[425] G. E. Boxer and De W. Stetten, Jr., *J. Biol. Chem.*, *153*, 607–616 (1944).
[426] De W. Stetten, Jr., and G. E. Boxer, *J. Biol. Chem.*, *156*, 271–278 (1944).
[427] H. E. Longenecker, *Biol. Symposia*, *5*, 99–115 (1941).
[428] De W. Stetten, Jr., and G. F. Grail, *J. Biol. Chem.*, *148*, 509–515 (1943).

site for the synthesis of fat from carbohydrate. The fact that adipose tissue containing glycogen has an R.Q. greater than unity[122,429] offers support for such an hypothesis. These observations are supported by the results of Henle and Szpingier,[121] who demonstrated that, in the isolated adipose tissue of the rat, the R.Q. rose above unity simultaneously with the disappearance of glycogen. Finally, the data of Shapiro and Wertheimer,[430] which indicated the incorporation of stably bound deuterium into the fatty acids of adipose tissue incubated *in vitro* in serum with D_2O, would seem to be excellent proof of the above assumption.

c. The Control of Fat Mobilization and Deposition. (*a*) *The Metabolism of Adipose Tissue as a Controlling Factor.* The factors which control the deposition or withdrawal of fat from the adipose tissue are not clearly understood. Apparently the level of fat in the blood is not a deciding factor since, in conditions of fat depletion, as for example in fasting phlorhizinized rats, a marked hyperlipemia obtains concomitantly with a mobilization of the fat from the depots.

On the other hand, Shapiro and his collaborators[431] reported that the balance between deposition and mobilization is controlled by a factor acting directly within the fat cell. Thus, it was shown by *in vitro* tests that the fat-depleted tissue, but not the fat-laden cells, can readily take up fat from serum at 38°C.; however, this uptake of fat can readily be prevented by heating the tissue to 80°C., by the addition of sodium cyanide and sodium fluoride, or by lowering of the temperature to 20°C. Moreover, azide did not prevent the transfer of fat from the incubation medium to the cells. The penetration of fats into the cells is therefore an active process depending upon the metabolism of the cells. It would seem logical to suppose that the rate of exchange could also be subject to hormone control, as well as to nervous regulation. These possibilities are discussed below.

(*b*) *Hormone Control of Fat Mobilization and Deposition in Adipose Tissue.* The endocrine control of fat deposition and mobilization has been demonstrated in the case of the anterior pituitary hormone, the adrenocortical hormones, insulin, and the thyroid hormone. These are discussed in turn below.

a′. The Regulation by the Anterior Pituitary Gland: Barrett, Best, and Ridout,[432] and Stetten and Salcedo,[433] using deuterio-labeled fats, dem-

[429] T. Ruska and T. Oestreicher, *Arch. exptl. Pathol. Pharmakol.* (*Naunyn-Schmiedeberg's*), *177*, 42–52 (1934).

[430] B. Shapiro and E. Wertheimer, *J. Biol. Chem.*, *173*, 725–728 (1948).

[431] B. Shapiro, D. Weissmann, V. Bentor, and E. Wertheimer, *Nature*, *161*, 482 (1948).

[432] H. M. Barrett, C. H. Best, and J. H. Ridout, *J. Physiol.*, *93*, 367–381 (1938).

[433] De W. Stetten, Jr., and J. Salcedo, Jr., *J. Biol. Chem.*, *156*, 27–32 (1944).

onstrated that the fats in the fatty livers produced in mice by the injection of extracts of the anterior pituitary gland had been transported to that organ from the adipose storage depots. The identity of the specific hormone responsible for this fat-mobilizing action is not entirely established. Best and Campbell[434] prepared a fat-mobilizing hormone from the anterior pituitary which, when injected into fasted mice, increased the quantity of liver fat linearly with the dose administered. This was called adipokinin.[435] It was subsequently shown that this product increases not only liver lipid but also that in the kidney.[436] Its mobilizing action is confined to the glyceride portion of the fat, and the amount of lipid transferred is a function of the glyceride content of the fat depots.[436]

If adipokinin is a separate entity among the hormones of the anterior pituitary gland, its fat-mobilizing action is shared by other pituitary hormones. Thus, Dobyns[437] reported the complete exhaustion of fat from the fat depots of mice and its replacement by a gelatinous material when the thyrotropic hormone of the anterior lobe of the pituitary was injected. Since this effect was noted even in the absence of the thyroid gland, it may indicate that the action was due to an impurity in the preparation of the thyrotropic hormone. It might likewise suggest that this hormone had a dual action. According to Reiss,[438] the lactogenic hormone of the anterior lobe of the pituitary also reduces the fat content of adipose tissue in the rat and in man. It is altogether possible that this may be a physiologic reaction which aids in providing fat during the lactation process. It is thus uncertain whether adipokinin, the thyrotropic hormone, and the lactogenic hormone, are all active as fat-mobilizing agents, or whether adipokinin alone is active. Under the latter conditions, the positive response of other anterior pituitary hormones would be the result of an impurity in the adipokinin.

There is a definite indication that such fat-mobilizing hormones play a role in normal metabolism. Weil and Stetten[435] isolated a component, capable of increasing the liver lipid in the mouse, from the urine of fasted but not from that of normally fed rabbits. It is not known whether or not this material is adipokinin. However, it resembles the latter compound in that it is precipitable at *p*H 5.2 by 2 volumes of ethanol.

The mechanism by which pituitary hormones invoke fat mobilization would seem to be connected with a nerve stimulation of the adipose tissues.

[434] C. H. Best and J. Campbell, *J. Physiol.*, *86*, 190–203 (1936).
[435] R. Weil and De W. Stetten, Jr., *J. Biol. Chem.*, *168*, 129–132 (1947).
[436] J. Campbell and C. C. Lucas, *Biochem. J.*, *48*, 241–245 (1951).
[437] B. M. Dobyns, *Surg. Gynecol. Obstet.*, *82*, 609–617, 717–722 (1946).
[438] M. Reiss, *Endocrinology*, *40*, 294–298 (1947).

Thus, Clément[439] reported that section of the nerve supply to the interscapular fat body or to the perinephric fat depot abolished the fat-mobilizing action of pituitary extracts.

The relation of the pituitary gland to fat mobilization has also been followed by the use of hypophysectomized animals. Thus, Lee and Ayres[440] reported that hypophysectomized rats kept on a suboptimal diet lost less depot fat than did control animals on the same diet. On the other hand, forced feeding of hypophysectomized rats was shown to lead to excessive adiposity.[441–443]

b′. The Regulation by the Adrenal Cortex and Medulla: Cortical hormones are of importance in both the mobilization and the deposition of adipose tissue. The original observation of Verzár and Laszt[444] that fatty livers cannot be produced in adrenalectomized animals has been confirmed by a number of workers.[445–448] In fact, Hartman and co-workers[449] reported the isolation of a new cortical factor, distinct from the sodium and carbohydrate factors, which causes the deposition of fat in the livers of fasting adrenalectomized animals.

However, the inability to elicit fatty livers in adrenalectomized animals cannot be attributed solely to an inadequate fat mobilization from the depots. If this were the only defect after extirpation of the adrenals, the adipose tissues should be fully charged. Tuerkischer and Wertheimer[120] reported that the opposite condition obtained, and that the fat depots of such adrenalectomized animals were largely depleted. The low level of storage could not be caused by an inadequate intake of food, since fat levels in adrenalectomized rats were always lower than those in pair-fed normal controls.[450] When desoxycorticosterone acetate or adrenal cortical extracts were injected into adrenalectomized animals, normal fat storage was restored. The adrenocortical extract was the less effective of the two.

Further experiments in support of this hypothesis have been reported by

[439] G. Clément, *Compt. rend. soc. biol.*, *141*, 317–320 (1947).
[440] M. Lee and G. B. Ayres, *Endocrinology*, *20*, 489–495 (1936).
[441] L. T. Samuels, R. M. Reinecke, and H. A. Ball, *Endocrinology*, *31*, 35–41 (1942).
[442] L. T. Samuels, R. M. Reinecke, and K. L. Bauman, *Endocrinology*, *33*, 87–95 (1943).
[443] L. Levin, *Am. J. Physiol.*, *141*, 143–150 (1944).
[444] F. Verzár and L. Laszt, *Biochem. Z.*, *288*, 356–358 (1936).
[445] E. M. MacKay and R. H. Barnes, *Am. J. Physiol.*, *118*, 525–527 (1937).
[446] E. M. MacKay, *Am. J. Physiol.*, *120*, 361–364 (1937).
[447] E. G. Fry, *Endocrinology*, *21*, 283–291 (1937).
[448] D. J. Ingle, *J. Clin. Endocrinol.*, *3*, 603–612 (1943).
[449] F. A. Hartman, K. A. Brownell, and J. S. Thatcher, *Endocrinology*, *40*, 450 (1947).
[450] F. Schiffer and E. Wertheimer, *J. Endocrinol.*, *5*, 147–151 (1947).

Baker and associates.[451] These workers demonstrated that an increase in the glyceride content of brown adipose tissue resulted when intact or castrated rats were injected with ACTH (adrenocorticotropic hormone). However, the pituitary gland, also, appears to be necessary to produce this effect; following the injection of even as much as three times the effective dose of ACTH, it was impossible to maintain the normal fat content of the interscapular gland of hypophysectomized rats.[451]

Another explanation for the fat deficit in animals following adrenalectomy is the failure to synthesize fats from glycogen in the adipose tissue under these conditions.[120] Only negligible amounts of glycogen are stored in the fat depots of animals after removal of the adrenals, whereas normally the tissues would be heavily charged with this polysaccharide. However, glycogen deposition could be brought about either by the administration of pure desoxycorticosterone acetate or by cortical extracts. It is therefore suggested that the basic reason for the decreased fat storage following adrenalectomy is the fact that the carbohydrate → fat change does not occur normally in the absence of the cortical hormones. Consequently, the fat depots fail to become filled.

Another suggestion which has been advanced to account for the absence of fatty livers in the adrenalectomized animals is that this organ cannot retain fat, or that it has a higher rate of fat metabolism than the normal. It has been found that, following fat ingestion, animals without adrenals store less fat in their livers than do normal controls.[446] These results all add up to the conclusion that fat storage in both the liver and the fat depots is inhibited after extirpation of the adrenal glands.

There also appears to be some indication that the hormone of the medullary portion of the adrenal gland, namely epinephrine, may have some effect on fat mobilization. Clément and Schaeffer[452] have demonstrated that epinephrine possesses this action. It is postulated that the effect of the pituitary hormones might be due to the adrenotropic hormone. However, if this is the case, the influence would be an indirect one, which is exerted through the nervous system.

c′. The Regulation by Insulin: Wertheimer[453] was the first to demonstrate that insulin is able to counteract the fatty livers which develop in animals having phlorhizin diabetes. This hormone also partially prevents the production of fatty livers resulting from the injection of anterior pitui-

[451] B. L. Baker, D. J. Ingle, and C. H. Li, *Proc. Soc. Exptl. Biol. Med.*, *73*, 337–339 (1950).

[452] G. Clément and G. Schaeffer, *Compt. rend. soc. biol.*, *141*, 320–322 (1947).

[453] E. Wertheimer, *Arch. ges. Physiol.* (*Pflüger's*), *213*, 298–320 (1926).

tary extracts.[454] It is believed that this insulin effect is an indirect one which causes glycogen deposition in the intrascapular fat of the normally fed rat, even during fasting.[455] It has also been shown that insulin restores the ability of alloxan-diabetic rats to store glycogen in their adipose tissue.[456]

Pauls and Drury,[457] and Stetten and Klein,[458] have demonstrated that insulin participates in the carbohydrate → fat transformation. It has been reported that fat synthesis proceeds to the extent of only 5% of the normal rate in the diabetic animal.[458] Since adipose tissue participates in fat formation,[430] and insulin is a requirement for glycogen deposition in such tissues, it is evident that normal fat deposition and mobilization will be interrupted in the absence of this hormone. However, it is difficult to agree with the interpretation of Wertheimer and Shapiro[401] that the fatty livers result from a lack of insulin. The failure of the carbohydrate → fat synthesis in the adipose tissue has been cited as the cause for the failure of fat mobilization after adrenalectomy.

d′. The Regulation by Thyroid Hormones: Although it is generally assumed that the normal result of thyroidectomy is obesity, MacKay and Sherril[459] reported that the total fat content of adult rats, fed on a fat-rich diet, was actually decreased after thyroidectomy. The rats weighed approximately the same as the controls. In no case could these workers induce obesity by removal of the thyroid gland. On the other hand, Tuerkischer and Wertheimer[120] found that the deposition of glycogen in the adipose tissue was enhanced in thyroid-fed rats, but that the carbohydrate level quickly reached zero thereafter. Thus, thyroxine did not interfere with fat formation, but speeded up the transformation of carbohydrate to fat.

(c) *Nervous Control of Fat Mobilization and Deposition in Adipose Tissue.* Numerous experimental data have demonstrated that nerve stimuli play a profound role in fat mobilization and deposition in the storage depots. Goering[412] pointed out, on the basis of clinical experience, that excessive nerve stimulation provokes a fat loss in the adipose tissue, while paralysis results in a deposition of fat in these depots. Proof of these statements has been obtained experimentally on animals. Thus, it was demonstrated[412,414,460] that section of the femoral nerve of the rabbit caused an increase in

[454] J. Campbell, *Am. J. Physiol.*, *147*, 742–747 (1946).
[455] E. Wertheimer, *J. Physiol.*, *103*, 359–366 (1945).
[456] E. Tuerkischer and E. Wertheimer, *J. Physiol.*, *104*, 361–365 (1946).
[457] F. Pauls and D. R. Drury, *J. Biol. Chem.*, *145*, 481–485 (1942).
[458] De W. Stetten, Jr., and B. V. Klein, *J. Biol. Chem.*, *162*, 377–382 (1946).
[459] E. M. MacKay and J. W. Sherril, *Endocrinology*, *28*, 518 (1941).
[460] J. Mansfeld and F. Müller, *Arch. ges. Physiol. (Pflüger's)*, *152*, 61–67 (1913).

the fat content of the denervated leg, as compared with the control leg, in which the nerve supply was normal. Wertheimer[461] proved that section of the spinal cord above the sixth thoracic segment prevented the development of fatty livers in fasting phlorhizinized dogs, while scission below this level did not prevent the fatty infiltration of this organ. On the other hand, section of the nerves to the liver likewise failed to prevent the accumulation of hepatic fat. The failure of fat mobilization after denervation is also illustrated by the tests of Erben and Hasselbach[462] with phosphorus-poisoned rats, and by those of Mill[463] with rats following hemorrhage. These observations can be interpreted to mean that, in the absence of nervous irritation of the adipose tissue itself, fat mobilization, but not fat deposition, is inhibited.

Normal fat synthesis has been shown to take place following denervation of adipose tissues. Thus, Hausberger[464] reported an influx of glycogen into the denervated interscapular fat body of the mouse, while the symmetrically located organ which possessed its normal innervation had a much lower glycogen level. The accumulation of fat was likewise found to take place more rapidly on the denervated side.[414,464] The denervated interscapulary fat body in the mouse had a higher fat content under all conditions, including fasting, than did the normally innervated control fat body. Moreover, it required long periods of time to deplete the denervated adipose tissue of its fat reserves.

There is some evidence that different nerves may exert their control either on the mobilization or on the deposition phases of fat metabolism in adipose tissue. Beznák and Hasch[465] found that the sympathetic nerves were involved in both mobilization and deposition of fat in the perinephric adipose tissue. According to Kuré and associates,[466] the sympathetic nerves in dogs act as inhibitors of fat deposition, while stimulation of the parasympathetic fibers brings about an acceleration of fat deposition. However, following complete denervation, both phases of fat storage are reduced. Such denervated tissues have a reduced metabolic rate. The deposition of fat following injury to the hypothalamus is likewise believed to be of neurogenic origin. For a discussion of hypothalamic obesity, see page 625.

Wertheimer and Shapiro[401] concluded that a normal innervation of adi-

[461] E. Wertheimer, *Arch. exptl. Pathol. Pharmakol. (Naunyn-Schmiedeberg's)*, *160*, 177–188 (1931).

[462] F. Erben and H. Hasselbach, *Z. ges. exptl. Med.*, *75*, 145–166 (1931).

[463] E. Mill, *Arch. ges. Physiol. (Pflüger's)*, *224*, 304–314 (1930).

[464] F. X. Hausberger, *Z. ges. exptl. Med.*, *102*, 169–177 (1937).

[465] A. B. L. Beznák and Z. Hasch, *Quart. J. Exptl. Physiol.*, *27*, 1–15 (1937).

[466] K. Kuré, T. Oi, and S. Okinaka, *Klin. Wochschr.*, *16*, 1789–1793 (1937).

pose tissue is necessary if this tissue is to maintain its dynamic equilibrium. The maintenance of a "tonus" in adipose tissues is dependent upon constant innervation; it may be changed by pathological conditions. Nerve section is ordinarily followed by a deposition of fat in the tissue involved. It is believed that the status of an animal as regards obesity or leanness, as well as the localized deposition of fat, is related to the extent to which it is subject to nervous stimuli.

(4) Glycogen Deposition in Adipose Tissue

It is only in recent years that the possibility of the presence of glycogen in adipose tissue has been recognized. As early as 1906, Gierke[467,468] noted glycogen in histologic preparations of adipose tissue. Other authors, however, were unable to demonstrate, by chemical tests, the presence of glycogen in the fatty tissues of healthy dogs and rats, under normal conditions.[120]

On the other hand, Wertheimer *et al.*[120,469,470] reported that glycogen could be demonstrated chemically in adipose tissues if the animals were undernourished or fasted, and were then placed for a short time on a high carbohydrate diet. However, the glycogen deposition was shown to be transient, and it could no longer be demonstrated after carbohydrate feeding had been continued for some time. The same conditions which augment fat storage in adipose tissues were found to enhance glycogen deposition; the fact that the deposition of glycogen precedes fat deposition, and that it ceases when fat is no longer laid down in increasing amounts, is evidence that these two phenomena are directly interconnected.

The presence of glycogen in depot fats can be demonstrated in a number of other nutritional states, in addition to the early stages of carbohydrate feeding following a period of undernutrition. Thus, it was found to occur in rats trained to consume their entire daily ration in one hour,[119] in animals fed and starved on alternate days,[455,471] in rats after excessive carbohydrate consumption[120] and in rats which had received appropriate dosages of insulin.[455] In newborn rats, the glycogen supply, which is present in adipose tissues at birth, rapidly decreases, concurrently with the increase in fat in

[467] E. Gierke, *Ergeb. allgem. Pathol., pathol. Anat. (Lubarsch-Ostertag), 11, Part 2,* 871–900 (1907).

[468] E. Gierke, *Verhandl. deut. pathol. Ges., 10th meeting,* Stuttgart, 1906, 182–185 (Jena, 1907).

[469] A. Hoffmann and E. Wertheimer, *Arch. ges. Physiol. (Pflüger's), 217,* 728–746 (1927).

[470] E. Wertheimer, *Arch. ges. Physiol. (Pflüger's), 219,* 190–201 (1928).

[471] E. M. MacKay and D. R. Drury, *Am. J. Physiol., 132,* 661–665 (1941).

these tissues.[472,473] Finally, Hausberger and Gujot[473] demonstrated the presence of glycogen granules in adipose tissue after the suture of nerves to this tissue.

The extent of deposition of glycogen varies in the different fat depots. It is highest in the interscapular brown fat tissues of the rat, where the concentration may be within the same range as in the liver. The adipose tissue in the mesenteric wall is likewise high in glycogen, while the lowest amounts occur in groin fat and in perinephric fat.[401]

Wertheimer and Shapiro[401] are of the opinion that glycogen is synthesized in the adipose tissue. Otherwise it must be carried in the blood stream to the fat depots, and must then diffuse through the cell membranes. However, well-preserved histological preparations of adipose tissue contain glycogen only within the cells, and not in the intercellular spaces. These facts can best be explained on the basis of the assumption that glycogen synthesis occurs *in situ*. Since glycogen appears in such tissues only when fat synthesis is most active, it is believed that its occurrence there is directly concerned with fat synthesis, in which it may be acting as an intermediary product. That this is the main function of the adipose tissue glycogen is shown by the fact that it cannot serve as a source of blood glucose, as is the case with glycogen deposited in other tissues.[122]

(5) *Normal Metabolism in Adipose Tissue*

There is general agreement that adipose tissue exhibits a definite metabolism characteristic of living protoplasm, rather than an absence of respiration, as one would expect if it were simply dead storage tissue. The respiratory quotient of adipose tissue has been reported[474] as 1.0 when it is obtained from normally fed or fasted animals, and over unity,[474,475] especially when glucose is added,[122] in which case it is reported as 1.15, or in adipose tissue containing glycogen,[122] when the figure is 1.27. In fact, Mirski[122] reported that a high R.Q. was maintained for many hours in glycogen-containing adipose tissue, and that a value as high as 1.6 was sometimes noted. These high R.Q. levels are characteristic when a transformation of carbohydrate to fat is taking place (see page 539). They offer con-

[472] F. X. Hausberger and O. Gujot, *Arch. exptl. Pathol. Pharmakol.* (*Naunyn-Schmiedeberg's*), *187*, 655–662 (1937).
[473] F. X. Hausberger and O. Gujot, *Arch. exptl. Pathol. Pharmakol.* (*Naunyn-Schmiedeberg's*), *187*, 647–654 (1937).
[474] G. Scoz, *Arch. sci. biol.* (*Italy*), *17*, 262–273 (1932).
[475] H. Ruska and A. Quast, *Arch. exptl. Pathol. Pharmakol.* (*Naunyn-Schmiedeberg's*), *179*, 217–225 (1935).

firmation of the hypothesis that the carbohydrate → fat change occurs in adipose tissue.

On the other hand, the R.Q. of adipose tissue obtained from fasting animals has been found in most cases to approach that of fat, namely, 0.71. Mirski[122] reported a figure of 0.64, while a quotient of 0.78 was noted when adipose tissue was suspended in serum. In the case of the ground-squirrel, Hook and Barron[476] found a respiratory quotient of 0.80 for the normal animal, and one of 0.67 in the adipose tissue of the hibernating animal, after eight weeks of fasting.

Most observers have reported fairly concordant results on oxygen consumption. The following figures have been reported for Q_{O_2} (mm.^{3} O_2/hr./mg. fresh tissue) of adipose tissue[122]: from fasting rats, 0.12; from adipose tissue containing glycogen, 0.18; from adipose tissue obtained from fasting rats and suspended in serum, 0.18 and, with added glucose, 0.22; and in other fat tissues containing glycogen, 0.38. The variation in R.Q. with and without glycogen, as well as the increase in metabolic activity accompanying fat synthesis, are in accordance with the hypothesis that a synthesis of fat from carbohydrate normally takes place in the adipose tissues.[430]

Although the experiments on rats with experimentally produced lesions of the hypothalamus were not made on adipose tissue but on the whole animal, the data of Tepperman *et al.*[119] indirectly support the conclusion that adipose tissue is one site for the carbohydrate → fat change. Rats having hypothalamic hyperphagia transformed carbohydrate to fat at a markedly accelerated rate as compared with unoperated animals. However, it was also found that, when normal rats consumed as much food as did the hyperphagic rats, within a short interval, correspondingly high R.Q. levels occurred. The augmented rate of fat formation was therefore due to the large accumulation of carbohydrate at one time, in the animal, rather than directly to the hypothalamic injury. However, the speed of the carbohydrate → fat transformation was shown to be accelerated in the liver, as shown by the R.Q. levels in *in vitro* tests obtained on slices prepared from livers of normal rats trained to consume their food within a short interval.[477]

(6) *Enzyme Activity in Adipose Tissue*

There is some disagreement as to whether the enzymatic activity noted in extracts of adipose tissue arises from enzymes elaborated by this tissue or whether it represents activity on the part of enzymes carried there by the

[476] W. E. Hook and E. S. G. Barron, *Am. J. Physiol.*, *133*, 56–63, 334 P (1941).
[477] V. C. Dickerson, J. Tepperman, and C. N. H. Long, *Yale J. Biol. Med.*, *15*, 875–892 (1943).

blood. The presence of diastase,[478] phosphatase,[479] lipase,[401] and dehydrogenase[420] in adipose tissue has been demonstrated. In support of the hypothesis that the diastase obtained from fatty tissues is an enzyme characteristic of adipose tissue, Hausberger[464] demonstrated an increased content in denervated tissue; moreover, the activity of phosphatase is enhanced on recovery feeding.[378] These data are interpreted to mean that the enzymes are formed *in situ* in adipose tissue. On the other hand, Mirski[122] reported that blood diastase and "adipose diastase" are apparently the same, since they act on glycogen in a similar manner to yield as hydrolysis products non-fermentable low polysaccharides, rather than glucose. However, Mirski was able to demonstrate a second pathway of glycogen degradation in brown adipose tissue, which involves phosphorylation of glycogen and the subsequent formation of the Cori ester through the mediation of phosphoglucomutase. The enzyme systems responsible for this change were not found in white adipose tissue.

The presence of dehydrogenases in adipose tissue is more firmly established[419,422] than is that of the diastase and phosphatase. Yosii[420] reported that the dehydrogenase activity in adipose tissue is two to three times that in liver and kidney, and thirty times that in muscle. For an active enzyme preparation, phosphate and a boiled extract of adipose tissue or yeast must be added to the dialyzed extracts of adipose tissue.[421] The co-factor in the boiled extract has been shown to be adenosine-5-phosphoric acid.[421,480] In contradistinction to the properties of other dehydrogenases,[481] those from adipose tissue appear to be concerned only with desaturation and not with the total breakdown of the fatty acids.[480]

The presence of lipolytic enzymes (lipases) in the adipose tissue has been noted by a number of investigators. Quagliariello and Scoz[482] reported that the hydrolysis of both triolein and tributyrin was catalyzed by an extract of the adipose tissue of the dog. The activity of adipose lipase was only 1% that of the same weight of dried pancreas, and approximately the same as that of dried stomach, but it was twenty-five times that of the same weight of dried liver. On the basis of histologic tests, Gomori[483,484]

[478] F. X. Hausberger and N. Neuenschwander-Lemmer, *Arch. exptl. Pathol. Pharmakol. (Naunyn-Schmiedeberg's)*, *192*, 530–535 (1939).

[479] F. X. Hausberger and N. Neuenschwander-Lemmer, *Arch. exptl. Pathol. Pharmakol. (Naunyn-Schmiedeberg's)*, *193*, 110–116 (1939).

[480] O. S. A. K. Lang and H. Mayer, *Z. physiol. Chem.*, *261*, 249–252 (1939).

[481] L. F. Leloir and S. M. Muñoz, *Biochem. J.*, *32*, 299–307 (1938).

[482] G. Quagliariello and G. Scoz, *Arch. sci. biol. (Italy)*, *17*, 513–529 (1932).

[483] G. Gomori, *Arch. Pathol.*, *41*, 121–129 (1946).

[484] G. Gomori, *Proc. Soc. Exptl. Biol. Med.*, *58*, 362–364 (1945).

reported evidence of lipolytic activity in fatty tissues from the rat and rabbit, but not in the fatty tissues of other animals. The results on human fatty tissues have all shown definite lipolytic activity.[485–487] Renold and Marble[487] demonstrated lipolytic activity in the subcutaneous adipose tissue of both rat and man. On the other hand, human intra-articular fat had only about 30% of the activity of human subcutaneous fat. Moreover, a sex variation in lipase activity obtained; in all cases, the concentration of lipolytic enzymes in the adipose tissues of females exceeded that in corresponding tissues from males. In cases of diabetes mellitus, the male tissue had only one-fourth of the lipolytic activity of the corresponding fatty tissue from women.

In the case of rat adipose tissue, Renold and Marble[487] were unable to demonstrate any correlation between lipolytic activity and sex, age, or food intake. Brown fat from a young rat had a lower lipase content than did white fat. The lipolytic activity of rat adipose tissue was one-third of that of the pancreas and one-half that of the liver.

Shapiro and Wertheimer[421] have shown that enzymes capable of attacking phospholipids, glycerophosphoric acid, lactic acid, and succinic acid are present in adipose tissue extracts.

(7) *Brown vs. White Adipose Tissue*

Distinct differences obtain both qualitatively and quantitatively between brown and white adipose tissue.[488] Brown adipose tissue occurs in the adult mouse and rat, and in human embryos,[488] but it is not present in the rabbit, except in the embryonic form.[401] White fatty tissue, largely depleted of its fat, has properties similar to those of brown fat tissue; it also resembles the latter tissue morphologically. Wertheimer and Shapiro[401] suggest that brown adipose tissue may be considered to be embryonic tissue which has failed to develop further in postnatal life.

Eger[488] showed that brown tissue has a much higher metabolic activity than does its white counterpart. Moreover, it has been shown to store glycogen in much higher concentrations than does white adipose tissue; values for fatty tissue glycogen comparable with those of the liver have been reported by Tuerkischer and Wertheimer.[120] On the other hand, Clément[489] reported that fat mobilization is considerably depressed in brown adipose tissue.

[485] D. L. Oesterreicher, Thesis, Univ. Western Ontario, 1947; cited by A. E. Renold and A. Marble, *J. Biol. Chem.*, *185*, 367–375 (1950), p. 367.

[486] A. Marble and R. M. Smith, *Proc. Am. Diabetes Assoc.*, *2*, 173–186 (1942).

[487] A. E. Renold and A. Marble, *J. Biol. Chem.*, *185*, 367–375 (1950).

[488] E. Eger, *Klin. Wochschr.*, *17*, 1033–1038 (1938).

[489] G. Clément, *Compt. rend. soc. biol.*, *141*, 255–257 (1947).

Some investigators have ascribed to brown adipose tissue a special function during hibernation. The brown interscapular fat body is especially developed in hibernating animals. It has been called the "hibernating gland." Earlier literature on this subject has been reviewed by Ferdmann and Feinschmidt,[490] and by Rasmussen.[491]

Later experiments of Wendt[492] suggested that the brown fat from hibernating animals contains an active component which reduces metabolism. Thus, it was found that, when extracts of the brown fat tissue of hibernating hedgehogs were injected into rats, the metabolism was reduced to the extent of 20 to 30%. Hook[493] likewise reported that extracts from the brown fat of the hibernating woodchuck or ground-squirrel produced a similar decrease of metabolism in rats. On the other hand, control experiments indicated that extracts from white adipose tissues such as omentum or perinephric fat tissue from hibernating animals were inactive in altering the respiratory metabolism. There are no tests to prove the nature of this component of brown adipose tissue, or to demonstrate whether or not the concentration varies in this tissue in hibernating and in non-hibernating animals. These results do suggest that fat may play a greater role in the regulation of the metabolic activities of the body by a humoral mechanism than is currently realized.

8. Lipid Storage under Abnormal Conditions

Two different types of abnormal lipid deposition are well recognized. In the first instance, excessive amounts of fat are stored in the usual fat depots, with the result that obesity or adiposity occurs. Under these conditions, the fatty deposits consist chiefly of triglyceride fat having the composition ordinarily noted for storage fats. The second type of abnormal lipid deposition consists of an accumulation of non-glyceride lipids, usually in specific organs, frequently with the result that the function of the affected organ is impaired. In many cases, a considerable enlargement of the particular organ involved also occurs. Cholesterol is the lipid most frequently concerned with this type of abnormality, but sphingomyelin and cerebrosides may be the specific lipids present in abnormal amounts.

(1) *Abnormal Deposition of Triglyceride Fats in General*

Although there are many different theories as to the cause of obesity, one underlying fact is undisputed, namely, that this condition occurs only when

[490] D. Ferdmann and O. Feinschmidt, *Ergeb. Biol.*, *8*, 1–74 (1932).
[491] A. T. Rasmussen, *Endocrinology*, *6*, 760–770 (1922).
[492] C. F. Wendt, *Z. physiol. Chem.*, *249*, iv (1937).
[493] W. E. Hook, *Proc. Soc. Exptl. Biol. Med.*, *45*, 37–40 (1940).

the supply of ingested calories exceeds the quantity of outgoing calories. A situation of this nature may be associated with an excessive appetite, with disturbances of the endocrine glands, or with certain metabolic disorders which result in the conversion of an abnormally large amount of the foodstuffs into fat. The subject of obesity was reviewed in 1944 by Newburgh[494] from the standpoint of energy metabolism, and by Conn,[495] who considered the biological aspects. Pennington[496] suggested that the simple "overfeeding" hypothesis of obesity should be replaced either by one which attributes this condition to psychic stress exerted upon the hypothalamus by way of the autonomic nervous system, as suggested by Waife,[497] or by the hypothesis of the so-called "lipophilia"[498] (tendency of adipose tissue to accumulate fat). Since the same reduction in metabolism obtains in obese individuals subjected to undernutrition as is the case with normal individuals on low-caloric diets, Pennington[499-501] is of the opinion that the treatment of obesity should involve a decrease in dietary carbohydrate which will result in reducing excessive energy stores; this would allow a weight reduction without any decline in energy expenditure, and without the need for caloric restrictions.

a. Obesity Resulting from Overconsumption of Foodstuffs. Newburgh[494] defines obesity as the condition in which the body contains an abnormally large amount of adipose tissue. This excessive fat may be evenly distributed, or may be localized in one or more discrete, encapsulated masses. The latter condition is referred to as "lipomatosis." Most investigators regard excessive caloric intake as the primary cause of obesity. Although, in many cases, the overconsumption is voluntary, the continuation of this practice over an extended period is, in all probability, based upon certain well-defined physiologic principles. The consumption of high-carbohydrate diets results in an increase in insulin production. This brings about an efficient storage of the excess foodstuff in the form of glycogen and fat. However, it results in a concomitant rapid depression in blood sugar, and the resulting hypoglycemia constitutes a stimulus to the ingestion of additional food, which again stimulates the production of more insulin. As early as 1924, Bulatao and Carlson[502] proved that changes in

[494] L. H. Newburgh, *Physiol. Revs.*, *24*, 18–31 (1944).
[495] J. W. Conn, *Physiol. Revs.*, *24*, 31–45 (1944).
[496] A. W. Pennington, *J. Clin. Nutrition*, *1*, 100–106 (1953).
[497] S. O. Waife, *Am. Practitioner*, *2*, 47–50 (1947).
[498] J. Bauer, *Arch. Internal Med.*, *67*, 968–994 (1941).
[499] A. W. Pennington, *Delaware State Med. J.*, *23*, 79–86 (1951).
[500] A. W. Pennington, *J. Clin. Nutrition*, *1*, 343–348 (1953).
[501] A. W. Pennington, *Am. J. Digestive Diseases*, *20*, 268–274 (1953).
[502] E. Bulatao and A. J. Carlson, *Am. J. Physiol.*, *69*, 107–115 (1924).

the blood sugar level exert a pronounced effect upon the appetite. There is also some evidence that the hypothalamus is sensitive to changes in the blood sugar level.[503] The repeated stimulation of insulin production may develop into a vicious cycle, which becomes difficult to counteract. The success of the treatment of obesity by the use of a protein-fat diet, as employed by Pennington,[504] may be attributable not only to a reduced food consumption due to the satiety value of the high-fat diet, but also to the absence of a continued stimulation of the appetite, as a result of hypoglycemia produced by diets containing large proportions of carbohydrate. Ingle[505] reported that marked obesity can be produced in rats by the simple expedient of force-feeding.

(a) *Obesity Due to Hyperinsulinism.* MacKay and associates[506,507] demonstrated that rats grew excessively fat when protamine insulin was injected frequently, and they were given a high-starch diet *ad libitum.* Ordinary insulin did not influence either the food intake or the body weight.

Peters[508] cited a similar type of obesity, in human patients, which may occur spontaneously. Thus, an uncontrollable hunger has been noted in patients who have a spontaneous hypoglycemia, sometimes but not always referable to a tumor in the islands of Langerhans. It is suggested that the overeating which provokes the obesity is a self-protective response.

(b) *Obesity Due to Lesions of the Hypothalamus.* It has long been recognized that, when injury of the hypothalamus results from tumors or from related destructive lesions in this area of the brain, obesity is the logical consequence. As early as 1840, Mohr[509] reported a typical case of obesity suddenly occurring in a 57-year-old woman who had suffered from "mental deterioration" for the preceding three years. Following her sudden death, which occurred not long after the development of the obesity, a large pituitary tumor was demonstrated which had presumably affected the area of the hypothalamus.

The area of the brain injury which will cause obesity has been a matter of controversy over a number of years. Although the original case of *adiposa genitalis* described by Fröhlich[510] in 1901 involved a hypothalamic tumor,

[503] J. Mayer, J. J. Vitale, and M. W. Bates, *Nature, 167,* 562–563 (1951).
[504] A. W. Pennington, *Ind. Med. and Surg., 20,* 267–271 (1951).
[505] D. J. Ingle, *Endocrinology, 39,* 43–51 (1946).
[506] E. M. MacKay, J. W. Callaway, and R. H. Barnes, *J. Nutrition, 20,* 59–66 (1940).
[507] E. M. MacKay and J. W. Callaway, *Proc. Soc. Exptl. Biol. Med., 36,* 406–407 (1937).
[508] J. P. Peters and associates, unpublished observations; cited by J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry,* 2nd ed., Vol. I, Williams & Wilkins, Baltimore, 1946, p. 42.
[509] Mohr, *Wochschr. ges. Heilk., 1840, No. 35,* 565–571.
[510] A. Fröhlich, *Wien. klin. Rundschr., 15,* 882–886, 906–908 (1901).

the obesity was attributed to hypopituitarism. Over a number of years, it has been generally accepted that the so-called "Fröhlich's syndrome" results from dysfunction of the anterior lobe of the pituitary gland. Three years after the original report of Fröhlich, Erdheim[511] expressed the opinion that the syndrome resulted, not from a change in the function of the hypophysis, but rather from the effect of the tumor on the region of the base of the brain. The classical controversy of the two opposing viewpoints has continued until recently. It has now apparently been resolved by Hetherington and Ranson,[512] and also by Hetherington alone.[513] Thus, it was shown that hypothalamic lesions produced obesity in rats, irrespective of whether the anterior lobe of the pituitary was present or had been removed.[512] Moreover, it was shown that no amount of pituitary disorder could cause obesity, provided that the hypothalamus remained intact.[513] In fact, in 1940, Fröhlich[514] stated his conviction that he had been wrong, in 1901,[510] in ascribing the cause of the defect to the pituitary body rather than to the hypothalamus.

According to Brobeck,[380] the obesity resulting from injury to the hypothalamus can be experimentally produced in a number of species by several procedures. In the monkey, dog, cat, and rat, it has been induced either by surgical means or electrolytically by the use of the Horsley-Clarke stereotaxic instrument. Prior to 1940, hypothalamic obesity had been produced in only one or perhaps two dogs.[515,516] Hetherington and Ranson[376,517] observed this condition in the rat following the use of the Horsley-Clarke stereotaxic instrument modified for the rat by Clark.[518] The pioneer experiments of Hetherington were confirmed for the rat by Brobeck, Tepperman, and Long,[379] and by Brooks and associates,[519,520] for the cat by Wheatley[521] and by Ingram,[522] and finally for the monkey by Brooks *et al.*[523] and

[511] J. Erdheim, *Sitzber. Akad. Wiss. Wien., Math.-naturw. Klasse, Abt. III, 113,* 537–726 (1904).

[512] A. W. Hetherington and S. W. Ranson, *Endocrinology, 31,* 30–34 (1942).

[513] A. W. Hetherington, *Am. J. Physiol., 140,* 89–92 (1943).

[514] A. Fröhlich, discussion following paper by P. Bailey, *Research Publs. Assoc. Research Nervous Mental Disease, 20,* 713–724 (1940).

[515] P. Bailey and F. Bremer, *Endocrinology, 5,* 761–762 (1921).

[516] P. Bailey and F. Bremer, *Arch. Internal Med., 28,* 773–803 (1921).

[517] A. W. Hetherington and S. W. Ranson, *Proc. Soc. Exptl. Biol. Med., 41,* 465–466 (1939).

[518] G. Clark, *Science, 90,* 92 (1939).

[519] C. Mc C. Brooks, R. A. Lockwood, and M. L. Wiggins, *Federation Proc., 4,* 9 (1945).

[520] C. Mc C. Brooks, *Federation Proc., 5,* 12 (1946).

[521] M. D. Wheatley, *Arch. Neurol. Psychiat., 52,* 296–316 (1944).

[522] W. R. Ingram, 1945, personal communication to J. R. Brobeck, *Physiol. Revs., 26,* 541–559 (1946), pp. 543, 546, 549.

[523] C. Mc C. Brooks, E. F. Lambert, and P. Bard, *Federation Proc., 1,* 11 (1942).

by Ruch and co-workers.[524] In the case of man, the hypothalamic injury results from some neoplastic or infectious disease which involves either the diencephalon or the adjacent region, namely the pituitary gland. Identical symptoms result from any of these causes in the several species.

The most remarkable circumstance in regard to this type of obesity is the rapidity with which it develops. In the case of laboratory animals having experimentally produced lesions in the hypothalamic region, a dramatic increase in weight may occur within a few hours after the operation. Brobeck[380] states that an adult female rat may increase in weight by as much as 15% during the first twenty-four hours after hypothalamic injury, and may then continue to gain at ten times the normal rate for several weeks. The body weight then plateaus, and may remain constant for many months.[376–379] The period of rapid gain has been designated as the *dynamic* phase, and the plateau as the *static* phase of obesity.[519,525] When this condition obtains in the human subject, obesity begins quite abruptly, and may frequently be correlated with the signs and symptoms of a disease process near the base of the brain.

The most prominent symptom of hypothalamic injury is the obesity resulting from the rapid weight gain. This is to be ascribed to the deposition of excess fat. Smith[375] recorded an experiment in which the surplus fat amounted to 74% of the total carcass weight. The excess fat was distributed uniformly in the various fat depots, including the subcutaneous tissues, the omentum, the mesenteries, the perirenal tissues and around the pericardium.[376] The liver was twice its normal size, and contained approximately four times the normal content of neutral fat. The level of plasma lipids was also elevated, especially following feeding. Moreover, Graef *et al.*[526] reported cirrhosis of the liver in obese dogs with combined hypophyseal and hypothalamic injury. Several symptoms, other than the fat deposition, occur in hypothalamic injury or in tumors of the hypophysis. These include hypoplasia of gonads and genitalia,[380,510,515,516,527,528] which is a prominent symptom in Fröhlich's syndrome. Abnormalities in skin and fur have likewise been observed in rats, probably because the animals are unable to groom themselves as normal animals do.[529] In the case of dogs the fur becomes soft and luxuriant.[526] The obese animals frequently die as a result of vague non-specific diseases.[376,519,522] Generalized dilation and

[524] T. C. Ruch, H. D. Patton, and J. R. Brobeck, *Federation Proc.*, *1*, 76 (1942).
[525] H. R. Rony, *Obesity and Leanness*, Lea & Febiger, Philadelphia, 1940, p. 47.
[526] I. Graef, J. Negrin, Jr., and I. H. Page, *Am. J. Pathol.*, *20*, 823–855 (1944).
[527] M. J. Babinski, *Rev. neurol.*, *8*, 531–533 (1900).
[528] A. W. Hetherington, *Endocrinology*, *26*, 264–268 (1940).
[529] A. W. Hetherington and S. W. Ranson, *J. Comp. Neurol.*, *76*, 475–499 (1942).

hypertrophy of the gastrointestinal tract occur. Conspicuous abnormalities in kidney function and structure have been noted, which result in albuminuria, in the presence of erythrocytes and casts in the urine, in hyalinization of the renal glomeruli, in dilation and necrosis of the tubules, and also in a generalized increase in the amount of connective tissue around the nephrons.[379] In addition to the physical symptoms, abnormalities in behavior have been reported. These are particularly concerned with hyperexcitability and hyperirritability.[530]

a′. Metabolic Aspects of Hypothalamic Obesity: It is a moot question whether or not the obesity resulting from hypothalamic injury is to be ascribed solely to increased food intake. This accumulation of fat might conceivably occur without augmentation of the energy intake but with a decline in basal metabolism, a decrease in spontaneous activity, or a reduction in specific dynamic action.

Experimental results indicated that the excess fat deposition resulting from hypothalamic injury is largely if not entirely ascribable to increased food consumption. In 1933, Keller, Hare, and D'Amour[531] first called attention to the "enhanced appetite" of cats and dogs subjected to hypothalamic lesions; this increased appetite was later associated with the resulting obesity by Keller and Noble.[377] Since these first experiments, the "enhanced appetite" has been shown to be a prominent symptom in the rat,[277,519,520,532] the cat,[521,522] the dog,[533] and the monkey.[523] Brobeck *et al.*[379] coined the term, *hyperphagia*, to connote the increased appetite. They consider that this term is preferable to the terms "hunger," "appetite," "satiety," "bulimia," and "polyphagia."

According to Brobeck,[380] rats have an entirely abnormal attitude toward their food almost immediately after hypothalamic injury. They continuously exhibit symptoms of extreme hunger, and are described as "attacking" or "devouring" food instead of eating it normally. Wheatley[521] describes how cats with hypothalamic lesions "wolfed" their food, in contradistinction to normal cats which ate their food slowly. Ingram[522] likewise calls attention to the fact that the operated cats had "a high degree of voracity, eating their meals in a fraction of the time taken by most normal cats or by cats with control lesions."

[530] A. W. Hetherington and S. W. Ranson, *Am. J. Physiol.*, *136*, 609–617 (1942).
[531] A. D. Keller, W. K. Hare, and M. C. D'Amour, *Proc. Soc. Exptl. Biol. Med.*, *30*, 772–775 (1933).
[532] J. Tepperman, J. R. Brobeck, and C. N. H. Long, *Am. J. Physiol.*, *133*, 468P–469P (1941).
[533] P. Heinbecker, H. L. White, and D. Rolf, *Am. J. Physiol.*, *141*, 549–565 (1944).

Although there is general agreement that the onset of obesity is primarily associated with the hyperphagia,[379,519,522,530,534] Brobeck *et al.*[379] cited experimental evidence to indicate that other factors may be involved. When a comparison in weight gain was made between normal rats and operated animals, using the pair-feeding technic, it was found that the same rate of gain obtained in the two groups in 75% of the cases. However, in 25% of the tests, the rats subjected to the hypothalamic injury gained somewhat better than did their pair-fed controls.

A second factor, other than hyperphagia, which may be partly responsible for the obesity resulting from hypothalamic injury, is a decrease in the spontaneous locomotor activity. Although a brief period of extreme hyperactivity occurs after the animal has recovered from the anesthetic following the brain operation, this is followed by a period of inactivity, which persists for several months. This inactivity has been observed not only in the rat,[379,520,530] but also in the cat.[522] On the one hand, Hetherington and Ranson[530] considered that the inactivity was the primary source of the excess energy stored as fat by their animals, while the food intake was thought to be a factor of minor importance. However, on the other hand, most other workers[379,520,522] are of the opinion that the hyperphagia is the principal cause of obesity, particularly when *ad libitum* feeding is employed, while the inactivity is a contributory cause of obesity, important only under certain conditions. It has been pointed out by Brooks[520] that inactivity itself does not always lead to obesity. Moreover, although the extent of activity may be decreased in rats with injuries of the hypothalamus, the total energy expenditure for the decreased activity may exceed that of the controls, inasmuch as the animal must exert more force to move the fat-laden members of its body during the movements required for drinking, eating, respiration, and for like activities.

A third condition which may influence fat deposition in animals suffering from hypothalamic injury is the reduction of the total heat production. In this situation, the food would be more efficiently utilized and the proportion of energy available for storage as fat would be increased. However, this cannot account for the obesity of the rats receiving the *ad libitum* diet, because an actual increase of heat production obtains in the obese animals, which may become twice as great as it was before the operation.[379,534,535] Brobeck[380] is of the opinion that the energy surplus is mainly the result of the hyperphagia. In spite of the marked increase in total heat production in the obese rats following hypothalamic lesions, there is some evidence

[534] C. Mc C. Brooks and D. N. Marine, *Federation Proc.*, *5*, 12 (1946).
[535] J. M. Bruhn and A. D. Keller, *Am. J. Physiol.*, *133*, 229P–230P (1941).

that the metabolism is actually diminished as a result of injury to this portion of the brain. This can be demonstrated when the metabolism tests are made shortly after the operation, before an appreciable weight gain occurs, or in animals on a limited food intake, so that the excessive body weight is prevented.[532,534] However, it was concluded by Brobeck[380] that "quantitatively the amount of energy made available for extra storage by the reduced heat production is almost insignificant in contrast to the huge excess ordinarily taken in as food." The most complete review of the subject of the obesity resulting from hypothalamic lesions is that of Brobeck.[380]

(c) *Obesity Resulting from the Injection of Gold-Thioglucose.* Brecher and Waxler[536] were the first to call attention to the fact that obesity may be produced, in stock albino mice, by a single injection of gold salt of thioglucose. This is an unstable hydroscopic compound containing approximately 50% of gold. The weight gains were shown to be due to an increased proportion of adipose tissue. It was later demonstrated[537] that the increased fat deposition resulted from an augmented food intake. Owen, Parson, and Crispell[538] suggest that this type of obesity may result from serious interference with the "satiety mechanism," and may be associated with less serious effects on the "hunger mechanism," as has been postulated in the case of experimental hypothalamic obesity. It is suggested that the obesity resulting from gold-thioglucose may be mediated through hypothalamic dysfunction.

b. Obesity Resulting from Reduced Basal Metabolism. Although an increased food intake may account for a large proportion of the incidence of obesity, this condition may frequently arise from a reduced metabolic rate coupled with a normal food intake. The tendency toward the deposition of fat in myxedema and other types of hypothyroidism is an excellent example of this kind of obesity. A decrease in basal metabolism may likewise occur, following castration, or after the menopause, which may be a contributing factor in increasing fat deposition. Finally, the reduction of the basal metabolism which is a normal concomitant of increasing age (after puberty) may likewise play an important role in bringing about the increased body weight frequently occurring in older individuals.

c. Obesity Resulting from Reduced Activity. Ingle[539] reported that obesity can readily be produced in rats by restriction of their activity. Under these conditions, the development of obesity is related to the level

[536] G. Brecher and S. H. Waxler, *Proc. Soc. Exptl. Biol. Med.*, *70*, 498–501 (1949).
[537] S. H. Waxler and G. Brecher, *Am. J. Physiol.*, *162*, 428–433 (1950).
[538] J. A. Owen, Jr., W. Parson, and K. R. Crispell, *Metabolism*, *2*, 362–366 (1953).
[539] D. J. Ingle, *Proc. Soc. Exptl. Biol. Med.*, *72*, 604–605 (1949).

of energy output, as well as to the level of food intake. According to Ingle,[539] it represents a principle long recognized in medicine and in animal husbandry. When adult male rats were confined in cages 9 inches long, 4.5 inches wide, and 4.5 inches deep, and were given an appetizing fluid diet *ad libitum*, the maximum body weights attained all exceeded 700 g.; one rat reached a maximum body weight of 1090 g. In contradistinction to these levels, Ingle[539] states that he has never recorded a weight greater than 550 g. for adult male rats on the stock diet, nor one greater than 600 g. when they were fed on a fluid diet.

d. Hereditary Obesity in Mice. Certain strains of mice are known to be especially susceptible to obesity. Thus, Fenton and Carr[540] and Fenton and Dowling[541] noted that, although it is the rule for mice of many inbred strains to become obese late in life without special nutritional manipulations, this rarely occurred in the case of younger animals. However, when mice were placed on diets of increasing fat content, an increased weight (which was interpreted as synonymous with a higher fat deposition) could be produced in rats of the C_3H and A strains, but not in the case of animals from the C_{57} and I strains.[540] Whereas the average fat content of mice of the A strain at three months on two representative diets was 24.6 and 25.2%, the mean value for mice of the I strain on these diets was only 13.1 and 8.7% at this age.[541] In a later study from the same laboratory,[542] it was shown that mice of the C_{57} and C_3H strains both consumed considerably more calories when fed a 50% fat diet than when given the 5% fat regimen. Moreover, the oxygen consumption was higher in mice of the C_{57} strain on a 50% fat intake than on a diet containing 5% of fat, indicating that the increased calories consumed on the high-fat diet were at least partly oxidized. On the other hand, no greater oxygen consumption occurred in the case of the C_3H strain on the high-fat diet than in those on the low-fat diet, in spite of the fact that the intake of calories was greater in the former case. Thus, the extra calories consumed were deposited as carcass fat. This difference in ability to oxidize extra fat exhibited by these two strains of mice may well explain the predisposition of the C_3H strain to develop obesity.

According to Mayer *et al.*,[543] the obese-hyperglycemic syndrome in mice is a Mendelian recessive condition, characterized by adult weights in the 60 to 115 g. range, atrophy and ulceration of the skin, hyperglycemia, and glycosuria. The obese animals were shown to exhibit atypical distribution

[540] P. F. Fenton and C. J. Carr, *J. Nutrition, 45,* 225–233 (1951).
[541] P. F. Fenton and M. T. Dowling, *J. Nutrition, 49,* 319–331 (1953).
[542] J. B. Lyon, Jr., M. T. Dowling, and P. F. Fenton, *J. Nutrition, 51,* 65–70 (1953).
[543] J. Mayer, D. N. Silides, and M. W. Bates, *Federation Proc., 12,* 423 (1953).

of nutrient selection, a low overall oxygen consumption, low activity, and an impaired resistance to cold. The rate of oxidation of injected radioacetate was likewise found to be depressed, as indicated by an increased accumulation of fatty acids and cholesterol. The hyperglycemia is extremely resistant to insulin and is sensitive to diet and growth hormone. In a later report, Mayer and co-workers[544] suggest that a main feature of hereditary obesity in mice is their failure to oxidize C_2 fragments, with the result that the pyruvate derived from carbohydrate is converted to fat instead of being oxidized. It is postulated that a blocking of the hexokinase reaction obtains under these conditions.

These workers[543] are of the opinion that this hereditary obesity results from an oversecretion of an alpha cell hormone of the islets of Langerhans. When the alpha cells are destroyed by diethyl dithiocarbamate, the metabolism of such mice is completely changed. Thus, some weight loss takes place, hyperglycemia disappears, blood glucose is no longer increased by the administration of growth hormone, the oxidation of acetate becomes normal, and insulin resistance is greatly decreased. Mayer and Hagman[545] note that only 12% of the excess weight of obese animals is water; the total proportion of the weight represented by water is similar in young obese and non-obese animals of the same weight.

Mayer[546] points out that obesity has a multiple etiology. According to the glycostatic mechanism of regulation of food intake, appetite is regulated by the rate of passage of glucose-phosphate into centers situated in the lateral parts of the anterior hypothalamus ("feeding centers"). More central centers, or "obesity" centers, act in part indirectly through the control of the autonomic nervous system on the rate of lipogenesis and energy metabolism. It is suggested that hyperphagia results from the activation of the lateral centers, inactivation of the central centers, lesions of the frontal or thalamic association fibers or frontal centers, hypoglycemia, a block of the hexokinase reaction or, indirectly, from an increased lipogenesis, metabolic inertia of depot fat, or from abnormalities in metabolism resulting from immobilization. In the case of the hereditary obese-hyperglycemic syndrome, the role of the endocrine glands also appears to be fundamental in the etiology of the obesity. Hypersecretion of a pancreatic hormone other than insulin is believed to be primarily responsible for this condition.

[544] J. Mayer, R. E. Russell, M. W. Bates, and M. M. Dickie, *Metabolism, 2,* 9–21 (1953).

[545] J. Mayer and N. C. Hagman, *Proc. Soc. Exptl. Biol. Med., 82,* 647–649 (1953).

[546] J. Mayer, *Abst. XIX Intern. Physiol. Congr.,* Montreal (Aug.–Sept., 1953), pp. 598–599.

(2) *Abnormal Deposition of Triglycerides in the Liver*

Although fatty livers have long been recognized, it is only comparatively recently that the factors related to the deposition of fat in this organ have been understood. The condition has been referred to both as a fatty degeneration and as a fatty infiltration. However, according to both viewpoints, the production of fatty livers is caused by an abnormal accumulation in this organ of lipids which have been transported there from other tissues. For a review of the subject of fatty livers, the reader is referred to the excellent treatises of McHenry and Patterson,[547] Frame,[548] and Peters and Van Slyke.[549] Best and Lucas[550] reviewed the subject from the standpoint of the importance of choline as a lipotropic agent.

a. Classification of Fatty Livers. Two main types of fatty livers can be differentiated, namely, physiologic and pathologic. In the case of the physiologic fatty livers, which develop whenever large amounts of fat are mobilized from fat depots to meet unusual requirements for fat combustion, there is merely an increase in the normal physiologic action of this organ. The proportion of the several lipid components is not altered, and the liver maintains its ordinary biochemical reactions satisfactorily.

On the other hand, the pathologic type of fatty livers occurs in the cases in which the partition of lipids differs from the normal, and in which, from a quantitative standpoint, the amounts of lipid are much higher than in the physiologic type. Such pathologic fatty livers occur not only when an animal receives an hepatotoxic agent such as chloroform, carbon tetrachloride, benzene, phosphorus, or phlorhizin, but also as a result of organic toxic agents which are attributable to severe infectious processes such as diphtheria, acute yellow atrophy, pernicious anemia, nephrosis, or diabetes mellitus, and also to the toxemia of pregnancy. Dallemagne *et al.*[551] reported that fatty liver and fatty biliary ducts resulted in the case of dogs subjected to chronic intoxication with γ-hexachlorocyclohexane. No nuclear or cellular destruction was observed as a result of this liver-fattening agent. Moreover, an even larger group of pathologic fatty livers owe their origin to the lack of some essential component such as choline, lipocaic, inositol, or other related "lipotropic" agents in the diet. A lipotropic agent or factor may be defined as "a substance which prevents or removes

[547] E. W. McHenry and J. M. Patterson, *Physiol. Revs.*, *24*, 128–167 (1944).

[548] E. G. Frame, *Yale J. Biol. Med.*, *14*, 229–255 (1942).

[549] J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, 2nd ed., vol. I, Williams & Wilkins, Baltimore, 1946.

[550] C. H. Best and C. C. Lucas, *Vitamins and Hormones*, *1*, 1–58 (1943).

[551] M. J. Dallemagne, M. A. Gerebtzoff, and E. Philippot, *Compt. rend. soc. biol.*, *144*, 457 (1950).

an accumulation of excess fat in the liver."[547] The term was first employed in 1935 by Best, Huntsman, and Ridout[552] to describe the action of choline in the prevention and cure of fatty livers.

Finally, physiologic and pathologic fatty livers can be differentiated by the resultant effects on metabolism. When a physiologic fatty liver develops as a result of fasting, hyperlipemia and ketosis appear. Anything which accelerates the metabolism of carbohydrate will tend to abolish the physiologic fatty liver. This is effected by the injection of insulin into the diabetic animal, or by the administration of glucose or other metabolizable carbohydrate to the animal having the physiologic type of fatty liver as a result of complete starvation or of carbohydrate starvation. However, lipotropic agents which ameliorate the condition of pathologic fatty livers are entirely ineffective in the physiologic type.[553,554]

On the other hand, it has been stated that the animal with the pathologic fatty liver does not ordinarily exhibit a ketosis or hyperlipemia, but frequently has a hypolipemia. However, Deuel *et al.*[277] demonstrated that a ketonuria does obtain in fasting rats having fatty livers as a result of a choline-deficient diet. This endogenous ketonuria is "physiological," since the same sex difference exists as in normal fasting human subjects, and in rats having an exogenous ketonuria. The cholesterol esters and phospholipids, particularly, are reported to be reduced[555] in the serum, while an unusually large proportion of the fat in the liver consists of phospholipids.[549] The pattern of the liver lipids varies consistently with the types of pathologic fatty livers, but depends upon the circumstance responsible for the origin of the condition.[520,556–559] The administration of carbohydrate has no effect in reducing the liver lipids. In fact, Best and Hershey[560] indicated that there is an increased glucose tolerance and a decrease of the insulin requirement, in depancreatized dogs, associated with the development of fatty livers. There is no increase in fat utilization when a pathologic fatty liver exists. When the appropriate lipotropic agent is given, the fat leaves the liver, the normal pattern of liver lipids is restored, the blood lipids return to normal, and the fat depots become filled with the characteristic depot fat.

552 C. H. Best, M. E. Huntsman, and J. H. Ridout, *Nature*, *135*, 821–822 (1935).

553 L. R. Dragstedt, C. Vermeulen, W. C. Goodpasture, P. B. Donovan, and W. A. Geer, *Arch. Internal Med.*, *64*, 1017–1038 (1939).

554 A. I. Lewin, *Z. ges. exptl. Med.*, *96*, 532–547, 548–560 (1935).

555 I. L. Chaikoff and A. Kaplan, *J. Biol. Chem.*, *106*, 267–279 (1934).

556 C. H. Best, H. J. Channon, and J. H. Ridout, *J. Physiol.*, *81*, 409–421 (1934).

557 C. H. Best, M. E. H. Mawson, E. W. McHenry, and J. H. Ridout, *J. Physiol.*, *86*, 315–322 (1936).

558 E. P. Ralli, S. H. Rubin, and C. H. Present, *Am. J. Physiol.*, *122*, 43–47 (1938).

559 De W. Stetten, Jr., and G. F. Grail, *J. Biol. Chem.*, *144*, 175–181 (1942).

560 C. H. Best and J. M. Hershey, *J. Physiol.*, *75*, 49–55 (1932).

b. Description of Fatty Livers. The pathologic fatty livers which develop as a result of the absence of choline in the diet have a characteristic appearance. They are greatly enlarged, due to their engorgement with fat. Whereas the liver usually accounts for approximately 3 to 4% of the total body weight, the average proportion of the body weight made up by the liver, in fifteen female rats receiving a choline-free diet with 2% cholesterol, was 6.68%, in the experiments of Deuel *et al.*[277] The actual amounts of water, protein, and ash remain constant in the fatty livers, although the percentage of these components decreases inversely with the increase in liver lipids. Liver lipids normally comprise 3 to 4% of the total moist weight of the liver, while the mean proportion of liver lipid in the group of female rats receiving 2% cholesterol was 37.9% of the moist liver weight, and the average water content was only 47.3%. Individual livers were found to have as much as 45% of lipids. The increased lipid in the fatty livers was composed almost exclusively of neutral fat. This fraction did not increase in the other tissues of animals with fatty livers, except for a slight increase in the brain, when cholesterol fatty livers, or hepatic fat deposits following liver feeding, were tested. Other lipid components remained constant in the fatty livers, except for choline, which was decreased.[561] Fatty livers are white, contain little blood, and are exceedingly brittle. They are so fragile that it is almost impossible to remove them whole.

Extremely fatty livers containing 68% of fat (dry-weight basis) have been obtained[562] in choline-deficient rats fed a 20% protein diet for one week, following seven days of fasting or twenty-one days of protein depletion. Under similar conditions, normal liver fat was maintained in rats receiving adequate dietary choline.

One of the most striking features of pathologic fatty livers produced by a choline-low diet is the rapidity with which they develop. When rats previously on a stock diet were fed the diet described by Best and Channon,[563] Deuel and Hallman[564] observed a marked increase in liver lipids within twenty-four hours; this was followed by a progressive rise in these components which did not reach the maximum value until more than sixteen days later. The data illustrating the rate of development of fatty livers are indicated in Table 21 (page 636).

The response of the female rats to the choline-free diet is much more pro-

[561] T. Tokushima, M. Yoshihara, and T. Shimojo, *J. Biochem. (Japan)*, *39*, 5 (1952).
[562] O. M. Hale and A. E. Schaefer, *J. Nutrition*, *46*, 479–487 (1952).
[563] C. H. Best and H. J. Channon, *Biochem. J.*, *29*, 2651–2658 (1935).
[564] H. J. Deuel, Jr., and L. F. Hallman, *J. Biol. Chem.*, *140*, 545–554 (1941).

TABLE 21

ANALYSES OF THE LIVERS OF MALE AND FEMALE RATS PREVIOUSLY FED A HIGH-FAT DIET FOR VARIOUS PERIODS AND THEN SACRIFICED[a]

	Male rats					Female rats				
Days on diet	Body wt., g.	Liver wt., g.	Liver, % body wt.	Liver water,[b] %	Liver lipid,[b] %	Body wt., g.	Liver wt., g.	Liver, % body wt.	Liver water,[b] %	Liver lipid,[b] %
0	227	9.32	4.10	69.4 ± 0.1	2.79 ± 0.2	153	6.06	3.99	70.4 ± 0.2	3.15 ± 0.1
1	202	7.33	3.63	68.7 ± 0.1	5.48 ± 0.2	158	6.22	3.94	67.8 ± 0.2	7.54 ± 0.2
3	201	7.36	3.67	68.5 ± 0.2	4.81 ± 0.2	164	6.33	3.85	67.2 ± 0.2	8.22 ± 0.5
6	187	7.49	4.03	65.7 ± 0.5	11.72 ± 0.7	144	6.26	4.36	64.8 ± 0.6	12.98 ± 0.8
9	182	7.48	4.11	61.5 ± 0.8	16.73 ± 0.8	139	6.04	4.36	60.8 ± 0.7	17.57 ± 1.0
12	154	7.24	4.75	60.1 ± 0.6	19.81 ± 1.2	135	6.75	5.01	58.4 ± 1.0	22.42 ± 1.4
16	161	8.24	5.09	55.1 ± 0.7	26.25 ± 0.9	139	8.44	6.04	50.1 ± 0.7	33.93 ± 1.0

[a] H. J. Deuel, Jr. and L. F. Hallman, *J. Biol. Chem.*, *140*, 545–554 (1941).
[b] Including the Standard Error of the Mean.

TABLE 22

LIVER WATER AND LIPIDS OF FASTED AND UNFASTED MALE RATS RECEIVING THE STOCK DIET, WITH OR WITHOUT BETAINE HYDROCHLORIDE, FOR VARIOUS PERIODS AFTER RECEIVING A CHOLINE-FREE DIET FOR 12 DAYS[a]

	Unfasted rats					Fasted rats					
		Liver					Liver				
Days on stock diet	No. of rats	Wt., g.	% body wt.	Water, %	Lipid, %	No. of rats	Wt., g.	% body wt.	Water, %	Lipid,[b] %	Av. acetonuria, mg./100 sq. cm.
Rats receiving stock diet but no betaine hydrochloride											
Control[c]	15	—	4.11	67.5	3.76	5	—	2.80	70.1	4.31	0.8
0	15	8.74	4.13	56.2	25.55	7	4.92	4.02	61.2	20.34	22.4 ± 1.7
7	5	8.61	3.61	67.7	7.22	4	5.85	2.86	65.5	12.83	13.7 ± 2.7
										+5 61	0.8 ± 0 3
14	5	9.95	3.93	67.7	6.02	5	5.81	2.79	67.0	9.42	
										+3.40	
21	5	10.14	3.54	69.9	4.73	5	5.59	2.71	68.7	7.90	3.1 ± 0.7
										+3.17	
Rats receiving stock diet with 100 mg. betaine hydrochloride daily											
7	3	9.65	4.19	68.7	6.40	5	5.50	2.71	69.8	6.18	9.0 ± 1.5
										(−0.22)	
14	5	9.53	3.87	67.3	4.04	5	5.62	2.70	68.2	7.47	0.8 ± 0.2
										(+3.43)	
21	5	8.87	3.66	69.8	2.90	5	5.49	2.72	69.5	6.26	1.8 ± 0.5
										(+3.36)	

[a] Adapted from H. J. Deuel, Jr., and L. F. Hallman, *J. Biol. Chem.*, *140*, 545–554 (1941); and from H. J. Deuel, Jr., L. F. Hallman, and S. Murray, *J. Biol. Chem.*, *119*, 257–268 (1937).

[b] The figures with + or − values represent changes in composition from that of the unfasted controls.

[c] Comparative results on male rats not subjected to the high-fat diet.

nounced than is that of the male rats. Even after the choline-free diet had been fed for only twenty-four hours, the liver lipids were increased from 3.15 to 7.54% in the female rats. Moreover, the values for liver lipid remained consistently higher in the female rats, and the water content of the liver was uniformly lower than in the male rats.

