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*A Practical Handbook*

FOR

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## PREFACE TO SECOND EDITION.

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FOR more than seven years no copies of this book have been obtainable, as owing to the author's ill-health it has been impossible to make the revision necessary to bring the work up to date. By lapse of time certain portions of the text had become obsolete, and these have been deleted and replaced by matter based on the latest investigations. It has been found necessary to re-write many sections and to insert methods which have been devised since the book was first published, and this has involved the re-setting of the work throughout, although no alteration has been made in its plan or scope. New illustrations have been introduced and the index greatly improved, and it is hoped that public analysts and other chemists, medical officers, dairy farmers, and those interested in the chemistry of milk, butter, and cheese will find the book of increased service.

H. D. R.

*July, 1914.*



## P R E F A C E .

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**THE** object of this work is to provide dairy chemists with a guide for the chemical control of dairy operations, the assumption being that a knowledge of dairying is already possessed; and public analysts, medical officers, dairy farmers, and Students with a practically useful manual.

The plan adopted is (1) to describe the chemical properties of the constituents of milk; (2) to make use of these properties in the practical analysis of the various milks and milk products; and (3) to apply analytical methods to the control of dairy operations.

In carrying out this work the author would especially notice that its value may be largely attributed to two factors. The first is that Dr. Paul Vieth (now Professor in and Director of the Dairy Institute at Hameln) during his twelve years occupation as Analyst to the Aylesbury Dairy Company, accumulated a large number of observations, which, as he remarked, contained very little theory, but a good deal of fact. This valuable material was handed over to me as his successor, and has been made full use of in this work. The second factor is that the author owes much of his training as a dairy chemist to Mr. Otto Hehner, who enjoys so high a reputation in connection with butter analysis.

It may also be stated that Mr. L. K. Boseley has kindly read the Section on the Analytical Characters of Sterilised Milk, that Mr. F. R. O'Shaughnessy has revised the mathematical portions of the book, and that Mr. A. W. Stokes has supplied the information respecting those methods which bear his name.

*June, 1899.*



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# DAIRY CHEMISTRY.

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## CHAPTER I.

### INTRODUCTORY—THE CONSTITUENTS OF MILK.

CONTENTS—General Composition—Fat—Sugar—Proteins—Salts—Colour—Reaction—Milk-Sugar—Glucose—Products derived from Milk-Sugar—The Proteins of Milk—Products of Hydrolysis—General Action of Hydrolysis—Mineral Constituents—Other Constituents of Milk—The Gases of Milk—The Fat of Milk—Products of Hydrolysis—Fatty Acids—Other Compounds—Rancidity.

**General Composition.**—Milk is the normal secretion of the mammary glands of a mammal; the milk of all mammals has a similar composition, consisting of fat, sugar, protein, mineral constituents, and small quantities of other compounds. The milk of the cow has been studied in greater detail than that of any other animal on account of the extended use of this animal's milk and the products derived from it as human food; the greater portion of this work will, therefore, be devoted to the consideration of the chemical properties of cow's milk, and the expressions "milk," "butter," etc., must be taken as applying to the products derived from the cow, unless described to the contrary. Much, however, that is stated with regard to the cow may be taken as applying equally to the milk of other animals; but our knowledge of the chemical composition of the milk of any animal, except the cow, is very incomplete. Studies, more or less incomplete, have been made of the milk yielded by woman, the goat, the ass, the mare, the gamoose, and the sheep, and analyses, few in number, have been made of the milk of other mammals, both terrestrial and marine. It is probable that there exist wider differences than are yet recognised between the milk yielded by different animals.

**Fat.**—The fat in milk is of peculiar and complex composition; it differs from all other fats in that it contains compound glycerides partly built up of fatty acids of low molecular weight.

It exists in milk in the form of small globules. Many have thought that a true membrane surrounds each globule, and Béchamp considers this view as proved by the behaviour of milk when treated with ether. He finds that milk is capable of dissolving a very large quantity of ether, much more than would be dissolved in the aqueous portion of the milk, and he explains this by the theory that the ether is dissolved by the fat contained in the membranes. His theory assumes that the ether has passed into the membrane by the process known as "endosmose," and that the endosmose is stopped only when the pressure exerted by the distended membrane is equal to the osmotic pressure; the presence of fat to small amount in the excess of ether which separates is explained as partly due to a process of exosmose of the fat within the membrane concurrently with

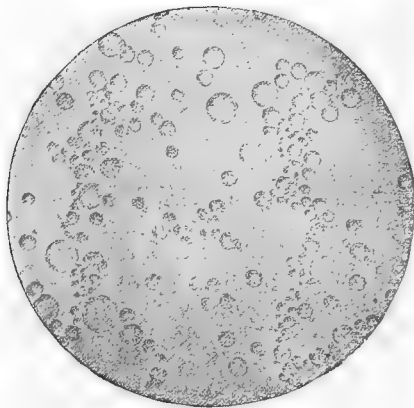


Fig. 1.—Fat Globules in Milk.

the endosmose, and partly to the bursting of a number of the globules. The opponents of his theory urge that the amount of fat in the excess of ether which separates, if this be great, is too large to be explained by assuming exosmose, or the bursting of the globules, which should not take place to a greater degree with a large excess of ether than with a small.

Storch has put forward the view that no real membrane exists round the fat globules, but that a gelatinous "mucoid" membrane (*slim-membran*, in Danish) surrounds them; this consists, according to him, of a combination of 6 parts of a "mucoid" protein with 94 parts of water (*membran-slim*). He bases his view—

(1) On the fact of the existence of this mucoid substance in

cream and butter, and therefore presumably in milk; he proves its existence, and, in fact, isolates it, by washing cream with water and separating the layer of globules till milk-sugar, casein, etc., are all removed.

(2) On the behaviour of milk with ether; his experience differs from that of Béchamp, as, while the latter finds that ether expands the globules to several times their normal size, Storch states that they are not swollen.

(3) On the appearance of the fat globules under the microscope when the milk has been stained by ammoniacal picric-acid, and the layer of cream treated with successive quantities of water till all the milk-sugar has been removed; he notices that a stained layer is present round each fat globule.

There is much to be said in favour of Storch's reasoning, and other evidence may be adduced in favour of it. Butter, in which the globules are certainly more naked than in milk, can be prepared with about 85 to 86 per cent. of fat; this is solid, because the solid fat globules are in close proximity; cream, on the other hand, cannot be prepared with more than about 72 per cent. of fat, and as this has the same consistency as butter at the same temperature, it may be assumed that the globules are in equally close proximity. This would agree with the view that each globule was surrounded by a layer, which increased the effective size. Storch himself has, however, shown that the fat in butter does not exist in the form of globules, but as a nearly homogeneous mass, containing water globules. Storch has adduced evidence, based on the property of ether to emulsify this mucoid substance, that butter-milk contains a larger amount than milk, and on this has based a theory that churning consists of rubbing off the membrane, with the effect that the globules coalesce. The author has experimentally proved the fact that buttermilk is richer in mucoid substance than milk by separating it with a cream separator. Storch considers this as confirmatory evidence of the presence of a membrane.

The evidence is, however, inadequate to settle the question, and in some respects may be held to show that a membrane does not exist. As the author has succeeded in isolating the protein, he has no doubt of its existence; by estimating in butter and buttermilk the water, fat, milk-sugar, protein, and ash, Storch finds that there is much less milk-sugar and more protein in proportion to the water in butter than in buttermilk; he calculates the proportion of protein and water equivalent to the milk-sugar in butter on the supposition that the milk-sugar is an index of the buttermilk left in the butter, and finds a residue of the following percentage composition in three series of experiments:—

	I.	II.	III.
Water, . . . . .	93.15	90.37	92.55
Protein, . . . . .	5.67	8.27	6.42
Ash, . . . . .	1.18	1.36	1.03

The results from the three experiments agree very well, considering the smallness of the actual quantities.

From the results of experiments, in which cream was treated with a 33 per cent. solution of cane-sugar (used to promote the separation of the layer of fat globules), and the fatty layer separated, and the procedure repeated several times, Storch deduces the proportion of mucoid substance to fat as 38.4 to 100.

From these experiments it is evident that cream containing 50 per cent. of fat should contain 19 per cent. of mucoid substance, and only 31 per cent. of the other constituents of milk; in other words, cream containing 50 per cent. of fat should, if Storch's hypothesis be true, only contain  $\frac{31}{100}$  of the milk-sugar in the skim milk. Analysis, however, shows that it actually contains  $\frac{50}{100}$ .

If a membrane be present, the ratio of solids not fat to water in cream should differ from the ratio found in milk (except in the case that the ratio of solids to water in the membrane is the same as that in milk); the author has shown, and is confirmed by Smith and Leonard, that the ratio remains the same.

The experiments of Storch and Béchamp on the mixing of ether with milk are capable of an explanation quite different from that which they attach to it when the laws governing the distribution of a substance between two immiscible solvents are taken into account. We may regard milk as a mixture of an aqueous solution with a large number of fat globules; on gently shaking up with ether it is evident that very few, if any, of the fat globules come into contact with the ether, but only with an aqueous solution of ether. According to the law, the ether should distribute itself between the fat and the aqueous solution in proportion to the solubility in each; if the fat is liquid we should expect a large proportion of the ether to pass into the globules, and they would naturally swell; if, on the other hand, the fat is solid we would not expect it to take up any appreciable proportion of ether, and the globules would remain the same size. In neither case would any appreciable amount of fat pass into the excess of ether which separates, as fat is very little soluble in an aqueous solution of ether.

Béchamp used milk which had not been strongly cooled, but

which was freshly drawn, and cooled simply by radiation. Under these conditions the author has obtained evidence that the fat globules are liquid. Storch gives no indication as to the condition of the milk used by him, but it is the common practice in Denmark to cool all milk to a very low temperature with ice immediately after milking. These conditions, according to the author's experience, facilitate the solidification of the fat. It is not improbable that the apparent discrepancy between Béchamp and Storch is due to a difference of conditions.

In concluding that the staining of a layer round the fat globules proved the presence of a solid membrane, Storch appears to have overlooked the surface energy of small particles, which would cause a layer composed wholly of liquid to be formed round each globule. The appearance noticed by Storch is quite explicable without the assumption that a membrane exists, and, indeed, is somewhat at variance with this view. If there were a solid or mucoid layer it should have a sharply defined outer edge. According to Storch's description this is wanting; the staining is deepest nearest to the globule, and fades imperceptibly away, an appearance quite compatible with the view that a condensed liquid layer is present.

Though data do not exist for calculating the force with which a semi-solid layer would be held by surface energy, it appears reasonable to suppose that it would be impossible to remove this by churning—*i.e.*, friction between globules—therefore butter could not be made were Storch's hypothesis correct.

By homogenising cream—*i.e.*, breaking up the fat into very minute globules—it is found that it is impossible to churn the fat into butter; this operation would certainly remove a membrane, and according to Storch's theory should facilitate churning.

If Storch's view were correct, it would be expected that the membrane would bear such a proportion to the smallest fat globules that their density would be equal to that of the milk serum, and the last traces of fat could not be removed by centrifugal force. By means of an efficient separator it is possible, on running milk twice, to obtain samples of separated milk in which the percentage of fat is so small that it does not reach the second place of decimals per cent.; this fact, while not definitely disproving Storch's view, is further evidence against it.

The following facts seem to definitely disprove Storch's view:—

(1) The ratio of the milk-sugar and protein in cream is the same as in the separated milk.

(2) When milk or cream is stained no layer can be detected round the globules until the aqueous portion is washed away. There are, however, many stained particles (probably mucoid protein) quite independent of the fat globules.



(3) The mucoid protein can only be separated with the fat globules if the density of the serum is increased (by the addition of cane sugar) till it is greater than the density of mucoid protein (1.0228); this proves that it is independent of the fat globules. 114

Milk is regarded by others as an emulsion, and they see no reason why an emulsion of fat containing ether should not exist of the same nature as that of fat alone; these regard the fact that while a small quantity of ether does not extract the fat to any extent from milk, a large quantity does so with a much nearer approach to completeness, to favour the idea that a membrane does not exist round the globules. If milk is precipitated with a solution of nitrate of mercury, which coagulates all the protein of milk, the whole of the fat is removed from suspension, even if it exceeds the protein in weight many times. That this is not due to the precipitation of substances distributed throughout the solution, and the enclosing of the fat therein, is shown by the fact that when the casein, which is equally distributed throughout the solution, is precipitated by means of rennet a considerable proportion of the fat is not enclosed. This fact can hardly be used as an argument that a membrane exists, as the two modes of precipitation differ essentially; the mercury precipitate commences to settle immediately, leaving the solution clear, while rennet gradually reduces the milk to a semi-solid mass, which does not yield a precipitate until the whole has received considerable agitation. As a strong argument against the existence of a membrane may be cited the possibility of preparing artificial emulsions of fats in a finely divided state with milk from which the natural fat has been removed; these emulsions partake very largely of the character of milk. Emulsions of a similar nature can be prepared with other substances, and their behaviour in a great number of respects resembles that of milk. The general consensus of opinion among chemists who may be regarded as authorities on this point is that the fat in milk is not surrounded by a membrane, and, therefore, that it is a true emulsion. There is very little doubt that a layer exists round each fat globule; this is probably formed by an attraction due to surface energy, a force akin to that which causes the phenomenon known as capillary attraction. Much of the evidence which has been taken as proof of the existence of a membrane round the globules is only evidence of the presence of a layer of some sort, but not necessarily membranous.

Bauer concludes from surface tension experiments that a solid layer exists round the fat globules, and that this probably contains some fat.

The author and S. O. Richmond have obtained evidence that the fat globules in milk solidify when cooled below their melting

point; the solidification is, however, a process which takes a considerable amount of time (some hours), and it appears probable that an apparent reversal of the laws of nature takes place. When a substance cools heat is given out or energy is evolved. When a very small globule cools it contracts, and the surface energy is increased—that is to say, energy is absorbed. As this energy can only be supplied as heat abstracted from the aqueous portion of the milk, it follows that, as the fat globules and aqueous portion are at approximately the same temperature, the passage of energy from one to the other will be slow, and, therefore, that solidification of the globules will be but a slow process.

Burri and Nussbaumer find that the surface tension of milk is diminished by cooling, and Bauer attributes this to the solidification of the fat, as the original value can be very nearly restored by heating above the melting point of fat.

**Sugar.**—The sugar in milk is of a peculiar nature; that of cow's milk is called "lactose," or, more commonly, sugar of milk. It is generally assumed that all milks contain the same sugar, but of this there is some doubt. The author, in conjunction with Pappel, has identified in the milk of the "gamoose," or Egyptian buffalo, a sugar distinct from lactose, to which the name of "tewfikose" has been given. The sugar of the milk of the mare has the property of easily undergoing alcoholic fermentation, a property not possessed by lactose. According to the experiments of Carter and the author, the sugar of human milk is not identical with that of the milk of the cow.

The **sweetness** of milk is entirely due to the sugar contained in it; the sugar of milk is many times less sweet than cane sugar. It is very easy of digestion, even by young children.

**Proteins.**—It is in the proteins that the milk of different animals show the most marked variations. They may be divided broadly into two classes—those which give a curd on the addition of an acid, and those which do not. In the first class are included the milk yielded by the cow, the goat, the gamoose, etc.; and in the second human milk, that of the mare, and that of the ass may be cited as examples. In the first class the curd is composed of **casein**, which is combined with phosphates of the alkaline earths; while in the second this is replaced by a similar protein, which is not, however, combined with phosphates. It is possible that the difference between the proteins of the two classes is simply dependent on the presence or absence of the phosphates, but the chemistry of these bodies is not yet sufficiently advanced to decide this. Besides casein, or a similar body, there exists in all milks a second protein called **albumin**; this differs from casein by not being precipitated by acids, and by being coagulated

by heat. Other proteins have been described in milk, but many of them are only decomposition products of casein or albumin, which were formed during the process adopted for the removal of the other proteins. Evidence has been adduced of a **third protein** in milk; Storch's **mucoïd protein** has already been referred to. Béchamp has described a **starch-liquefying enzyme**, and lately Babcock and Russell have separated a **proteolytic enzyme**. There also exists an enzyme which has the properties of a peroxydase, which may be identical with those of Béchamp and Babcock and Russell.

The casein in milk is not in a state of true solution; it is probably in the state described by Picton and Linder as "pseudo-solution." They have shown that this state is due to the existence of particles in the solution not sufficiently large to settle under the influence of gravity, but which will interfere with the passage of light; they can also be separated by a current of electricity, or by passage of the solution through a porous jar. They show also that there is no sharp dividing line between crystalloids and colloids in solution, substances in pseudo-solution, and substances in suspension. In milk we have the four states represented—the fat is in suspension, the casein in pseudo-solution, the albumin in solution as a colloid, and the milk-sugar in solution as a crystalloid; these four states are probably due to the size of the conglomerates of molecules or particles.