The disappearance of the lipid from the liver occurred at a much slower rate than did its accumulation. Thus, after five days of fasting, in the case of rats which had fatty livers, a further increase of fat was observed in all experiments with male rats.[564] The increases were as follows for the several groups: 0 days (controls), 1.49; 1 day, 3.25; 3 days, 4.75; 6 days, 3.43; 9 days, 2.83; 12 days, 0.53; and 16 days, 2.59%. However, in the case of female rats, the increases in liver lipids during the fasting period were invariably less than in the male animals. This reflects the higher metabolism of fats in the female. The changes in the several groups of female rats after a five-day fasting period were as follows: 0 days (control), +1.59; 1 day, −1.82; 3 days, +2.11; 6 days, −2.65; 9 days, +2.31; 12 days, +0.49; and 16 days, −5.76%.

On the other hand, when male rats having fatty livers were given the stock diet without fasting, the liver fat decreased rapidly, but was still slightly elevated after twenty-one days. When betaine hydrochloride, which possesses a lipotropic action, was also administered, the decrease in liver lipids was much more precipitous. However, even in this case, the lipid metabolism remained quite susceptible to inanition, since the response to fasting as indicated by increased liver lipids was greater than in the case of normal rats. Finally, another index of fat metabolism, namely fasting ketonuria, has been shown to remain elevated for seven days after rats with fatty livers were placed on the stock diet, although the response was reduced more rapidly in rats receiving betaine hydrochloride. These results are summarized in Table 22 (page 637).

The source of the lipid in fatty livers is also of interest. Li and Freeman[565] demonstrated that the fat accumulating in the liver of a protein-deficient dog is largely exogenous in origin. This was indicated by the fact that the fat content of the livers was higher on the high-fat, protein-deficient diet, with or without cholesterol, when bile salts were added. This hypothesis is also supported by the fact that the fat content of the liver increased when the dietary fat was augmented. Channon *et al.*[566] have concluded, on the basis of experiments on rats, that the extent of fatty

[565] T. W. Li and S. Freeman, *Am. J. Physiol.*, *145*, 667–675 (1946).

[566] H. J. Channon, S. W. F. Hanson, and P. A. Loizides, *Biochem. J.*, *36*, 214–220 (1942).

infiltration in the liver is related to the proportion of C_{14} to C_{18} saturated acids in the diet. Solid unsaturated fatty acids were shown to exert no effect. On the other hand, elaidic acid was distributed normally, and appeared in the liver in proportion to its amount in the diet. Choline, however, plays no role in the deposition of carcass fat.[567]

Another characteristic of fatty livers is the fact that, apparently, there is a difference in susceptibility as related to species. Although mice, rats, and dogs readily develop fatty livers on diets deficient in choline, Handler[568] observed that guinea pigs did not accumulate abnormal levels of liver fat on these regimens. This fact can apparently be correlated with the lack of hepatic choline oxidase activity in the guinea pig.

Age plays a prominent role in the hepatic response to choline and cystine deficiencies. Young rats succumb much more readily than do older rats when these supplements are absent from the diet. While choline deficiency almost invariably results in hepatic fibrosis in young rats, Handler and Fallis[569] state that, in the case of rats eight months of age or older, fatty livers alone result. Although the incidence of hepatic necrosis due to cystine deficiency was found to be about the same in young and in old rats, the rate of development was about three times as rapid in the former case.

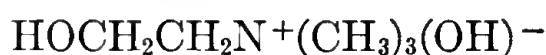
c. Conditions Related to the Development of Pathologic Fatty Livers. Many factors will result in the development of pathologic fatty livers but, for the most part, they are related either directly or indirectly to the base, choline. Since this compound is the most important substance related to fatty livers, both in the historical development and in more recent practical application, it will be considered at length.

(*a*) *Choline and Choline Precursors as Lipotropic Agents.* a′. The Historical Development of Knowledge of the Lipotropic Action of Choline: The realization that choline acts to prevent the accumulation of fats in the liver is a direct outgrowth of the discovery of insulin. The first relatively short-term experiments of Banting and Best on the maintenance of depancreatized dogs by means of insulin were signally successful in clarifying diabetic conditions. However, it was noted simultaneously by Fisher[570] and by Allan, Bowie, MacLeod, and Robinson[571] that, when the operated animals were maintained for periods up to two years on this hormone, the dogs had large yellow livers containing an excess of fat. The development

[567] C. S. Raman, *Biochem. J.*, *52*, 320–324 (1952).
[568] P. Handler, *Proc. Soc. Exptl. Biol. Med.*, *70*, 70–73 (1949).
[569] P. Handler and R. H. Fallis, Jr., *Proc. Soc. Exptl. Biol. Med.*, *75*, 567–570 (1950).
[570] N. F. Fisher, *Am. J. Physiol.*, *67*, 634–643 (1924).
[571] F. N. Allan, D. J. Bowie, J. J. R. MacLeod, and W. L. Robinson, *Brit. J. Exptl. Pathol.*, *5*, 75–83 (1924).

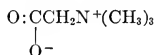
of fatty livers in insulin-treated depancreatized dogs could be prevented by the feeding of raw pancreas. Hershey alone[572] and later with Soskin[573] reported that crude egg yolk "lecithin" could be used to combat fatty livers as effectively as could raw pancreas. Hershey used lecithin in place of raw pancreas because he considered that the development of fatty livers was a result of failure in fat transport rather than of a disturbance in its digestion. Since the phospholipids were known to be primarily concerned with the former function, lecithin was selected to test its effectiveness in removing fat from the liver.[573]

A renewed stimulus to the development of the problem followed the discovery by Best, Hershey, and Huntsman[574] that fatty livers could readily be produced in normal rats by feeding a diet high in saturated fats. The availability of the white rat for such studies made it possible to carry out many more experiments at a fraction of the cost than was possible with depancreatized dogs. The rats responded to lecithin feeding by maintaining normal liver lipids when put on the liver-fattening diet. In determining the fraction of lecithin which is responsible for the lipotropic action,[575,576] it was found that sodium oleate, glycerophosphoric acid, and ethanolamine[576] were inactive; however, it was noted that choline and a closely related compound, betaine, were both highly active in preventing the accumulation of fat in the liver. It was also reported that mice developed fatty livers on an appropriate dietary regimen, and that they could be prevented by choline.[577] Choline is trimethylhydroxyethylammonium hydroxide:



$$HOCH_2CH_2N^+(CH_3)_3(OH)^-$$

while betaine is the anhydride of the corresponding acid with the structure:



$$O{:}\underset{\underset{O^-}{|}}{C}CH_2N^+(CH_3)_3$$

For a complete discussion of the chemical properties of these compounds, the reader is referred to the review of Best and Lucas,[550] and to pages 426 to 432 of Volume I.

b′. The Effect of Choline on Various Types of Fatty Livers: The effectiveness of choline in preventing fatty livers due to high-fat diets was con-

[572] J. M. Hershey, *Am. J. Physiol.*, *93*, 657P–658P (1930).
[573] J. M. Hershey and S. Soskin, *Am. J. Physiol.*, *98*, 74-85 (1931).
[574] C. H. Best, J. M. Hershey, and M. E. Huntsman, *J. Physiol.*, *75*, 56–66 (1932).
[575] C. H. Best, J. M. Hershey, and M. E. Huntsman, *Am. J. Physiol.*, *101*, 7P (1932).
[576] C. H. Best and M. E. Huntsman, *J. Physiol.*, *75*, 405–412 (1932).
[577] C. H. Best, M. E. Huntsman, and O. M. Solandt, *Trans. Roy. Soc. Can., Sect. V*, *26*, 175–176 (1932).

firmed by Best in collaboration with Channon.[563] It was found that, when fatty livers were developed by the administration of a diet high in cholesterol, large doses of choline were required to prevent the fatty infiltration.[370,556,578–580] Moreover, although choline was able to prevent the accumulation of glycerides in such livers,[578] it was not possible to prevent the storage of cholesterol esters in this organ. When choline was given to rats which had developed fatty livers as a result of a high-cholesterol diet, the triglyceride fraction of the liver was rapidly decreased, while the cholesterol esters were only slowly affected.[556,580]

Junkersdorf and Kohl[581] reported indirectly that, in the physiologic type of fatty livers occurring after fasting, the fatty infiltration receded following the eleventh day of fasting when choline was given. However, in the rat, which is much more resistant to the development of fatty livers on fasting than is the dog, Best[582] was unable to demonstrate any positive action on the part of choline in reducing liver lipids. Moreover, Best and Ridout[583] found that, in the rabbit, guinea pig, or mouse, choline was likewise ineffective in preventing the development of fatty livers as a result of starvation.

Best and co-workers[584] found that, when the fatty infiltration of the liver was produced by the administration of a toxic substance such as phosphorus, choline did not inhibit the infiltration of fat or the ensuing degenerative changes in that organ. However, after recovery, choline was shown to increase the rate of removal of lipid from the liver. This result was confirmed by Laszt and Verzár.[585] In the case of the hepatotoxic agent, carbon tetrachloride, Barrett *et al.*[586] reported that large doses of choline were able to inhibit fatty infiltration of the liver, although small doses were ineffective. In other work, Barrett, Best, and Ridout[432] were able to trace to the tissue depots the fat which reached the liver after carbon tetrachloride poisoning.

The ineffectiveness of choline in preventing fatty infiltration of the liver in rabbits, after the administration of the "ketogenic" hormone from the anterior lobe of the pituitary, has been reported.[587] MacKay and Barnes[588]

[578] H. J. Channon and H. Wilkinson, *Biochem. J.*, *28*, 2026–2033 (1934).
[579] C. H. Best and J. H. Ridout, *J. Physiol.*, *78*, 415–418 (1933).
[580] C. H. Best and J. H. Ridout, *J. Physiol.*, *86*, 343–352 (1936).
[581] P. Junkersdorf and A. Kohl, *Arch. ges. Physiol. (Pflüger's)*, *211*, 612–635 (1926).
[582] C. H. Best, *Lancet*, *226*, 1274–1277 (1934).
[583] C. H. Best and J. H. Ridout, *J. Physiol.*, *94*, 47–66 (1938).
[584] C. H. Best, D. L. MacLean, and J. H Ridout, *J. Physiol.*, *83*, 275–284 (1935).
[585] L. Laszt and F. Verzár, *Biochem. Z.*, *285*, 356–367 (1936).
[586] H. M. Barrett, C. H. Best, D. L. MacLean, and J. H. Ridout, *J. Physiol.*, *97*, 103–106 (1939).
[587] B. Mukerji and R. C. Guha, *Indian J. Med. Research*, *26*, 295–302 (1938).
[588] E. M. MacKay and R. H. Barnes, *Proc. Soc. Exptl. Biol. Med.*, *38*, 803–805 (1938).

also confirmed the fact that choline could not counteract the development in rats of fatty livers caused by the injection of extracts of the pituitary hormone.[432,434,435,589] However, they did note a reduction in the ketonuria.[588] Although Julian, Dragstedt, and associates[590] stated that this type of fatty liver can be prevented by lipocaic, MacKay and Barnes[588] disputed this finding.

Dietary cirrhosis of the liver, in which fatty infiltration is an invariable concomitant,[591–593] was shown by György and Goldblatt[593] to respond to choline in doses of 10 to 20 mg. per rat. Since this type of cirrhosis invariably developed on the deficient diet when casein was present to the extent of only 10%, while its occurrence was quite erratic when a level of 18 to 20% of casein was employed,[594,595] the suggestion was made that casein had acted as a lipotropic agent in the latter instance. Blumberg and McCollum[596,597] were also able to produce a liver cirrhosis in rats on 10% casein or skim milk diets. Since a number of workers[596,598–600] have agreed that conditions which result in the production of fatty livers in short-term experiments lead to the development of cirrhosis when the experimental regimen is continued over a long period, it is evident that the level of dietary protein is related to the availability of choline. There is some evidence that dietary carbohydrate may influence the lipotropic action of choline. Thus, Artom and Fishman[601] found that the effectiveness of choline supplementation in reducing fatty infiltration of the liver, and in augmenting the level of liver lecithin, is considerably greater on a lactose diet than on one containing dextrin and sucrose as the dietary carbohydrates.

c′. The Lipotropic Action of Proteins: The first experimental evidence that protein possesses a lipotropic action was that of Best and Huntsman,[602] who noted that casein had an effect similar to that of choline or betaine in

[589] C. H. Best and J. Campbell, *J. Physiol.*, *92*, 91–110 (1938).
[590] O. C. Julian, D. E. Clark, J. van Prohaska, C. Vermeulen, and L. R. Dragstedt, *Am. J. Physiol.*, *138*, 264–268 (1943).
[591] C. L. Connor, *Am. J. Pathol.*, *14*, 347–364 (1938).
[592] C. L. Connor, *J. Am. Med. Assoc.*, *112*, 387–390 (1939).
[593] P. György and H. Goldblatt, *J. Exptl. Med.*, *75*, 355–368 (1942).
[594] P. György and H. Goldblatt, *J. Exptl. Med.*, *70*, 185–192 (1939).
[595] P. György and H. Goldblatt, *Proc. Soc. Exptl. Biol. Med.*, *46*, 492–494 (1941).
[596] H. Blumberg and E. V. McCollum, *Science*, *93*, 598–599 (1941).
[597] H. Blumberg, *U. S. Pub. Health Repts.*, *55*, 531–537 (1940).
[598] G. Webster, *J. Clin. Invest.*, *20*, 440 (1941).
[599] R. D. Lillie, F. S. Daft, and W. H. Sebrell, Jr., *U. S. Pub. Health Repts.*, *56*, 1255–1258 (1941).
[600] F. S. Daft, W. H. Sebrell, Jr., and R. D. Lillie, *Proc. Soc. Exptl. Biol. Med.*, *48*, 228–229 (1941).
[601] C. Artom and W. H. Fishman, *J. Biol. Chem.*, *170*, 587–595 (1947).
[602] C. H. Best and M. E. Huntsman, *J. Physiol.*, *83*, 255–274 (1935).

preventing the accumulation of fat in the liver. On the other hand, Channon and Wilkinson[603] were the first workers to attach significance to this finding; they investigated the effect of casein, when included in the diet in amounts varying from 5 to 50%, on the fatty infiltration in the liver. Fatty livers developed in the two groups receiving the lowest casein intake, namely 5%. These authors suggested that certain amino acids might be transformed by the rat into choline. This hypothesis was not an improbable one; Rosenfeld[604] had suggested that glycine might be the mother substance of choline, while Engeland[605] had proposed that betaine might originate in the tissues by a methylation of some of the amino acids. The hypothesis that a protein constituent controls liver fat was further supported by Best and Channon.[563]

In an extension of the work on casein, Beeston, Channon, and Wilkinson[606] found that, whereas increased amounts of casein in the diet caused a decrease in neutral fat in the liver, higher levels of phospholipid and cholesterol oleate obtained. On the other hand, when the casein intake was at 50%, the level of cholesterol esters in the liver was reduced. As a result of these experiments, it seemed probable that the effect of protein in preventing fatty infiltration of the liver might differ from that of choline. The maximum effect of casein was exerted when it was present at a 30% level in a diet containing 40% fat. One gram of casein was calculated to have the same lipotropic action as 7 to 8 mg. of choline.[607,608]

However, all proteins are not active in preventing fatty livers. Thus, arachin, a protein from the peanut, was found by Singal and Eckstein[609] to lack a lipotropic action. Gelatin was likewise reported by Best *et al.*[607] to have no significant activity, although Channon and associates[610] later reported that gelatin had a slight but demonstrable action on liver fats. The lipotropic activity of a number of proteins was shown by Channon *et al.*[610] to be in the following descending order: gromax and whale muscle protein, casein (caseinogen), albumin, beef muscle protein and edestin, fibrin and gliadin, and gelatin and zein. In general, according to Tucker

[603] H. J. Channon and H. Wilkinson, *Biochem. J.*, *29*, 350–356 (1935).

[604] G. Rosenfeld, *Biochem. Z.*, *218*, 48–53 (1930).

[605] R. Engeland, *Ber.*, *42*, 2962–2969 (1909).

[606] A. W. Beeston, H. J. Channon, and H. Wilkinson, *Biochem. J.*, *29*, 2659–2667 (1935).

[607] C. H. Best, R. Grant, and J. H. Ridout, *J. Physiol.*, *86*, 337–342 (1936).

[608] A. W. Beeston, H. J. Channon, J. V. Loach, and H. Wilkinson, *Biochem. J.*, *30*, 1040–1046 (1936).

[609] S. A. Singal and H. C. Eckstein, *Proc. Soc. Exptl. Biol. Med.*, *41*, 512–513 (1939).

[610] H. J. Channon, J. V. Loach, P. A. Loizides, M. C. Manifold, and G. Soliman, *Biochem. J.*, *32*, 976–985 (1938).

and Eckstein,[611] lipotropic activity parallels the methionine content as reported for proteins by Baernstein.[612] The lack of potency exhibited by arachin, and the low potency reported for gelatin, were later shown to be traceable to their low methionine contents. On the other hand, Sauberlich[613] reported that the supplementation of a 7% casein diet with 10% of zein, lactalbumin, or casein prevented the development of fatty livers in rats, while 10% gelatin was also partially effective. Harper *et al.*[614,615] likewise noted that the addition of 6% gelatin to the diet reduced the accumulation of liver fat in rats.

d′. The Lipotropic Action of Amino Acids: The explanation for the lipotropic action of protein is now known to reside in the amino acids of which they are composed. There are three categories to which these amino acids may be assigned.

(*a*′) Amino Acids Having No Lipogenic or Lipotropic Action: In the first group, the amino acids are completely inactive in producing fatty livers or in preventing the accumulation of fat in the liver. Thus, Beeston and Channon[616] reported that aspartic acid, glutamic acid, glycine, lysine, phenylalanine, and serine were ineffective in influencing liver fat deposition. Beeston and co-workers[617,618] found that alanine, arginine, histidine, hydroxyproline, leucine, proline, and valine were likewise inactive, and confirmed the fact that aspartic acid and lysine belong in this category. Tyrosine was found to have some effect in preventing glyceride deposition.[618] The inactivity, from a lipotropic standpoint, of *DL*-leucine and *DL*-valine was confirmed by Singal and Eckstein,[619] who demonstrated that djenkolic acid and *DL*-isoleucine behave likewise. In a later study of fourteen pure amino acids, Channon and co-workers[620] revised their earlier conclusions to include tyrosine as a possible lipotropic agent acting on fatty livers containing fat or cholesterol. There was some evidence, also, that tryptophane may possess a slight lipotropic action. On the other hand, Eckstein[621] was unable to demonstrate lipotropic effects on the part of any essential amino acids other than methionine.

[611] H. F. Tucker and H. C. Eckstein, *J. Biol. Chem.*, *121*, 479–484 (1937).
[612] H. D. Baernstein, *J. Biol. Chem.*, *97*, 669–674 (1932); *115*, 25–32 (1936).
[613] H. E. Sauberlich, *Federation Proc.*, *12*, 263 (1953).
[614] A. E. Harper, W. J. Monson, D. A. Benton, and C. A. Elvehjem, *J. Nutrition*, *50*, 383–393 (1953).
[615] A. E. Harper, W. J. Monson, D. A. Benton, and C. A. Elvehjem, *Federation Proc.*, *12*, 416 (1953).
[616] A. W. Beeston and H. J. Channon, *Biochem. J.*, *30*, 280–284 (1936).
[617] A. W. Beeston and A. P. Platt, *J. Soc. Chem. Ind.*, *58*, 557 (1939).
[618] A. W. Beeston, H. J. Channon, and A. P. Platt, *J. Soc. Chem. Ind.*, *56*, 292 (1937).
[619] S. A. Singal and H. C. Eckstein, *J. Biol. Chem.*, *140*, 27–34 (1941).
[620] H. J. Channon, G. T. Mills, and A. P. Platt, *Biochem. J.*, *37*, 483–492 (1943).

(*b'*) Amino Acids Having Lipogenic (Antilipotropic) Action: The second category in classifying amino acids as to their action in regard to liver lipids includes those which tend to increase the level of liver fats. These are classed as *lipogenic* or *antilipotropic* agents. Curtis and Newburgh[622] reported, as early as 1927, that cystine possesses this property. This observation was confirmed by Beeston and Channon,[616] who demonstrated that, when an amount of cystine as small as 0.2% was added to a diet containing 5% of casein and 40% of fat, the content of liver fat was doubled. When the casein content of the diet was increased to 30%, the antilipotropic effect of cystine was abolished. Tucker and Eckstein[611] confirmed the antilipotropic effect of cystine, while Singal and Eckstein[609] demonstrated, several years later, with mice, that cysteine, $HSCH_2CH(NH_2)COOH$, and homocystine, $HOOCCH(NH_2)CH_2CH_2S—SCH_2CH_2CH(NH_2)COOH$, were antilipotropic. Salmon[623] likewise demonstrated that the deficiency of labile methyl groups in diets containing less than 18% of casein was accentuated by the feeding of cystine alone or with fat. Griffith,[624] as well as Griffith and Mulford,[625] ascribed the antilipotropic action of cystine to the increased choline requirements due to a general improvement in the nutritional state. Associated with this is the augmented demand for methionine to be used for protein synthesis, which diverts a considerable amount of this amino acid from acting as a lipotropic agent. Smythe[626] ascribed this antilipotropic action of cystine to the toxic action of H_2S liberated as a result of the metabolism of cystine.

However, Stetten and Salcedo[433] concluded that cystine actually increases fat formation in the animal body. When deuterium was given to rats on a choline-free diet, deuterio-fats were found only in the liver. Under such conditions there is a blocking of the passage of fat from this organ to the tissues. On the other hand, when cystine was administered, the deuterium was found in depot fat as well as in liver fat. This result is interpreted as proving that cystine promotes fat formation.

(*c'*) Amino Acids Having Lipotropic Action: The most important category of amino acids which influence the level of liver lipids is the third one, which includes only methionine, $CH_3SCH_2CH_2CH(NH_2)COOH$. Tucker and Eckstein made the brilliant discovery that methionine is lipotropic.[611] These workers suggested that the lipotropic action of casein—and pre-

[621] H. C. Eckstein, *J. Biol. Chem.*, *195*, 167–174 (1952).
[622] A. C. Curtis and L. H. Newburgh, *Arch. Internal Med.*, *39*, 828–832 (1927).
[623] W. D. Salmon, *J. Nutrition*, *33*, 155–168 (1947).
[624] W. H. Griffith, *J. Nutrition*, *21*, 291–306 (1941).
[625] W. H. Griffith and D. J. Mulford, *J. Nutrition*, *21*, 633–646 (1941).
[626] C. V. Smythe, *J. Biol. Chem.*, *142*, 387–400 (1942).

sumably of any other protein—was due to "a resultant effect of the simultaneous opposing influences of the cystine and the methionine of the diet." This work was almost immediately confirmed by Channon, Manifold, and Platt.[627,628] Methionine was found to be active on both the glyceride and the cholesterol fractions of liver; this amino acid was first calculated to have a choline equivalent of one-twelfth,[627] but this figure was later revised to one-fifth.[628] The *D*- and *L*-isomers were found to have equal potency as lipotropic agents.[629] Singal and Eckstein[609] found that methionine exerts a lipotropic action on mice similar to that on the rat. Harper *et al.*[614] reported that *DL*-threonine is capable of reducing fat in the liver of rats when incorporated in the diet to the extent of 0.18%.

(*d'*) The Quantitative Relationship of Lipotropic and Antilipotropic Action: Treadwell[630] suggested that the requirement for methionine in the rat is based upon two considerations, namely, that for the growth demands, and that for lipotropism. The latter requirement is obviously conditioned by the amount of other lipotropic factors in the diet. The total methionine necessary for optimal growth in the rat was set at 600 mg. per day, and that for lipotropic action at a level of 600 mg. per 100 g. of food. When the diet contained 100 milligram per cent of cystine,[631] together with 500 milligram per cent of methionine, and 18.6% of protein, fatty livers and hemorrhagic kidneys developed in young white rats. Under these conditions, the methionine requirement was reported to be 1300 to 1500 mg. per 100 g. of diet. When the diet contained 200 milligram per cent of cystine, an antilipotropic effect was noted, and a significant increase in growth rate. At the highest level at which cystine was tested (400 milligram per cent), death occurred within fourteen days in 85% of the rats.

On the other hand, 100 milligram per cent of choline in the diet produced the maximum reduction of liver fat.[630] Treadwell[631] reported the greatest stimulation of growth in young rats at the 200 milligram per cent level. Liver fat was also found to be normal when this diet was fed. It is believed that the growth-stimulating effect of choline is due to its methionine-sparing action.

Best and collaborators[632] pointed out the necessity for preparing dose-response curves for the accurate estimation of lipotropic requirements un-

[627] H. J. Channon, M. C. Manifold, and A. P. Platt, *Biochem. J.*, *32*, 969–975 (1938).
[628] H. J. Channon, M. C. Manifold, and A. P. Platt, *Biochem. J.*, *34*, 866–878 (1940).
[629] C. H. Best and J. H. Ridout, *J. Physiol.*, *97*, 489–494 (1940).
[630] C. R. Treadwell, *J. Biol. Chem.*, *160*, 601–607 (1945).
[631] C. R. Treadwell, *J. Biol. Chem.*, *176*, 1141–1147 (1948).
[632] C. H. Best, C. C. Lucas, J. H. Ridout, and J. M. Patterson, *J. Biol. Chem.*, *186*, 317–329 (1950).

der stated dietary and environmental conditions. These workers are of the opinion that a comparison of lipotropic agents at any one dosage level has no general applicability.

e′. The Lipotropic Action of Proteins as Related to Their Amino Acid Content: Although one would naturally suppose that the lipotropic action of a protein would be the sum of the effects of the several amino acid components, it may not always be the case. This relationship may not even exist for a protein digest. Thus, Rose, Machella, and György[633] reported that methionine at a 50 mg. dosage (0.8% of the diet) did not prevent the development of fatty livers in rats when a mixture of essential fatty amino acids replaced the protein. Even when methionine constituted 2.4% of the diet, liver lipids were still at a level of 10% on the diet containing the amino acid mixture. However, when choline chloride was fed, the liver lipids were reduced to essentially normal values.[634]

Further reports indicate that the lipotropic action of proteins may not be entirely dependent upon their methionine and cystine content. Thus, Channon *et al.*[610] found that albumin, which contains more methionine than does casein, had a lower lipotropic effect. Moreover, Best and Ridout[629] found that methionine and cystine, equivalent to that present in a 30% casein diet, had a lower lipotropic action; these latter results were directly opposed to those of Tucker *et al.*[635] In their later work, Treadwell *et al.*[636] attributed the inferior lipotropic action of casein to the fact that the rats on the higher level of protein grew better and thus had less methionine available to act as a lipotropic agent. Finally, Best and Ridout[629] reported that methionine had a maximum lipotropic action at 0.5%; Channon and coworkers[628] confirmed this result and reported that casein continued to exert an increasing lipotropic activity up to 30%, which corresponded to a much higher methionine level than the highest effective methionine dosage. These results led Channon *et al.*[628] to postulate that "since methionine is probably not the only lipotropic constituent of caseinogen, two possibilities arise, either that some other amino acid also exerts a lipotropic action, or, alternatively, added methionine is incapable of exerting its full action in the absence of some other protein constituent." Although at the time there was no direct evidence of the second possibility, the authors mention that

[633] C. S. Rose, T. E. Machella, and P. György, *Proc. Soc. Exptl. Biol. Med., 64,* 352–354 (1947).

[634] C. S. Rose, T. E. Machella, and P. György, *Proc. Soc. Exptl. Biol. Med., 67,* 198–199 (1948).

[635] H. F. Tucker, C. R. Treadwell, and H. C. Eckstein, *J. Biol. Chem., 135,* 85–90 (1940).

[636] C. R. Treadwell, M. Groothuis, and H. C. Eckstein, *J. Biol. Chem., 142,* 653–658 (1942).

ethanolamine, $HOCH_2CH_2NH_2$, may act as a structural unit in the synthesis of choline, and hence be a limiting factor. This has later been found to be the case. Moreover, Cantoni[637] obtained evidence which suggests that methionine must be activated as a prerequisite for the transfer of its methyl group. The rate at which the radioactive methyl group of dietary methionine is oxidized to $C^{14}O_2$ has been shown by Mackenzie and du Vigneaud[638] to be increased by choline, and reduced to its original value by cystine.

In the later work of Beveridge *et al.*,[639,640] the differences between the lipotropic effect of casein and of a corresponding amount of methionine were related to the protein level of the diet. When casein made up less than 22% of the diet, the activity of free methionine as a lipotropic agent exceeded that of casein containing a similar amount of methionine. On the other hand, conditions were reversed when the dietary casein exceeded 22%; in this situation, casein exhibited a lipotropic effect superior to that of a corresponding amount of free methionine. These investigators demonstrated that the differences between the two rations were equalized when the quantities of essential amino acids in the two diets were identical. The lipotropic activity of a protein is determined not only by its methionine and cystine contents but also by the nature and quantity of the sulfur-free essential amino acids. The lipotropic effects of the essential amino acids are not the result of a direct action, but rather of an effect on growth and maintenance which influences the amount of new tissue, thus modifying the quantity of methionine left available for lipotropic purposes.

Treadwell *et al.*[641] have demonstrated that growth has first priority as regards demand on the methionine available in the organism. When the need for this function is satisfied, the remaining methionine can be used for lipotropic purposes. Thus, it was found that, when rats were raised on a diet containing 20% of arachin (which is deficient in methionine) and 0.5% methionine, the rats grew normally but developed fatty livers. On the other hand, when the proportion of methionine was increased to 1%, the rats grew normally and the liver lipids were only slightly above normal. Since less methionine is required for growth in older rats than in rapidly growing animals, more of the ingested methionine remains, in the former case, to act lipotropically.[642]

[637] G. L. Cantoni, *J. Biol. Chem.*, *189*, 745–754 (1951).

[638] C. G. Mackenzie and V. du Vigneaud, *J. Biol. Chem.*, *195*, 489–491 (1952).

[639] J. M. R. Beveridge, C. C. Lucas, and M. K. O'Grady, *J. Biol. Chem.*, *160*, 505–518 (1945).

[640] J. M. R. Beveridge, C. C. Lucas, and M. K. O'Grady, *J. Biol. Chem.*, *154*, 9–19 (1944).

[641] C. R. Treadwell, H. C. Tidwell, and J. H. Gast, *J. Biol. Chem.*, *156*, 237–246 (1944).

[642] M. G. Horning and H. C. Eckstein, *J. Biol. Chem.*, *155*, 49–53 (1944).

f′. The Lipotropic Action of Compounds Related to Methionine: Evidence that the action of methionine is relatively specific is afforded by the fact that the closely related methoxybutanoic acid is inactive. Thus, methoxinine or oxymethionine, $CH_3OCH_2CH_2CH(NH_2)COOH$, which was synthesized by Roblin *et al.*,[643] was shown to have a growth-inhibitory action on bacteria which was somewhat counteracted by methionine.[644] However, Shaffer and Critchfield[644] reported it to be lipotropic, although it possessed nephrotoxic properties.

Roberts and Eckstein[645] investigated the activity of other sulfur compounds when included in the rations of rats on diets producing fatty livers. Dimethyl sulfide, $(CH_3)_2S$, dimethyl disulfide, $CH_3S\text{—}SCH_3$, methyl xanthogenate, $CH_3O\overset{S}{\overset{\|}{C}}S\text{—}S\overset{S}{\overset{\|}{C}}OCH_3$, and *S*-methylisothiourea, $HN{:}C(NH_2)SCH_3$, were all found to be lipotropic when administered intraperitoneally. Although mercaptan formation could be detected only after dimethyl disulfide was given, it was suggested that the formation of this compound might likewise occur in the case of the other lipotropic sulfur compounds, and that this might be the active lipotropic agent. On the other hand, sodium sulfide, Na_2S, and methionine sulfone $CH_3O\overset{O}{\overset{\uparrow}{S}}CH_2CH_2CH(NH_2)COOH$, when given intraperitoneally, and trimethylsulfonium chloride, $(CH_3)_3SCl$, when administered orally, had neither a lipotropic nor an antilipotropic action.

Jensen *et al.*[646] demonstrated that, in contradistinction to the strong lipotropic activity of methionine, ethionine, $C_2H_5SCH_2CH_2CH(NH_2)COOH$, does not induce a lipotropic effect but, on the contrary, actually exhibits a liver-fattening action. Choline and other lipotropic agents were found to have little or no effect on this type of fatty liver.[647] Farber and associates[647] consider this finding to be inconsistent with the assumption that ethionine produces fatty livers by blocking the demethylation of methionine. Carbohydrate in large doses was found to prevent or to cure this type of fatty liver. On the other hand, Simmonds *et al.*,[648] employing deuterio-labeled

[643] R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole, and J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, *67*, 290–294 (1945).

[644] C. B. Shaffer and F. H. Critchfield, *J. Biol. Chem.*, *174*, 489–493 (1943).

[645] E. Roberts and H. C. Eckstein, *J. Biol. Chem.*, *154*, 367–375 (1944).

[646] D. Jensen, I. L. Chaikoff, and H. Tarver, *J. Biol. Chem.*, *192*, 395–403 (1951).

[647] E. Farber, M. V. Simpson, and H. Tarver, *J. Biol. Chem.*, *182*, 91–99 (1950).

[648] S. Simmonds, E. B. Keller, J. P. Chandler, and V. du Vigneaud, *J. Biol. Chem.*, *183*, 191–195 (1950).

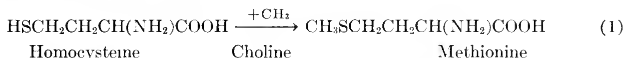
methionine, proved that, when ethionine was present, a decrease of about 20% obtained in the extent of transmethylation from methionine to choline. However, the amount of creatine synthesized from dietary methionine was not decreased by the presence of ethionine in the diet. Hardwick and Winzler[649] also reported a lipotropic action on the part of ethionine when it was fed along with carbohydrates. Levine and Fopeano[650] likewise reported a reduction of liver fat in male rats receiving ethionine as compared with control animals; in this case, the decrease is attributed to a decreased food consumption. On the other hand, the feeding of ethionine to female rats caused an increase in liver lipid, irrespective of whether or not choline was also given. It is postulated that the administration of methionine results in the formation and accumulation of abnormal protein in the liver.

g'. The Biological Synthesis of Choline: The demonstration of the mechanism of synthesis of choline in the animal body has been one of the brilliant accomplishments of the last decade. At the same time, it has solved many mysteries in the understanding of the effect of amino acids on liver fats. The biosynthesis of choline was reviewed by du Vigneaud.[651,652]

The clue to the mechanism responsible for the synthesis of choline developed as the result of diametrically opposite conclusions obtained by two groups of workers who were investigating whether or not homocystine, in place of methionine, could support the growth of rats. Womack and colleagues[653] had previously demonstrated that the amino acid, methionine, was essential for growth. Although du Vigneaud *et al.*[654] showed that homocystine could not support the growth of rats on a diet containing all the essential amino acids except methionine, together with a vitamin B supplement consisting of thiamine chloride, riboflavin, nicotinic acid, and ryzamin B, they did find that growth occurred when a tikitiki extract (rice polishings) and milk concentrate were added. Similar results were obtained by Rose and Rice.[655] It was suggested that the factor permitting growth when these supplements were added to the homocystine diet might be choline.[656] It was then shown that, when choline was added to the deficient diet, growth resulted. Presumably growth occurs because choline

[649] V. L. Hardwick and R. J. Winzler, *Proc. Soc. Exptl. Biol. Med.*, *69*, 217–219 (1948).
[650] M. Levine and J. F. Fopeano, *Federation Proc.*, *12*, 238 (1953).
[651] V. du Vigneaud, *Biol. Symposia*, *5*, 234–247 (1941).
[652] V. du Vigneaud, *Harvey Lectures*, *38*, 39–62 (1942-1943).
[653] M. Womack, K. S. Kemmerer, and W. C. Rose, *J. Biol. Chem.*, *121*, 403–410 (1937).
[654] V. du Vigneaud, H. M. Dyer, and M. W. Kies, *J. Biol. Chem.*, *130*, 325–340 (1939).
[655] W. C. Rose and E. E. Rice, *J. Biol. Chem.*, *130*, 305–323 (1939).
[656] V. du Vigneaud, J. P. Chandler, A. W. Moyer, and D. M. Keppel, *J. Biol. Chem.*, *131*, 57–76 (1939).

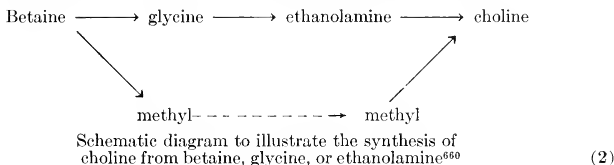
transfers the methyl groups necessary for transforming homocysteine into methionine, as shown in (1).[652]



It was demonstrated by Simmonds, du Vigneaud, and co-workers[657] that this change actually takes place. The deuteriomethionine isolated after the feeding of homocystine and deuteriocholine contained the labeled methyl group, thus proving that it had been transferred from the choline molecule to that of homocysteine.

On the other hand, the lipotropic action of methionine is concerned with the reverse action, namely the synthesis of choline from precursors such as aminoethyl alcohol and methionine, the latter serving as a donor of methyl groups. That methionine can act in this capacity has been proved by du Vigneaud and his group,[658,659] who fed methionine labeled with deuterium in the methyl group; the choline subsequently isolated from the tissue phospholipids contained sufficient deuterium in the methyl groups to suggest that they had originated from methionine.

(*a′*) The Importance of Ethanolamine and of Serine in Choline Synthesis: Ethanolamine is the structure from which choline is derived by the process of methylation, as has been adequately proved by Stetten.[660] When ethanolamine or choline containing N^{15} was fed to rats, phosphatides containing choline rich in the nitrogen isotope resulted. It was also shown that ethanolamine was readily formed from glycine; the latter amino acid originates from betaine. The series of reactions shown in (2) occur.



Schematic diagram to illustrate the synthesis of choline from betaine, glycine, or ethanolamine[660] (2)

Serine serves as another source of the ethanolamine molecule necessary as the precursor of choline. Thus, *L*-serine is readily converted to the

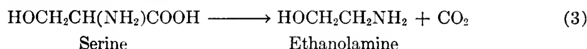
[657] S. Simmonds, M. Cohn, J. P. Chandler, and V. du Vigneaud, *J. Biol. Chem.*, *149*, 519–525 (1943).

[658] V. du Vigneaud, J. P. Chandler, M. Cohn, and G. B. Brown, *J. Biol. Chem.*, *134*, 787–788 (1940).

[659] V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck, and S. Simmonds, *J. Biol. Chem.*, *140*, 625–641 (1941).

[660] De W. Stetten, Jr., *J. Biol.Chem.*, *140*, 143–152 (1941).

ethanolamine moiety by decarboxylation; on the other hand, *D*-serine does not act as a precursor of choline.[661–663] The conversion of *L*-serine to ethanolamine is illustrated in (3).



$$HOCH_2CH(NH_2)COOH \longrightarrow HOCH_2CH_2NH_2 + CO_2 \quad (3)$$

Serine Ethanolamine

Barrenscheen and Papadopoulou[664] called attention to an entirely new and unsuspected factor in choline synthesis. Thus, it was shown that carbohydrate metabolism plays a role in this change. It was found that a very limited synthesis of choline obtains when the liver glycogen is at a low level.

From this discussion, the reason is now clear why casein serves as a better source of the lipotropic factor than does a like amount of methionine. Not only is methionine required for the synthesis of choline, but also one or two additional amino acids are involved, *i.e.* glycine and/or serine, which are likewise supplied by casein; one would therefore expect the most efficient synthesis of choline to obtain when all the necessary components are simultaneously present.

(*b′*) The Role of the Citrovorum Factor in Choline Synthesis: Dubnoff[665] first demonstrated that the presence of choline oxidase is essential for the transfer of choline methyl to homocysteine. Thus, the transfer of this group does not occur unless the alcohol group of choline has been oxidized; this oxidation requires the intermediation of the enzyme, choline oxidase. Dubnoff believes that the extent of utilization of choline methyl is regulated by this enzyme system. Muntz[666] confirmed the hypothesis that choline oxidase is necessary for transmethylation.

Folic acid has been shown to play an important part in the functioning of choline oxidase. Dinning, Keith, and Day[667] suggest that this vitamin functions as a constituent of the prosthetic group of this enzyme. It was likewise observed by Dinning *et al.*[668] that the choline oxidase activity of monkey liver and of the bone marrow of chickens was decreased when aminopterin was injected. The authors explain this effect as due to displacement of folic acid in the choline oxidase molecule by aminopterin.

[661] H. R. V. Arnstein, *Biochem. J.*, *47*, xviii–xix (1950).
[662] H. R. V. Arnstein, *Biochem. J.*, *48*, 27–32 (1951).
[663] S. Jansson and W. A. Mosher, *J. Am. Chem. Soc.*, *72*, 3316 (1950).
[664] H. K. Barrenscheen and D. Papadopoulou, *Z. physiol. Chem.*, *284*, 236–242 (1949).
[665] J. W. Dubnoff, *Arch. Biochem.*, *24*, 251–262 (1949).
[666] J. A. Muntz, *J. Biol. Chem.*, *182*, 489–499 (1950).
[667] J. S. Dinning, C. K. Keith, and P. L. Day, *Arch. Biochem.*, *24*, 463–464 (1949).
[668] J. S. Dinning, C. K. Keith, P. L. Davis, and P. L. Day, *Arch. Biochem.*, *27*, 89–93 (1950).

Nichol and Welch[669] suggested that the mechanism which accounts for the inhibitory action of aminopterin toward folic acid may consist in preventing its conversion to CF (LCF) (*Leuconostoc citrovorum factor*). LCF is considered to be the active form of folic acid. Sauberlich and Baumann[670] first presented evidence for this factor, based upon microbiological assays. This substance was reported to be essential for the optimal growth of the fungoid coccus which uses citric acid in milk (*Leuconostoc citrovorum* 8081). The above workers suggested that a relationship exists between folic acid and the natural agents active in the organism. Shive and coworkers[671] succeeded in demonstrating the relationship of synthetic folic acid to natural folinic acid. An active agent was prepared by formylation of synthetic folic acid, followed by catalytic hydrogenation in the presence of ascorbic acid. Williams[672,673] noted that choline oxidase activity was significantly increased by LCF in rats fed aminopterin, while the stimulation of activity caused by the addition of folic acid and ascorbic acid to choline oxidase is inhibited by aminopterin added to the incubation mixture *in vitro*. Williams postulated that LCF may itself be incorporated into another molecule which is the specific coenzyme for choline oxidase.

The relation of folic acid to choline utilization in the chick was investigated by Dinning *et al.*[674] Livers of folic acid-deficient chicks had a decreased ability to form methionine from homocystine or homocysteine in the presence of added choline or betaine. The addition of folic acid to replete chick livers *in vitro* enhanced methionine synthesis, but the same condition could not be produced in livers deficient in folic acid. Moreover, Verly and associates[675] reported that rats deficient in folic acid synthesized less choline from C^{14}-labeled methanol than did animals supplemented with folic acid or LCF.

The citrovorum factor occurs in dried brewer's yeast, largely in the bound form.[676] It can be liberated from the bound form by a CF-liberating factor; this appears to be identical with folic acid conjugase, which has previously been described. The CF-liberating enzyme is activated by ascorbic acid.[677]

[669] C. A. Nichol and A. D. Welch, *Proc. Soc. Exptl. Biol. Med.*, *74*, 403–411 (1950).
[670] H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, *176*, 165–173 (1948).
[671] W. Shive, T. J. Bardos, T. J. Bond, and L. L. Rogers, *J. Am. Chem. Soc.*, *72*, 2817–2818 (1950).
[672] J. N. Williams, Jr., *J. Biol. Chem.*, *191*, 123–127 (1951).
[673] J. N. Williams, Jr., *J. Biol. Chem.*, *192*, 81–85 (1951).
[674] J. S. Dinning, C. K. Keith, and P. L. Day, *J. Biol. Chem.*, *189*, 515–520 (1951).
[675] W. G. Verly, J. M. Kinney, and V. du Vigneaud, *J. Biol. Chem.*, *196*, 19–23 (1952).
[676] C. H. Hill and M. L. Scott, *J. Biol. Chem.*, *196*, 189–193 (1952).
[677] C. H. Hill and M. L. Scott, *J. Biol. Chem.*, *196*, 195–199 (1952).

(*c'*) The Importance of Vitamin B_{12} in Choline Synthesis: Drill and McCormick[678] were the first to demonstrate that vitamin B_{12} exerts a lipotropic effect, which is not attributable to its choline content, when injected into rats on a high-fat diet. The choline requirement necessary to maintain a normal liver fat level was shown by Strength *et al.*[679] to be markedly reduced by vitamin B_{12} and folacin. The effectiveness of vitamin B_{12} in preventing fatty livers in rats has also been demonstrated by Bennett and associates.[680] This vitamin functions in a similar manner in dogs.[681]

The mechanism by which vitamin B_{12} acts on fatty livers is not entirely understood. One of the suggested methods by which the vitamin functions is by its effect on transmethylation. Oginsky[682] reported that the livers of vitamin B_{12}-deficient rats exhibited a lower capacity for synthesizing methionine from homocysteine plus choline or betaine than was observed in the case of control animals. Bennett *et al.*[680] likewise state that vitamin B_{12} is concerned with the synthesis of methionine, but not with its demethylation.

Another suggestion as to the mechanism of action of vitamin B_{12} is that it aids in the synthesis of the active methyl group. Arnstein and Neuberger believe that vitamin B_{12} is essential for the synthesis of methyl from glycine,[683] as well as from unknown precursors.[684] Vitamin B_{12} may be active in the synthesis of labile methyl not only from glycine but also from serine,[685] although Stekol *et al.*[686] now believe that it is concerned only with glycine.

As a result of *in vitro* studies, Dubnoff[687] concluded that vitamin B_{12} is concerned with the reduction of homocystine to homocysteine, which is the direct methyl acceptor in the synthesis of methionine. In subsequent studies with a mutant of *Escherichia coli* (Escherich's intestinal bacillus) which required vitamin B_{12} or methionine, it was found that the bacteria can grow and synthesize methionine in the absence of vitamin B_{12} if homocysteine or certain reducing agents which can act on homocystine are present.[688] It is believed that these data implicate vitamin B_{12} in main-

[678] V. A. Drill and H. M. McCormick, *Proc. Soc. Exptl. Biol. Med.*, *72*, 388–390 (1949).

[679] D. R. Strength, E. A. Schaefer, and W. D. Salmon, *J. Nutrition*, *45*, 329–343 (1951).

[680] M. A. Bennett, J. Joralemon, and P. L. Halpern, *J. Biol. Chem.*, *193*, 285–291 (1951).

[681] M. M. Burns and J. M. McKibbin, *J. Nutrition*, *44*, 487–499 (1951).

[682] E. L. Oginsky, *Arch. Biochem.*, *26*, 327–329 (1950).

[683] H. R. V. Arnstein and A. Neuberger, *Biochem. J.*, *48*, ii–iii (1951).

[684] H. R. V. Arnstein and A. Neuberger, *Biochem. J.*, *50*, xxxviii (1952).

[685] J. A. Stekol and K. Weiss, *J. Biol. Chem.*, *186*, 343–350 (1950).

[686] J. A. Stekol, S. Weiss, and K. Weiss, *Arch. Biochem. Biophys.*, *36*, 5–10 (1952).

[687] J. W. Dubnoff, *Arch. Biochem.*, *27*, 466–467 (1950).

[688] J. W. Dubnoff, *Arch. Biochem. Biophys.*, *37*, 37–45 (1952).

taining homocysteine in the reduced state; they exclude the concept that vitamin B_{12} functions as a coenzyme in transmethylation in the organism.

Vitamin B_{12}, together with folacin, also improves the efficiency of betaine, *DL*-methionine, dimethylaminoethanol and methylaminoethanol as dietary replacements for choline in the rat.[679] The chick was able to utilize methylaminoethanol plus betaine HCl or *DL*-methionine for growth or for the prevention of perosis only when vitamin B_{12} was present.[689] Moreover, dimethylaminoethanol could not serve as a replacement for choline in growth, or to prevent perosis, unless the diet contained vitamin B_{12}.[689]

In the later tests of Jukes and Stokstad[690] with chickens, it was found that the addition of homocystine to a vitamin B_{12}-free, choline-free diet actually depressed growth. However, the growth response of the birds was markedly improved by vitamin B_{12}. Although the administration of choline or betaine without vitamin B_{12} resulted in an improvement in the condition of the chicks, the response was no greater than the effects of the supplements individually. However, when vitamin B_{12} was also given, the growth stimulus was further increased over that on the diet free from the vitamin. Choline apparently acts as a methylating agent for homocystine on a diet deficient in methionine only when vitamin B_{12} is included in the ration.

(*d'*) "Labile Methyl" Groups and Choline Synthesis: The possibility that other than preformed methyl groups may serve as sources of such groups in the choline molecule has been demonstrated. It was reported that, on oxidation of acetone, acetate[691] and formate[692] were formed. Formate is known to contribute to the serine molecule.[693]

A number of workers, including Sakami,[692] du Vigneaud *et al.*,[694,695] and others,[696,697] demonstrated that formate is converted to labile methyl. This labile methyl has been detected in newly formed choline and methionine.[692] Sakami and Welch[696] reported that the conversion of formate to methyl groups could be detected not only in the intact rat but also in rat liver slices. It is suggested that folic acid is concerned with the metabolism of the one-carbon compounds. Ressler and co-workers[697] noted that a quantitative

[689] A. E. Schaefer, W. D. Salmon, and D. R. Strength, *J. Nutrition, 44,* 305–311 (1951).
[690] T. H. Jukes and E. L. R. Stokstad, *J. Nutrition, 48,* 209–229 (1952).
[691] E. Borek and D. Rittenberg, *J. Biol. Chem., 179,* 843–845 (1949).
[692] W. Sakami, *J. Biol. Chem., 187,* 369–378 (1950).
[693] W. Sakami, *J. Biol. Chem., 176,* 995–996 (1948).
[694] V. du Vigneaud, W. G. Verly, and J. E. Wilson, *J. Am. Chem. Soc., 72,* 2819–2820 (1950).
[695] V. du Vigneaud, W. G. Verly, J. E. Wilson, J. R. Rachele, C. Ressler, and J. M. Kinney, *J. Am. Chem. Soc., 73,* 2782–2785 (1951).
[696] W. Sakami and A. D. Welch, *J. Biol. Chem., 187,* 379–384 (1950).
[697] C. Ressler, J. R. Rachele, and V. du Vigneaud, *J. Biol. Chem., 197,* 1–5 (1952).

conversion of formate to the labile methyl group obtains after the subcutaneous administration of sodium deuterio-C^{14}-formate to rats. Formic acid is able to give rise to methyl, not only when it is present as the sodium salt, but also when it is administered as an ester such as formyl-*L*-phenylalanine.[695]

Methanol is another one-carbon compound which can be changed to a labile methyl group. Positive results were obtained not only by Arnstein[662] but also by the du Vigneaud group.[695,698] Formaldehyde has likewise been found to be a precursor of the methyl radicle.[694,695] However, the C of sodium bicarbonate[694] cannot serve as a source of the methyl group. Moreover, CO_2 arising from oxidation of *D*-β-C^{14}-serine or the —$C^{14}OOH$ of glycine cannot be transformed to —CH_3.[662]

Arnstein,[662] and Jansson and Mosher[663] proved that the α-carbon atom of glycine and the β-carbon of *L*-serine both appear in the labile methyl fraction. Toporek, Miller, and Bale[699] have also reported that the *L*-histidine may contribute the number 2 carbon of its molecule to the labile methyl groups of the liver, blood, and body choline, as well as of the body creatine. These workers showed that, when a choline deficiency existed, the synthesis was accelerated both *in vivo* and *in vitro*. When additional amino acids (both essential and non-essential) were given to rats, the conversion of the histidine carbon 2 to methyl was retarded. It is suggested that other sources of synthesis of methyl are now available, so that those arising from histidine are not so critically required, although they are an important dietary source.

Du Vigneaud and associates[700] reported that the synthesis of small amounts of methyl continues to occur in the tissues even when the diet contains an adequate amount of preformed methyl groups. There is no distinction between the direct synthesis of choline in the tissues and synthesis by intestinal bacteria, with the subsequent utilization of such newly formed methyl groups in the tissues. However, the bacteria are not required either primarily or secondarily for the synthesis of labile methyl.

Although bacteria are not necessary for the synthesis of the labile methyl radicle, animals which could utilize homocystine for growth on a methyl-free diet lost this ability after receiving the diet plus 2% sulfasuxidine over a five-month period. The capacity to utilize homocystine for growth was restored after the animals were put back on the preexperimental diet.

[698] V. du Vigneaud and W. G. Verly, *J. Am. Chem. Soc.*, *72*, 1049 (1950).

[699] M. Toporek, L. L. Miller, and W. F. Bale, *J. Biol. Chem.*, *198*, 839–851 (1952).

[700] V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn, *J. Biol. Chem.*, *159*, 755–756 (1945).

Bennett[701] interprets her findings as supporting the theory that a bacterial synthesis of unknown factors influencing homocystine utilization obtains. The ability of rats to grow on a homocystine-containing diet devoid of other sulfur-containing amino acids and other known methyl donors has been observed in a number of strains of rats. According to Bennett and Toennies,[702] this capacity depends upon preexperimental nutritional conditions. When the rats are prefed on sulfonamide, the intestinal flora are so modified that methionine, but not homocystine, can be used. If choline or certain liver fractions are fed to such animals, homocystine can be utilized. It is obvious that bacteria, under certain conditions, may play a role in the utilization of homocystine. However, du Vigneaud and co-workers[703] demonstrated that the degree of synthesis of the methyl group in choline and creatine was comparable in bacteria-free rats and in control multicontaminated animals.

Kelley *et al.*[704] have demonstrated the lipotropic effect of folic acid. They explain this action as related to stimulation of the conversion of glycine to serine, which has previously been demonstrated.[705] In this manner, the drain on labile methyl groups is reduced, and more are available for choline synthesis.

(*e′*) The Intracellular Distribution of Choline Oxidase: Further evidence for the interrelationship of LCF, folic acid, and vitamin B_{12} with choline oxidase can be inferred from their similar distribution in the cell. Williams,[706] using Schneider's method of differential centrifugation for the separation of the cell particles,[707] proved that choline oxidase was highly concentrated in the mitochondria, while only small amounts were present in the nuclei and microsome fractions. All of the vitamin fractions and choline oxidase are more frequently localized in the mitochondria than in other portions of the particulate matter; Williams believes that this indicates their close relationship to each other.

h′. Modification of the Lipotropic Action of Choline in the Presence of Methyl Acceptors: The synthesis of choline by the methylation of ethanolamine is merely one of a series of methylations which can occur in the animal body. Thus, Stetten and Grail[559] demonstrated that guanidoacetic

[701] M. A. Bennett, *J. Biol. Chem.*, *163*, 247–250 (1946).

[702] M. A. Bennett and G. Toennies, *J. Biol. Chem.*, *163*, 235–245 (1946).

[703] V. du Vigneaud, C. Ressler, J. R. Rachele, J. A. Reyniers, and T. D. Luckey, *J. Nutrition*, *45*, 361–376 (1951).

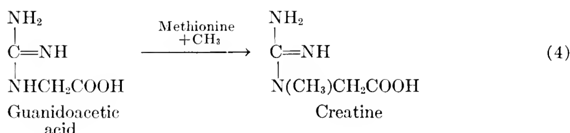
[704] B. Kelley, J. R. Totter, and P. L. Day, *J. Biol. Chem.*, *187*, 529–535 (1950).

[705] J. R. Totter, B. Kelley, P. L. Day, and R. R. Edwards, *J. Biol. Chem.*, *186*, 145–151 (1950).

[706] J. N. Williams, Jr., *J. Biol. Chem.*, *194*, 139–142 (1952).

[707] W. C. Schneider, *J. Biol. Chem.*, *176*, 259–266 (1948).

acid produces an extreme fattening of the liver, even when protective doses of choline are being administered. The reason for the antilipotropic action of this compound is that it exerts a higher priority for methyl groups than does ethanolamine, with the result that methionine and also the administered choline transfer their methyl groups to guanidoacetic acid to form creatine.[708,709] Du Vigneaud *et al.*[659] demonstrated that the methyl group in methionine may be used for the methylation of guanidoacetic acid to form creatine, as shown in (4).[652]



Borsook and Dubnoff[710] reported that *DL*-homocystine plus choline accelerates the methylation of guanidoacetic acid as effectively as does *DL*-methionine; however, homocystine is ineffective in the absence of choline, just as choline is inactive without homocystine. It was found that *DL*-homocystine is more potent than is *DL*-homocysteine.

On the other hand, a number of common physiologic compounds other than methionine, choline, and betaine, may be used to supply methyl groups for transmethylation. Thus, Heppel *et al.*[711] reported that the methyl purines, caffeine, theobromine, and theophylline, exert a lipotropic action when choline is absent from the diet. It is suggested that these compounds may act as methylating agents under these circumstances.

Sarcosine, $(CH_3)NHCH_2COOH$, may likewise serve as a methyl donor for creatine and choline, but its effectiveness as a transmethylating agent is so low that it has little influence on liver fattening. By using sarcosine containing N^{15}, du Vigneaud *et al.*[712] demonstrated a transfer of methyl groups to choline and creatine, but the rate at which the methyl groups appeared was slow as compared to that when deuteriocholine was fed.

It is evident that the body not only possesses a limited ability to synthesize the methyl radical but also has the power to transfer this group intact from one molecule to another. The process of transferring the

[708] K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, *138*, 167–194 (1941).
[709] H. Borsook and J. W. Dubnoff, *J. Biol. Chem.*, *132*, 559–574 (1940).
[710] H. Borsook and J. W. Dubnoff, *J. Biol. Chem.*, *160*, 635–636 (1945).
[711] L. A. Heppel, V. T. Porterfield, and E. G. Peake, *Arch. Biochem.*, *15*, 439–443 (1947).
[712] V. du Vigneaud, S. Simmonds, and M. Cohn, *J. Biol. Chem.*, *166*, 47–52 (1946).

methyl group from a methyl "donor" to a methyl "acceptor" is now commonly known as *transmethylation*.

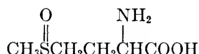
i'. The Comparative Lipotropic Action of Choline Analogues: Following the discovery of the lipotropic action of choline, betaine was the next compound which was proved to have a lipotropic action. Best and Huntsman[576] reported that betaine has a lipotropic activity; this behavior has been confirmed by a number of investigators,[713–715] whose results suggested that the lipotropic activity of betaine was from one-third to one-half that of choline. This would indicate that only one methyl group in betaine is available for transmethylation. Platt[714] is of the opinion that the lower potency of betaine, as compared with choline, may be explained by the fact that it must first be converted to choline in the liver. However, du Vigneaud and associates,[716] using betaine labeled with N^{15} and D, found that it was an extremely effective methyl donor. Methyl groups appeared in tissue choline as rapidly after the administration of betaine as following that of choline. Since a disparity in the amount of N^{15} and D obtained, it was concluded that the betaine molecule is not converted as a whole to choline. In addition to ordinary betaine (glycine-betaine), only alanine-betaine[717] and cystine-betaine[619] have lipotropic activity. Negative results were obtained on a large number of other betaines, including ergothionine (thiolhistidine-betaine),[656,717,718] trigonelline (nicotinic acid-betaine),[717] and the betaines of glutamic acid,[717] serine, threonine, and allothreonine.[719] The fact that colamine (ethanolamine) is not lipotropic would seem to indicate that cephalins are of little importance in the metabolism of fatty acids.[714] Creatine, likewise, has been shown to be devoid of activity in removing fats from the liver.

Mawson and Welch,[720] as well as Barrenscheen and Pantlitschko,[721] demonstrated that the hydroxyl group of choline is necessary for its lipotropic action. Esterification abolishes the lipotropic activity.[721] Trimethyl ammonium chloride actually caused an increase in liver fat, while trimethylethyl-, tetramethyl-, and trimethylphenylammonium chloride were too toxic to be tested for lipotropic activity. The length of the

[713] W. H. Griffith and D. J. Mulford, *J. Am. Chem. Soc.*, *63*, 929–932 (1941).
[714] A. P. Platt, *Biochem. J.*, *33*, 505–511 (1939).
[715] A. D. Welch and M. S. Welch, *Proc. Soc. Exptl. Biol. Med.*, *39*, 7–9 (1938).
[716] V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn, *J. Biol. Chem.*, *165*, 639–648 (1946).
[717] A. W. Moyer and V. du Vigneaud, *J. Biol. Chem.*, *143*, 373–382 (1942).
[718] C. H. Best and J. H. Ridout, *Ann. Rev. Biochem.*, *8*, 349–370 (1939).
[719] H. E. Carter and D. B. Melville, *J. Biol. Chem.*, *133*, 109–116 (1940).
[720] E. H. Mawson and A. D. Welch, *Biochem. J.*, *30*, 417–418 (1936).
[721] H. K. Barrenscheen and M. Pantlitschko, *Z. physiol. Chem.*, *284*, 250–256 (1949).

groups attached to the nitrogen is a deciding factor in lipotropic action; thus, the triethylhydroxyethylammonium hydroxide, or the so-called triethylcholine ($HOCH_2CH_2N(C_2H_5)_3OH$), has two-thirds of the lipotropic activity of choline, weight for weight,[722,723] but the corresponding tripropyl compound is practically devoid of lipotropic action.

The presence of the quaternary nitrogen appears to be unnecessary. Trimethylaminoxide, $(CH_3)_3NO$, has been shown to play an important role as a lipotropic agent.[721] It is believed that its behavior in choline transmethylation is similar to that of methionine sulfonide:



in the methyl transfer from methionine. However, the mechanism differs for these two reactions. The methionine-attacking pherase is a lyoenzyme which is cyanide-sensitive, while the choline pherase is a cyanide-insensitive desmoenzyme. Du Vigneaud and associates[724] found that dimethylaminoethanol ($(CH_3)_2NCH_2CH_2OH$) prevented fatty livers, although it was decidedly inferior to choline in promoting the growth of rats on a homocystine diet. Both the dimethyl compound and the monomethylaminoethanol ($CH_3NHCH_2CH_2OH$) were effective precursors of choline, as demonstrated by the transfer of deuterium-labeled methyl groups from the amines to newly synthesized choline. On the other hand, dimethylglycine ($(CH_3)_2NCH_2COOH$) was relatively ineffective as a precursor of choline or creatine.[716] Monomethylaminoethanol was found to have a high degree of toxicity. It is not a direct methyl donor. Although it is not considered that either di- or monomethylaminoethanol enters directly into transmethylation reactions, such as would lead to the formation of methionine or creatine, these compounds presumably act as lipotropic agents by virtue of their ready conversion to choline.

When the alcohol group is inactivated, as for example by the formation of an ether, the lipotropic activity is destroyed.[714] Welch and Welch[715] reported that the phosphorus and arsenic analogues of choline are about one-half as effective in removing fat from the liver as are the corresponding choline compounds. The results are summarized in Table 23 (page 661).

From the data presented in Table 23, it is evident that the general configuration of the choline molecule is essential for lipotropic activity. How-

[722] H. J. Channon and J. A. B. Smith, *Biochem. J.*, *30*, 115–120 (1936).
[723] H. J. Channon, A. P. Platt, and J. A. B. Smith, *Biochem. J.*, *31*, 1736–1742 (1937).
[724] V. du Vigneaud, J. P. Chandler, S. Simmonds, A. W. Moyer, and M. Cohn, *J. Biol. Chem.*, *164*, 603–613 (1946).

TABLE 23

THE LIPOTROPIC ACTIVITY OF COMPOUNDS RELATED TO CHOLINE[a]

Compound	Formula	Ref.
	Positive lipotropic activity	
Arsenocholine chloride	$(CH_3)_3As^+CH_2CH_2OH(Cl)^-$	b,c
Betaine	$(CH_3)_3N^+CH_2CO(O^-)$	c,d
Betaine aldehyde chloride	$(CH_3)_3N^+CH_2CHO(Cl)^-$	b
Betaine aldehyde acetal chloride	$(CH_3)_3N^+CH_2CH(OC_2H_5)_2(Cl)^-$	b
Calcium phosphorylcholine chloride	$[(CH_3)_3N^+CH_2CH_2OPO_3]_2Ca(Cl)_2^-$	b
Diethylmethylhydroxyethylammonium chloride	$(C_2H_5)_2CH_3N^+CH_2CH_2OH(Cl)^-$	e
Dimethylethylhydroxyethylammonium chloride	$(CH_3)_2C_2H_5N^+CH_2CH_2OH(Cl)^-$	e
Homocholine	$(CH_3)_3N^+CH_2CH_2CH_2OH(HCl)^-$	f
α-Methylbetaine hydrochloride	$(CH_3)_3N^+CH(CH_3)COO^-\cdot HCl$	b
Phosphocholine chloride	$(CH_3)_3P^+CH_2CH_2OH(Cl)^-$	b
Triethyl-β-hydroxyethylammonium hydroxide	$(C_2H_5)_3N^+CH_2CH_2OH(OH)^-$	g,h
Dimethylaminoethanol	$(CH_3)_2NCH_2CH_2OH$	i
	No lipotropic activity	
Arsenobetaine hydrochloride	$(CH_3)_3As^+CH_2COOH(Cl)^-$	b
Choline methyl ether	$(CH_3)_3N^+CH_2CH_2OCH_3(Cl)^-$	j
β-Methylcholine chloride	$(CH_3)_3N^+CH_2CH(CH_3)OH(Cl)^-$	b
β-Methylcholine ethyl ether chloride	$(CH_3)_3N^+CH_2CH(CH_3)OC_2H_5(HCl)^-$	b
Phosphobetaine hydrochloride	$(CH_3)_3P^+CH_2COO^-\cdot HCl$	b
Tetra-β-hydroxyethylammonium chloride	$(HOCH_2CH_2)_4N^+(Cl)^-$	j
Trimethylamine oxide hydrochloride	$(CH_3)_3N^+O^-\cdot HCl$	b
Trimethylammonium chloride	$(CH_3)_3N^+Cl^-$	k
Tripropyl-β-hydroxyethylammonium hydroxide	$(CH_3CH_2CH_2)_3N^+CH_2CH_2OH(OH)^-$	f
	Toxic compounds	
Tetramethylammonium chloride	$(CH_3)_4N^+(Cl)^-$	k
Trimethylethylammonium chloride	$(CH_3)_3(C_2H_5)N^+(Cl)^-$	k
Trimethylphenylammonium chloride	$(CH_3)_3(C_6H_5)N^+(Cl)^-$	k

[a] Adapted from E. W. McHenry and J. M. Patterson, *Physiol. Revs.*, *24*, 128–167 (1944), p. 152.
[b] A. D. Welch and M. S. Welch, *Proc. Soc. Exptl. Biol. Med.*, *39*, 7–9 (1938).
[c] A. D. Welch, *Proc. Soc. Exptl. Biol. Med.*, *35*, 107–108 (1936).
[d] C. H. Best and M. E. Huntsman, *J. Physiol.*, *75*, 405–412 (1932).
[e] A. W. Moyer and V. du Vigneaud, *J. Biol. Chem.*, *143*, 373–382 (1942).
[f] H. J. Channon, A. P. Platt, and J. A. B. Smith, *Biochem. J.*, *31*, 1736–1742 (1937).
[g] H. J. Channon and J. A. B. Smith, *Biochem. J.*, *30*, 115–120 (1936).
[h] H. J. Channon, A. P. Platt, J. V. Loach, and J. A. B. Smith, *Biochem. J.*, *31*, 2181–2186 (1937).
[i] V. du Vigneaud, J. P. Chandler, S. Simmonds, A. W. Moyer, and M. Cohn, *J. Biol. Chem.*, *164*, 603–613 (1946).
[j] A. P. Platt, *Biochem. J.*, *33*, 505–511 (1939).
[k] E. H. Mawson and A. D. Welch, *Biochem. J.*, *30*, 417–418 (1936).

ever, the segments may be substituted (as ethyl for methyl), and nitrogen may be replaced by other elements of suitable valency, for example phosphorus or arsenic. The hydroxyl group must also be present if the compound is to be active.

j′. The Physiologic Role of Choline and Its Method of Action: The most important function of choline is its lipotropic action. Although this influence on fatty livers is obviously related to its occurrence in lecithin, other more complicated explanations have been offered. These include the hypothesis that choline acts by virtue of an undefined role in liver function, or that it controls carbohydrate metabolism. These theories have not been substantiated, so it would appear that choline is needed for the synthesis of lecithin. Choline is also of importance in kidney growth and development, as a precursor of acetylcholine, as a required dietary constituent during lactation, and as one of the required dietary agents for preventing perosis.

(*a′*) The Mode of Action of Choline as a Lipotropic Agent: The prevention of fatty livers is undoubtedly connected with the maintenance of an adequate transport mechanism between the liver and the fat depots, and *vice versa*. Since the original work of Leathes and Raper,[13] it has generally been accepted that phospholipids function in fat transport. This hypothesis has been strengthened by the experimental results summarized by Bloor,[117] and by Sinclair,[369] in their articles in *Physiological Reviews*.

The results of Fishman and Artom[725] furnish circumstantial evidence of the importance of choline in lecithin synthesis. Thus, it was found that the level of lecithin in the liver and tissues of young rats was lower than normal when the animals had received a choline-low diet from weaning. Proof that choline plays a direct part in phospholipid synthesis is afforded by the data of Welch,[726] of Welch and Landau,[727] and by those of Stetten,[660,728] which demonstrated the incorporation of this substance into phospholipid. Thus, following the administration of arsenocholine, which proved to be lipotropic, the arsenic analogue of lecithin was found in the tissues.[726,727] The finding that the methyl groups in arsenocholine are not labile[717] would seem to constitute further evidence that the lipotropic action depends upon the availability of the entire choline molecule (or arsenocholine molecule) for incorporation into a newly-formed phospholipid molecule. Furthermore, triethylcholine was also found by Welch,[726] by McArthur *et*

[725] W. H. Fishman and C. Artom, *J. Biol. Chem.*, *154*, 109–115 (1944).
[726] A. D. Welch, *Proc. Soc. Exptl. Biol. Med.*, *35*, 107–108 (1936).
[727] A. D. Welch and R. L. Landau, *J. Biol. Chem.*, *144*, 581–588 (1942).
[728] De W. Stetten, Jr., *J. Biol. Chem.*, *138*, 437–438 (1941).

al.,[729] and others[722,723] to be lipotropic,[717] and it has been proved that it does not furnish labile methyl groups.[652] This latter compound must act by being incorporated into the phospholipid molecule, although Channon *et al.*[730] were unable to detect it in the liver phospholipids under such circumstances. McArthur and co-workers[729] reported significant differences between the microchemical and the microbiological assays for choline in the hydrolysates of the phospholipids isolated from the livers of rats fed triethylcholine. Subsequently these workers were able to fractionate the phospholipids and to obtain chemical proof that the triethyl homologue had been incorporated in the phospholipid molecule.

The importance of choline in the synthesis of phospholipids is likewise shown by its effect on the rate of turnover of this component. When choline was fed, the half-life of phosphatide choline was found by Boxer and Stetten[731] to be six days, with a daily replacement of choline in the phospholipids amounting to 3.9 mg. per rat per day. On the other hand, when no choline was fed, the half-life of choline was increased to eighteen days, and the daily replacement of choline in the phospholipids was reduced to 1.3 mg. daily. Although the total quantity of choline in such phospholipids was not influenced by choline-free diets, the *rate* of the interchange was profoundly affected.

Further evidence that choline plays a role in phospholipid synthesis was brought forward by Perlman and Chaikoff,[732] and by Perlman *et al.*,[733] who based their conclusions upon the use of P^{32}. These workers showed that an increased formation and a more rapid removal of phospholipids occurred in the livers of rats fed choline, as compared with those which did not receive this compound. The effect of a single dose of ingested choline was noted within one hour, and its effect was dissipated within ten to twelve hours. Moreover, the increase in phospholipid was found to be proportional to the amount of choline administered. In the experiments of Horning and Eckstein,[734] the lipotropic action of methionine and of choline could be demonstrated within eight hours after their ingestion. Using P^{32}, they found that an increased transport of radiophosphorus to the liver phospholipids occurred. This was not invariably associated with a decrease in liver lipids, and may not be related to it. Cornatzer and Cayer[735] report

[729] C. S. McArthur, C. C. Lucas, and C. H. Best, *Biochem. J.*, *41*, 612–618 (1947).
[730] H. J. Channon, A. P. Platt, J. V. Loach, and J. A. B. Smith, *Biochem. J.*, *31*, 2181–2186 (1937).
[731] G. E. Boxer and De W. Stetten, Jr., *J. Biol. Chem.*, *153*, 617–625 (1944).
[732] I. Perlman and I. L. Chaikoff, *J. Biol. Chem.*, *127*, 211–220 (1939).
[733] I. Perlman, N. Stillman, and I. L. Chaikoff, *J. Biol. Chem.*, *133*, 651–659 (1940).
[734] M. G. Horning and H. C. Eckstein, *J. Biol. Chem.*, *166*, 711–720 (1946).
[735] W. E. Cornatzer and D. Cayer, *J. Clin. Invest.*, *29*, 542–551 (1950).

that the benefit of lipotropic agents is to be traced to their effect in stimulating phospholipid synthesis. Increased amounts of phospholipids were found, when methionine or choline was given to patients with cirrhotic livers, as compared with the amount noted in untreated individuals. The beneficial effects of these lipotropic agents on phospholipid synthesis could be observed only if the animals had previously been receiving a choline-deficient diet. Cayer and Cornatzer[736] reported that the beneficial effect of methionine or of choline on the phospholipid turnover of two cirrhotic patients was noted only at the initiation of the treatment. It is also evident that, in this connection, the lipotropic agent is active in stimulating phospholipid turnover only when a relative deficiency in lipotropic agents exists. Patterson and associates[737] also demonstrated that choline deficiency decreases the rate of turnover of phospholipid.

Another fact supports the lecithin theory in explaining the lipotropic effect of choline, *i.e.*, the antilipotropic action of cholesterol has been shown by Aylward *et al.*[370] to be associated with a decreased amount of phospholipid. It was found that a reduction of the phospholipid content of the liver preceded the fatty infiltration. When choline was administered, the phospholipid content was maintained, and the fatty liver did not develop. Finally, the finding of Welch and Welch[715] that phosphoryl choline, which would be expected to act as an intermediate in phospholipid synthesis, is unaffected by liver phosphatase and hence is protected from oxidation, would offer support to the assumption of the direct utilization of choline in the synthesis of lecithin.

Only one report is in disagreement with the hypothesis that choline owes its lipotropic activity to a stimulation of the rate of phospholipid turnover. That is the report of Perlman *et al.*,[738] which indicates that two decidedly antilipotropic substances, *i.e.*, cystine and cysteine, also stimulate phospholipid turnover in the liver. However, as a possible explanation for these discrepancies, it has been suggested[547] that the phospholipid turnover was studied several hours after the administration of cystine, and the lipotropic activity was investigated after the daily feeding of the cystine for several weeks.

The weight of evidence thus preponderantly supports the hypothesis that the action of choline precursors in preventing fatty livers is related to their function as an essential component in the *in vivo* synthesis of phospholipid.

[736] D. Cayer and W. E. Cornatzer, *Science*, *109*, 613–615 (1949).

[737] J. M. Patterson, N. B. Keevil, and E. W. McHenry, *J. Biol. Chem.*, *153*, 489–493 (1944).

[738] I. Perlman, N. Stillman, and I. L. Chaikoff, *J. Biol. Chem.*, *135*, 359–364 (1940).

The lipotropic action of choline is associated with the intact molecule rather than with its methyl groups. In the absence of this essential building stone, phospholipids such as the lecithins cannot be formed. Neutral fats then accumulate in the liver as a result of inadequate means for transporting them to the fat depots.

(*b'*) The Effect of Choline and Related Compounds on Growth: Although choline exerts no pronounced effect on growth under ordinary conditions, a definite correlation between increased growth and choline intake can be demonstrated in animals receiving diets deficient in labile methyl groups. In fact, the original discovery of du Vigneaud *et al.*[656] that homocystine can support the growth of the rat in the absence of methionine when choline is present is a demonstration of the growth-promoting action of choline. However, this effect is obviously an indirect one which results from the synthesis of an essential amino acid through the mediation of the methyl groups of choline. Chandler and du Vigneaud[739] have shown that the choline analogue, betaine, is likewise able to support the growth of rats receiving homocystine diets, although it is not as effective as choline itself. On the other hand, ethionine has a toxic effect on the growth of rats,[649,740] as does also the ethyl analogue of choline, namely triethylcholine.[741,742] The failure of weanling rats receiving triethylcholine to grow may possibly explain the apparent lipotropic action of this compound.

Although du Vigneaud reported that dimethylaminoethanol, $(CH_3)_2$-NCH_2CH_2OH, could not substitute for choline in effecting growth when homocystine was fed, Jukes and Oleson[743] found that this compound exhibited a choline-like effect in promoting growth and in preventing perosis in the chick, on a diet deficient in choline. Moreover, deuterium-containing methyl groups appeared in choline from rats fed deuteriodimethylaminoethanol which had the isotope in the methyl group.[652]

(*c'*) The Effect of Choline on the Kidney: Griffith and Wade[744] were the first to present evidence of the important effect of choline in preventing hemorrhagic degeneration of the kidneys. Young rats, placed on a choline-low diet, developed this deficiency within ten days; in addition to the renal enlargement and the fatty livers, hypertrophy of the spleen and a regression of the thymus were likewise noted. Only 2 mg. of choline per day

[739] J. P. Chandler and V. du Vigneaud, *J. Biol. Chem.*, *135*, 223–229 (1940).
[740] H. M. Dyer, *J. Biol. Chem.*, *124*, 519–524 (1938).
[741] J. A. Stekol and K. Weiss, *J. Biol. Chem.*, *185*, 585–587 (1950).
[742] C. S. McArthur and C. C. Lucas, *Biochem. J.*, *46*, 226–231 (1950).
[743] T. H. Jukes and J. J. Oleson, *J. Biol. Chem.*, *157*, 419–420 (1945).
[744] W. H. Griffith and N. J. Wade, *Proc. Soc. Exptl. Biol. Med.*, *41*, 188–190, 333–334 (1939).

were required to prevent kidney degeneration, but 10 mg. were needed to avoid fatty infiltration of the liver. Methionine as well as choline prevented kidney injury. Moreover, when rats were fed on a choline-low diet in which the protein was arachin (a protein low in methionine), kidney lesions developed.[745] Cystine exaggerated the kidney injury.[744]

Griffith and Wade[746] suggest that the role of choline in preventing kidney injury is a more fundamental one than is its function as a lipotropic agent. Presumably, it is related to its function in cell structure, which is supported by the widespread distribution and the constancy of phospholipids in various tissues,[349,352] and especially in the liver.[747] This effect on the kidney presumably reflects a generalized condition, since, in the above experiments, hemorrhages were shown to develop not only in the kidneys but also in the adrenal glands, lungs, and eyes. Total liver homorrhages have likewise been reported in rats receiving diets deficient in methionine and cystine.[748] These studies serve to emphasize the importance of choline in maintaining the cellular structure intact.

Marked variations in susceptibility to kidney injury due to choline deficiency are related to the sex, weight, and age of the rats.[746,749] Only six to ten days are required to produce the injury in male rats weighing 40 g., and twenty to thirty days old, while it becomes progressively more difficult to bring about the deficiency in older and heavier rats. In fact, Griffith[750] noted a spontaneous partial recovery from kidney damage in animals which had survived the initial ten-day period of the choline-deficient diet. Engel,[751] however, stated that symptoms of choline deficiency can be produced in the rat within seven to fourteen days, at any time during the growth period. In the absence of choline, the severity of the kidney injury is augmented by cystine, fat, or cholesterol.[752] The histologic changes have been described by Christensen[753] and by György and Goldblatt.[754]

Since transmethylation was early recognized to be an important function of choline, it is only natural that Griffith *et al.*[624,713,755,756] offered the ex-

[745] R. W. Engel and W. D. Salmon, *J. Nutrition, 22,* 109–121 (1944).
[746] W. H. Griffith and N. J. Wade, *J. Biol. Chem., 131,* 567–577 (1939).
[747] P. L. MacLachlan and H. C. Hodge, *J. Biol. Chem., 127,* 721–726 (1939).
[748] T. E. Weichselbaum, *Quart. J. Exptl. Physiol., 25,* 363–367 (1935).
[749] W. H. Griffith, *J. Nutrition, 19,* 437–448 (1940).
[750] W. H. Griffith, *Biol. Symposia, 5,* 193–212 (1941).
[751] R. W. Engel, *Proc. Soc. Exptl. Biol. Med., 50,* 193–196 (1942).
[752] W. H. Griffith, *J. Biol. Chem., 132,* 639–644 (1940).
[753] K. Christensen, *J. Biol. Chem., 133,* xx (1940).
[754] P. György and H. Goldblatt, *J. Exptl. Med., 72,* 1–9 (1940).
[755] W. H. Griffith and N. J. Wade, *J. Biol. Chem., 132,* 627–637 (1940).
[756] W. H. Griffith and D. J. Mulford, *Proc. Soc. Exptl. Biol. Med., 45,* 657–658 (1940).

planation that hemorrhagic injury of the kidney might result from a deficiency of the labile methyl supply. The fact that either betaine or methionine can prevent the kidney degeneration would seem to support such an hypothesis. However, McHenry and Patterson[547] consider that methyl groups are needed merely for the synthesis of choline and not *per se* for the prevention of hemorrhagic kidneys. They cite as evidence for this conclusion the fact that triethylcholine[757] and arsenocholine,[758] neither of which contributes to the supply of labile methyl groups, prevent the onset of renal hemorrhage in rats on a low choline diet. However, dimethylglycine was ineffective in preventing hemorrhagic kidneys in young rats.[716] Monoethyl and diethylcholine chlorides are quite potent as antihemorrhagic agents, but the tripropyl homologue is inactive. Other active compounds include betaine aldehyde chloride and betaine hydrochloride, as well as two sulfur analogues of betaine, namely dimethylthetin hydrochloride ($(CH_3)_2SCH_2COOH(Cl)$) and methylethylthetin hydrochloride ($CH_3(C_2H_5)$-$SCH_2COOH(Cl)$).[759]

The requirement for choline in preventing renal hemorrhagic damage was ascribed by Griffith[750] to the essential character of choline phospholipids, since lecithin is an intermediate in fat metabolism and an important component in the cell structure. This viewpoint is strengthened by the report of Patterson and McHenry,[760] and of Patterson *et al.*,[737] that the renal damage which results from a choline-low diet is preceded by a decreased concentration of phospholipids in both the kidney and the liver. These investigators noted that either choline or triethylcholine maintains the phospholipid within normal limits, and likewise prevents the kidney injury. The suggestion is therefore made that the kidney lesions result from an inability of the animal to keep pace with the rapid phospholipid turnover needed to maintain the structure of the growing kidney. Moreover, since the rate of phospholipid turnover is much higher in young rats than in older ones,[761] the possibility of a deficiency at this period of rapid growth is intensified. Since all substances which protect the kidney are lipotropic agents, it seems probable that their behavior is traced simply to their stimulation of the production of an increased phospholipid supply, with consequent improvement in fat transport. A comprehensive review of the antihemorrhagic action of choline-like compounds has been written by Welch.[759]

[757] A. D. Welch, *J. Biol. Chem.*, *137*, 173–181 (1941).
[758] R. L. Landau and A. D. Welch, *Federation Proc.*, *1*, 156–157 (1942).
[759] A. D. Welch, *J. Nutrition*, *40*, 113–131 (1950).
[760] J. M. Patterson and E. W. McHenry, *J. Biol. Chem.*, *145*, 207–211 (1942).
[761] G. W. Changus, I. L. Chaikoff, and S. Ruben, *J. Biol. Chem.*, *126*, 493–500 (1938).

Baxter and Campbell[762] demonstrated that the renal lesions and mortality caused by a purified diet deficient in choline could largely be prevented by supplementing the diet with crystalline aureomycin. This treatment somewhat increased the choline level in liver and kidney tissue, as well as in the feces.

(*d'*) The Effect of Choline Intake on Acetylcholine Formation: Since acetylcholine plays such a vital role in the transmission of nerve impulses (see page 26), it would seem probable that a decreased efficiency in this function might occur in choline deficiency.[763] Solandt and Best[764] were able to demonstrate that this was the case. Whereas a given stimulation of the vagus nerve produced a reduction of the heart rate to 30% of the normal value in rats on a normal diet, it was reduced to 45% in rats on a choline-low diet when a choline supplement was being administered, and only to 75% in rats on the choline-low diet which was unsupplemented. The action of the vagus nerve on the heart muscle in the deficient group was increased after the intravenous injection of choline. It is believed that this function of choline proceeds entirely independently of the lipotropic action.

(*e'*) The Effect of Choline on Lactation: Sure[765] reported that choline is an essential component in the diet of the suckling rat; it is likewise required after weaning. When lactating rats were deprived of choline, their young stopped growing within thirteen days, and died shortly thereafter, having developed a characteristic paralysis. The deficiency was overcome by the administration of a dose of 15 mg. of choline daily to the mothers, or of 5 mg. to the mothers together with 10 mg. to the young each day. The high requirement of the weanling rats for choline is a reflection of the increased rate of phospholipid turnover during this period, as shown by Sinclair[766] and by Fries *et al.*[767] It has been demonstrated by Artom and Fishman[768] that, when a choline-low diet is given, choline must be administered if a normal concentration of choline phospholipid is to be maintained in the liver of the newly weaned rat.

(*f'*) The Effect of Choline on Perosis: Perosis, also referred to as "slipped tendon," which occurs in chicks, results from a dietary deficiency. Although one cause of perosis has been shown to be a manganese

[762] J. H. Baxter and H. Campbell, *Proc. Soc. Exptl. Biol. Med., 80,* 415–419 (1952).
[763] D. Y. Solandt, *Can. Chem. Process Inds., 23,* 280 (1939).
[764] D. Y. Solandt and C. H. Best, *Nature, 144,* 376 (1939).
[765] B. Sure, *J. Nutrition, 19,* 71–76 (1940).
[766] R. G. Sinclair, *J. Biol. Chem., 88,* 575–587 (1930).
[767] B. A. Fries, G. W. Changus, and I. L. Chaikoff, *J. Biol. Chem., 132,* 23–34 (1940).
[768] C. Artom and W. H. Fishman, *J. Biol. Chem., 148,* 423–430 (1943).

deficiency,[769–771] Jukes reported that, in turkeys, it was not prevented when manganese was given.[772] However, when choline was administered together with manganese, to turkeys and chicks,[773,774] perosis was prevented. Arsenocholine was shown to have an antiperotic effect, while gelatin was completely ineffective. In the case of chicks, perosis did not develop on a choline-low diet unless the ration contained an excess of creatine or gelatin (high in glycine, which is a precursor of creatine).[775] In the latter case, 0.1% of choline was required to prevent perosis. According to Jukes and Welch,[776] the antiperotic activity of analogues of choline is distinct from their growth-promoting effect. Moreover, perosis occurs without a concomitant fatty liver.[777] This would indicate that these two effects of choline differ from each other. Although Hogan and co-workers[778] are in agreement with the statement that choline is important in preventing perosis, it is believed that it is not the only natural perosis-inhibiting component in liver. In fact, Jukes and Oleson[743] reported that dimethylaminoethanol prevented perosis in the chicken. It is not known how choline functions in alleviating perosis. Nichol and Welch[669] called attention to the importance of vitamin B_{12} in preventing this condition. However, in spite of the fact that vitamin B_{12} was shown to increase the effectiveness of choline in promoting growth, Kratzer[779] reported that this vitamin decreased the action of choline in preventing perosis.

(*g'*) The Beneficial Effect of Choline on Nutritional Edema: Alexander and Engel[780] showed that choline was able to counteract the nutritional edema produced when rats were fed low-protein, low-choline diets over a prolonged period. In the case of rats suffering from edema, a substantial increase in body moisture occurred, with a resultant diminution in the concentration of protein and fat. However, on a dry-weight basis, the rats without choline presented low protein values, but identical body fat values were noted, irrespective of whether or not choline was included in the diet.

[769] T. H. Jukes, *J. Nutrition, 13,* 359–387 (1937).
[770] H. S. Wilgus, L. C. Norris, and G. F. Heuser, *Poultry Sci., 16,* 232–237 (1937).
[771] H. S. Wilgus, L. C. Norris, and G. F. Heuser, *Science, 84,* 252–253 (1936).
[772] T. H. Jukes, *Poultry Sci., 18,* 405–406 (1939).
[773] T. H. Jukes, *J. Biol. Chem., 134,* 789–790 (1940).
[774] T. H. Jukes, *J. Nutrition, 20,* 445–458 (1940).
[775] T. H. Jukes, *Proc. Soc. Exptl. Biol. Med., 46,* 155–157 (1941).
[776] T. H. Jukes and A. D. Welch, *J. Biol. Chem., 146,* 19–24 (1942).
[777] D. M. Hegsted, R. C. Mills, C. A. Elvehjem, and E. B. Hart, *J. Biol. Chem., 138,* 459–466 (1941).
[778] A. G. Hogan, L. R. Richardson, H. Patrick, and H. L. Kempster, *J. Nutrition, 21,* 327–340 (1941).
[779] F. H. Kratzer, *J. Nutrition, 48,* 201–207 (1952).
[780] H. D. Alexander and R. W. Engel, *J. Nutrition, 47,* 361–373 (1952).

It was found that the edematous condition of the animals on the choline-free diet was associated with fatty infiltration and cirrhosis of the liver.

k′. The Effect of Excessive Doses of Choline and of Methionine: Luecke and Pearson[781] found that the daily administration of 40 g. of choline chloride to dogs and to sheep, over a period of six days, did not increase the free or total choline content of the liver, kidney, or plasma. The choline was apparently metabolized, inasmuch as only 0.7 to 2.5% of that fed could be recovered in the urine of the sheep, and only 0.5% in that of the dog. The urine nitrogen was increased by an amount equivalent to that of the choline nitrogen ingested. The mechanism by which this large amount of choline is destroyed is problematical. Handler and Bernheim[782] demonstrated that the activity of choline oxidase is depressed in the case of the fatty liver. Luecke and Pearson[781] postulate that a reverse mechanism may be set up, and that the feeding of large quantities of choline stimulates the activity of this enzyme, thereby preventing any increase in the choline content of the tissues or of the blood.

In contradistinction to the lack of toxicity of massive doses of choline, methionine may have a harmful effect if given in large amounts. Roth and Allison[783] demonstrated that, when 4.8% of methionine was added to a diet containing 12% of casein, a marked loss of weight occurred in the case of the rats. This represents the fat stores of the animal. In later work, Roth and Allison[784] reported that the injury caused by a diet containing 4.8% methionine was aggravated when choline was omitted from the diet. Under these conditions, atrophy of the seminal vesicles was also observed. When the dietary methionine was increased to 7%, the excretion of creatinine was augmented, and marked hypertrophy of the kidney occurred. This suggests that the catabolism of methionine or of homocysteine requires the simultaneous catabolism of fat.

(*b*) *Fatty Livers Resulting from a Deficiency of Lipocaic.* a′. Experimental Evidence of Lipocaic in Tests on Dogs: After the early work of Hershey,[572] in which it was found that raw pancreas prevented the development of fatty livers in depancreatized dogs, it was at first generally accepted that this effect could be traced to the lecithin or choline content of the gland. The first view opposing this explanation was that of Ralli, Flaum, and Banta,[785] who indicated that the pancreas exerted a more beneficial effect in controlling liver lipid than could be accounted for on the

[781] R. W. Luecke and P. B. Pearson, *J. Biol. Chem.*, *158*, 561–566 (1945).
[782] P. Handler and F. Bernheim, *J. Biol. Chem.*, *144*, 401–403 (1942).
[783] J. S. Roth and J. B. Allison, *Proc. Soc. Exptl. Biol. Med.*, *70*, 327–330 (1949).
[784] J. S. Roth and J. B. Allison, *J. Biol. Chem.*, *183*, 173–178 (1950).
[785] E. P. Ralli, G. Flaum, and R. Banta, *Am. J. Physiol.*, *110*, 545–551 (1935).

basis of its choline content. Following this work, Dragstedt and his co-workers[786,787] arrived at similar conclusions; they believed that 1 g. of choline was required daily to prevent fatty livers in depancreatized dogs, while the amount administered in their pancreatic preparation amounted to only 60 mg. These workers coined the term "lipocaic" to designate the component which prevented the development of fatty livers. It was considered to be a hormone, found only in the pancreas, and concerned exclusively with the transport and utilization of fat. Further support for the hypothesis that another factor, in addition to choline, is involved in controlling fatty livers came from the experiments of Kaplan and Chaikoff.[788–790] These workers confirmed the fact that liver fat was reduced and blood fat increased when raw pancreas was given to depancreatized dogs. These two effects could not be produced by means of choline alone or with choline plus autoclaved pancreas. On the basis of this work it was suggested that pancreas contains two factors active in lipid metabolism, namely, a heat-labile fraction controlling blood lipids, and a heat-stable component (probably choline) which controls the deposition of lipids in the liver. These workers recognized that the factors controlling fatty livers in depancreatized dogs and in rats on a low-protein, high-fat diet may be entirely different, and that the conclusions obtained in one case may not be applicable to the other. The numerous controversies in the interpretation of the results in this field are to a considerable degree due to the failure to recognize these differences.

b′. Experimental Evidence of Lipocaic Activity toward Fatty Livers Resulting from Choline Deficiency: Shortly after the report of Dragstedt on lipocaic, a number of workers attempted to investigate the lipotropic action of this compound on rats with fatty livers produced by a low choline regimen. Best and Ridout[791] were the first to report that lipocaic had no specific activity in removing liver fat other than that attributable to its choline and protein content. Aylward and Holt,[792] MacKay and Barnes,[793] and Wick and Laurence[794] reached essentially similar conclusions when the

[786] J. Van Prohaska, L. R. Dragstedt, and H. P. Harms, *Am. J. Physiol.*, *117*, 166–174 (1936).

[787] L. R. Dragstedt, J. Van Prohaska, and H. P. Harms, *Am. J. Physiol.*, *117*, 175–181 (1936).

[788] A. Kaplan and I. L. Chaikoff, *Proc. Soc. Exptl. Biol. Med.*, *34*, 606–607 (1936).

[789] A. Kaplan and I. L. Chaikoff, *J. Biol. Chem.*, *119*, 435–449 (1937).

[790] A. Kaplan and I. L. Chaikoff, *J. Biol. Chem.*, *120*, 647–657 (1937).

[791] C. H. Best and J. H. Ridout, *Am. J. Physiol.*, *122*, 67–72 (1938).

[792] F. X. Aylward and L. E. Holt, Jr., *J. Biol. Chem.*, *121*, 61–69 (1937).

[793] E. M. MacKay and R. H. Barnes, *Proc. Soc. Exptl. Biol. Med.*, *38*, 410–414 (1938).

[794] A. N. Wick and E. Laurence, *Arch. Biochem.*, *20*, 113–117 (1949).

type of fatty liver tested was that produced in the rat on a low-protein, high-fat, and essentially choline-free diet. On the other hand, Eilert and Dragstedt[795] stated that the lipocaic effect on dietary fatty livers in rats must be due to some constituent other than choline, methionine, inositol, or the non-specific action of protein. Further work is obviously required to resolve the inconsistencies in this field.

c′. Experimental Evidence of Lipocaic Activity toward Fatty Livers Resulting from Cholesterol: On the other hand, Channon, Loach, and Tristram[796] have found that lipocaic is active in removing fat from the liver of rats in which the fatty infiltration was induced by cholesterol feeding. The average value for the non-choline activity of the pancreatic preparation was equivalent to 426 mg. choline per 100 g. pancreas. Ralli *et al.*[558] found an increased content of cholesterol esters in the livers of depancreatized dogs, but the level of the free cholesterol fraction was not uniformly affected. They suggest that this type of fatty liver may differ from the type produced on a choline-low diet. Moreover, Montgomery, Chaikoff, *et al.* [797–800] reported that fatty livers of depancreatized dogs contain a large proportion of cholesterol. One is led to postulate that the activity of lipocaic in removing the fat from the livers of pancreatectomized dogs may be related to its action toward fatty livers having a high percentage of cholesterol.

d′. Lipocaic as a Hormone: Dragstedt and colleagues[786,787] reached the conclusion that lipocaic is a hormone, distinct from insulin, which is elaborated by the pancreas. These workers are of the opinion that the lipotropic action is not associated with the presence of pancreatic juice in the intestine. Canepa, Grossman, and Ivy[801] found that crystalline trypsin, chymotrypsin, and carboxypeptidase, when given by mouth, are not lipotropic following ligation of the pancreatic duct of the dog. However, these conclusions are not borne out by other workers (see following section).

A number of facts lead one to question whether or not lipocaic can be classified as a hormone in the usual sense of the word. The primary cir-

[795] M. L. Eilert and L. R. Dragstedt, *Am. J. Physiol.*, *147*, 346–351 (1946).

[796] H. J. Channon, L. V. Loach, and G. R. Tristram, *Biochem. J.*, *32*, 1332–1344 (1938).

[797] M. L. Montgomery, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, *128*, 387–398 (1939).

[798] C. Entenman, M. L. Montgomery, and I. L. Chaikoff, *J. Biol. Chem.*, *135*, 329–335 (1940).

[799] M. L. Montgomery, C. Entenman, I. L. Chaikoff, and C. Nelson, *J. Biol. Chem.*, *137*, 693–698 (1941).

[800] C. Entenman, I. L. Chaikoff, and M. L. Montgomery, *J. Biol. Chem.*, *137*, 699–706 (1941).

[801] J. F. Canepa, M. I. Grossman, and A. C. Ivy, *Am. J. Physiol.*, *156*, 387–395 (1949).

cumstance which argues against this concept is the time required to produce a fatty liver deficiency resulting from the lack of lipocaic. Hormones ordinarily act with dispatch. Secretin results in the production of pancreatic juice within thirty seconds after its injection. Insulin has an almost immediate effect in reducing blood sugar. Fat accumulates in the liver of rats, following the administration of a choline-low diet, within twenty-four hours after the initiation of the diet.[564] However, it requires weeks or even months for fatty livers to develop in the absence of lipocaic. The prolonged time required for lipocaic deficiency to become manifest would lead one to class it as a dietary deficiency.

Another result, which is opposed to the view that lipocaic is a hormone, is the apparently wide distribution of this substance in different tissues. A hormone is characterized by the fact that it is produced and concentrated in a single endocrine organ. On the other hand, Gavin and McHenry,[802] using extracts of liver, kidney, muscle, wheat germ, and rice polishings prepared by the method described by Dragstedt *et al.* for the preparation of lipocaic, were able to demonstrate that these extracts, as well as pancreatic extract, prevented the biotin type of fatty livers. However, it is admitted by McHenry and Patterson[547] that this proof is not decisive, since tests were not made with other types of fatty livers in rats, or on the fatty livers of depancreatized dogs. However, the results argue against the specific nature of the lipotropic substance, which would be necessary if it were to be classed as a hormone.

e′. Lipocaic as an Enzyme: The first suggestion that the effect of pancreas on liver lipids might be enzymatic in nature was the demonstration that ligation of the pancreatic duct produced fatty livers in a manner analogous to that following removal of the gland. Thus, Ralli *et al.*[558] noted that ligation of the pancreatic duct (and hence the removal of pancreatic juice from the lumen of the gut) resulted in fatty livers. Moreover, Montgomery, Chaikoff, and their associates,[799,800] reported a number of experiments which indicate that the feeding of pancreatic juice is just as effective in reducing the fat in the fatty livers of depancreatized dogs as is raw pancreas. In later work[803] it was reported that as small an amount as 5 mg. of trypsin in each lean meat meal fed to insulin-treated, depancreatized dogs was sufficient to prevent the development of fatty livers. Trypsin is thus identified as a possible intrinsic anti-fatty-liver factor in the dog. In addition to trypsin, other proteolytic enzymes, such as chymotrypsin, car-

[802] G. Gavin and E. W. McHenry, *J. Biol. Chem.*, *139*, 485 (1941).

[803] M. L. Montgomery, C. Entenman, I. L. Chaikoff, and H. Feinberg, *J. Biol. Chem.*, *185*, 307–310 (1950).

boxypeptidase, papain,[803,804] and ficin[804] may possibly serve in a similar capacity.

Proof that the anti-fatty-liver fraction in the pancreas is an enzyme has likewise been adduced from the behavior of preparations from the pancreatic gland. Thus, Entenman *et al.*[805] were able to prepare an anti-fatty-liver fraction from pancreas which maintained normal livers in depancreatized dogs for as long as six months when the material was given in doses of 60 mg. daily. This substance was prepared by a procedure differing from that of lipocaic. Moreover, it was shown that lipocaic as prepared by Dragstedt *et al.*[787] was a relatively poor source of lipotropic material.[806] The active lipotropic fraction prepared from pancreas did not owe its activity to its choline content.[807] It caused an increase in plasma choline either by inducing a shift of this substance from the tissues to the plasma or, more probably, by stimulating the synthesis from its precursors.[808] However, Entenman *et al.*[798] did report that the appearance of fatty livers in dogs deprived of the external excretion of the pancreas by ligation of the duct could be completely prevented by the daily ingestion of 2 g. of choline chloride.

Methionine was later shown by Chaikoff *et al.*[809] to prevent fatty livers in insulin-treated, depancreatized dogs. The conclusion was therefore drawn that the action of the anti-fatty factor in the pancreas is not concerned with the synthesis of choline in the body, or with its action upon the liver as a lipotropic agent, but rather with the liberation of bound methionine contained in dietary protein. Chaikoff *et al.*[809] postulate that the anti-fatty-liver factor might be a proteolytic enzyme without which ingested proteins cannot exert their lipotropic action. This viewpoint is supported by subsequent evidence supplied by these workers,[810] to the effect that, whereas unhydrolyzed casein failed to maintain a normal level of liver lipids in insulin-treated, depancreatized dogs, hydrolyzed casein was effective. Since the methionine content of the latter preparation was sufficient to account for this action, it is suggested that the anti-fatty-liver factor of pancreas

[804] H. Feinberg, I. L. Chaikoff, and C. Entenman, *Proc. Soc. Exptl. Biol. Med., 80,* 161–162 (1952).

[805] C. Entenman, I. L. Chaikoff, and M. L. Montgomery, *J. Biol. Chem., 155,* 573–578 (1944).

[806] C. Entenman, M. L. Montgomery, and I. L. Chaikoff, *Am. J. Physiol., 141,* 221–226 (1944).

[807] C. Entenman and I. L. Chaikoff, *J. Biol. Chem., 138,* 477–485 (1941).

[808] I. L. Chaikoff, C. Entenman, and M. L. Montgomery, *J. Biol. Chem., 160,* 387–395 (1945).

[809] I. L. Chaikoff, C. Entenman, and M. L. Montgomery, *J. Biol. Chem., 160,* 489–492 (1945).

[810] I. L. Chaikoff, C. Entenman, and M. L. Montgomery, *J. Biol. Chem., 168,* 177–181 (1947).

acts by rendering the methionine from ingested protein available for lipotropic purposes.

Confirmation of the mechanism of action of the pancreatic preparations has been obtained from recent studies on rats whose pancreatic ducts had been ligated. According to Clowes and Macpherson,[811] these animals develop degeneration of the acinar tissue within a month, and exhibit a significant decrease in intestinal proteolysis, as well as a reduction in the absorption of nitrogenous products and of fats. A "pancreatic factor" containing proteolytic enzymes was shown to restore the normal growth activity of the pancreatic fraction, as a result of the enzymatic liberation of methionine from ingested protein after the reactivation of the proteolytic enzyme in the small intestine.

According to Rhoads and co-workers,[812] the lipotropic fraction of the pancreas is markedly active in doses of 60 mg. daily. It appears to be in the category of enzymes, since it is destroyed by boiling its solutions for three minutes. Haanes and György[813] demonstrated, by *in vitro* experiments, the presence of a proteolytic enzyme, probably trypsin, in the pancreatic anti-fatty-liver preparation. These workers likewise postulate that the lipotropic action is based upon enzymatic liberation of methionine from ingested protein. It is suggested that these findings are in harmony with the assumption that one of the functions of the external pancreatic secretion is to release lipotropic precursors of choline from the protein of the diet.

(*c*) *Fatty Livers Resulting from an Inositol Deficiency.* Inositol, $C_6H_{12}O_6$, is a cyclic hexahydric alcohol. Its importance in nutrition stems from the discovery of Eastcott,[814] in 1928, that it was identical with bios I. Further evidence of its importance in the animal body is that of Woolley,[815] who proved that it is an essential dietary factor for the mouse. A deficiency in this factor produces alopecia in the mouse,[815] and the so-called "spectacle-eye" syndrome in the rat.[816]

The relation of inositol to the lipids was first suggested by Anderson,[817] who reported its presence in the lipid isolated from the tubercle bacilli. Klenk and Sakai[818] later identified it as a component of soybean phospho-

[811] G. H. A. Clowes, Jr., and L. B. Macpherson, *Am. J. Physiol.*, *165*, 628–638 (1951).
[812] J. E. Rhoads, O. Liboro, S. Fox, P. György, and T. E. Machella, *Am. J. Physiol.*, *166*, 436–440 (1950).
[813] M. L. Haanes and P. György, *Am. J. Physiol.*, *166*, 441–450 (1950).
[814] E. V. Eastcott, *J. Phys. Chem.*, *32*, 1094–1111 (1928).
[815] D. W. Woolley, *Science*, *92*, 384–385 (1940).
[816] P. L. Pavcek and H. M. Baum, *Science*, *93*, 502 (1941).
[817] R. J. Anderson, *J. Am. Chem. Soc.*, *52*, 1607–1608 (1930).
[818] E. Klenk and R. Sakai, *Z. physiol. Chem.*, *258*, 33–38 (1939).

lipids, and Woolley[819] prepared an inositol-containing phospholipid from soybean oil which contained as much as 16% of inositol. The great importance of this compound in the animal body has been recognized only since the discovery by Folch[820] alone and with Woolley[821] that the inositol phosphatides belong in one of the three classes of components which make up the lipids formerly referred to as cephalins. Since this inositol phospholipid comprises an important part of such an essential organ as the brain, it is not unexpected to find it classified as an essential dietary component.

Gavin and McHenry[802] were the first to indicate that inositol has a lipotropic action in rats. When this substance was added to the diet of rats with fatty livers induced by the administration of biotin in conjunction with thiamine, riboflavin, pantothenic acid, and pyridoxine, the increase of fatty acids and of cholesterol was prevented. Thus, this lipotropic action resembles that of lipocaic, although the authors do not state that the active agent in lipocaic is necessarily inositol. Engel[822] obtained a lipotropic action by as small an amount as 3 mg. of inositol, in the case of livers refractory to choline. However, he did not carry out tests with biotin fatty livers. In a later study by Gavin *et al.*,[823] it was found that, whereas choline is effective in reducing the fat in the case of thiamine fatty livers, and is partially effective against cholesterol fatty livers, it is almost completely inactive toward biotin fatty livers. On the other hand, inositol and lipocaic are both lipotropic toward the biotin fatty liver. Lipocaic differs from inositol in the fact that it is ineffective against fatty livers produced by feeding cholesterol with a high-fat diet. Inositol is entirely inactive as a lipotropic agent in the case of the thiamine fatty liver. The addition of other B vitamins is thus required before inositol exhibits its lipotropic action.

Beveridge[824] reported that the feeding of corn oil actually obliterated the lipotropic action of inositol. This result was confirmed by Beveridge and Lucas.[825] Engel[822] had previously noted that some augmentation of the action of inositol could be produced by feeding corn oil. On the other hand, Blewett and associates[826] noted that the injection of inositol together with

[819] D. W. Woolley, *J. Biol. Chem.*, *147*, 581–591 (1943).
[820] J. Folch, *J. Biol. Chem.*, *146*, 35–44 (1942).
[821] J. Folch and D. W. Woolley, *J. Biol. Chem.*, *142*, 963–964 (1942).
[822] R. W. Engel, *J. Nutrition*, *24*, 175–185 (1942).
[823] G. Gavin, J. M. Patterson, and E. W. McHenry, *J. Biol. Chem.*, *148*, 275–279 (1943).
[824] J. M. R. Beveridge, *Science*, *99*, 539–540 (1944).
[825] J. M. R. Beveridge and C. C. Lucas, *J. Biol. Chem.*, *157*, 311–321 (1945).
[826] M. Blewett, I. G. Campbell, and J. Olley, *Nature*, *164*, 621–622 (1949).

oleic acid reduced the rate at which P was introduced into phospholipids containing choline and into those lacking choline.

Inositol has been reported to exert a lipotropic effect in the case of depancreatized dogs,[827,828] although the results were not as marked as those produced by a similar amount of lipocaic. McHenry and Patterson[547] state, however, that the lipocaic preparations made in their laboratories contain sufficient inositol to account for the lipotropic potency in rats.

In later work, Best and co-workers[829] failed to confirm the findings of McHenry and Patterson[547] that biotin produces a selective deposition of cholesterol esters in the liver, that inositol has a specific effect on bound cholesterol, or that the biotin fatty liver is particularly resistant to the action of choline. In fact, Beveridge and Lucas[825] reported that choline brings about a greater reduction in cholesterol esters than does inositol. However, Best *et al.*[278] did report that inositol exerts a limited but clear-cut lipotropic effect when added to a hypolipotropic diet devoid of fat. The lipotropic action disappears when tested with fatty livers produced on a diet containing the saturated fat fraction from beef drippings, a hydrogenated fat, or an unsaturated fat. Subsequently, it was concluded[830] that the action of inositol is strictly limited, and that there are no indications that inositol possesses unique lipotropic properties under any dietary conditions. Balatre and Merlen[831] confirmed the lipotropic action of inositol, but noted that it was completely inactive in preventing experimental arteriosclerosis. Both inositol acetate and nitrate were found to be superior to free inositol as lipotropic agents. These authors also reported that the acetate provided partial protection against atherosclerosis. MacFarland and McHenry[832] are of the opinion that the inositol effect is unique, and is characteristic of the biotin fatty livers. A comparison of the action of the several lipotropic agents in different types of fatty livers is given in Table 24 (page 678).

Ridout and collaborators[833] have demonstrated that a synergism exists between choline and inositol, exhibited by their relative lipotropic effects in

[827] F. M. Owens, J. G. Allen, D. Stinger, and L. R. Dragstedt, *Federation Proc.*, *1*, 65 (1942).

[828] S. H. Rubin and E. P. Ralli, *Federation Proc.*, *1*, 76 (1942).

[829] C. H. Best, C. C. Lucas, J. M. Patterson, and J. H. Ridout, *Biochem. J.*, *40*, 368–373 (1946).

[830] C. H. Best, C. C. Lucas, J. M. Patterson, and J. H. Ridout, *Biochem. J.*, *48*, 452–458 (1951).

[831] P. H. Balatre and J. F. Merlen, *Compt. rend. soc. biol.*, *145*, 579–581 (1951).

[832] M. L. MacFarland and E. W. McHenry, *J. Biol. Chem.*, *176*, 429–434 (1948).

[833] J. H. Ridout, C. C. Lucas, J. M. Patterson, and C. H. Best, *Biochem. J.*, *40*, 494–499 (1946).

TABLE 24

COMPARISON OF THE EFFECTS OF CHOLINE, LIPOCAIC, AND INOSITOL ON DIFFERENT TYPES OF FATTY LIVERS[a]

Regimen used for production of fatty livers	Lipotropic action[b] by		
	Choline	Lipocaic	Inositol
Depancreatized dogs	++*[c]	++*	−?
Rats:			
High-fat diet			
Thiamine	++*	0	−
All B vitamins	++*	−	−
Cholesterol	+*	0	+
Fat-free diet			
Thiamine	++*	−	0
Thiamine and riboflavin	++*	−	−
Thiamine, riboflavin, pantothenic acid, pyridoxine	+*	−	+*
The 4 vitamins mentioned above, biotin	0	++	++
B vitamins, cholesterol	+	+	+

[a] Adapted from E. W. McHenry and J. M. Patterson, *Physiol. Revs.*, *24*, 128–167 (1944), p. 160.
[b] ++, Strong lipotropic action; +, moderate lipotropic action; 0, no lipotropic action; −, lack of data.
[c] * indicates verification in two or more laboratories.

rats on cholesterol-containing diets. In contradistinction to the action of choline, inositol does not exert any preferential effect in removing cholesterol. Moreover, according to McHenry and Patterson[547] and Handler,[834] in the case of rats which had received a diet free from vitamin B_2 during the preliminary period, choline was uniformly more effective than inositol in decreasing liver glycerides or cholesterol esters in cholesterol-fed rats receiving diets either with or without fat.[833] Inositol does not have a favorable effect on hemorrhagic kidneys produced by acute choline deficiency.[835] There appears to be little doubt that the mechanism of action of choline and that of inositol as lipotropic agents are entirely distinct.

(*d*) *Fatty Livers Resulting from a Deficiency of Essential Acids.* There is a general opinion that lecithins are composed of equal proportions of saturated and unsaturated acids.[836,837] Since the iodine numbers of most lecithins greatly exceed the value of 32.7 for palmityl-oleyllecithin, or 31.5 for stearyl-oleyllecithin, it would appear that they must have either 2 mono-

[834] P. Handler, *J. Biol. Chem.*, *162*, 77–85 (1946).
[835] C. H. Best, F. Hoffmann, C. C. Lucas, and J. Talesnik, *J. Physiol.*, *105*, 27P (1946).
[836] H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930.
[837] P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *46*, 193–207 (1921).

ethenoid acids or a single di- or polyethenoid acid as the unsaturated acid component. Since the presence of palmitic or stearic acid to the extent of about 50% of the total acid content seems to be well established, the high iodine number can be reconciled only with the second alternative given above, namely that unsaturated acids containing more than one unsaturated linkage must be present to a considerable extent in the mixed lecithin acids. In addition to oleic acid, linoleic, linolenic, arachidonic, and clupanodonic acids, other unsaturated acids have been isolated from various lecithin preparations. It is quite possible that lecithins are unable to function without the presence of a certain proportion of the highly unsaturated acids in the molecules. For a further discussion of the composition of the lecithin molecule and its fatty acid composition, the reader is referred to pages 408 to 426 of Volume I.

When the essential fatty acids (linoleic, linolenic, and arachidonic) are absent from the diet, not only does malnutrition develop, but a failure of growth ensues, and fatty livers develop.[571,822] Burr and Burr[838] also noted lesions in the kidneys, which were sometimes fatal. It is not certain whether or not the pathological effect is similar to that resulting from choline deficiency, but this would seem probable. Smedley-MacLean and Nunn[839] have reported that essential fatty acid-deficient rats, likewise, have an impaired ability to lay down depot fat. Moreover, although fat absorption is not reduced out of porportion to the size of the animals, phosphorylation of fats (and lecithin synthesis) in the intestinal mucosa is at a subnormal level.[840] Presumably the phospholipid turnover in the liver is decreased, due to inability to synthesize phospholipids, with the result that fat metabolism and fat transport are blocked. Hence, fatty acids accumulate in this organ. Fatty livers, which originate because of an essential fatty acid deficiency, are readily explained by a failure in the biosynthesis of phospholipid in the absence of the highly unsaturated fatty acid which is needed for incorporation into this molecule.

(*e*) *Fatty Livers Resulting from an Excess of Cholesterol.* Best and Ridout[579,841] confirmed the findings of Schönheimer and Yuasa,[842] of Bailey,[843] and of Sperry and Stoyanoff[844] that the administration of cholesterol in moderate amounts to rats results in fatty livers. These livers contain in-

[838] G. O. Burr and M. M. Burr, *J. Biol. Chem.*, *86*, 587–621 (1930).
[839] I. Smedley-MacLean and L. C. A. Nunn, *Biochem. J.*, *34*, 884–902 (1940).
[840] R. H. Barnes, E. S. Miller, and G. O. Burr, *J. Biol Chem.*, *140*, 773–778 (1941).
[841] C. H. Best and J. H. Ridout, *Am. J. Physiol.*, *105*, 6P (1933).
[842] R. Schönheimer and D. Yuasa, *Z. physiol. Chem.*, *180*, 5–16 (1929).
[843] C. H. Bailey, *J. Exptl. Med.*, *23*, 69–85 (1916).
[844] W. M. Sperry and V. A. Stoyanoff, *J. Nutrition*, *9*, 131–155 (1935).

creased amounts of both glycerides and cholesteryl esters,[845] but essentially normal amounts of phospholipids.

Although all types of fatty livers contain a certain proportion of cholesterol, the quantity of this sterol stored in the liver, chiefly in the form of the ester, is greatly increased after cholesterol is introduced into the diet.[556,845–847] Concomitantly, the cholesterol ester fraction is reduced in the serum.[554,789,847] Li and Freeman[848] have shown that cholesterol feeding also results in a higher cholesterol and lipid content in the liver of the protein-deficient dog. In opposition to the results on rats, these workers report an increase in the amount of free cholesterol, as well as of cholesterol ester, in the livers of these animals. According to Clément and associates,[849] the fatty infiltration occurring when 3 to 5% of cholesterol is included in the diet consists entirely of free lipids, not linked to cellular structures.

Fatty livers resulting from cholesterol are more resistant to choline[579] than are other types of fatty livers, although the fatty infiltration can be prevented if sufficiently large doses of choline are given.[823] Whereas 2 g. of casein per lb. of body weight sufficed to prevent fatty livers in dogs on a high-fat diet, this amount was found to be insufficient to prevent the cholesterol type of fatty liver.[848] The cholesterol esters appeared to be especially resistant to all lipotropic agents. On the one hand, Ridout and collaborators[845] were able to control the triglyceride content of the livers of rats on lipotropic cholesterol diets; on the other hand, the cholesterol esters in this organ were slightly increased when the choline intake was highest, even when the cholesterol level in the diet was lowest. However, Benard *et al.*[847] reported that, when rats having fatty livers of dietary origin or due to poisoning were injected with choline or methionine, the ratio of ester to total cholesterol was reduced from 0.419 to 0.185 and 0.192, respectively, as compared with a normal ratio of 0.165.

The mechanism of action of cholesterol in producing fatty infiltration of the liver has not been explained. It has been suggested that cholesterol acts as a vehicle for transporting fatty acids from the intestinal wall to the liver, but this leaves open to question the mode of transfer of the fatty acid to another alcohol in the liver. Only a limited amount of cholesterol is

[845] J. H. Ridout, C. C. Lucas, J. M. Patterson, and C. H. Best, *Biochem. J.*, *52*, 79–83 (1952).

[846] R. P. Cook, *Biochem. J.*, *30*, 1630–1636 (1936).

[847] H. Benard, A. Gajdos, and M. G. Török, *Compt. rend. soc. biol.*, *144*, 762–764 (1950).

[848] T. W. Li and S. Freeman, *Am. J. Physiol.*, *145*, 645–659 (1946).

[849] G. Clément, J. Clément, and E. Le Breton, *Compt. rend.*, *234*, 2006–2008 (1952).

absorbed, and the fatty acid content of the liver is far greater than that present as cholesterol ester. A clue to the difficulty was given by Perlman and Chaikoff,[732,850] who found that the incorporation of P^{32} into the livers of rats given cholesterol was diminished; however, the rate of phospholipid turnover could be increased in the cholesterol-fed rats by choline.[850] The effect of lipocaic on cholesterol fatty livers has been discussed in an earlier section.

(*f*) *Fatty Livers Resulting from the Administration of Liver or of Liver Extracts* (*Biotin Fatty Livers*). Blatherwick and his associates[851] reported, in 1931, that fatty livers developed in rats fed a diet of dried whole liver. The fatty livers were shown to contain large proportions of cholesterol esters and of fat, and it was suggested that this effect might be attributed to the cholesterol in the liver fed. However, McHenry and Gavin[852] were able to produce fatty infiltration in the liver by the administration of aqueous extracts of liver, which, of course, ruled out cholesterol as the causative agent. The hypothesis was then adduced that biotin in the liver extract was the factor responsible for producing the fatty livers, since the type of fatty liver produced by the injection of biotin resembled that which developed after liver or liver extract had been given. Both types were resistant to choline, but responded to lipocaic, as well as to extracts prepared in a manner similar to that employed for lipocaic derived from liver, kidney, muscle, wheat germ, rice polishings, and yeast.[802] They also responded to egg white and to inositol.[853] In the more recent work of MacFarland and McHenry[854] it was demonstrated that biotin fatty livers were partially responsive to choline or to inositol, and that the fatty infiltration could be entirely prevented by their presence in conjunction. On the other hand, fatty livers produced by a beef liver fraction were resistant to choline. This would seem to indicate that the fattening agent in the liver includes some factor other than, or in addition to, biotin.

(*g*) *Fatty Livers Resulting from Imbalances of the B Vitamins.* In addition to the fatty livers resulting from increased concentrations of biotin, or from a lack of inositol, as described above, fatty livers are associated with a number of other members of the B vitamins. Some of these are active because of a secondary effect on appetite, as, for example, thiamine, while others owe their effectiveness to a resulting increase in the choline or inositol requirement.

[850] I. Perlman and I. L. Chaikoff, *J. Biol. Chem.*, *128*, 735–743 (1939).
[851] N. R. Blatherwick, E. M. Medlar, P. J. Bradshaw, A. L. Post, and S. D. Sawyer, *Proc. Soc. Exptl. Biol. Med.*, *29*, 345–346 (1931).
[852] E. W. McHenry and G. Gavin, *J. Biol. Chem.*, *134*, 683–692 (1940).
[853] G. Gavin and E. W. McHenry, *J. Biol. Chem.*, *141*, 619–625 (1941).
[854] M. L. MacFarland and E. W. McHenry, *J. Biol. Chem.*, *159*, 605–609 (1945).

a′. Thiamine Fatty Livers: McHenry[855] found that fatty infiltration of the liver failed to occur on choline-free diets if thiamine was also absent. Rats on such a dietary regimen lost weight. However, if thiamine supplements were given to the animals, their appetites were restored, the animals gained weight, and fatty livers developed. These fatty livers could be prevented by choline.[856] The intensity of the fatty infiltration was to some extent dependent upon the amount of fat in the diet,[856,857] but it also occurred on fat-low diets. The fatty infiltration was increased by the simultaneous administration of riboflavin and pyridoxine.

Although McHenry[856] concluded that a possible explanation for the action of thiamine in fattening livers in rats receiving high carbohydrate diets would be that this vitamin promotes the synthesis of fat from carbohydrate, Peters and Van Slyke[549] prefer to explain it by the increased food intake resulting from the stimulation of appetite. There are several facts which favor the latter explanation. In the first place, the extent of liver fattening in one series of tests by McHenry[856] was proportional to the food intake. Moreover, thiamine does not cause fatty infiltration in rats when the food intake is restricted to that consumed by the control rats on a thiamine-free diet. Engel[822] reported substantially the same results.

b′. Pyridoxine Fatty Livers: Engel[822] reported that fatty livers develop in rats following a prolonged deficiency in pyridoxine. Halliday[858] noted a similar effect, which she found was partially counteracted by choline and was completely prevented by pyridoxine, given in the form of a liver extract. However, Gavin and McHenry[857] could detect no lipotropic action on the part of crystalline pyridoxine. They suggest that the discrepancy between their results and those of Halliday might be attributable to the fact that they used a pure pyridoxine preparation, while Halliday employed a liver concentrate which may have contained other factors as well. According to Engel,[822] fatty livers developing as a result of pyridoxine deficiency are counteracted by inositol. It is suggested by Peters and Van Slyke[549] that the demand for choline and inositol is increased in pyridoxine deficiency.

c′. Pantothenic Acid Fatty Livers: Scudi and Hamlin[859] reported that a pantothenic acid deficiency in dogs results in a hypolipemia (cholesterol, phospholipids, total lipids), which occurs concomitantly with a fatty infiltration of the liver. These results are in accord with the finding that

[855] E. W. McHenry, *J. Physiol.*, *85*, 343–349 (1935).
[856] E. W. McHenry, *J. Physiol.*, *89*, 287–295 (1937).
[857] G. Gavin and E. W. McHenry, *J. Biol. Chem.*, *132*, 41–46 (1940).
[858] N. Halliday, *J. Nutrition*, *16*, 285–290 (1938).
[859] J. V. Scudi and M. Hamlin, *J. Nutrition*, *24*, 273–282 (1942).

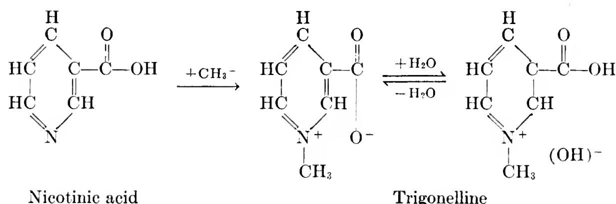
pantothenic acid is a component in the coenzyme A molecule, which is apparently necessary for the synthesis of fat. For a further discussion of the relationship of coenzyme A to fat synthesis, the reader is referred to Chapter II, Volume III.

d′. Nicotinic Acid Fatty Livers: Fatty livers may occur on low-fat, choline-free diets, in association with either a deficiency or an excess of nicotinic acid. The mechanism of the action is completely different under these two conditions. Thus, Salmon[623] reported that, on low-fat, lipogenic diets containing less than 18% of casein, a nicotinic acid deficiency was present; this was counteracted by a high level (30%) of dietary fat. This effect is believed to be due to the nicotinic acid-sparing action ensuing when the energy metabolism is shifted from carbohydrate to fat.

On the other hand, a number of workers have demonstrated the liver-fattening action of diets high in nicotinic acid. Handler and Dann[860] found that the inclusion of nicotinic acid in the diets of rats on a low casein diet, at a 1% level, resulted in the production of fatty livers, although it did not inhibit growth. However, when nicotinic acid was present in the diet at a 2% level, a slight inhibition of growth was noted. Niacinamide, when incorporated in the diet in a 1% proportion, almost completely inhibited growth. The fatty liver formation could be prevented by the administration of methionine, or by that of choline and homocystine, but not by choline, betaine, homocystine, or cystine alone. Foà *et al.*[861] confirmed the lipogenic action of nicotinic acid and of nicotinamide reported by Handler and Dann, and demonstrated that these vitamins likewise increased the severity of hemorrhagic degeneration of the kidneys. Both effects were reversed by choline.

A number of workers[862–864] had previously shown that the excess nicotinic acid or nicotinamide is excreted by rats, dogs, and man in the urine as trigonelline. The reaction is pictured in (5). On the basis of these facts, Handler and Dann[860] explained their results by showing that nicotinamide had removed the available methyl groups in the trigonelline molecule, and thus had deprived the body of the ability to form choline. When an adequate excess of methyl groups was available, the fatty livers did not occur. It is thus evident that neither excess nor deficiency of nicotinic acid *per se* can be considered to be the causative factor in fatty infiltration of the liver.

[860] P. Handler and W. J. Dann, *J. Biol. Chem.*, *146*, 357–368 (1942).
[861] P. P. Foà, N. L. Foà, and H. Field, Jr., *Arch. Biochem.*, *6*, 215–224 (1945).
[862] J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, *142*, 401–416 (1942).
[863] H. P. Sarett, *J. Nutrition*, *23*, 35–45 (1942).
[864] H. P. Sarett, J. W. Huff, and W. A. Perlzweig, *J. Nutrition*, *23*, 23–34 (1941).



Nicotinic acid Trigonelline

The detoxication of nicotinic acid in the animal body (5)

Tyner, Lewis, and Eckstein[865] reported that the fattening effect of cystine on the liver is exerted only when the animals have a low nicotinic acid intake.

The physiologic effect of nicotinic acid in producing fatty livers, on diets low in choline or in methionine, can be reversed when the N′-methylnicotinamide is given to rats receiving glycocyamine as a lipogenic agent. The lipotropic action of N′-methyl-nicotinamide was shown by Najjar and Deal[866] to be associated with a demethylation resulting in a marked increase in the daily urinary output of nicotinic acid. Rats which received the N′-methylnicotinamide averaged half the amount of liver fat which was present in the controls.

(*h*) *Fatty Livers Resulting from Hormones.* a′. Pancreatic Hormones: Insulin has been shown to bring about a six-fold increase in the fatty acid content of the liver of the alloxan-diabetic rat within four days.[867] Presumably this condition results largely because of an increased hepatic lipogenesis, as demonstrated by the rate at which C^{14} is incorporated into such fatty acids when pyruvate-2-C^{14} is metabolized. However, Osborn, Felts, and Chaikoff[867] noted that this insulin-induced fatty infiltration is transitory; the fatty acid content of the liver decreases abruptly after the fourth day of insulin injections, and returns almost to the original diabetic level within six days. It is suggested that a homeostatic control regulates the rate of hepatic lipogenesis. The effect of lipocaic has been discussed earlier (see page 670).

b′. Hormones of the Anterior Pituitary Gland: (*a′*) Ketogenic Hormone.—Best and associates[432,434,589] demonstrated that a pronounced fat mobilization occurs in the liver following the injection of the ketogenic hormone into mice, rats, and guinea pigs. The fattening of the liver was not prevented by choline, although this lipotropic agent accelerated the removal

[865] E. P. Tyner, H. B. Lewis, and H. C. Eckstein, *J. Biol. Chem.*, *187*, 651–654 (1950).
[866] V. A. Najjar and C. C. Deal, *J. Biol. Chem.*, *162*, 741–742 (1946).
[867] M. J. Osborn, J. M. Felts, and I. L. Chaikoff, *J. Biol. Chem.*, *203*, 173–181 (1953).

of lipid from the liver during the recovery phase. However, Campbell[454] reported that insulin partially prevents the increase in liver fat, in fasting mice, rats, and guinea pigs, which occurs after the administration of anterior pituitary extracts. After labeling body fats with deuterium, Stetten and Salcedo[433] were able to prove that the fatty infiltration following the injection of the anterior pituitary substance is produced by the increase in the rate of fat transfer from the fat depots to the liver. Conversely, when no ketogenic hormone is available, as occurs after hypophysectomy, fat transfer to the liver is inhibited, even when such powerful stimuli as the liver poisons, phosphorus and carbon tetrachloride, are administered.[868] Weil and Stetten[435] gave the name *adipokinin* to this fat-mobilizing agent of the anterior lobe of the pituitary.

(*b′*) Growth Hormone.—In addition to the ketogenic hormone of the anterior lobe of the pituitary gland, growth hormone also causes a rapid mobilization of lipid to the liver; the peak of mobilization occurs within six hours after treatment with the hormone, and the blood lipids return to normal within twenty-four hours.[869] According to Greenbaum and McLean,[869] the changes are mainly reflected in the neutral fat fraction. The synthesis of phospholipids in the liver is likewise stimulated. These compounds are then released to the plasma. When the purified growth hormone was continuously injected into adult female rats, a progressive decrease in the fat content of the carcass occurred. Greenbaum[870] suggests that one of the primary effects of the growth hormone is in the catabolism of fats.

c′. Adrenal Hormones: The injection of adrenocortical extracts has been shown to cause an increase in the fat content of the liver[281,871]; on the other hand, the animal loses its ability to transport fat to the liver following adrenalectomy. Thus, LeBlond and co-workers[872] reported that fatty livers could not be produced after extirpation of the adrenal glands, while Verzár and Laszt[444] found that even the powerful hepatic poison, phosphorus, was unable to elicit fat mobilization in the liver under such circumstances. Barnes, Miller, and Burr[281] likewise found that adrenalectomized animals which had been maintained in good physical condition by the administration of salt had an impaired ability to deposit absorbed fat in the neutral fat stores in the liver.

[868] B. v. Issekutz and F. Verzár, *Arch. ges. Physiol.* (*Pflüger's*), *240*, 624–635 (1938).
[869] A. L. Greenbaum and P. McLean, *Biochem. J.*, *54*, 407–413 (1953).
[870] A. L. Greenbaum, *Biochem. J.*, *54*, 400–407 (1953).
[871] C. H. Li, M. E. Simpson, and H. M. Evans, *Arch. Biochem.*, *23*, 51–54 (1949).
[872] C. P. LeBlond, N. Van Thoai, and G. Segal, *Compt. rend. soc. biol.*, *130*, 1557–1559 (1939).

Fat mobilization to the liver produced by anterior pituitary extracts is completely abolished following adrenalectomy.[873] However, Levin and Farber[873] demonstrated that, when adrenalectomized mice were pretreated with cortisone, the response to the pituitary hormone by a mobilization of fat in the liver was equal to that of intact mice, or even greater. On the other hand, cortisone and other cortical steroids were found to be ineffective by themselves in increasing the liver fat in these animals. The loss of liver fat usually occurring after adrenalectomy could be prevented by these cortical hormones. Li *et al.*[874] demonstrated an increase in liver fat in normal male rats which were force-fed a fluid containing carbohydrate, and were given injections of 3 mg. of ACTH (adrenocorticotropic hormone) daily for ten days.

The pronounced effect of adrenalectomy on liver fat is in line with the alterations in ketosis which result from adrenalectomy (see Volume III).

d′. Sex Hormones: In 1942, MacBryde and co-workers[875] reported that the administration of female sex hormones, estrone and estradiol, was followed by the production of fatty livers in dogs. However, the later evidence of György and collaborators[876–879] indicated rather that a lipotropic action follows the injection of these hormones. As small an amount as 30 μg. per day of estrone was shown to have a lipotropic action when administered to intact and to castrated female rats fed a diet normally producing fatty livers. Estrone had a synergistic effect on the lipotropic action of methionine.[876] Following ovariectomy, the fat content in the liver was increased.[877] Ethinyl estradiol was more active than estradiol benzoate or estrone, and was effective whether or not methionine was given.[878] The lipotropic effect is apparently mediated by an extrahepatic factor, which may be activated through the anterior pituitary gland.

e′. Thyroid Hormones: It is known that blood cholesterol is increased in thyroid deficiency and is reduced with increasing thyroid activity. Handler[880] reported that a thyroid deficiency, produced either by extirpation of the gland or by the administration of thiouracil, results in an increase in liver

[873] L. Levin and R. K. Farber, *Proc. Soc. Exptl. Biol. Med.*, *74*, 758–763 (1950).

[874] C. H. Li, D. J. Dingle, H. M. Evans, M. C. Prestruda, and J. E. Nezamis, *Proc. Soc. Exptl. Biol. Med.*, *70*, 753–756 (1949).

[875] C. M. MacBryde, D. Castrodale, E. B. Helwig, and O. Bierbaum, *J. Am. Med. Assoc.*, *118*, 1278–1281 (1942).

[876] P. György, C. S. Rose, and R. A. Shipley, *Arch. Biochem.*, *12*, 125–133 (1947).

[877] R. A. Shipley, E. B. Chudzik, and P. György, *Arch. Biochem.*, *16*, 301–307 (1948).

[878] P. György, C. S. Rose, and R. A. Shipley, *Arch. Biochem.*, *22*, 108–118 (1949).

[879] R. A. Shipley, E. B. Chudzik, P. György, and C. S. Rose, *Arch. Biochem.*, *25*, 309–315 (1950).

[880] P. Handler, *J. Biol. Chem.*, *173*, 295–303 (1948).

cholesterol, but in only a small increase in liver fat. On diets which would normally produce fatty livers, no deposition of excess liver fat occurred following thyroidectomy.[877] Moreover, Handler and Fallis[881] found that a decrease in thyroid activity prevented or retarded the development of hepatic necrosis and fibrosis associated with choline and cystine deficiencies. Thyroid feeding was shown to hasten the death of choline-deficient or cystine-deficient animals. In the first case, the liver contained little fat, and presented no necrosis. However, in the second instance, the livers exhibited acute necrosis. The existence of an already established fatty liver appeared to protect the rat against thyrotoxicosis.