**Salts.**—The salts of milk are not yet fully studied; the presence of chlorides, phosphates, and sulphates of sodium, potassium, calcium, and magnesium is generally admitted. Salts of organic acids are also present; Henkel has described citric acid, and Béchamp acetic acid, but this latter result is not universally accepted. Béchamp also maintains that the casein and albumin exist in milk as salts of alkalies; there is much to recommend this view. A solution greatly resembling milk can be prepared in which casein undoubtedly exists combined with an alkali, while it has not been found possible to dissolve casein to an appreciable extent unless an alkali is present; milk does not taste sour until an appreciable acidity has developed; at about the same point it curdles on heating; it is proved that this is due to the acid developed displacing the alkali from its compound with casein. It is also found impossible to coagulate the albumin in milk unless a certain amount of free acid is added, and this fact accords well with the theory of Béchamp. Söldner has also adduced evidence in proof of this view.

Besides the constituents enumerated above, milk contains traces of other compounds; among these may be mentioned urea and other bases, an odoriferous principle, and a colouring-

matter; these two latter occur in very small amount, and are of unknown composition.

**Colour.**—The colour of milk is nearly white, due not to the presence of the colouring-matter just mentioned, which accumulates in the fat, but to the interference with the passage of light by the casein in pseudo-solution. When milk is viewed in thin layers, especially if the bulk of the fat has been removed, it has a bluish tint: the bluish tint can hardly be called a colour; it partakes more of the nature of a fluorescence, and the transmitted light is polarised to a slight degree.

The fat globules, being very much lighter than the medium in which they are suspended and being of sufficient mass to overcome the viscosity of the fluid, have a tendency to rise and form a layer of cream on the surface of the milk when left to rest.

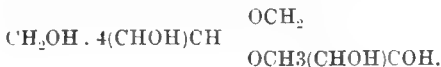
**Reaction.**—Milk has always, when fresh, an amphoteric reaction—*i.e.*, it turns blue litmus paper slightly red and red litmus slightly blue. A similar reaction is possessed by certain phosphate solutions, and it is to the presence of such in milk that this reaction is due. The true explanation is that the acidity of milk is due to mono- and di-acid phosphates, and the strength of these acid salts is of the same order as the strength of the acid of litmus. When blue litmus is used for testing, a substance more alkaline than the milk is introduced and equilibrium is set up; alkali passes from the litmus to the milk, and consequently the blue litmus is reddened.

When red litmus is used an acid substance is introduced, and for the attainment of equilibrium alkali must pass from the milk to the litmus, thereby turning it slightly blue.

This reaction has acquired a false importance, owing to the erroneous idea that neutrality as measured by the action of litmus is chemical neutrality; with the recognition of the fallacy of this idea the importance of the amphoteric reaction vanishes.

## THE CHEMICAL PROPERTIES OF THE CONSTITUENTS OF MILK.

**Milk-Sugar, Lactose** (*Lacton* or *Lacto-biose*),  $C_{12}H_{22}O_{11}$   $\cdot$   $OH_2$ .  
**—Properties.**—This sugar is found in the milk of the cow and probably in that of most other mammals. It is a hexa-biose, and belongs to the class of aldehydes (aldoses), or rather aldehydrols. It has the constitution of a galactose-glucoside, and on hydrolysis by acids yields a mixture of galactose and glucose. Fischer assigns the following constitution to it:—



The aldehyde group of the galactose has been eliminated in milk-sugar, while that of the glucose remains. This is shown by the reactions of several derivatives of milk-sugar; by heating milk-sugar with phenylhydrazine and acetic acid, phenyl-lactosazone is formed, which yields an osone on treatment with strong hydrochloric acid; this, by boiling with hydrochloric acid, yields a mixture of galactose and glucosone. By treatment again with phenylhydrazine, the glucosone forms phenylglucosazone almost immediately, and, on warming, phenylgalactosazone is precipitated. A clear demonstration is thus afforded that the aldehyde group of the glucose only remains.

By oxidation with bromine, lactobionic acid is formed, which is hydrolysed by acids to gluconic acid and galactose; again showing that the galactose group is modified.

The reactions of milk-sugar, which are all displayed in solution, are those of an aldehyde, but from its formation of stable hydrated compounds it appears more correct to regard it as an aldehydrol.

**Modifications.**—Milk-sugar exists in several modifications which are distinguished from each other chiefly by their behaviour towards polarised light.

The best known modification is the hydrated  $\alpha$ -milk-sugar, usually known as crystallised milk-sugar; this is the form in which it crystallises from water. The  $\alpha$ -modification exhibits multi-rotation—*i.e.*, when dissolved in water it has a much higher specific rotation than that which it attains after a lapse of time. The author has found that when more milk-sugar than will immediately dissolve is shaken with water it causes a lowering of the temperature of the solution by about  $0.55^{\circ}$  C. By shaking up the finely powdered sugar with water, a solution is obtained containing about 7.5 grammes per 100 c.c. at  $15^{\circ}$  C., the quantity dissolved increasing roughly about 0.1 gramme per 100 c.c. for each degree above  $15^{\circ}$  C. No thermal change was detected in this solution by a thermometer reading to  $0.01^{\circ}$  C., but the temperature rose steadily till it attained that of the surrounding atmosphere, which was kept constant; the rate of rise was identical with that of a previously prepared solution of milk-sugar of the same strength, which had been cooled to the same temperature. Brown and Pickering have, however, shown that a slight thermal change takes place with change of rotatory power (+ 0.19 calorie per gramme). No change in density or molecular weight indicated by freezing point determination was observed on keeping solutions of milk-sugar, though the specific rotation varied very widely.

It is usually stated that a freshly prepared solution of  $\alpha$ -milk sugar contains 14.55 per cent. at  $10^{\circ}$  C.; while by long standing

in contact with milk-sugar, or by boiling, a saturated solution containing 21·64 per cent. can be obtained. The author is unable to confirm the figure for the freshly prepared solution.

The density of well formed crystals is  $1\cdot545$  at  $\frac{15\cdot5}{15\cdot5}$ , bad crystals—i.e., those which are strained, have, however, a lower density.

The hydrated  $\alpha$ -modification is practically insoluble in alcohol, ether (in ether saturated with water it dissolves to the extent of 0·00075 gramme per 100 c.c.), chloroform, benzene, and other organic solvents. It is slightly, but distinctly, soluble in amyl alcohol on boiling, but is probably dehydrated.

It is unaffected by heating to  $100^{\circ}$  C., but the water of hydration is given off at  $130^{\circ}$  C.; at  $170^{\circ}$  a change takes place with formation of lacto-caramel, and it melts at  $213\cdot5$  C.

When dissolved in water the specific rotatory power remains constant for a short period, 3 minutes at  $20^{\circ}$  C., 6 minutes at  $15^{\circ}$  C., and 15 minutes at  $10^{\circ}$  C.; the rotation then gradually falls.

The following series of observations (Table I.) will show the nature of the change in rotation:—

TABLE I.—CHANGE OF ROTATION OF  $\alpha$ -MILK-SUGAR IN SOLUTION.

Time T.	Observed Rotation, $R_T$ .	Calculated Rotation	Difference.
2·2 min.	12·73°	12·75°	+ 0·02
2·7 "	12·78°		- 0·03
3·2 "	12·68°		+ 0·07
4·2 "	12·83°		0·08
4·7 "	12·73°		+ 0·02
6·0 "	12·73°		+ 0·02
11·0 "	12·48°	12·49°	+ 0·01
16·25 "	12·23°	12·23°	...
23·0 "	11·83°	11·91°	+ 0·08
32·5 "	11·53°	11·51°	- 0·02
43·0 "	11·23°	11·11°	- 0·12
54·5 "	10·73°	10·72°	0·01
360·0 "	8·03°	8·03°	...
24 hrs.	$7\cdot95 = R_{\infty}$	7·95	..

The solution used was examined in a 198·4 mm. tube using the sodium light; two determinations gave 7·090 and 7·072 per cent. of anhydrous milk-sugar, and, as the solution had a density of 1·0265 at  $17^{\circ}$  (the temperature of observation), it contained 7·651 grammes of hydrated milk-sugar per 100 c.c.

It is seen that the rotation is approximately constant for the first 6 minutes, and averages  $12.75^\circ$ , which corresponds to  $[\alpha]_D = 83.99^\circ$ . After 24 hours the rotation is constant at  $7.95^\circ$ , which corresponds to  $[\alpha]_D = 52.37^\circ$ .

The figures given in the "calculated" column are deduced by the formula—

$$\log_{10} (R_T - R_\infty) = 0.68124 - 0.00491 (T - 6).$$

The fact that the fall in rotation is expressed by a logarithmic curve shows that the rate of change is proportional to the amount of unchanged substance in solution; this is Harcourt's law of mono-molecular change.

The ratio between the initial rotation and the final rotation, which may be called the bi-rotation ratio, is  $\frac{83.99}{52.37} = 1.604$ .

The mean of several determinations has led to the value for  $[\alpha]_D$  of the hydrated  $\alpha$ -modification =  $84.0^\circ$ , and the bi-rotation ratio 1.6; these are the figures given by Schmoeger, who, however, assigned to them an approximate value only.

The small amount of thermal change during change of rotation and absence of change in density and freezing point show, with a considerable degree of probability, that the change manifested by alteration in rotation is intra-molecular. It is probably caused by the migration of the water of hydration from one carbon atom to another.

The anhydrous modification of the  $\alpha$ -modification is obtained by heating the hydrated modification to  $130^\circ$  C. It is hygroscopic and dissolves in water with evolution of heat; the solubility is much greater than that of the hydrated modification. The optical properties are stated by Schmoeger to be the same as those of the hydrated modification.

Both the  $\alpha$ - and  $\beta$ -modifications are converted on dissolving in water into a stable equilibrium form. Schmoeger gives the specific rotatory power  $[\alpha]_D$  as  $52.53^\circ$  at  $20^\circ$  C., diminishing  $0.075^\circ$  for each degree C. above and this increasing for lower temperatures. The author can absolutely confirm these numbers. It has never been prepared pure in the solid state, though considerable evidence of its existence, both in the hydrated and anhydrous modifications, has been obtained by the author.

By the addition of alcohol or, better, ether to a very highly supersaturated hot solution of milk-sugar, it sets to a solid mass, which may be dried *in vacuo*, and does not then lose weight at  $100^\circ$  C., but which contains, however, a certain amount—2 to 4 per cent.—of water of hydration which is lost at  $130^\circ$  C. There is no appreciable change in rotation on dissolving this product

in water and taking readings at intervals; as some of the readings obtained have been above, and some below, that ultimately obtained, it is probable that the very slight differences noticed were due to errors of observation.

By precipitating less strong solutions of milk-sugar by alcohol, products can be obtained which contain very nearly, if not quite, the same percentage of water as the hydrated  $\alpha$ -modification, but which have a much smaller, but not constant, bi-rotation ratio. These also give a constant rotation for a few minutes on dissolving in water and behave as mixtures of the  $\alpha$  and  $\beta$ -modifications. They have a less density than the  $\alpha$ -modification, but it is not certain whether this may not be due to the very imperfect crystallisation which takes place, the products appearing nearly amorphous.

By evaporating aqueous solutions of milk-sugar on the water bath, an anhydrous sugar can be obtained, which has a very slight bi-rotation ratio, which is not constant. As this varies from 1.09 to 1.02, such sugar probably consists of the equilibrium form, mixed with a small amount of the  $\alpha$ -modification. A specimen having a bi-rotation ratio of 1.03 had a density of 1.585 at  $\frac{15.5^\circ}{15.5^\circ}$  and dissolved in water with a slight evolution of heat.

There is some evidence that the hydrated  $\beta$ -modification dissolves in water with a greater absorption of heat than the  $\alpha$ -modification, as the mixtures obtained by precipitation with alcohol cause a greater lowering of temperature than the  $\alpha$ -modification. The solubility appears to be greater.

There exists also a  $\beta$ -modification, which is obtained in the anhydrous form by the rapid evaporation of aqueous solutions in metallic vessels. It has a specific rotatory power of  $32.7^\circ$  at the moment of solution. Schmoeger states that it has a bi-rotation ratio of  $\frac{1}{1.6}$ , which Tanret confirms. The rate of change of the  $\alpha$ - and  $\beta$ -modifications is the same for the same temperature.

By the addition of ammonia, the change which the  $\alpha$ - and  $\beta$ -modifications slowly undergo on solution in water becomes almost instantaneous. By raising the temperature, the rate of change is increased and is practically instantaneous on boiling.

The solubility of milk-sugar in water is small compared with the solubilities of other carbohydrates: owing to the tendency of milk-sugar to form supersaturated solutions it is difficult to determine its exact solubility, but the mother liquors from which crystals have deposited usually contain about 21 per cent. The  $\alpha$ -modification crystallises in wedge-shaped forms which



often have the face at the end of the wedge greatly prolonged. The  $\beta$ -modification crystallises in needles.

The taste of the  $\alpha$ -modification is not sweet, and from its comparative insolubility it appears to be gritty. In solution milk-sugar has a sweet taste of about a quarter the sweetness of cane-sugar.

As already stated, on heating to  $170^{\circ}$  C. it turns brown, and lacto-caramel is formed; a similar change takes place by heating an aqueous solution to  $100^{\circ}$  C. for some hours; the presence of small amounts of alkali greatly increase the browning of the solution. The rotatory power is greatly diminished.

**Chemical Properties.**—Milk-sugar, in common with other aldoses and ketoses, reduces alkaline solutions of copper, silver, and mercury, forming cuprous oxide, and metallic silver and mercury respectively. On this fact the well-known Fehling's test for sugar is based. The amount of reduction is constant for fixed amounts of milk-sugar under the same conditions, and is nearly proportional to the amount of milk-sugar. Each sugar shows a definite amount of reduction in the same way, and a valuable method for distinguishing them is thus available. The difference between reduction by various sugars is not due to any difference in the reaction with the metallic salt, but depends on their relative stability towards alkalis.

On warming with dilute nitric acid (sp. gr. 1.2) an energetic action takes place, mucic acid, together with saccharic, oxalic, and other acids being formed; the mucic acid, which can be separated by its relative insolubility, amounts to about 32 per cent. of the weight of the milk-sugar. This is due to the galactose portion of the milk-sugar. Strong nitric acid (sp. gr. 1.5) mixed with sulphuric acid, to absorb the water formed in the reaction, gives rise to the formation of tri- and penta-nitrates; both these compounds have explosive properties. The penta-nitrate is a constituent of certain high explosives.

On heating with an excess of precipitated copper oxide gummy acids are formed, such as galactinic and pecto-galactinic acids, compounds which are also formed from galactose.

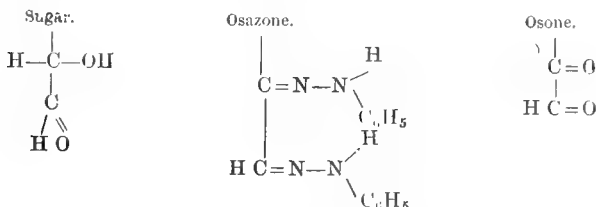
By oxidation with bromine lacto-bionic acid is formed, in which the COH group is converted into COOH. Potassium permanganate in acid solution oxidises it to carbonic acid, but the reaction is not complete, not more than 80 per cent. of the theoretical quantity of carbon dioxide being obtained.

By heating with phenylhydrazine acetate two compounds are formed; one of these—phenyl-lactosazone—is sparingly soluble in cold water, but in 80 to 90 parts of hot water, from which it separates on cooling in fine yellow needles melting at  $200^{\circ}$  C. with decomposition. It is also soluble in alcohol and ether; the latter

solvent extracts it from aqueous solution. The second compound is an anhydride of phenyl-lactosazone, and is almost insoluble in hot water; but can be crystallised from hot alcohol in yellow needles which melt at  $223^{\circ}$  to  $224^{\circ}$  C. Milk-sugar is distinguished from other sugars by its osazone forming an anhydride.

By treating with strong cold hydrochloric acid the phenyl-hydrazone groups are removed, and lactosone is formed.

The relation between these compounds is shown by the following formulæ:—



The osone is readily reconverted into osazone by treatment with phenylhydrazine acetate in the cold.

By reduction with sodium amalgam a mixture of mannitol and dulcitol, hexahydric alcohols of the formula  $\text{C}_6\text{H}_{14}\text{O}_6$ , with lactic acid and methyl, iso-propyl and hexyl alcohols is formed.

On heating with acetic anhydride and sodium acetate an oct-acetyl-lactose is formed. This crystallises in stout prisms from a mixture of alcohol and chloroform, and has an ill-defined melting point about  $90^{\circ}$  C. Its solution in chloroform is optically inactive or very slightly *levo*-rotatory.

Milk-sugar dissolves lime, baryta, lead, copper, and mercuric oxides, and probably forms compounds with them. No compound with sodium chloride is known.

Ammoniacal lead acetate precipitates milk-sugar from an aqueous solution.

It is not fermentable by ordinary yeast, and is unacted on by invertase, diastase, rennet, pepsin, and trypsin. There exists, however, an enzyme, which has been called lactase, which is found in fresh kephir grains, which hydrolyses it to glucose and galactose. The enzyme does not appear to be present in dried kephir grains, but is probably found in other substances.

The action of acids generally is to convert it into glucose and galactose. Some organic acids, such as citric, are, however, without action on milk-sugar when heated for moderate periods.

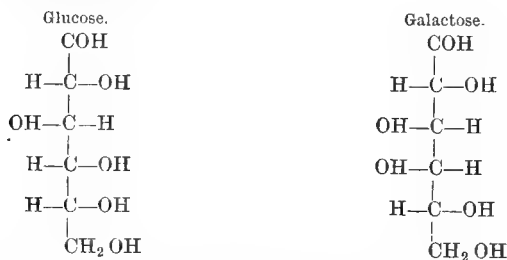
**Preparation.**—Milk-sugar is prepared on a large scale by evaporating whey *in vacuo*, after neutralisation of any acid with lime and clarification with alum or other means, and allowing it

to crystallise. The product is purified by re-solution, treatment with animal charcoal and re-crystallisation. In countries where alcohol used for manufacturing purposes is free from duty the sugar is precipitated from solution by this means instead of being crystallised from water.

On a small scale it is best to precipitate the protein from milk or whey by as small a quantity of acid mercuric nitrate (p. 90) as possible. The clear filtrate is neutralised with dilute caustic soda solution till a very faint tinge is given with phenolphthalein; it is filtered from the precipitate thus produced, which consists of mercury salts. Sulphuretted hydrogen is passed through the clear solution to remove the mercuric oxide dissolved by the sugar, and, after filtration from mercuric sulphide, the sulphuretted hydrogen is expelled by boiling. On evaporating the solution, milk-sugar crystallises out; crystallisation may be hastened by vigorously stirring the concentrated solution while it is being rapidly cooled.

**Glucose and Galactose.**—These are two isomeric sugars of the monose type. Both are aldoses or aldehydrols, and have been obtained in three modifications ( $\alpha$ - and  $\beta$ -modifications and a stable equilibrium form).

Their constitution is given by E. Fischer as



Wohl and List have confirmed the constitution of galactose.

They are thus isomeric sugars differing only in the third asymmetric carbon atom from the aldehyde group.

It is not known whether these sugars on solution in water give a constant rotation for a short time as in the case of milk-sugar. Their specific rotatory powers  $[\alpha]_D$  are

	Glucose.	Galactose.
Equilibrium form, . . . . .	52.7°	80.3°
$\alpha$ -modification, . . . . .	105°	120°
Bi-rotation ratio, . . . . .	2	1.5

Both sugars give, on treatment with phenylhydrazine acetate, nearly insoluble osazones. Phenylglucosazone crystallises from dilute alcohol in fine yellow needles melting at 204° to 205° C.

to a dark red liquid: phenylgalactosazone is obtained in yellow needles from alcoholic solutions, which melt at  $188^{\circ}$  to  $191^{\circ}$  C. with decomposition. They are converted into osones by treatment with strong cold hydrochloric acid.

**Products derived from Milk-Sugar.**—The most important of these products is formed by the action of certain micro-organisms on milk-sugar during the so-called lactic fermentation. By their action the milk-sugar is split up into **lactic** or oxypropionic acid almost quantitatively, a certain portion, however, being converted into other products, of which carbon dioxide is the most important. The micro-organisms which produce lactic acid are acted on inimically by acids, so that not much more than 1 per cent. of lactic acid is usually formed, unless the solution is kept neutralised by chalk or other means.

$$\begin{array}{c} \text{H} \\ | \\ \text{CH}_3-\text{C} \\ | \\ \text{OH} \end{array} \text{COOH,}$$

and it contains an asymmetric

carbon atom: it is not, however, optically active, though the isomeric sarcolactic acid possesses this property. It appears to be a racemic compound; but both the dextro- and levo-acids are produced by certain micro-organisms. It has a remarkable tendency to form compounds which contain less water.

On evaporating aqueous solutions of lactic acid, dehydrolactic acid is formed,  $\text{C}_6\text{H}_{10}\text{O}_5$ , which, by further evaporation (especially at a high temperature), gives lactide,  $\text{C}_6\text{H}_8\text{O}_4$ .

Lactic acid acts as a monobasic acid: while dehydrolactic acid behaves as a monobasic acid, monohydric alcohol and an ethereal salt at the same time; lactide is a neutral substance.

Sarcolactic acid gives the same lactide, which, on boiling with water, is converted into the inactive modification.

The so-called syrupy lactic acid is a mixture of lactic and dehydrolactic acids with probably a little lactide. Wislicenus has shown that by direct titration with alkali lactic and dehydrolactic acids are estimated, while by further boiling with excess of alkali one molecule of lactic acid is produced for each molecule of dehydrolactic acid, and two for each molecule of lactide. Dehydrolactic acid has not been obtained pure, but appears to be amorphous and nearly insoluble in water.

Lactide can be prepared by subliming syrupy lactic acid at  $150^{\circ}$  in a current of dry air. It is insoluble in water, but can be crystallised from alcohol in colourless rhombic plates melting at  $124.5^{\circ}$  C. It boils at  $255^{\circ}$  C.

Syrupy lactic acid is said to have a specific gravity of 1.2485. Lactic acid is not appreciably volatile in dilute solution, but

passes over with water to a slight extent as the solution becomes concentrated.

Lactic acid is soluble in and miscible in all proportions with water, alcohol, ether, and glycerol. It is insoluble in petroleum ether. Fats also dissolve it. It is probable that the lactic acid present in milk is, partially at all events, dissolved in the fat.

As milk almost immediately after milking contains organisms which produce lactic acid, it may be considered as a normal constituent of milk; indeed Béchamp has held that it is produced from milk by organisms (micro-zymes) derived from the udder itself. That this view is erroneous is shown by the fact that Lister, Pohl, Warrington, and others have succeeded in preserving milk, drawn direct into sterilised vessels, for a considerable length of time without the development of acidity.

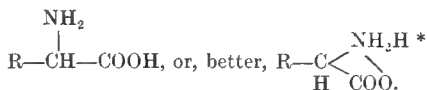
Lactic acid probably exists in milk, not in the free state, but as a salt, at all events until the acidity is sufficient to curdle the milk on boiling.

**The Proteins—Properties.**—Our present knowledge of the proteins of milk is far from complete, though much work has been done on the subject. This is due to the fact that it is extremely difficult to obtain these compounds in anything like a state of purity. The method of crystallisation, which is so largely depended on in the case of other bodies, is only available in the case of albumin, and as proteins are altered in their essential properties by very many reagents, the choice of methods of purification is limited. The difficulty is still further increased by the peculiar behaviour of casein in retaining calcium salts, once it has been brought into contact with them, as is the case in milk. The proteins of milk have been prepared in as pure a state as possible by the general method of precipitating them by some reagent, dissolving them, reprecipitating as many times as may be thought necessary, and, finally, by eliminating such impurities as may have been introduced during the process. As there is no means of knowing when all the impurities have been eliminated, it is possible that we are yet unacquainted with the proteins of milk in a state of purity. This should not be forgotten during their study.

The proteins are composed of carbon, nitrogen, hydrogen, oxygen, and usually sulphur and phosphorus. The exact mode of combination of the elements in any protein is not known, but recent researches, notably by Hofmeister, Schiff, Curtius, Kühne, Neumeister, Hammarsten, E. Fischer, Abderhalden, Chittenden, Osborne, and Skraup, have thrown much light on the types on which proteins are formed.

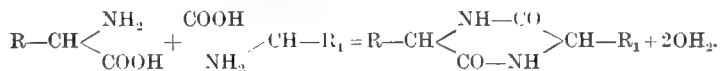
Of the ultimate products obtained by the continued breaking down of proteins either by enzymes, acids or other hydrolysing

agents, the most important, both in character and amount, are amino-acids; it has been found that nearly, if not quite, all the amino-acids obtained from proteins are  $\alpha$ -compounds of the type—

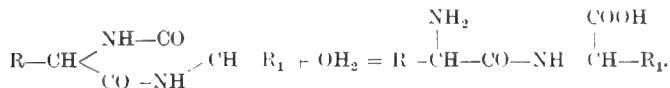


Amino-acids of this type have also formed the starting point in the synthesis of compounds having many of the characteristic reactions of proteins, and identical with those formed by their hydrolysis—the polypeptides.

These acids condense readily to form what were called “anhydrides,” but which really have no anhydride grouping; the condensation takes place between the amino-group of one molecule and the carboxyl-group of another, and di-keto-piperazine compounds are formed, thus—

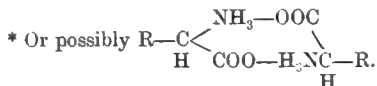


By boiling with concentrated hydrochloric acid the heterocyclic compound is converted into an open chain, thus—



These compounds form the simplest polypeptides. By the action of an acid chloride on the silver salt of a polypeptide, or by the condensation of acid-azides with amino-acids, employed by Curtius, it was possible to link up three or more amino-acids; or by protecting the amino-group of a polypeptide by the introduction of a carboxethyl-group Fischer was able to condense the carboxyl-group with an amino-group of another amino-acid, and by introducing an  $\alpha$ -halogen acid chloride into the amino-group and substituting an amidogen radicle for the halogen he was able to condense the amino-group with the carboxyl-group of another amino-acid.

By these and other means polypeptides containing two, three, four, and five amino-acid radicles have been synthesised, and some of these have been identified with products of hydrolysis of proteins, or with their optical isomerides. There is little



doubt that the grouping—CH—NH—CO—CH—is characteristic of proteins; Fischer also considers it proved that the di-keto-piperazine group also occurs.

Fischer has recently prepared a polypeptide containing 18 molecules of amino-acids; it was *l*-leucyl-triglycyl-*l*-leucyl-triglycyl-*l*-leucyl-octoglycyl-glycine, and gave many of the protein reactions: this compound was hydrolysed by trypsin, but not by pepsin.

**Classification of Proteins.**—A joint committee of the Chemical and Physiological Societies has proposed the following classification for proteins:—

1. Protamines. These are characterised by being free from sulphur, and containing large amounts of arginine. Strongly basic.
2. Histones. These are very basic substances, and are precipitated by ammonia. They contain little sulphur.
3. Albumins. Soluble in water; coagulated by heat.
4. Globulins and their derivatives. Insoluble in water, but soluble in salt solutions.
5. Sclero-proteins; insoluble proteins which form the supporting structures or connective tissues of animals.
6. Phospho-proteins; derivatives of para-nucleic acid; do not contain purine or pyrimidine derivatives; are distinctly acid substances.
7. Conjugated proteins, subdivided into—
  - (a) Nucleo-proteins; derivatives of nucleic acid; contain purine and pyrimidine derivatives.
  - (b) Gluco-proteins; containing a carbohydrate radicle. Mucins.
  - (c) Chromo-proteins; coloured protein substances as hæmoglobin.

The proteins of milk are:—

Class 3.	Lactalbumin,	.	.	.	about 0·4 to 0·5 per cent.
„	4. Lacto-globulin,	.	.	.	traces.
„	7b. Storch's mucoid protein,	.	.	.	traces.
„	6. Casein,	.	.	.	about 3·0 per cent.

**General Reactions of Proteins.**—The following are the general reactions of proteins:—

1. **The Biuret Reaction.**—Add to a solution an excess of caustic alkali, and one drop of copper sulphate solution. Proteins give a violet colour, and proteoses and other products of hydrolysis a reddish tint.

This reaction is characteristic of proteins.

2. **The Xantho-protein Reaction.**—On heating with strong nitric acid proteins yield a yellow colour, darkened by alkalis. This reaction is due to the presence of homocyclic ring compounds, chiefly tryptophane and tyrosine, and to a less degree by phenylalanine.

3. **Millon's Reaction.**—On adding Millon's reagent (a solution of mercurous and mercuric nitrates in nitric acid) to a protein a red colour is produced. This is produced by all oxy-phenyl compounds, and is given by the tyrosine group in proteins.

4. **Adamkiewicz' Reaction.**—On dissolving proteins in glacial acetic acid, and adding strong sulphuric acid coloured rings are formed at the junction of the two liquids. This reaction is due to the presence of glyoxylic acid in the acetic acid, which compound gives a blue or bluish-violet colour with tryptophane. Hopkins and Cole, to make the test more certain, add glyoxylic acid.

5. **Lieberman's Reaction.**—Proteins which have been extracted with ether give a blue or bluish-violet colour on boiling with strong hydrochloric acid. This reaction is really the same as the above, as it is due to the presence of glyoxylic acid or other aldehydic compounds in the ether.

6. **Ehrlich's Diazo Reaction.**—On adding a diazonium salt to a soluble protein, and making alkaline, a red colour is produced, if histidine or tyrosine be present in the molecule. A diazotised solution of sulphanilic acid is convenient. Other radicles give a yellow colour.

7. **Richmond and Miller's Diazo Reaction.**—On diazotising a solution of a protein, and adding an alkaline solution of  $\beta$ -naphthol, a colour (usually yellow) is produced, and gas is given off in the cold. This test proves the presence of aryl and acyl amino-groups respectively.

8. **The Halogen Reaction.**—Chlorine and bromine give insoluble compounds with all soluble proteins. Iodine gives a brown coloration.

9. **Aldehyde Reactions.**—On adding a solution of formaldehyde to a solution of a protein neutral to phenolphthalein it becomes acid. This is characteristic of  $\alpha$ -amino-acids, the basic amino-group being converted into a very feebly basic methylene-amino-group.

10. **Ehrlich's Aromatic Aldehyde Reaction.**—Certain aromatic aldehydes when added to proteins in acid solution give a well-marked coloured condensation product. *p*-dimethyl-amino-benzaldehyde and vanillin (*p*-hydroxy-*m*-methoxy-benzaldehyde) give a red colour (the latter, however, tinged with blue) and *p*-nitro-benzaldehyde a green colour. This reaction appears to be characteristic of the tryptophane radicle.

11. On boiling most proteins with an alkali, a portion of the sulphur is transformed into sulphide, which may be conveniently demonstrated by the black colour given on adding a solution of a lead salt.



The presence of protein may be considered as proved if reactions 1, 8, and 9 are given; many, though not necessarily all, of the other reactions will also be obtained should proteins be present.

### PRODUCTS OF HYDROLYSIS OF PROTEINS.

By the action of hydrolysing agents—acids, enzymes—proteins are gradually split up into simpler compounds. These are classified as:—

(a) *Meta-proteins.* Products which have undergone but little change and still have most of the protein characters; the product formed by heating lactalbumin in slightly acid solution to its coagulating point comes under this class. The curd formed by the action of rennet on casein will also come under this heading, and may be termed meta-casein (the usual name is para-casein).

(b) *Proteoses*, which may be again subdivided into—

1. *Proto-proteoses.* Insoluble in ammonium sulphate solution, 24 to 42 per cent., saturated. Hetero-proteose contains phenyl-alanine, proline, and glycine, but is free from tyrosine and tryptophane. Insoluble in 32 per cent. alcohol. Hemi-proteose contains tyrosine and tryptophane. Soluble in alcohol.

2. *Deutero-proteoses A.* Insoluble in ammonium sulphate solution, 54 to 62 per cent., saturated. May be further fractionated by their solubility in alcohol.

3. *Deutero-proteoses B.* Insoluble in ammonium sulphate, 70 to 95 per cent., saturated. Those soluble in alcohol contain no sulphur. Those insoluble in alcohol contain sulphur.

4. *Deutero-proteoses C.* Insoluble in saturated ammonium sulphate solution and acid. Free from sulphur.

(c) *Peptones.* Soluble in saturated ammonium sulphate solution and acid. Those insoluble in 96 per cent. alcohol contain no tyrosine nor tryptophane. Those soluble contain both these substances.

(d) *Polypeptides*, subdivided into—

1. *Kyrins.* Rich in basic substances—lysine and arginine.

2. *Peptides.* Simple condensation products of amino-acids. Diketo-piperazines.

(e) *Amino-acids* obtained on continued hydrolysis.

This classification is not quite satisfactory, as the distinction between the various classes is somewhat arbitrary, and there is no sharp distinction between them. There is, however, a steady fall in molecular complexity.