(*i*) *Fatty Livers Resulting from the Ingestion of Alcohol.* Ashworth[882] found that fatty infiltration of the liver occurred in rats when they were given alcohol daily, with either a high or a low casein diet. The experiments were interpreted as indicative of the fact that alcohol exerts an effect which permits an accumulation of fat within the liver cells. This effect is separate from that of the extrinsic deficiency of lipotropic factors. Phillips *et al.*[883] arrived at a somewhat different conclusion, on the basis of their study of three alcoholics suffering from fatty cirrhotic livers. No improvement in liver function was observed when a purified diet was given, but a marked improvement in hepatic function, with a decrease in hepatic fat, resulted from the administration of an adequate diet for eight to ten days. This ration consisted of 2000 to 2200 Calories made up of 80 to 100 g. protein, 200 to 250 g. carbohydrate, and 110 g. fat daily. The improvement observed was considered to be mainly due to the adequate diet, and not merely to withdrawal of the alcohol, which played only a subsidiary role.[883]

(*j*) *Fatty Livers Resulting from Irradiation.* Chevallier and his associates[884] demonstrated that fatty livers develop in rats receiving various sublethal doses of x-irradiation daily.

(*k*) *Miscellaneous Lipotropic Factors.* In addition to the common lipotropic agents, several less well-known compounds have been shown to exert a similar action. Maw and du Vigneaud[885] demonstrated that dimethylpropiothetin isolated from the red marine alga, *Polysiphonia fastigiata*, has lipotropic properties. Amellin, a substance, reputedly of therapeutic value in diabetes mellitus, obtained from the sweet broomwort (*Scoparia dulcis*

[881] P. Handler and R. H. Fallis, Jr., *J. Nutrition, 35,* 669–687 (1948).
[882] C. T. Ashworth, *Proc. Soc. Exptl. Biol. Med., 66,* 382–385 (1947).
[883] G. B. Phillips, G. J. Gabuzda, Jr., and C. S. Davidson, *J. Clin. Invest., 31,* 351–356 (1952).
[884] A. Chevallier, C. Burg, and H. Spehler, *Compt. rend. soc. biol., 147,* 497–500 (1953).
[885] G. A. Maw and V. du Vigneaud, *J. Biol. Chem., 176,* 1037–1045 (1948).

TABLE 25

CLASSIFICATION OF FATTY LIVERS, AND LIPOTROPIC AGENTS THAT PREVENT THEM[a]

Cause of fatty liver	Nature of pathologic effect	Lipotropic agent[b]
Deficiencies:		
1. Essential fatty acids	Interfere with phospholipid synthesis	E
2. Choline	Interferes with phospholipid synthesis	C; B; M
3. Pyridoxine	Increases demand for inositol and choline	I, ++; C+; M+
4. Pantothenic acid	Unknown	C(?); M(?)
5. Thyroid hormone	Depresses metabolism	—
Excesses:		
6. Cystine	Diverts methionine by stimulating growth	C; M
7. Cholesterol	Competes for essential fatty acids required for phospholipid synthesis	C; M
8. Guanidoacetic acid	Diverts methyl groups from choline synthesis for creatine synthesis	C; M
9. Thiamine	Diverts methionine by stimulating appetite and growth	C; M
10. Biotin	Increases demand for inositol	I; L
11. Riboflavin	Diverts methionine by stimulating appetite and growth	C; M
12. Niacin	Diverts methyl groups from choline synthesis for trigonelline synthesis	C; M
13. Ketogenic hormone	Possibly due to fat-mobilizing factor, adipokinin	—
14. Adrenocortical hormones	Increase mobilization of fat to liver	—
15. Female sex hormones	Uncertain	—
Liver poisons		
(CCl_4, $CHCl_3$, P)	Injure tissues and decrease capacity to metabolize lipids brought to liver	C[c]

[a] Adapted from E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951.

[b] The following is the explanation of the letters: B, betaine; C, choline; E, essential fatty acids; I, inositol; L, lipocaic; M, methionine.

[c] Does not prevent but aids in recovery.

Linn.),[886,887] has been reported by Nath *et al.*[887–889] to counteract fatty livers. The lipotropic action is presumably due to increased desaturation of the liver fats,[887] and may be attributable to the thiomethyl pentose in its molecule.

Manganese has likewise been considered to exert a lipotropic action. Thus, Amdur *et al.*[890] noted that, at a given level of choline, more fat was present in the liver of manganese-deficient rats than in the livers of animals receiving adequate amounts of this element. Since the lipotropic action of manganese was greater when the choline content of the diet was low, an interaction between manganese and choline would seem to be indicated.

Sellers and You[891] were able to prevent an excessive fat deposition in the livers of rats receiving a hypolipotropic diet, by maintaining the animals in a cold room. It is believed that the increased metabolic rate is responsible for the prevention of fat deposition in this case. However, this so-called "pseudolipotropic" action of cold is a matter of degree, inasmuch as fatty livers do develop in animals kept in the cold when the low-choline diet contains 50% of fat.[892] On the other hand, the fat deposition in the liver at low temperatures, on this diet, was found to be less pronounced than in the case of animals kept at room temperature. This pseudolipotropic effect can be demonstrated equally well in acclimatized and in normal animals; apparently it is not mediated by either the thyroid or the adrenal glands.

The extent of hepatic fat deposition is influenced by the kidney, as well. Thus, Ludewig and Chanutin,[893] in confirmation of earlier tests,[894] found that the amount of total lipids in the livers of partially nephrectomized rats was less than in pair-fed intact animals, irrespective of whether or not choline was included in the diet. Hall and Drill[895] reported that liver extract prevented fatty livers and the subsequent diffuse, progressive hepatic fibrosis which resulted in the case of rats fed a diet containing 16% casein and 51% fat for twenty-nine weeks. The lipotropic effect of the liver ex-

[886] M. C. Nath, *Science & Culture*, *8*, 427 (1943).
[887] M. C. Nath, *Ann. Biochem. Exptl. Med.* (*India*), *3*, 55–62 (1943).
[888] M. C. Nath and H. D. Brahmachari, *Ann. Biochem. Exptl. Med.* (*India*), *9*, 13–16 (1949).
[889] M. C. Nath, J. S. Gadgil, and S. Pontremoli, *Nature*, *169*, 711 (1952).
[890] M. O. Amdur, L. C. Norris, and G. F. Heuser, *J. Biol. Chem.*, *164*, 783–784 (1946).
[891] E. A. Sellers and R. Wen You, *Science*, *110*, 713 (1949).
[892] E. A. Sellers and R. Wen You, *Biochem. J.*, *51*, 573–576 (1952).
[893] S. Ludewig and A. Chanutin, *J. Biol. Chem.*, *167*, 35–48 (1947).
[894] J. C. Hortenstine, A. Chanutin, and S. Ludewig, *J. Biol. Chem.*, *125*, 455–459 (1938).
[895] C. A. Hall and V. A. Drill, *Proc. Soc. Exptl. Biol. Med.*, *69*, 3–6 (1948).

tract was not due to a stimulation of appetite, nor to the small choline content of the liver extract.

(*l*) *Summary of Factors Related to Fatty Livers.* The factors which are responsible for the production of fatty livers are numerous, but they fall into a limited number of categories, *viz:* (A) Conditions in which fat becomes the chief source of calories, as in starvation or carbohydrate deprivation, due either to lack of carbohydrate in the diet or to failure to utilize it, as is the case in diabetes (physiologic fatty liver); (B) conditions involving the failure of fat transport and utilization due to dietary deficiencies which prevent lecithin synthesis (*1,2*); (C) conditions involving the prevention of fat transport and utilization by removal of components essential for lecithin synthesis with competing reactions (*6,7,8,9,11,12*); (D) conditions involving an increased requirement for lipotropic agents (*3,10*); and (E) conditions causing an increased mobilization of fat (*13,14*). Table 25 summarizes the causes for the several types of fatty livers and the effect of lipotropic agents in combatting them (page 688).

d. The Effect of Fatty Livers on the Functioning of the Liver. One might expect that a difference in metabolism would obtain between the dietary type of fatty livers and those resulting from injury to the hepatic tissue. It appears highly probable that abnormalities in metabolism may occur when the fatty liver has resulted from liver poisons involving an injury to the cell structure. However, it is less certain that a metabolic upset would result in the animal having the dietary type of fatty liver; in this latter condition, the cells remain intact, although they are engorged with fat. However, it will be observed that a derangement occurs in a number of hepatic functions, even when this latter type of fatty infiltration of the liver is present.

(*a*) *Metabolic Changes Occurring in Animals Having Fatty Livers Produced by Hepatic Poisons.* Although profound changes occur almost immediately in animals subjected to hepatectomy, and death ensues within a limited time interval, the presence of even small amounts of functioning liver tissue will prevent such difficulties. Thus, although urea synthesis was completely inhibited and blood amino nitrogen consequently increased proportionally in dogs[896] and monkeys[897] following the excision of the liver, McMaster and Drury[898] reported that these reactions were not appreciably impaired until 90% of the liver had been removed. Moreover,

[896] J. L. Bollman, F. C. Mann, and T. B. Magath, *Am. J. Physiol.*, *69*, 371–392 (1924).
[897] S. Maddock and A. Svedberg, *Am. J. Physiol.*, *121*, 203–208 (1938).
[898] P. D. McMaster and D. R. Drury, *J. Exptl. Med.*, *49*, 745–758 (1929).

Whipple and Van Slyke[899] found that the blood amino acid of dogs with Eck fistulas was not appreciably affected, even when the protein metabolism was augmented by the administration of meat or by toxic autolysis of the tissues.

In view of the fact that only limited amounts of liver are required for normal function of the organ, it is not surprising that little evidence of abnormality in function as a result of hepatic poisons is available. Thus, Levene and Van Slyke[900] were unable to detect any increased proportion of amino nitrogen in the urine of dogs which had undergone extreme liver degeneration as a result of phosphorus or chloroform poisoning, although Marshall and Rowntree[901] did note that an increase in total non-protein nitrogen, urea, and amino acids in the blood serum occurred in phosphorus poisoning, and to a lesser extent in chloroform poisoning. Moreover, in rabbits whose livers were injured by hydrazine, a decreased ability to transform glycine to glucose was reported.[902]

(*b*) *Metabolic Changes Resulting from Dietary Fatty Livers.* a′. Hypolipemia: The disturbances resulting from fatty livers are characterized, not only by a decrease in the quantity of lipids in the blood, but also by a qualitative change in the proportion of the several blood lipids. Usually there is a considerable decrease in the amount of phospholipids in the blood, which occurs concomitantly with a rise in the cholesterol and neutral fat fractions. The specific changes in these fractions vary with the cause of the fatty infiltration of the liver. A hypolipemia (lipopenia) usually occurs in types in which deficiencies obtain as, for example, in choline, vitamin, or essential fatty acid deficiency. On the other hand, in conditions in which the nutrition is preserved, *i.e.*, those in which fatty livers are induced by excessive amounts of cystine or vitamins, a hyperlipemia rather than a hypolipemia may be the normal response.[549]

b′. The Production and Composition of Bile: Colwell[903] reported that the bile production of rats with an abnormal accumulation of liver fat due to choline deficiency was less than was the case in rats receiving choline. Although Colwell concludes that choline is necessary for normal chologenesis, it appears probable that its effect is only indirect. By preventing the engorgement of the liver cells with fat, it permits them to function in a

[899] G. H. Whipple and D. D. Van Slyke, unpublished results; cited by J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, 2nd ed., vol. I, Williams & Wilkins, Baltimore, 1946, p. 806.

[900] P. A. Levene and D. D. Van Slyke, *J. Biol. Chem.*, *12*, 301–312 (1912).

[901] E. K. Marshall, Jr., and L. G. Rowntree, *J. Exptl. Med.*, *22*, 333–346 (1915).

[902] H. B. Lewis and S. Izume, *J. Biol. Chem.*, *71*, 33–49 (1926).

[903] A. R. Colwell, Jr., *Am. J. Physiol.*, *164*, 274–283 (1951).

TABLE 26

COMPOSITION OF THE LIVERS OF RATS RECEIVING A LOW-PROTEIN, HIGH-FAT, CHOLINE-FREE DIET FOR DIFFERENT INTERVALS, TOGETHER WITH THE AVERAGE ACETONE BODY EXCRETION OVER A SUBSEQUENT FIVE-DAY FAST[a]

Days on diet	Male rats			Female rats		
	Liver lipid, %		Urine acetone bodies,[c] mg./100 sq. cm.	Liver lipid, %		Urine acetone bodies,[c] mg./100 sq. cm.
	At start[b]	After 5-day fast		At start[b]	After 5-day fast	
0	2.79 ± 0.18	4.28 ± 0.10	0.3 ± 0.1 (39)	3.15 ± 0.10	4.74 ± 0.27	2.6 ± 0.4 (40)
1	5.48 ± 0.15	8.73 ± 0.21	6.0 ± 0.7 (40)	7.54 ± 0.21	5.72 ± 0.37	10.3 ± 0.9 (40)
3	4.81 ± 0.17	9.56 ± 0.48	13.3 ± 1.0 (39)	8.22 ± 0.48	10.33 ± 0.48	23.0 ± 1.5 (40)
6	11.72 ± 0.73	15.15 ± 0.78	15.1 ± 1.0 (40)	12.98 ± 0.78	10.33 ± 1.11	24.6 ± 1.0 (40)
9	16.73 ± 0.82	19.56 ± 1.06	23.4 ± 1.2 (40)	17.57 ± 1.01	19.88 ± 1.26	32.2 ± 1.2 (32)
12	19.81 ± 1.20	20.34 ± 1.40	22.4 ± 1.7 (28)	22.42 ± 1.40	22.91 ± 1.46	28.3 ± 1.3 (39)
16	26.25 ± 0.93	28.85 ± 0.95	22.5 ± 1.4 (40)	33.93 ± 0.95	28.17 ± 0.93	30.4 ± 1.0 (37)

[a] H. J. Deuel, Jr., and L. F. Hallman, *J. Biol. Chem.*, *140*, 545–554 (1941).
[b] Values for control group. Ten rats in each group.
[c] Averages from second to fifth days. Ten rats in each group. Figures in parentheses are total number of individual determinations.

normal manner. Bile produced by rats having fatty livers was likewise shown to be low in total lipid, as well as in cholesterol.

c′. Ketonemia and Ketonuria: Although fat deposits occurring in fatty livers are generally regarded as metabolically inactive, there is some evidence that they may stimulate fat oxidation. This deduction is based upon the fact that the level of starvation ketonuria is markedly increased in animals having fatty livers.[277,904] Since ketogenesis has been shown to be almost exclusively a function of the liver, the appearance of increased ketone bodies in the blood and urine of animals having fatty livers would be circumstantial evidence of an increased fat breakdown. On the other hand, one might argue that the rate of ketone body production was unchanged in the fasting animal with a fatty liver, as compared with a normal fasting animal. The reason why a ketonuria of considerable magnitude would develop in the first case, while only a slight and irregular ketonuria would occur under the second condition, would then be referable to the inability of the rats with fatty livers to oxidize the ketone bodies as effectively as do normal fasting animals. However, the liver is not the principal organ for the utilization of ketone bodies; it is the extrahepatic tissues which serve in this capacity.[905,906] Moreover the ketone bodies disappear immediately from the urine when glucose is administered.[907] For a further discussion of this phase of the subject, the reader is referred to the section on ketosis (see Chapter III, Volume III).

The comparative endogenous ketonuria produced in male and female rats during fasting, together with the liver fat values after the administration of the fatty-liver-producing diets for different time intervals, are summarized in Table 26.

Although no exact relationship exists between the level of liver lipid and ketonuria, a greater decrease in the liver lipids of the females occurs during fasting, coincident with the higher ketonuria in this sex. MacKay *et al.*[908] are of the opinion that neither the level of liver fat *per se* nor any of the agents such as choline, methionine, or cystine which are known to influence the amount of fat in the liver have a significant effect on the level of fasting ketosis. However, Roberts and Samuels[909] have arrived at opposite con-

[904] D. L. MacLean, J. H. Ridout, and C. H. Best, *Brit. J. Exptl. Pathol.*, *18*, 345–354 (1937).

[905] I. L. Chaikoff and S. Soskin, *Am. J. Physiol.*, *87*, 58–72 (1928–1929).

[906] D. R. Drury, R. Barnes, P. O. Greeley, and A. Wick, *Am. J. Physiol.*, *129*, 348P–349P (1940).

[907] H. J. Deuel, Jr., L. F. Hallman, and S. Murray, *J. Biol. Chem.*, *124*, 385–393 (1938).

[908] E. M. MacKay, H. O. Carne, A. N. Wick, and F. E. Visscher, *J. Biol. Chem.*, *141*, 889–896 (1941).

[909] S. Roberts and L. T. Samuels, *J. Biol. Chem.*, *151*, 267–271 (1943).

TABLE 27

THE COMPOSITION OF THE LIVERS OF RATS THAT RECEIVED A LOW-PROTEIN, HIGH-FAT DIET FOR 12 DAYS, AND THAT SUBSEQUENTLY RECEIVED A STOCK DIET WITH OR WITHOUT BETAINE, TOGETHER WITH THE AVERAGE ACETONE BODY AND TOTAL NITROGEN EXCRETION OVER A FIVE-DAY FAST PERIOD[a]

	Rats receiving no supplement				Rats receiving betaine hydrochloride			
	Liver lipid				Liver lipid, %			
Days on stock diet	At start[b]	After 5-day fast	Urine acetone bodies,[c] mg./100 sq. cm.	Urine N, mg./100 sq. cm.	At start[b]	After 5-day fast	Urine acetone bodies,[c] mg./100 sq. cm.	Urine N, mg./100 sq. cm.
Control[d]	—	—	0.3 ± 0.1 (39)	41.1 ± 0.7	—	—	—	—
0	25.55	20.34 (7)	22.4 ± 1.7 (28)	29.0 ± 0.5	—	—	—	—
7	7.22	12.83 (4)	13.7 ± 2.7 (15)	27.6 ± 0.9	6.40	6.18 (5)	9.0 ± 1.5 (20)	31.4 ± 0.7
14	6.02	9.42 (5)	0.8 ± 0.3 (19)	34.3 ± 0.6	4.04	7.47 (5)	0.8 ± 0.2 (20)	34.1 ± 0.7
21	4.73	7.90 (5)	3.1 ± 0.7 (20)	37.3 ± 1.0	2.90	6.26 (5)	1.8 ± 0.5 (20)	35.8 ± 0.7

[a] H. J. Deuel, Jr. and L. F. Hallman, *J. Biol. Chem.*, *140*, 545–554 (1941).
[b] Values for control group. Values in parentheses are for the number of rats.
[c] Averages from second to fifth fast days. Figures in parentheses are the total number of individual determinations.
[d] Rats not fed fatty-liver-producing diet.

clusions, on the basis of experiments in which fat or carbohydrate was forcibly fed over periods of three to six weeks. Ketonuria occurred only in the fat-fed rats, in spite of the fact that the protein intake and nitrogen excretion during fasting had been the same in the two groups. These workers concluded[909] that the fasting ketosis is a reflection of accelerated fat metabolism initiated during feeding, and continued after withdrawal of the food. This phenomenon is referred to as a *preferential utilization.*

The administration of betaine to rats having fatty livers but receiving an ordinary diet resulted in a more rapid reduction in liver lipids than occurred when no supplement was given. Moreover, the rats did not respond to a subsequent fast period with such a marked rise in liver lipid as did those animals which had not received the lipotropic agent. However, the effect of betaine on ketonuria was not immediate. After the feeding of betaine for seven days a marked endogenous ketonuria still obtained over a subsequent five-day fasting period. The average level of acetone bodies was somewhat reduced below the control value.

Table 27 summarizes the data on the liver fats and ketonuria of rats, fed a stock diet, which developed fatty livers.

d′. Effect on Glucose Tolerance Tests: One would expect that, when the liver cells are congested with lipid, the ability to store glycogen would be reduced and a decrease in glucose tolerance might be observed. Bodansky[910] reported that a lower tolerance for glucose existed in dogs whose livers had previously been damaged with chloroform or phosphorus than in normal dogs.

In the case of animals having the dietary type of fatty liver, MacLean *et al.*[904] found that, while the liver was able to store appreciable quantities of glycogen in the presence of large amounts of fat, the glycogen storage resulting from given amounts of carbohydrate was less when the liver was extremely fat. Treadwell and associates[911] noted a marked decrease in glucose tolerance by rats on a diet high in fat and low in lipotropic factors. Their results were confirmed by Deuel and Davis.[912] The latter workers found that the fasting blood sugar levels were significantly higher in rats of both sexes which had fatty livers than in animals having a normal content of liver lipid. The rise in blood sugar level after glucose feeding was significantly greater in female rats with fatty livers than in those having a normal level of liver lipids. However, these workers were unable to demonstrate abnormal glucose tolerance curves in male rats with fatty livers.

[910] M. Bodansky, *J. Biol. Chem., 58,* 515–522 (1923-1924).

[911] C. R. Treadwell, W. C. King, K. C. Bebb, and H. C. Tidwell, *J. Biol. Chem., 143,* 203–209 (1943).

[912] H. J. Deuel, Jr., and A. Davis, *J. Biol. Chem., 146,* 649–653 (1942).

e′. Effect on the Transformation of Sorbitol to Glucose: Johnston and Deuel[913] reported that the ketolytic effect of the hexitol, sorbitol, is only about 25% of that of glucose when tested with endogenous ketonuria, while a figure of 50% of the potency of glucose was noted when the tests were based upon exogenous ketonuria. Since glycogen was found to be higher in the liver and lower in the muscle following the administration of sorbitol than after that of glucose, it was postulated that the liver is probably the site for the conversion of sorbitol to glucose. These authors state: "The lower effect of sorbitol in endogenous compared with exogenous ketonuria is believed to be caused by the failure of the fatty liver to convert sorbitol to glycogen at its normal rate, owing to a derangement in its function in animals with fatty livers."

It is apparent from a consideration of the reactions of fatty livers when tested by some common physiologic procedures that the dietary fatty livers, as well as those arising from hepatic poisons, are abnormal. The extent to which abnormalities in metabolism will occur would be expected to increase as the proportion of liver fat becomes greater. However, the development of one function, namely that of producing a ketonuria, does not change with a further increase in liver lipids after a moderate fatty infiltration has occurred.

f′. Effect on Uric Acid Oxidation: In contradistinction to the higher primates and man, the dog possesses the enzyme, uricase, present in the liver. Consequently, instead of uric acid being the ultimate product of purine metabolism, as it is in man, this substance is oxidized to allantoin in the dog; this is, in turn, excreted in the urine.

Some years ago it was demonstrated by Bollman, Mann, and Magath,[914,915] at the Mayo Clinic, that uric acid appears in the blood and urine of the dog following hepatectomy. A similar phenomenon has recently been observed by Groen,[916] as a result of liver damage following fatty infiltration. Nine dogs were fed exclusively bacon for a period of months. This diet provoked the production of fatty livers. Of the nine dogs, seven died within a relatively short period. Postmortem examination revealed the presence of uric acid stones in the kidneys, bladders, and ureters of these dogs. The author suggests that the continued presence of fat in the liver had produced a condition akin to "chemical hepatectomy."

g′. Choline Synthesis and Destruction in the Liver: Barrenscheen and

[913] C. Johnston and H. J. Deuel, Jr., *J. Biol. Chem., 149,* 117–124 (1943).
[914] J. L. Bollman, F. C. Mann, and T. B. Magath, *Am. J. Physiol., 72,* 629–646 (1925).
[915] J. L. Bollman and F. C. Mann, *Am. J. Physiol., 104,* 242–246 (1933).
[916] J. Groen, *Science, 107,* 425–426 (1948).

Papadopoulou[664] have noted that the synthesis of choline is significantly reduced in fatty livers, even when this organ is adequately supplied with glycogen. When the fatty degeneration is produced by phosphorus poisoning, choline synthesis from methionine and colamine is completely abolished.

According to Handler and Bernheim,[782] the destruction of choline is decreased in animals with fatty livers. These workers demonstrated that the activity of choline oxidase was depressed in the fatty liver. Since this is the principal enzyme concerned with the degradation of choline, it is evident that the capacity for destroying this substance is reduced in animals with fatty livers.

h′. Liver Function Tests: Li and Freeman[565] reported that the hepatic dye clearance test is reduced during the first weeks when dogs are given a protein-deficient diet with cholesterol. This retardation can be largely attributed to the accumulation of lipids in the liver.

(3) Abnormal Deposition of Essential Lipids (Lipidoses)

Thannhauser[917] coined the term, *lipidoses* (sometimes spelled *lipoidoses*) to designate a group of diseases in which lipid deposition occurs in the reticulum and in the histiocytes. The term, *xanthomatoses*,[918] was originally applied to many of the diseases which are now classed as lipidoses. This second term was first employed as descriptive of the yellow coloration of the affected organs. However, the terminology of Thannhauser[917] seems more appropriate, and it has been generally accepted by most workers in the field. The term, xanthomatoses, is accordingly reserved for those types of lipidoses in which cholesterol is the predominant lipid. The several types of lipidoses differ from each other in the nature of the lipid component and in its site of storage. For a more complete discussion of the subject of lipidoses, the reader is referred to the monograph of Thannhauser,[917] and to the review articles of Pick,[919] Sperry,[920] Sobotka,[921] and Thannhauser and Schmidt.[922]

a. Xanthomatoses. Although cholesterol is the principal constituent in the deposits which occur in xanthomatosis, considerable amounts of phospholipid likewise occur, while only an extremely limited proportion

[917] S. J. Thannhauser, *Lipidoses: Diseases of the Cellular Lipid Metabolism*, 2nd ed., Oxford Univ. Press, London-New York, 1950.

[918] S. J. Thannhauser and H. Magendantz, *Ann. Internal Med., 11*, 1662–1746 (1938).

[919] L. Pick, *Am. J. Med. Sci., 185*, 453–469, 601–616 (1933).

[920] W. M. Sperry, *J. Mount Sinai Hosp., 9*, 799–817 (1942).

[921] H. Sobotka, *J. Mount Sinai Hosp., 9*, 795–798 (1942).

[922] S. J. Thannhauser and G. Schmidt, *Physiol. Revs., 26*, 275–317 (1946).

of neutral fat is present. The absence of appreciable amounts of neutral fat distinguishes the xanthomas from depot fat.[923] The cholesterol usually occurs largely in the form of the esters.[918,920,923] An analysis of specimens of xanthomatous tissue obtained from skin by Ciaccio[924] was as follows: total lipids, 11.1%; phospholipids, 0.63%; free cholesterol, 0.98%; total cholesterol, 2.0%; and neutral fats, 7.74%.

(*a*) *Primary Xanthomatosis.* According to Thannhauser and Schmidt,[922] there are two types of primary xanthomatosis. In the first class, referred to as primary essential xanthomatosis of the hypercholesteremic type, the following sub-groups are listed: (*1*) xanthelasma of eyelids and *xanthoma tuberosum et planum*; (*2*) tendon xanthoma; (*3*) *xanthoma tuberosum et planum* and tendon xanthoma; (*4*) xanthoma of the blood-vessels and endocardium; (*5*) *forme fruste* of essential xanthomatosis; and (*6*) xanthomatous biliary cirrhosis. In this first category, an accumulation of cholesterol obtains in a transparent clear serum, but the neutral fat is normal.[925] Lipid phosphorus is high. Serum cholesterol values as high as 600 milligram per cent have been reported, but the proportion of ester to alcohol is normal.[917,918,925,926] In this type of xanthomatosis, the deposition of lipid appears to have a predilection for tendons, skin, and subcutaneous tissue. It differs from other types of xanthomatosis in that it does not respond to diet.[917,918,927] It is believed that the increased cholesterol reflects an increased synthesis of this sterol, together with an imbalance in the excretory mechanism for the substance.

The second division of primary xanthomatoses is designated by Thannhauser and Schmidt[922] as primary essential xanthomatosis of the normocholesteremic type (eosinophilic granuloma). In turn, it is divided into the following categories: (*1*) *xanthoma disseminatum* of the skin; (*2*) *xanthoma disseminatum* and diabetes insipidus; (*3*) osseous xanthoma; (*4*) Schüller-Christian's syndrome; and (*5*) generalized xanthoma of the normocholesteremic type. As one would surmise from the title of this subdivision, normal cholesterol as well as normal lecithin and normal neutral fat occur in the serum in xanthomatosis of the normocholesteremic type.

(*b*) *Secondary Xanthomatosis.* This type of xanthomatosis is characterized by a hyperlipemia, which may contribute to its formation. The level

[923] A. Chanutin and S. Ludewig, *J. Lab. Clin. Med.*, *22*, 903–911 (1937).

[924] I. Ciaccio, *Bol. soc. ital. biol. sper.*, *19*, 225–227 (1944).

[925] E. B. Man, unpublished observations; cited by J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, vol. I, 2nd ed., Williams & Wilkins, Baltimore, 1946, p. 541.

[926] G. Klatskin, *New Intern. Clinics*, [*n.s. 4*], *3*, 13–39 (1941).

[927] W. M. Sperry and B. Schick, *Am. J. Diseases Children*, *51*, 1372–1384 (1936).

of blood cholesterol is doubled or trebled, while there is a ten- to sixty-fold increase in the neutral fat concentration in the serum.[917]

Peters and Van Slyke[549] are of the opinion that the designation, "secondary xanthomatosis," should be confined to cases in which the xanthomatosis is secondary to another disease characterized by a hyperlipemia, such as diabetes. The following sub-groups are listed in this category, by Thannhauser and Schmidt[922]: (*1*) idiopathic or familial hyperlipemia with hepatosplenomegaly and secondary xanthoma; (*2*) secondary xanthomatosis due to diabetic hyperlipemia; (*3*) hyperlipemia with secondary xanthomatosis occurring in chronic pancreatitis; (*4*) hyperlipemia in glycogen storage disease; and (*5*) hyperlipemia in lipoid nephrosis.

(*c*) *Localized Xanthoma Formation in Inflamed Tissue and in True Tumors.* In addition to primary and secondary xanthomatosis, which are generalized types of the disease, xanthoma formation may originate in localized areas. The first division of this group are the xanthoma cells in inflamed tissue. This is subdivided as follows: (*1*) inflamed tissue showing xanthoma cells; (*2*) inflammatory xanthoma of the breast; (*3*) xanthoma cells in *osteitis fibrosa cystica disseminata* (fibrous dysplasia); (*4*) xanthomatous transformation of the mesentery, intestinal lipodystrophy of Whipple; and (*5*) xantholipoma.

The second division is made up of xanthoma cells in tumors. This category includes the following: (*1*) nervoxanthoendotheliomas; (*2*) xanthomatous polycystic lymphangiomas; (*3*) single xanthomatous giant-cell tumors; and (*4*) epithelial tumors with xanthoma cells.

A third division, comprising xanthoma cells in other conditions, includes only two sub-groups, *viz.*, (*1*) lipoid proteinosis and (*2*) *necrobiosis lipoidica diabeticorum.*

b. Niemann-Pick's Disease (Reticular and Histiocytic Sphingomyelinosis). Niemann[928] first discovered this disease in 1914. Thirteen years later, Pick[929] differentiated the syndrome from that of Gaucher's disease. Comprehensive studies of the disease have been reported by Baumann, Klenk, and Scheidegger,[930] and by Thannhauser.[917]

The nature of the chemical disturbance in Niemann-Pick's syndrome was clarified by Klenk.[931,932] He discovered that the lipid present in the large pale cells was the phospholipid, sphingomyelin. These sphingomyelin-

[928] A. Niemann, *Jahrb. Kinderheilk.*, *79*, 1–10 (1914).
[929] L. Pick, *Med. Klin.* (*Munich*), *23*, 1483–1488 (1927).
[930] T. Baumann, E. Klenk, and S. Scheidegger, *Ergeb. allgem. Pathol. u. pathol. Anat.*, *30*, 183–323 (1936).
[931] E. Klenk, *Z. physiol. Chem.*, *235*, 24–36 (1935).
[932] E. Klenk, *Z. physiol. Chem.*, *229*, 151–156 (1934).

filled cells were not limited to the reticuloendothelial system, but occurred in all organs. Menten and Welton[933] reported the following composition of liver and spleen lipids (based on dry weight), respectively, in the tissues of an infant nine months of age who had died of Niemann-Pick's disease: lecithin, 13.0 and 9.7%; cephalin, 4.1 and 3.4%; crude sphingomyelin, 28.0 and 32.1%. Although the sphingomyelin content of all organs was greatly increased, the amount of this phospholipid in the brain remained unchanged.[932,934,935] The fatty acids present in the sphingomyelin obtained from the different organs in Niemann-Pick's disease were those normally present.[932,936] However, in contradistinction to these findings, Klenk[931] reported only stearic acid in the sphingomyelin of diseased brains, while stearic, palmitic, and lignoceric acids occur in the brain sphingomyelin of normal individuals. However, Tropp and Eckardt[937,938] reported that, although stearic acid was found to be the main fatty acid component of the sphingomyelin obtained from the brain, liver, and spleen of patients who had died as a result of Niemann-Pick's disease, some lignoceric and palmitic acids were also found. It is quite possible that, in these latter cases, the disease was not as severe, or had not continued for as long a period, as in the case reported by Klenk. One might well assume that only a partial replacement of lignoceric acid and palmitic acid had been completed when death occurred. However, Thannhauser and Schmidt[922] do not consider the experiments indicating the unusual fatty acid composition of brain sphingomyelin in Niemann-Pick's syndrome to be conclusive, since the presence of hydrolecithin was not excluded. Sperry[920] reported that the level of brain cerebrosides was depressed in this disease. Moreover, Klenk[931,936] demonstrated the presence of variable amounts of Substance X, which is a galactoside.

The sphingomyelin content of the serum of patients suffering from Niemann-Pick's disease has been reported as normal by Thannhauser,[917] by Sperry,[920] and by Chargaff.[939] The latter worker found that the sphingomyelin content in the serum was 120 milligram per cent, which is within the normal range reported by Thannhauser and Setz[940] as 100 to 180 milligram per cent. However, this finding does not support the hypothesis of

[933] M. L. Menten and J. P. Welton, *Am. J. Diseases Children, 72*, 720–727 (1946).
[934] H. Sobotka, E. Z. Epstein, and L. Lichtenstein, *Arch. Pathol., 10*, 677–686 (1930).
[935] E. Epstein and K. Lorenz, *Z. physiol. Chem., 211*, 217–230 (1932).
[936] E. Klenk, *Z. physiol. Chem., 262*, 128–143 (1939–1940).
[937] C. Tropp and B. Eckardt, *Z. physiol. Chem., 243*, 38–42 (1936).
[938] C. Tropp and B. Eckardt, *Z. physiol. Chem., 245*, 163–167 (1936–1937).
[939] E. Chargaff, *J. Biol. Chem., 130*, 503–511 (1939).
[940] S. J. Thannhauser and P. Setz, *J. Biol. Chem., 116*, 533–541 (1936).

Pick[919,929] that the pathogenesis of this disease involves a constant removal of sphingomyelin from the circulating blood by the several tissues in which storage of this phospholipid takes place. On the other hand, Thannhauser and Schmidt[922] suggest that the pathogenesis of Niemann-Pick's disease corresponds to that of essential xanthomatosis of the normocholesteremic type. This increased tissue sphingomyelin is believed to result from a decreased rate of breakdown, due to a disturbance in the intracellular enzymes responsible for the disintegration of this phospholipid, or to an increased rate of synthesis.

However, neutral fat is greatly increased in the blood.[930] Pick[919] claims that a hypercholesterolemia, as well as a general hyperlipemia, usually occurs. Other workers have not confirmed the fact that hypercholesterolemia is present.[917,920]

Niemann-Pick's disease is also familial, and has usually been observed in infants. At the onset of the disease, there is loss of appetite, and a corresponding decrease in weight results. Death usually occurs between the first and second year.

c. Tay-Sachs' Disease (Amaurotic Idiocy, Juvenile Type). This disease corresponds in many respects to Niemann-Pick's disease, but the chemical changes in the brain lipids differ markedly in the two conditions. Sachs,[941] in 1896, was the first to report a "family form of idiocy, generally fatal, associated with early blindness"; the ophthalmological features of the disease were already known, since they had been described fifteen years earlier by Tay.[942] Although certain changes in the retina appeared to be similar in Niemann-Pick's disease and in Tay-Sachs' disease,[930] Rintelen[943] found that they differed histologically. Moreover, Thannhauser[917] observed that there was no increase in sphingomyelin in the organs of infants suffering from Tay-Sachs' disease, in contradistinction to the marked rise which is observed in Niemann-Pick's disease.

On examination of the lipids in the brain of infants succumbing to the Tay-Sachs syndrome, Klenk[936,944] and Klenk and Langerbeins[945] reported the presence of a new group of lipids, which was first called "Substance X." These substances were later named "gangliosides." They were found to contain neuraminic acid.[944,945]

Although Klenk and Schumann[946] later demonstrated that the ganglio-

[941] B. Sachs, *J. Nervous Mental Disease, 21* [*n.s.*], 475–479 (1896).
[942] W. Tay, *Trans. Ophthalmol. Soc. United Kingdom, 1,* 55–57 (1881).
[943] F. Rintelen, *Arch. Augenheilk., 109,* 332–345 (1935–1936).
[944] E. Klenk, *Z. physiol. Chem., 268,* 50–58 (1941).
[945] E. Klenk and H. Langerbeins, *Z. physiol. Chem., 270,* 185–193 (1941).
[946] E. Klenk and E. Schumann, *Z. physiol. Chem., 267,* 128–144 (1940).

sides were present in normal brain as well, their concentration in this tissue was increased in Tay-Sachs' disease, in which they partially replaced the cerebrosides. Thus, the gangliosides made up 4 to 8% of the solids in the brain of the Tay-Sachs infant, while they are normally present to the extent of only 0.3%. In a sub-type of this disease, known as the juvenile type of amaurotic idiocy, the ganglioside content was increased to 1.5%; slightly higher than normal amounts of ganglioside were also noted in Niemann-Pick's disease.

d. Gaucher's Disease (Reticular and Histiocytic Cerebrosidosis). The first recognition of Gaucher's disease is to be credited to E. Gaucher who, in 1882, described a disease characterized by enlargement of the spleen.[947] However, it was not until twenty-three years later that Brill, Mandelbaum, and Libmann[948] and Schlaugenhaufer[949] called attention to the fact that the liver and osseous system were affected, in addition to the spleen. It is now known that the lymph-nodes are also involved. Before a clear picture of the nature of this disorder was available, it was considered to be neoplastic in nature. After Schlaugenhaufer[949] discovered that Gaucher's disease was centered in the lymphohemopoietic system, it was proved by histologic examination that it was not neoplastic in origin, and did not arise as a result of simple hyperplasia of the tissues. The enlargement of the organs was a result of deposition of a foreign substance in the area involved.

Lieb,[950] Lieb and Mladenovíc,[951] and Epstein and Lorenz[952] identified the foreign substance as a cerebroside, which was believed to be identical with cerasine. This finding was confirmed by Cushing and Stout,[953] by Bloom and Kern,[954] and by Pick.[919] These workers were the first to associate Gaucher's disease with xanthomatosis and Niemann-Pick's disease; each of these three abnormalities was characterized by the deposition of a particular and specific type of lipid. Although the storage of lipids in the usual form of Gaucher's disease is confined to the reticulum, Oberling and Woringer[955]

[947] E. Gaucher, *De l'epithelioma primitif de la rate, Thèse de Paris,* 1882; cited by S. J. Thannhauser and G. Schmidt, *Physiol. Revs., 26,* 275–317 (1946), p. 305.
[948] N. E. Brill, F. S. Mandelbaum, and E. Libmann, *Am. J. Med. Sci., 129,* 491–504 (1905).
[949] F. Schlaugenhaufer, *Arch. pathol. Anat. u. Physiol. (Virchow's), 187,* 125–163 (1907).
[950] H. Lieb, *Z. physiol. Chem., 140,* 305–313 (1924); *170,* 60–67 (1927).
[951] H. Lieb and M. Mladenovíc, *Z. physiol. Chem., 181,* 208–220 (1929).
[952] E. Epstein and K. Lorenz, *Z. physiol. Chem., 192,* 145–170 (1930).
[953] E. H. Cushing and A. P. Stout, *Arch. Surg., 12,* 539–560 (1926).
[954] W. Bloom and R. Kern, *Arch. Internal Med., 39,* 456–461 (1927).
[955] C. Oberling and P. Woringer, *Rev. franç. pediat., 3,* 475–532 (1927).

described a variation in an infant; in this case the Gaucher cells were also found in the pulmonary and cerebral tissue, lymphatic glands, liver, bone-marrow, and thymus.

Pick[919] postulated that Gaucher's disease is a storage disease analogous to xanthomatosis. It was believed that an extra amount of cerasine was supplied in the blood stream to the several organs which were able to remove it and accumulate it. However, this hypothesis seems unlikely, since cerasine and other cerebrosides are not normal components of the blood,[956,957] and have not been demonstrated in the serum in Gaucher's disease.[957,958] Although Erickson *et al.*[959] reported that cerebrosides are present in normal serum, their methods were based upon indirect analyses and did not involve isolation of the cerebrosides. Thannhauser and Schmidt[922] are convinced that cerebrosides do not occur in normal blood, or in that of patients suffering from Gaucher's disease. However, when highly purified cerebrosides were injected intraperitoneally into rabbits,[960] morphologic changes resembling those of Gaucher's disease in man were found, although they were less extensive. Moreover, increased levels of cerebrosides were observed in the liver, spleen, and lymph-nodes of the rabbits, even after the oral administration of cerebrosides.

On the other hand, Thannhauser[917] suggested that the cerebrosides originate in the same cells in which they are found. This would imply an overproduction, and a resultant accumulation of this metabolite in the cells of the several organs in which the cerebrosides are concentrated. This condition might arise from a disturbance of the intracellular enzymes concerned with either the anabolism or the catabolism of the cerebrosides. This explanation is analogous to that proposed for the etiology of essential xanthomatosis of the normocholesteremic type, and for that of Niemann-Pick's disease.

Another finding which has been interpreted as evidence against the transport theory accounting for the presence of an excess amount of cerebrosides in the spleen in Gaucher's disease is the discovery that the cerebrosides in the latter organ may differ from those in the brain. Thus, Halliday *et*

[956] S. J. Thannhauser, J. Benotti, and H. Reinstein, *J. Biol. Chem.*, *129*, 709–716 (1939).

[957] J. Brückner, *Z. physiol. Chem.*, *268*, 251–256 (1941).

[958] E. Dworaček and H. Pesta, *Wien. klin. Wochschr.*, *52*, 332–337 (1939).

[959] B. N. Erickson, H. J. Souders, M. L. Shepherd, D. M. Teague, and H. H. Williams, *Proc. Soc. Exptl. Biol. Med.*, *45*, 153–156 (1940).

[960] O. O. Christianson, *Arch. Pathol.*, *32*, 369–377 (1941).

al.,[961,962] Klenk *et al.*,[946,963] and others [964–967] demonstrated that the cerebroside isolated from spleen contained glucose instead of galactose. However, galactose may sometimes occur also.[967] In the case of Gaucher's disease reported by Lieb,[950] galactose was found to be the component sugar in the cerebroside. Brown and Morris,[968] in 1890, suggested that cerebrose is

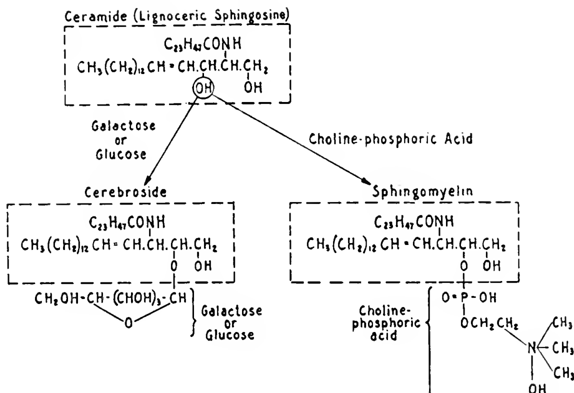


Fig. 5. A possible explanation for the synthesis of cerebrosides or sphingomyelin in the tissues from the intermediate, lignocerylsphingosine.[917,922]

identical with galactose. In view of the fact that both types of cerebroside occur in normal spleen,[969] it is not permissible to consider the glucose-containing cerebroside as abnormal. However, the fact that the newly formed cerebroside in the tissues other than the brain in Gaucher's disease is usually the glucose type, while that in the brain is the galactose variety, speaks against the transportation and deposition theory in this disease.

[961] N. Halliday, H. J. Deuel, Jr., L. J. Tragerman, and W. E. Ward, *J. Biol. Chem.*, *132*, 171–180 (1940).

[962] N. Halliday, *Proc. Soc. Exptl. Biol. Med.*, *75*, 659 (1950).

[963] E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, *272*, 280–282 (1942).

[964] I. S. Danielson, C. H. Hall, and M. R. Everett, *Proc. Soc. Exptl. Biol. Med.*, *49*, 569–571 (1942).

[965] Aghion, *La maladie de Gaucher dans l'enfance, Thèse, Paris*, 1934; cited by E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, *273*, 253–268 (1942).

[966] J. Polonovski, *Compt. rend.*, *215*, 443–445 (1942).

[967] B. Ottenstein, G. Schmidt, and S. J. Thannhauser, *Blood*, *3*, 1250–1258 (1948).

[968] H. T. Brown and G. H. Morris, *J. Chem. Soc.*, *57*, 57–59 (1890).

[969] E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, *273*, 253–268 (1942).

If the cerebrosides in the spleen and other organs do not accumulate from the blood, another source of starting material must necessarily be available. Thannhauser[917] and Thannhauser and Schmidt[922] suggested that lignocerylsphingosine or other ceramides might be the starting materials. A compound of this nature could be converted to the glucose or galactose type of cerebroside by condensation with the appropriate sugar, or to sphingomyelin by condensation with choline phosphate. This suggestion is supported by the fact that lignocerylsphingosine has been found to be present in these organs, together with cerebrosides and sphingomyelin.[970] Moreover, a glucoside-splitting enzyme has been detected in the spleen[971]; if its action is reversible, as is believed to be the case with most enzymes, it would be able to catalyze the synthesis of cerebroside by the combination of lignocerylsphingosine and glucose or galactose. Figure 5 affords a graphic explanation of this hypothesis.

(4) *Atheroma in Arteriosclerosis*

The formation of atheroma in the walls of the blood vessels has long been recognized to be a concomitant of arteriosclerosis. A number of different investigators[972–976] proved that the atheromatous vessels contain excessive amounts of lipids, especially cholesterol; these lipid deposits accumulate in the atheromatous patches.

The question as to the cause of the deposition of cholesterol and of other lipids in the walls of the blood vessels is still unsolved. Apparently, although hypercholesterolemia is a condition which sometimes accompanies hypertension, it is not a constant symptom. When hypercholesterolemia can be demonstrated, the increase in this component is slight and variable. In certain cases of xanthomatosis, much larger deposits of lipids may occur without the occurrence of a hyperlipemia. In the late stages of arteriosclerosis, calcium deposits replace the lipids in the atheroma, without the development of hypercalcemia. Although Bürger,[977] Rosenthal,[974] and Faber[978] have demonstrated increasing cholesterol concentrations in the

[970] E. Fränkel, F. Bielschowsky, and S. J. Thannhauser, *Z. physiol. Chem.*, *218*, 1–11 (1933).
[971] S. J. Thannhauser and M. Reichel, *J. Biol. Chem.*, *113*, 311–317 (1936).
[972] T. Leary, *Arch. Pathol.*, *32*, 507–555 (1941).
[973] C. S. McArthur, *Biochem. J.*, *36*, 559–570 (1942).
[974] S. R. Rosenthal, *Arch. Pathol.*, *18*, 473–506, 660–698, 827–842 (1934).
[975] R. Schönheimer, *Z. physiol. Chem.*, *160*, 61–76 (1926).
[976] R. Schönheimer, *Z. physiol. Chem.*, *177*, 143–157 (1928).
[977] M. Bürger, *Ergeb. inn. Med. Kinderheilk.*, *34*, 683–701 (1928).
[978] M. Faber, *Arch. Pathol.*, *48*, 342–350 (1949).

aorta of human subjects which parallel their advancing age, no corresponding increase in blood cholesterol has been noted. Moreover, Faber and Lund[979] found that the progressive increase in the cholesterol content of the aorta, which occurs with advancing age, is not altered by obesity. These results have led many investigators to question whether atherosclerosis is a result of hypercholesterolemia or whether it results from abnormal metabolism occurring locally in the intima of the aorta.

On the other hand, there does appear to be evidence that hypercholesterolemia favors the production of atheromatous lesions in the rabbit. After large amounts of cholesterol are given,[980] a deposition of lipids, consisting mainly of cholesterol, occurs in the wall of the aorta. Although Leary[980] states that the individual lesions found in the intima of the rabbit are similar to those found in arteriosclerosis in man, Rosenthal[974] considers that they differ, in that they are far more diffuse in the rabbit. Moreover, in the latter species, they are not confined to the aortic and systemic vessels, but also occur in the pulmonary system, as well as in the veins.[981,982] However, Duff[983] showed that, even in the rabbit, the injury to the aorta may precede the deposition of cholesterol. This would indicate that the condition of hypercholesterolemia and of atherosclerosis can be dissociated in the rabbit. However, the subsequent rate of deposition may be augmented or exaggerated in the presence of high levels of cholesterol in the serum. These facts would seem to indicate that the lipid metabolism of the rabbit differs from that of man and, in fact, from that of most other animals which fail to develop atherosclerosis when cholesterol is fed. This leads one to question whether or not the results on rabbits may be applicable to man. A discussion of the relationship of the plasma cholesterol level to atherosclerosis is included in Chapter V.

[979] M. Faber and F. Lund, *Arch. Pathol., 48,* 351–361 (1949).
[980] T. Leary, *Arch. Pathol., 17,* 453–492 (1934).
[981] R. Schönheimer, *Arch. pathol. Anat. Physiol. (Virchow's), 249,* 1–42 (1924).
[982] R. Schönheimer, *Arch. pathol. Anat. Physiol. (Virchow's), 251,* 732–738 (1924).
[983] G. L. Duff, *Arch. Pathol., 20,* 81–123, 259–304 (1935).

CHAPTER VII

LIPID DISTRIBUTION IN SPECIFIC TISSUES AND IN THEIR SECRETIONS

1. Introduction

Although there are marked variations in the composition of the fat of the entire animal as related to species, much greater differences exist between the lipids present in the several tissues. Since some of the tissues which play important roles in metabolism are relatively insignificant in the total mass of the body, information concerning the lipids in these tissues is unobtainable on the basis of the lipid pattern of the animal as a whole. For this reason, a detailed review of the composition of the separate tissues is of importance. The carotenoid and fat-soluble vitamin components of these tissues are discussed more fully in Volume III.

2. Lipids Present in the Liver

The liver plays an important and unique role in the metabolism of the various lipid components. Although, under normal circumstances, the liver lipids consist primarily of phospholipids and neutral fats, this organ may, under certain conditions, contain larger accumulations of cholesterol (up to 9.6%)[1]; in Niemann-Pick's disease, sphingomyelin comprises a larger than usual proportion of the lipids.[2,3] In Gaucher's disease, the amount of cerebrosides in the liver is somewhat increased. In various types of fatty livers, on the other hand, the proportion of triglyceride fat to the non-glyceride moiety is greatly increased, due to the large accumulation of the former fraction. These types of abnormalities in the storage of liver lipids are discussed in Chapter VI.

It is believed that newly absorbed fat is worked over in the liver to alter

[1] N. R. Blatherwick, E. M. Medlar, P. J. Bradshaw, A. L. Post, and S. D. Sawyer, *J. Biol. Chem.*, *103*, 93–106 (1933).

[2] E. Klenk, *Z. physiol. Chem.*, *235*, 24–36 (1935).

[3] C. Tropp and B. Eckardt, *Z. physiol. Chem.*, *243*, 38–42 (1936).

the nature of its fatty acid pattern so that it will conform to the type usually stored by that particular species; the reworked fat is then transferred to the fat depots for storage. Hepatic cells, also, have the ability to synthesize the fatty acids,[4] as well as cholesterol.[5-7] The liver is the chief organ in which the fatty acids are oxidized, with the resultant production of ketone bodies. Thus, the liver is the only organ in which a production of ketone bodies can be demonstrated by the higher content of acetone bodies in the venous blood coming from the organ, as contrasted with that in the arterial blood supplying this organ.[8,9] Fats are likewise desaturated in the liver, as shown by the fact that the iodine values of the neutral fat and phospholipid fractions are invariably higher than the corresponding fractions in other tissues.[10]

Although phospholipid synthesis takes place in a number of organs, the liver is one of the most important sites for its synthesis.[11] It is now recognized that a number of tissues may bring about esterification of free cholesterol; however, the master tissue which appears to mediate the change is the liver.[12] Another more striking transformation of cholesterol, brought about by hepatic tissue, is its conversion to bile acids.[13] Carotene occurs in maximum concentration in the liver of man and of animals which are capable of absorbing it without changing it to vitamin A[14,15]; furthermore, this organ is the site of transformation of the pigment to vitamin A in these species. The liver is almost universally regarded as the chief storehouse for vitamin A. This is true not only for fishes,[16] in which vitamin A plays an especially important role, but also for a large variety of reptiles, birds, and mammals, including man.[17] The liver also serves as a site for the storage of vitamin D.[18] In addition to all of the functions which concern the

[4] K. Bloch and W. Kramer, *J. Biol. Chem.*, *173*, 811–812 (1948).
[5] H. N. Little and K. Bloch, *J. Biol. Chem.*, *183*, 33–46 (1950).
[6] I. Zabin and K. Bloch, *J. Biol. Chem.*, *185*, 131–138 (1950).
[7] P. A. Srere, I. L. Chaikoff, S. S. Treitman, and L. S. Burstein, *J. Biol. Chem.*, *182*, 629–634 (1950).
[8] I. L. Chaikoff and S. Soskin, *Am. J. Physiol.*, *87*, 58–72 (1928–1929).
[9] H. E. Himwich, W. Goldfarb, and A. Weller, *J. Biol. Chem.*, *93*, 337–342 (1931).
[10] W. R. Bloor, *Ann. Rev. Biochem.*, *1*, 267–298 (1932).
[11] I. Perlman, S. Ruben, and I. L. Chaikoff, *J. Biol. Chem.*, *122*, 169–182 (1937).
[12] M. L. Nieft and H. J. Deuel, Jr., *J. Biol. Chem.*, *177*, 143–150 (1949).
[13] K. Bloch, B. N. Berg, and D. Rittenberg, *J. Biol. Chem.*, *149*, 511–517 (1942).
[14] H. Willstaedt and T. Lindqvist, *Z. physiol. Chem.*, *240*, 10–18 (1936).
[15] C. L. Connor, *Am. J. Pathol.*, *4*, 293–308 (1928).
[16] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York-London, 1945.
[17] H. B. Jensen and T. K. With, *Biochem. J.*, *33*, 1771–1786 (1939).
[18] W. Heymann, *J. Biol. Chem.*, *118*, 371–376 (1937).

several lipids, the liver plays a commanding role in the metabolism of carbohydrate[19,20] and of protein.[21]

(1) *The Lipid Composition of Normal Liver*

The liver of man and of most mammals contains 3 to 5% of total lipids. Theis[22] reported that the lipids of beef liver comprised 4.6% of the total weight of the organ; of this, 55% consisted of phospholipids and 45% of acetone-soluble components (neutral fat, cholesterol, etc.). On the other hand, Bloor[23] reported a figure of 4.2% for the total lipid content of beef liver, 73% being classified as phospholipids and the remaining 27% as the acetone-soluble portion. Deuel *et al.*[24] recorded values for total liver lipid between 3.5 and 4.0% for normal rats on stock diet. Sperm whale liver lipid was shown by Tsujimoto and Kimura[25] to comprise about 6% of the total weight of the organ, of which 69.3% was fatty acid and 16.4% was non-saponifiable. Jones *et al.*[26] found that the liver, unlike the pancreas, contains only traces of α-monoglycerides.

a. Fatty Acids Present in the Liver. The outstanding discovery in the investigation of the liver fatty acids was the demonstration by Hartley[27,28] that arachidonic acid occurs, as well as a 12,13-octadecenoic acid. The presence of the latter acid could not be confirmed by Channon and collaborators.[29] The mean molecular weight of the fatty acids in liver reported by Hartley[28] was 308 to 312, compared with a theoretical value of 282 for oleic acid. According to Brown,[30] arachidonic acid is the only highly unsaturated acid present; it comprises 2 to 7.7% of the total fatty acids in the liver. In the case of rats, Wesson[31] reported that the administration of cod-liver oil increased the arachidonic acid four-fold; this increase occurred primarily in the liver. A C_{22}-unsaturated acid has been

[19] F. C. Mann and T. B. Magath, *Am. J. Physiol.*, *55*, P 285–P 286 (1921).
[20] F. C. Mann and T. B. Magath, *Arch. Internal Med.*, *30*, 73–84 (1922).
[21] F. C. Mann and T. B. Magath, *Am. J. Physiol.*, *55*, P 286–P 287 (1921).
[22] E. R. Theis, *J. Biol. Chem.*, *76*, 107–114 (1928).
[23] W. R. Bloor, *J. Biol. Chem.*, *80*, 443–459 (1928).
[24] H. J. Deuel, Jr., L. F. Hallman, and S. Murray, *J. Biol. Chem.*, *119*, 257–268 (1937).
[25] M. Tsujimoto and K. Kimura, *Chem. Umschau Fette, Öle, Wachse, u. Harze*, *35*, 317–318 (1928); *Chem. Abst.*, *23*, 723 (1929).
[26] M. E. Jones, F. C. Koch, A. E. Heath, and P. L. Munson, *J. Biol. Chem.*, *181*, 755–760 (1949).
[27] P. Hartley, *J. Physiol.*, *36*, 17–26 (1907).
[28] P. Hartley, *J. Physiol.*, *38*, 353–374 (1909).
[29] H. J. Channon, E. Irving, and J. A. B. Smith, *Biochem. J.*, *28*, 1807–1811 (1934).
[30] J. B. Brown, *J. Biol. Chem.*, *80*, 455–460 (1928).
[31] L. G. Wesson, *J. Biol. Chem.*, *65*, 235–250 (1925).

detected by Klenk and von Schoenebeck[32] in beef liver fat. Hilditch and Shorland[33] likewise noted the presence of C_{20}- and C_{22}-unsaturated acids in the liver fat of New Zealand cattle. Except for these unsaturated acids and a hexadecenoic acid, the liver fat and the depot fat were similar. A still more unsaturated acid, clupanodonic acid, has been demonstrated in fat from the European brown frog (*Rana temporaria*).[34] Presumably it is present largely in the liver.

The percentages of the fatty acids in terms of the total mixed fatty acids in pig liver were found to be the following[35]: *n*-decanoic and lauric (C_{12}), 0.4%; myristic (C_{14}), 0.7%; palmitic (C_{16}), 14.0; stearic (C_{18}), 18.8%; arachidic (C_{20}), 1.7%; palmitoleic (C_{16}), 1.5%; oleic (C_{18}), 28%; linoleic (C_{18}), 5%; C_{20}-acid, 20%; and C_{22}-acid, 7.5%. These figures compare with those of Klenk and von Schoenebeck[32] for solid acids in beef liver, which were cited as follows: C_{14}, trace; C_{16}, 25%; C_{18}, 20%; C_{20}, trace; C_{22}, trace. For liquid acids the values were: C_{14}, trace; C_{16}, 9%; C_{18}, 37%; C_{20}, 8%; and C_{22}, 1%.

b. Phospholipids in the Liver. The total phospholipids in the livers of a large number of animals range from 1.5 to 3.7% of the net weight of the organ. Lecithin and cephalin occur in about the same proportion. Sphingomyelin has been shown to comprise less than 5% of the total phospholipids in human liver tissue (0.38% for sphingomyelin compared with 9.80% for total phospholipids, based upon dry weight),[36] while Schmidt *et al.*[37] recorded a value for sphingomyelin of 2.6% of the total phospholipids in rat liver.

The presence of glycerophosphoric acid in the liver has been suggested by the results of Cahn and collaborators.[38] These workers indicate that part of the phosphoric acid is in the form of glycerophosphoric acid, rather than of the more complicated phospholipids; they base their conclusion upon the fact that the total amount of fatty acid is insufficient for the phospholipids, calculated from the amount of their other constituents.

Glycerylphosphorylcholine, also, has been shown to be present in animal tissues. Thus, after developing a method for its quantitative determina-

[32] E. Klenk and O. von Schoenebeck, *Z. physiol. Chem.*, *209*, 112–133 (1932).
[33] T. P. Hilditch and F. B. Shorland, *Biochem. J.*, *31*, 1499–1515 (1937).
[34] E. Klenk, *Z. physiol. Chem.*, *221*, 259–264, 264–270 (1933).
[35] E. Irving and J. A. B. Smith, *Biochem. J.*, *29*, 1358–1368 (1935).
[36] S. J. Thannhauser, J. Benotti, A. Walcott, and H. Reinstein, *J. Biol. Chem.*, *129*, 717–719 (1939).
[37] G. Schmidt, J. Benotti, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, *166*, 505–511 (1946).
[38] T. Cahn, J. Houget, and R. Agid, *Bull. soc. chim. biol.*, *31*, 766–778 (1949).

tion, Schmidt *et al.*[39] proved that this component accounted for more than one-half of the phospholipid choline in fresh lamb liver. On the other hand, only negligible amounts of water-soluble choline compounds occurred in the fresh livers obtained from weanling or adult rats. These results indicate that glycerylphosphorylcholine is an intermediate in the metabolism of lecithin. The analyses of the classical phospholipids in the liver of man are included in Table 1.

TABLE 1
THE PHOSPHOLIPID CONTENT OF THE LIVER OF MAN AND OF VARIOUS OTHER ANIMALS, EXPRESSED AS PER CENT OF MOIST ORGAN WEIGHT

Source of tissue	Total phospholipid	Lecithin	Cephalin	Sphingo-myelin	Acetone-soluble fraction
Human[a]	2.94[b]	1.44[b]	1.19[b]	0.11[b]	—
Beef[c]	3.08	1.56	1.50	—	0.90
Horse[d]	3.7	—	—	—	—
Guinea pig[d]	2.9	—	—	—	—
Rat[d]	2.9	—	—	—	—
Pigeon[d]	2.9	—	—	—	—
Frog[d]	3.1	—	—	—	—
Carp[d]	2.5	—	—	—	—
Dogfish[d]	1.7	—	—	—	—

[a] S. J. Thannhauser, J. Benotti, A. Walcott, and H. Reinstein, *J. Biol. Chem.*, *129*, 717–719 (1939).
[b] Calculated from dry weight on basis of 70% water content.
[c] W. R. Bloor, *J. Biol. Chem.*, *80*, 443–454 (1928).
[d] M. Javillier, A. Crémieu, and H. Hinglais, *Bull. soc. chim. biol.*, *10*, 327–337 (1928). Figures recalculated by W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 199.

c. Plasmalogens in the Liver. In addition to the usual phosphatides shown to be present in the liver, Klenk and Friedrichs[40] have demonstrated the presence of plasmalogens in this organ. In contradistinction to the lipids of the heart, pancreas, kidney, and adrenal glands, in which the plasmalogen content is determined directly, and can then be confirmed by the isolation of the dimethylacetal fraction, the plasmalogen in the liver has a dimethylacetal value corresponding to 64% of that determined directly. This discrepancy is probably to be ascribed to an inaccuracy inherent in the method when small amounts of plasmalogen are present. In fatty livers

[39] G. Schmidt, L. Hecht, P. Fallot, L. Greenbaum, and S. J. Thannhauser, *J. Biol. Chem.*, *197*, 601–609 (1952).
[40] E. Klenk and E. Friedrichs, *Z. physiol. Chem.*, *290*, 169–171 (1952).

resulting from a diet of raw liver, there was a marked increase in the fatty aldehydes in this organ.[41]

d. Lipoprotein in the Liver. Some of the lipid components in the liver occur in combination with protein. Carver and Thomas[42] made an electrophoretic study of the lipoprotein of calf liver, and found that it consisted of only one component. A firm combination was shown to exist between the protein and the lipid component.

e. Free Fatty Acids in the Liver. A number of workers have reported the presence of free fatty acids of high molecular weight in liver. Thus, Bloor and Snider[43] noted that they comprise about one-third of the acetone-soluble extract of liver, while Hilditch and Shorland[33] have also recorded the occurrence of free fatty acids in hepatic tissues. Their presence in liver tissue does not appear to be unique, as they have been found in blood (see page 364), as well as in heart, lung, and kidney,[43] and also in the intestinal mucosa.[44,45]

Fairbairn[46] cites a value of 2.2 mg./g. of dry fat-free mouse tissue and 2.3% of the acetone-soluble fraction of cat liver for the quantity of free fatty acids. There would appear to be some question as to whether or not even these minimum values represent true figures, since a rapid autolysis occurs immediately on removal of the liver, with the formation of free fatty acids. Thus, when rat or cat liver was extirpated, a part of the constituent phospholipids was found to be rapidly hydrolyzed by intracellular phospholipases. The extent of hydrolysis amounted to 8% within a few minutes after hepatectomy. When the liver tissue was ground, hydrolysis to the extent of 15% was noted; after the ground tissue was suspended in buffer at *p*H 7.2 for four hours, as much as 40% of the phospholipid was hydrolyzed. It is thus questionable whether or not the free fatty acids are normal components of liver tissue; if they are, they represent an extremely minor component, from the quantitative standpoint.

f. Unsaponifiable Components Present in the Liver. (*a*) *Cholesterol and the Sterols.* The sterols constitute the main components of the unsaponifiable fraction of liver lipids, from a quantitative standpoint. Freytag and Smith[47] reported that the sterols account for 64% of the total non-

[41] T. Tokushima, M. Yoshihara, and T. Shimojo, *J. Biochem. (Japan)*, *39*, No. 5, P5 (1952).

[42] M. J. Carver and L. E. Thomas, *Arch. Biochem. Biophys.*, *40*, 342–345 (1952).

[43] W. R. Bloor and R. H. Snider, *J. Biol. Chem.*, *87*, 399–413 (1930).

[44] J. A. Lovern and R. A. Morton, *Biochem. J.*, *33*, 330–337 (1939).

[45] J. A. Lovern, T. H. Mead, and R. A. Morton, *Biochem. J.*, *33*, 338–343 (1939).

[46] D. Fairbairn, *J. Biol. Chem.*, *157*, 645–650 (1945).

[47] F. C. Freytag and H. G. Smith, *J. Biol. Chem.*, *100*, 309–317 (1933).

saponifiable extract in the case of beef liver. Cholesterol was shown to comprise the largest proportion of the total sterols, although small amounts of dihydrocholesterol and ergosterol were also present. According to Chanutin and Ludewig,[48] free cholesterol comprises over 80% of the total cholesterol in rat liver.

The levels of liver cholesterol approximate 200 to 300 milligram per cent of the wet weight of the organ (or 2 to 3 mg./g. liver). This applies to the figure reported by Bloor[49] for beef liver, which is 0.93% on the dry basis, or 2.8 mg./g. liver on the wet basis (assuming that the water content of normal liver is 70%). In the case of normal rats, the figures per gram liver include 1.0 mg.,[50,51] 2.7 mg.,[48] 2.8 mg. (assuming that the water content of normal liver is 70%),[52] and values ranging from 2.1 to 3.1 mg.[53] Similar figures have been recorded for the cholesterol in the liver of chickens (2.6 mg.),[54] of cats (5.05 mg.),[51] and of mice (2.2 mg.).[51] Free cholesterol was also shown to constitute the major portion of the total in the livers of chickens (93%).[54]

(*b*) *Hydrocarbons.* Hydrocarbons may constitute a considerable portion of the liver lipids, particularly in the case of certain fishes. Thus, Tsujimoto[55] first reported the presence of an unsaturated hydrocarbon in the liver oil of the black shark of the genus *Zameus.* This compound was later shown to have an empirical formula of $C_{30}H_{50}$, and was named squalene.[56] It has later been reported as present in the livers of sixteen of thirty-six species of elasmobranch fishes from Japanese waters.[57] Klenk[58] also demonstrated the presence of squalene in the liver oil of the dogfish, a shark of the *Squalidae* (*Etmopterus spinax*). It comprises a large portion of the 50% of the unsaponifiable fraction in this liver oil.