During hydrolysis, the hydrolysts are not destroyed, or are destroyed with extreme slowness, and appear to be able to act on a relatively enormous quantity of the hydrolyte. The time

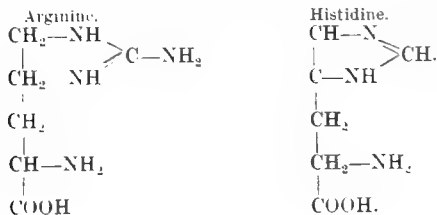
taken to produce a given change on a given quantity of hydrolyte is inversely proportional to the quantity of hydrolyst. Each hydrolyst has a certain optimum temperature at which it acts most rapidly, the action being diminished at both higher and lower temperatures. Certain substances—*e.g.*, acids—affect the rate of hydrolysis by enzymes; their influence, however, follows laws which are not fully known. This may be perhaps due to the hydrolysing effects of the acid combined with those of other hydrolysts taking a course influenced by both of them.

Fischer separates the amino-acids obtained by long boiling with acids by esterifying, crystallising out the glycine ester, and distilling the others *in vacuo*, and extracting with ether. Fischer and Bergell also convert the amino-acids into their  $\beta$ -naphthalene sulphonates, which are very slightly soluble. These methods involve, however, some loss, but they give a rough estimate of the proportions of the amino-acids.

A large number of amino-acids have been separated from the products of continued hydrolysis of proteins; these are—

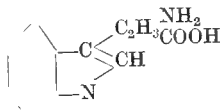
- A. Mono-amino-mono-carboxylic acids.  
 Amico-acetic acid, or glycine.  
 Amino-propionic acid, or alanine.  
 Amico-butyric acid.  
 Amino-valeric acid or valine.  
 Iso-butyl-amino-acetic acid, or leucine.
- B. Hydroxy-mono-amino-mono-carboxylic acids.  
 Hydroxy-amino-propionic acid, or serine.  
 Tetra-hydroxy-amino-caproic acid.
- C. Mono-amino-di-carboxylic acids.  
 Amino-succinic acid, or aspartic acid.  
 Amino-glutaric acid, or glutamic acid.
- D. Hydroxy-mono-amino-di-carboxylic acids.  
 Hydroxy-amino-succinic acid.  
 Hydroxy-amino-suberic acid.
- E. Di-amino-mono-carboxylic acids.  
 $\alpha$ - $\beta$ -diamino-propionic acid.  
 $\alpha$ - $\epsilon$ -diamino-caproic acid, or lysine.  
 $\alpha$ - $\delta$ -diamino-valeric acid, or ornithine.
- F. Substituted-mono-amino-mono-carboxylic acids.  
 $\alpha$ -amino- $\delta$ -guanidine-valeric acid, or arginine.  
 $\alpha$ -amino- $\beta$ -iminazoly-propionic acid, or histidine.

The constitution of arginine and histidine is as below :—



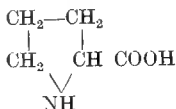
$\beta$ -phenyl- $\alpha$ -amino-propionic acid, or phenyl-alanine.  
 $\beta$ -*p*-oxy-phenyl- $\alpha$ -amino-propionic acid, or tyrosine.  
 Indole-amino-propionic acid, or tryptophane.

This has the constitution

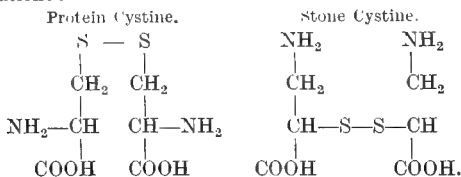


- G. Hydroxy-diamino-mono-carboxylic acid.  
 Tri-hydroxy-diamino-dodecanoic acid.
- H. Diamino-di-carboxylic acids.  
 Diamino-glutaric acid.  
 Diamino-adipic acid.
- I. Hydroxy-diamino-di-carboxylic acids.  
 Hydroxy-diamino-sebacic acid.  
 Dihydroxy-diamino-suberic acid, and probably others.
- J. Pyrrolidine compounds.  
 $\alpha$ -pyrrolidine-carboxylic acid, or proline.  
 Oxy-pyrrolidine-carboxylic acid, or oxy-proline.

Proline has the constitution

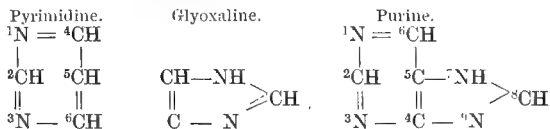


- K. Thio-amino-acids.  
 Protein-cystine and stone-cystine.  
 These are derived respectively from  
 $\alpha$ -amino- $\beta$ -thio-lactic acid, and  
 $\alpha$ -thio- $\beta$ -amino-lactic acid, and are represented by the following  
 constitutions :—



Among other compounds obtained by the hydrolysis of proteins are :—

\*L. Purine bases. These are contained in the nucleo-proteins; they are products containing a pyrimidine and a glyoxaline (or imidazolyl) ring, thus—



\* It is not certain whether purine or pyrimidine derivatives occur in milk proteins.

Four purine bases are obtained in which substitution occurs only in pyrimidine ring; these are—

Hypo-xanthine, . . . . .	6-oxy-purine.
Xanthine, . . . . .	2-6-dioxy-purine.
Adenine, . . . . .	6-amino-purine.
Guanine, . . . . .	2-amino-6-oxy-purine.

The nucleic acids are derivatives of purine bases, being compounds in which a phosphoric acid radicle is condensed in the 8 position.

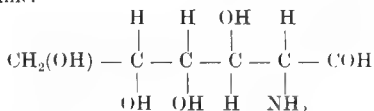
Uric acid, which is excreted by the kidneys, has the composition of 8-oxy-xanthine.

\*M. Pyrimidine derivatives.

Three pyrimidine derivatives are obtained from the nucleo-proteins :—

Uracil, . . . . .	2-6-dioxy-pyrimidine.
Cytosin, . . . . .	2-oxy-6-amino-pyrimidine.
Thymin, . . . . .	2-6-dioxy-5-methyl-pyrimidine.

N. Glucosamine.



is obtained from the gluco-proteins.

Glycine. Monoclinic crystals, soluble in 4.3 parts cold water. Nearly insoluble in alcohol. M.P., 232° to 236° C., with dark purple colour. Best obtained by the action of ammonia on mono-chlor-acetic acid, or by the hydrolysis of glue.

Alanine. Needles. Soluble in 4.6 parts cold water. Nearly insoluble in alcohol. May be prepared from 2 parts aldehyde-ammonia and 1 part hydrocyanic acid with excess of HCl.

Leucine. Volatilises at 210° to 220° C. without melting, or melts in a closed tube at 270° C. Moderately soluble in water, and crystallises in soft nacreous scales consisting of concentrically grouped rhombic prisms.

Serine. Bunches of monoclinic crystals soluble in 32 parts water at 10° and 24 at 20° C. Insoluble in alcohol. Active form polarises + 6, and the lævo-form occurs in proteins.

Aspartic acid. Prepared by the hydrolysis of asparagin, or it may be separated from beet molasses. Polarises to the left in water, and to the right in strong acids. Very soluble in hot water, and little so in cold.

Glutamic acid. Rhombic-spheroidal-hemihedric crystals, melts with decomposition at 202° to 202.5° C. One part soluble in 100 parts water at 16° C.; less soluble in alcohol. May be prepared by heating casein with HCl and ZnCl<sub>2</sub>.

\* It is not certain whether purine or pyrimidine derivatives occur in milk proteins.

Tyrosine,  $C_9H_{11}NO_3$ , is an oxyphenyl derivative of amino-propionic acid. It crystallises in fine glistening needles usually grouped in bundles, soluble in about 150 parts of boiling water. It gives a red precipitate with a solution of mercuric nitrate containing nitrous acid, and is capable of forming metallic salts.

By tryptic digestion more tyrosine is produced than leucine; other hydrolytic actions produce greater quantities of leucine.

A. J. Brown and J. H. Millar have shown that tyrosine is separated in the early stages of tryptic digestion, but the tyrosine nucleus is very resistant to peptic digestion.

*Proximate Determination of the Constitution of Proteins.*—Hausmann's method consists in boiling 1 gramme of protein with strong hydrochloric acid for some hours and determining

1. The nitrogen split off as ammonia by distillation with magnesia, preferably at a low temperature.
2. The nitrogen in the insoluble portion. Melanin nitrogen.
3. The nitrogen in the phospho-tungstic acid precipitate; basic (lysine, histidine, or arginine) or diamino-nitrogen.
4. The soluble nitrogen. Mono-amino-nitrogen.

The only milk protein which has been thus examined is casein, and the mean of the results of Gumbel, Osborne and Harris, and Kutscher is—

Ammonia Nitrogen.	Melanin Nitrogen.	Diamino- Nitrogen.	Mono-amino- Nitrogen.
1.63	0.27	3.87	9.98

**The Proteins of Milk.**—The number of proteins present in milk (of the cow) has been variously stated at from one to eight by different observers. The most recent work has tended to reduce the number to not more than four, the larger number described having been obtained by faulty methods of separating these bodies or by the action of some reagent used on the protein. Many of the products described are now known to be mixtures of one or more of the proteins with various impurities, or decomposition products obtained during the separation of the proteins one from another.

The theories of leading observers are briefly given as follows :—

Duclaux maintains that there is only one protein in milk, which exists in two forms—the coagulable and non-coagulable; he gives to this protein the name of **casein**. The first modification is not in a state of solution, and can be separated by filtration through a porous jar; it is combined with the phosphates of the alkaline earths, and this causes it to differ in its properties from the other modification, which is in a state of true solution and passes through the porous jar. Were this view correct, the coagulable modification should gradually lose its distinctive properties as it is purified from phosphates; and, on

the other hand, the non-coagulable modification should be capable of being converted into the other by associating it with phosphates; neither alternative has as yet been found possible, and, as two proteins having distinct properties can be separated from milk, Duclaux's view is hardly tenable.

Hammarsten describes two proteins; one, casein, corresponding to Duclaux's coagulable casein; the other, **lactalbumin**, corresponding to Duclaux's non-coagulable casein. He shows that lactalbumin has the properties of a true albumin, approaching very closely to serum-albumin, but differing from it in certain physical constants, which entitles it to rank as a distinct body. Sebelein has shown that there exist in milk traces of a **globulin**, in addition to the casein and albumin of Hammarsten.

Halliburton describes the proteins of milk as **caseinogen** and lacto-albumin; there is no essential difference between the casein of Hammarsten and the caseinogen of Halliburton, except a difference of name. He reserves the name casein for the curd produced by the action of rennet.

Hewlett has confirmed Sebelein's statement as to the existence of globulin in milk, though he has shown that Sebelein's globulin was probably contaminated with small amounts of casein.

Musso and Menozzi have claimed the presence in milk of a body midway between casein and albumin: this is probably the globulin of Sebelein in an impure state, as their description is in fair accordance with a statement of the properties of the latter.

Radenhausen and Danilewsky have described many proteins in milk. Hammarsten and—later—Chittenden and Painter have shown that their view that casein is a mixture of two compounds is untenable, while the various lacto-protein bodies have been shown to be the result of their method of separating casein and albumin.

Wynter Blyth has described a body called **galactin** in milk; this is essentially lacto-protein, perhaps contaminated with some organic salts, and has no real existence in milk, being portions of the casein and albumin which had escaped separation, together with products of their decomposition during the process used for their removal.

Béchamp supposes that the proteins of milk number three—casein, albumin, and a body having the properties of an enzyme, which he calls **galacto-zymase**; this enzyme he finds liquefies starch paste, evidently not its normal function in milk: his results have not been confirmed. He also supposes the casein and albumin to exist in milk in combination with bases (soda, lime, or potash).

Biel has described **syntonin** as a normal constituent of milk, but the existence of this must be considered doubtful at present.

Palm has stated that **albumoses** are found in milk; this is probably not wholly correct; it is possible that traces of albumoses are formed during the decomposition to which milk is prone, but no other observer has identified more than traces, while Palm gives 1.5 per cent. as occurring in milk. True peptone has been proved to be absent. Storch's researches have been referred to (p. 2). Babcock has found very small amounts of **nuclein**, but the presence of this has now been disproved, and the same observer has with Russell separated a proteolytic enzyme. There are also a peroxydase, a catalase, and a reductase.

From the above list of the various proteins described as existing in milk we may select the four of whose existence we have the strongest evidence; these are casein, lactalbumin, lacto-globulin, and Storch's mucoid; the last two, however, are only found in traces in milk, and, practically, the proteins may be reduced to the former two. The other compounds described, except the enzymes whose proteinic nature is not fully established, are hypothetical.

The main reactions that distinguish the four proteins of milk are as follows:—Casein is precipitated by saturating the solution with sodium chloride, magnesium sulphate, and ammonium sulphate; globulin is soluble in a saturated solution of sodium chloride, but is precipitated by magnesium and ammonium sulphates; albumin is soluble in saturated solutions of sodium chloride and magnesium sulphate, but is precipitated by saturation with ammonium sulphate, while Storch's mucoid is not in solution; albumin is, however, precipitated from a saturated solution of magnesium sulphate by acidifying slightly, and is redissolved by neutralisation of the solution. Casein and globulin are precipitated by the addition of acid, while albumin (and globulin, if much salt is present) is not so precipitated. Casein has the remarkable property of being acted on by chymase, the enzyme of rennet, with the formation of an insoluble product; albumin is coagulated by the action of heat, the raising of the solution to about 70° C. under suitable conditions of acidity being sufficient to precipitate a great portion. Casein is gradually removed by filtration through paper, and completely through coarse porcelain; filtration through fine porcelain removes all the proteins. Properties common to the three proteins are solubility in alkalis, insolubility of their copper, mercury, and other salts, insolubility in alcohol; all are precipitated by tannin and phospho-tungstic acid.

**Casein.**—This protein, when pure, is a white amorphous

body without taste or smell: it is practically insoluble in water, dissolving in this menstruum to the extent of about 0·1 per cent. : it is quite insoluble in alcohol and ether. Very dilute acids seem to diminish the solubility; but it is soluble in stronger acids, becoming, however, changed; a solution of casein in acetic acid has been used as glue; it is completely soluble in caustic alkaline solutions even when very dilute; the solutions of the carbonates, bi-carbonates, and phosphates of the alkalis also dissolve it, and from these solutions, as well as those of the alkalis, it is precipitated unchanged by the addition of sufficient acid to neutralise the alkali. It has the property of forming an opalescent solution when it is dissolved in the least possible excess of sodium phosphate, and the addition of small quantities of calcium chloride is made; it gives then a solution having the appearance of milk. It is highly probable that milk contains casein in this form. Casein has a peculiar affinity for calcium salts, especially the phosphate. It is extremely difficult to free it from this body, the purest preparations that have been prepared having always been contaminated with small amounts of this compound. Casein yields a comparatively small amount of sulphide if boiled with an alkali, and contains less of this element than either globulin or albumin; it also differs from these compounds by containing phosphorus; on analysis, like other proteins, it does not yield very concordant results; the most probable composition is as follows:—

Carbon, . . . . .	Per cent.	53·13	Sulphur, . . . . .	Per cent.	0·77
Hydrogen, . . . . .	7·06	Phosphorus, . . . . .	0·86		
Nitrogen, . . . . .	15·78	Oxygen, . . . . .	22·40		

The composition of casein is variously stated by different authorities. The following are the most reliable results:—

Authority.	Hammarsten.	Chittenden and Painter.	Ellenberger.	Stolmann.	Lehmann.	Ritt-hausen.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
C	52·96	53·30	53·07	54·08	54·00	54·22
H	7·05	7·07	7·13	7·09	7·04	7·17
N	15·65	15·91	15·64	15·57	15·60	15·49
S	0·72	0·82	0·76	0·77	0·77	0·91
P	0·85	0·87	0·80	..	0·85	..
O	22·78	22·03	22·60	..	21·70	..

The author has calculated the two following formulæ as possible for casein; the first agrees with the results of Hammarsten, Chittenden and Painter, and Ellenberger, while the second



represents the figures obtained by Stohmann, Lehmann, and Ritthausen; it is probable that the last three observers did not remove Storch's mucoid protein so completely as the others:—

	I. $C_{162}H_{258}N_{41}SPO_{52}$	II. $C_{170}H_{268}N_{42}SPO_{51}$
	Per cent.	Per cent.
C	52.96	54.04
H	7.03	7.10
N	15.64	15.56
S	0.86	0.84
P	0.84	0.82
O	22.41	21.60

Hammarsten and Chittenden purified their casein by solution in alkali and reprecipitation several times; Ritthausen worked on the copper salt; and Lehmann used what he designated "genuine" casein, which was separated from milk by the use of a porous plate.

Lehmann found that in "genuine" casein 1.45 to 1.75 parts of lime were combined with 100 parts of casein; one molecule (CaO) to one molecule  $C_{162}H_{258}N_{41}SPO_{52}$  requires a proportion 1.53. Söldner has also shown that two lime compounds exist containing 1.55 and 2.39 per cent. CaO respectively, which correspond with CaO and 1.5 CaO to one molecule of casein.

The author has found that  $\frac{N}{100}$  sodium and potassium carbonate solutions treated with an excess of casein dissolve 1.86 and 1.83 parts per 100 c.c. respectively. The first formula would give 1.84.

It appears probable that casein exists in milk as a calcium sodium salt combined with one molecular proportion of tricalcium phosphate. The author has found that by filtration through a porous cell the compound separated has the composition:—

	Calculated for 0.477% N and $C_{162}H_{258}N_{41}SPO_{52}$ $CaNa \cdot \frac{1}{2}(Ca_3P_2O_8)$ .
Casein nitrogen, . . . . .	0.477
CaO, . . . . .	0.116
$P_2O_5$ , . . . . .	0.123
Alkalies, . . . . .	0.031
Total ash, . . . . .	0.27
	0.266

It was also found that on adding 8.6 c.c. of normal hydrochloric or sulphuric acid per litre the casein was precipitated on boiling, and the acidity of the serum was equal to the acidity of the milk after boiling. The quantity of acid required by the

above formula to replace the atom of sodium by hydrogen in the above formula is 8.3 c.c. The precipitated casein contained all the lime and phosphoric acid, the quantities carried down being (calculated as before for 0.477 nitrogen)—

CaO,	0.120
P <sub>2</sub> O <sub>5</sub> ,	0.120
Total ash, . . . . .	0.23

From the above figures it is seen that the formula given fairly well represents the composition of casein, though, of course, it is only an approximation; the real formula is almost certainly a multiple of this.

It is noticed that the sulphur is lower than that calculated from either of the above formulæ. By treating casein with alkalis a portion of the sulphur is removed as sulphide. It is possible that in the purification of the casein by solution in dilute alkali and precipitation by acids that a small amount of decomposition sets in.

The following are the amounts of various acids (calculated as c.c. of normal solution per litre of milk) required to precipitate the casein on boiling:—

Hydrochloric and sulphuric,	8.6 c.c.
Acetic and lactic, . . . . .	9 to 10 c.c.
Citric, . . . . .	13.5 c.c.
Oxalic, . . . . .	28.5 c.c.
Phosphoric, . . . . .	34 to 35 c.c.

That large amounts of the weaker acids are required is only to be expected; the behaviour of oxalic and phosphoric acids is anomalous, and appears to be due to the fact that oxalic acid removes the lime from the casein complex, while phosphoric acid forms an acid phosphate with the tricalcium phosphate. In either case the formation of a mono-acid calcium salt of casein combined with calcium phosphate is prevented.

Revis and Payne consider that casein exists in milk combined with calcium phosphate, and that on acidifying the milk with small progressive amounts of acid the calcium phosphate is removed from the combination; when the casein is precipitated by the acid practically all the calcium phosphate is precipitated. Their results show that calcium is removed from the complex in direct proportion to the acid added, but their figures with regard to phosphoric acid are less definite.

They also show that it is very improbable that any appreciable amount of lactates of casein are formed in milk as it turns sour, as supposed by van Slyke, Hart, and Laxa.

Casein behaves as a tribasic acid; it has also basic properties, and combines with acids giving salts easily decomposed by water. The acid functions are much more strongly marked than the

basic ones. On hydrolysis it ultimately yields, according to Fischer and others, especially Osborne and Greed, the following (the estimations of leucine, glutamic acid, tyrosine, lysine, arginine, and histidine are probably nearly correct; the other figures probably low):—

Glycine,	. . . . .	none
Alanine,	. . . . .	1.5
Leucine,	. . . . .	10.5
Phenyl-alanine,	. . . . .	3.2
Proline,	. . . . .	6.7
Glutamic acid,	. . . . .	10.50
Aspartic acid,	. . . . .	1.39
Cystine,	. . . . .	0.065
Serine,	. . . . .	0.43
Oxy-proline,	. . . . .	1.5
Tyrosine,	. . . . .	4.5
Lysine,	. . . . .	5.8
Histidine,	. . . . .	2.6
Arginine,	. . . . .	4.84
Tryptophane,	. . . . .	1.5
Ammonia,	. . . . .	1.61
Cystein,	. . . . .	none
Amino-valeric acid,	. . . . .	7.2
Glucosamine,	. . . . .	none
Diamino-trioxy-dodecanoic acid,	. . . . .	0.75

Levites has also shown that casein contains 0.93 per cent. of nitrogen removed by the action of nitrous acid—*i.e.*, as free  $\text{NH}_2$  group—this compound, however, yielded as much nitrogen as ammonia (1.67 per cent.) as the original casein.

When dissolved in dilute alkali it has a lævo-rotatory action on polarised light. J. H. Long gives the specific rotation of casein when 5 grammes are dissolved in 100 c.c. of water with the number of c.c. of  $\frac{\text{N}}{10}$  alkali given as follows:—

NaOH,	. . . . .	22.5	95.2°
„	. . . . .	45.0	—103.5°
„	. . . . .	67.5	—107.6°
„	. . . . .	90	—111.8°
KOH,	. . . . .	45	—104.4
LiOH,	. . . . .	22.5	—94.8°
„	. . . . .	45	—100.8°
$\text{NH}_4\text{OH}$ ,	. . . . .	45	—97.8°

It is completely precipitated from milk by copper sulphate; if the solution be neutral, a definite compound containing about 1 per cent. of copper is obtained; basic compounds are probably obtained if the solution be alkaline. Mercury salts precipitate casein completely, even in acid solution; it is also precipitated by meta-phosphoric acid. Casein has probably a higher molecular weight than the other proteins existing in milk.

On boiling milk with the quantity of acid just sufficient to curdle, the whole of the casein is not precipitated; thus the author found that 2.91 per cent. was thrown down out of a total of 3.05 per cent.

Casein on treatment with strong sulphuric acid gives off carbon monoxide.

**Preparation of Casein.**—Casein is prepared from milk by diluting it to about five times its volume, and adding sufficient acetic acid to give 0.1 per cent. of the acid in the solution; the casein is precipitated, carrying down with it the fat: the precipitate is well washed by decantation some ten times, collected on a cloth filter, washed on the filter, and then dried, as far as possible, by pressure. This precipitate is dissolved in the least possible excess of ammonia, the solution allowed to stand for some time (to allow the fat to rise), then syphoned off and filtered, and the filtrate precipitated, as before, by acetic acid; the precipitate washed and redissolved in ammonia; and this treatment repeated three or four times. The casein is now rubbed up in a mortar with 80 per cent. alcohol, and the alcohol poured off; the treatment with alcohol is repeated several times, using, finally, absolute alcohol; it is then treated two or three times with ether which has been freshly distilled from some reagent which removes aldehydes (*e.g.*, casein, sodium phenyl-hydrazine-sulphonate) in the same manner, and then extracted for some hours in a Soxhlet extractor to remove the fat, and the ether evaporated off at as low a temperature as possible. This casein may, if a very pure product is required, be redissolved, reprecipitated, and the treatment with alcohol and ether repeated; the casein is finally dried at 100 to 105° C., and is then a white amorphous powder; if casein containing water is dried it forms horny masses.

**Products of Hydrolysis of Casein.**—The following composition is given by Chittenden and Painter to the products of hydrolysis of casein:—

CASEOSES PRODUCED BY THE ACTION OF PEPSIN.

	Casein.	Dys-Caseose.	Proto-Caseose.		Deutero-Caseoses.		
			Weak Pepsin.	Strong Pepsin.	Mixed.	α	β
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
C	53.30	51.16	52.89	54.40	51.79	52.20	47.61
H	7.07	7.07	7.10	7.04	7.05	6.94	6.76
N	15.91	15.31	15.94	15.84	16.00	15.95	15.95
S	0.82	0.72	0.95	0.95	1.17		
P	0.87	none					
O	22.03	25.74	...	...			

## CASEOSES PRODUCED BY THE ACTION OF TRYPSIN.

	Casein.	$\alpha$ -Deutero- caseose.	$\beta$ -Deutero- caseose.	Caseone.
	Per cent.	Per cent.	Per cent.	Per cent.
C	53·30	56·17	53·56	50·28
H	7·07	6·90	6·70	6·53
N	15·91	14·80	15·07	15·95
S	0·82	...	0·93	0·78 *

## CASEOSES PRODUCED BY THE ACTION OF ACIDS.

	Casein.	Dys-caseose.	Proto-caseose.	$\alpha$ -Deutero- caseose.	$\beta$ -Deutero- caseose.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
C	53·30	54·40	56·20	54·55	52·93
H	7·07	6·80	7·08	6·84	6·87
N	15·91	14·80	15·36	15·33	15·66

Siegfried has prepared a caseino-kyrine by restricted acid hydrolysis. It has the composition  $C_{23}H_{47}N_9O_8$ , and on further hydrolysis yields 1 molecule of arginine, 2 of lysine, and 1 of glutamic acid. Skraup and Witt, however, consider this to be a mixture.

## CASEOSES PRODUCED BY THE ACTION OF RENNET.

Hammarsten gives the following composition for the curd produced by rennet, and the caseose of the whey :—

	Curd.		Whey Caseose.	
	Found.	Calculated.	Found.	Calculated.
Carbon, . . . .	52·88	52·95	50·33	49·72
Hydrogen, . . . .	7·00	7·00	7·00	6·97
Nitrogen, . . . .	15·84	15·88	13·25	13·18
Phosphorus, . . . .	0·99†	0·98	..	..

From these figures the author has deduced formulæ, not as

\* The figure 0·68 occurs in the original; from the weighings given it is evident that 0·78 is the correct figure.

† Determined by the author.

absolutely correct, but probable near approximations. They are :—

Curd, . . . . .	$C_{140}H_{222}N_{36}SPO_{44}$
Whey caseose,	$C_{22}H_{37}N_5O_{10}$

The curd produced by rennet (calculated for 0.477 casein nitrogen, *cf.* p. 30) contains :—

		Calculated.
Nitrogen, . . . . .	0.411	0.419
CaO, . . . . .	0.119	0.119
P <sub>2</sub> O <sub>5</sub> , . . . . .	0.117	0.121
Ash, . . . . .	0.23	0.24

and the quantity of caseose nitrogen left in the whey is

	0.061	0.058
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The acidity of the whey was found to be 8.4 c.c. of normal alkali per litre less than that of the milk.

These figures are in accordance with the view that casein in milk,  $C_{162}H_{275}N_{41}SPO_{52}CaNa \cdot \frac{1}{2}(Ca_3P_2O_8)$ , is split by rennet into—

and  $C_{140}H_{220}N_{36}SPO_{44}Ca \cdot \frac{1}{2}(Ca_3P_2O_8)$  (curd)  
 $C_{22}H_{37}N_5O_{10}$  (whey caseose).

The whey caseose is free from tyrosine and tryptophane. When acted on by lactic acid the curd protein forms lactates, which have the property of becoming stringy when heated.

If calcium be removed from milk the action of rennet differs, and a whole series of caseoses is formed, and no curd is produced. Casein and its immediate derivatives appear to have the power of forming with tricalcium phosphate very insoluble salts.

### Reactions of the Caseoses.

**Dys-caseoses.**—These products in the pure state are soluble in water; they combine with calcium salts, especially phosphates, to form insoluble compounds.

The following reactions are given by dys-pepto-caseose; the other dys-caseoses behave similarly.

Acetic acid in moderate excess gives an insoluble white precipitate, soluble in large excess on heating.

Hydrochloric and sulphuric acid give precipitates, also soluble in large excess on heating. Even 0.2 per cent. hydrochloric acid produces complete precipitation.

Nitric acid gives a precipitate far more easily soluble in excess of acid. On warming, the solution turns yellow, and, with ammonia, gives the orange-yellow colour of the xantho-protein reaction.

With a little copper sulphate and an excess of caustic potash the violet colour of the biuret reaction is given.

Cupric sulphate and ferric chloride precipitate dys-caseose.

Ammonium sulphate added to saturation precipitates dys-caseose, but sodium chloride does not. Addition of acetic acid, however, to the salt-saturated fluid gives the usual precipitate of dys-caseose.

The insoluble compound with lime salts is dissolved with more or less readiness by an alkaline trypsin solution, giving finally a caseone, presumably trypto-caseone.

**Proto-caseoses.**—Proto-caseoses are soluble in water, and precipitated incompletely by the addition of acetic, hydrochloric, sulphuric, and nitric acids. They are soluble in 0.4 per cent. hydrochloric acid, but are precipitated by stronger solutions. The portion not precipitated by acetic acid gives a precipitate on saturation with salt solution and with potassium ferrocyanide.

Copper sulphate gives a heavy precipitate, as does ferric chloride, but the latter is soluble in excess of the reagent. They give the xantho-protein reaction with nitric acid.

Proto-caseoses are precipitated by saturation with sodium chloride.

**$\alpha$ -Deutero-caseoses** are soluble in water, not precipitated by acids nor by saturation of a neutral solution with sodium chloride; on adding acetic acid to the salt-saturated solution,  $\alpha$ -deutero-caseose is incompletely precipitated; it is precipitated by saturation with ammonium sulphate in the cold.

$\beta$ -deutero-caseose is precipitated by saturation of the solution with ammonium sulphate and boiling.

Cupric sulphate gives a precipitate with  $\alpha$ -deutero-caseose soluble in excess, but none with  $\beta$ -deutero-caseose.

Potassium ferrocyanide in acetic acid solution gives a precipitate with both deutero-caseoses.

**Caseone.**—Only the trypto-caseone has been prepared; it is not precipitated by acids; nor by saturation of its solution by sodium chloride; nor by ammonium sulphate, even on boiling; nor by zinc sulphate. Caseone and peptones generally are very hygroscopic. Caseone is dialysable and only precipitated by such reagents as tannin and phospho-tungstic acid.

All the caseoses and caseones give the biuret reaction with copper sulphate and caustic potash.

It must be remembered that the separation of the caseoses is by no means sharp; thus proto-caseose is not completely precipitated by sodium chloride and the residue is obtained with the  $\alpha$ -deutero-caseose.

Besides the above products another caseose, resembling proto-caseose, but soluble only in dilute acid and salt solutions, is also formed; this is called hetero-caseose and is precipitated by dialysis.

**Lactalbumin.**—This protein has the property characteristic of albumins of being coagulated by raising the temperature of its solution to 70° C.; the precipitation is never complete, since as much as 12 per cent. may be left in solution, according to Sebelein.

Lactalbumin, like other albumins, is not precipitated by saturating its solution with magnesium sulphate; but on the addition of acetic acid to the solution a precipitate of albumin is obtained, and this is redissolved on neutralisation of the acid. It can be obtained in a crystalline form by diluting the saturated magnesium sulphate solution with an equal bulk of water, adding acetic acid till permanently turbid, and setting aside. Gentle shaking assists the crystallisation. It is, like other albumins, precipitated by sodium sulphate added to saturation, and also by ammonium sulphate. It is also precipitated by tannin, phospho-tungstic acid, and other general reagents. The salts of albumin with copper, mercury, and lead are insoluble. Alcohol precipitates it and the precipitated albumin is soluble in water. It has a specific rotatory power  $[\alpha]_D$  of  $-67.5$ ? (*Béchamp*).

Lactalbumin has the following composition, according to Sebelein:—

Carbon, .	Per cent. 52.19	Sulphur, .	Per cent. 1.73
Hydrogen, .	7.18	Oxygen, .	23.13
Nitrogen,	15.77		

It differs from casein by containing no phosphorus and about twice as much sulphur. When boiled with an alkaline solution of lead acetate it gives a very strong sulphur reaction. There appears to be no difference in elementary composition between soluble and coagulated albumin. *Abderhalden* and *Hunter* state that the mixed coagulable proteins of milk contain 1.2 per cent. of glycine.

**Preparation of Lactalbumin.**—Milk is saturated with magnesium sulphate and filtered. To the clear filtrate is added as much acetic acid as will give  $\frac{1}{4}$  per cent. of acetic acid: lactalbumin is precipitated, and is filtered off. The precipitate, with the filter, is stirred up with water, and the acid neutralised: the lactalbumin dissolves. The solution is filtered, and reprecipitated by saturating with magnesium sulphate and adding  $\frac{1}{4}$  per cent. of acetic acid; this is repeated three or four times; the solution of lactalbumin is then dialysed to remove salts. The solution is precipitated by alcohol, the precipitate washed with alcohol and ether and, finally, dried at a low temperature. Lactalbumin, prepared in this way, is a white powder without taste, and completely soluble in water.

The albumoses are bodies analogous to the caseoses; they are



not, however, precipitated by acids, and are less readily precipitated by copper sulphate.