Pristane, $C_{18}H_{38}$, is a somewhat similar hydrocarbon, which Tsujimoto[59,60]

[48] A. Chanutin and S. Ludewig, *J. Biol. Chem.*, *102*, 57–65 (1933).

[49] W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

[50] D. Yuasa, *Beitr. pathol. Anat. u. allgem. Pathol.* (*Zeigler's*), *80*, 570–594 (1928).

[51] R. Schönheimer and D. Yuasa, *Z. physiol. Chem.*, *180*, 5–16 (1929).

[52] W. M. Sperry and V. A. Stoyanoff, *J. Nutrition*, *9*, 131–155 (1935).

[53] R. Alfin-Slater, M. C. Schotz, S. M. Greenberg, and H. J. Deuel, Jr., *unpublished results*, 1952.

[54] W. M. Sperry and V. A. Stoyanoff, *J. Nutrition*, *9*, 157–161 (1935).

[55] M. Tsujimoto, *J. Soc. Chem. Ind., Japan*, *9*, 953 (1906). Cited by M. Tsujimoto, *J. Ind. Eng. Chem.*, *8*, 890 (1916).

[56] M. Tsujimoto, *J. Ind. Eng. Chem.*, *8*, 889–896 (1916).

[57] M. Tsujimoto, *J. Ind. Eng. Chem.*, *12*, 63–73 (1920).

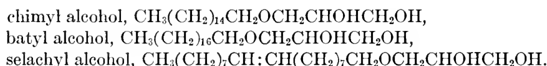
[58] E. Klenk, *Z. physiol. Chem.*, *217*, 228–236 (1933).

[59] M. Tsujimoto, *Bull. Chem. Soc. Japan*, *10*, 144–148, 149–153 (1935); *J. Chem. Soc. Japan*, *55*, 702–711 (1934); *Chem. Abst.*, *28*, 6484 (1934).

[60] M. Tsujimoto, *J. Ind. Eng. Chem.*, *9*, 1098–1099 (1917).

has shown to be present in the liver oil of the basking shark (*Cetorhinus maximus*) to the extent of 6 to 10%. A still more unsaturated hydrocarbon, gadusene, $C_{18}H_{32}$, has been isolated from the liver of the Japanese ishinagi (*Stereolepis ishinagi*).[61] Strangely enough, this latter compound is likewise present in the unsaponifiable fraction of soybean oil, rice germ oil,[62] wheat germ oil,[63] and in other fish liver oils.[62] For a more complete exposition of the chemistry and distribution of these lipids, the reader is referred to Volume I, pages 400–404.

(*c*) *Glyceryl Ethers.* The glyceryl ethers constitute very important components in the liver lipids of certain fishes. The three members of this group include:



chimyl alcohol, $CH_3(CH_2)_{14}CH_2OCH_2CHOHCH_2OH$,
batyl alcohol, $CH_3(CH_2)_{16}CH_2OCH_2CHOHCH_2OH$,
selachyl alcohol, $CH_3(CH_2)_7CH{:}CH(CH_2)_7CH_2OCH_2CHOHCH_2OH$.

They represent combinations of palmityl, stearyl, and oleyl alcohols, respectively, with the α-hydroxyl of glycerol, to form water-insoluble ethers.

The glyceryl ethers were discovered by Tsujimoto and Toyama[64] in the unsaponifiable fraction of the liver oil of such elasmobranch fishes as the shark and ray. The glyceryl ethers may be present in a relatively high concentration. Thus, practically all of the non-saponifiable fraction of ratfish liver oil (*Chimaera monstrosa*), which constitutes 37% of the total liver lipid, consists of selachyl alcohol.[65] However, the glyceryl ethers are present not only in the elasmobranch family, but also in the Japanese crab (*Paralithoides camtschatica* Tilesius).[66] In fact, batyl alcohol has been isolated from the bone marrow of cattle,[67] from the spleen of pigs,[68] and from the arteriosclerotic arteries of human beings,[69] although it has not been detected in the liver lipids of the higher animals. For a more detailed

[61] M. Tsujimoto, *Bull. Chem. Soc. Japan, 6,* 237–239 (1931); *Chem. Abst., 26,* 612–613 (1932).

[62] Z. Nakamiya, *Sci. Papers Inst. Phys. Chem. Research (Tokyo), 28,* 16–26 (1935).

[63] J. C. Drummond, E. Singer, and R. J. MacWalter, *Biochem. J., 29,* 457–471 (1935).

[64] M. Tsujimoto and Y. Toyama, *Chem. Umschau Fette, Öle, Wachse, u. Harze, 29,* 27–29, 35–37, 43–45 (1922); Y. Toyama, *Ibid., 29,* 237–240, 245–247 (1922); *31,* 13–17, 61–67, 153–155 (1924).

[65] T. P. Hilditch, *The Chemical Constitution of Natural Fats,* Wiley, New York, 1947.

[66] M. Tsujimoto, *J. Soc. Chem. Ind. Japan, 32,* 362B–364B (1929); *Chem. Abst., 24,* 4650 (1930).

[67] H. N. Holmes, R. E. Corbet, W. B. Geiger, N. Kornblum, and W. Alexander, *J. Am. Chem. Soc., 63,* 2607–2609 (1941).

[68] V. Prelog, L. Ruzicka, and P. Stein, *Helv. Chim. Acta, 26,* 2222–2242 (1942).

[69] E. Hardegger, L. Ruzicka, and E. Tagmann, *Helv. Chim. Acta, 26,* 2205–2221 (1943).

discussion of the glyceryl ethers, the reader is referred to Volume I, pages 392–396.

(2) *Physiologic Factors Altering the Lipid Composition of Normal Livers*

a. The Effect of Age on Liver Lipids. In studies of guinea pigs, Imrie and Graham[70] found that the level of liver lipid of the embryo pigs approximated the value found in the liver of the mother, *i.e.*, 2 to 3%. After the fetuses had reached a weight of 35 to 40 g., a rapid increase in liver lipids obtained, until the maximum figure of 16 to 18% was reached at birth. The excess fat stored in the livers was rapidly used in the first two or three days of life. Lang[71] reported a higher level of liver lipid in the rat fetus and in young animals than in the adult animal; the human fetus and newborn, also, have been shown to contain more fat than is present in the liver and other organs after birth.[72] Dimter[73] also found that the cholesterol content of the fetal liver of the horse, cattle, and man was higher than that of the adult organ. On the other hand, the non-cholesterol component of the non-saponifiable fraction of the liver was much higher in the adult animal.

Lang[71] found that, in growing rats concerning which data were available from birth to maturity, the liver phospholipid was approximately constant. On the other hand, liver cholesterol increased to a maximum at the age of fifteen days, after which it again decreased. The decrease was attributed to the rapid growth period beginning at twenty days.

Williams *et al.*[74] reported that the total liver lipids of rats fifteen days old amounted to 19.85% of the dry weight; the essential lipids totaled 13.50% on an average, while the balance of 6.35% consisted of neutral fat. The percentages of the essential lipids based upon the dry weight of the liver of the fifteen-day rat were as follows: phospholipids, 11.67%; cerebrosides, 0.12%; cholesterol esters, 1.44%; and free cholesterol, 0.27%. At the age of seventy days, there was a slight decrease in neutral fat to 5.22%, and somewhat of an increase in essential lipids to 14.98%. The principal changes in the individual products were a marked increase in phospholipid coupled with a concomitant reduction of the cholesterol esters.

[70] C. G. Imrie and S. G. Graham, *J. Biol. Chem.*, *41*, xlviii–xlix (1920).
[71] A. Lang, *Z. physiol. Chem.*, *246*, 219–223 (1937).
[72] L. Aschoff, *Zentr. allgem. Pathol. u. pathol. Anat.*, *8*, 861–862 (1897).
[73] A. Dimter, *Z. physiol. Chem.*, *271*, 293–315 (1941).
[74] H. H. Williams, H. Galbraith, M. Kaucher, E. Z. Moyer, A. J. Richards, and I. G. Macy, *J. Biol. Chem.*, *161*, 475–484 (1945).

Age may be an important consideration in the determination of the level of polyunsaturated acids in the liver. Klein and Johnson[75] noted that no change obtained in the level of dienoic, trienoic, tetraenoic, and pentaenoic acid content in three cytoplasmic particulate fractions of the livers of old rats maintained on a diet considered to be adequate with respect to the essential fatty acids. However, there was a marked decrease in the level of pentaenoic acid, concurrently with an increase in trienoic acid in the liver fractions of old rats raised on a diet which had an inadequate essential fatty acid content.

The concentration of vitamin A is considerably lower in the fetal liver, as well as in fetal plasma, than it is in the liver and plasma of the mother.[76–84] Williamson[82] reported that, when 2.5 to 5% of cholesterol was incorporated in the diet of pregnant rats, the lipid content of the placenta was increased over that of the controls. However, the fetuses from such cholesterol-fed mothers contained significantly less vitamin A than was present in the maternal organism. This led to the conclusion that the placenta inhibits the transfer of vitamin A from the maternal to the fetal organism.

b. The Effect of Sex on Liver Lipids. Although the sex variation in lipid metabolism is a generalized phenomenon, the clearest expression of this variation is to be noted in the lipid composition of the liver. Higher liver glycogen values occur in male rats than in female rats, while the opposite obtains insofar as lipids are concerned. Moreover, the extent of the ketonuria produced by fasting is much greater in women than in men, and the same relationship is true in the case of endogenous ketonuria in the rat. Likewise, fasting female rats excrete much higher amounts of the ketone bodies when ketogenic acids are fed to them than do male rats.

c. The Effect of Fasting on Liver Lipids. Since the liver is the principal organ involved in the degradation of fats, as well as of most other lipids, it is only natural that the lipid content of this organ should rise during starvation, when the maximum energy is being engendered by the combustion of fats. This situation also obtains in rats and mice[85] when they are given carbohydrate-free diets.

[75] P. D. Klein and R. M. Johnson, *Arch. Biochem. Biophys.*, *48*, 172–177 (1954).
[76] W. J. Dann, *Biochem. J.*, *26*, 1072–1080 (1932).
[77] W. J. Dann, *Biochem. J.*, *28*, 634–637 (1934).
[78] W. Neuwiler, *Z. Vitaminforsch.*, *13*, 275–280 (1943).
[79] G. H. Wise, M. J. Caldwell, and J. S. Hughes, *Science*, *103*, 616–618 (1946).
[80] C. J. Lund and M. S. Kimble, *Am. J. Obstet. Gynecol.*, *46*, 207–221 (1943).
[81] L. Portes and J. Varangot, *Compt. rend. soc. biol.*, *136*, 166–168 (1942).
[82] M. B. Williamson, *J. Biol. Chem.*, *174*, 631–636 (1948).
[83] J. B. Ellison and T. Moore, *J. Soc. Chem. Ind.*, *55*, 236 (1936).
[84] J. B. Ellison and T. Moore, *Biochem. J.*, *31*, 165–171 (1937).
[85] A. Hynd and D. L. Rotter, *Biochem. J.*, *24*, 1390–1399 (1930).

The lipid content of the liver increases as long as there are available supplies of reserve fat, after which a decrease occurs. The increase in liver lipids in response to fasting varies considerably in different species. Thus, the mouse, which is extremely susceptible to fasting, was shown to have the following total lipids during inanition,[86] expressed in mg. per liver: 0 day (unfasted), 66; first day, 107; second day, 110; third day, 42; and fourth day, 20. When expressed on the basis of milligrams per gram moist liver, the figures are 52, 100, 115, 52, and 31 mg. for the prefast day and the four successive fast days, respectively. In later work, Hodge *et al.*[87] confirmed the fact that liver lipids first increase, then decrease, in the fasted mouse. It was also found[88] that the proportion of phospholipids remains essentially constant in fasting mice. However, since the liver weight decreased by 50% during inanition, it is evident that the total phospholipids were also reduced by 50%. In the rat, the variations in liver lipid during fasting are less striking than in the mouse, but the increased values for liver lipids are maintained at the elevated level over a longer period. Thus, Deuel and co-workers[89] found that the values for liver lipid (expressed in % of moist liver weight) were the following in male rats: 0 day (unfasted), 3.06; first fast day, 3.90; second fast day, 4.22; third fast day, 4.44; fourth fast day, 3.98; and fifth fast day, 3.89. The values were slightly higher on corresponding days in the case of female rats; these percentages were 3.34, 4.53, 4.92, 4.55, 5.02, and 5.10, respectively, for the unfasted rats and for those fasted for one to five days. Similar responses to fasting were reported by other investigators.[90,91] Hynd and Rotter[85] noted that cats did not respond to fasting with as pronounced an increase in liver lipids as did mice and rats. During fasting the liver fats rapidly assume the character of the depot fats[86,90,92] whereas, in unfasted animals, the liver lipids tend to display a marked similarity to the food fats.[93]

(*a*) *Fat Turnover in the Liver.* The rapidity with which the quantity of fat changes in the liver is a reflection of its rapid turnover in this organ.

[86] P. L. MacLachlan, H. C. Hodge, W. R. Bloor, E. A. Welch, F. L. Truax, and J. D. Taylor, *J. Biol. Chem.*, *143*, 473–490 (1942).

[87] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. Welch, S. L. Kornberg, and M. Falkenheim, *Proc. Soc. Exptl. Biol. Med.*, *67*, 137–139 (1948).

[88] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. Welch, S. L. Kornberg, and M. Falkenheim, *Proc. Soc. Exptl. Biol. Med.*, *68*, 332–334 (1948).

[89] H. J. Deuel, Jr., M. Gulick, C. F. Grunewald, and C. H. Cutler, *J. Biol. Chem.*, *104*, 519–530 (1934).

[90] H. M. Barrett, C. H. Best, and J. H. Ridout, *J. Physiol.*, *93*, 367–381 (1938).

[91] J. H. Dible, *J. Pathol. Bacteriol.*, *35*, 451–466 (1932).

[92] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, C. A. Stoneburg, M. C. Oleson, and R. Whitehead, *J. Biol. Chem.*, *139*, 897–916 (1941).

[93] R. G. Sinclair, *J. Biol. Chem.*, *111*, 515–526 (1935).

Fat synthesis occurs in the liver before it can be demonstrated in any other organ when animals are fed on fat-low diets in the presence of deuterium.[90] Under such conditions, no comparable reaction can be demonstrated in the depot fats. However, Bernhard and Schoenheimer[94] were able to prove that some fatty acids are synthesized in tissues other than the liver, although the rate was found to be markedly higher in hepatic tissue. These workers concluded that the half-life of average saturated fatty acids in the liver is only about one day, as contrasted with an average figure of five to nine days in other tissues. In later work from this same laboratory,[95] it was indicated that the half-life of liver fats in mice is 2.6 to 2.8 days, while that of depot fats is five to six days.

Variations have been shown to obtain in the rate of turnover of liver and carcass phospholipids similar to those previously noted for the entire lipid content. Thus, Sinclair[96] showed that the iodine number of the liver phospholipids increased within one day to 65% of that reached at equilibrium, which occurred at five days when a cod-liver oil diet was employed. On the other hand, the rise in carcass phospholipids was only 27% of the maximum in one day, and the equilibrium value was not observed until thirty-five days later. Cavanagh and Raper[97] reported that the D_2 content of liver phospholipids observed after the feeding of deuterated linseed oil was such as to indicate a replacement of 14% of the fatty acids in liver phospholipids within 6 hours after the feeding of this compound. Tolbert and Okey[98] calculated the turnover time of the phosphate of the choline-containing phospholipids in the livers of fasted rats as 10.9 hours; that for the total phospholipids was estimated as 8.9 hours. The same order of turnover prevails in carcass lipids; however, in the brain, the fatty acids exhibit the most rapid interchange. The average values for the half-life of these lipids are given in Table 2.

TABLE 2

THE RATE OF TURNOVER OF LIPIDS IN RAT TISSUES (half-life in days)[a]

Lipid	Liver	Carcass	Brain
Fatty acids	1–3	6–9	10–15
Phospholipids	1–2	5–7	>200
Cholesterol	5–7	12–15	>100

[a] E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951.

[94] K. Bernhard and R. Schoenheimer, *J. Biol. Chem.*, *133*, 713–720 (1940).
[95] De W. Stetten, Jr., and G. F. Grail, *J. Biol. Chem.*, *148*, 509–515 (1943).
[96] R. G. Sinclair, *J. Biol. Chem.*, *95*, 393–408 (1932).
[97] B. Cavanagh and H. S. Raper, *Biochem. J.*, *33*, 17–21 (1939).
[98] M. E. Tolbert and R. Okey, *J. Biol. Chem.*, *194*, 755–767 (1952).

A number of factors may alter the rate of turnover of liver lipids. Thus, in the case of rats with fatty livers due to choline deficiency, Boxer and Stetten[99] reported that, in spite of the fact that the amount of choline may be normal,[100,101] the rate of turnover of choline is depressed. The normal half-life of choline, instead of being six days, was shown to be eighteen days in rats with severe fatty livers. The daily rate of replacement decreased from a normal value of 3.9 mg. per rat per day to a level of 1.3 mg. per rat per day. Thus, phospholipids are not effectively utilized in rats having fatty livers; apparently, the animal requires a continuous supply of choline derived either from the diet or by synthesis, to insure a normal rate of turnover of phospholipids and so to maintain a normal level of liver fat.

Bollman, Flock, and Berkson[102] determined that the proportional turnover rate for hepatic phospholipid approximates 5% per hour. Since the hepatic phospholipid phosphorus is 132 mg./100 g. liver, the mass turnover rate is 6 mg. P/hr./100 g. liver, or 0.2 mg. P/100 g. body weight. In a later communication from the above workers,[103] it was reported that the rate of phospholipid turnover in the liver of rats was increased by thyroxine and decreased by thiouracil. The rate of turnover of phospholipid was likewise found to be essentially the same in young rats as in adult animals. However, since the relative size of the liver is greater in young rats than in older ones, more phospholipid is formed in proportion to body weight in the young rat than in the adult.

There has been some question as to the effect of palmitic acid on phospholipid turnover in the liver. When a large amount was given to rats along with a low-protein, high-fat diet, liver fat was shown by Campbell *et al.*[104] to be increased, together with a concomitant release of depot fats. The metabolism of oleic acid did not impose a similar demand upon the fat depots. However, the rate of phospholipid turnover as determined with P^{32} did not show any correlation with the amount of liver lipids or with gross changes in fat metabolism caused by palmitic acid feeding. Either the phospholipid turnover rates are not involved, or the rate of exchange of P^{32} is not a measure of that of fatty acid in the phospholipid molecule.

d. Diurnal Variation in Liver Lipids. It has already been shown that liver lipids have an especially short half-life as contrasted with carcass

[99] G. E. Boxer and De W. Stetten, Jr., *J. Biol. Chem.*, *153*, 617–625 (1944).
[100] H. P. Jacobi, C. A. Baumann, and W. J. Meek, *J. Biol. Chem.*, *138*, 571–582 (1941).
[101] H. P. Jacobi and C. A. Baumann, *J. Biol. Chem.*, *142*, 65–76 (1942).
[102] J. L. Bollman, E. V. Flock, and J. Berkson, *Proc. Soc. Exptl. Biol. Med.*, *67*, 308–313 (1948).
[103] E. V. Flock, J. L. Bollman, and J. Berkson, *Am. J. Physiol.*, *155*, 402–408 (1948).
[104] I. G. Campbell, J. Olley, and M. Blewett, *Biochem. J.*, *45*, 105–112 (1949).

fats. Thus, the suggestion of Ohlsson and Blix[105] that the lipids in the liver undergo a cyclic variation is not surprising. These workers report that the neutral fat of rat liver varies with the time of day, accumulating during the early hours of the day and decreasing during the afternoon. It has been stated that this variation occurs independently of food intake. Moreover, the high-fat phase of the liver coincides with the low-glycogen phase of this organ, and *vice versa*. It is possible that a physiologic control is exerted which causes fat to pass into the liver when the glycogen store becomes depleted.

Forsgren,[106] on the basis of histochemical evidence, first described a diurnal variation in liver glycogen in the rabbit. This observer was convinced that an assimilatory cycle occurs during the night when the glycogen storage is at its maximum and bile production is at its minimum. These results were confirmed by Ågren *et al.*[107] on rabbits, rats, and mice by the demonstration that increased resistance to insulin occurs concomitantly with the period of increased glycogen retention. Moreover, an increased excretion of urinary nitrogen was noted in fasted as well as in non-fasted rabbits during the night, as contrasted with the values during the day. On the other hand, Higgins, Berkson, and Flock[108] challenged the hypothesis that such diurnal variations are independent of nutritional differences. They reported that, although diurnal variations were noted in rats fed at 9 to 11 A.M., this phenomenon was not repeated on the following day if the animals were fasted. Moreover, the liver glycogen was practically constant in various groups of rats, six hours after feeding periods spaced at two-hour intervals during the day and night.[109] These results were confirmed by Deuel and collaborators,[110] who concluded that the cyclic variations were solely of dietary origin.

Thus, although it is possible that the diurnal variation in the neutral fat content of the liver may be independent of ingested fat, it may be related to the liver glycogen level. Under such conditions, it would be linked only secondarily to the nutritional status as reflected in the cyclic content of liver glycogen.

e. The Effect of Hormones on Liver Lipids. A number of hormones are of importance in controlling fat distribution and utilization, in addi-

[105] B. Ohlsson and G. Blix, *Skand. Arch. Physiol.*, *69*, 182–188 (1934).

[106] E. Forsgren, *Skand. Arch. Physiol.*, *53*, 137–151 (1928).

[107] G. Ågren, O. Wilander, and E. Jorpes, *Biochem. J.*, *25*, 777–785 (1931).

[108] G. M. Higgins, J. Berkson, and E. Flock, *Am. J. Physiol.*, *102*, 673–682 (1932).

[109] G. M. Higgins, J. Berkson, and E. Flock, *Am. J. Physiol.*, *105*, 177–186 (1933).

[110] H. J. Deuel, Jr., J. S. Butts, L. F. Hallman, S. Murray, and H. Blunden, *J. Biol. Chem.*, *123*, 257–265 (1938).

tion to those of the sex glands, which have already been discussed. These include the hormones of the anterior and posterior lobes of the hypophysis, those of the adrenal cortex and of the thyroid gland.

(*a*) *The Effect of Extracts of the Anterior Lobe of the Hypophysis on Liver Lipids.* The important effect which the pituitary gland exerts on the fat content of the liver was shown by the fact that the injection of pituitrin caused a doubling of the fat content of the liver, which returned to normal in about thirty hours.[111] Insulin inhibited this action on the part of pituitrin.[112] The marked effect of pituitrin and pitressin on liver fat was demonstrated by Hynd and Rotter,[113,114] who found that an increase in liver fat occurred within a few hours after the injection of these agents, concomitantly with a decrease in the carbohydrate level in this organ. Of the two extracts, pitressin produced the greater effect on the fatty infiltration of the liver.[114]

The relationship of the hormones of the anterior lobe of the hypophysis to fat metabolism (and transport) was first established by Burn and Ling,[115,116] who reported that an increased ketonuria followed their injection into rats. These results were confirmed in rats and in man by Hoffmann and Anselmino,[117,118] as well as by many others.[119–128] Anselmino and Hoffmann[118] and also Weil and Stetten,[120] reported that an increase in liver fat also occurred; these latter workers postulated that the anterior pituitary gland assists in the regulation of the fatty acids of the blood. It was suggested that these phenomena are to be traced to the action of a hormone governing fat metabolism, adipokinin, which was assumed to be

[111] R. Coope and E. N. Chamberlain, *J. Physiol.*, *60*, 69–78 (1925).
[112] R. Coope, *J. Physiol.*, *60*, 92–94 (1925).
[113] A. Hynd and D. L. Rotter, *Biochem. J.*, *26*, 578–585 (1932).
[114] A. Hynd and D. L. Rotter, *Biochem. J.*, *26*, 1633–1639 (1932).
[115] J. H. Burn and H. W. Ling, *J. Physiol.*, *69*, xix (1930).
[116] J. H. Burn and H. W. Ling, *Quart. J. Pharm. Pharmacol.*, *6*, 31–38 (1933).
[117] F. Hoffmann and K. J. Anselmino, *Klin. Wochschr.*, *10*, 2380–2383, 2383–2386 (1931).
[118] K. J. Anselmino, F. Hoffmann, and E. Rhoden, *Arch. ges. Physiol. (Pflüger's)*, *237*, 515–516 (1936).
[119] B. O. Barnes and J. F. Regan, *Endocrinology*, *17*, 522–528 (1933).
[120] R. Weil and De W. Stetten, Jr., *J. Biol. Chem.*, *168*, 129–132 (1947).
[121] C. H. Best and J. Campbell, *J. Physiol.*, *92*, 91–110 (1938).
[122] J. S. Butts, C. H. Cutler, and H. J. Deuel, Jr., *J. Biol. Chem.*, *105*, 45–58 (1934).
[123] P. T. Black, J. B. Collip, and D. L. Thomson, *J. Physiol.*, *82*, 385–391 (1934).
[124] V. G. Foglia and P. Mazzocco, *Compt. rend. soc. biol.*, *127*, 150–152 (1938).
[125] E. G. Fry, *Endocrinology*, *21*, 283–291 (1937).
[126] E. M. MacKay and R. H. Barnes, *Am. J. Physiol.*, *118*, 525–527 (1937).
[127] A. H. Neufeld and J. B. Collip, *Endocrinology*, *25*, 768–774 (1939).
[128] C. H. Gray, *Biochem. J.*, *32*, 743–755 (1938).

secreted into the blood stream and subsequently excreted in the urine whenever the metabolism of fat was accelerated.[120] Several workers proved that the production of ketone bodies is accompanied by an increased amount of liver fat,[118,124,127,128] as well as by a depression of blood lipids,[129] and also by a lowering of the R.Q.[130–132]

The principle in the anterior lobe responsible for this effect has been referred to as the "ketogenic" or "diabetogenic" hormone. The mechanism by which it brings about its action is uncertain. Although Bloor[49] suggests that the ketogenic action must be entirely related to its retardation of carbohydrate metabolism and especially of protein metabolism, and not to its action as a fat-metabolizing hormone, certain experimental data render this conclusion open to question. Since it is known that only a slight ketonuria develops in the rat after prolonged fasting,[133–135] the high level of ketonuria evoked by the injection of the hormone cannot be attributed entirely to the removal of carbohydrate. Moreover, there is no experimental evidence that a suppression of protein metabolism occurs, since urinary nitrogen was found to be essentially normal in the rat when a marked ketonuria was produced by the injection of the anterior pituitary extract.[122] On the other hand, the ketonuria is not produced in fed rats,[120,136] and it is likewise counteracted by the administration of small amounts of glucose.[122] These facts lead the present author to conclude that the action of the extract of the anterior pituitary lobe in producing ketonuria is to be traced to a direct action on fat.

According to Greaves *et al.*[137] the ketogenic hormone is a heat-labile, nondiffusible compound which is salted out by the addition of ammonium sulfate to make a 0.2 to 0.45% solution, or by full saturation with sodium chloride. Butts and co-workers[122] likewise demonstrated that the hormone is heat-labile and precipitable by alcohol; moreover, it was shown to be completely inactive when given orally. Although the isoelectric point is at *p*H 6.7 to 5.75, the hormone is more stable at a *p*H of 9.5 to 11. The unit has been defined as the quantity which causes a lowering of the R.Q.

[129] O. B. Houchin and C. W. Turner, *Endocrinology, 24,* 638–644 (1939); *25,* 216–220 (1939).
[130] R. E. Fisher and R. I. Pencharz, *Proc. Soc. Exptl. Biol. Med., 34,* 106–107 (1936).
[131] R. E. Fisher, J. A. Russell, and C. F. Cori, *J. Biol. Chem., 115,* 627–634 (1936).
[132] H. S. Meyer, L. J. Wade, and C. F. Cori, *Proc. Soc. Exptl. Biol. Med., 36,* 346–348 (1937).
[133] J. S. Butts and H. J. Deuel, Jr., *J. Biol. Chem., 100,* 415–428 (1933).
[134] H. Levine and A. H. Smith, *J. Biol. Chem., 75,* 1–22 (1927).
[135] G. T. Cori and C. F. Cori, *J. Biol. Chem., 72,* 615–625 (1927).
[136] R. A. Shipley and C. N. H. Long, *Biochem. J., 32,* 2242–2256 (1938).
[137] J. D. Greaves, I. K. Freiberg, and H. E. Johns, *J. Biol. Chem., 133,* 243–259 (1940).

to 0.80 from a level of 0.86 to 0.94. It contains 0.17 mg. of protein. The ability of the material to reduce the R.Q. and the tendency to produce ketonuria run parallel.

The close interrelationship of the hormones of the adrenal cortex with those of the anterior pituitary gland is shown by the fact that ketonuria is suppressed by adrenalectomy[125,138]; it has also been demonstrated that this operation prevents the accumulation of fat in the liver.[126]

Further information as to the behavior of the pituitary in regulating fat metabolism can be gleaned from investigations on hypophysectomized animals. The fatty infiltration which normally follows pancreatectomy is not decreased after removal of the pituitary gland.[139] However, fatty livers do not ensue as the result of the toxic effects of carbon tetrachloride or phosphorus on the liver.[140] Peters and Van Slyke[141] suggest that the absence of fat mobilization may be the result of a failure of the hypophysectomized animals to eat. On the other hand, the hyperlipemia which may follow the injection of pituitary extracts is not changed by pancreatectomy.[124,142]

An interrelationship exists between the pancreas and the hypophysis. Thus, *lipocaic*, a preparation made from pancreas, was found by Julian *et al.*[143] to negate the high liver lipid produced by injection of the anterior pituitary hormone into fasting guinea pigs. They likewise showed that lipocaic prevented the development of fatty livers in depancreatized-hypophysectomized dogs (the so-called Houssay dog). The thyroid gland also influences the action of the pituitary. After removal of both the thyroid and the pituitary glands, a severe fatty infiltration results, with an increase in phospholipids and cholesterol; this eventually leads to a condition of cirrhosis.[144]

(*b*) *The Effect of Extracts of the Posterior Lobe of the Hypophysis on Liver Lipids.* The effects of the extracts of the posterior lobe on liver lipids are more ephemeral than are those of the anterior lobe. Pitressin, which is the pressor principle, appears to be the only hormone in the posterior lobe which

[138] E. M. MacKay and R. H. Barnes, *Am. J. Physiol.*, *118*, 184–189 (1937).

[139] I. L. Chaikoff, G. E. Gibbs, G. F. Holton, and F. L. Reichert, *Am. J. Physiol.*, *116*, 543–550 (1936).

[140] B. v. Issekutz and F. Verzár, *Arch. ges. Physiol. (Pflüger's)*, *240*, 624–635 (1938).

[141] J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, Vol. I, 2nd ed., Williams & Wilkins, Baltimore, 1946.

[142] J. M. Muñoz, *Compt. rend. soc. biol.*, *127*, 156–157 (1938).

[143] O. C. Julian, D. E. Clark, J. van Prohaska, C. Vermeulen, and L. R. Dragstedt, *Am. J. Physiol.*, *138*, 264–268 (1943).

[144] I. L. Chaikoff, C. Entenman, J. F. Rinehart, and F. L. Reichert, *Proc. Soc. Exptl. Biol. Med.*, *54*, 170–171 (1943).

influences the fat content of the liver.[113,114] When pitressin is injected into rats, a marked, rapid but transient increase in liver lipids ensues,[145] coincident with a decrease in liver glycogen.[113,114] Since the fatty infiltration is not inhibited by choline,[145] Peters and Van Slyke[141] consider that the lack of carbohydrate in the liver is the contributing factor in bringing about the increase in liver lipid.

Raab[146] reported the isolation of a substance from both the anterior and the posterior lobes of the pituitary gland, which he called *lipoitrin*. This is a hormone-like compound which lowers blood fat, decreases ketosis, and simultaneously increases liver fat. Thus, to some extent, the action of this principle is antagonistic to that of the ketogenic hormone.

(*c*) *The Effect of the Pancreas and of Insulin on Liver Lipids.* It is well known that hyperlipemia occurs following pancreatectomy. It has been proved by Gibbs and Chaikoff[147] and by Seo[148] that the liver lipids are also increased. Although the injection of insulin into depancreatized dogs abolishes the attendant ketonemia and ketonuria,[141] as well as the hyperlipemia, it does not eliminate the high concentration of liver lipids, but rather changes their character.[147] Moreover, insulin does not greatly alter the liver lipid concentration in normal rabbits[149] or dogs, in the presence of adequate glycogen stores.[150]

(*d*) *The Effect of the Adrenal Cortex on Liver Lipids.* Although the adrenocortical hormones are known to have a pronounced effect on carbohydrate metabolism, they also exert an influence on liver lipids. Thus, it has been demonstrated that the ketonuria induced by extracts of the anterior lobe of the hypophysis is suppressed. This is presumably related to the failure of fat mobilization in the liver of adrenalectomized animals as a result of the administration of extracts of the anterior lobe.[125,126] Adrenalectomy also interferes with the normal transfer of fat from the depots to the liver.[151] When adrenocortical extracts are injected, the effectiveness of the anterior lobe extracts in inducing fatty infiltration of the liver and in producing ketonuria is restored. Adrenalectomy likewise interferes with the slight fasting ketonuria in rats.[138] The relation of the adrenal cortex to the control of fat metabolism exerted by the sex glands is indicated by the fact

[145] B. Mukerji and H. B. Van Dyke, *Chinese J. Physiol.*, *9*, 69–74 (1935).
[146] W. Raab, *Klin. Wochschr.*, *13*, 281–285 (1934).
[147] G. E. Gibbs and I. L. Chaikoff, *Endocrinology*, *29*, 877–884, 885–889 (1941).
[148] Y. Seo, *Arch. exptl. Pathol. Pharmakol.* (*Naunyn-Schmiedeberg's*), *61*, 1–6 (1909).
[149] I. H. Page, L. Pasternak, and M. L. Burt, *Biochem. Z.*, *231*, 113–122 (1931).
[150] H. R. Rony and T. T. Ching, *Endocrinology*, *14*, 355–363 (1930).
[151] L. T. Samuels and R. F. Conant, *J. Biol. Chem.*, *152*, 173–179 (1944).

that adrenalectomy abolishes the sex differences in ketonuria.[152] However, adrenocortical extracts are not able to provoke a ketonuria when injected into fasting rats.[153]

f. The Effect of Diet on Liver Lipids. The liver lipids are more susceptible to alteration than are those in the several fat depots. Thus, they rapidly assume the character of the food fats during alimentation, and they likewise mirror the composition of the storage fats during periods of inanition.

In 1910, Joannovics and Pick[154] demonstrated that the iodine numbers of the liver phospholipids increased from 100 to 150 within a few hours after the feeding of the highly unsaturated cod-liver oil. Differences in the iodine values of liver phospholipids were found when coconut oil[155] or linseed oil,[156] respectively, was the dietary fat. Sinclair demonstrated that at least one-third of the fatty acids in the liver of rats can be replaced by elaidic acid when this acid or trielaidin is included in the diet,[93] and this substitution occurs within eighteen hours. On the other hand, elaidic acid is taken up somewhat more slowly in the liver and other tissues of the cat.[157]

Rieckehoff and co-workers[158] reported that the polyunsaturated fatty acid content of the tissues of rats was somewhat influenced by fat deficiency, and by the feeding of fat diets, respectively. When rats on a fat-deficient diet were given a single dose of the dienoic acid, linoleic acid, the amount of arachidonic acid was found to be markedly increased in a number of tissues.[158,159] Linoleic acid could not serve as a source of hexaenoic acids,[158,159] except in the heart.[158] On the other hand, while the trienoic acid, linolenic acid, was incapable of affording a source of the tetraenoic acid, arachidonic acid,[159] it did give rise to pentaenoic and hexaenoic acids.[159] The greatest deposition of polyunsaturated acids following the feeding of a normal stock ration was shown to occur in the heart, followed in decreasing order in the liver, brain, kidney, blood, and skeletal muscle.[159] Only negligible amounts were found in the adipose tissue.

Although extreme deprivation of choline may lead to fatty livers, choline-low diets may also result in differences in the lipid pattern, without the development of fatty livers. A number of workers reported a signifi-

[152] E. M. MacKay and R. H. Barnes, *Endocrinology, 22,* 351–353 (1938).
[153] R. A. Shipley and E. G. Fry, *Am. J. Physiol., 135,* 460–463 (1942).
[154] G. Joannovics and E. P. Pick, *Wien. klin. Wochschr., 23,* 573–577 (1910).
[155] E. Shioji, *J. Biochem. (Japan), 4,* 43–72 (1924).
[156] K. Koizumi, *J. Biochem. (Japan), 5,* 171–185 (1925).
[157] R. G. Sinclair, *J. Biol. Chem., 134,* 71–81 (1940).
[158] I. G. Rieckehoff, R. T. Holman, and G. O. Burr, *Arch. Biochem., 20,* 331–340 (1949).
[159] C. Widmer, Jr., and R. T. Holman, *Arch. Biochem., 25,* 1–12 (1950).

cant decrease in liver phospholipids,[160-165] as well as in kidney phospholipids,[160,161] when animals were subjected to a choline-deficient diet. Although decreases in lipid choline derivatives may account for some of the reduction in liver phospholipids,[161-163,165,166] increases in non-choline phospholipids may partially offset this loss, and may markedly alter the lipid pattern. McKibbin and Taylor[167] noted that, in the case of puppies on a choline-low diet, a decrease in liver lecithin obtained, but that this was compensated for by an increase in sphingomyelins. Consequently no actual decrease in phospholipids was found. Lipid choline was also reduced in the skeletal muscle, but little or no change was observed in the lipids of the cerebrum, spleen, pancreas, heart, kidney, or lung. Fishman and Artom[162] reported that, when choline was added to the diet of rats previously on a low-fat diet, no change in liver phospholipids occurred. However, when choline was added to the diet of rats receiving a 20% fat diet, the choline phospholipids were increased, while the non-choline phospholipids were decreased. These data are interpreted as demonstrating that both choline and fat are essential for lecithin synthesis.

On the other hand, the amount of cholesterol present in the liver is directly related to the cholesterol content of the diet. In the hamster, the proportion of cholesterol is increased from a basal level of 220 milligram per cent to 8.68% by a diet containing 1% of cholesterol and 0.25% of bile salts.[168]

Sinclair and Chipman[169] have shown not only that dietary cholesterol and liver cholesterol are related to each other, but also that the fatty acid composition of the cholesterol esters is related to the dietary fat. When elaidin was fed along with cholesterol, elaidic acid made up a higher proportion of the total acids in the cholesterol esters than in the glycerides in the liver. Although the cholesterol esters were always found to contain a lower percentage of saturated acids than did the glycerides, variations occurred which were related to diet. Levy and co-workers[170] noted that,

[160] J. M. Patterson, N. B. Keevil, and E. W. McHenry, *J. Biol. Chem.*, *153*, 489–493 (1944).

[161] J. M. Patterson and E. W. McHenry, *J. Biol. Chem.*, *156*, 265–269 (1944).

[162] W. H. Fishman and C. Artom, *J. Biol. Chem.*, *164*, 307–312 (1946).

[163] W. H. Fishman and C. Artom, *J. Biol. Chem.*, *154*, 109–115, 117–127 (1944).

[164] M. G. Horning and H. C. Eckstein, *J. Biol. Chem.*, *166*, 711–720 (1946).

[165] De W. Stetten, Jr., and G. F. Grail, *J. Biol. Chem.*, *144*, 175–181 (1942).

[166] R. W. Engel, *Federation Proc.*, *1*, 189 (1942).

[167] J. M. McKibbin and W. E. Taylor, *J. Biol. Chem.*, *185*, 357–366 (1950).

[168] W. Marx, L. Marx, and H. J. Deuel, Jr., *Am. Heart J.*, *42*, 124–128 (1951).

[169] R. G. Sinclair and L. Chipman, *J. Biol. Chem.*, *167*, 773–779 (1947).

[170] M. Levy, G. Amat, and J. Legrand, *Compt. rend.*, *236*, 2267–2268 (1953).

when trielaidin was fed to rats, equal amounts were incorporated into the several types of phospholipids in the liver.

The most marked alterations in liver lipids occur in conditions favorable to the development of fatty livers. This is discussed in Chapter VI.

g. Miscellaneous Factors Affecting Liver Lipids. Terbrüggen[171] reported, in 1938, that the livers of scorbutic guinea pigs contained 10 to 19% of fat, in contrast to a normal level of 3.4 to 3.8%. However, Baldwin and collaborators[172] failed to confirm Terbrüggen's findings. No significant differences were found in the lipid content of the liver and carcass of normal *vs.* scorbutic animals. Moreover, no major changes in the phospholipid or cholesterol of the liver accompanied the development of scurvy. No appreciable alterations in adrenal cholesterol resulted from vitamin C deficiency.

Artom *et al.*[173] reported that diethanolamine, $NH(CH_2CH_2OH)_2$, administered to rats which had been injected with isotopic phosphate, stimulated the formation of both choline and non-choline phospholipids. After long-continued administration of diethanolamine to rats, the isotope content of the total phospholipid was lower than in the controls, due to inhibition of the formation of choline containing phospholipids. Diethanolamine acts as an antagonist of ethanolamine in the formation of both lecithins and cephalins. Although a marked decrease in the choline-containing phospholipids obtains, the total and non-choline lipid fractions are increased. There is some evidence that the liver fats of the rats fed diethanolamine contained considerable amounts of this compound, incorporated in an atypical phospholipid. Such unnatural phospholipids are less readily metabolized than are their natural analogues, and hence accumulate in the liver.

In cirrhosis of the liver, the levels of phosphatides and of cholesterol are lower than normal.[174] When choline is administered, the level of liver phosphatides is restored to normal. The cholesterol content of the liver was found to be increased in some cases of the Indian disease, "kwashiorkor" (infantile pellagra, or malignant malnutrition), and also in the nutritional edema of adults.[175]

[171] A. Terbrüggen, *Verhandl. deut. pathol. Ges.*, *31*, 114–121 (1938); *Chem. Abst.*, *33*, 9384 (1939).

[172] A. R. Baldwin, H. E. Longenecker, and C. G. King, *Arch. Biochem.*, *5*, 137–146 (1944).

[173] C. Artom, W. E. Cornatzer, and M. Crowder, *J. Biol. Chem.*, *180*, 495–503 (1949).

[174] E. Polli and G. Ratti, *Biochem. Z.*, *321*, 166–179 (1950).

[175] V. Ramalingaswami, S. Sriramachari, and P. G. Tulpule, *Lancet*, *263*, 661–662 (1952).

3. Lipids Present in the Kidney

Although the perirenal depots represent an important site for the storage of neutral fat, the proportion of this component within the kidney is minimal. In the analyses reported by Williams and co-workers[74] on the kidneys of rats fifteen days and seventy days of age, the total lipid comprised 19.62 and 21.59%, respectively, of the dry weight of the tissue. Of this total, neutral fat accounted for only 4.43% in the kidney of the fifteen-day rat and 3.16% in the kidney of the seventy-day animal. More than 85% of the lipid in the kidneys of the older rats was composed of essential lipids which, obviously, must be concerned with the structure of the organ, and hence would be classified as the constant component. The distribution of the essential lipids in the kidneys of the mature rats was as follows: phospholipids, 15.19%; cerebrosides, 1.30%; free cholesterol, 1.00%; and cholesterol ester, 0.94%. The phospholipids were composed of cephalin, 7.40%, lecithin, 5.96%, and sphingomyelin, 1.83%. Srere *et al.*[7] demonstrated that cholesterol synthesis normally occurs in rat kidney.

Variations in the lipid content of the kidney are associated with species. Mottram[176] and Turner[177] called attention to the relatively high level of total lipids in the kidneys of the cat. The total fatty acids were found to average 4.9% (based upon moist weight) in the cat kidney, while the figure for beef kidney was only 2%, and that for the human kidney was 1.9%. In a compilation of the composition of human kidneys, made by Cowie and Magee,[178] the average figures for total lipids, recorded in two series of tests on normal individuals, were 2.06 and 1.87% of the moist tissue.

When one examines the kidney lipids qualitatively, a certain variability, ascribable to species, is apparent. Javillier and associates[179] listed the total phospholipid content in the kidneys of the several species as follows: pigeon, 3.3% (moist weight); frog, 2.9%; rat, 2.9%; guinea pig, 2.8%; and horse, 2.6%. Variations in iodine number have been noted by Turner[177]; the values for cat kidney were from 53 to 113, while figures of 134 and 131 were reported for the fatty acids of beef and human kidneys, respectively. Thannhauser *et al.*[36] have cited the following figures for the distribution of phospholipids in two normal human kidneys: total phospholipid, 8.38% (based upon dry weight); lecithin, 4.34%; cephalin,

[176] V. H. Mottram, *J. Physiol.*, *50*, 380–390 (1916).

[177] K. Turner, *Biochem. J.*, *25*, 49–56 (1931).

[178] D. M. Cowie and M. C. Magee, *Arch. Internal Med.*, *53*, 391–399 (1934).

[179] M. Javillier, A. Crémieu, and H. Hinglais, *Bull. soc. chim. biol.*, *10*, 327–337 (1928); also cited by W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 199.

3.26%; and sphingomyelin, 0.78%. According to Cowie and Magee,[178] the average cholesterol values reported for the kidney of different series of normal individuals average 0.293% of moist weight (0.272, 0.250, 0.310, 0.340, and 0.293).

Bloor[23,49] made a detailed analysis of the lipids of beef kidney, and obtained the following values: total lipids, 2.53% (moist basis); lecithin, 0.88%; cephalin, 0.74%; acetone-soluble fraction, 0.90%; and unsaponifiable fraction, 0.19%. The total fatty acids had iodine numbers of 109, 110, and 82 in the cephalin, lecithin, and acetone-soluble fractions, respectively. Moreover, the liquid fatty acids in these fractions were found to be exceedingly highly unsaturated; the values for iodine numbers found in the liquid fatty acids were 179, 156, and 136, respectively, in the corresponding fractions.

A slight increase in the proportion of total lipids in the kidney occurs with increasing age. However, when one compares the values for the increase in essential lipids, it is observed that a 21% greater content is present in the seventy-day rats than in the fifteen-day animals; in the same period, a 29% decline occurs in the neutral fat fraction, which decreases from 4.43 to 3.16%. The greatest proportional increase occurs in the phospholipids (27%), sphingomyelin increasing 93%, cephalin increasing 27%, and the quantity of lecithin being augmented[74] to the extent of 14%. For a further analysis of these changes, see page 573.

One of the most important factors concerned with the nature of the kidney lipids is diet. The kidneys are almost as active as the liver in the incorporation of ingested fatty acids into the phospholipid. Perlman *et al.*[11] found that the maximum level of labeled phospholipid was reached in the kidney about fifteen hours after it was fed, while it required only ten hours to reach the peak in the case of the liver. The phospholipid was retained longer in the kidney than in the liver or intestine. Similar results were reported by Artom *et al.*[180,181] On the other hand, the rate of incorporation of radioactive phosphorus into the phospholipid of the kidney is uninfluenced by the feeding of fat, in contradistinction to the response of the phospholipids in the intestine and liver after fat is administered.[11] Sinclair,[157] using elaidic acid as a tracer, reported that the time required for a complete turnover of phospholipid in the kidney of the rat is approximately a week, as contrasted with an interval of eighteen hours for the liver.

[180] C. Artom, G. Sarzana, C. Perrier, M. Santangelo, and E. Segrè, *Arch. internat. physiol.*, *45*, 32–39 (1937).

[181] C. Artom, G. Sarzana, C. Perrier, M. Santangelo, and E. Segrè, *Nature*, *139*, 836–837, 1105–1106 (1937).

Chargaff and associates[182] noted that the rate of synthesis of lecithin exceeds that of cephalin in the kidney, as was also the case in the liver and in the intestine, but not in the brain. It has been found by Weissberger[183] that kidney phospholipid is concerned with the regulation of blood and body acidity by the renal excretion of acid phosphate. She suggests that this physiologic function may explain the rapid turnover of phospholipid in this organ. Further studies in this field, made with deuterio-fats, have been reported by Cavanagh and Raper.[97] According to Ellis and Gardner,[184] the cholesterol content of the kidneys is uninfluenced by diet.

The kidney, like the liver, is quite susceptible to injury when lipogenic diets are fed.[185] Presumably, due to the rapid turnover of phospholipids, these organs require a continuous supply of phospholipids for replenishing this loss. If such compounds are not available, damage to the cell structure occurs, and the injury is soon reflected by a disordered function. Phospholipid deficiency, *i.e.*, choline deficiency, is especially readily produced in weanling rats, in whose case kidney injury will appear within a ten-day interval. Patterson and McHenry[186] proved that kidneys from these choline-deficient rats have a lower than normal phospholipid content. The liver phospholipids from choline-deficient rats are also reduced, and it is suggested[186] that the renal hemorrhagic lesions may be due to this deficiency. A kidney injury somewhat similar to that resulting from choline deficiency has been reported in rats which have received a diet devoid of essential acids, over a prolonged period.[187] In this case also, the injury appears to be related to the inability of the rat to synthesize enough phospholipids to serve as the structural portion of the kidney when the essential fatty acids are absent from the diet.

Although large amounts of fat associated with the kidney consist of perirenal fat located outside of the organ, definite concentrations occur within the kidney itself. Thus, Gairns and Morrison[188] were able to demonstrate, histologically and chemically, large accumulations of lipids in the tubule cells of the nephrons of cats. The concentration of the fat increased with age, and its location and the size of the intracellular fat globules varied widely with increasing age. Dallemagne and associates[189] found

[182] E. Chargaff, K. B. Olson, and P. F. Partington, *J. Biol. Chem.*, *134*, 505–514 (1940).
[183] L. H. Weissberger, *J. Biol. Chem.*, *132*, 219–226 (1940).
[184] G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc.*, *B85*, 385–393 (1912).
[185] W. H. Griffith and N. J. Wade, *J. Biol. Chem.*, *131*, 567–577 (1939).
[186] J. M. Patterson and E. W. McHenry, *J. Biol. Chem.*, *145*, 207–211 (1942).
[187] G. O. Burr and M. M. Burr, *J. Biol. Chem.*, *82*, 345–367 (1929).
[188] F. W. Gairns and S. D. Morrison, *J. Physiol.*, *110*, 17P (1949).
[189] M. J. Dallemagne, M. A. Gerebtzoff, and E. Philippot, *Science*, *112*, 148 (1950).

that, in the case of dogs, fat deposition occurred in the proximal tubules of the kidney as well as in other organs, following chronic intoxication with γ-hexachlorocyclohexane. The kidney effect of the δ-isomer was less marked, while D.D.T. did not cause any fat deposition in these organs.

4. Lipids Present in the Muscles

(*1*) *Lipids in Different Types of Muscles*

The composition of the muscle lipids varies with the type of muscle involved. There are three types, voluntary, cardiac, and involuntary. The characteristic physiologic properties differ for each type. Bloor[190] describes these properties as follows:

Voluntary or *skeletal* muscles are able to contract quickly and strongly, and can be tetanized, but are not spontaneously active; they are capable of large expenditures of energy; they have a single nerve supply, which is from the outside.

Cardiac or *heart* muscles possess a spontaneous rhythmic activity; they have an absolute refractory period, so that tetany is impossible, an "all or none" contraction, an intrinsic nerve supply, and a regulatory nerve supply from the outside.

Involuntary or *smooth* muscles have spontaneous rhythmic activity, ordinarily with small tensions and slow motions requiring relatively small energy expenditure; there is an intrinsic nerve net which insures spontaneous activity, and a regulatory nerve supply from the outside.

The lipids vary qualitatively and quantitatively with the type of muscle, with the species of animal, with age, and with the amount of activity. Of these three types of muscle, the cardiac muscle invariably has the highest phospholipid content, while involuntary muscle has the highest and voluntary muscle the lowest cholesterol content. Although the heart may, in some cases, have as high a cholesterol value as involuntary muscle, the phospholipid:cholesterol ratio between these two muscles is quite different. The P:C ratio for cardiac and voluntary muscle approximates 15:1, while involuntary muscle has a 4:1 ratio. The neutral fat in the muscles is similar to that in other fat depots.[191] Quagliariello[192] believes that the lipids in the muscles are localized in the fibrils, and that they are absent from sarcoplasm.

Perry[193] was able to sediment lipoprotein granules by high-speed centrifugation of muscle homogenates from which the myofibrils and nuclei had been removed. These lipoprotein granules possessed ATPase (adenosine

[190] W. R. Bloor, *J. Biol. Chem.*, *114*, 639–648 (1936).
[191] E. Klenk and F. Ditt, *Z. physiol. Chem.*, *226*, 213–220 (1934).
[192] G. Quagliariello, *Arch. internat. physiol.*, *16*, 239–250 (1921).
[193] S. V. Perry, *Biochim. et Biophys. Acta*, *8*, 499–509 (1952).

triphosphatase) activity on activation with Mg. It is believed that this preparation is identical with the ATPase in the Kielley-Meyerhof system. However, it differs from myosin ATPase, which is localized in the isolated myofibrils.

In addition to the lipids normally noted in other tissues, several aldehydes are also contained in muscle. Thus, Klenk and co-workers[194] isolated a mixture of C_{14}, C_{16}, and C_{18} aldehydes, in the form of the dimethyl acetals, from the phospholipids of horse muscle and beef heart, as well as Δ^9-octadecenal. However, no Δ^{11}-octadecenal was detected, although this is known to be a component of brain phosphatides. These plasmalogens, as they are called, were found to account for 6.5% of the total phospholipids of swine heart.[40] In a later study, Klenk and Gehrmann[195] reported that the plasmalogens of beef heart are concentrated in the lecithin fraction, and that only a relatively minor proportion are present in the several cephalin fractions.

Different lipid patterns have been found in the conductive and connective tissues of beef heart, as contrasted with the heart muscle. One important variation reported by Mallov, McKibbin, and Robb[196] was in the cerebroside content, which was 0.01 to 0.02% of the fresh weight in the heart muscle, and 0.03% of the fresh weight in the conducting tissue of this organ.

(2) *The Effect of Species on the Composition of Muscle Lipids*

Bloor and Snider,[197] and later Bloor alone,[190] recorded the lipid composition of the different types of muscle from a wide variety of animals. Table 3 gives the average figures for the lipids in the various types of muscle from different groups of animals, while Table 4 furnishes individual data on a large number of animals. Table 5 is a summary of the phospholipid and cholesterol content of the different types of muscle of various species of birds and of cold-blooded animals (see pages 734, 735).

Several other studies have been made of muscle composition. One of these is reported by Javillier *et al.*,[179] who cited the following figures for phospholipids (expressed in percentage of moist weight); pigeon, 2.4%; horse, 1.7%; guinea pig, 1.5%; frog, 1.2%; and rat, carp, and dogfish, each 1.1%.

[194] E. Klenk, W. Stoffel, and H. J. Eggers, *Z. physiol. Chem.*, *290*, 246–251 (1952).
[195] E. Klenk and G. Gehrmann, *Z. physiol. Chem.*, *292*, 110–117 (1953).
[196] S. Mallov, J. M. McKibbin, and J. S. Robb, *J. Biol. Chem.*, *201*, 825–838 (1953).
[197] W. R. Bloor and R. H. Snider, *J. Biol. Chem.*, *107*, 459–470 (1934).

TABLE 3
AVERAGE CHOLESTEROL AND PHOSPHOLIPID CONTENT OF SKELETAL, CARDIAC, AND SMOOTH MUSCLES OF GENERAL CLASSES OF ANIMALS[a]

Category	Mammals	Birds	Cold-blooded animals
Skeletal muscle (thigh)			
Phospholipid, %	4.59	4.34	4.14
Cholesterol, %	0.27	0.31	0.23
P:C ratio	17	14	18
Cardiac muscle (ventricle)			
Phospholipid, %	7.65	7.56	6 00
Cholesterol, %	0.51	0.54	0.75
P:C ratio	15	14	8
Smooth muscle			
Phospholipid, %	3.52[b]	2.84[c]	—
Cholesterol, %	0.88[b]	0.71[c]	—
P:C ratio	4	4	—

[a] Adapted from W. R. Bloor, *J. Biol. Chem.*, *114*, 639–648 (1936).
[b] Tissue includes uterus, stomach, and intestine.
[c] Gizzards of hen, pigeon, owl, and sparrow.

Kaucher and her associates[198] reported an exceedingly comprehensive study on the lipid composition of the voluntary muscle of a number of mammals, of chickens, of amphibia, and of fishes, as well as of the smooth muscle from the intestine and stomach of cattle. Their data are summarized in Table 6 (page 736).

Although the species examined are for the most part different from those studied by Bloor,[190] the results of Kaucher *et al.*[198] offer excellent confirmation for the former work. This is especially the case with the phospholipid: cholesterol ratio, which averages 15 for the skeletal muscle of mammals and 4 for the smooth muscle of cattle. The shrimp, whose muscles are largely of the smooth type, has a similarly low P:C ratio. The cholesterol values are exceedingly high in the smooth muscle, which largely accounts for the low P:C ratio.

Bloor[199] reports that lecithin and cephalin are present in skeletal muscle in about the same amounts, lecithin being somewhat higher; MacLachlan[200] also found a considerably higher lecithin:cephalin ratio for smooth muscle (chiefly uterus). The results of Kaucher *et al.*[198] substantiate these results: the lecithin:cephalin ratios in the skeletal muscle of the four mammals

[198] M. Kaucher, H. Galbraith, V. Button, and H. H. Williams, *Arch. Biochem.*, *3*, 203–215 (1943).
[199] W. R. Bloor, *J. Biol. Chem.*, *72*, 327–343 (1927).
[200] P. L. MacLachlan, unpublished results; cited by W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 201.

TABLE 4

THE PHOSPHOLIPID AND CHOLESTEROL CONTENT (% DRY WEIGHT) OF SKELETAL MUSCLE (THIGH), CARDIAC MUSCLE (VENTRICLE), AND OF SEVERAL SMOOTH MUSCLES OF DIFFERENT MAMMALS[a]

Systematic name	Common name	Skeletal muscle (thigh)			Cardiac muscle (heart)			Smooth muscle		
		Phospholipid	Cholesterol	P:C[b]	Phospholipid	Cholesterol	P:C[b]	Phospholipid	Cholesterol	P:C[b]
Homo sapiens	Man	—	—	—	7.00	0.70	10	3.50[c]	1.00	3.5
Zalophus californianus	California sea lion	—	—	—	5.60	0.40	14	—	—	—
Macropus rufus	Red kangaroo	2.38	0.17	14	6.72	0.32	21	—	—	—
Canis familiaris	Dog	8.00	0.32	25	8.54	0.61	14	3.00[c]	1.00	3
Felis domestica	Cat	2.47	0.19	13	5.72	0.44	13	3.24[d]	0.81	4
Lepus, spp.	Hare, rabbit	1.70	0.17	10	9.12	0.57	16	4.40[c]	1.10	4
Sylvilagus floridanus	"Cottontail," wild rabbit (Florida)	3.75	0.25	15	7.65	0.45	17	2.50[e]	0 50	5
Lepus townsendii	White-tailed jack-rabbit	8 75	0.35	25	7.22	0.38	19	—	—	—
Cavia cobaya	Guinea pig	3.30	0.33	10	5.72	0.44	13	—	—	—
Citellus richardsonii	American ground squirrel, souslik, pouched marmot	7.50	0.30	25	9.60	0.80	12	—	—	—
Rattus, spp.	Rat	3.50	0.25	14	7.95	0.53	15	—	—	—
Peromyscus maniculatus	White-footed mouse	6.72	0.42	16	—	—	—	—	—	—
Average		*4.81*	*0.28*	*17*	*7.35*	*0.51*	*15*	*3.33*	*0.88*	*3.9*

[a] Adapted from W. R. Bloor, *J. Biol. Chem.*, *114*, 639–648 (1936).
[b] Phospholipid:Cholesterol ratio. [c] Uterus. [d] Intestine. [e] Stomach.

TABLE 5

PHOSPHOLIPID AND CHOLESTEROL CONTENT (% DRY WEIGHT) OF SKELETAL MUSCLE (THIGH), AND CARDIAC MUSCLE (VENTRICLE) OF BIRDS AND OF COLD-BLOODED ANIMALS, AND OF SMOOTH MUSCLE (GIZZARD) OF BIRDS[a]

Category	Birds						Cold-blooded animals				
	Arctic wild duck[b]	Hen[c]	Pigeon[d]	House sparrow[e]	Screech-owl[f]	Av.	Turtle[g]	Leopard frog[h]	Alligator[i]	Grasshopper[j]	Av.
Skeletal muscle (thigh)											
Phospholipid	3.78	3.75	4.51	4.40	3.64	4.02	3.50	3.80	2.04	5.40	3.68
Cholesterol	0.21	0.25	0.41	0.40	0.28	0.31	0.35	0.20	0.17	0.18	0.22
P:C[k]	18	15	11	11	13	14	10	19	12	30	18
Cardiac muscle											
Phospholipid	7.80	7.56	7 28	8.55	6.16	7.47	6.40	4.20	7.50	—	6.03
Cholesterol	0.52	0.54	0.52	0.57	0.56	0.54	0 80	0.70	0.75	—	0.75
P:C[k]	15	14	14	15	11	14	8	6	10	—	8
Smooth muscle (gizzard)											
Phospholipid	—	2.00	2.52	2.88	3.50	2.75	—	—	—	—	—
Cholesterol	—	0.50	0.63	0.72	1.00	0.71	—	—	—	—	—
P:C[k]	—	4	4	4	3.5	3.9	—	—	—	—	—

[a] Adapted from W. R. Bloor, *J. Biol. Chem.*, *114*, 639–648 (1936).
[b] Red-throated diver, *Colymbus septentrionalis*. [c] *Gallus gallus*. [d] *Columbidae* family. [e] *Passer domesticus*. [f] Mottled American, *Scops asio*. [g] Scribe, *Pseudemys scripta elegans*. [h] *Rana pipiens*. [i] North American, *Alligator mississippiensis*. [j] Lubber, buffalo hopper, *Brachystola magna*. [k] Phospholipid:cholesterol ratio.

TABLE 6

LIPID CONTENT OF THE MUSCLE TISSUE OF VARIOUS SPECIES, EXPRESSED IN PER CENT DRY WEIGHT[a]

Muscle	Phospholipids				Cholesterol			P:C[b]	Cerebrosides	Total essential lipids	Neutral fat	Total lipids
	Total	Lecithin	Cephalin	Sphingomyelin	Total	Free	Ester					
Beef	3.08	1.72	1.12	0.24	0.22	0.19	0.03	14	0.94	4.24	9.97	14.21
Pork	3.06	1.69	1.25	0.12	0.20	0.14	0.06	15	1.17	4.43	17.97	22.40
Veal	5.04	2.70	2.15	0.19	0.32	0.16	0.16	16	0.62	5.98	5.86	11.84
Lamb	4.74	2.55	1.90	0.29	0.31	0.13	0.18	15	1.95	7.00	4.19	11.19
Chicken (dark)	4.36	1.70	2.39	0.27	0.40	0.28	0.12	11	1.26	6.02	6.65	12.67
Chicken (light)	2.72	1.91	0.81	0.00	0.25	0.17	0.08	11	1.99	4.96	1.99	6.95
Turtle	5.25	2.98	2.27	0.00	0.37	0.26	0.11	14	0.91	6.53	10.89	17.42
Frog	7.14	2.79	4.16	0.19	0.26	0.18	0.08	27	1.76	9.16	1.72	10.88
Salmon	4.39	2.53	1.86	0.00	0.19	0.11	0.08	23	3.96	8.54	9.76	18.30
Codfish	4.28	3.31	0.49	0.48	0.35	0.22	0.13	12	2.64	7.27	2.20	9.47
Shrimp	3.89	2.63	1.03	0.23	0.77	0.70	0.07	5	1.22	5.88	2.24	8.12
Beef (intestine)	6.93	3.81	1.86	1.26	1.49	0.76	0.73	4.5	0.32	8.74	2.89	11.63
Beef (stomach)	3.10	1.60	0.88	0.62	0.89	0.64	0.25	3.5	0.39	4.38	3.60	7.98

[a] Adapted from M. Kaucher, H. Galbraith, V. Button, and H. H. Williams, *Arch. Biochem.*, *3*, 203–215 (1943).

[b] Phospholipid:cholesterol ratio.

were 1.53, 1.35, 1.26, and 1.34, respectively, as contrasted with figures of 2.05 and 1.82 in the two samples of smooth muscle.

A relatively high proportion of liquid (unsaturated) fatty acids to solid (saturated) fatty acids is present in muscle phospholipid. The proportion found by Snider[201] was 73 for the liquid acids to 27 for the saturated acids. This is much higher than the 55:40 proportion reported for liver.[202] The constancies of these ratios have been confirmed by Sinclair.[203] The iodine absorption value for the liquid fatty acids of muscle averaged 173, which is lower than that found for the corresponding fraction in the liver (2.22).

In the various muscles examined, the cerebrosides are the highest in salmon and codfish, where they amount to 3.96 and 2.64%, respectively. Cerebrosides are the lowest in the smooth muscles of the stomach and intestine. Neutral fat reaches the highest level in pork and the lowest in the frog; however, the essential lipids are highest in the latter animal.

(3) *The Effect of Age on Muscle Lipids*

Whereas practically every tissue of seventy-day rats examined by Williams and his co-workers[74] had a higher lipid content than did those of fifteen-day rats, skeletal muscle was unique in presenting the opposite picture. The total lipids of the skeletal muscle of fifteen-day rats averaged 27.79%, while those of the rats sacrificed fifty-five days later amounted to only 15.84%. However, during this period, the essential lipids had increased from 8.21 to 12.40%; this is a rise of 51%. The decrease in total lipids resulted from a decline in neutral fat from 19.58% at fifteen days to 3.44% at seventy days. The muscle was found to differ from all other tissues examined in that the fat content in the young animal was high. Another especially noticeable change was the increase in cerebrosides, which amounted to 146% (1.45 to 3.57%). In the seventy-day rat, only the brain and testes had a higher cerebroside content than did the skeletal muscle. These changes occurred in spite of the fact that the total proportion of the body weight composed of muscle tissues increased from 24% at birth to 41% at the age of seventy days.[204] In contradistinction to the conditions existing in skeletal muscle, the fat content of cardiac muscle increases somewhat as the rat matures, and falls in line with the changes in most other tissues during the growth interval from fifteen to seventy days.

[201] R. H. Snider, *J. Biol. Chem.*, *116*, 503–510 (1936).
[202] R. H. Snider and W. R. Bloor, *J. Biol. Chem.*, *99*, 555–573 (1933).
[203] R. G. Sinclair, *J. Biol. Chem.*, *111*, 261–273 (1935).
[204] H. H. Donaldson, *The Rat*, Memoirs Wistar Inst. Anat. Biol., 2nd ed., Philadelphia, 1924, p. 184.

For further information as to the changes in the muscle lipids of rats, as related to age, see Tables 9 and 10 of Chapter VI. The cholesterol content has been reported to be greater in the muscles of the calf than in the corresponding muscles of the cow.[205]

(4) *The Effect of Increased Metabolic Activity and of Exercise on Muscle Lipids*

Pasternak and Page[206] reported that the injection of thyroxine markedly increased the phospholipids of muscle, but not the cholesterol content. This observation is difficult to correlate with the results of Onizawa,[207] who noted that a decrease in muscle cholesterol occurred concomitantly with the removal of the thyroid gland.

The muscle is apparently responsive to increased activity in the form of exercise, and reacts by changes in both phospholipids and cholesterol. Not only the amount of lipids but also their character varies with the extent of exercise to which the muscle is subjected. Reed and her coworkers[208] found that forced activity and voluntary nocturnal activity increased the proportion of intermuscular lipids in rats. Bloor and Snider[197] also reported a correlation between the extent of activity of beef muscle and its phospholipid content. The higher the degree of activity of the muscle, the higher was the level of phospholipids. Embden and Lawaczeck[209] noted that the cholesterol content of the different muscles of the normal rabbit varied with the activity of the muscle.