**Lacto - Globulin.**—This protein is coagulated by heat and precipitated by neutral sulphates, tannin, etc.; rennet does not coagulate it. It coagulates at  $72^{\circ}$  C. It only occurs in traces in milk, but in larger amounts in colostrum. It is not known whether it differs chemically from serum-globulin. Its chief characteristic is its solubility in sodium chloride solutions even when acidified.

**Storch's Mucoïd Protein.**—The following properties are given by Storch:—Washed with alcohol and afterwards with ether, and dried in air at the ordinary temperature, it forms a loose, fine, hygroscopic powder of a greyish-white colour. It is insoluble in dilute ammonia and acetic or hydrochloric acids; it swells considerably without dissolving in weak solutions of alkalis, and is only partly soluble in dilute potassium or sodium hydroxide. It gives the reactions of proteins—*i.e.*, red coloration with Millon's reagent, brown colour with iodine and yellow with nitric acid and ammonia (xantho-protein reaction). When heated with dilute hydrochloric acid it yields a substance which reduces Fehling's solution; the amount of copper reduced is 6.5 parts for each 100 parts of dry ash-free substance. It gives also the biuret reaction.

It contains 14.76 per cent. of nitrogen and 2.2 per cent. of sulphur, of which only a small portion is removed by boiling with alkalis.

**Preparation of Mucoïd Protein.**—(i.) The author has found the easiest method is to centrifuge sweet butter milk and wash the deposit several times with water made faintly alkaline with ammonia, the deposit being separated each time by centrifugal action. The mass is treated with strong alcohol, and afterwards with ether, and dried *in vacuo*.

(ii.) Storch has prepared it from butter, by melting 1 to 2 lbs. at a low temperature; the fat is carefully decanted; and the liquid rinsed twice with benzene, diluted with distilled water and mixed with one and a half times its volume of strong alcohol. The precipitate is washed with 60 per cent. alcohol and extracted with ether till all fat is removed, and air dried.

(iii.) Fresh cream (about 30 per cent. fat) is diluted with four times its volume of a 33 per cent. solution of cane sugar and placed in a large separating funnel; after a day's repose, the sugar solution is drawn off, and the remaining cream again mixed with four times its volume of the sugar solution; this process is repeated four times and the washed cream is shaken with an equal volume of strong alcohol, and twice as much ether and some benzene are added. A gelatinous precipitate separates

from the clear ethereal solution, which is separated by filtration, washed with strong alcohol and afterwards with ether, and dried in the air at the ordinary temperature. Storch found that if the cream was mixed with water at 35° C. and separated in a cream separator, and this process repeated several times, the protein could be prepared from the washed cream. This method was, however, more difficult than that involving the use of cane sugar solution.

The density of the mucoid substance containing 6.42 per cent. of mucoid protein and 1.03 per cent. ash was found to be 1.0228 at 15° C.

This substance appears to be identical with a product described some years ago as  $\beta$ -casein by Struve; he separated it from his  $\alpha$ -casein by dissolving in ammonia, when the  $\beta$ -casein was left; it was found in traces only in milk.

**Mineral Constituents.**—On burning milk a white ash is left: this contains the mineral constituents of milk, altered, however, to some extent by the oxidation of some of the compounds present in milk; thus the phosphorus and sulphur of the proteins give rise to phosphoric and sulphuric acids; and carbon dioxide is also formed by the oxidation of organic carbon. The ash does not truly represent the mineral constituents of milk.

The average composition of the ash of milk is—

	Per cent
Lime, . . . . .	20.27
Magnesia, . . . . .	2.80
Potash, . . . . .	28.71
Soda, . . . . .	6.67
Phosphoric acid,	29.33
Chlorine,	14.00
Carbon dioxide	0.97
Sulphuric acid, . . . . .	trace
Ferric oxide, &c., . . . . .	0.40
	—
	103.15
Less O = Cl, . . . . .	3.15
	—
	100.00

The amount of insoluble ash—*i.e.*, ash insoluble in hot water—amounts to about 0.52 per cent. of the milk; and the soluble ash to 0.23 per cent. The soluble ash consists mainly of the chlorides of the alkalis, with a little carbonate and a mere trace of phosphates.

The insoluble ash is mainly composed of double phosphates of the formula  $\text{CaKPO}_4$ , the lime being partially replaced by magnesia and the potash by soda; double carbonates of the formula  $\text{CaNa}_2(\text{CO}_3)_2$  also exist in traces: these compounds are insoluble in water, and this accounts for the fact that the insoluble ash is always higher than the sum of the calcium and magnesium phosphates.

An ash of this composition is only formed when the milk is homogeneous; if it is curdled, by natural souring or by the addition of acids, the precipitated lumps do not contain sufficient alkali metals to form these compounds, and much calcium and magnesium phosphates are formed; on dissolving in water, soluble alkaline phosphates go into solution, and calcium and magnesium phosphates, together with varying proportions of double phosphates, are left insoluble. Curdled milk gives the same total proportion of ash as fresh milk, but the soluble ash is higher and the insoluble ash lower.

About 8 per cent. of the phosphoric acid present in the ash is derived from the phosphorus of the casein; the traces of carbonic acid present are not true mineral constituents of the milk.

Deducting these, we have a considerable excess of bases over acids; in the milk these bases are combined partly with the proteins to form soluble salts, and partly with citric acid to form citrates.

Citric acid is contained in milk to the extent of 0·1 to 0·15 per cent.; its most characteristic salt is the calcium citrate, which is fairly soluble in cold water, but insoluble in boiling water. It is a tribasic acid, and forms three classes of salts.

Söldner deduces the following composition as most probable for the salts existing in milk:—

	Per cent.
Sodium chloride, NaCl, . . . . .	10·62
Potassium chloride, KCl, . . . . .	9·16
Mono-potassium phosphate, $\text{KH}_2\text{PO}_4$ , . . . . .	12·77
Di-potassium phosphate, $\text{K}_2\text{HPO}_4$ , . . . . .	9·22
Potassium citrate, $\text{K}_3(\text{C}_6\text{H}_5\text{O}_7)$ , . . . . .	5·47
Di-magnesium phosphate, $\text{Mg}_2\text{HPO}_4$ , . . . . .	3·71
Magnesium citrate, $\text{Mg}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ , . . . . .	4·05
Di-calcium phosphate, $\text{Ca}_2\text{HPO}_4$ , . . . . .	7·42
Tri-calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$ , . . . . .	8·90
Calcium citrate, $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ , . . . . .	23·55
Lime combined with protein, . . . . .	5·13
	100·00

The mineral salts, as stated above, would amount to 0·90 per cent., as against 0·75 per cent. of ash obtained.

According to Söldner 36 to 56 per cent. of the phosphoric acid and 53 to 72 per cent. of the lime are not in solution, but are in the colloidal form.

The following shows the distribution of the phosphoric acid of the milk according to the author's experiments:—

$\text{P}_2\text{O}_5$ as casein, combined with CaNa, . . . . .	0·0605 per cent.
$\text{P}_2\text{O}_5$ as $\text{Ca}_3(\text{PO}_4)_2$ , . . . . .	0·0625 "
$\text{P}_2\text{O}_5$ as $\text{R}_2\text{HPO}_4$ , . . . . .	0·077 "
$\text{P}_2\text{O}_5$ as $\text{RH}_2\text{PO}_4$ , . . . . .	0·020 "
	0·220 "
Total $\text{P}_2\text{O}_5$ , . . . . .	

The author's conclusions differ from those of Söldner, and are :—

(i.) One-third of the base with which casein is combined in milk is soda and not lime.

(ii.) Casein forms a molecular compound with calcium phosphate.

(iii.) The citrates are dibasic, and not tribasic.

**Other Constituents of Milk.**—Besides the constituents mentioned, minute traces of silica, iodine, fluorine, acetates, and thio-cyanates have been described.

None of the salts of milk require a detailed description. They, together with the acids and bases composing them, are described in any elementary book on chemistry.

Among the other substances present in traces in milk the following have been described :—Urea, hypoxanthine and other nitrogenous basic substances, a colouring-matter, odorous substances and alcohol (described by Béchamp, but certainly not ordinarily present).

**Lecithin**,  $C_{11}H_{20}O_2PN$ , exists in small quantities in milk: on saponification it gives glyceryl phosphoric acid, fatty acids and choline; it contains 3·84 per cent. of phosphorus, and gives 8·8 per cent. of  $P_2O_5$  on oxidation.

**The Gases of Milk.**—It is extremely probable that the gases of milk are derived from the air by absorption during and after milking. Oxygen, nitrogen (probably argon), and carbon dioxide are present in fresh milk. As the milk is kept the amount of oxygen decreases and that of the carbon dioxide increases; this is probably due to aerobic micro-organisms, which absorb the oxygen and give out carbon dioxide.

The gases of milk may also include products of decomposition; thus in decomposed milk, volatile sulphur compounds of evil odour are present. If such, as is probably the case in dirty surroundings, were present during milking they would be absorbed to some extent by the milk.

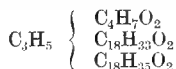
The gases have no practical importance.

Milk is sometimes charged with carbon dioxide under high pressure to form an effervescing drink. In this case, and in koumiss and kephir, products of fermentation of milk, the carbon dioxide is an important constituent.

### THE FAT OF MILK.

**Constitution.**—The fat in milk is found in the shape of small globules varying in size, according to Besana, Fleischmann, and other authorities, from 0·01 mm. to 0·0016 mm. in diameter. There is some probability that the total weight of globules of any size is equal to the total weight of globules of any other size.

The fat consists of a mixture of glycerides—*i.e.*, ethereal salts of glycerol. It appears most probable that there are three acid radicles in combination with each glycerol residue, thus—



which represents glyceryl butyro-oleo-stearate. This view has been formed from the following facts:—(1) Were the fat a mixture of glyceryl tributyrate with other glycerides, it would be possible to dissolve out the glyceryl tributyrate by means of alcohol, leaving nearly the whole of the other glycerides behind. This is not the case. The portion soluble in alcohol contains a notable quantity of the higher glycerides.

(2) If glyceryl tributyrate existed as such in milk fat it should be possible to distil it off under reduced pressure, but this cannot be done.

We know nothing of the way in which the fatty acids are combined with glycerol; it is convenient, however, to state the composition as if each glyceride existed separately.

**Composition.**—The average composition of the fat of milk appears to be, from the mean results obtained by different observers, as follows:—

Butyrin,	3.85	per cent.	yielding	3.43	% Fatty acids and	1.17	% Glycerol.
Caproin,	3.60	"	"	3.25	"	0.86	"
Caprylin,	0.55	"	"	0.51	"	0.10	"
Caprin,	1.9	"	"	1.77	"	0.31	"
Laurin,	7.4	"	"	6.94	"	1.07	"
Myristin,	20.2	"	"	19.14	"	2.53	"
Palmitin,	25.7	"	"	24.48	"	2.91	"
Stearin,	1.8	"	"	1.72	"	0.19	"
Olein, &c.,	35.0	"	"	33.60	"	3.39	"
Total,	100.00		Insoluble,	87.65	Total,	12.53	
			Total,	94.84			

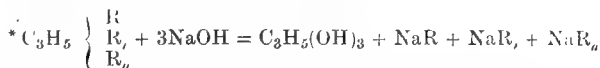
C. A. Browne states that 1.0 per cent. of dioxy-stearic acid occurs in butter, and 0.1 per cent. of unsaponifiable matter.

In this table, butyric, caproic, and caprylic acids have been classed as soluble in water, and the others insoluble; this is not, strictly speaking, correct, as capric and, probably, lauric acids are also slightly soluble; on the other hand, caprylic acid possesses so slight a solubility in water that it probably is not wholly dissolved.

The figure 87.65 per cent. is, however, a near approximation to the mean found for the insoluble fatty acids. The figure for the total amount of glycerol 12.53 also agrees with that found.

Besides the constituents enumerated above, there also exist in the fat of milk traces of cholesterol (which doubtless replaces a portion of the glycerol), lecithin, a colouring-matter, and possibly also a hydrocarbon.

**Saponification.**—On boiling with a solution of caustic alkali, the fat undergoes hydrolysis, thus—



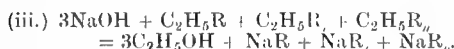
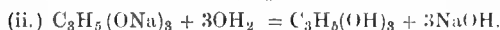
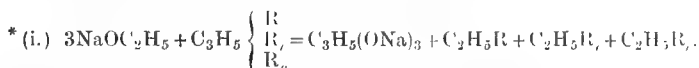
R, R', and R'' representing radicles of the fatty acids.

If the hydrolysis be carried out in presence of alcohol a portion of the caustic alkali is converted into an alkali ethoxide (alcoholate) thus—



This acts in a slightly different manner from the hydroxide, though the ultimate products of hydrolysis are identical.

The actions are probably as follows:—



In the first stage, sodium ethoxide and fat form sodium glyceroxide and ethyl salts (esters).

In the second, the glyceroxide is decomposed by the water present into glycerol and sodium hydroxide: while, in the third, the esters are hydrolysed by the hydroxide into alcohol and sodium salts of the fatty acids (soaps).

The action between the sodium hydroxide and the alcohol is never complete, and it is probable that the formation of esters is only partial: evidence of the formation of ethyl butyrate can be obtained by warming a little of the fat with alcoholic soda, when the characteristic pine-apple odour of ethyl butyrate is at once developed. By carefully avoiding any excess of alkali and distilling the ethyl butyrate as soon as possible, Wanklyn and Fox have succeeded in obtaining about 3 per cent. of volatile acid (probably chiefly butyric) in the form of ester.

It is probable that the equation (ii.) may not represent the way in which sodium glyceroxide acts on the substances present: a portion may follow this equation—



\* These reactions probably take place in stages, one acid radicle at a time being attacked.

The glyceroxide may act on alcohol forming ethoxide, instead of on water forming hydroxide.

Allen and Homfray have shown that by the action of a very small quantity of caustic soda on acetin (glyceryl tri-acetate), in the presence of alcohol, a very large proportion of ethyl acetate is formed, many molecules of ethyl acetate being produced by each molecule of sodium ethoxide; this can only be explained by the action shown in equation (iv.).

Duffy has shown that ethyl and amyl stearate may be produced from glyceryl stearate and sodium ethoxide and amoxide respectively.

The action of sodium ethoxide on milk fat has a practical bearing on butter analysis, owing to the volatility of ethyl butyrate, which, unless precautions are taken, is liable to cause loss of butyric acid on saponification.

From their sodium salts the acids may be set at liberty by the addition of a mineral acid.

**Properties.**—Milk fat is insoluble in water, but dissolves about 0·2 per cent. of this substance. It is not volatile, though when heated to 100° C. a loss of weight is noticed owing to the dissolved water being volatilised. On further heating at this temperature, in a current of hydrogen, no change is noticed; but if oxygen be allowed access a gradual increase of weight, due to oxidation, is found; if the heating be prolonged, say for a week, the weight again decreases, and profound changes, the nature of which has not been elucidated, take place.

**Solid and Liquid Portions.**—The fat of milk being an undoubted mixture has no sharply defined melting point. If rapidly cooled to a low temperature it becomes solid, and melts on warming at from 29·5 to 33° C. By slow cooling it does not solidify as a whole, but behaves as a solution of fat of a high melting point in fat of a low melting point.

The author obtained the following figures (Table II.) by allowing a sample to cool down gradually to about 25° C., and separating the liquid portion from the deposited solid:—

TABLE II.—PROPERTIES OF MILK FAT (*Richmond*).

	Original Fat.	Liquid Fat at 25° C.	Solid Fat.
Sp. gr. at 15·5°, .	...	0·922	...
Reichert-Wollny, .	26·1 c.c.	31·6 c.c.	19·9 c.c.
Iodine absorption, .	...	37·5	27·6
	Original Fat.	Liquid Fat at 17° C.	Liquid Fat at 0° C.
Reichert-Wollny, .	39·0 c.c.	41·3 c.c.	45·0 c.c.

Pizzi has also recorded the following experiments (Table III.):—

TABLE III.—PROPERTIES OF MILK FAT (*Pizzi*).

	Original	Liquid at 26.2°	Liquid at 21.2°	Liquid at 17.0°	Liquid at 12.4°	Liquid at 11.0°	Liquid at 6.5°	Solid at 26.2°
Sp. gr. at 26.2°	0.908	0.912	0.916	0.923	0.927	...	...	...
Melting point,	36°	...	...	...	...	...	...	44°
Solidifying „	25°	...	...	...	...	...	...	35°
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Reichert-Wollny, } Iodine absorption, }	26.96 32.96	28.05 34.50	29.81 38.80	31.57 40.75	30.36 42.75	32.34 52.25	34.10 58.50	19.91 25.38

	Solid Fat deposited from Liquid at 26.2° by cooling to 21.2°.	Solid Fat deposited from Liquid at 21.2° by cooling to 17.0°.	Solid Fat deposited from Liquid at 17.0° by cooling to 12.4°.	Solid Fat deposited from Liquid at 12.4° by cooling to 11.0°.	Solid Fat deposited from Liquid at 11.0° by cooling to 6.5°.
Melting point,	43.5	31	18	17	10.5
Solidifying „	21	16.5	13	14	8.5°
Reichert-Wollny, } Iodine absorption, }	29.13 c.c. 28.13	27.59 c.c. 38.70	29.70 c.c. 41.25	29.37 c.c. 45.50	31.02 c.c. 50.55

The above figures all show that the liquid fat is somewhat richer in both volatile and unsaturated acids than the original fat, while the solid portion is correspondingly poorer in these constituents. There is, however, no sharp separation.