In a further investigation of this problem, Bloor[210] found that the muscles of active rats and pigeons generally presented higher phospholipid values than did the corresponding muscles of inactive animals. In the case of muscles from chickens which had suffered from "range paralysis," it was found that the phospholipid was invariably low, and the cholesterol higher than normal. The P:C ratios were as follows, as compared with normal values (in parentheses): ventricle, 5(14); thigh, 6(16); pectoralis major, 5(8); gizzard, 1.1(4). Furthermore, in muscles from patients suffering from muscular dystrophy, Bloor[210] noted P:C ratios varying from 1 to 8 instead of a normal value of 14. In later studies Bloor[211] found that, after

[205] K. Hotta, *Z. physiol. Chem.*, *125*, 220–228 (1923).

[206] L. Pasternak and I. H. Page, *Biochem. Z.*, *274*, 122–145 (1934).

[207] J. Onizawa, *J. Biochem.* (*Japan*), *10*, 425–434 (1928–1929).

[208] L. L. Reed, F. Yamaguchi, W. E. Anderson, and L. B. Mendel, *J. Biol. Chem.*, *87*, 147–174 (1930).

[209] G. Embden and H. Lawaczeck, *Z. physiol. Chem.*, *125*, 199–209 (1923).

[210] W. R. Bloor, *J. Biol. Chem.*, *119*, 451–465 (1937).

[211] W. R. Bloor, *J. Biol. Chem.*, *132*, 77–82 (1940).

three generations, phospholipid and especially cholesterol were increased in the muscles of rats which had been strenuously exercised. Hypertrophy also occurred in most cases. These data are interpreted as indicating that the increased levels of phospholipid and cholesterol may to some extent be inherited. Table 7 summarizes the data of the Bloor tests (page 740).

(5) *The Effect of Atrophy on Muscle Lipids*

Morgulis and collaborators[212] reported that, when nutritional dystrophy was induced in rabbits, a marked increase in cholesterol resulted. An increase in phospholipid content was also noted in skeletal muscle, but no change in phospholipid occurred in cardiac muscle. The changes in lipid content were most marked in the gastrocnemius muscle, and least marked in the abdominal muscles. The rise in cholesterol occurred first, and was followed by an increase in phospholipid, and finally in neutral fat. Under conditions of muscular dystrophy, a rise in ester cholesterol was found; proportions of cholesterol ester as high as 27% were noted in dystrophic muscles, as compared with values of 4 to 8% in the muscles of normal animals.

In the case of muscles undergoing atrophy, following denervation, Humoller *et al.*[213] found that the lipids of the affected muscle were broken down at a rate faster than they could be replenished. In muscles which had been denervated for longer than three days, the total fat and phospholipid content gradually increased. The total fat exceeded that of the contralateral control muscles within fifteen days after the operation. In contradistinction to the behavior of other lipids, cholesterol does not exhibit the initial drop in the early phases of atrophy, but it begins to increase in amount immediately after neurotomy. It is suggested that the metabolic disturbances in the muscle resulting from nerve section may affect the cholesterol in a different manner than they do the phospholipids and total fat.

(6) *The Effect of Fasting on Muscle Lipids*

Although it is generally accepted that fat stored intermuscularly may decrease during fasting, most workers have agreed with Terroine[214] that

[212] S. Morgulis, V. M. Wilder, H. C. Spencer, and S. H. Eppstein, *J. Biol. Chem.*, *124*, 755–766 (1938).

[213] F. L. Humoller, D. Hatch, and A. R. McIntyre, *Am. J. Physiol.*, *169*, 654–658 (1952).

[214] E. F. Terroine, *Contribution à la connaissance de la physiologie des substances grasses et lipoidiques*, Masson, Paris, 1919; *Ann. sci. nat. Zool.* [*10*], *4*, 5–397 (1920).

TABLE 7

EFFECT OF EXERCISE ON THE SIZE OF MUSCLES AND ON THE PHOSPHOLIPID AND CHOLESTEROL CONTENT OF THE FIRST (I), SECOND (II), AND THIRD (III) GENERATION RATS[a]

Muscle	Change in muscle size, %		Phospholipid content[b]			Change in phospholipids, %		Cholesterol content[b]			Change in cholesterol, %	
	I	II–III	Resting	I	II–III	I	II–III	Resting	I	II–III	I	II–III
Heart	+16.5	+68.2	1.68	1.94	1.67	+15.5	− 0.6	0.119	0.135	0.156	+ 7.4	+31.1
Diaphragm	+38.5	+36.2	1.05	0.92	1.12	−14.1	+ 6.7	0.091	0.077	0.148	−18.2	+62.6
Thigh	+12.9	−55.5	0.84	0.87	0.97	+ 3.6	+15.3	0.064	0.060	0.088	− 6.6	+37.5
Front leg	+16.1	+20.6	0.89	1.03	1.09	+15.7	+22.5	0.072	0.077	0.111	+ 6.9	+54.2
Abdominal wall	+ 8.0	+36.9	0.73	0.77	0.84	+ 5.5	+15.2	0.066	0.071	0.082	+ 7.6	+24.4
Gastrocnemius	+21.1	+53.2	0.85	0.97	1.09	+14.1	+28.2	0.068	0.076	0.119	+11.8	+75.0
Back and loin	+17.9	+91.6	0.73	0.82	0.85	+12.3	+16.2	0.059	0.062	0.091	+ 5.1	+54.2
Pectoralis	+ 8.9	+60.1	0.89	0.97	1.07	+ 9.0	+21.3	0.074	0.077	0.105	+ 4.1	+41.8

[a] Adapted from W. R. Bloor, *J. Biol. Chem.*, *132*, 77–82 (1940).

[b] Expressed in per cent moist weight.

other lipids change only to a minimum extent during fasting. On the other hand, Cahn[215] reported that the value for phospholipids decreased during inanition. De Boer[216] found only insignificant changes in the lipid content of the skeletal muscles in dogs when dehydration was produced by withholding food and water.

5. Lipids Present in the Brain and Nervous Tissue

Brain and other nervous tissue is characterized by its high lipid content; in fact, adipose tissue is the only tissue which contains a higher proportion of lipids than does brain. According to West and Todd,[217] Donaldson has indicated that the brain represents about 2% of the total body weight in man, and the spinal cord and nerves account for an additional 0.4%. The weight of the nervous tissue in a man weighing 68 kg. has been estimated at 1620 g.; the brain accounts for 1400 g. of this amount; the spinal nerves are responsible for 151 g.; the sympathetic nerves weigh approximately 30 g.; the spinal cord amounts to 27 g.; and the cranial nerves make up 12 g. of the total. Although the function of the lipids of the central nervous system has generally been assumed to be purely structural, certain brain preparations are able to break down phospholipids. This fact led Stanley[218] to postulate that there may be a direct connection between the metabolism of certain brain lipids and nervous and mental activity.

(1) The Lipid Composition of Normal Brain

The classical experiments to determine the composition of brain were carried out by the German chemist, Thudichum, whose first report[219] was made in 1874; this was extended and amplified 10 years later in a subsequent publication.[220]

[215] T. Cahn, *Ann. physiol. physicochim. biol., 3,* 4–60 (1927).

[216] B. de Boer, *Am. J. Physiol., 147,* 49–53 (1946).

[217] E. S. West and W. R. Todd, *Textbook of Biochemistry,* Macmillan, New York, 1951, pp. 197, 421, 425.

[218] G. H. S. Stanley, *Biochem. J., 50,* xxiv (1952).

[219] J. L. W. Thudichum, *Researches on the Chemical Constitution of the Brain,* Report of the Medical Officer of the Privy Council and Local Government Board, *n.s. 3, No. 5,* 113–247 (1874); cited by H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide,* Springer, Berlin, 1930, p. 63.

[220] J. W. L. Thudichum, *A Treatise on the Chemical Composition of the Brain,* Baillière, Tindall, and Cox, London, 1884; cited by H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide,* Springer, Berlin, 1930, pp. 65 ff., and by G. Blix, *Z. physiol. Chem., 219,* 82–98 (1933).

The composition of the whole brain, as well as of the gray and white matter, is given in Table 8 and that of the nerves in Table 9.

TABLE 8

THE COMPOSITION OF WHOLE ADULT BRAIN, OF THE GRAY MATTER OF THE CORTEX, AND OF THE WHITE MATTER OF THE CORPUS CALLOSUM[a]

Substance	Fresh adult brain, %	Gray matter, %	White matter, %
Water	77	84	70
Solids	23	16	30
Proteins	8–9	7.5[b]	8.7
Total lipids	12–15	2.5[b]	19.5
Cholesterol	3.6–4.8	1.2[b]	3.1
Phospholipids	5.7–6.9	0.8[b]	9.0
Cerebrosides	1.3–2.6	0.4[b]	5.1
Sulfolipids	0.66–1.8	—	2.3
Extractives (Total)	2.1–2.9	3.0[b]	(1.0)
Organic	1.1–2.0	—	—
Inorganic	0.9–1.0	—	—
Ash	1.5	1.0	1.75

[a] Adapted from I. H. Page, *Chemistry of the Brain*, C. C Thomas, Springfield, Ill., 1937.

[b] Figures cited by E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951, p. 422.

The composition of the brain shows marked variation in various portions of its structure. For example, the white matter has about twice the content of total solids that is found in the gray matter. The high solid content of the white matter is accounted for largely by the increased proportion of lipid as compared with gray matter. The chief lipids in the brain and nervous tissues are cholesterol, phospholipids, cerebrosides, and ill-defined compounds known as sulfolipids or sulfatides. Practically no neutral fat is present in the brain and nerves of the adult.

a. Phospholipid Components in the Brain. In the early studies of Thudichum,[221] the cephalin and lecithin content of the moist gray matter was given as 0.33 and 1.59%, and that of the moist white substance as 0.09 and 0.73%, respectively. Bloor[23] recorded the following figures for whole beef brain: cephalin, 2.36%; lecithin, 2.23%; and acetone-soluble products, mostly cholesterol, 1.88%.

The lecithins and cephalins in brain consist of both the α- and the β-

[221] J. W. L. Thudichum, *Die chemische Konstitution des Gehirns des Menschen und der Tiere*, Pietzcker, Tübingen, 1901; cited by W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 203.

TABLE 9

THE LIPID DISTRIBUTION IN NERVE STRUCTURES[a]

Category	Gray matter, %					White matter, %		
				Cerebral cortex				
	Claw nerve (lobster)[b]	Brain (bee)[c]	Splenic nerve (cow)[b]	Human fetus (4 1/2 mo.)[b]	Adult human[b]	Human brain[b]	Spinal cord (cow)[b]	Ischiatic nerve (rabbit)[b]
Total solids[d]	13.9	—	16.7	9.6	15.1	29.2	36.2	35.4
Total lipids[e]	13.7	39.0	15.5	22.5	35.1	61.2	75.8	55.9
Cholesterol[e]	1.9	1.3	2.1	2.4	5.1	13.8	15.9	11.5
Cerebrosides[e]	0.8	—	1.6	2.4	4.2	16.0	19.7	10.9
Phospholipids[e]	7.3	17.0	8.3	12 4	20.4	26.5	33.7	27.4
Lecithin	6.2	2.0	2.5	4.4	7.3	5.7	6.3	3.6
Cephalin A	1.1	12 0	3.4	7.1	9.3	14.1	17.1	13.4
Cephalin B	—	—	0.8	—	2.0	3.0	5.2	4.1
Sphingomyelin	0	1.5	1.6	0.9	1.8	3.7	5.1	6.5
Diglycerides[e]	—	2.5[f]	0.9	—	1.1	—	0	2.0

[a] E. Klenk, *Der chemische Aufbau der Nervenzellen und der Nervenfasern*, Colloquium Ges. physiol. Chemie, Mosbach, Baden, April 26–27, 1952, p. 35.

[b] G. Brante, *Acta Physiol. Scand., 18, Suppl. 63*, 7–189; Appendix A, 1–24; Appendix B, i-xxviii (1949).

[c] E. K. Patterson, M. E. Dumm, and A. G. Richards, Jr., *Arch. Biochem., 7*, 201–210 (1945).

[d] % Moist weight.

[e] % Dry weight.

[f] Neutral fat.

varieties. Using a new technic for the differential estimation, Welch[222] showed that the α-type is the more common, in the case of both lecithins and cephalins. Thus, in rat brain, the phospholipids were fractionated as follows: α-lecithins, 35%; β-lecithins, 22%; α-cephalins, 20.5%; and β-cephalins, 13%. That this distribution is not confined to the rat is shown by the fact that a similar situation obtains in beef brain. In a crude cephalin fraction of beef brain, 59% was accounted for as the α-isomer and 28% as the β-component. Similarly, in a crude lecithin preparation, the amounts of the pure α- and β-fractions isolated were 73 and 20%, respectively.

In a further study of brain cephalin fractions, Burmaster[223] showed that inositol phospholipid is the principal component of the β-cephalin fraction of calf brain, while phosphatidylserine and phosphatidylethanolamine are

TABLE 10
THE COMPOSITION OF THE FATTY ACID MIXTURES OF THE ESTER PHOSPHATIDES OF THE BRAIN IN PER CENT OF TOTAL FATTY ACIDS[a]

Type of acid	Number of carbons	Fatty acid composition in % of total		
		Lecithin	Phosphatidyl-ethanolamine	Phosphatidyl-serine
Saturated.........	14	Trace	Trace	—
	16	29.0	9.7	3.2
	18	9.0	9.7	32.7
	20	1.2	1.5	—
	22	—	0.9	—
Unsaturated.......	16	1.2	2.3	—
	18	48.0	34.7	51.6
	20	7.3	15.1	7.1
	22	4.3	24.4	5.4
	24	—	1.7	—

[a] E. Klenk and P. Böhm, *Z. physiol. Chem.*, *288*, 98–107 (1951); E. Klenk, *Der chemische Aufbau der Nervenzellen und der Nervenfasern.* Colloquium Ges. physiol. Chemie, Mosbach, Baden, April 26–27 (1952).

the main components of the α-cephalin fraction. The fatty acid composition of these latter two cephalin fractions has recently been proved by Klenk and Böhm[224] to be quite different. These data are recorded in Table 10.

In a review article by Klenk[225] on the composition of nervous tissue, the

[222] E. A. Welch, *J. Biol. Chem.*, *161*, 65–69 (1945).
[223] C. F. Burmaster, *J. Biol. Chem.*, *165*, 577–583 (1946).
[224] E. Klenk and P. Böhm, *Z. physiol. Chem.*, *288*, 98–107 (1951).
[225] E. Klenk, *Der chemische Aufbau der Nervenzellen und der Nervenfasern*, Colloquium Ges. physiol. Chemie, Mosbach, Baden, April 26–27 (1952).

following unsaturated acids are listed as being present under certain conditions, in addition to oleic acid and hexadecenoic acid[226–229]:

C_{20} acids	Δ^{11}-Eicosenoic acid
	$\Delta^{11,14}$-Eicosadienoic acid
	$\Delta^{6,8,11}$-Eicosatrienoic acid
	$\Delta^{5,8,11,14}$-Eicosatetraenoic acid
C_{22} acids	$\Delta^{7,10,13}$-Docosatrienoic acid
	$\Delta^{4,7,10,13}$-Docosatetraenoic acid
	$\Delta^{7,10,13,16}$-Docosatetraenoic acid
	$\Delta^{4,7,10,13,16}$-Docosapentaenoic acid
	$\Delta^{7,10,13,16,19}$-Docosapentaenoic acid
	$\Delta^{4,7,10,13,16,19}$-Docosahexaenoic acid

The high proportion of unsaturated acids in the cephalin fractions in the brain is of interest. Although the C_{24}-acids are the principal acids in the case of cerebrosides, they are practically absent in the phospholipids. Beauvallet and Manuel[230] have called attention to the high proportion of arachidonic acid occurring in the phospholipids of nervous tissue; these workers suggest that this acid plays a structural rather than a dynamic role in this case.

Sphingomyelin is another important phospholipid in nervous tissue. According to Johnson *et al.*,[231] both white and gray matter are characterized by a relatively high concentration of sphingomyelin, together with cholesterol and cerebroside. The white matter in beef brain has been shown to have four times as much sphingomyelin as the gray matter.[37] According to Thannhauser and co-workers,[36] sphingomyelin accounts for 20% of the total phospholipids in the brain and for only 5 to 10% of these lipids in most other organs.

The great difference in sphingomyelin content in the brain and in the peripheral nerves is evident from the studies of Schmidt, Benotti, Hershman, and Thannhauser.[37] In the case of the rat, this compound comprised 5% of the total phospholipids in the brain, compared with 22% in the sciatic nerve. In the cat, sphingomyelin accounted for 24% of the brain phospholipids while, in the sciatic nerve, it made up 46.3 of the total phospholipid content. The variation in sphingomyelin content between the brain and the spinal cord is emphasized in the studies of Carter and asso-

[226] E. Klenk, *Z. physiol. Chem.*, *200*, 51–68 (1931).
[227] E. Klenk, *Z. physiol. Chem.*, *206*, 25–40 (1932).
[228] K. Schuwirth, *Z. physiol. Chem.*, *277*, 147–158 (1943).
[229] E. Klenk and W. Bongard, *Z. physiol. Chem.*, *291*, 104–118 (1952).
[230] M. Beauvallet and S. Manuel, *Compt. rend. soc. biol.*, *144*, 1599–1602 (1950).
[231] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *43*, xxii (1948).

ciates.[232] While these workers prepared 1300 to 1600 g. of a crude sphingomyelin-cerebroside mixture from beef brain, they were able to separate approximately twice this amount from spinal cords. Johnson *et al.*[231] suggest that sphingomyelin, together with cholesterol and cerebroside, rather than lecithins and cephalins, form the basis of "myelin."

Sphingomyelin prepared from the brain apparently has a different composition from that obtained from other tissues. Thus, Thannhauser and Boncoddo[233] proved that brain sphingomyelin, prepared in such a manner as to exclude hydrolecithin, which had previously been an invariable contaminant in preparations made by the earlier procedures, contained stearic, nervonic, and lignoceric acids. In contradistinction to this finding, sphingomyelin from lung or spleen contained only palmitic and lignoceric acids. Both spleen and brain sphingomyelin contained dihydrosphingosine, as well as the usual unsaturated sphingosine. According to Carter *et al.*,[234] dihydrosphingosine is present in a higher concentration in spinal cord than in brain.

The phospholipid fatty acids in white and in gray matter have been shown to differ in saturation.[235] Thus, the iodine value of the fatty acids isolated from the phospholipids of frontal white matter was found to be 84, as contrasted with a figure of 129 for the phospholipid fatty acids prepared from frontal gray matter.

A definite proportion of the phospholipids of beef brain has been shown to consist of acetal phospholipids. Thannhauser and co-workers[236] were able to demonstrate that these acetal phospholipids could be split catalytically into aldehydes and glycerylphosphorylcolamine (glycerylphosphorylethanolamine) in the presence of mercury salts. Under these conditions of hydrolysis, no migration of phosphoric acid was noted. The compound was entirely of the α-configuration. Klenk and Böhm[224] also reported that definite amounts of brain phospholipids consist of acetal derivatives (plasmalogens). The acetal phosphatides were found to occur chiefly in the phosphatidylethanolamine fraction, in which the fatty acid: aldehyde ratio was 2:1. In the case of phosphatidylserine, only small amounts of the acetal compounds were found, corresponding to a fatty

[232] H. E. Carter, W. J. Haines, W. E. Ledyard, and W. P. Norris, *J. Biol. Chem., 169*, 77–82 (1947).

[233] S. J. Thannhauser and N. F. Boncoddo, *J. Biol. Chem., 172*, 141–147 (1948).

[234] H. E. Carter, W. P. Norris, F. J. Glick, G. E. Phillips, and R. Harris, *J. Biol. Chem., 170*, 269–283 (1947).

[235] L. O. Randall, *J. Biol. Chem., 124*, 481–488 (1938).

[236] S. J. Thannhauser, N. F. Boncoddo, and G. Schmidt, *J. Biol. Chem., 188*, 423–426 (1951).

acid:aldehyde ratio of 20:1 in this fraction. On the other hand, Klenk, Debuch, and Daun[237] reported that choline-containing acetal compounds (lecithins) do not occur in the brain.

The aldehyde components of the plasmalogens of the brain consist exclusively of C_{14}, C_{16}, and C_{18} compounds.[225] These have been identified as follows[238,239]:

Saturated aldehydes:	Tetradecanal (trace)
	Hexadecanal
	Octadecanal
Unsaturated aldehydes:	Hexadecenal (trace)
	Δ^9-Octadecenal
	Δ^{11}-Octadecenal

Thannhauser *et al.*[240] reported that the acetal α-phospholipids of brain do not contain unsaturated aldehydes. Palmitic aldehyde was present in a greater proportion than was stearyl aldehyde.

b. Cerebrosides in the Brain. Cerebrosides were discovered by Thudichum,[219] who coined the name because he prepared these substances from the cerebrum. Although phrenosine and cerasine (kerasine) were the only two types of cerebrosides originally described, two additional compounds, namely nervone[241] and oxynervone,[242] have since been discovered. Klenk and Faillard[243] demonstrated the presence of two new additional isomeric unsaturated hydroxy acids in brain cerebrosides which had hitherto been unrecognized. These acids were proved to be:

Δ^{15}-*n*-α-hydroxytetracosenoic acid, $CH_3(CH_2)_7CH:CH(CH_2)_{12}CHOHCOOH$,

Δ^{17}-*n*-α-hydroxytetracosenoic acid, $CH_3(CH_2)_5CH:CH(CH_2)_{14}CHOHCOOH$.

This brings to six the known varieties of cerebrosides, which vary only in the composition of the C_{24}-acid.

In 1940, Halliday and co-workers[244] demonstrated that two general classes of cerebrosides occur in animal tissues, one containing galactose as the carbohydrate component—called galactolipids—and the second class containing glucose and referred to as the glucolipids. Presumably each

[237] E. Klenk, H. Debuch, and H. Daun, *Z. physiol. Chem.*, *292*, 241–250 (1953).

[238] E. Klenk, *Z. physiol. Chem.*, *282*, 18–21 (1945).

[239] F. Leupold, *Z. physiol. Chem.*, *285*, 182–200 (1950).

[240] S. J. Thannhauser, N. F. Boncoddo, and G. Schmidt, *J. Biol. Chem.*, *188*, 427–430 (1951).

[241] E. Klenk and R. Härle, *Z. physiol. Chem.*, *189*, 243–253 (1930).

[242] E. Klenk, *Z. physiol. Chem.*, *157*, 291–298 (1926).

[243] E. Klenk and H. Faillard, *Z. physiol. Chem.*, *292*, 268–275 (1953).

[244] N. Halliday, H. J. Deuel, Jr., L. J. Trageman, and W. E. Ward, *J. Biol. Chem.*, *132*, 171–180 (1940).

of the six fatty acids may form a glucose-containing and a galactose-containing cerebroside, making a total of twelve possible cerebrosides.

Galactose was identified as the carbohydrate component in the original preparations from brain made by Thierfelder[245] and by Brown and Morris.[246] On the other hand, glucose was recognized as the sugar present in the cerebroside isolated from the spleen in Gaucher's disease by a number of workers.[244,247–252] However, the galactose-containing cerebroside was also identified in the spleen,[252–255] and its presence was confirmed many years after the original finding.[247] The current opinion, which is concurred in by Klenk and Rennkamp,[248] is that both the glucose and the galactose types of cerebrosides occur in Gaucher's disease; the former type is the more common one. The galactose type of cerebroside has been isolated from normal beef spleen,[256] and from beef lung.[257]

Although the glucose-containing cerebroside occurs primarily under abnormal conditions, the question arises as to whether or not it may also be classed as a normal type of cerebroside. The latter viewpoint would seem to be the correct one, since all normal and pathologic specimens examined recently have contained small amounts of the glucose-containing type. Under such conditions, it is a moot question whether or not brain cerebroside contains the glucose type of cerebroside.

According to Kimmelstiel,[258] brain contains about 2–3% of cerebroside, which is slightly higher than the limits cited by Page.[259] The cerebrosides are found in a much higher concentration in white matter than in gray matter.[259,260] Cerebrosides are also widely distributed in tissues other than brain. They have been observed in the liver, as well as in the spleen, in

[245] H. Thierfelder, *Z. physiol. Chem.*, *14*, 209–216 (1890).
[246] H. T. Brown and G. H. Morris, *J. Chem. Soc.*, *57*, 57–59 (1890).
[247] E. Klenk and E. Schumann, *Z. physiol. Chem.*, *267*, 128–144 (1940).
[248] E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, *272*, 280–282 (1942).
[249] I. S. Danielson, C. H. Hall, and M. R. Everett, *Proc. Soc. Exptl. Biol. Med.*, *49*, 569–571 (1942).
[250] Aghion, *La maladie de Gaucher dans l'enfance, Thesis*, Paris, 1934; cited by E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, *273*, 253–268 (1942), p. 253.
[251] J. Polonovski, *Compt. rend.*, *215*, 443–445 (1942).
[252] B. Ottenstein, G. Schmidt, and S. J. Thannhauser, *Blood*, *3*, 1250–1258 (1948).
[253] H. Lieb, *Z. physiol. Chem.*, *140*, 305–313 (1924); *170*, 60–67 (1927).
[254] H. Lieb and M. Mladenovíc, *Z. physiol. Chem.*, *181*, 208–220 (1929).
[255] H. Lieb and V. Günther, *Z. physiol. Chem.*, *271*, 211–213 (1941).
[256] E. Walz, *Z. physiol. Chem.*, *166*, 210–222 (1927).
[257] S. J. Thannhauser and J. Benotti, unpublished observations; cited by S. J. Thannhauser and J. Benotti, *Physiol. Revs.*, *26*, 275–317 (1946), p. 307.
[258] P. Kimmelstiel, *Biochem. Z.*, *212*, 359–362 (1929).
[259] I. H. Page, *Chemistry of the Brain*, C. C Thomas, Springfield, Ill., 1937.
[260] A. Noll, *Z. physiol. Chem.*, *27*, 370–397 (1899).

Gaucher's disease. Thierfelder and Klenk[261] are of the opinion that cerebrosides are to be considered as general cell constituents under normal conditions.

c. Cholesterol in the Brain and Nervous Tissue. The white matter of brain contains approximately four times as much cholesterol as does the gray matter. Fieser and Fieser[262] cite a figure of 17% for the cholesterol content of brain, based upon dry substance. On the water-free basis, the values for cholesterol given in Table 8 are calculated as 15.6% in gray matter and 10% in white matter. Yasuda[263] cites values for cholesterol in gray and white matter, respectively, of 1.1 and 4.3% on the moist basis, and of 6.3 and 13.4% on the dry basis. Although the brain of the newborn rat was shown to possess the ability to synthesize cholesterol, Srere and co-workers[7] were unable to demonstrate any synthesis whatsoever of cholesterol from acetate in surviving brain slices of adult rats. According to West and Todd,[217] the cholesterol content of spinal cord approximates that of white brain matter, being 10 to 15% on the dry basis. Practically all the cholesterol present in the brain is in the form of the free alcohol (see Tables 13 and 14, on page 756). The amount of cholesterol is not influenced by inanition.[184]

d. The Presence of Proteolipids and of Strandin in the Brain. Folch and Lees[264] described a new class of lipids which they call proteolipids. This new type of compounds is made up of proteins and lipids, but instead of the protein properties predominating, as is the case with lipoproteins, the lipid properties prevail. Thus, in contradistinction to the behavior of lipoproteins, proteolipids are insoluble in water, but are freely soluble in chloroform-methanol-water mixtures. Although proteolipids were reported in a number of tissues, they were present in the greatest concentration in brain white matter. They were found to occur in descending order in brain tumors, brain gray matter, heart, kidney, liver, lung, smooth muscle, and skeletal muscle. They were, however, absent from blood plasma.

In a further study of the nature of proteolipids, Folch *et al.*[265] were able to prepare proteolipids and strandin separately. Strandin, so named be-

[261] H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930.

[262] L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949, p. 94.

[263] M. Yasuda, *J. Biochem. (Japan)*, *26*, 203–210 (1937).

[264] J. Folch and M. Lees, *J. Biol. Chem.*, *191*, 807–817 (1951).

[265] J. Folch, I. Ascoli, M. Lees, J. A. Meath, and F. N. LeBaron, *J. Biol. Chem.*, *191*, 833–841 (1951).

cause of its tendency to form strands, on drying from aqueous solution, which show good orientation under polarized light, is freely soluble in water and chloroform.[266] It was reported to be present in gray matter to the extent of 0.6 to 0.7% on a wet basis. Among its constituents are fatty acids, sphingosine or a sphingosine-like substance, a carbohydrate, a primary amine combined with the strandin molecule through its NH_2 group, and a chromogenic group. The minimal molecular weight as estimated by its behavior on ultracentrifugation[266] has been placed at 250,000.

e. Miscellaneous Components in the Brain. There are a number of water-soluble components in the brain fraction defined as extractives, which are related to the lipids. The extractives from brain are similar qualitatively to those from muscle and other tissues; however, from a quantitative standpoint, they exhibit marked variations.

(*a*) *Inositol and Diphosphoinositide.* The quantity of inositol is unexpectedly large in brain. It constitutes 0.19% of the gray matter and 0.22% of the white matter.[217] Its function is unknown. A related compound, from which inositol undoubtedly originates, is diphosphoinositide. This substance, which is bound to protein by a bond which is resistant to solvent action, can be extracted from a brain homogenate with a 2:1 chloroform-methanol mixture.[265] It appears to be a constant component of brain lipids.

(*b*) *Ethanolamine-O-Phosphoric Acid.* Ethanolamine-*O*-phosphoric acid is a second water-soluble product, recently isolated from brain by Ansell and Dawson.[267] On the basis of the proportion of this compound labeled after the injection of a solution containing P^{32}, it was suggested that the main portion of the ethanolamine phosphate had originated by synthesis rather than by a degradation of phosphatidylethanolamine. However, Ansell and Dawson were unwilling to exclude this acid as a possible precursor for phosphatidylethanolamine.

(*c*) *Acetylcholine.* This is the most important constituent in the water-soluble extractives which is related to lipids. Since the classical experiments of Loewi,[268] it has been recognized that this substance is set free in heart muscle when the vagus nerve is stimulated. Subsequently, it was demonstrated that a similar reaction occurs at the nerve endings of the parasympathetic nerves when a voluntary muscle is stimulated, resulting in the liberation of acetylcholine. Apparently, acetylcholine can likewise be produced in the brain on stimulation of the nerve cells, but the

[266] J. Folch, A. Arsove, and J. A. Meath, *J. Biol. Chem., 191,* 819–831 (1951).
[267] G. B. Ansell and R. M. C. Dawson, *Biochem. J., 50,* 241–246 (1951).
[268] O. Loewi, *Arch. ges. Physiol.* (*Pflüger's*), *189,* 239–242 (1921).

exact mode of its formation is not understood. After being formed in the brain, it is set free at the neuronal surface. It is closely associated with the propagation of nerve impulses. The acetylcholine content in the brain of man and dog, as well as in the peripheral nerves of the dog, is given in Table 11.

TABLE 11
THE ACETYLCHOLINE CONTENT IN THE BRAIN OF DOG AND MAN, AND IN THE PERIPHERAL NERVES OF THE DOG[a]

Tissues	Man, μg./g. tissue	Dog, μg./g. tissue
Peripheral nerves:		
Splanchnic	—	10.5
Vagosympathetic	—	5.6
Hypogastric	—	4.0
Sciatic	—	2.5
Brachial plexus	—	2.3
Optic chiasma	—	1.0
Central nervous system:		
Optic thalamus	0.85	1.7
Cerebrum (gray)	0.75	0.9
Corpus striatum	0.5	1.1
Crus cerebri	0.5	1.1
Spinal cord	0.45	0.75
Cerebrum (white)	0.27	0.55
Pons	0.20	0.5
Medulla oblongata	0.16	0.5
Cerebellum	0.12	0.4

[a] I. H. Page, *Chemistry of the Brain,* C. C Thomas, Springfield, Ill., 1937, p. 71.

The acetylcholine level in the central nervous system of the dog is about double that in the central nervous system of man. However, a surprisingly uniform parallelism obtains between the relative acetylcholine content of the several parts of the nervous system in the two species. In only one instance (cerebellum, gray matter) does the descending order of acetylcholine content fall out of line in the brains of the two species.

Cholinesterase, which is the enzyme by which the level of acetylcholine is regulated, occurs not only in the nerve endings but also in the brain. This enzyme causes the destruction of the acetylcholine by catalyzing its hydrolysis to acetic acid and choline. According to Ord and Thompson,[269] all areas of the human brain studied contained measurable amounts of this enzyme, as judged by the hydrolysis of benzoylcholine and butyrylcholine.

[269] M. G. Ord and R. H. S. Thompson, *Biochem. J., 50,* xxxii–xxxiii (1952).

The enzyme appears to be essentially a butyrylcholinesterase, and resembles the pseudoenzyme of human plasma. More of the enzyme was present in the white matter than in the gray matter. It is suggested that the action of this enzyme may be related to processes occurring in the myelin sheaths or connective tissue, rather than to those of the axis cylinders.

(*d*) *Sulfolipids.* Thudichum,[220] as early as 1884, reported the presence of a sulfur-containing lipid in a phrenosine mixture obtained from brain. The sulfur content was of such magnitude as to preclude the possibility that it was an impurity. The purified product of Thudichum had a sulfur content of 6.19%. At a later date, Koch[270] prepared a sulfate from the ether-insoluble residue which contained not only sulfur (1.91%) but also phosphorus (1.80%) and sugar (12.8%). Because of the parallelism between the sulfur and the sugar content, Koch assumed that the sulfuric acid formed an ester with the alcohol groups of the sugar. Fränkel and co-workers[271,272] likewise reported the preparation of two sulfur-containing lipids, called "Hirnsäure" (brain acid), and "Hypohirnsäure," which they obtained by Thudichum's method. The two preparations contained 1.86 and 1.70% of sulfur, respectively. Levene[273] prepared a sulfatide from brain which was phosphorus-free, but which contained 2.66% sulfur. Later, with Landsteiner,[274–276] he isolated a sulfur-containing lipid from the protagon of brain and kidney which yielded 1.32% S on analysis. These experimental results are sufficiently confirmatory to convince one that sulfatides exist in nervous tissue.

Subsequently, Blix[277] extended the earlier observations by a comprehensive study of the lipids in human brain. This investigator was able to isolate cerebron-sulfuric acid, as the potassium salt, in the amount of 20 to 25% of the total cerebrosides. The potassium-cerebron-sulfuric acid was assumed to have an empirical formula of $C_{48}H_{92}NSO_{12}K$; the composition of this compound, as determined by hydrolysis, agreed well with the theoretical values for a molecule made up of one part each of fatty acid (cerebronic), 40.7%, sphingosine, 31.6%, galactose, 19.0%, sulfuric acid, 10.4% and potassium, 4.1%.

[270] W. Koch, *Z. physiol. Chem.*, *70*, 94–97 (1910).
[271] S. Fränkel and O. Gilbert, *Biochem. Z.*, *124*, 206–215 (1921).
[272] S. Fränkel and O. Karpfen, *Biochem. Z.*, *157*, 414–424 (1925).
[273] P. A. Levene, *J. Biol. Chem.*, *13*, 463–464 (1912).
[274] K. Landsteiner and P. A. Levene, *J. Immunol.*, *10*, 731–733 (1925).
[275] K. Landsteiner and P. A. Levene, *Proc. Soc. Exptl. Biol. Med.*, *23*, 343–344 (1926); *24*, 693–695 (1927).
[276] P. A. Levene and K. Landsteiner, *J. Biol. Chem.*, *75*, 607–612 (1927).
[277] G. Blix, *Z. physiol. Chem.*, *219*, 82–98 (1933).

As early as 1907, Koch[278] reported the presence of sulfolipids in the submaxillary glands, liver, testes, and muscle; he[279] later demonstrated that the highest concentrations of these lipids were in the white matter of the brain.[278] Sulfur-containing lipids have likewise been demonstrated in the kidney[274] and in the lung.[280] Blix[277] suggested that cerebroside sulfuric acid is the sulfur-containing component of "jecorin," isolated by Drechsel[281] and by Baldi[282] from dog and rabbit liver, beef spleen, horse blood and muscle, as well as from human brain, and by Manasse[283] from the adrenal gland of cattle and horses, although this author expressed some doubt as to the identity of the substance. It may likewise be related to the "ovins" present in egg yolk.[284]

(*e*) *Lipid Anticoagulant in Brain Tissue.* Tocantius and associates[285] called attention to a new heat-labile lipid inhibitor of blood coagulation which could be extracted from brain tissue, and which differed from any known anticoagulants. It is not certain whether or not it is related to the fatty acid which has been reported to exhibit hemolytic action. Laser and Friedman[286] were the first to isolate a solid acid from human blood, which possessed a strong hemolytic activity. It was later found to be widely distributed, particularly in the brain.[287] Morton and Todd[288] have now isolated an unsaturated acid from horse brain which exhibits a considerable hemolytic action. It was identified as *cis*-octadec-11-enoic acid, and has not hitherto been reported in nature. Oleic acid was present as a contaminant. The synthetic *cis*-acid was shown to possess a physiologic activity similar to that of the natural product. Goddard and Alexander,[289] on the basis of monolayer properties, confirmed the identity of *cis*-octadec-11-enoic acid with the hemolytic acid. However, they concluded that the natural hemolytic acid is a mixture of oleic acid and the afore-mentioned *cis*-acid.

278 W. Koch, *Z. physiol. Chem.*, *53*, 496–507 (1907).
279 W. Koch and M. L. Koch, *J. Biol. Chem.*, *31*, 395–410 (1917).
280 U. Sammartino, *Biochem. Z.*, *124*, 234–243 (1921).
281 E. Drechsel, *J. prakt. Chem.* [*n.s.*], *33*, 425–432 (1886).
282 D. Baldi, *Arch. Physiol., Suppl.*, *1887*, 100–108.
283 P. Manasse, *Z. physiol. Chem.*, *20*, 478–488 (1895).
284 N. A. Barbieri, *Compt. rend.*, *145*, 133–135 (1907).
285 L. M. Tocantius, R. T. Carroll, and T. J. McBride, *Proc. Soc. Exptl. Biol. Med.*, *68*, 110–117 (1948).
286 H. Laser and E. Friedman, *Nature*, *156*, 507 (1945).
287 H. Laser, *J. Physiol.*, *110*, 338–355 (1949).
288 I. D. Morton and A. R. Todd, *Biochem. J.*, *47*, 327–330 (1950).
289 E. D. Goddard and A. E. Alexander, *Biochem. J.*, *47*, 331–335 (1950).

(2) *The Lipid Distribution in Brain Cells*

Tyrrell and Richter,[290] using the Richter and Hullin technic[291] for the isolation of cell nuclei from the rest of the cell, determined the percentage composition of the nuclei of brain cells, and calculated the composition of the cytoplasm. Their data, as well as those of Brante,[292] are summarized in Table 12.

TABLE 12
THE DISTRIBUTION OF LIPIDS BETWEEN THE NUCLEI AND THE CYTOPLASM IN HUMAN BRAIN CORTEX[a]

Substance analyzed	Medullary sheath,[b] % moist wt.	Whole cortex,[c] % dry wt.	Cell nuclei,[c] % dry wt.	Cytoplasm of nerve cells and axis cylinders,[c] % dry wt.
Cholesterol	8.4	5.3	4.1	0
Cerebrosides	9.8	4 8	10.0	0
Total phospholipids	15.3	19.7	13.0	18.4
Lecithins	2.4	5.2	4.4	9.2
Cephalins	4 2	11.6	6.5	9.2
Phosphatidylethanolamine	0	—	—	9.2
Phosphatidylserine	4.2	—	—	0
Acetal phosphatides	3.9	—	—	0
Sphingomyelin	4.8	2.9	2.1	0
Gangliosides	0	—	—	4.1

[a] Adapted from E. Klenk, *Der chemische Aufbau der Nervenzellen und der Nervenfasern,* Colloquium Ges. physiol. Chemie, Mosbach, Baden, April 26–27, 1952.
[b] G. Brante, *Acta Physiol. Scand., 18, Suppl. 63,* 7–189, Appendix A, 1–24; Appendix B, i-xxviii (1949).
[c] L. W. Tyrrell and D. Richter, *Biochem. J., 49,* li–lii (1951).

The most striking variation in the distribution of lipids in the brain cell is the lower concentration of all phospholipids in the cell nuclei as compared with the cytoplasm, and the higher concentration of cerebrosides in the nuclei as contrasted with their level in the rest of the cell. This may indicate that some specific function in given areas of the cell is mediated by particular lipids.

In recent studies of Petersen and Schou,[293] it was found that the concen-

[290] L. W. Tyrrell and D. Richter, *Biochem. J., 49,* li–lii (1951).
[291] D. Richter and R. P. Hullin, *Biochem. J., 48,* 406–410 (1951).
[292] G. Brante, *Acta Physiol. Scand., 18, Suppl. 63,* 7–189, Appendix A, 1–24, Appendix B, i–xxviii (1949).
[293] V. P. Petersen and M. Schou, *XIX Intern. Physiol. Congress* (Montreal, Aug.-Sept., 1953), *Abst.,* 676–677 (1953).

tration of all phospholipid fractions was lower in the supernatant fluid than in any of the particle fractions. Sphingomyelin and cephalin were localized mainly in the mitochondria, whereas the concentration of lecithin was highest in the microsomes.

(3) Physiologic Factors Altering the Composition of Normal Brain

a. The Effect of Species on Brain Lipids. Lanfranchi[294] reported that the proportion of cerebroside in the brain increases with phylogenetic evolution. Cerebrosides were shown to be absent from the brain of the octopus (*Octopus vulgaris*); in the teleost scorpion-fish, or toad-fish (*Scorpaena porcus*), they averaged 3.37% of the dry weight, while a mean value of 3.69% was noted in cartilaginous fishes, for instance the small-spotted dogfish (*Scyllium canicula*). The following figures (based on dry weight) for cerebroside were noted in the terrestrial animals: common toad (*Bufo vulgaris*), 3.10%; Greek tortoise (*Testudo graeca*), 6.9%; fowl (*Gallus domesticus*), 6.02% and, representing the mammals, the black rat (*Mus rattus*), 10.23%. Study of the chicken embryo showed that the cerebroside content increased with ontogenetic development also; at ten days the value was 0.12% (moist weight), at twenty days, 1.37%. Patterson and associates[295] were unable to demonstrate the presence of cerebrosides in the head portion of the honey bee.

Argiris[296] reported, in 1908, that cerebrosides were present in only extremely small amounts in the brain tissue of codfish (*Gadus morrhua*) and haddock (*Gadus aeglefinus*); however, as early as 1893, Kossel and Freytag[297] had isolated cerebrosides from the brain of the sturgeon (*Acipenser sturio*). The presence of cerebrosides in the brain of chickens and ducks has also been reported.[296]

The concentration of various lipids in the brain of a number of mammals has been investigated by Johnson, McNabb and Rossiter.[298] These data are summarized in Tables 13 and 14, on page 756.

Practically no esterified cholesterol occurs in any of the several species reported in Tables 13 and 14. Moreover, Ellis and Gardner,[184] in their classical studies on cholesterol, could find no trace of esterified cholesterol

[294] F. Lanfranchi, *Arch. sci. biol. (Italy)*, *24*, 120–124 (1938).
[295] E. K. Patterson, M. E. Dumm, and A. G. Richards, Jr., *Arch. Biochem.*, *7*, 201-210 (1945).
[296] A. Argiris, *Z. physiol. Chem.*, *57*, 288–295 (1908).
[297] A. Kossel and F. Freytag, *Z. physiol. Chem.*, *17*, 431–456 (1893).
[298] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *43*, 573–577 (1948).

TABLE 13
COMPARATIVE AMOUNTS OF LIPIDS IN THE WHOLE BRAIN OF THE GUINEA PIG, CAT, AND RABBIT (EXPRESSED IN PER CENT OF FRESH TISSUE)[a]

Substance analyzed	Guinea pig	Cat	Rabbit
Cerebroside	2.29	1.88	2.74
Cholesterol			
Total	1.76	1.94	2.21
Free	1.61	1 89	2 16
Ester	0.15	0.05	0.05
Phospholipids			
Total	4.82	4.57	5.40
Monoamino-	3.79	3.69	4.32
Lecithin	—	1.18	1.39
Sphingomyelin	0.94	0.93	1.08

[a] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *43*, 573–577 (1948).

TABLE 14
COMPARISON OF LIPID COMPOSITION OF THE GRAY AND WHITE MATTER OF THE BRAIN OF THE BEAVER, CAT, DOG, AND MAN (EXPRESSED IN PER CENT OF FRESH TISSUE)[a]

Substance analyzed	Gray matter				White matter			
	Beaver	Cat	Dog	Man	Beaver	Cat	Dog	Man
Cerebroside	0.68	1.86	1.54	1.20	4.85	5.37	7.43	4.61
Cholesterol								
Total	1.18	1.20	1.30	1.00	4.16	4.64	4.86	4.00
Free	1.16	1.19	1.29	1.00	4.16	4.64	4.86	3.69
Ester	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.31
Phospholipid								
Total	3.70	4 03	4.03	3.12	7.22	7.73	7.99	6.24
Monoamino-	2.90	3 29	3 28	2.82	4.54	3.95	4.95	3.64
Lecithin	0.69	1.35	1.23	0.61	1.13	1.36	1.62	0.90
Sphingomyelin	0.80	0.74	0.91	0.30	2.68	3.78	3.04	2.60
Total essential lipids	5.56	7.09	7.09	5.32	16.23	17.74	20.27	14.85

[a] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *43*, 573–577 (1948).

in the brain of the rabbit after the injection of cholesterol, or following inanition. The average cholesterol level in rabbit brain was given as 2.32% based upon the fresh tissue; this is in harmony with the mean value recently cited by Johnson *et al.*[298] for this species.

Kaucher and her collaborators[198] reported the following values (expressed on a dry basis) for the composition of lipids from beef brain: fat, 2.97%; cerebroside, 12.01%; total cholesterol, 10.25%; cholesterol esters, 0.25%; free cholesterol, 10.00%; total phospholipid, 26.37%;

lecithin, 7.05%; cephalin, 14.35%; sphingomyelin, 4.96%; total essential lipids, 48.62%; total lipids, 51.59%.

No important differences would appear to obtain in the distribution of the lipids in the brain of the higher mammals similar to the variation in cerebroside content noted with phylogenetic evolution. Moreover, Blancher *et al.*[299] noted that a significant amount of a hexaenoic acid is present in the gray matter of both monkey and human brain, but is absent from the white matter of both species.

b. The Effect of Age on Brain Lipids. (*a*) *The Composition during Prenatal Development.* Most workers have reported that cerebrosides are lacking in the brain at birth; their appearance and their subsequent increase parallel the development of the myelin sheath. Sphingomyelin, another lipid component largely present in the myelin sheath, is one of the last phospholipids to appear in the development of the brain and nervous tissue.

Backlin[300] reported the absence of cerebrosides from the rabbit brain at birth. In the case of the human fetus, Schuwirth[301] found only traces of cerebrosides and no sphingomyelin in the seventh and eighth months of intrauterine life, and even at birth.

Mihara[302] observed that cephalin made up the principal part of the central nervous system of the early fetus. During the course of fetal growth, lipids were shown to increase, while the total nitrogen decreased in the cerebrum, medulla oblongata, and spinal cord. The greatest increase in lipids occurred in the cerebrum. The most distinct changes in cholesterol and cerebrosides did not occur until the last two months before term. Bieth and Mandel[303] reported that, during the primary phase in the development of the brain of the chick embryo, cephalin is the chief phospholipid. Lecithins increase during the next stage, while sphingomyelins appear last.

(*b*) *The Composition during Postnatal Development.* During the extrauterine development, the dry substance of the brain increases; the total lipids constitute a greater portion of the dry matter, and all lipid fractions are increased in terms of moist weight.

As already indicated, the greatest increase occurs in the cerebrosides.

[299] G. Blancher, E. Le Breton, and P. May, *Compt. rend. soc. biol., 146,* 219–222 (1952).

[300] E. Backlin, *Beiträge zur quantitativen Kenntnis der Gehirnlipoide,* Inaugural Dissertation, Almquist, Upsala, 1930; cited by W. R. Bloor, *Biochemistry of the Fatty Acids,* Reinhold, New York, 1943, p. 207.

[301] K. Schuwirth, *Z. physiol. Chem., 263,* 25–36 (1940).

[302] T. Mihara, *J. Biochem. (Japan), 39,* 155–161 (1952).

[303] R. Bieth and P. Mandel, *Bull. soc. chim. biol., 32,* 109–115 (1950).

and the least in the phospholipids. Little or no change takes place in the case of the *unsaturated* phospholipids; however, the *saturated* phospholipids (including sphingomyelin), together with cholesterol and the cerebrosides, increase markedly.

McConnell and Sinclair[304] recorded variations in the lipid content of rats as related to age. Thus, the values for brain lecithin plus cephalin on the moist basis were as follows: newborn rats, 1.14%; rats three weeks

TABLE 15
THE COMPARATIVE COMPOSITION OF HUMAN BRAIN AS INFLUENCED BY AGE[a]

Substance	Whole brain (child), % dry matter	Whole brain (adult), % dry matter
Phospholipids	24.2	27.3
Cerebrosides (glycolipids)	6.9	13.6
Cholesterol	1.8	10.9
Lipid sulfur	0.1	0.3
Protein	46.6	37.1
Extractives	12.1	6.7
Ash	8.3	4.1

[a] W. Koch, *Z. physiol. Chem.*, *63*, 432–442 (1909).

TABLE 16
THE COMPOSITION OF BRAIN FROM CHILDREN AND FROM ADULTS AS INFLUENCED BY AGE (EXPRESSED IN PER CENT OF DRY TISSUE)[a]

Category	Source of sample				
	Fetus (7–8 mo.)	New-born	Child 1 (13 mo.)	Child 2 (14 mo.)	Old man
No. of samples examined	21	13	1	1	1
Fat plus cholesterol, %	6.8	4.4	8.0	5.6	13.4
Glycerophosphatides					
Ether-soluble, %	16.4	15.5	22.9	19.1	25.0
Ether-insoluble, %	0.5	0.3	1.9	1.1	0.0
Sphingomyelin, %	0	0	—	—	0.9
Cerebroside, %	0.02	0.05	1.5	2.7	6.4
Lignocerylsphingosine, %	0.07	0	0	0	0
Substance X, %	0.1	0.4	0.3	0.4	0

[a] K. Schuwirth, *Z. physiol. Chem.*, *263*, 25–36 (1940).

old, 2.32%; and rats three months of age, 3%. Monde and Bieth[305] likewise reported a regular increase in phospholipids and fatty acids in the brain of rats during postnatal development. Cerebrosides were present as

[304] K. P. McConnell and R. G. Sinclair, *J. Biol. Chem.*, *118*, 131–136 (1937).
[305] P. Monde and R. Bieth, *Bull. soc. chim. biol.*, *33*, 973–981 (1951).

early as the second day, and sphingomyelin appeared eight to sixteen days after birth.

Waldemar Koch,[306] many years ago, demonstrated similar variations in the composition of the human brain as related to age. His data are summarized in Table 15, while those of Schuwirth[301] are included in Table 16.

Although neutral fat does not occur in appreciable amounts in adult brain tissue, definite quantities are present in the brain of the newborn. Tuthill[307] reported that the fat content of the brains of forty-six infants ranging in age from newborn to two years contained varying amounts of neutral fat. Fat was found around the blood vessels and the glial cells of the centrum ovale, corpus callosum, and white substance of the lower part of the gyri of all infants who had died at the age of less than four months. The disappearance of the fat after four months occurred concomitantly with the completion of myelinization. Fat formed around the large subcortical vessels from the first month to the second year of life.

In an extension of their studies on the composition of nervous tissue, Johnson and co-workers[308] noted that the water content of both white and gray matter of infant brain was greater than that of adult brain. In the infant, the water content of the gray matter, also, was greater than that of white matter. In the adult brain, a higher concentration of free cholesterol, cerebroside, and sphingomyelin was found both in the gray and in the white matter. Less lecithin was present in the adult brain than in that of the infant.

Gorodisskay[309] found no definite relation between age and the composition of the brain of mature individuals up to forty-five years of age. In older individuals, the unsaturated phospholipids and proteins decreased, while cholesterol increased. No change was observed in the level of saturated phospholipids and of cerebrosides. The changes in composition were most marked in the association centers.

c. The Effect of Brain Areas on Brain Lipids. Although it has been demonstrated that there is a marked variation in composition between the gray and the white matter, Gorodisskay[309] was able to prove that variations in composition are concomitant with variations in psychic function. Cholesterol was the component most affected. Saturated phospholipids were somewhat less variable, while the unsaturated phospholipids showed the least variability. Based upon total nitrogen as a reference, the lowest

[306] W. Koch, *Z. physiol. Chem.*, *63*, 432–442 (1909).
[307] C. R. Tuthill, *Arch. Pathol.*, *25*, 336–346 (1938).
[308] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *44*, 494–498 (1949).
[309] H. Gorodisskay, *Biochem. Z.*, *164*, 446–480 (1925).

cholesterol levels were noted in areas of high psychic function, while the highest values of this alcohol were found in the motor areas. Variations in composition occurred in both sides of the brain.

In general, the more active the portion of the nervous system examined, the lower was the lipid content and the higher the protein level. The highest lipid content was noted in the motor centers and the lowest in the association areas. Cholesterol, cerebrosides, and saturated phospholipids reached an especially low level in the association areas. Peripheral nerves had the highest lipid content of the nervous system, while the spinal cord presented a lower value and the brain had the lowest lipid content.

d. The Effect of Diet on Brain Lipids. There is a general agreement that the brain is one of the organs least influenced by diet.[310–312] This is especially true in the case of brain lipids. Stoesser *et al.*[311] demonstrated only minimal variations in the composition of rat brains. No differences in the ratio of phosphatides to total lipids in the brain could be demonstrated by Beauvallet[312] in the case of the adult or of the growing rat.

However, McConnell and Sinclair[304] were able to introduce a certain proportion of the unnatural fatty acid, elaidic, into the brain of the young rat, either through the placenta or through the milk of its mother. However, the extent of elaidic acid substitution in the brain lipids was only about one-fourth of that which obtained in the liver and muscle lipids of the same animals. This indicates that the brain exhibits a greater degree of selection than do other tissues.

Other experiments indicate that the composition of the brain lipids may be influenced by diet. Changus and collaborators[313] found that radioactive phosphorus could be incorporated into brain phospholipids, but that the change in the brain components was slower than that for any of the other tissues. The increase in uptake was progressive for 200 hours, after a single dose, following which it decreased at a correspondingly slow rate for four weeks. The rate of uptake was more rapid in the brains of young animals than in those of older ones. The rate at which radiophosphorus was taken up by brain phospholipids of mice was reduced by 25% by emotional excitement or by direct electrical stimulation of the brain.[314] Torda[315] noted that the rate of phospholipid synthesis was markedly decreased in mice during convulsions induced by electroshock or by

[310] C. Artom, G. Sarzana, and E. Segrè, *Arch. intern. physiol., 47,* 245–276 (1938).
[311] A. V. Stoesser, K. A. Petri, and I. McQuarrie, *Proc. Soc. Exptl. Biol. Med., 32,* 761-762 (1935).
[312] M. Beauvallet, *Compt. rend. soc. biol., 144,* 1596–1599 (1950).
[313] G. W. Changus, I. L. Chaikoff, and S. Ruben, *J. Biol. Chem., 126,* 493–500 (1938)
[314] R. M. C. Dawson, *J. Physiol., 109,* 21P–22P (1949).
[315] C. Torda, *Federation Proc., 12,* 145 (1953).

intraperitoneal administration of pentylene tetrazole and physostigmine salicylate. Within fifteen minutes after the injection of ACTH, the rate of phospholipid synthesis in the brain was increased. These results are interpreted as indicating that the brain phospholipids are utilized during activity. Moreover, they prove that the turnover of phospholipids is controlled by a humoral mechanism.

In addition, the brain is especially susceptible to vitamin E deficiency. Heinrich and Mattill[316] demonstrated that marked changes in the total lipids occurred at various stages of the vitamin E deficiency induced by diet; furthermore, there was a marked increase in the cholesterol content. The proportion of free cholesterol was increased to a much greater extent than was that of the total cholesterol.

e. The Effect of Hyperinsulinism on Brain Lipids. Randall[317] was the first to demonstrate a reduction in brain phospholipid and neutral fat, following a prolonged insulin hypoglycemia. Page and associates[149] were unable to note that a single dose of insulin exerted any significant effect upon the total fat in the brain of rabbits, although the fatty acids were decreased by 14% and the cholesterol was increased by 10%. McGhee *et al.*[318] demonstrated a consistent and significant decrease in brain lipid phosphorus of about 10%, following massive doses of insulin. The decrease could not be reversed by the administration of glucose or of lecithin. The authors consider this decrease to be a direct and perhaps irreversible effect of hyperinsulinism.

f. The Effect of Choline Deficiency on Brain Lipids. Choline deficiency has been shown by Foà and associates[319] to have no effect upon the total lipid, total phospholipid, sphingomyelin, lecithin, cephalin, or cholesterol content of rat brain. This constancy in brain lipid values is maintained in spite of the fact that choline deficiency in the diet results in a depressed value for lecithin in the liver, which can be reversed by choline supplements. On the other hand, the level of sphingomyelin, cephalin, or cholesterol in the liver is not influenced by the choline-free diet.

(4) The Lipid Composition of Nerves

Palladin and his associates[320] compared the lipid and nitrogen content in various parts of the nervous system of dogs. The highest amount of

[316] M. R. Heinrich and H. A. Mattill, *Proc. Soc. Exptl. Biol. Med.*, *52*, 344–346 (1943).
[317] L. O. Randall, *J. Biol. Chem.*, *133*, 129–136 (1940).
[318] E. C. McGhee, E. Papageorge, W. L. Bloom, and G. T. Lewis, *J. Biol. Chem.*, *190*, 127–132 (1951).
[319] P. P. Foà, H. R. Weinstein, and B. Kleppel, *Arch. Biochem.*, *19*, 209–212 (1948).
[320] A. V. Palladin, E. I. Rashba, and R. M. Helman, *Ukrain. Biochem. Zhur.*, *8*, No. 1, 5–26 (1935); *Chem. Abst.*, *30*, 5277 (1936).

cholesterol and of unsaturated phospholipids was present in the spinal cord, followed by the nucleus caudatus, the cortex cerebri, and finally the cortex cerebelli. The content of cerebrosides and of saturated phospholipids was less in the spinal cord than in the other nerve structures.

In later experiments of the Palladin group,[321] the composition of the vegetative nervous system of cows was investigated. The following data were given for the ganglion coeliacus (I), for the ganglia of the sympathetic trunk (II), and for the ganglion nodosum of the vagus nerve (III): *dry residue*, I, 24.4%, II, 21.1%, and III, 20.5%; *total phosphorus*, I, 1.777%, II, 0.939%, and III, 0.836%; *proportion of unsaturated phospholipids and of acid-soluble phosphorus*, in the decreasing order, I, II, and III; and *amount of saturated phospholipid*, I, III, and II. The ganglion nodosum of the vagus nerve (III) had the highest cholesterol content.

Johnson, McNabb, and Rossiter[322] have indicated that the distribution of essential lipids, including that of the individual phospholipids, in the peripheral nerves of rats, cats, dogs, beavers, and human subjects, more closely resembles that in the white matter than it does that in the gray matter or in the whole brain. However, relatively more sphingomyelin and less cephalin and cerebroside were found in the peripheral nerve than in the white matter of the brain.

Falk[323] has shown that, in non-medullated nerves, the phospholipids are the principal lipid constituents while, in medullated nerves, cerebrosides are the characteristic lipids. Cephalins are high in both types of nerves. On the other hand, the various normal human nerves were found by Randall[324] to have a practically identical composition. These results are summarized in Table 17.

Further information on nerve composition was obtained by investigation of the effect of Wallerian degeneration on the chemistry of peripheral nerves.[325,326] After section of the nerve, the myelin sheath is completely destroyed. Since free cholesterol, cerebrosides, and sphingomyelin have previously been identified as the principal lipid components of the sheath, one would anticipate a relative decrease in these substances in degenerated nerve. After section of the sciatic nerve of the cat, it was found that the total lipid content of the nerve decreased steadily throughout the course

[321] A. V. Palladin, E. I. Rashba, and R. M. Helman, *Ukrain. Biochem. Zhur.*, *8*, No. 1, 27–46 (1935); *Chem. Abst.*, *30*, 5277–5278 (1936).

[322] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *43*, 578–580 (1948).

[323] F. Falk, *Biochem. Z.*, *13*, 153–172 (1908).

[324] L. O. Randall, *J. Biol. Chem.*, *125*, 723–728 (1938).

[325] A. C. Johnson, A. R. McNabb, and R. J. Rossiter, *Biochem. J.*, *45*, 500–508 (1949).

[326] N. S. Burt and A. R. McNabb, *Biochem. J.*, *47*, 318–323 (1950).

TABLE 17
THE COMPARATIVE LIPID COMPOSITION (IN PER CENT) OF HUMAN AND OF BOVINE MEDULLATED AND NON-MEDULLATED NERVES, AND OF DIFFERENT PERIPHERAL NERVES OF HUMAN SUBJECTS

	Compn. of lipid extract of nerve on dry basis[a]		Compn. of normal human nerves[b]		
Substance	Non-medullated (splenic, bovine)	Medullated (ischial, human)	Femoral (23 nerves)	Sciatic (3 nerves)	Post-tibial (12 nerves)
Water	—	—	65.8[c]	68.0[c]	68.3[c]
Total extract	11.5	46.6	—	—	—
Total phospholipids	—	—	13.36[d]	13.58[d]	13.13[d]
Lecithin	9.8	2.9	—	—	—
Cephalin	23.7	12.4	—	—	—
Cholesterol	47.0	25.0	4.37[d]	4.59[d]	4.37[d]
Cerebrosides	6.0	18.2	5.36[d]	4.52[d]	4.24[d]
Fat	—	—	9.05[c]	9.03[c]	8.04[c]

[a] F. Falk, *Biochem. Z.*, *13*, 153–172 (1908).
[b] L. O. Randall, *J. Biol. Chem.*, *125*, 723–728 (1938).
[c] Calculated on moist basis.
[d] Calculated on dry basis.

of the degeneration. Neutral fat decreased during the first four to eight days, and returned to normal within thirty-two days. Little change was noted in the free cholesterol, sphingomyelin, and cephalin content during the first eight days, but a rapid decrease was noted in from eight to thirty-two days. Cholesterol ester, which was absent from the normal nerve, made an appearance and reached a maximum value after sixteen days. It is suggested that some of the acids liberated by the other lipids may combine with free cholesterol to form the ester, while others may be converted into neutral fat. In a related series of tests, Burt and McNabb[326] noted that a somewhat similar pattern was followed after the nerve was crushed (but not cut). The wet weight of the nerve increased after the operation, reaching the maximum at thirty-two days and returning to normal after 144 days. The total lipid decreased for the first sixteen days, remained constant for from sixteen to forty-eight days, and then gradually increased. However, after 144 days, the total lipid content was still less than it was in control nerves. Neutral fat had returned to normal at the end of forty-eight days, but the myelin lipids were present in the amount of only 44% of their original content at the end of 144 days. May and Thillard[327] reported that the cerebroside content in the degenerated sciatic nerve of the dog first increased to 69% during the three days following sciaticotomy,

[327] R. M. May and M. J. Thillard, *Bull. soc. chim. biol.*, *35*, 307–311 (1953).

and then decreased to less than 56% twelve days after the operation. Thus the amount of cerebrosides present during degeneration changed in a manner opposite to that occurring during the growth of the nerve.

May[328] noted that normal dry nerve contained 7.8% of cholesterol. During the three weeks following section of the nerve, the cholesterol content increased to 24%. However, measured on a wet basis, an actual decrease in cholesterol obtained. Randall[324] reported that the peripheral nerves from diabetic and arteriosclerotic patients exhibited a marked decrease in phospholipids, cholesterol, and cerebrosides, and an increase in fat content, but no change in the proportion of water, when compared with corresponding nerves from normal individuals. The posterior tibial nerve displayed the most extensive change in composition as compared with the normal, followed in order by the sciatic and the femoral nerves.

The concentration of phospholipids in the peripheral nerves of the rat was found to be a function of the weight of the animal rather than of the age. Thus, Mannell[329] observed that the mean concentration of phospholipids in the nerves of protein-depleted rats was greater than in controls of the same age, but did not differ significantly from that of controls of the same weight.

(5) *The Lipid Composition of the Nerve Structures of Invertebrates*

In general, the same type of lipids makes up the nervous tissue of invertebrates as has been shown to exist in that of the higher animals.

TABLE 18
THE LIPID COMPOSITION (IN PER CENT MOIST WEIGHT) OF THE NERVE TISSUE OF SOME INVERTEBRATES

Substance present	Esophageal ring		Ventral nerve cord	
	Loligo[a]	*Limulus*[b]	*Limulus*[b]	*Libinia*[b]
Total phosphatide	3.21	1.36	0.99	0.75
Lecithin	1.17	0.61	0.42	0.34
Monoaminophospholipid	2.81	0.84	0.56	0.52
Total cholesterol	0.73	0.28	0.21	0.16
Free cholesterol	0.72	0.26	0.20	0.14
Cerebroside	0.03	0.07	0.09	0.64

[a] J. D. McColl and R. J. Rossiter, *J. Exptl. Biol.*, *28*, 116–124 (1951).
[b] J. D. McColl and R. J. Rossiter, *J. Cellular Comp. Physiol.*, *36*, 241–250 (1950).

[328] R. M. May, *Bull. soc. chim. biol.*, *30*, 562–566 (1948).
[329] W. A. Mannell, *Federation Proc.*, *12*, 242 (1953).

Table 18 summarizes these data for *Loligo pealii* (Atlantic squid or cuttlefish, calamary), *Limulus* spp. (King crab), and *Libinia* spp. (spider crab).

6. Lipids Present in the Adrenal Glands

The importance of the lipid composition of the adrenal glands is entirely out of line with the relatively small weight of the organ. Not only does the adrenal cortex have a high concentration of cortisone and related steroids and also of ascorbic acid (vitamin C), but, in addition, large amounts of cholesterol and an unusually high concentration of arachidonic acid are also present in this portion of the gland. Although the adrenal medulla is of considerable importance as a site of the production of epinephrine (adrenalin), it is of less interest insofar as its lipid composition is concerned than is the cortex.

The adrenal gland and the pancreas are unique in containing appreciable amounts of monoglycerides. Reichstein,[330] as well as Wintersteiner and Pfiffner,[331] identified α-monopalmitin as a minor constituent of the adrenal lipids. Although Jones and her co-workers[26] were unable to confirm these earlier results, they attribute their failure to the small amount of tissue which was available.