The density of the fat of milk is as follows:—

Temperature.	Mean.	Limits.	Authorities.
15°	0.9307 (solid)	...	Fleischmann.
15°			
37.8°	0.9118 (liquid)	0.9094—0.9140	{ Bell, Allen, Muter, &c.
37.8°			
39.5°	0.9113 ( „ )	0.9104—0.9117	Author.
39.5°			
100° (in glass)	0.8667 ( „ )	0.8650 - 0.8685	Numerous.
15.5°			

The last figure is not a true density, as it is not corrected for



the expansion of glass between  $15.5^{\circ}$  and  $100^{\circ}$ ; it has been assumed that the volume of the glass instrument used to determine the density is the same at  $100^{\circ}$  as at  $15.5^{\circ}$ . The error has no practical importance when the figures thus obtained from different samples are compared, as they are all subject to the same correction.

From the average specific gravities given above the author has calculated the true specific gravities and specific volumes; these are—

Temperature	Specific Gravity.	Specific Volume.	Calculation.
$\frac{15^{\circ}}{4^{\circ}}$	0.9300	1.0753	1.0844
$\frac{37.8^{\circ}}{4^{\circ}}$	0.9057	1.1041	1.1041
$\frac{39.5^{\circ}}{4^{\circ}}$	0.9045	1.1056	1.1056
$\frac{100^{\circ}}{4^{\circ}}$	0.8637	1.1578	1.1578

The figures calculated are based on the assumption that the expansion is regular between  $15^{\circ}$  and  $100^{\circ}$ , and that it averages 0.000863 for each degree Centigrade; on this assumption the specific volume of liquid fat at  $15^{\circ}$  is higher, and the specific gravity is lower than that of the solid.

The specific gravity of liquid fat at  $\frac{15.5^{\circ}}{15.5^{\circ}}$  (calculated) is 0.922.

It is interesting to note that the specific gravity of the liquid fat obtained by the author (*above*) had a specific gravity of 0.922. The specific gravities of the liquid fats obtained by Pizzi appear to have been taken at the temperature at which the fat was separated, and on calculating to  $15^{\circ}$  have values from 0.921 to 0.925.

On the whole the evidence available appears to show that the specific gravity of solid fat is greater than that of liquid fat at the same temperature.

In connection with this, it may be mentioned that E. W. T. Jones has shown that the specific gravity of other fats is greater when partially solidified at  $37.8^{\circ}$  than when liquid at this temperature.

The index of refraction of the fat of milk averages 1.4566 at  $35^{\circ}$ , and the limits observed have been 1.4550 to 1.4586.

Stohmann has determined the heat of combustion of butter fat as 9.231 calories per gramme, while Atwater found from

9·320 to 9·362 calories in three samples of butter, which appear from the analytical results of Schweinitz and Emery to have been of doubtful purity.

The fat of milk is soluble in all hydrocarbons which are liquid at the ordinary temperature, in their halogen derivatives, in ether, carbon bisulphide, nitro-benzene, and acetone. It is slightly soluble in alcohol and to a considerable extent in amyl alcohol when cold, but in all proportions when hot. Glycerol, when hot, dissolves it to a very small extent. It appears to mix in all proportions with esters. Fatty acids have a limited solvent effect, those of higher molecular weight dissolving more than the lower homologues. Phenol also dissolves it to some extent. On cooling a strong solution of the fat in any solvent, the portion deposited has not the same composition as the original fat, but is of the same nature as the solid portion obtained by slow cooling of the melted fat.

The molecular weight of the fat has been determined by Garelli and Carcano by Raoult's cryoscopic method in benzene solution to be from 696 to 716. That calculated from the amount of alkali necessary for saponification is 720 to 740.

#### PRODUCTS OF HYDROLYSIS.

**Glycerol.**—This is the simplest tri-hydric alcohol and has

the constitution—
$$\begin{array}{c} \text{CH}_2\text{OH} \\ | \\ \text{CHOH} \\ | \\ \text{CH}_2\text{OH} \end{array}$$
 It was discovered by Scheele in

1779 in olive oil, and was first recognised in butter in 1784.

The anhydrous product is a thick syrupy liquid, which can be obtained in crystals by cooling to a low temperature; the melting point of the solid glycerol is given as 17° C. by Henniger and 20° C. by Nitsche. It boils at 290° C. under the ordinary pressure, but undergoes slight decomposition; it can be readily distilled without change under reduced pressure. It is not volatile with steam. An aqueous solution containing less than 75 per cent. glycerol can be boiled without loss; but from solutions containing more than 75 per cent. glycerol it is somewhat volatile (*Hehner*). Anhydrous glycerol volatilises slowly at 100° C. When heated above 150° C. it is inflammable.

The density at  $\frac{15\cdot5^\circ}{15\cdot5^\circ}$  is 1·2675; the refractive index at the same temperature is 1·4748.

When heated to its boiling point, especially if not pure, various

products, of which acrolein ( $C_3H_4O$ ) is the most important, are given off. Di- and tri-glyceric alcohols are also formed.

By the action of acid oxidising agents—*e.g.*, chromic acid and potassium permanganate in acid solution—it is wholly converted into carbon dioxide and water. Alkaline permanganate converts it quantitatively into oxalic acid. By the action of bromine in the cold glycerose is formed, which is an aldehyde; by further oxidation with bromine at a high temperature, or by boiling with

dilute nitric acid, glyceric acid  $\begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ COOH \end{array}$  is produced.

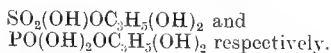
Tartronic acid  $\begin{array}{c} CHOH \\ | \\ COOH \end{array}$  is, under certain conditions, also

produced together with glycolic, glyoxylic, oxalic, and formic acids.

Glyceric acid appears to have a constitution similar to lactic acid (*q.v.*), from which it differs only by containing the group  $CH_2(OH)$  in place of  $CH_3$ . It forms a lactone and a dehydro-derivative, and contains an asymmetric carbon atom.

By the action of fuming nitric acid (mixed with sulphuric acid to preserve its strength) glyceryl tri-nitrate (nitro-glycerine), usually mixed with small quantities of di- and mono-nitrates, is formed. This is a heavy explosive liquid of specific gravity 1.6 and of limited solubility in water. This compound is best known as a powerful explosive.

With strong sulphuric and phosphoric acids glyceryl mono-hydrogen sulphate and mono-glyceryl di-hydrogen phosphate are produced. These have the composition—

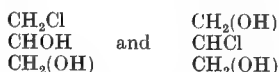


When glycerol is heated with alkalis above  $250^\circ C.$  a variety of products are formed; among these are formic, acetic, acrylic, and lactic acids. The oxygen of the air seems to play an important part in these changes, as all the products contain more oxygen and less hydrogen. No change takes place below  $250^\circ$ , especially in the absence of air.

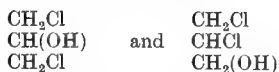
Several glyceroxides are known—*i.e.*, bodies in which the hydrogen of the hydroxyl groups is replaced by metals. By heating lead oxide with glycerol, lead glyceroxide is formed. Glycerol also dissolves lead oxide.

By treating glycerol with sodium dissolved in alcohol a crystalline deposit of the composition  $C_3H_7NaO_3$ ,  $C_3H_6O$  is formed, which, when heated at  $100^\circ$  in a current of hydrogen, loses alcohol. It is a white amorphous powder, very hygroscopic and immediately decomposed by water. Calcium, strontium, and barium hydroxides are freely soluble in glycerol, and form glyceroxides, which may be dissolved in water; the aqueous solutions do not give precipitates with carbonic anhydride.

By the action of hydrochloric and hydrobromic acids, mono- and dichlor-hydrin and mono- and dibrom-hydrin are produced. There are two possible mono-chlor- and mono-brom-hydrins: thus—



and similarly two di-hydrins: thus—



Both compounds are simultaneously produced.

By the action of alkalies on both the dichlor-hydrins, epichlor-hydrin  $\text{CH} \begin{array}{l} \text{CH}_2\text{Cl} \\ \text{CH}_2 \end{array} > \text{O}$  is produced.

A mono-iod-hydrin also appears to be produced by the action of hydriodic acid.

The penta-chloride and penta-bromide of phosphorus produce trichlor- and tribrom-hydrins, which are the  $\alpha$ - $\beta$ - $\gamma$ -trichlor- and  $\alpha$ - $\beta$ - $\gamma$ -tribrom-derivatives of propane.

Phosphorus tri-iodide or concentrated hydriodic acid produce a mixture of allyl and iso-propyl iodides with propylene.

By the action of dehydrating agents acrolein, acrylic aldehyde,  $C_3H_4O$  is formed.

Glycerol is very soluble in water and alcohol, but insoluble in ether and chloroform.

**Cholesterol**,  $C_{27}H_{43}OH$ , is a mono-hydric alcohol, containing one unsaturated bond. This is shown by its combination with two atoms of bromine to form dibrom-cholesterol,  $C_{27}H_{43}Br_2OH$ , unchanged. It is lævo-rotatory, having an  $[\alpha]_D - 36.6$  (*Dragendorff*) or  $-31.6$  (*Lindenmeyr*).

It is easily soluble in hot alcohol, crystallising out on cooling in characteristic plates; occasionally from alcohol and more often from ether it is obtained in needles.

Cholesteryl acetate, melting point  $92^\circ$ , is obtained by the action of acetic anhydride on cholesterol. The benzoate is obtained by

heating cholesterol with benzoic acid under pressure, and melts at  $150^{\circ}$  to  $151^{\circ}$  C.

The most characteristic reaction is the following, due to Sal-kowski:—About 10 milligrammes of cholesterol are dissolved in 2 c.c. of chloroform, and the solution shaken with an equal measure of strong sulphuric acid in a corked test tube. The chloroform layer becomes blood-red, passing to cherry-red and purple, the last colour being permanent for several days. The sulphuric acid acquires a well-marked green fluorescence. If the test tube be not corked, or if the chloroform solution be poured into a basin, the colour changes to blue, green, and, finally, yellow, probably due to moisture. On addition of water the solution becomes paler, then blue, and, finally, nearly colourless, while showing a fine green fluorescence.

By cautiously heating cholesterol with a drop of strong nitric acid and adding ammonia before the product has cooled completely, a yellowish-red coloration is produced.

If a mixture of 3 measures of concentrated hydrochloric acid and 1 of a solution of ferric chloride be evaporated with a little cholesterol, a reddish-violet coloration changing to blue is produced. By substituting sulphuric acid for hydrochloric acid, a carmine colour is produced, passing gradually to violet, which is changed to scarlet on treatment with ammonia.

### Fatty Acids.

**Acids of the Series,  $C_nH_{2n+1}COOH$ .**—As far as is known, only the normal acids of this series, in which  $n$  is an odd number, occur in the fat of milk.

**Butyric Acid,  $CH_3CH_2CH_2COOH$ .**—Grunzweig has proved that the butyric acid of the fat of milk is normal. This acid is a liquid with a characteristic smell, which is specially developed in dilute solution; the anhydrous acid has a sharp acid smell, the characteristic smell being hardly perceptible.

The acid solidifies at  $-19^{\circ}$  C. The solidified acid melts at  $-2^{\circ}$  to  $+2^{\circ}$  C. according to Linnemann, and at  $-4.5^{\circ}$  to  $-2^{\circ}$  C. according to Zander. The boiling point is variously stated according to different authorities. Thus—

Linnemann gives . . . . .	162.3° C.
Lieben and Rossi, . . . . .	163.2°
Kahlbaum, . . . . .	161.5°
Bruhl, . . . . .	161.5°-162.5°
Zander, . . . . .	162.3°
The author finds . . . . .	161.5°-162.5°

The density is given as 0.9587 at  $\frac{20^{\circ}}{4^{\circ}}$  by Bruhl, 0.9746 at  $0^{\circ}$  by Zander, and 0.9886 at  $0^{\circ}$  by Linnemann.

It is very difficult to prepare the anhydrous acid by distillation alone, the last traces of water being retained with great obstinacy; dehydrating agents remove this water, and the acid is somewhat hygroscopic. It is soluble in all proportions in water, but is separated as an oily layer on saturating the solution with calcium chloride. It is extracted from aqueous solution by ether.

From dilute solutions it distils 2.1 times as fast as water—*i.e.*, the vapour arising from a dilute solution contains 2.1 times the proportion of butyric acid contained in the solution. Its solubility in the mixture of higher fatty acids of milk fat is very small.

By the action of strong chromic acid at the boiling point it is oxidised to a mixture of carbon dioxide and water, but dilute solutions are unaffected. Alkaline permanganate oxidises it to carbon dioxide; Johnstone states that oxalic acid is formed from butyric acid by the action of alkaline permanganate, but other observers are unanimous in denying this.

The salts of butyric acid are all soluble in water. When ignited they leave a residue of the carbonate of the metal (except the silver and mercury salts, which leave metallic silver and no residue, respectively). The calcium salt has the following solubility:—

100 parts of water at 0° C. dissolve	19.4 parts.
"    "    20°    "    "	17.6    "
"    "    60°-85°    "    "	15.0    "
"    "    100°    "    "	15.8    "

A cold saturated solution is precipitated by heat. It crystallises in rhombic needles from cold solutions, and in rhombic prisms from hot solutions.

The barium salt is much used for determining the molecular weight of the acid; it cannot be dried at 100° C. without slight loss of butyric acid, but is quite permanent at 90° C. One thousand parts of absolute alcohol dissolve 11.7 parts at 30° and 2.45 parts at 14° according to Luck, who has used this method of separating it from barium formate, acetate, etc.

Silver butyrate crystallises by cooling a hot solution in needles, but by spontaneous evaporation in monoclinic prisms. 100 parts of water at 16° dissolve 0.413 part.

Both the acid and calcium salt form molecular compounds with calcium chloride.

Butyric acid occurs in the free state in perspiration and as ethyl salt in the oils of *Heracleum giganteum* and *H. spondylium*, hexyl butyrate being also present in the latter; the oil from the seeds of the parsnip (*Pastinaca sativa*) consists chiefly of octyl

butyrate. Ethyl butyrate is a volatile liquid of a smell recalling the odour of pine apples.

**Caproic Acid**,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$ .—Kœfoed has proved that the caproic acid of the fat of milk is normal. This acid is an oily liquid with an unpleasant goat-like smell. It boils at  $205^\circ\text{C}$ ., solidifies at  $-18^\circ\text{C}$ ., and melts at  $-1.5^\circ$ . Its density at  $0^\circ$  is 0.9446 according to Zander.

It hardly mixes with water, is extracted by ether from an aqueous solution, and possesses considerable solubility in the mixed higher fatty acids of milk fat. From a dilute aqueous solution it distils four times as fast as water.

The calcium salt differs from calcium butyrate by increasing in solubility on heating; 100 parts of water dissolve at  $11^\circ$  to  $12^\circ$  2.36 parts, at  $17.5^\circ$  2.58 parts, and at  $18.5^\circ$  2.71 parts of calcium caproate. It crystallises in needles. The barium salt dissolves in 100 parts of water to the extent of 12 parts at  $11^\circ$  to  $12^\circ$ , and the solubility decreases on heating.

**Caprylic Acid**,  $\text{C}_7\text{H}_{15}\text{COOH}$ .—This acid crystallises in plates or needles melting at  $16.5^\circ\text{C}$ ., and boils at about  $236^\circ\text{C}$ . It has a faint unpleasant odour of sweat, and a sharp rancid taste; it is difficultly soluble even in hot water, from which it crystallises in plates. From dilute solutions it distils eight times as fast as water.

Barium caprylate crystallises in anhydrous plates, and is soluble to the extent of 6 parts in 100 parts of water at  $20^\circ\text{C}$ . The calcium salt crystallises in long thin needles, and is less soluble than the barium salt—0.6 part per 100.

**Capric Acid**,  $\text{C}_9\text{H}_{19}\text{COOH}$ .—This acid has a faint goat-like odour, and is only very slightly soluble in water. It crystallises in brilliant plates, melting at  $30^\circ\text{C}$ .; it boils at  $268^\circ$  to  $270^\circ$ . The barium and calcium salts are nearly insoluble in water, even on boiling, and the salts of the alkalies are the only ones appreciably soluble.