(1) Cholesterol in the Adrenal Glands

As early as 1915, Borberg[332] reported that the lipids in the adrenal cortex (of horses, guinea pigs, rabbits, and cats) consisted principally of cholesterol esters and free fatty acids, most of which were unsaturated; no triglycerides were recorded as present. On the other hand, Sorg and Jaffé[333] found no cholesterol esters in the adrenal cortex of cattle. Bär and Jaffé[334] report that cholesterol is normally absent, or that it occurs only in minimal amounts, in rabbit suprarenal cortex; the principal lipids found are phospholipids and cerebrosides. After cholesterol was fed, large amounts of this steroid were found in the suprarenals, as well as in the ovaries, of rabbits.[334]

The cholesterol content of the adrenal cortex varies markedly with the species. In the cow and sheep, cholesterol has been reported to account for 0.45% of the moist weight of the suprarenal cortex.[335] Brown *et al.*[336]

[330] T. Reichstein, *Helv. Chim. Acta*, *19*, 29–63 (1936).
[331] O. Wintersteiner and J. J. Pfiffner, *J. Biol. Chem.*, *116*, 291–305 (1936).
[332] N. C. Borberg, *Skand. Arch. Physiol.*, *32*, 287–354 (1915).
[333] K. Sorg and R. Jaffé, *Zentr. allgem. Pathol.*, *35*, 353–359 (1924).
[334] R. Bär and R. Jaffé, *Z. Konstitutionslehre*, *10*, 321–328 (1925).
[335] C. I. Parhon and M. Cahane, *Compt. rend. soc. biol.*, *107*, 836–837 (1931).
[336] J. B. Brown, R. A. Knouff, M. M. Conlin, and B. M. Schneider, *Proc. Soc. Exptl. Biol. Med.*, *37*, 203–205 (1937).

cited an even lower figure for the beef cortical tissue (0.255%). On the other hand, Parhon and Cahane[335] reported a value as high as 10.9% for the cholesterol content of the suprarenal cortex of the cat. The cholesterol content of the cortical tissue of guinea pigs is intermediate between these extremes; it is recorded as 3.5 to 6.7% by Parhon and Cahane,[335] and as 3.25% by Whitehead *et al.*[337] The figure is reported by Oleson and Bloor[338] as 1.84% for the whole gland. Although the ester cholesterol comprises 81% of the total adrenal cholesterol in the guinea pig,[338] it makes up only 10% of the total in the cortex, and 15% in the medullary tissue of cattle.[334]

As early as the first week of life, the cholesterol content of the suprarenals was high in comparison to its level in the spleen, liver, and lungs of the rat; it increased further as the animals became older. Fasting caused some decrease in free cholesterol, but mostly in ester cholesterol, in the cortical tissue.[338] Parhon and co-workers[335,339] found that the cholesterol content of the adrenals varied inversely with the water content, but that thyroidectomy, splenectomy, and thyroxine injections caused only slight changes in this component.[339,340] However, when guinea pigs were anesthetized with chloroform or nitrous oxide, not only did the cholesterol level of the suprarenal gland fall, but a similar situation obtained with respect to the lecithin content.[341]

Although the cortex of the adrenal gland is of prime importance in the case of cholesterol, actually the differences in lipid composition of the cortex and medulla of cattle are minimal. It is not known whether or not a similar uniformity in composition in the two portions of the gland, respectively, obtains in species other than cattle. The data on cattle are summarized in Table 19.

An increase in the amount of cholesterol in the adrenal gland of rats has been shown to result from the feeding of rapeseed oil.[342] Carroll[343] proved that a similar phenomenon results when erucic acid, which is the chief component of rapeseed oil, is given to rats. Of the other fatty acids tested, only nervonic acid (present in cerebrosides and in sphingomyelin) was shown to elicit a response comparable to that associated with erucic acid.

[337] R. Whitehead, M. C. Oleson, and W. R. Bloor, unpublished observations; cited by W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943, p. 221.

[338] M. C. Oleson and W. R. Bloor, *J. Biol. Chem.*, *141*, 349–354 (1951).

[339] C. I. Parhon, A. Blinov, and M. Cahane, *Compt. rend. soc. biol.*, *109*, 239–240 (1932).

[340] C. I. Parhon, C. Parhon-Stefanescu, and I. Ornstein, *Compt. rend. soc. biol.*, *121*, 187–189 (1936).

[341] P. Manceau, *Compt. rend. soc. biol.*, *92*, 1507–1510 (1925).

[342] K. K. Carroll, *Endocrinology*, *48*, 101–110 (1951).

[343] K. K. Carroll, *J. Biol. Chem.*, *200*, 287–292 (1953).

TABLE 19
LIPID ANALYSES OF THE SUPRARENAL CORTEX AND MEDULLA[a]

Fraction	Cortex, % moist wt.	Medulla, % moist wt.
Total phospholipid	3.09	2.66
Total fatty acid	1.76	1.46
Total cholesterol	0.255	0.354
Free cholesterol	0.230	0.300
Ester cholesterol	0.025	0.054

[a] J. B. Brown, R. A. Knouff, M. M. Conlin, and B. M. Schneider, *Proc. Soc. Exptl. Biol. Med.*, *37*, 203–205 (1937).

The mechanism responsible for the mobilization of adrenal cholesterol following the feeding of rapeseed oil is unknown.

The level of cholesterol in the adrenal gland is intimately connected with that of vitamin C. Thus, in scorbutic guinea pigs, not only is vitamin C reduced in the suprarenal gland, but a concomitant decrease in cholesterol occurs in this gland. Mouriquand and Leulier[344] were the first to report a decrease in cholesterol in scurvy, while Banerjee and Deb,[345] and Oesterling and Long,[346] confirmed the decrease of both components in this deficiency disease. However, the latter workers have shown that, in early scurvy, suprarenal cholesterol may actually be increased during a period when the vitamin C is reduced to 5% of its normal level. However, in late scurvy the cholesterol level is markedly below normal.[346]

The cholesterol and vitamin C levels in the adrenal glands of both guinea pigs and rats are reduced by the injection of ACTH.[347] The administration of glutathione, in dosages which offer protection against irradiation damage, also lowers both ascorbic acid and cholesterol levels in rats and guinea pigs.[348] Cholesterol in the adrenal gland of the guinea pig is also decreased by infectious diseases, but no significant changes in phospholipid or neutral fat are brought about under these conditions.[349]

Although Parhon and co-workers[339,340] stated that splenectomy had only a slight effect on the lipids in the suprarenals of guinea pigs, Marino[350] reported diametrically opposed results. An increase in cholesterol began

[344] G. Mouriquand and A. Leulier, *Compt. rend. soc. biol.*, *93*, 1314–1315 (1925).
[345] S. Banerjee and C. Deb, *J. Biol. Chem.*, *190*, 177–180 (1951).
[346] M. J. Oesterling and C. N. H. Long, *Science*, *113*, 241–242 (1951).
[347] G. Sayers, M. A. Sayers, T. Y. Liang, and C. N. H. Long, *Endocrinology*, *38*, 1–9 (1946).
[348] M. M. Carey, E. P. Vollmer, R. L. Zwemer, and D. L. Spence, *Am. J. Physiol.*, *164*, 770–773 (1951).
[349] E. J. Baumann and O. M. Holly, *J. Biol. Chem.*, *63*, lxiii–lxiv (1925).
[350] S. Marino, *Arch. farmacol. sper.*, *55*, 243–254 (1933).

immediately after the operation, reached a maximum in sixty days, began to decline after 270 days, and returned to normal after one year. Fatty acids and phosphatides first showed a reduction following extirpation of the spleen, but approximately normal levels were obtained after one year. These data are interpreted as indicating a relationship between the spleen and the suprarenal glands. Marino[350,351] also reported that hemorrhage produced a decrease in cholesterol, along with a rise in other lipids in the adrenal glands. During blood regeneration all lipids increased in this organ. After the administration of hemolytic poisons, cholesterol increased at first, but later diminished to a subnormal value in the suprarenal capsule. When blood regeneration was taking place following the hemolysis, the low cholesterol level persisted in the adrenal glands. Adams and Baxter[352] reported that the cholesterol and total lipid content of the adrenal glands, as determined in seventy-seven autopsy specimens from individuals who had had hypertension or arteriosclerosis, were not elevated above normal values.

The reproductive cycle in the rat is without profound effect on the level of the free cholesterol in the adrenal gland. Andersen and Sperry[353] reported constant values during this cycle. On the other hand, the level of ester cholesterol was lower in ovariectomized, pregnant, and parturient rats than it was in normal females during estrus, diestrus, or lactation. These workers failed to demonstrate any relationship between the cholesterol level in the serum and in the adrenal gland. Moreover, Simonnet *et al.*[354] noted no differences in the cholesterol content of the adrenals from normal and from castrated rats. The cholesterol levels were given as 2.3 and 2.4 milligram per cent, respectively. Moreover, castration did not influence the ratio of free to esterified cholesterol.

Adrenalin, which is produced by the medullary tissue, is believed to control the lipid level in the suprarenal cortex. Thus, Vogt[355] reported that repeated injections of adrenalin over an eight-hour period brought about a decrease in the lipid material in the cortical tissue of the rat and cat. A similar depletion of lipids in the adrenals of rats occurred following the injection of insulin. Since the latter effect could usually be prevented by denervation of the adrenal glands, it is postulated that the loss of cortical lipids following insulin injection in normal animals results from the liberation of adrenalin.

[351] S. Marino, *Arch. farmacol. sper.*, *55*, 183–206 (1933).
[352] E. Adams and M. Baxter, *Arch. Pathol.*, *48*, 13–26 (1949).
[353] D. H. Andersen and W. M. Sperry, *J. Physiol.*, *90*, 296–302 (1937).
[354] H. Simonnet, E. Michel, and V. Segal, *Compt. rend. soc. biol.*, *142*, 579–580 (1948).
[355] M. Vogt, *J. Physiol.*, *106*, 394–404 (1947).

The reasons for the high cholesterol levels in the suprarenal cortex are not entirely clear. Chauffard *et al.*[356] were of the opinion that the suprarenals were concerned with synthesis and esterification of cholesterol. Moreover, Srere *et al.*[357] demonstrated the synthesis of cholesterol from labeled acetate in the adrenal cortex of the rat. However, Baumann and Holly[358] reported that no significant change in the blood cholesterol or lipid phosphorus obtained in adrenalectomized rats up to the time that the animals were moribund. This would indicate that the adrenal gland was not the organ of synthesis or even an important site of storage of cholesterol. Similar results on cholesterol were reported by Randles and Knudson.[359] On the other hand, this hypothesis is open to question, in view of the finding of Joelson and Schorr,[360] and of Yeakel and Blanchard,[361] that a hypocholesterolemia and a general hypolipemia obtained after adrenal excision. A possible explanation for these divergent results may be that adequate provision was not made to prevent hemoconcentration and the attendant disturbance of circulation resulting from the upset in the salt balance following adrenalectomy.

Another possible explanation for the role of cholesterol in the adrenal cortical tissue is that it serves as the precursor of the adrenocortical hormones. That this may be the case is indicated by the fact that the injection of ACTH results in a marked decrease in the cholesterol content of cortical tissue.[347] Moreover, Marx and his collaborators,[362] as well as Ershoff and Marx,[363] demonstrated a marked protective action on the part of dietary cholesterol in experimental thyrotoxicosis in rats. One possible explanation for these findings is that the adrenocortical hormones are produced at such a high rate, in response to the stimulus of the thyroid hormone, as to result in a depletion in cortical cholesterol. When the rats can no longer produce adequate adrenocortical hormones, death ensues. However, when cholesterol is administered simultaneously with thyroid, a sufficient amount of the precursor of the adrenocortical hormones is made available to the cortical tissue to insure a continued supply of these hormones, even at an elevated level.

[356] A. Chauffard, G. Laroche, and A. Grigaut, *Ann. méd. (Paris)*, *8*, 149–172 (1920).
[357] P. A. Srere, I. L. Dauben, and W. G. Dauben, *J. Biol. Chem.*, *176*, 829–833 (1948).
[358] E. J. Baumann and O. M. Holly, *J. Biol. Chem.*, *55*, 457–475 (1923).
[359] F. S. Randles and A. Knudson, *J. Biol. Chem.*, *76*, 89–93 (1928).
[360] J. J. Joelson and E. Schorr, *Arch. Internal Med.*, *34*, 841–866 (1924).
[361] E. H. Yeakel and E. W. Blanchard, *J. Biol. Chem.*, *123*, 31–38 (1938).
[362] W. Marx, E. Meserve, and H. J. Deuel, Jr., *Proc. Soc. Exptl. Biol. Med.*, *67*, 385–387 (1948).
[363] B. H. Ershoff and W. Marx, *Exptl. Med. Surg.*, *6*, 145–148 (1948).

(2) *Phospholipids and Plasmalogens in the Adrenal Glands*

Brown and collaborators[336] reported that the phospholipids comprise the chief lipid in both the cortex and the medulla of beef adrenal tissue, where they are present to the extent of 3.09 and 2.66%, respectively, of the moist weight. The total lipid content of the beef suprarenal cortex is given as 5.36%, while that of the medullary tissue was found to be 4.83%. No neutral fat was present in this tissue. Although Bloor reports a comparable level of phospholipids for whole adrenal glands of the guinea pig (4.03%), the total lipid in this organ was 15.84%. Klenk and Friedrichs[40] reported that the plasmalogens accounted for 5.5% of the total phosphatide in beef adrenal tissue; the latter amounted to 1.13% of the moist organ.

(3) *Arachidonic Acid in the Adrenal Glands*

Brown, after demonstrating that arachidonic acid is the only highly unsaturated acid in liver lipids,[30] reported the presence of this tetraenoic acid in thyroid, spleen, and suprarenal lipids to the extent of 0.4, 4.0, and 5.5%, respectively, of the total fatty acids.[364] In a later communication, the figure for the arachidonic acid concentration of the fatty acids of the whole suprarenal glands was revised upward to 11.2%.[365] The fatty acids in suprarenal lipids other than arachidonic acid were principally palmitic, stearic, and oleic. In a later study, Ault and Brown[366] noted that arachidonic acid made up 22% of the suprarenal phosphatides, which is the richest source of this acid thus far reported. The saturated acids were isolated in the following amounts: palmitic, 23.8%; stearic, 11.1%; arachidic 2.0%; and myristic, 1.2%. Oleic acid made up about 40% of the total.

7. Lipids Present in the Spleen

Under normal conditions, the spleen presents no unusual features is lipid composition. Pfeiffer[367] reported a cholesterol content of 95 milligram per cent based upon moist weight; of this, about two-thirds was present as the free alcohol. Fresh spleen was found to contain only 2.9% of the total lipids, and most of this belonged to the ether-soluble fraction.[368] No cerebroside is present in normal spleen, although Tropp and Wiedersheim[369]

[364] J. B. Brown, *J. Biol. Chem.*, *83*, 777–782 (1929).

[365] J. B. Brown, unpublished data; cited by W. C. Ault and J. B. Brown, *J. Biol. Chem.*, *107*, 607–614 (1934), p. 607.

[366] W. C. Ault and J. B. Brown, *J. Biol. Chem.*, *107*, 607–614 (1934).

[367] G. Pfeiffer, *Biochem. Z.*, *231*, 239–243 (1931).

[368] L. Bouisset and C. Soula, *Compt. rend. soc. biol.*, *110*, 673–674 (1932).

[369] C. Tropp and V. Wiedersheim, *Z. physiol. Chem.*, *222*, 39–43 (1933).

did find minimal amounts of lignocerylsphingosine (0.016 to 0.034% of moist weight) in this organ. Klenk and Friedrichs[40] reported that beef spleen contained 0.78% of phospholipids (moist basis), of which 1.7% consisted of plasmalogens.

In contradistinction to the lipid composition of the normal spleen, that occurring in several types of lipidoses presents marked variations from the composition noted in most other tissues. Under these pathologic conditions, not only may the organ be markedly enlarged due to the incorporation of large amounts of lipids, but also the type of lipid deposited is that usually characteristic of nervous tissue. For a discussion of the effect of lipidoses on the composition of the spleen, see Chapter VI, page 702.

8. Lipids Present in the Reproductive Organs

Cholesterol is of primary importance, either directly or indirectly as a building stone in the synthesis of the male and female hormones. However, other lipids also play an important role, both in the structure of the cells of the sex organs and also in their metabolic behavior.

(1) Lipids in the Testes

Phospholipids and cerebrosides have been demonstrated in the seminal cells and in the interstitial tissue of the male.[370] Although the lipids were present extracellularly in the latter case, as storage products, and thus were involved in the total lipid metabolism, they were intracellular and intrinsic components of the seminal cells. The role of the testicular phospholipids is problematical, in view of the finding of Sinclair[371] that they do not enter into the same metabolic interplay as do the phospholipids of other tissues. When elaidic acid, which was used as a tracer, was given to rats during the entire period of growth, from weaning on, practically none was incorporated in the phospholipids of the testes, although it was taken up by most other tissues. The absence of this acid from the lipids of the testicles was even more complete than was its absence from the brain lipids. Arachidonic acid has been found to occur in an especially high concentration in bull testes.[372]

Ward and Moore[373] have demonstrated the presence of 7-dehydrocholesterol in the male genitals of the rat. These workers reported the

[370] K. Sorg, *Z. Konstitutionslehre, 10*, 67–78 (1924).
[371] R. G. Sinclair, *J. Biol. Chem., 134*, 89–94 (1940).
[372] R. T. Holman, personal communication to the author, 1953.
[373] R. J. Ward and T. Moore, *Biochem. J., 52*, v (1952); *55*, 295–298 (1953).

occurrence of this unusual sterol in the preputial glands, in the caput epididymis, and in the corpus epididymis, but not in the testes, cauda epididymis, seminal vesicles, coagulating glands, or in the prostate. The presence of 7-dehydrocholesterol in the lipids of the male genital organs seems to be peculiar to the rat. Srere and associates[7] have demonstrated that cholesterol can be synthesized from acetate in the rat testicles.

(2) Lipids in the Ovaries

a. The Lipid Composition of Resting Ovaries. In the female, the chief interest has centered in the composition of the lipids of the corpus luteum and placenta. Since the lipid composition differs with the various phases of the estrus cycle, as well as with those of pregnancy, it is difficult to assign a definite composition to the lipids of the ovary. Fenger[374] noted that the phospholipid content of the corpus luteum was comparable to that of other endocrine organs (except the thyroid), and that it was about fifteen times as high as in muscle tissue. Cartland and Hart[375] reported that about one-half of the acetone-soluble portion of the lipids consisted of glycerides, and the balance of fatty acids and soaps. The fatty acids in the total extract, 35% of which were saturated and 65% unsaturated, consisted of the following: palmitic, 25%; stearic, 11%; oleic, 33%; linoleic, 17%; arachidonic, 8%; and 4.8% of a new, hexaunsaturated acid of the C_{20} series, having the formula $C_{20}H_{24}O_2$. These workers reported the presence of a considerable quantity of cholesterol, both in the form of the free alcohol and as the palmitic acid ester. On the other hand, considerable variation in the fatty acid make-up of lecithin from that of the triglycerides was noted by Hart and Heyl.[376] Although they found palmitic, oleic, arachidonic, and another 20-carbon acid having 3 double bonds, in the lecithin from corpus luteum, no linoleic or linolenic acid was present, and stearic acid occurred only to a minimal extent, if at all. The cephalin fraction appeared to be similar to that obtained from liver, heart, and brain, and consisted chiefly of phosphatidylethanolamine. Swine ovaries contained about 0.80% of phospholipids (moist basis), of which approximately 0.4% could be isolated as pure plasmalogens.[40]

b. The Lipid Composition of the Ovaries as Related to the Estrus Cycle. Cholesterol and phospholipids have been shown to undergo marked changes during the estrus cycle. As early as 1912, Chauffard and his collab-

[374] F. Fenger, *J. Biol. Chem.*, *27*, 303–307 (1916).
[375] G. F. Cartland and M. C. Hart, *J. Biol. Chem.*, *66*, 619–637 (1925).
[376] M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, *70*, 663–674, 675–682 (1926).

orators[377] reported that the amount of cholesterol continually increased in the corpora lutea of sows and cows during the estrus cycle. It was suggested that this organ was a site for the storage and possibly even for the synthesis of the sterol. On the other hand, Bloor and colleagues[378] found practically no change in the total cholesterol during estrus, in the sow. However, the amount of free cholesterol increased up to the time of ovulation; during the period following the active functioning of the gland, the cholesterol ester increased. In fact, these authors state that the content of cholesterol esters in the corpus luteum varies inversely with the functional activity of the gland, a high content thus being characteristic of the degenerated organ. Kaufmann[379] reported similar results. According to Boyd and Elden,[380] the progestin content of the corpus luteum is proportional to the free cholesterol content; the estrin content corresponds to that of phospholipids, but not to that of other lipid fractions of the gland.

Phospholipids present more marked alterations during the functioning of the corpus luteum than do other lipid components. In the sow, the percentage content of the phospholipid in the corpus luteum was two to three times as high during the period of activity as it was during the formation of ova or after retrogression. Hermstein[381] found that, in human subjects, phospholipids were highest at the time of maximum activity of the gland. Kaufmann and Raeth[382] likewise reported that the lecithin content was highest at the time of ovulation in twenty-four cases, while the amount of this phospholipid decreased as menstruation approached. Cerebrosides as well as phospholipids have been reported to increase during the functional stage.[383]

Boyd[384] reported a generalized two- to seven-fold increase in all lipids of the ovaries of the leopard frog (*Rana pipiens*), concomitantly with the production of ova. The increase in lipid content included not only total lipids, free and esterified cholesterol and phospholipids, but also neutral fat and fatty acids.

c. The Lipid Composition of the Ovaries as Affected by Pregnancy. According to Boyd,[385] the changes in the several lipid components in the

[377] A. Chauffard, G. Laroche, and A. Grigaut, *Compt. rend. soc. biol.*, *72*, 223–225, 265-267 (1912).

[378] W. R. Bloor, R. Okey, and G. W. Corner, *J. Biol. Chem.*, *86*, 291–306 (1930).

[379] C. Kaufmann, *Z. Geburtshülfe Gynäkol.*, *91*, 668–681 (1927).

[380] E. M. Boyd and C. A. Elden, *Endocrinology*, *19*, 599–602 (1935).

[381] Hermstein, *Arch. Gynäkol.*, *124*, 739–770 (1925).

[382] C. Kaufmann and K. Raeth, *Arch. Gynäkol.*, *130*, 128–151 (1927).

[383] F. v. Mikulicz-Radecki, *Arch. Gynäkol.*, *116*, 203–251 (1923).

[384] E. M. Boyd, *J. Physiol.*, *91*, 394–397 (1938).

[385] E. M. Boyd, *J. Biol. Chem.*, *108*, 607–617 (1935).

ovary depend upon the stage of pregnancy. Up to the fourteenth to sixteenth days of pregnancy in the rabbit, phospholipid and free cholesterol increased; a maximum rise of as much as 300% was sometimes observed. The variations in cholesterol ester and neutral fat content during this period were found to be negligible. After the pregnancy had progressed to the halfway mark, the concentration of phospholipid and free cholesterol in the ovaries began to decrease, and this decrease continued until, at term, the values were similar to those at the start of pregnancy. On the other hand, the level of cholesterol ester and neutral fat remained high during the last half of pregnancy; in fact, the value for cholesterol ester did not begin to decline until the last day of pregnancy. The author interprets these findings as indicative of the fact that the ovary reaches its maximum activity about the middle of pregnancy, after which the activity decreases to the low figure reached at term. Boyd[386] was unable to demonstrate any pattern for the changes in cholesterol and phospholipid in the ovaries of the Brazilian guinea pig (*Cavia porcellus*) during pregnancy, corresponding to that observed in the case of the sow. The phospholipids remained at the control average of 1250 milligram per cent, and free cholesterol at a proestrus mean of 300 milligram per cent, during the entire pregnancy.

The results obtained by Bloor *et al.*[378] for the pregnant sow are likewise characteristic for this species. Phospholipid values continue, during the entire course of pregnancy, at the high levels obtained at ovulation. On the other hand, the total cholesterol level remains somewhat lower than the proestrus level, during the whole sequence of pregnancy. A greater decrease apparently occurs in the cholesterol esters than in the free cholesterol. Hence, the ratio of lecithin:free cholesterol rises to a lesser degree than that of lecithin:ester cholesterol.

(3) *Lipids in the Uterine Mucosa*

Marked structural changes occur in the uterine mucosa which are associated with the phenomena of estrus, menstruation, implantation of the embryo, pregnancy, and the puerperium. It has been established through the studies of Bouin and Ancel,[387] and especially of Corner and Allen,[388] that the pregestational proliferation of the uterine mucosa can be traced to the action of a hormone elaborated by the corpus luteum.

[386] E. M. Boyd, *J. Biol. Chem.*, *112*, 591–595 (1936).
[387] P. Bouin and P. Ancel, *J. physiol. et pathol. gén.*, *12*, 1–16 (1910).
[388] G. W. Corner and W. M. Allen, *Am. J. Physiol.*, *88*, 326–339, 340–346 (1929).

Okey and her collaborators[389] compared the cyclic changes in the lipid composition of the corpus luteum and of the uterine mucosa of the pig. It was found that the total lipid content of the uterine mucosa (based upon dry weight) was approximately the same as that of the lung, kidney, or pancreas of beef. During the period of greatest elaboration of endometrium, a small but definite increase in lecithin obtained, together with an increase in free cholesterol. The lecithin:cholesterol ratios were consequently lower than those existing simultaneously in the corpus luteum. Moreover, a minimum amount of cholesterol ester was found in the endometrium in all cases.

(4) Lipids in the Placenta

According to Watanabe,[390] the total lipids present in the placenta are higher in early pregnancy than they are as term approaches. Phospholipid, chiefly lecithin, was found to the extent of approximately 30%, while neutral fat accounted for 27% of the total lipid. Cholesterol was likewise present in both free and ester form. Čmelik[391] reported that the total fat content of human placenta, obtained at term, was only 0.5% of the moist weight. Slightly more than 50% of the fatty acids obtained from the placenta lipid were water-soluble, which is a most unusual feature for any tissue. It is suggested that higher fatty acids may be built up in the epithelial cells and then excreted into the surrounding fluid. The water-insoluble acids consisted largely of unsaturated acids, although stearic acid was also identified. Cholesterol accounted for 88% of the unsaponifiable fraction. However, no squalene could be demonstrated chromatographically. This hydrocarbon had previously been shown to be a component in ovarian dermoid cysts[392] and in the depot fat of women.[393] Čmelik and his co-workers[394,395] likewise demonstrated the presence of squalene in the vernix caseosa (waxy substance covering the fetus at birth). This compound was absent from the lipids present in skin atheroma.[396]

[389] R. Okey, W. R. Bloor, and G. W. Corner, *J. Biol. Chem.*, *86*, 307–314 (1930).
[390] H. Watanabe, *J. Biochem. (Japan)*, *2*, 369–397 (1923).
[391] S. Čmelik, *Biochem. Z.*, *322*, 150–153 (1951).
[392] A. Dimter, *Z. physiol. Chem.*, *270*, 247–265 (1941).
[393] E. Calandra and P. Cattáneo, *Rev. Soc. argent. Biol.*, *24*, 275–285 (1948); *Chem. Abst.*, *43*, 8494 (1949).
[394] S. Čmelik, N. Petrak-Longhino, and F. M. Mihelić, *Biochem. Z.*, *322*, 355–359 (1952).
[395] S. Čmelik, N. Petrak-Longhino, and F. M. Mihelić, *Arhiv. Kem. (Zagreb)*, *22*, 236–237 (1950); *Chem. Abst.*, *45*, 10352 (1951).
[396] S. Čmelik, *Biochem. Z.*, *322*, 497–501 (1952).

9. Lipids Present in the Skin and Its Appendages

(1) General Distribution of the Lipids

The skin not only represents one of the most extensive organs of the body, but it also possesses varied physiologic functions and specialized metabolic patterns which contribute to its importance. Lipids play an important role in these several functions of the skin. The metabolism and permeability of normal skin was reviewed in 1946 by Calvery *et al.*[397]

The first comprehensive study of the composition of skin lipids was that of Unna and Golodetz,[398] who reported chiefly on the cholesterol distribution in human skin, as affected by various metabolic processes. According to Jono,[399] human skin contains 2% of lipids. Eckstein and Wile[400] found that cholesterol occurred in exfoliated human skin to the extent of 13 to 24% of the total lipids; approximately 90% of the cholesterol was present in free form. Phospholipid was found to constitute only about 3% of the total skin lipids. Pachur[401] reported similar analyses of the human skin surface. He found 88.26% neutral fat, 4.2% cholesterol esters, phosphatides and soaps, and 7.54% cholesterol.

However, differences in the results of several investigators have been difficult to evaluate because of the variations in the source of the samples. Differences exist in the lipid nature of the various strata of the skin. In order to obtain a serial picture of the composition of skin lipids as related to the portion of the skin from which they were obtained, Koppenhoefer[402,403] compared the lipid composition in six histologically distinct horizontal layers. These included the epidermal region consisting of hair (*1*), horny (*2*) and basal area (*3*), the transition region (*4*), and the corium region, which is made up of the corium major (*5*) and of the corium base (*6*). In these studies, the skin of steer was employed, since this offered the advantage of being available in large amounts and of being readily separable mechanically into the several different layers. A striking similarity exists between the lipids of human skin and those of steer skin, particularly in regard to their nature and to the pattern of their distribution. Table 20 summarizes the data on the composition of lipids in the several layers of steer skin.

[397] H. O. Calvery, J. H. Draize, and E. P. Laug, *Physiol. Revs.*, *26*, 495–540 (1946).
[398] P. G. Unna and L. Golodetz, *Biochem. Z.*, *20*, 469–502 (1909).
[399] Y. Jono, *J. Biochem.* (*Japan*), *10*, 311–323 (1928–1929).
[400] H. C. Eckstein and U. J. Wile, *J. Biol. Chem.*, *69*, 181–186 (1926).
[401] R. Pachur, *Dermatol. Z.*, *60*, 486–490 (1931).
[402] R. M. Koppenhoefer and J. H. Highberger, *J. Am. Leather Chem. Assoc.*, *29*, 598–623 (1934).
[403] R. M. Koppenhoefer, *J. Biol. Chem.*, *116*, 321–341 (1936).

TABLE 20
THE LIPID COMPOSITION OF THE DIFFERENT LAYERS OF THE SKIN OF THE STEER (BASED UPON DRY WEIGHT)[a]

Category	Epidermal region			Transition region	Corium region	
	Hair	Horn	Base		Corium major	Corium base
Proportionate dry wt., %	3.0	6.2	12.7	7.3	58.7	12.1
Total lipid						
%	4.60	8.72	7 28	1.95	2.44	7.15
% skin lipid	3.39	13.4	22.8	3 52	35.6	21.4
Cholesterol						
%	0.67	1.01	0.97	0.22	0.06	0.08
% skin lipid	14.5	11.6	13.4	11.2	2.60	1.11
Phospholipid, %	0.00	0.78	2.35	0.42	0.10	0.10
Wax						
%	0.97	2.21	2.55	—	—	—
% skin lipid	21.0	25.3	35.1	—	—	—
Free fatty acids						
%	1.24	2.09	0.49	0.21	0.06	0.15
% skin lipid	26.8	17.7	8.3	11.1	4.0	2.05

[a] R. M. Koppenhoefer, *J. Biol. Chem.*, *116*, 321–341 (1936).

The lipid concentration is greatest toward the upper and lower extremities of the skin. In the epidermal region, cholesterol, phospholipids, and waxes constitute the main lipids, while in the corium base a considerable deposition of triglycerides obtains; this deposit is distinct from the subcutaneous fat layer.

a. Cholesterol and Other Sterols in the Skin. The greater proportion of the skin cholesterol occurs in the epidermal region.[400,403–405] The cholesterol present in the outer skin layers is largely in the form of esters, while that in the corium consists principally of the free alcohol. Esterification of cholesterol is believed to take place concomitantly with the keratinization of epidermal cells, which occurs only in the epidermal region.

In the corium, the distribution of cholesterol is uniform, as is also that of the phospholipids. This has led to the hypothesis that these lipid constituents have a mutual physiologic relationship, and that they are associated with cellular elements in the corium. The cholesterol concentration begins to increase in the transition region, and reaches its maximum in the epidermal portion of the skin. Although the phospholipid:chole-

[404] D. J. Kooyman, *Arch. Dermatol. and Syphilol.*, *25*, 444–450 (1932).

[405] M. F. Engman and D. J. Kooyman, *Arch. Dermatol. and Syphilol.*, *29*, 12–19 (1934).

sterol proportion remains uniform in those divisions of the outer layers in which keratinization processes are absent, the ratio decreases in the outer layers in which keratinization is taking place. In the latter regions, phospholipids are apparently destroyed, but less destruction of cholesterol[404] takes place. Since no phospholipids are present in the hair division, a more rapid destruction of phospholipids than of cholesterol takes place at the skin surface.

Taylor *et al.*[406] noted that a sex variation occurred in the lipid composition of rat skin. Total lipids made up 22% of the dry weight in the female skin, and only 12.5% in that of the male rat. On the other hand, total cholesterol was higher in the skin tissues of the male (0.354%) than in those of the female (0.248%), but free cholesterol was higher in the female (0.132%) than in the male (0.118%).

Roffo[407] reported that the cholesterol content of the skin of men is greater than that of women. It is suggested that the higher susceptibility of men to cutaneous neoplasm, especially of the face, may be related to this phenomenon, inasmuch as neoplasms contain more cholesterol than does healthy, normal tissue.

The cholesterol content varies with the particular area of the skin involved. Roffo[407,408] reported that the cholesterol content of skin from an exposed area such as the face was greater than in that obtained from an area normally covered, such as the abdomen. In the case of the adult, more cholesterol occurred in the skin of the face than in that of the back; this was also the case in the skin of the fetus or newborn, but to a considerably lesser degree.[409] The cholesterol in the facial skin of the infant was shown to increase with age, as a result of the effect of light.[409] Thus, Roffo[410,411] found that irradiation of the skin with sunlight caused an increase in the cholesterol content of rat skin, while a decrease occurred in the skin content of this sterol when the animals were kept in the dark.[411,412] Kawaguchi[413] reported that an increase in skin cholesterol also resulted in guinea pigs and rabbits subjected to ultraviolet irradiation, although the response was not as marked as that to sunlight. Roffo[407,408] found a rela-

[406] J. D. Taylor, H. E. Paul, and M. F. Paul, *Arch. Biochem.*, *17*, 421–428 (1948).

[407] A. H. Roffo, *Néoplasmes*, *7*, 344–351 (1928).

[408] A. H. Roffo, *Bol. inst. med. exptl. estud. cáncer (Buenos Aires)*, *6*, 370–382 (1929); *Chem. Abst.*, *26*, 1304 (1932).

[409] A. H. Roffo, *J. physiol. et pathol. gén.*, *30*, 345–349 (1932).

[410] A. H. Roffo, *Bol. inst. med. exptl. estud. cáncer (Buenos Aires)*, *8*, 32–38 (1931); *Chem. Abst.*, *29*, 5871 (1935).

[411] A. H. Roffo and F. Pilar, *J. physiol. et pathol. gén.*, *28*, 854–856 (1930).

[412] A. H. Roffo, *J. physiol. et pathol. gén.*, *29*, 739–741 (1931).

[413] S. Kawaguchi, *Biochem. Z.*, *221*, 232–240 (1930).

tionship between the cholesterol level of the skin and the frequency of skin tumors. Gould and Taylor,[414] and Srere *et al.*,[7] making use of C^{14}-acetate, demonstrated that the skin possesses the ability to synthesize cholesterol at a rate comparable with that of the liver.

In addition to cholesterol, several other sterols have been identified as components of the skin. The most important of these is 7-dehydrocholesterol, or provitamin D_3. Although this compound has been shown to be a constant contaminant of cholesterol,[415] it usually occurs in a higher proportion in the skin lipids than in those obtained from inner tissues.[16] Windaus and Bock[416] were able to separate it in amounts as high as 3 to 6% of the total sterols in pigskin. Rosenberg[16] reported the following percentages of provitamins D in the sterols obtained from the skin of several species: man, 0.15 to 0.43; cattle, 0.18; calf, 0.68; mouse, 0.87; and chicken (feet) 1.0 to 4.0. Rosenberg[417] recently confirmed the presence of high concentrations of 7-dehydrocholesterol (provitamin D_3) in the feet of the chicken. However, no evidence could be obtained indicating the presence of this or of any other provitamin D in the preen gland (*glandula uropygialis*) of ducks, geese, or chickens. It is therefore evident that our concept of the synthesis of vitamin D from the secretion of the preen gland of fowl must be revised.

It is generally believed that 7-dehydrocholesterol originates from cholesterol in the skin, and that it can be further transformed to vitamin D_3 by ultraviolet radiation. The latter transformation can readily be demonstrated in an *in vitro* system; it is believed that the protection from rickets afforded to rats by sunlight or ultraviolet light is to be traced to a similar activation of the provitamins D within the skin, resulting in the *in vivo* production of vitamin D.

Moore and Baumann[418] have reported evidence for the presence of sterols other than cholesterol in the cholesterol fraction obtained from rat skin. Whereas the sterols isolated from most tissues of the body reacted with the modified Schoenheimer-Sperry reagents in a manner similar to the reaction of pure cholesterol, those isolated from the skin formed an intense blue color almost immediately. 7-Dehydrocholesterol and 7-hydroxycholesterol are known to react with this reagent at a rapid rate. The skin was found to contain 22 to 36% of a fast-reacting sterol, although spectro-

[414] R. G. Gould and C. B. Taylor, *Federation Proc.*, *9*, 179 (1950).

[415] A. G. Boer, E. H. Reerink, A. van Wijk, and J. van Niekerk, *Proc. Acad. Sci., Amsterdam*, *39*, 622–632 (1936).

[416] A. Windaus and F. Bock, *Z. physiol. Chem.*, *245*, 168–170 (1936).

[417] H. R. Rosenberg, *Arch. Biochem. Biophys.*, *42*, 7–11 (1953).

[418] P. R. Moore and C. A. Baumann, *J. Biol. Chem.*, *195*, 615–621 (1952).

scopic analyses revealed the presence of only 1% of 7-dehydrocholesterol. The major component of the fast-reacting sterol fraction which was isolated by chromatographic separation of its azoyl ester on silica was shown to be Δ^7-cholestene-3β-ol.[419]

b. Phospholipids in the Skin. The phospholipid level present in the two corium layers does not vary with the triglyceride content of the skin, but remains fairly constant. Koppenhoefer[403] suggests that this indicates that the phospholipids are associated with the fundamental protoplasmic structure, namely the fibroblasts of the corium.

Much higher amounts of phospholipids occur in the basal layer of the epidermal region. This is also the location in which the nucleated cells present maximum development. In Koppenhoefer's studies, an appreciable decline in phospholipids occurred in the horny layer; none could be identified in the lipid isolated from hair.[403] As indicated earlier, the disappearance of the phospholipid from this area is believed to reflect a destruction which occurs during keratinization. The hydroxylated fatty acids present in the wax produced by the sebaceous glands probably originate from the phospholipid fatty acids.

Both lecithin and cephalin are present in the several skin layers; in general, the lecithin represents the phospholipid present in the higher concentration. Thus, the proportions of the acetone-insoluble phospholipid precipitated as lecithin and as cephalin, respectively, were found to be as follows[403]: horn division, 41.3 and 6.2%; basal epidermal region, 67.0 and 9.9%; transitional region, 7.2 and 18.0%; corium major layer, 68.9 and 4.2%; and corium basal layer, 34.5% and not determined.

The iodine number of the lecithin fraction decreases in the outer layers. Koppenhoefer[403] found the following iodine numbers for the several phospholipid fractions: corium major, 83.0; basal epidermal region, 77.2; and horn layer, 66.5. In contradistinction to this progressive decrease in iodine number occurring toward the outer layers of the skin, the acetyl value showed the opposite response (51.2, 52.1, and 74.9, respectively). The cephalin fraction contained fatty acids with a greater degree of unsaturation and higher molecular weights than those present in lecithin.

c. Waxes in the Skin. Koppenhoefer and Highberger[402] were able to prepare isohydroxystearic acid, stearic acid, and *n*-eicosanol (arachyl alcohol) from the waxes prepared from steer skin. The last compound has been isolated by Ameseder[420] from a dermoid cyst. The presence of

[419] D. R. Idler and C. A. Baumann, *J. Biol. Chem.*, *195*, 623–628 (1952).
[420] F. Ameseder, *Z. physiol. Chem.*, *52*, 121–128 (1907).

myristyl alcohol (*n*-tetradecanol) and lauryl alcohol (*n*-dodecanol) also appears probable.

The wax fraction can be divided into two distinct types. One group, present in a large amount in the epidermal region, consists of esters of aliphatic alcohols with a saturated hydroxy acid. The second group is made up of cholesterol combined with unsaturated acids.

The site of formation of the epidermal wax is uncertain. Because of the saturated nature of the wax, it was first thought to be formed as a result of oxidation processes occurring at the epidermal surface. However, the results of Koppenhoefer,[403] which indicated the presence of only 30% of the total wax in the horn division as contrasted with 65% of the total in the lower basal region, would seem to refute this earlier hypothesis. The best suggestion as to the site of origin of the epidermal wax is that it is formed in the sebaceous secretion.

d. Triglycerides in the Corium. Although neutral fats are practically absent from the outer skin layers, appreciable amounts are present in the corium, where they occur as cellular deposits distributed among and between the corium fiber elements.[403] The deposition of fat is more pronounced along the subcutaneous border, and is identical in histologic structure with that in the cells of this layer.[421] The amount of the fat deposits in the corium region varies considerably from skin to skin, probably depending upon the nutritional status of the animal from which the skin was obtained. The corium triglycerides are made up of approximately 65% liquid and 35% solid fatty acids. Tripalmitin has been isolated from the corium lipids.[402]

e. Free Fatty Acids in the Skin. Free fatty acids were shown by Engman and Kooyman[405] to be present in a considerable concentration on the surface of the human skin. This worker attributed his finding to the lipolytic action of bacteria and enzymes, followed by an atmospheric oxidation at the skin surface. According to the results of Koppenhoefer,[403] there was a progressive increase in the free fatty acids as one approached the outer surface of the skin regions. The origin of the free acids appears to be in doubt. Although the fatty acids of the horn division resembled those of the phospholipids, except that the free fatty acids had a higher acetyl value, the composition of the free fatty acids of the basal region differed markedly from that of the combined acids.

De Boer[216] reported that chronic dehydration of the dog, produced by withholding food and water, resulted in a 4% decrease in the fat in the skin.

[421] F. O'Flaherty and W. T. Roddy, *J. Am. Leather Chem. Assoc.*, *30*, 290–311 (1935).

Less water was lost under these conditions by obese dogs than by lean animals.

(2) *Lipids Present in the Hair and Other Appendages*

Eckstein[422] reported that the hair of young adult albino rats contained 4.5% of total lipids; cholesterol constituted 12% and phospholipids (lecithin) only 0.8% of the total lipids. These values are in line with those obtained by Koppenhoefer[403] for the hair layer of steer skin. Eckstein[422] found that 80% of the cholesterol was present as the free alcohol, and only 20% occurred as the ester. Table 21 summarizes the total lipid and cholesterol content of hair from several animals, of wool and of feathers.

TABLE 21
THE LIPID COMPONENTS (IN PER CENT DRY WEIGHT) IN HAIR, FEATHERS, AND WOOL

Substance	Animal source	No. of tests	Total lipid	Total cholesterol	Free cholesterol
Hair	Human, adult[a]	5	6.1	0.15	0.08
	Human, child[a]	20	3.6	0.60	0.23[b]
	Rabbit[a]	5	1.5	0.57	0.53
	Rabbit[c]	1	—	0.45	—
	Rat[a]	1	4.3	0.57	0.41
	Cat[a]	1	5.3	0.55	0.46
	Dog[a]	1	1.8	0.56	—
Wool	Sheep[a]	8	10.8	0.82	0.47
Feathers	Duck[a]	1	3.2	0.29	0.18
	Goose[a]	1	1.9	0.27	
	Turkey[a]	2	1.5	0.27	—
	Wild birds[d]	12	—	0.54–0.06	—
Quills	Duck[a]	1	3.6	0.38	—
	Goose[a]	1	4.6	0.30	—
	Turkey[a]	1	2.2	0.37	—

[a] H. C. Eckstein, *J. Biol. Chem.*, *73*, 363–369 (1927).
[b] Values for 13 children only are included in this average.
[c] M. Kawaguchi, *Nagoya J. Med. Sci.*, *10*, 348–349 (1937); *Chem. Abst.*, *31*, 7099 (1937).
[d] R. Salgues, *Compt. rend. soc. biol.*, *124*, 923–925 (1937).

Cholesterol has been found to be higher in the feathers of young birds,[423] as well as in the fur of young rabbits,[424] than in adult animals; moreover,

[422] H. C. Eckstein, *Proc. Soc. Exptl. Biol. Med.*, *23*, 581 (1926).
[423] R. Salgues, *Compt. rend. soc. biol.*, *124*, 923–925 (1937).
[424] M. Kawaguchi, *Nagoya J. Med. Sci.*, *10*, 348–349 (1937); *Chem. Abst.*, *31*, 7099 (1937).

the observations of Eckstein[425] indicate that the same phenomenon applies to man. The cholesterol content of the fur of the rabbit was found to vary with the season, being higher in the fall and winter and lower in spring and summer.[424] No difference in the amount of this component was attributable to color, although curly hair had a lower cholesterol content than did straight hair.

In addition to the presence of cholesterol in hair, the so-called "isocholesterol" has likewise been reported as a component of this fat. As early as 1872, Schulze[426–428] described this cholesterol-like component which is present in lanolin or wool grease. It has likewise been identified in the petroleum ether extract of merino wool.[429] Isocholesterol is now known to consist of at least two separate compounds,[430] which are referred to as agnosterol and lanosterol. Agnosterol has the empirical formula, $C_{30}H_{47}OH$, while that for lanosterol is $C_{30}H_{49}OH$. Neither of these products is isomeric with cholesterol. In fact, they do not possess the steroid nucleus. They belong to the triterpene group. The names, "isocholesterol," lanosterol, and agnosterol, are therefore misleading and should not be used to describe these compounds. In addition to cholesterol and the triterpenes, two higher aliphatic alcohols, cetyl and ceryl alcohols, were reported by Drummond and Baker[429] to be present in the lipids extracted from merino wool. However, no glycerol was found in the wool fat.

The fatty acids in wool wax are characteristic of this tissue. Weitkamp[431] classified the fatty acids in wool wax into the following categories: (*1*) normal saturated acids; (*2*) 2-hydroxy-acids; (*3*) iso- (or branched-chain) acids corresponding to the general formula, $CH_3CH(CH_3)(CH_2)_{2n}$-COOH, in which n is 3 to 11 inclusive; and (*4*) anteiso-acids with the general structure, $CH_3CH_2CH(CH_3)(CH_2)_{2n}COOH$, in which n is 2 to 13 inclusive.

Group *1* comprises not only palmitic and stearic acid, but also the C_{26} acid, cerotic acid.[429] Acids identified in Group *2* include 2-hydroxymyristic,[431] 2-hydroxypalmitic,[431,432] and lanopalmic acid,[432–434] which is an

[425] H. C. Eckstein, *J. Biol. Chem.*, *73*, 363–369 (1927).
[426] E. Schulze, *Ber.*, *5*, 1075–1078 (1872).
[427] E. Schulze, *Ber.*, *6*, 251–254 (1873).
[428] E. Schulze, *J. prakt. Chem.* [*n.s.*], *7*, 163–178 (1873).
[429] J. C. Drummond and L. C. Baker, *J. Soc. Chem. Ind.*, *48*, 232–238T (1929).
[430] A. Windaus and R. Tschesche, *Z. physiol. Chem.*, *190*, 51–61 (1930).
[431] A. W. Weitkamp, *J. Am. Chem. Soc.*, *67*, 447–454 (1945).
[432] T. Kuwata, *J. Am. Chem. Soc.*, *60*, 559–560 (1938).
[433] E. Darmstaedter and J. Lifschütz, *Ber.*, *29*, 1474–1477, 2890–2900 (1896).
[434] E. E. U. Abraham and T. P. Hilditch, *J. Soc. Chem. Ind.*, *54*, 398–404T (1935).

isomer of 2-hydroxypalmitic acid. Lanocerotic acid is a dihydroxy acid with an empirical formula, $C_{30}H_{60}O_4$, which has also been identified in wool wax.[433,434]

The principal fatty acids in wool fat and hair fat are the iso- and anteiso-acids listed as Groups *3* and *4*. In Group *3*, the iso-acids have the methyl group in the penultimate position; the acids have an even number of carbons, although the fatty acid chains contain an odd number of carbons. In Group *4*, in which the methyl side chain is attached to the antepenultimate carbon, the compounds have an odd number of carbon atoms, although the fatty acid chain is an even one.

Weitkamp, Smiljanic, and Rothman[435] have reported that the free fatty acid fraction of human hair fat consists of normal saturated and unsaturated acids ranging in chain length from 7 to 22 carbon atoms. The 6,7-position was found to be the characteristic location of the double bond in the unsaturated acids, although some $\Delta^{8,9}$-acids, as well as other isomers, were found. The following proportions of acids in Group *3* were noted: C_8, 0.15%; C_{10}, 0.33%; C_{12}, 3.5%; C_{14}, 9.5%; C_{16}, 36%; C_{18}, 23%; C_{20}, 8.5%; C_{22}, 2.0%; and higher than C_{22}, 4.0%. The anteiso-acids (Group *4*)[435] were present as follows: C_7, 0.07%; C_9, 0.20%; C_{11}, 0.15%; C_{13}, 1.4%; C_{15}, 6.0%; and C_{17}, 6.0%. For a further discussion of the nature of these wool fatty acids, the reader is referred to Volume I, pages 36, 37, and 370.

(*3*) *Lipids Present in the Sebum*

The sebum represents the secretion of the sebaceous glands of the skin. In the fur-bearing animals, this secretion emerges at the roots of the hair. In birds, the coccygeal or preen gland, located at the base of the tail, has a function similar to that of the sebaceous gland; it secretes lipids important in the functioning of feathers.

The nature of the secretion of the sebaceous glands varies with the position in which the glands are located. *Cerumen*, the waxy secretion of the outer ear, is a specialized type of sebum. The sebaceous glands located in the feet, armpits, and anus secrete more neutral fat than do glands from other parts of the skin; the unpleasant odor from these areas (*bromidrosis*) is believed to be due to the rancidity of this fat. In the disease known as *seborrhea*, the secretion of the sebaceous glands is increased.[436]

The composition of sebum is ill-defined because of the difficulty of ob-

[435] A. W. Weitkamp, A. M. Smiljanic, and S. Rothman, *J. Am. Chem. Soc.*, *69*, 1936–1939 (1947).

[436] M. R. Everett, *Medical Biochemistry*, 2nd ed., Hoeber, New York, 1946, p. 229.

taining it uncontaminated with other materials present on the surface of the skin. Koppenhoefer[403] prepared a fairly pure sample from steer skin, by first removing the horny layer and later pressing out the oily secretion

TABLE 22
THE COMPOSITION OF SEBUM[a]

Component	Total amt., %	Analytical values: Mol. wt.	Sapon. No.	Iodine No.	Acetyl value	Acid value
Total lipids	100.0	—	154.8	32.6	—	10.1
Fatty acid	57.4	249.1	—	27.3	74.9	—
Unsapon. fraction	42.7	—	—	36.0	—	—
Cholesterol, total	14.4	—	—	—	—	—
Cholesterol, esterified	13.7	—	—	—	—	—
Phospholipids	4.0	—	—	—	—	—

[a] Adapted from R. M. Koppenhoefer, *J. Biol. Chem.*, *116*, 321–341 (1936), p. 335.

TABLE 23
THE CALCULATED AVERAGE COMPOSITION OF HUMAN FOREARM SEBUM[a]

Component of sebum	Per cent of total lipid
Free fatty acids	
Saturated	15.0
Unsaturated	15.0
Triglycerides	32.5
Waxes (including cholesterol esters)	15.0
Sterols	
Free cholesterol	2.5
Combined cholesterol[b]	(2.5)
Other sterols	2.5
Squalene	5.0
Paraffins	7.5
Unidentified compds. (including oxidized squalene)	5.0

[a] Adapted from V. R. Wheatley, *Livre jubilaire, 1901–1951, de la Société Belge de Dermatologie et de Syphilographie*, 91–103; Imprima méd. sci., Brussels (1952); cited by R. P. Cook, "Comparative Aspects of Lipid Absorption and Excretion," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, 14–36, Cambridge Univ. Press (1952).
[b] Included in waxes.

from the exposed sebaceous glands. Table 22 lists data on the composition of sebaceous secretion obtained from steer skin, while Table 23 gives an analysis of human sebum.

In studies of the sebaceous secretion of the human subject, MacKenna

et al.[437] presented the following analysis: free fatty acids, 29%; combined fatty acids (as glycerides, waxes, and esters), 36%; and unsaponifiable fraction, 32%. In a later study,[438] the unsaponifiable fraction was found to contain 30 to 46% of hydrocarbons, 14 to 19% of cholesterol, and 20% of aliphatic alcohols, and also other unidentified products. The hydrocarbon fraction contained 30 to 40% of squalene and possibly pentacosane. No vitamin A, carotene, vitamin K, or provitamins D_2 and D_3 could be detected, by chemical tests, in the sebum or in the separated fractions. However, appreciable amounts of vitamin E were believed to be present in a concentration of 20 milligram per cent.

Pritchard and associates[439] obtained samples of skin lipids from human subjects by the use of an apparatus consisting of a filter paper attached to sponge rubber pads fastened to plungers and operated by compressed gas. This device enabled them to obtain all available lipids from a specified area of the skin. The over-all range of values for the forehead samples varied between 0.05 and 0.50 mg./sq. cm., with a mean value of 0.19 mg. for men, and 0.17 mg. for women.

Cerumen is produced in increased amounts in hamsters on a fat-free diet. Christensen and Dam[440] noted that the *membrani tympani* were covered by a thick layer of a light yellow secretion, which proved to be cerumen. In some cases there was sufficient secretion to plug the external ear canal completely. This secretion did not occur in the case of hamsters fed 10% of lard or of those animals which received a supplement of linoleic acid (up to 28 mg. daily). It is therefore postulated that the abnormal secretion of cerumen is probably due to a deficiency of essential fatty acids in the diet.

Weitzel and Lennert[441] reported on the composition of the secretion of the preen gland of ducks. When this secretion is spread over the feathers, it produces the hydrophobic effect which prevents the feathers from becoming wet; in this manner it assists in affording buoyancy in the case of aquatic birds. The nonsaponifiable fraction, which was composed entirely of octadecyl alcohol, constituted about 50% of the total lipid. The balance of the lipids in the secretion were fatty acids. Of these, 36% were

[437] R. M. B. MacKenna, V. R. Wheatley, and A. Wormall, *J. Investigative Dermatol.*, *15*, 33–47 (1950).

[438] R. M. B. MacKenna, V. R. Wheatley, and A. Wormall, *Biochem. J.*, *52*, 161–167 (1952).

[439] J. E. Pritchard, L. D. Edwards, and J. E. Christian, *J. Am. Pharm. Assoc.*, *38*, 546–549 (1949).

[440] F. Christensen and H. Dam, *Acta Physiol. Scand.*, *27*, 204–205 (1952).

[441] G. Weitzel and K. Lennert, *Z. physiol. Chem.*, *288*, 251–265 (1951).

steam-volatile, optically active, and branched. The main constituent of this group was found to be methylhexanoic acid. A large proportion of liquid, optically active, branched-chain acids having C_9 to C_{18} were also present. The higher non-volatile acids included palmitic, stearic, and an unsaturated C_{18} acid. The solid acids were not branched. However, the secretion did not contain provitamin D_3 (7-dehydrocholesterol), as was formerly believed.[417]

Wheatley[442,443] concluded that ovarian dermoid cyst fat differs significantly from human sebum. Free fatty acids are absent, and *n*-paraffin hydrocarbons occur only in traces in the cyst fat. On the other hand, *n*-alcohols such as eicosanol are present in much larger amounts in the cyst fat than in sebum. It is suggested that the cyst fat is sebum in which the formative process has not been completed. As noted earlier Koppenhoefer[403] reported the presence of squalene in ovarian dermoid cysts.

10. Lipids Present in Lung Tissue

Bloor[23] reported the following lipid composition of moist lung tissue of cattle: lecithin, 0.68%; cephalin, 0.57%; acetone-soluble fraction, 0.76%; and unsaponifiable fraction, 0.25%. Thannhauser *et al.*[36] further analyzed the phospholipid components in normal human lungs. These workers

TABLE 24
THE LIPID COMPONENTS IN THE FLUID FROM THE RESPIRATORY TRACT OF THE RABBIT, CAT, AND DOG[a]

Substance determined	Lipid concentration in mg./100 ml.		
	Rabbit	Cat	Dog
Total lipid	65	60	197
Neutral fat	18	12	53
Total fatty acids	42	33	126
Cholesterol			
Total	13	19	41
Ester	8	12	28
Free	5	7	13
Phospholipid	28	22	85

[a] E. M. Boyd, S. Jackson, M. MacLachlan, B. Palmer, M. Stevens, and J. Whittaker, *J. Biol. Chem., 153*, 435–438 (1944).

found that, in the dried lung tissue, the total phospholipids amounted to 6.65%, while lecithin was present to the extent of 3.85%, cephalin to that of

[442] V. R. Wheatley, *Nature, 168*, 1128–1129 (1951).
[443] V. R. Wheatley, *Biochem. J., 53*, xxi (1953).

1.34% (recalculated), and sphingomyelin in the amount of 1.46% (recalculated). In later studies, Thannhauser and his collaborators[444] isolated hydrolecithin as a component of lung phospholipids. It was identified as a dipalmityl lecithin. The amount of hydrolecithin present in the lung tissue varied between 20 and 40% of the sphingomyelin content of this organ.

The fluid isolated from the respiratory tract of the rabbit, cat, and dog was shown by Boyd *et al.*[445] to contain all the lipids present in the blood, but in smaller amounts. Table 24 summarizes these data (page 787).

11. Lipids Present in the Pancreas

Pancreas differs from other glandular tissues in having a considerably higher content of neutral fat (acetone-soluble fraction), in relation to the other lipid components. Thus, Bloor[23] reported that fresh beef pancreas had the following composition: lecithin, 1.05%; cephalin, 0.82%; acetone-soluble fraction, 3.90%; and unsaponifiable residue, 1.29%.

In addition to the usual tissue lipids, a number of partial hydrolysis products of the phospholipids have been prepared from pancreatic tissue. Klenk and Friedrichs[40] reported that plasmalogens comprised 4% of the total phospholipids in swine pancreas. Jones and co-workers[26] demonstrated the presence of monopalmitin in hog pancreas, in amounts of 1.7 to 1.9% of the fresh tissue weight. Glycerophosphorylcholine (choline glycerophosphate) is another component of pancreatic tissue. This compound originates by the splitting of two fatty acid residues from lecithin, which may be accomplished by the action of lecithinase B on lecithin. Schmidt *et al.*[446] and King and Aloisi[447] reported the presence of glycerophosphorylcholine in beef pancreas. Both the α- and the β- forms of this compound have been isolated from pancreas.[447,448]

King and Small[449] isolated an intermediate of sphingomyelin from pancreas which is believed to be sphingosine choline phosphoric ester. This is the compound formed after the fatty acid residue has been removed.

[444] S. J. Thannhauser, J. Benotti, and N. F. Boncoddo, *J. Biol. Chem.*, *166*, 669–675 (1946).

[445] E. M. Boyd, S. Jackson, M. MacLachlan, B. Palmer, M. Stevens, and J. Whittaker, *J. Biol. Chem.*, *153*, 435–438 (1944).

[446] G. Schmidt, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, *161*, 523–536 (1945).

[447] E. J. King and M. Aloisi, *Biochem. J.*, *39*, 470–473 (1945).

[448] M. Aloisi and P. Buffa, *Biochem. J.*, *43*, 157–160 (1948).

[449] E. J. King and C. W. Small, *Biochem. J.*, *33*, 1135–1139 (1939).

12. Lipids Present in Bone-Marrow

The phospholipid content of bone-marrow has been shown by Bolle[450] to vary within a narrow range in different animals, *viz.*, beef, 0.46%; pig, 0.43%; sheep, 0.40%; dog, 0.83%; and cat, 1.16%. The lecithin content of bone-marrow is a function of the age of the animals, being highest in the young, and decreasing to a minimum value with increasing age.[450–452] On the other hand, total lipid was shown by Dietz[452] to increase with advancing age; there is an inverse relationship between the lipid and the water content in the bone-marrow. The unsaturated fatty acid content has been found to be higher in gelatinous marrow than in the yellow marrow. The saturated acids consist of palmitic and stearic acids, while the main unsaturated acid is oleic; small amounts of arachidonic acid are also present.[453]

Newlin and McCay[454] have shown that the marrow cavities in the bones of rabbits serve as storage space for mobile supplies of lipids. It was found that the iodine number of the marrow fat reflected that of the diet, especially when the fat stores of the rabbits were first depleted and then flooded with unsaturated lipid.

13. Lipids Present in the Stomach and Intestine

Gastric mucosa was shown to contain lecithins, cephalins, and small amounts of cerebrosides.[455] In contradistinction to the intestinal mucosa, in which the lipid composition varies markedly during the absorption of lipids, the mucosae of the stomach walls have a relatively constant composition. This is due to the fact that the stomach is not the site of absorption of lipids, and the lipids do not pass into these cells to the extent that occurs in the intestinal mucosa. However, it should be remembered that Inouye[456] recorded that fat droplets could be observed in the gastric mucosa of dogs after the administration of olive or mineral oil.

In the case of intestinal mucosa, the amount of lipid material which can be isolated depends upon whether or not fatty foods have been fed several hours before the tissues are excised. It is well known that neutral fat, phospholipids, cholesterol, and the fat-soluble vitamins become concentrated in the mucosal cells following their absorption from the lumen of the

[450] A. Bolle, *Biochem. Z.*, *24*, 179–190 (1910).
[451] W. Glikin, *Biochem. Z.*, *4*, 235–243 (1907).
[452] A. A. Dietz, *J. Biol. Chem.*, *165*, 505–511 (1946).
[453] L. T. Cheng, *Z. physiol. Chem.*, *201*, 209–218 (1931).
[454] H. E. Newlin and C. M. McCay, *Arch. Biochem.*, *17*, 125–128 (1948).
[455] B. Uhnoo, *Z. physiol. Chem.*, *256*, 104–110 (1938).
[456] T. Inouye, *Am. J. Physiol.*, *69*, 116–124 (1924).

gut; ordinarily these substances are rather rapidly transferred to the lacteals for transport to the liver and other tissues. The interval during which the elevated lipid concentration would be expected would depend upon the amount of lipid fed. In general, it would not be prolonged for more than three to six hours. Although considerable amounts of plasmalogens were indicated in the 0.27% of phosphatides found in pig intestine, practically no pure material could be separated, and these compounds were probably not present in appreciable amounts.[40]

The cholesterol content of intestinal mucosa varies according to the portion of the intestine from which it is obtained. Thus, in samples of human intestine, material from the upper portion was shown to have a cholesterol concentration of 0.37%,[457] while that from the sigmoid had a concentration of 0.65%, based upon dry weight.[457] On the other hand, the cholesterol content of the large intestine was 0.65% (dry weight).[458] This sterol was present largely in the mucosa, as is shown by the fact that the concentration in the latter case was 0.82%. Bloor[49] calculates that the total mucosa of the large intestine of man would contain only 78 mg., which is less than the amount excreted daily on a cholesterol-free diet. Variations in cholesterol content may be related to the fact that it can be synthesized from acetate in the wall of the gut.[7] This fact would emphasize the mobile nature of the cholesterol stores in the mucosa.

The presence of phospholipids in intestinal mucosa has been repeatedly demonstrated. For example, Sinclair[459] reported an average of 10.4% of phospholipid (based upon dry weight) in the intestinal mucosa of the fasted cat. No change in the amount of phospholipid could be demonstrated after fats were fed; however, marked variations occurring in the composition of the phospholipid fatty acids were referable to diet. This led Sinclair to postulate that fatty acids are transformed into phospholipids within the intestinal mucosa as an essential step in the resynthesis of fat. In a recent study, Morehouse[460] demonstrated that only 66% of the total fatty acids absorbed pass through the phospholipid stage in the intestinal wall. Moreover, Zilversmit *et al.*[461] and Flock and associates[462] found that the phospholipid turnover in the intestinal wall is too slow to allow all

[457] M. Bürger and H. D. Oeter, *Z. physiol. Chem.*, *182*, 141–147 (1929).
[458] M. Bürger and H. D. Oeter, *Z. physiol. Chem.*, *184*, 258–260 (1929).
[459] R. G. Sinclair, *J. Biol. Chem.*, *82*, 117–136 (1929).
[460] M. G. Morehouse, unpublished observations, 1953.
[461] D. B. Zilversmit, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, *172*, 637–650 (1948).
[462] E. V. Flock, J. C. Cain, J. H. Grindlay, and J. L. Bollman, *Federation Proc.*, *6*, 252 (1947).

fats to pass through the phospholipid stage. Lovern and Morton[44] demonstrated the presence of free fatty acids in the intestinal mucosa.

14. Lipids Present in Milk Fat

(1) *The Composition of Milk Fat*

a. Fatty Acids in Milk Fat. In most species of animals, the composition of the milk fat differs from that of the depot fat. Although there are minor differences in the fatty acid patterns in milks obtained from different types of animals, by and large milk fats are characterized rather by their similarities. Thus, myristic and palmitic acids are consistently present in mature milk from several species of animals, within the relatively narrow ranges of 7 to 12% and 22 to 32%, respectively. A considerable uniformity likewise obtains in the oleic acid content, which usually ranges between 28 and 41% in the different milks. The most characteristic components in milk fat are the volatile acids, which comprise 7% of the total fatty acids, although values more than twice this figure have been recorded for milk fats from at least one species of animal.

The first comprehensive analysis of butterfat was that of Bosworth and Brown.[463] These investigators reported the presence of the monoethenoid acids with an even number of carbons from C_{10} to C_{14}. Hilditch and Longenecker[464] have shown that the double bond is present in the 9–10 position; this can be interpreted to mean that these acids originated from oleic acid by combined oxidation and reduction.

Linoleic acid is another acid which has been demonstrated in butterfat. Although Bosworth and Brown[463] failed to detect the presence of this acid in butterfat, Eckstein[465] found that it was present to the extent of 0.17 to 0.25% in cow's butter. Linolenic acid was also demonstrated in this fat in amounts of 0.07 to 0.17%. Using a new spectrophotometric method for the determination of the dienoic and trienoic acids, Schaffer and Holm[466] obtained values of 2.62 to 2.71% for linoleic acid in summer milk fat, while octadecatrienoic acid values varied between 0.77 and 1.17%. These results are within the range of total essential acid content for butters reported by Deuel *et al.*,[467] who employed a new bioassay method for the determination. It is probable that the divergent results concerning the presence and

[463] A. W. Bosworth and J. B. Brown, *J. Biol. Chem.*, *103*, 115–134 (1933).
[464] T. P. Hilditch and H. E. Longenecker, *J. Biol. Chem.*, *122*, 497–506 (1938).
[465] H. C. Eckstein, *J. Biol. Chem.*, *103*, 135–140 (1933).
[466] P. S. Schaffer and G. E. Holm, *J. Dairy Sci.*, *33*, 865–869 (1950).
[467] H. J. Deuel, Jr., S. M. Greenberg, L. Anisfeld, and D. Melnick, *J. Nutrition*, *45*. 535–550 (1951).

proportion of these acids may be related to variations in species or to dietary differences. Hydroxypalmitic and cerotic acids are two other unusual fatty acids present in milk fats.[468,469] As much as 0.5% of elaeostearic acid has been reported in the milk fat of cows after the feeding of tung oil.[470]

b. Phospholipids in Milk Fat. Phospholipids occur in milk fat to the extent of 0.03 to 0.1%. Apparently there is no relationship between the phospholipid content and the proportion of fat in the milk. Although Bloor[49] considers that the phospholipids are adventitious products in this fluid, it is possible that they may function in promoting emulsification of the fat, since they combine with protein, to form a protective covering around the fat particles in cream.[471] Koch[472] reported values of 0.036 to 0.049% for lecithin, and 0.072 to 0.086% for cephalin in cow's milk. Chapman[473] published figures for total phospholipid as follows: whole milk, 0.045%; cream, 0.20%; skim milk, 0.016%; and buttermilk, 0.13%. According to Hess and Helman,[474] the phospholipid content of cow's milk is twice that of human milk, while the total amount of phosphorus in cow's milk is four times the level obtained in human milk, and that in goat's milk is six times that of human milk. The lecithin and cephalin fatty acids of cow's milk are stearic, palmitic, and oleic acids.[475] None of the lower fatty acids are present.[476,477]

c. Cholesterol in Milk Fat. Cholesterol is known to be an invariable component of milk from various species of animals. In 1913, Meigs and Marsh[478] recorded an average value of 0.021% for the cholesterol content of whole cow's milk. However, reports of various workers since that time have consistently given lower values. The results of a number of workers are summarized in Table 25.

In the extensive investigations of the Keys' group,[479] it was found that the average cholesterol content of milk was 11.4 ± 1.50 milligram per cent in the winter and approximately the same in the summer (11.3 ± 1.56

[468] A. W. Bosworth and G. E. Helz, *J. Biol. Chem.*, *112*, 489–492 (1936).
[469] G. E. Helz and A. W. Bosworth, *J. Biol. Chem.*, *116*, 203–208 (1936).
[470] J. Houston, A. G. Cotton, and S. K. Kon, *Biochem. J.*, *33*, 1626–1629 (1939).
[471] L. S. Palmer and H. F. Wiese, *J. Dairy Sci.*, *16*, 41–57 (1933).
[472] W. Koch, *Z. physiol. Chem.*, *47*, 327–330 (1906).
[473] O. W. Chapman, *J. Dairy Sci.*, *11*, 429–435 (1928).
[474] A. F. Hess and F. D. Helman, *J. Biol. Chem.*, *64*, 781–796 (1925).
[475] W. Diemair, B. Bleyer, and M. Ott, *Biochem. Z.*, *272*, 119–132 (1934).
[476] F. E. Kurtz, G. S. Jamieson, and G. E. Holm, *J. Biol. Chem.*, *106*, 717–724 (1934).
[477] T. P. Hilditch and L. Maddison, *Biochem. J.*, *35*, 24–30 (1941).
[478] E. B. Meigs and H. L. Marsh, *J. Biol. Chem.*, *16*, 147–168 (1913).
[479] B. Nataf, O. Mickelsen, A. Keys, and W. E. Petersen, *J. Nutrition*, *36*, 495–506 (1948).

TABLE 25
THE FAT AND CHOLESTEROL CONTENT OF COW'S MILK[a]

Investigators	Date	Methods used	Fat content, %	Cholesterol content, mg. %
Denis and Minot[b]	1918	Colorimetric (Bloor)	3.2–5.3	10.5–17.6
Wacker and Beck[c]	1921	Digitonin pptn. (Windaus)	3.65	12.58
Nakanishi[d]	1931	Modified (Authenrieth and Funk)	3 85	12.67
Coccheri[e]	1932	Modified (Grigaut)	—	13–14
Dam[f]	1934	Digitonin pptn.	—	13.36
		(Liebermann-Burchard)	—	12.50
Mühlbock[g]	1934	Digitonin pptn.	1.80	9.18
Torrisi[h]	1940	Liebermann-Burchard	—	10–12
Nataf *et al.*[a]	1948	Digitonin pptn. (Schoenheimer-Sperry)[i]		
Holstein cows			2.87	9.6
Jersey cows			4.70	12.9
Guernsey cows			3.98	11.5

[a] Adapted from B. Nataf, O. Mickelsen, A. Keys, and W. E. Petersen, *J. Nutrition*, *36*, 495–506 (1948), p. 502.
[b] W. Denis and A. S. Minot, *J. Biol. Chem.*, *36*, 59–61 (1918).
[c] L. Wacker and K. F. Beck, *Z. Kinderheilk.*, *27*, 288–292 (1921).
[d] H. Nakanishi, *Nagoya J. Med. Sci.*, *5*, 190–191 (1931).
[e] P. Coccheri, *Lattante*, *3*, 284–304 (1932).
[f] H. Dam, *Biochem. Z.*, *270*, 112–115 (1934).
[g] O. Mühlbock, *Z. Kinderheilk.*, *56*, 303–306 (1934).
[h] D. Torrisi, *Arch. fisiol.*, *39*, 431–457 (1940).
[i] R. Schoenheimer and W. M. Sperry, *J. Biol. Chem.*, *106*, 745–760 (1934).

milligram per cent). The lowest values were obtained for the Holsteins (9.6 milligram per cent), the highest for the Jersey cows (12.9 milligram per cent), and intermediate values for the Guernseys (11.5 milligram per cent). There is considerable disagreement as to whether or not cholesterol is present in the esterified form. According to Wacker and Beck,[480] 100% of the milk cholesterol is esterified; smaller amounts of the esters were reported by other workers,[481] and nothing but free cholesterol was found by a number of investigators.[479,482,483] Nataf *et al.*[479] are of the opinion that no fixed relationship exists between the fat level and the cholesterol level of the diet.

Marked variations in the milk cholesterol are associated with species. Table 26 records data on the cholesterol and fat content of milk obtained from a number of common animals.

[480] L. Wacker and K. F. Beck, *Z. Kinderheilk.*, *27*, 288–292 (1921).
[481] F. W. Fox, and J. A. Gardner, *Biochem. J.*, *18*, 127–135 (1924).
[482] S. Ansbacher and G. C. Supplee, *J. Biol. Chem.*, *105*, 391–404 (1934).
[483] O. Mühlbock, *Z. Kinderheilk.*, *56*, 303–306 (1934).

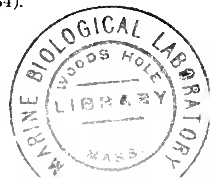


TABLE 26
THE FAT AND CHOLESTEROL CONTENT OF MILK OF SEVERAL SPECIES[a]

Animal	Fat content, %	Cholesterol content, mg. %	Animal	Fat content, %	Cholesterol content, mg. %
Sow	8.03	145 0	Human	3.36	16.4
Rabbit	10.45	109.0	Goat	4.42	14.0
Cat	3.33	63.0	Cow	3.85	12.7
Dog	9.26	55.0	Mare	0.97	11.5
Sheep	7.84	22.5			

[a] Adapted from H. Nakanishi, *Nagoya J. Med. Sci.*, *5*, 190–191 (1931).

In the case of human milk, Denis and Minot[484] reported a range in cholesterol content from 0.038 to 0.0096%, while the average proportion in human milk fat was given as 0.42%. Other figures for the cholesterol content of human milk are a low value of 0.014% (with a daily secretion of 2 liters),[485] and 0.026%; cholesterol made up 1% of the total milk fat.[483] No relationship between the total fat content of human milk and the cholesterol level was observed. Cholesterol in human milk was found to be present exclusively as the ester.[480] Ansbacher and Supplee[482] found that a considerable amount of milk cholesterol was combined with lactalbumin. Lange,[486] in his extensive compilation of the cholesterol content of different biological materials, recorded the following average values in milligram per cent: whole milk, 13,[487] whole milk powder, 88,[487] butter, 185,[487] 280,[488] 71,[489] and 242.[490]

(2) *Factors Altering the Composition and Amount of Milk Fat*

a. Species Variations in Milk Fat. Although marked similarities obtain in the composition of milk fat of different species, certain differences are likewise known. For example, the most outstanding variation between human milk fat and that of other species is in the proportion of butyric and other short-chain acids. Whereas Indian buffalo butterfat contains as much as 15.4% of butyric acid,[491] and the butterfat from the milk of

[484] W. Denis and A. S. Minot, *J. Biol. Chem.*, *36*, 59–61 (1918).
[485] H. Knauer, *Jahrb. Kinderheilk., Suppl. vol. 22, Abhandl. Kinderheilk.*, 1–164 (1928).
[486] W. Lange, *J. Am. Oil Chemists' Soc.*, *27*, 414–422 (1950).
[487] A. Winterstein and K. Schön, "*Die Sterine*," in G. Hefter and H. Schönfeld, *Fette und Fettprodukte*, Vol. I, 2nd ed., Springer, Vienna, 1936, 111–144.
[488] R. Okey, *J. Am. Dietet. Assoc.*, *21*, 341–344 (1945).
[489] M. Klostermann and H. Opitz, *Z. Untersuch. Nahr. u. Genussm.*, *27*, 713–723 (1914).
[490] P. Berg and J. Angerhausen, *Z. Untersuch. Nahr. u. Genussm.*, *28*, 145–149 (1914).
[491] K. T. Achaya and B. N. Banerjee, *Biochem. J.*, *40*, 664–669 (1946).

cows, sheep, goats, and camels contains approximately 3% of this acid, mature human milk fat contains only 0.4% of this 4-carbon acid,[492] or none at all.[493] The milk fat of the mare most nearly approaches that of the human in butyric acid content.[494] Another difference between human milk fat and that of other species is its octadecadienoic acid content. This value is considerably higher in human milk fat than in that of any animal except the mare.[494] Human milk is also unique in containing a small proportion of octadecatrienoic acid, although Eckstein[465] reported its presence in cow's milk fat. The milk fat of the Atlantic gray seal (*Halichoerus grypus*) exhibits the greatest divergence in composition from the usual fatty acid make-up of milk fats of any milk fat investigated. According to Meara,[495] the total fat content of this milk is ten times the level of fat in the milks of land animals. Only those fatty acids are present in the milk of this seal which also occur in the blubber oil. However, this milk fat is more saturated than is the body fat, particularly because of the higher palmitic acid content. The opposite relationship between the saturation of blubber fat and of milk fat obtains in the case of the blue whale (*Balaenoptera musculus*). For an analysis of the composition of the milk fats of several species, the reader is referred to pages 194 and 195 of Vol. I, *The Lipids*.

b. Colostrum vs. Mature Milk Fat. Marked variations in the composition of milk fat occur at different stages of lactation. The colostrum, which is the first milk produced immediately after parturition, shows the greatest variability from the mean average composition. Anantakrishnan *et al.*[496] found that the composition of buffalo colostrum fat differed from that of mature milk in having a lower content of butyric, myristic, and palmitic acids, and an increased proportion of stearic and oleic acids. The following figures (in per cent) were obtained for the composition of colostrum milk fat and mature milk fat, respectively, of the buffalo: butyric acid, 7.4, 12.1; caproic acid, 0.3, 0.7; caprylic acid, 1.3, 2.4; capric acid, 1.5, 1.5; lauric acid, 1.7, 1.8; myristic acid, 9.1, 12.8; palmitic acid, 19.5, 28.2; stearic acid, 15.9, 11.5; arachidic acid, 0.9, 0.7; and total saturated acids, 57.6, 71.7. In the group of unsaturated acids, the following differences in composition were noted between colostrum and mature butterfat: decenoic acid, 0.1, 0.1; dodecenoic acid, 0.1, 0.1; tetradecenoic

[492] A. R. Baldwin and H. E. Longenecker, *J. Biol. Chem.*, *154*, 255–265 (1944).
[493] T. P. Hilditch and M. L. Meara, *Biochem. J.*, *38*, 29–34 (1944).
[494] T. P. Hilditch and H. Jasperson, *Biochem. J.*, *38*, 443–447 (1944).
[495] M. L. Meara, *Biochem. J.*, *51*, 190–193 (1952).
[496] C. P. Anantakrishnan, V. R. B. Rao, T. M. Paul, and M. C. Rangaswamy, *J. Biol. Chem.*, *166*, 31–33 (1946).

acid, 0.4, 0.6; hexadecenoic acid, 5.8, 4.4; oleic acid, 34.1, 21.6; linoleic acid, 0.7, 0.2; C_{20}–C_{22} unsaturated acids, 1.2, 1.3; and total unsaturated acids, 42.4, 28.3.

In the case of cow's milk, Baldwin and Longenecker[497] found that the fatty acid composition of colostrum and that of mature milk were practically identical. On the other hand, human colostrum was found to have a higher phospholipid content and a larger proportion of high molecular weight acids than mature milk, while the opposite condition obtained in the case of the low molecular weight fatty acids.[492,497] Phospholipids decreased from 6.1% of total lipids on the second day to 0.5% of the total in human milk fat obtained between the 22nd and 43rd days.[492]

Bartley *et al.*[498] reported that the greatest unsaturation of cow-milk fat occurs at the peak of production, irrespective of the season of the year. There is a decrease in unsaturation up to the fourth or fifth month, followed by a slight increase in unsaturation toward the end of lactation. Variations in the oleic acid content of the milk were shown to account for the changes in saturation.

c. Seasonal Changes in the Composition of Milk Fat. Cyclic changes in the fatty acid composition of milk fats occur independently of the variations due to the stage of lactation. For example, Hansen and Shorland[499] reported that, beginning in July, there was a progressive increase in the content of C_6–C_{14} saturated acids up to November, followed by a slow decline to the end of the season. The proportion of C_{18}-unsaturated acids and of butyric acid varied in the reverse direction, while palmitic acid increased along with the shorter-chain fatty acids. The similarity between the constants of butterfats sampled during the same months of different years gave support to the hypothesis that the changes were seasonal. Since the work of Hansen and Shorland was carried on in New Zealand, it would be of interest to determine whether or not the variations were reversed in the northern hemisphere.

Summer butters have been reported to have nutritive properties not shared by winter butters. Boer and associates[500,501] reported that butterfat is superior to vegetable oils in promoting growth; this they ascribed to the presence of vaccenic acid ($\Delta^{11,12}$-octadecenoic acid). However, the results

[497] A. R. Baldwin and H. E. Longenecker, *J. Biol. Chem.*, *155*, 407–412 (1944).

[498] E. E. Bartley, J. H. Zaletel, E. W. Bird, C. Y. Cannon, G. H. Wise, and O. Kempthorne, *J. Dairy Sci.*, *34*, 536–547 (1951).

[499] R. P. Hansen and F. B. Shorland, *Biochem. J.*, *52*, 207–216 (1952).

[500] J. Boer, B. C. P. Jansen, and A. Kentie, *J. Nutrition*, *33*, 339–358 (1947).

[501] J. Boer, B. C. P. Jansen, A. Kentie, and H. W. Knol, *J. Nutrition*, *33*, 359–360 (1947).

of the Boer group were not confirmed by von Euler *et al.*[502,503] The Dutch workers explain this discrepancy by the possibility that the butter employed by the Swedish investigators did not contain vaccenic acid, since it was not summer butter. However, negative results as to the growth-stimulating effect of vaccenic acid have been reported by a number of workers,[504–506] including the latest communication of Boer *et al.*[507] If such a seasonal variation in milk composition does actually exist insofar as vaccenic acid is concerned, it probably has a dietary origin. Variations in the proportion of the carotenoids and vitamin A in milk, which are likewise seasonal, are the result of differences in the content of these components in the feed.

Although seasonal variations in the composition of milk fat can be largely ascribed to the indirect effect of season on the food available to the cows, Ragsdale and Brody[508] stated that low environmental temperature is the direct cause of an increased fat level in cow's milk. This is in line with a comprehensive study by Ragsdale and Turner[509] on the fat content of different breeds. It was shown that the lowest levels of fat obtained in the summer and the highest content occurred in the winter. These latter workers reported that low temperatures *per se* were the cause of high fat content.

d. The Effect of Hormones on Milk Fat. The most important hormone related to milk production is that secreted by the anterior lobe of the pituitary gland; it has been called *prolactin, luteotropin,* or simply the *lactogenic hormone.* It is a protein, slightly soluble in water, having an isoelectric point of 5.5, and a molecular weight of about 30,000. Preparations of the hormone from the pituitaries of sheep and cattle cannot be differentiated electrophoretically; presumably they are identical. The pure hormone has been prepared by a number of investigators.[510–513] An excellent re-

[502] B. von Euler, H. von Euler, and I. Säberg, *Ernährung, 7,* 65–74 (1942).
[503] B. von Euler, H. von Euler, and I. Säberg, *Ernährung, 8,* 257–264 (1943); *Chem. Abst., 39,* 4921–4922 (1945).
[504] H. J. Deuel, Jr., S. M. Greenberg, E. E. Straub, D. Jue, C. M. Gooding, and C. F. Brown, *J. Nutrition, 35,* 301–314 (1948).
[505] H. Nath, V. H. Barki, C. A. Elvehjem, and E. B. Hart, *J. Nutrition, 36,* 761–772 (1948).
[506] B. von Euler, H. von Euler, and G. Lindeman, *Ark. Kemi Mineral., Geol., 26B, No. 3,* 1–5 (1948).
[507] J. Boer, E. H. Groot, and B. C. P. Jansen, *Voeding, 9,* 60–62 (1948); *Chem. Abst., 42,* 7847 (1948).
[508] A. C. Ragsdale and S. Brody, *J. Dairy Sci., 5,* 212–215 (1922).
[509] A. C. Ragsdale and C. W. Turner, *J. Dairy Sci., 5,* 544–554 (1922).
[510] C. H. Li and H. M. Evans, "Chemistry of Anterior Pituitary Hormones," in G. Pincus and K. V. Thimann, *The Hormones,* Vol. I, Academic Press, New York, 1948, p. 648.
[511] A. White, *Ann. New York Acad. Sci., 43,* 341–381 (1943).
[512] A. White, *Vitamins and Hormones, 7,* 253–292 (1949).
[513] W. E. Petersen, *Recent Advances in Hormone Research, 2,* 133–158 (1948).

view on the chemistry and physiology of prolactin was compiled by White, in 1949.[512]

In addition to luteotropin, other hormones are necessary for the production of milk. Thus, although luteotropin is effective in developing the mammary tissue in preparation for lactation, thyroxine is also required if milk secretion is actually to occur. Apparently the ovaries secrete an inhibitory substance; in ovariectomized rats prolactin caused secretion of milk, irrespective of whether or not thyroxine was present.[514] However, Desclin[514] was unable to demonstrate that prolactin alone or with thyroxine exerted an effect upon the mammary gland of the hypophysectomized rat. After parturition, this pituitary hormone (prolactin) is released, resulting in a stimulation of milk production.[515] Folley and Young[516] reported that an increase in milk yield occurred in cows following the injection of prolactin; this was accompanied by a substantial increase in the milk fat content. The average daily production of milk fat rose nearly 50% during the period of five successive injections of prolactin. According to Smith and Dastur,[517] thyroxine also increased the yield of milk and of milk fat in lactating cows. However, there was no important alteration in the nature of the milk fat during the period when its yield was being enhanced through the administration of thyroxine.

(3) *The Origin of the Milk Lipids*

Comparatively large amounts of fats are secreted by the mammary gland during periods of high milk production. According to Folley,[518] a cow yielding 50 to 60 pounds of milk daily will secrete about 1 kg. of fat per day; this is about one-fourth of the total dry weight of the udder. Several possible sources of this fat have been suggested, namely that it is derived from dietary fat, from body fat transported to the mammary gland in the blood, and also from fat synthesized by the mammary gland *in situ*. The first suggestion as to the source of milk fat is not adequate to explain the marked differences in fatty acid composition and in glyceride structure between milk fats and food fats, as well as the several body fats.

a. Dietary Fat as a Source of Milk Fat. Smith and associates[519] have

[514] L. Desclin, *Compt. rend. soc. biol.*, *142*, 1172–1174 (1948).

[515] W. O. Nelson, *Physiol. Revs.*, *16*, 488–526 (1936).

[516] S. J. Folley and F. G. Young, *Biochem. J.*, *33*, 192–197 (1939).

[517] J. A. B. Smith and N. N. Dastur, *Biochem. J.*, *34*, 1093–1107 (1940).

[518] S. J. Folley, "Aspects of Fat Metabolism in the Ruminant," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 52–65 (1952).

[519] F. H. Smith, C. A. Wells, and P. V. Ewing, *Georgia Expt. Sta., Bull. No. 122*, 95–111 (June, 1916).

reviewed the influence of food fat on the composition of milk fat. Although ingested fat was shown to have an effect on the nature of the milk lipids, its action was not immediate, and a number of days were required before the effect was noted. After cottonseed oil had been included in the diet of cows, the fat content of milk showed a gradual increase to the seventh day, although certain components present in cottonseed oil appeared in the milk as early as twelve to thirty-six hours after initiation of the diet. In the case of goats, some food fat also appeared in the milk within twelve hours.[520] The transfer of ingested fat to the milk has been shown to vary with species; Mendel and Daniels[521] noted that several fat-soluble dyes, such as Sudan III, were able to pass into the milk of animals accustomed to fat diets. Thus, they appeared in the milk of rats and cats, as well as in the egg of the hen, but they did not appear in the milk of the cow after they had been introduced into the food. Similar results were reported by Gage and Fish.[522] They concluded that, in the cow and in other herbivorous animals, the milk fat is derived largely from the carbohydrates and protein in the food, rather than from dietary fat.

Contrary to this view, there is considerable evidence in the literature that the secretion of milk fat is reduced in cows receiving a low-fat diet. However, Maynard and McCay[523] found that, under these conditions, the fat content of the milk produced remained fairly constant, but that the amount of the milk production was reduced.[523,524] Concomitantly with the fat output in the milk, reduction in the plasma fatty acid and cholesterol took place. Furthermore, the milk fat was more saturated. Phospholipid and total phosphorus were also shown to decrease under these conditions.[524] The interpretation which has been offered for these and similar findings is that cows which are secreting large quantities of milk fat cannot synthesize the fat rapidly enough to supply the need; if the outside source is removed, a decrease in fat output occurs, along with decreased milk production. A fall in blood lipids also ultimately ensues.

In addition to the effect of diet on the amount of fat in the milk, it may also change its composition. Buschmann[525] reported that the addition of 0.4 to 1.0 kg. of oil per day per 1000 kg. body weight affected the milk composition in a manner similar to that which would occur if 18% of oil were added directly to the milk. Coconut oil was shown to reduce the

[520] O. C. Bowes, *J. Biol. Chem.*, *22*, 11–13 (1915).
[521] L. B. Mendel and A. L. Daniels, *J. Biol. Chem.*, *13*, 71–95 (1912).
[522] S. H. Gage and P. A. Fish, *Am. J. Anat.*, *34*, 1–85 (1924).
[523] L. A. Maynard and C. M. McCay, *J. Nutrition*, *2*, 67–81 (1929).
[524] C. M. McCay and L. A. Maynard, *J. Biol. Chem.*, *92*, 273–280 (1931).
[525] A. Buschmann, *Tierernähr.*, *1*, 129–178 (1930); *Chem. Abst.*, *25*, 1558 (1931).

iodine value of milk fat, usually about 4 to 5 points,[526,527] while less oleic and more lauric and myristic acids were present in the milk fat.[528]

Brown *et al.*[526] found that, when soybean oil was given, the iodine value of the butterfat was increased as much as 40%. The iodine value of milk fat was also increased by the feeding of linseed oil.[529] When linseed or rapeseed oil was fed, the amount of palmitic acid in the milk fat was decreased, while that of oleic and of octadecadienoic acid was increased.[529] After the administration of cod-liver oil, Hilditch and Thompson[529] found an increased proportion of C_{20}- and C_{22}-unsaturated fatty acids, as well as of oleic acid, together with a decrease of butyric acid by as much as 50%. Peanut oil feeding resulted in an increase in the proportion of oleoglycerides, together with a decrease of the butyric to decanoic acid glycerides.[530] On the other hand, when palm kernel oil was fed, the C_{12}- and C_{14}-glycerides were increased, while the C_{16}-glycerides and the oleate content were decreased. Smith and Dastur[531] reported that a low dietary level resulted in a reduction of the proportion of C_4- to C_{14}-saturated glycerides, by about 80%, together with a decrease in the C_{14}- and C_{16}-unsaturated acids. On a low dietary level, oleic acid was increased. Maynard *et al.*[532] reported that the administration of unsaturated fats to cows was reflected by a change of iodine number of the milk fat within eighteen hours; the maximum effect of the ration was noted within three or four days.

In the case of goats, increased saturation of the milk fat resulted from the administration of a low-fat diet or of one containing coconut oil; in the latter case the molecular weight of the average fatty acids was lower than it was on high-fat diets.[533]

The relationship of ingested fat to milk fat has also been demonstrated by the presence of certain "marked" fatty acids in milk fat after they had been fed to lactating animals. The best examples of this type of proof have been obtained with iodized fats by Aylward and co-workers,[534] with odd-chain synthetic fats by Appel and collaborators,[535] and with cod-liver oil, which contains characteristic highly unsaturated fatty acids, by Hilditch and Thompson.[529]

[526] W. C. Brown, R. B. Dustman, and C. E. Weakley, *J. Dairy Sci.*, *24*, 265–275 (1941).
[527] O. J. Hill and L. S. Palmer, *J. Dairy Sci.*, *21*, 529–544 (1938).
[528] T. P. Hilditch and J. J. Sleightholme, *Biochem. J.*, *24*, 1098–1113 (1930).
[529] T. P. Hilditch and H. M. Thompson, *Biochem. J.*, *30*, 677–691 (1936).
[530] T. P. Hilditch and H. Jasperson, *Biochem. J.*, *37*, 238–243 (1943).
[531] J. A. B. Smith and N. N. Dastur, *Biochem. J.*, *32*, 1868–1876 (1938).
[532] L. A. Maynard, C. M. McCay, and L. L. Madsen, *J. Dairy Sci.*, *19*, 49–53 (1936).
[533] R. C. Bender and L. A. Maynard, *J. Dairy Sci.*, *15*, 242–253 (1932).
[534] F. X. Aylward, J. H. Blackwood, and J. A. B. Smith, *Biochem. J.*, *31*, 130–137 (1937).
[535] H. Appel, H. Böhm, W. Keil, and G. Schiller, *Z. physiol. Chem.*, *282*, 220–244 (1947).

b. Blood Lipids as a Source of Milk Fat. In addition to the indirect evidence of the relationship of food fat to milk fat as described in the previous section, data of a more direct nature are available which demonstrate the effect of blood lipids on milk fat production. The blood lipids may, of course, be traced not only to food fats but also to those originating in the liver or derived from body fat. It was reported by Leroy *et al.*[536] that a close relationship exists between the total fatty acids of the blood and the butterfat content of the milk. Maynard and associates[537] likewise pointed out that a close correlation exists during the lactation cycle between the level of blood lipids and the amount of milk produced. Thus, blood lipids rise to a high level following parturition, and remain high during the period of greatest milk production; the level of blood lipids gradually declines as milk production diminishes, reaching a low level as the dry period approaches. The effect of lactation on the level of blood lipids was found to be independent of the effect of dietary fat. In another study, Maynard[538] confirmed these findings by demonstrating that blood lipids are invariably higher in lactating than in non-lactating cows.

In the early studies of Meigs, Blatherwick, and Cary,[539] it was suggested that the phospholipids in the blood were the source of the milk fat. This hypothesis was based upon the observation that the blood leaving the mammary gland contained less phospholipids and more inorganic P than were present in the general circulation. In some cases, Meigs *et al.*[539] were able to account for the total milk fat secreted by the decrease in phospholipid in the blood as it passed through the mammary glands.

However, later workers have been unable to obtain decisive evidence that blood phospholipids are the mother substances of milk fats. Although Doulkin and Helman[540] did find a positive correlation between blood lecithin and milk fat, neither Lintzel,[541] working with six goats as well as with a cow, nor McCay and Maynard[542] found any significant difference in phospholipid nor in inorganic P between the jugular venous and arterial blood and that of the mammary gland. Similar results have also been recorded by Graham and co-workers,[543] who found little difference between these levels in

[536] A. Leroy, R. Lecoq, M. Veline, Mme. Valissant, and G. Barjot, *Lait, 11,* 12–20, 144–155, 234–244, 359–368 (1931); *Chem. Abst., 25,* 5457 (1931).

[537] L. A. Maynard, E. S. Harrison, and C. M. McCay, *J. Biol. Chem., 92,* 263–272 (1931).

[538] L. A. Maynard, *Lait, 12,* 761–775 (1932).

[539] E. B. Meigs, N. R. Blatherwick, and C. A. Cary, *J. Biol. Chem., 37,* 1–75 (1919).

[540] A. Doulkin and S. Helman, *Lait,* 14, 797–808 (1934); *Chem. Abst., 29.* 835 (1935).

[541] W. Lintzel, *Z. Zücht., Reihe B, Z. Tierzücht. Züchtungsbiol., 29,* 219–242 (1934).

[542] C. M. McCay and L. A. Maynard, *J. Biol. Chem., 109,* 29–37 (1935).

[543] W. R. Graham, Jr., T. S. G. Jones, and H. D. Kay, *Proc. Roy. Soc., B120,* 330–346 (1936).

the arterial and venous blood, respectively. Both of these groups of investigators suggest that fat itself is the source of the milk fat.

In the more recent studies in which a comparison of the lipid content of the arteriovenous system of the mammary gland has been followed, it has been shown that the gland absorbs blood neutral fat and glycerides, but probably no other fraction, unless it is the cholesterol ester fraction.[543–545] However, all workers realize that the blood fat cannot directly serve as a source of milk fat without a considerable modification in its glyceride structure. Not only is enough fat made available by the blood supplying the udder to account for the milk fat, but also an additional supply is removed from the blood to be oxidized and so to supply the energy requirements of the mammary gland.[546] It is suggested that, by this process,[547] the short-chain acids are formed which are present in milk fat but which do not occur in the blood lipids. However, the later results of Shaw and collaborators[544,547] failed to confirm the fact that the mammary gland removed fat from the blood in excess of that excreted in the milk.

There has been considerable corroborative evidence that milk fat originates from the neutral fat fraction of blood, in addition to data obtained by a study of arteriovenous differences in the lactating udder.

Hilditch[548] developed a theory to account for the formation of the typical glyceride structure in ruminant milk, particularly the oleoglycerides, by chemical transformation of the neutral fat fraction of blood. It is suggested that the oleic acid residues in glyceride combination are broken down in the udder by a series of oxidations and reductions to give rise to the short-chain acids and to the $\Delta^{9,10}$-unsaturated acids of the C_{10}–C_{16} series which appear to be characteristic of milk fat. The proportion of totally saturated glycerides occurring with certain proportions of saturated fatty acids are characteristic of milk fats, as well as of mammalian depot fats, but are not found in other natural fats. This hypothesis was summarized and extended by Achaya and Hilditch,[549] in 1950. Although the hypothesis of short-chain acids arising from the long-chain acids is attractive, it is not supported by the results of Appel *et al.*[535] When odd-numbered long-chain fatty acids were fed to a lactating sheep, no odd-chain acids below C_{11} were found in the milk. Moreover, the synthesis of the short-chain acids from acetate, as described below, would seem to offer an entirely logical explanation for the formation of the short-chain acids.

[544] J. C. Shaw and W. E. Petersen, *J. Dairy Sci.*, *23*, 538–539, 1045–1056 (1940).
[545] L. Voris, G. Ellis, and L. A. Maynard, *J. Biol. Chem.*, *133*, 491–498 (1940).
[546] J. C. Shaw and W. E. Petersen, *Am. J. Physiol.*, *123*, 183 P (1938).
[547] J. C. Shaw and C. B. Knodt, *J. Biol. Chem.*, *138*, 287–292 (1941).
[548] T. P. Hilditch, *Analyst*, *62*, 250–259 (1937).
[549] K. T. Achaya and T. P. Hilditch, *Proc. Roy. Soc.*, *B137*, 187–211 (1950).

c. Synthesis of Milk Fat in the Udder from Small Molecules. (*a*) *Carbohydrate as a Source of Milk Fats.* It is well known that fats can be readily synthesized from carbohydrates. Although it was formerly believed that the liver was the main if not the only site of this synthesis, more recent information has indicated that extrahepatic tissues, also, have the ability to bring it about. Considerable evidence has been adduced to prove that fat synthesis from carbohydrate proceeds readily in adipose tissue.[550] In view of these facts, it would seem logical to postulate that fat may also be synthesized from carbohydrate in the mammary tissue.

Several experimental findings indicate that fat can be formed from carbohydrate in the udder. In the first place, it has been proved by a number of investigators[551-553] that the lactating ruminant udder *in vivo* has a respiratory quotient (R.Q.) greater than 1.00. A metabolism of this nature has usually been interpreted as indicative of the carbohydrate → fat synthesis. The fact that, during fasting, the R.Q. drops promptly below unity, affords a satisfactory control test to increase the validity of the experiments on normal unfasted cows.[552] Smith and Dastur[531] showed previously that the short-chain fatty acids disappear from the milk fat during fasting, with a concomitant rise in oleic acid content. It has been suggested that the synthesis of this short-chain fatty acid fraction occurs in the udder.[552] This finding can be as readily interpreted to indicate that short-chain acids are intermediates in the synthesis of oleic acid as that they are formed from oleic acid.

Another proof of the synthesis of fat from carbohydrate in the mammary gland is obtained from *in vitro* tests. When slices of lactating mammary gland, obtained from rat, rabbit, mouse, or guinea pig, are incubated in glucose, they can utilize it as the sole substrate, with a resultant R.Q. in excess of one.[554] Although the mammary tissues of ruminants (sheep, goat, cow) are inert to glucose, they can utilize acetate with an elevated R.Q.[555,556] Folley and French[557] are of the opinion that carbohydrate can be used for fat synthesis if acetate is also present.

A third approach in determining whether or not mammary tissue can

[550] E. Wertheimer and B. Shapiro, *Physiol. Revs.*, *28*, 451–464 (1948).
[551] W. R. Graham, Jr., O. B. Houchin, V. E. Peterson, and C. W. Turner, *Am. J. Physiol.*, *122*, 150–153 (1938).
[552] E. P. Reineke, W. D. Stonecipher, and C. W. Turner, *Am. J. Physiol.*, *132*, 535–541 (1941).
[553] J. C. Shaw, *J. Dairy Sci.*, *29*, 183–197 (1946).
[554] S. J. Folley and T. H. French, *Biochem. J.*, *45*, 117–125 (1949).
[555] S. J. Folley and T. H. French, *Biochem. J.*, *43*, lv (1948).
[556] S. J. Folley and T. H. French, *Nature*, *163*, 174–175 (1949).
[557] S. J. Folley and T. H. French, *Biochem. J.*, *46*, 465–473 (1950).

transform carbohydrate into fat has been by the use of C^{14}-labeled glucose. French and Popják[558] were able to show that glucose so labeled is as satisfactory a source of fatty acids in the lactating rabbit as is acetate. In fact, Popják[559] reported that the glucose → fat reaction proceeds more effectively in the mammary tissue than in the liver itself. Balmain, Folley and Glascock[560] reported that slices of lactating mammary gland of rats actively incorporate C^{14} from C^{14}-labeled glucose into their fatty acids. These results, carried out by a variety of procedures, are completely convincing in demonstrating that the lactating mammary tissue is able to synthesize some of the fat required for its secretion from carbohydrate. Kleiber *et al.*[561] found that 2% of the C^{14}-carbon injected as isotopic sodium bicarbonate into cows appeared in the lipid fraction of the milk, while 10% was present in the lactose. The mechanism of CO_2-fixation is uncertain.

(*b*) *Acetate as a Source of Milk Fat.* It is logical to consider that acetate or some 2-carbon fragment will act as the intermediate in the synthesis of fat. Thus, acetate has been shown to play an essential role in the intermediary metabolism of fat.[562] Rittenberg and Bloch,[563] in 1945, indicated that acetate or some closely related compound was utilized for the synthesis of fatty acid chains in the animal body. This behavior on the part of acetate is not unexpected, in view of the fact that it has been shown to be a building stone for other more complicated compounds, including a complex molecule such as that of cholesterol.[564]

A number of facts support the hypothesis that acetate may serve as a source of a considerable amount of the milk fat in the lactating ruminant. The most important of these is the recognition that cellulose and other plant polysaccharides are degraded by the microorganisms in the rumen to lower fatty acids, including acetic acid. Proof for this statement has been reviewed by Elsden and Phillipson,[565] and it appears to be especially convincing. Another proof which supports the theory that acetate is the mother substance of milk fat is the fact that acetate is available in the peripheral blood of cattle and sheep in amounts of 2 to 14 milligram per cent, depend-

[558] T. H. French and G. Popják, *Biochem. J.*, *49*, iii–iv (1951).

[559] G. Popják, personal communication to S. J. Folley, 1952; cited by S. J. Folley, "Aspects of Fat Metabolism in the Ruminant," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ Press, 52–65 (1952), p. 55.

[560] J. H. Balmain, S. J. Folley, and R. F. Glascock, *Nature*, *169*, 447–449 (1952).

[561] M. Kleiber, A. H. Smith, and A. L. Black, *J. Biol. Chem.*, *195*, 707–714 (1952).

[562] K. Bloch, *Physiol. Revs.*, *27*, 574–620 (1947).

[563] D. Rittenberg and K. Bloch, *J. Biol. Chem.*, *160*, 417–424 (1945).

[564] K. Bloch and D. Rittenberg, *J. Biol. Chem.*, *143*, 297–298 (1942); *145*, 625–636 (1942); *159*, 45–58 (1945); *160*, 417–424 (1945).

[565] S. R. Elsden and A. T. Phillipson, *Ann. Rev. Biochem.*, *17*, 705–726 (1948).

ing upon the interval after feeding.[566,567] Thus, evidence is available not only that acetate is formed in the rumen of cattle and goats, but also that it is absorbed; it can also be demonstrated as a component of the peripheral blood. The final evidence of the incorporation of acetate into milk fat has been derived from feeding experiments with C^{14}-labeled acetate. Popják and associates[568] found that, when this compound was administered to a lactating goat, 80% was oxidized, and 10% participated in the synthesis of milk fat in the udder within six hours after its administration. The acetate hypothesis also offers the simplest explanation for the presence of the volatile fatty acids in milk fat.

Early experiments, which attempted to restore the normal short-chain fatty acid content to the milk fat in fasting cows by injecting acetate, gave negative or inconclusive results,[569–571] irrespective of whether the acetate was introduced intravenously or by infusion into the rumen. The reasons for these negative results have not been explained. However, experimental data obtained by means of various procedures have consistently yielded positive results bearing on the question of whether or not acetate is the mother substance of milk fats. The various proofs for this finding are classified below.

a′. Proof of the Synthesis of Milk Fat from Acetate Based upon the Respiratory Quotient of Mammary Gland Slices: Folley and French[557] reported that surviving mammary gland slices from cow, sheep, and goat were able to utilize acetate as the sole substrate with an R.Q. > 1. These data are interpreted as indicating that the mammary tissue of the ruminant is capable of utilizing acetate carbon as the sole source of carbon for fatty acid synthesis, and thus of effecting a net synthesis of fat from acetate. It is believed that the short-chain fatty acids represent intermediate stages in long-chain acid formation which have been "trapped" and stabilized by incorporation into triglycerides.

On the other hand, slices of mammary tissue from non-ruminants cannot utilize acetate alone for fat formation. In the presence of this substrate, the R.Q. was less than 1.00. However, in the presence of glucose, acetate is utilized for fat synthesis, and slices of the udder exhibit an R.Q. greater than unity. It would thus appear that glucose is able to stimulate the

[566] R. L. Reid, *Australian J. Agr. Research, 1*, 338–354 (1950).
[567] G. L. McClymont, *Australian J. Agr. Research, 2*, 158–180 (1951).
[568] G. Popják, T. H. French, and S. J. Folley, *Biochem. J., 48*, 411–416 (1951).
[569] F. H. Malpress, "Discussion on Digestion in the Ruminant," *Proc. Roy. Soc. Med., 39*, Sect. Comp. Med., 805 (1946).
[570] A. I. Mann and J. C. Shaw, *J. Dairy Sci., 30*, 183–196 (1947).
[571] G. L. McClymont, *Biochem. J., 45*, i–ii (1949).

transformation of acetate to fatty acid in the mammary tissue of non-ruminants in the same way as it has been shown to accelerate the formation of fat from acetate in liver slices.[4]

b′. Proof of the Synthesis of Milk Fat from Acetate Based upon Experiments with Acetate Containing C^{14}: Direct proof of the transformation of acetate to fatty acids has been afforded by the use of acetate labeled with C^{14} as a substrate for slices of mammary tissue.[572] Considerable amounts of C^{14} were found to be incorporated into the fatty acids.

c′. Proof of the Synthesis of Milk Fat from Acetate Based upon *in vivo* Experiments: The most convincing proof of the conversion of acetate to fatty acids by the mammary tissue has been obtained by the use of the intact animal. Thus, Popják and Beeckmans[573] found that fatty acids could be synthesized in the mammary tissue from short-chain acids; in fact, this synthesis could be demonstrated in the twenty-eight-day pregnant rabbit. It takes place primarily in the mammary gland rather than in the liver, as is proved by the fact that the concentration of C^{14}-labeled fat is much greater in the udder than in the liver after the administration of C^{14}-acetate. Moreover, the synthesis from small carbon compounds is indicated by the fact that the volatile (short-chain) fatty acids were found to contain much more radioactive carbon than did the long-chain acids.[573,574] These facts disprove the concept that the short-chain acids arise from the long-chain acids; moreover, they contradict the theory that the primary site of fat synthesis is in the liver, and that the milk fat represents fat which has been previously synthesized in the liver and carried to the mammary gland *via* the blood stream.

Further proof of the primary synthesis of the short-chain acids from acetate has been obtained by experiments in which C^{14}-acetate was injected into goats. The specific radioactivity time curves of the soluble and insoluble volatile fatty acids, as well as of the liquid and solid non-volatile fatty acids, showed maxima four hours after the injection. However, the highest activities were noted in the volatile fractions; this characterizes them as the primary products. Moreover, a higher specific activity was observed in the fatty acids of milk than in the fatty acid fraction in the blood. This result furnishes convincing evidence that the conversion of acetate into fatty acids is not exclusively a function of the liver or of other non-mammary tissues; rather it indicates that fat synthesis from acetate proceeds better in mammary tissue than in other tissues.[568,575]

[572] J. H. Balmain, S. J. Folley, and R. F. Glascock, *Biochem. J.*, *52*, 301–306 (1952).
[573] G. Popják and M. L. Beeckmans, *Biochem. J.*, *46*, 547–558 (1950).
[574] G. Popják, S. J. Folley, and T. H. French, *Arch. Biochem.*, *23*, 508–510 (1949).
[575] G. Popják, T. H. French, and S. J. Folley, *Biochem. J.*, *46*, xxviii–xxix (1950)

According to the results of Popják and co-workers,[576] which were also summarized by Popják alone,[577] the synthesis of fatty acids from acetate occurs by the addition of the 2-carbon group to the carboxyl group of an acetate molecule. The short-chain acids originate in this manner; furthermore, all acids up to and including palmitic acid apparently originate from acetate by this mechanism, although stearic and oleic acid in milk fat appear to arise principally by some other process.[578] Possibly the blood triglycerides are the source of stearic and oleic acids. Although the butyric acid in milk fat is known to arise in part from acetate, another possible source would be β-hydroxybutyrate, from which butyrate could readily be synthesized. Shaw and Knodt[547] demonstrated the presence of appreciable quantities of the β-hydroxybutyrate in the blood of the cow, and showed by the technic of arteriovenous differences that appreciable amounts (2.5 milligram per cent) were absorbed by the bovine udder. Butyrate itself is present in the blood of ruminants in too low a concentration to serve as a source of the butyrate in milk fat.[570]

It would thus appear that acetate plays an outstanding role in the metabolism of the udder; this compound is one source of the short-chain acids while, in part, they originate from β-hydroxybutyrate formed in other tissues. Both acetate formed catabolically and that brought in the blood to the mammary tissue can function in the synthesis of fat.

d′. Proof of the Synthesis of Milk Fat from Acetate Based upon Perfusion of the Udder: The *in vivo* experiments cited in the previous section are always subjected to the criticism that the liver may in some way have contributed to the fat synthesis from acetate, and that the change may not have been completely and independently mediated by the mammary tissue. This criticism is not valid as applied to the experiments of Cowie *et al.*,[579] who demonstrated that, when the isolated mammary gland was perfused with blood containing C^{14}-acetate, and with labeled bicarbonate, fatty acids were synthesized which contained the isotopic carbon. Similar results were obtained with C^{14}-acetate in the lactating cow,[580] as well as in the lactating rabbit.[558] The experiments of McClymont[567,571] likewise support

[576] G. Popják, T. H. French, G. D. Hunter, and A. J. P. Martin, *Biochem. J.*, *48*, 612–618 (1951).

[577] G. Popják, "Fat Synthesis from Small Molecules," in R. T. Williams, *Lipid Metabolism*, Biochem. Soc. Symposia, No. 9, Cambridge Univ. Press, 37–51 (1952).

[578] T. H. French, G. D. Hunter, A. J. P. Martin, and G. Popják, *Biochem. J.*, *48*, vi–vii (1951).

[579] A. T. Cowie, W. G. Duncombe, S. J. Folley, T. H. French, R. F. Glascock, L. Massart, G. J. Peeters, and G. Popják, *Biochem. J.*, *49*, 610–615 (1951).

[580] M. Kleiber, A. H. Smith, A. L. Black, M. A. Brown, and B. M. Tolbert, *J. Biol. Chem.*, *197*, 371–379 (1952).

the thesis that a direct synthesis of fatty acids from acetate takes place in the udder. Using the arteriovenous differences in the acetate content of blood as the criterion, McClymont found that the acetate content of arterial blood decreased from 2 to 8 milligram per cent as it passed through the udder. The actual decrease in acetate depended upon its original level in the arterial blood.

e′. Proof of the Synthesis of Milk Fat from Glucose and from Acetate, Based upon the Stimulatory Effect of Insulin on the Reaction, as Demonstrated by the Respiratory Quotient: It is now well known that one of the main functions of insulin is the stimulation of lipogenesis. A number of workers[581,582] demonstrated this effect by the use of liver slices. Since mammary tissue possesses such outstanding potentialities in synthesizing fat from small molecules *in vitro*, it should offer a favorable medium for studying the insulin effect.

On the basis of measurements of respiratory metabolism, Balmain and associates[583,584] demonstrated that crystalline insulin exerted a marked effect on the fat synthesis in mammary tissue of such non-ruminants as the mouse, rat, and rabbit, provided that glucose was present in the medium. Insulin increased the R.Q. and oxygen consumption. There was also a concomitant rise in the rate of disappearance of glucose and acetate. Only 0.1 μg. (0.0022 I.U.) of insulin was required per ml. to initiate this reaction.[518] On the other hand, insulin failed to exert a stimulatory effect on sheep udder slices, either with acetate alone or with acetate plus glucose. This indicates that a profound difference obtains in the action of insulin on fat synthesis in non-ruminants as contrasted with ruminants.[518]

f′. Proof of the Synthesis of Milk Fat from Glucose and from Acetate, Based upon the Stimulatory Effect of Insulin on the Reaction, as Demonstrated by Isotope Studies: The results of the respiration experiments have been confirmed by studies with isotopic acetate. Thus, Balmain *et al.*[572] found that, when slices of the lactating mammary gland of the rat were incubated with C^{14}-acetate and unlabeled glucose, a synthesis of fat occurred; the rate of this fat formation could be augmented with insulin in the *in vitro* experiments. Insulin was likewise able to cause an increase in the incorporation of tritium into the fatty acids of rat mammary slices incubated in tritiated water.[585] On the other hand, insulin was

[581] K. Bloch, *Cold Spring Harbor Symposia Quant. Biol., 13*, 29–34 (1948).
[582] R. O. Brady and S. Gurin, *J. Biol. Chem., 186*, 461–469 (1950).
[583] J. H. Balmain, T. H. French, and S. J. Folley, *Nature, 165*, 807–808 (1950).
[584] J. H. Balmain and S. J. Folley, *Biochem. J., 48*, i–ii (1951).
[585] J. H. Balmain, S. J. Folley, and R. F. Glascock, *Nature, 168*, 1083–1084 (1951).

shown to have no similar action on fat synthesis in the mammary tissue of the ruminant (sheep).[518]

Folley[518] proposed an interesting hypothesis to explain the variability of insulin in stimulating lipogenesis in *in vitro* tests in the mammary tissue of the non-ruminant as contrasted with that of the ruminant. It is suggested that there are differences in the energy-yielding systems in the two classes of animals. In the case of the ruminant, acetate is the preferred source of energy, while in the non-ruminant carbohydrate appears to be the energy-yielding substrate of choice. Since insulin does not influence the conversion of acetate to fat in the ruminant, and does so in the non-ruminant only when glucose is present as well as acetate, it is postulated that insulin stimulates fat synthesis by its effect on the utilization of carbohydrate for energy production.

(*c*) *The Importance of Glycerol in the Synthesis of Fats in the Mammary Gland.* In view of the fact that the main fatty end-products from the mammary gland excreted in milk are triglycerides, it is possible that glycerol may also present a bottleneck in the synthesis of this new fat. A plentiful supply of this alcohol would probably accelerate fat synthesis, and would tend to trap the fatty acids, thus preventing further changes. Folley and French[557] suggested that the stimulatory effect of glucose on the net fat synthesis by mammary tissue slices might in part be due to the formation of glycerol from carbohydrate. That this is the case has been proved by Balmain and Folley,[586] who reported that the addition of glycerol to a system containing lactating mammary gland slices incubated in an acetate plus glucose medium caused an insulin-like effect; the R.Q. was increased, the oxygen uptake and acetate utilization rose, but the glucose breakdown was not changed. In the last respect, the glycerol effect differed from the action of insulin. Balmain and associates[572] proved, by the use of isotopic acetate, that an increased fat synthesis resulted from the addition of glycerol. Glycerol was likewise shown to promote fat synthesis from acetate in media containing lactating sheep udder slices. In this case its stimulatory effect was exerted only when acetate alone was present in the medium, and not when both acetate and glucose were available.

Folley[518] has offered as a possible explanation for the above findings the hypothesis that the effect of insulin on the mammary synthesis of fat is mediated by a system which forms glycerol from glucose rather than by yielding energy derived from the breakdown of carbohydrate. The similarity of the stimulation of fat synthesis by glycerol and by insulin is indicated by the fact that the effects of glycerol and of insulin are not additive.[586]

[586] J. H. Balmain and S. J. Folley, *Biochem. J.*, *49*, 663–670 (1951).

Moreover, the glycerol effect has been shown to occur without an increased glucose uptake.

There is considerable evidence that glycerol can originate from glucose in the udder. French and Popják[558] demonstrated that the glycerol in the milk of lactating rabbits obtained six hours after C^{14}-glucose had been given contained a large proportion of the carbon isotope. Although a similar condition existed after labeled acetate had been given, the level of specific activity was much higher when glucose was used. The incorporation of C^{14} into lactose follows the administration of acetate containing isotopic carbon. However, Folley[518] is of the opinion that the glycerol synthesized from acetate arises from glucose rather than from lactose as the intermediate. Although the rat and sheep were able to produce radioactive glycerol from labeled acetate when the tests were carried out on the intact animal, Balmain and co-workers[572] were unable to duplicate the results in *in vitro* tests with slices of mammary tissue.

15. Lipids Present in Eggs

While the ova of mammals as well as the eggs of fishes contain a large proportion of nucleic acid and only small amounts of lipids, those of the fowl and of reptiles contain relatively large amounts of this foodstuff, in addition to the egg cell. During the course of multiplication of the single egg cell, with the corresponding development of the embryo, the components stored in the yolk and white of the egg furnish basic materials for the development of the cells, and at the same time provide the essential energy for such growth. Whereas milk provides the nutrients essential for the growth and development of the young animal which is·already completely formed at birth, the egg supplies the material essential for the embryo from the beginning of development. The chief variation in lipid composition between milk and eggs is that the latter supply a large proportion of cholesterol and phospholipids, while these components are largely absent in milk. Apparently, the embryo must be furnished with these requisites at an early stage in the process of development, until it is able to synthesize them. In the case of the mammal, these essential lipids are supplied to the fetus in the mother's blood[587]; since this is not possible in the case of the developing chick, the storehouse of reserve materials available in the egg yolk must contain an adequate supply.

[587] E. M. Boyd and K. M. Wilson, *J. Clin. Invest.*, *14*, 7–15 (1935).

(1) The Composition of Eggs

The lipids in egg are concentrated in the yolk, and only a trace is found in the white. The average composition of the egg is given in Table 27.

TABLE 27
THE APPROXIMATE AVERAGE COMPOSITION (IN PER CENT) OF HENS' EGGS[a]

Category	Whole egg Av. = 44 g.	Yolk Av. = 16 g.	White Av. = 28 g.
Water	73.0	49.0	85.0
Lipid	12.0	33.0	Trace
Protein	12.5	16.0	11.5
Carbohydrate	0.5	0.5	0.6
Ash	1.0	1.5	0.5

[a] Adapted from E. S. West and W. R. Todd, *Textbook of Biochemistry*, Macmillan, New York, 1951, p. 1215.

One-third of the yolk of the hen's egg is lipid. It consists of about one-third phospholipids and two-thirds cholesterol and neutral fat. The total cholesterol content is 0.2 to 0.4 g. per egg, and the average phospholipid is 2.0 g. per egg.[49]

a. Fatty Acids Present in Eggs. Riemenschneider *et al.*[588] reported the presence of oleic, linoleic, and clupanodonic acids in egg-yolk lecithin and glycerides, as well as small amounts of palmitoleic acid (9,10-hexadecenoic acid). Palmitic and stearic acids were also present. According to Riemenschneider *et al.*,[588] the average proportion of the saturated acids, by weight, in the glycerides and phospholipids of egg yolk are as follows: myristic, 0.7, not shown; palmitic, 25.2, 31.8; and stearic, 7.5, 4.1. In the unsaturated group, the following average proportions, by weight, were reported in the glyceride and phospholipid fractions, respectively: palmitoleic, 3.3, not shown; oleic, 52.4, 42.6; linoleic, 8.6, 8.2; clupanodonic, 2.3, 13.3; and total unsaturated acids, 66.6, 64.1. It appears that palmitoleic acid occurs only in the glycerides, while the highly unsaturated acid, clupanodonic, is concentrated mainly in the lecithin (phospholipid) fraction.

The mean figures for the iodine number of the egg lipids vary between 80 and 90. Mottram[589] reported that some variations existed between different hens, but that the iodine number of the fat from any particular fowl was extremely constant. It was likewise constant for hens of each breed, respectively, and for eggs of birds in any certain locality. Incubation

[588] R. W. Riemenschneider, N. R. Ellis, and H. W. Titus, *J. Biol. Chem.*, *126*, 255–263 (1938).
[589] V. H. Mottram, *J. Physiol.*, *47*, xviii–xix (1913).

of the egg had little effect upon its unsaturation, although the iodine value sometimes rose during the first week.

b. Phospholipids Present in Eggs. Levene and Rolf[590] used egg yolks as the source of production of lecithin. In addition to stearic, palmitic, and oleic acids, small quantities of linoleic and arachidonic acid were present. Yokoyama[591] was able to prepare over 6.9% of lecithin from egg yolk; it consisted of 73% of the β-form and 27% of α-lecithin. According to this investigator, α-lecithins yielded oleic, clupanodonic and "isopalmitic" acids in the proportions of 72:2:26, while only oleic and "isopalmitic" acids were present in the β-lecithins.

Nishimoto[592] prepared cephalins from egg yolk, and noted the presence of palmitic, oleic, and arachidonic acids. He reported a yield of only slightly over 0.4% of cephalin from fresh egg yolks. Riemenschneider *et al.*[588] confirmed the finding that only a minimal amount of cephalin was present.

Lecithin has been shown to comprise the main part of phospholipids in the eggs of a variety of fowl and fishes. Masuda and Hori[593] reported that approximately 20% of phospholipids were present in the dried yolks of birds' eggs. These workers recorded the following phospholipid:lecithin ratios in various types of eggs: hen, 1.45; duck, 1.3 to 1.37; quail, 1.77 to 1.93; peacock, 1.22; salmon, 1.11; prawn, 1.11; cod, 1.6; carp, 1.14; halibut, 1.40; herring, 2.39; shark, 1.0; and yellowtail, 2.41. The phospholipid in snake eggs was reported as 0.9%, which is about one-half of the amount in hens' eggs.[594]

Evidence that the lecithins and cephalins of eggs are similar to those prepared from other sources is afforded by the fact that they undergo similar reactions in both cases. For example, typical lysolecithin and lysocephalin were prepared by King and Dolan,[595] by the action of rattlesnake venom on egg yolk. These compounds exerted the hemolytic action which would usually be expected of them. Boyd[596] found that there is a decrease in choline in hens' eggs during incubation. The original quantity, which amounts to 180 mg., is reduced to 50% of the original amount by the date of hatching.

[590] P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *46*, 193–207 (1921); *51*, 507–513 (1922).
[591] Y. Yokoyama, *Proc. Imp. Acad.* (*Tokyo*), *10*, 582–585 (1934).
[592] U. Nishimoto, *Proc. Imp. Acad.* (*Tokyo*), *10*, 578–581 (1934).
[593] Y. Masuda and T. Hori, *J. Agr. Chem. Soc.* (*Japan*), *13*, 200–205 (1937); *Chem. Abst.*, *31*, 7548 (1937).
[594] S. Fukuda, *J. Biochem.* (*Japan*), *30*, 125–134 (1939).
[595] E. J. King and M. Dolan, *Biochem. J.*, *27*, 403–409 (1933).
[596] G. S. Boyd, *Biochem. J.*, *47*, xlvii–xlviii (1950).

c. Cholesterol Present in Eggs. Eggs contain a comparatively large proportion of cholesterol. Skarzyński[597] reported values for hens' eggs ranging between 0.38 and 0.56%; each egg was shown to have about 0.25 g. of cholesterol. Lange[486] cites the following analyses for the cholesterol content of egg yolk in milligram per cent: yolk, 1347;[487] yolk, vacuum-dried, 1750;[598] yolk, frozen, 1365;[599] Chinese dried yolk, 2810;[599] commercial dried eggs, 2870;[599] yolk, dried, 3900;[488] yolk, fresh, 2000.[488] Miyamori[600] found that the cholesterol content of egg yolks of twenty-two kinds of birds varied only slightly, being 1 to 2% in all but two species.

d. Lipoproteins Present in Eggs. Alderton and Fevold[601] isolated a lipoprotein, lipovitellin, from egg yolk. This protein was found to comprise 18% of the egg-yolk solids. About 16 to 18% of the lipoprotein consisted of phospholipid, which was largely lecithin. In a later study, Fevold and Lausten[602] isolated another lipoprotein, called lipovitellenin, which contained 36 to 41% of alcohol-extractable phospholipids. This was shown to comprise 40% of the total lipoprotein in egg yolk, and to make up 12 to 13% of the egg-yolk solids. Lipovitellenin is a phosphoprotein similar to lipovitellin, but it contains only one-third as much phosphorus as does lipovitellin. Lea and Hawke[603] showed that the combination of phospholipid with protein stabilized the lipid against the catalytic action of copper and hemin, but not against that of hemoglobin. The lipoprotein could be freeze-dried at a *p*H of 7.0 with little decomposition; it was comparatively stable at low temperatures or even at 37°C., if extremely dry.[604]

(2) Factors Altering the Composition of Egg Lipids

a. The Effect of Diet. It is well known that the fatty acids of the neutral fat and phospholipids of egg yolk are greatly influenced by the fat of the food. As early as 1888, Liebermann[605] demonstrated that the yolk was laid down in successive rings; each ring represented the deposition of yolk material for one day. The bulk of the yolk was shown to be laid

[597] B. Skarzyński, *Bull. intern. Acad. polon. sci., Classe sci. math. nat., 1936B*, II, 437–452.
[598] E. Fourneau and M. Piettre, *Bull. soc. chim. France* [*4*] *11*, 805–810 (1912).
[599] E. O. Haenni, *J. Assoc. Official Agr. Chemists*, *24*, 119–147 (1941); *25*, 365–368 (1942).
[600] S. Miyamori, *Nagoya J. Med. Sci.*, *8*, 176 (1934).
[601] G. Alderton and H. L. Fevold, *Arch. Biochem.*, *8*, 415–419 (1945).
[602] H. L. Fevold and A. Lausten, *Arch. Biochem.* *11*, 1–7 (1946).
[603] C. H. Lea and J. C. Hawke, *Biochem. J.*, *50*, 67–73 (1951).
[604] C. H. Lea and J. C. Hawke, *Biochem. J.*, *52*, 105–114 (1952).
[605] L. Liebermann, *Arch. ges. Physiol.* (*Pflüger's*), *43*, 71–151 (1888).

down in four days. A visible demonstration of the periodicity of fat deposition in the egg yolk was given by Gage and Fish,[522] who used a unique method. It was reported that, when stained fats were fed to laying hens on certain days, the yolk fat was stained in layers corresponding to the fat laid down during those particular periods.

A number of workers have recorded variations in the egg glycerides as affected by diet. Thus, after feeding linseed oil to laying hens, Henriques and Hansen[606] found that the iodine value of the glycerides was increased from 70 (on the basal carbohydrate diet) to 97; when hempseed oil was included in the diet, the iodine number of egg-yolk fat was 119 to 123. Cruickshank[607,608] likewise reported that the iodine number of egg-yolk fat, as well as of depot fat, may be raised by feeding a fat having a high iodine number. However, she found that the iodine number of egg yolk was practically unchanged when more saturated fats, such as mutton fat or palm oil, were fed. Reiser[609] observed that elaeostearic acid was deposited in egg yolk after tung oil had been fed to hens. On the other hand, the iodine values of both the glycerides and the phosphatides in egg yolk were found to be lower in hens on a fat-free diet than when the birds had received 3 to 4% of fat in the diet.[610,611]

Reiser[612] noted that, when fat-free diets were fed to laying hens, there was a marked decrease in the polyunsaturated acid content of the egg yolks. The 6 double-bond acids were found to disappear from the glycerides after the hens had received the fat-free regimen for four weeks. A period of eight weeks was required before the acids having 5 double bonds disappeared from the egg yolks. The acids having 2, 3, or 4 double bonds decreased in the yolks more slowly under these experimental conditions. After fourteen weeks they had declined to 16 to 25% of the control level. The variations in the phospholipid fatty acids resembled those in fatty acids from the triglyceride fraction. When cottonseed oil was included in a purified diet, the hexaenoic acids disappeared from the egg yolks, but the other polyunsaturated acids were still present. It was concluded that the hen cannot synthesize polyunsaturated acids from non-fat precursors, but that it can form acids with 3, 4, and 5, but not with 6 double bonds, from

[606] V. Henriques and C. Hansen, *Skand. Arch. Physiol.*, *14*, 390–397 (1903).

[607] E. M. Cruickshank, *Harper Adams Utility Poultry J.*, *17*, *No. 12*, 626–632 (1932).

[608] E. M Cruickshank, *Biochem. J.*, *28*, 965–977 (1934).

[609] R. Reiser, *Arch. Biochem. Biophys.*, *32*, 113–120 (1951).

[610] E. V. McCollum, J. G. Halpin, and A. H. Drescher, *J. Biol. Chem.*, *13*, 219–224 (1912).

[611] E. F. Terroine and P. Belin, *Bull. soc. chim. biol.*, *9*, 12–48 (1927).

[612] R. Reiser, *J. Nutrition*, *40*, 429–440 (1950).

linoleic acid.[612] No changes in the moisture, fat, phospholipid or cholesterol content of egg yolk were occasioned by the fat-free ration.

In addition to triglyceride fats, fat-soluble components may likewise be transferred to the yolk when they are present in the diet. Thus, Lorenz and associates[613,614] reported that the Halphen test, which is characteristic of malvaceous fats, was positive in both the depot fat and the egg-yolk fat when malvaceous plants such as the little mallow (*Malva parviflora*), the California tree-mallow (*Lavatera assurgentiflora*), the marsh-mallow (*Althaea*, spp.), or cottonseed meal were fed to the hens. However, it was later shown that the fatty material responsible for the Halphen test could not be transferred from the depot fat to that of the egg.[614] It is well known that the proportion of carotenols, vitamin A, and vitamin D present in the eggs is related to the quantity ingested by the hens. For a discussion of this subject, the reader is referred to Volume III.

b. The Effect of Incubation. Marked variations in lipid occur during the incubation of hens' eggs. Needham[615] reported that the fat content of the egg decreased 50% during incubation. A sudden inflection in the curves of all types of lipids occurred at fourteen days, when an activation of fat metabolism apparently took place. During the week before hatching, there was a considerable augmentation of the body fat of the embryo. According to Cahn,[616] an increase in triglyceride fat occurs during incubation. This rise is greatest after the fourteenth day. After the tenth day of incubation, an increase in unsaturation of fats obtains; this is apparently due to a selection of lipids to be retained, since the early embryo is incapable of desaturating the fatty acids.[617] During the later periods, from the thirteenth to the twentieth day, the iodine value increases.

Practically no change in the phospholipids occurs during the incubation of hens' eggs until the sixteenth day, after which they begin to diminish rapidly, with a concomitant increase in inorganic phosphorus.[618,619] Kugler[620] reported that the initial lecithin:cephalin ratio of 3:1 is maintained, both in the yolk, in which a decrease occurs, and in the embryo, in which the phos-

[613] F. W. Lorenz, H. J. Almquist, and G. W. Hendry, *Science*, *77*, 606 (1933).

[614] H. J. Almquist, F. W. Lorenz, and B. R. Burmester, *J. Biol. Chem.*, *106*, 365–371 (1934).

[615] J. Needham, *Chemical Embryology*, Vol. II, Cambridge Univ. Press, 1931, pp. 1163 ff.

[616] T. Cahn, Inaugural Dissertation, Paris, 1928; cited by J. Needham, *Chemical Embryology*, Vol. II, pp. 1167, 1169 ff.

[617] E. C. Eaves, *J. Physiol.*, *40*, 451–453 (1910).

[618] R. H. A. Plimmer and F. H. Scott, *J. Physiol.*, *38*, 247–253 (1909).

[619] Y. Masai and T. Fukutomi, *J. Biochem.* (*Japan*), *2*, 271–277 (1923).

[620] O. E. Kugler, *Am. J. Physiol.*, *115*, 287–291 (1936).

pholipid is increased. In the case of the Atlantic and Mediterranean loggerhead sea-turtle (*Thalassochelys corticata* (*caretta*), *Chelonia caouanna*), the total fat content of the egg remains fairly constant during incubation, but the free fatty acids increase markedly in the course of this developmental period.[621] On the other hand, Takahashi[622] reported that, although an increase in the free choline content took place during incubation, the total choline was decreased by 50% by the end of this period.

There are conflicting reports as to the change in the cholesterol in the egg during incubation. Kusui[623] reported a decrease in cholesterol until the fourteenth day, after which an increase occurred. This indicates that the embryo develops the capability of synthesizing cholesterol after the fourteenth day. On the other hand, Dam[624,625] observed considerable irregularity in the cholesterol content of different eggs. This precluded any statements as to the development of the synthetic capacity on the part of the chick. However, Hanes[626] noted that, on the fourteenth day, cholesterol esters appeared in the liver. It was believed that this occurred concomitantly with the decrease in yolk phospholipid. Although Mueller[627] reported that no change in the total cholesterol occurred over the period of incubation, he found that ester cholesterol increased from practically 0 up to the thirteenth day to about 40% of the total at hatching. Several workers have been unable to demonstrate any synthesis of cholesterol during the incubation of the hens' eggs,[628,629] but others produced some experimental evidence of synthesis.[625,630]

The composition of various types of eggs, as reflected by their fat metabolism, was exhaustively reviewed by Needham[615] in 1931.

[621] J. Karashima, *J. Biochem.* (*Japan*), *10*, 375–377 (1929).
[622] M. Takahashi, *J. Biochem.* (*Japan*), *10*, 443–449 (1929).
[623] K. Kusui, *Z. physiol. Chem.*, *181*, 101–106 (1929).
[624] H. Dam, *Biochem. Z.*, *194*, 188–196 (1928).
[625] H. Dam, *Biochem. Z.*, *215*, 475–492 (1929).
[626] F. M. Hanes, *J. Exptl. Med.*, *16*, 512–526 (1912).
[627] J. H. Mueller, *J. Biol. Chem.*, *21*, 23–28 (1915).
[628] A. H. Roffo and I. Azaretti, *Bol. inst. med. exptl., estud. cáncer* (*Buenos Aires*), *3*, 629–632 (1926).
[629] G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, *B81*, 129–132 (1909).
[630] S. J. Thannhauser and H. Schaber, *Z. physiol. Chem.*, *127*, 278–280 (1923).

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PLANT AND ANIMAL SOURCES OF LIPIDS

This section of the index is limited to generic names of the plant and animal sources from which lipids are derived. Where two generic names are applied, both are listed. The more usual common names of the plant and animal sources are also listed in the subject index.

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