**Lauric Acid**,  $\text{C}_{11}\text{H}_{23}\text{COOH}$ .—The acid is solid at ordinary temperatures, and is not soluble to any extent in water; it passes over to a very appreciable extent when distilled with steam. It crystallises from alcohol in needles, melting at  $43.6^\circ\text{C}$ . It cannot be distilled without decomposition at the atmospheric pressure, but at 100 mm. it has a boiling point of  $225^\circ\text{C}$ . The salts of the alkali metals yielded by the acids previously described are soluble in strong salt solution; the laurates of sodium and potassium are, however, precipitated by strong sodium chloride solutions, but not by weaker ones. Lauric acid is a leading constituent of cocoa-nut and palm-nut oils.

**Myristic Acid**,  $\text{C}_{13}\text{H}_{27}\text{COOH}$ .—This acid crystallises in laminae melting at  $53.8^\circ\text{C}$ . and boils at  $250.5^\circ$  under 100 mm.

pressure; it cannot be distilled alone. The acid is insoluble in water and its salts of the alkali metals are precipitated by salt.

**Palmitic Acid**,  $C_{15}H_{31}COOH$ .—The acid is quite insoluble in water; it crystallises from alcohol in tufts of finely crystallised needles; and the melted acid solidifies on cooling to a pearly crystalline mass. The melting point is  $62^{\circ}C.$ , and it boils under 100 mm. pressure at  $271.5^{\circ}C.$ ; it cannot be distilled under atmospheric pressure without decomposition.

According to Hehner and Mitchell a saturated solution of palmitic acid in alcohol of specific gravity 0.8183 contains from 1.32 to 1.03 grammes per 100 c.c. at  $0^{\circ}C.$  100 parts of absolute alcohol at  $19.5^{\circ}$  dissolve 9.32 parts; it is, however, readily soluble in boiling alcohol and crystallises out on cooling.

**Stearic Acid**,  $C_{17}H_{35}COOH$ .—This acid is quite insoluble in water; it crystallises from alcohol in white, nacreous laminae, melting at  $69.2^{\circ}$  (*Heintz*) or  $68.5^{\circ}$  (*Hehner and Mitchell*) to a colourless liquid, which on cooling solidifies to a crystalline whitish mass. Under 100 mm. pressure it boils at  $291^{\circ}C.$  It cannot be distilled under the atmospheric pressure without decomposition.

Hehner and Mitchell have shown that at  $0^{\circ}C.$  a saturated solution in alcohol of 0.8183 specific gravity contains from 0.142 to 0.158 gramme per 100 c.c. Absolute alcohol dissolves about 2.5 grammes per 100 c.c.

The salts of stearic acid are, with the exception of those of the alkali metals (soaps), insoluble in water and almost insoluble in alcohol. The salts of palmitic acid resemble them very much. The most marked difference between these two acids is the difference of solubility of the magnesium salt; that of stearic acid is practically insoluble in cold alcohol, while that of palmitic acid possesses a slight, but appreciable, solubility; the presence of magnesium palmitate causes, however, appreciable solubility of magnesium stearate.

All salts of stearic acid (and palmitic acid) are partly decomposed by water into basic and acid salts; the salts of the alkali metals (soaps) cannot be dissolved without becoming appreciably alkaline. They are, however, soluble in hot alcohol without decomposition, forming solutions which gelatinise on cooling.

Soaps of stearic and palmitic acids are quite insoluble in 12 per cent. solution of sodium chloride.

**General Properties of Acids of the Series**,  $C_nH_{2n+1}COOH$ .—The following table (IV.) gives a summary of the leading properties of these acids:—



TABLE IV.—PROPERTIES OF THE ACIDS OF SERIES  
 $C_nH_{2n-1}COOH.$ 

Acid.	Mol. Wt.	Sp. Gr.	B. P.	M. P.	Solubility of Calcium Salt.	Rate of Distillation Water=1.
Butyric,	88	0.9746 at 0°	162°	-2°	17.6 at 20°	2.1
Caproic,	116	0.9446 at 0°	205°	-1.5°	2.7 at 18.5°	4
Caprylic,	144	0.9270 at 0°	236°	16.5°	0.6 at 20°	8
Capric,	172	0.893 at 30°	269°	30°	0.1 at 20°	?
			at 100 mm.			
Lauric,	200	0.875 at 43.6°	225°	43.6°	0.039 at 15°	?
Myristic,	228	0.8622 at 53.8°	250.5°	53.8°	insoluble	..
Palmitic	256	0.8527 at 62°	271.5°	62°	"	..
				68.5°	"	..
Stearic,	284	0.8454 at 71°	291°	to	"	..
				69.2°	"	..

Acid.	Solubility in Water.	At 0°. Solubility in Alcohol Sp. Gr. 0.8183.	Viscosity. Dyne per sq. cm. at 20°.
Butyric,	all proportions	all proportions	0.1634
Caproic,	soluble	"	0.3263
Caprylic,	0.25 % at 100°	"	0.5860
Capric,	0.1	very soluble	...
Lauric,	very slight	"	...
Myristic,	insoluble	soluble	...
Palmitic.	"	1.2 %	...
Stearic,	"	0.15 %	...

None of the acids of this series absorb iodine or bromine, as they are saturated compounds, and are not appreciably attacked by strong sulphuric acid or fused alkalis; the lead, copper, and zinc salts of the lower members of the series (up to lauric) are soluble in ether, but lead, copper, and zinc myristate, palmitate, and stearate are not very soluble in this menstruum.

**Acids of the Series,  $C_nH_{2n-1}COOH$ —Oleic Acid,  $C_{17}H_{33}.COOH$ ,**  
 —This acid is probably a constituent of butter; it is extraordinarily difficult to prepare it in the state of purity, as it is altered by exposure to the air, and no well-defined stable compound is known. It is extremely doubtful whether it has ever been isolated; the formula given for the acid is to some extent a matter of conjecture, as there is great probability that the analyses from which it was deduced were made on impure

products. For the same reason there is some doubt as to its properties.

The following properties are assigned to oleic acid :—A colourless liquid free from smell, which moistens the skin, solidifies at 4° C., and melts at 14° C. Specific gravity at 14° C. 0·898, and at 100° C. 0·876. It cannot be distilled under atmospheric pressure, but boils under 100 mm. pressure at 286° C. It can be easily distilled below 270° in a current of superheated steam.

Oleic acid is insoluble in water, but very soluble in alcohol even if considerably diluted. To a solution of 1 c.c. of oleic acid in 95 per cent. alcohol 2·2 c.c. of a mixture of equal parts of acetic acid and water can be added without causing precipitation; further quantities, however, throw the oleic acid out of solution.

On exposure to the air it turns yellowish, and becomes rancid; it then reddens blue litmus paper, while pure oleic acid is said not to do so.

By the oxidation of oleic acid dioxystearic acid is formed.

By the action of bromine di-brom-stearic acid is formed: this is a heavy yellow oil, which has not been crystallised. By reduction with zinc and hydrochloric acid oleic acid is again formed. Oleic acid also absorbs iodine from Hubl's reagent, and is said to form chlor-iodo-stearic acid.

Strong sulphuric acid acts on oleic acid, forming stearo-sulphonic acid or sulpho-stearic acid; on boiling with water, sulphuric acid is split off and hydroxystearic acid is formed, with other products.

When oxidised by alkaline potassium permanganate di-oxy-stearic acid is formed.

The salts of oleic acid behave with water in much the same way as the salts of stearic and palmitic acids. All the oleates are soluble in alcohol, and those of copper, lead, and zinc are soluble in ether.

By the action of fused alkalis oleic acid is split up into salts of acetic and palmitic acids.

Nitrous fumes act on oleic acid in a characteristic manner; the liquid oleic acid is transformed into the isometric solid elaidic acid melting at 45° and boiling at 288° C. at 100 mm. pressure.

Baruch has proposed the following formulæ for oleic and elaidic acids :—



**Acids of the Series.**  $\text{C}_n\text{H}_{2n-3}\text{COOH}$  — **Linolic Acid**,  $\text{C}_{17}\text{H}_{31}\text{COOH}$ .—It is not known whether this acid exists normally in butter fat; if so, the proportion is probably not large.

It, however, is present in many vegetable oils, and will thus be a constituent of margarine.

Linolic acid is an oily substance of slight yellow colour, having a faint acid reaction; it dissolves readily in alcohol and ether. It remains fluid at low temperatures.

The salts of linolic acid resemble those of oleic acid, but are more soluble in alcohol and ether. Nitrous acid does not produce a solid acid. Both the acid and its salts readily absorb oxygen from the air, and form resinous substances.

Linolic acid absorbs 4 atoms of bromine, forming tetra-brom-stearic acid, which is a crystalline substance melting at  $114^{\circ}$  to  $115^{\circ}$ ; from this linolic acid can be prepared by reduction with zinc in a solution of hydrochloric acid in alcohol.

**Acids of the Series,  $C_nH_{2n-5}COOH$ —Linolenic Acid.**—This occurs in vegetable oils. It is a liquid, even at very low temperatures, and has a fishy odour. It forms a hexa-brom-compound melting at  $177^{\circ}C$ .

**Comparison of the Acids of the four Series.**—The following table (V.) will show the main differences between stearic, oleic, linolic, and linolenic acids, the corresponding members of the four series:—

TABLE V.—COMPARISON OF THE FOUR TYPICAL FATTY ACIDS.

Acid.	Condit.on.	Melting Point.	Bromine Compound.	Melting Point.	Behaviour with Nitrous Acid.
Stearic, Oleic,	Solid Liquid	$68.5^{\circ}$ $14^{\circ}$	None 2 Br.	... Liquid	No action Solid elaidic acid
Linolic, Linolenic,	,, ,,	Below $-18^{\circ}$ Very low	4 Br. 6 Br.	$114^{\circ} - 115^{\circ}$ $177^{\circ}$	Liquid product ,, ,,

Acid.	Behaviour with Sulphuric Acid.	Product formed by Alkaline Permanganate.	Melting Point.	Character of Product.
Stearic,	No action	No characteristic product	...	...
Oleic,	Sulphonic acid formed	Dihydroxystearic acid	$134^{\circ}$	Insoluble in cold water. Very little soluble in ether.
Linolic,	Great action	Sativic acid	$172^{\circ}$	Soluble in hot water. Insoluble in ether.
Linolenic,	,, ,,	Linusic acid	$204^{\circ}$	More soluble in hot water than sativic acid. Insoluble in ether.

**Rancidity—Products of Decomposition.**—But little is known of the real nature of the changes which take place when butter becomes rancid. The following statement will give an idea of the probable nature of the changes :—

The first action seems to be hydrolysis of the fat, splitting it up into fatty acids and glycerol; the latter, perhaps, is not liberated as such, but is oxidised, yielding aldehydes and acids soluble in water, but not so volatile as the soluble acids of butter; the volatile acids are liberated, and the smell of these can be detected in rancid butter. The odoriferous principle is destroyed. The unsaturated fatty acids are oxidised to form hydroxy acids, which are perhaps slightly soluble in water, but not volatile; the total capacity for combining with bromine is reduced by this cause. It is probable that the fatty acids of the series,  $C_nH_{2n+1}COOH$ , are but slightly affected.

The effect of rancidity is—

- (1) To diminish the glycerol produced on saponification.
- (2) To increase the soluble acids.
- (3) To increase slightly the volatile acids.
- (4) To decrease the insoluble acids.
- (5) To increase the total molecular proportion of acids.
- (6) To greatly increase the free acids.
- (7) To diminish the unsaturated acids by
- (8) The formation of hydroxy acids.

If the change takes place in the presence of water, some of the products are soluble therein; hence the fat separated from the water will not have the same characters as fat which has become rancid alone. If freely exposed to the air, some of the products may be volatile.

## CHAPTER II.

## ANALYSIS OF MILK.

CONTENTS.—Specific Gravity of Milk—Estimation of Total Solids—of Ash—of Citric Acid—of Milk-Sugar—of Cane-Sugar—of Starch—of Fat and Cream—of Proteins—of Total Acidity—Analysis of Milk Products.

**The Specific Gravity of Milk—Modes of Expression.—**

Specific gravity is the weight of a unit of volume. The unit of volume is a cubic centimetre, the unit of weight one gramme, and the specific gravity of any substance is the weight of one cubic centimetre in grammes. As one cubic centimetre of water at 4° C. weighs one gramme, the specific gravity of water at 4° C. is exactly 1; at temperatures higher and lower than 4° C. water expands, and therefore has a specific gravity less than 1. Table VI. gives the specific gravity of water at different temperatures:—

TABLE VI.—SPECIFIC GRAVITY OF WATER.

Temperature.	Specific Gravity.	Temperature.	Specific Gravity.
0° C.	0·99988	40°	0·99236
4°	1·00000	50°	0·98817
10°	0·99974	60°	0·98334
15·55° (60° F.)	0·99908	70°	0·97789
20°	0·99827	80°	0·97190
30°	0·99577	90°	0·96549
37·78° (100° F.)	0·99313	100°	0·95856

As at 4° C. one cubic centimetre of water weighs one gramme, a practical method of ascertaining the specific gravity at this temperature is to take the weight of any volume of the liquid and to compare it with the weight of an equal bulk of water. To ascertain the specific gravity at any other temperature—say, for example, 30° C.—the weight of any volume of liquid is ascertained and compared with the weight of water at that temperature

divided by the specific gravity at that temperature—in the case taken 0.99577.

The following formula may be used for determining specific gravity:—

$$\text{Sp. gr.} = \frac{\text{wt. of a known vol. of liquid} \times \text{sp. gr. of water at same temp.}}{\text{wt. of same volume of water at same temperature.}}$$

In practice it is customary to assume that water at 15.55° C. (60° F.) has the specific gravity 1.

Thus, to ascertain the specific gravity at 15.55° C. (60° F.) it is customary to weigh a known volume of liquid, and to compare it with the weight of an equal volume of water at that temperature. All specific gravities in this volume are stated in this way unless otherwise mentioned. In order to avoid confusion the symbol specific gravity at  $\frac{15.55^\circ}{15.55^\circ}$  is often used to express this mode of expression. This means that the weight of a volume of liquid at 15.55° is compared with the weight of an equal volume of water at 15.55°.

Similarly, the expression specific gravity at  $\frac{15.55^\circ}{4^\circ}$  or  $\frac{20^\circ}{4^\circ}$  is used to express the true specific gravities at 15.55° and 20° respectively.

It is occasionally convenient to compare the weight of a liquid at some other temperature with the weight of water at the same temperature; thus the specific gravity of fats is taken sometimes at 100° C., and the expression specific gravity at  $\frac{100^\circ}{100^\circ}$  is used to express the value obtained by dividing the weight of a volume of fat at 100° C. by the weight of an equal volume of water at 100° C.

If we ascertain the weight of water held by a certain vessel at a definite temperature, we can ascertain the specific gravity of any liquid by filling it with the liquid at the same temperature and weighing it. If we fill the vessel with the liquid at any other temperature, the volume contained will not be the same as that of the water, owing to the expansion of the vessel itself altering the capacity. Nevertheless, specific gravity is frequently ascertained on the assumption that the vessel does not alter in capacity by change of temperature. As the vessel is usually made of glass, this mode of expression of specific gravity may be termed the “apparent specific gravity in glass at  $\frac{20^\circ}{15.55^\circ}$ ” (or whatever the temperature may be).

As a matter of fact, specific gravities of milk are usually

determined as "apparent specific gravities in glass at  $\frac{x^{\circ}}{15.55^{\circ}}$ ."

**Determination of Specific Gravity.**—There are two methods of determining specific gravity, which is, as above stated, the weight of unit volume, or, expressed as an equation—

$$S = \frac{W}{V}.$$

We may either determine the weight of a known volume, or the volume of a known weight. Both methods are used in practice, the first in two ways:—

(1) A vessel of known volume is filled with the liquid and its weight taken.

(2) A plummet of known volume is immersed completely in the liquid, and the loss of weight due to the displacement of an equal volume of liquid noted.

The second method is applied (3) by immersing a float of

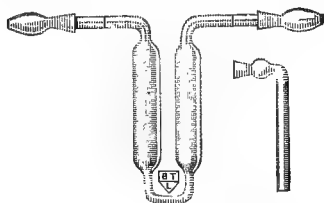


Fig. 2.—Sprengel Tube.

known weight, and noting the volume immersed; the volume immersed will be equal to a volume of the liquid of weight equal to that of the float.

Determinations of specific gravity by method (1) are made by specific gravity bottles and Sprengel tubes, by method (2) by a Westphal balance, and by method (3) by hydrometers, of which lactometers are special forms of limited range suited for milk.

For exact determinations of the specific gravity of milk, a Sprengel tube (Fig. 2) presents many advantages. It is a U-shaped tube with narrow capillary ends bent outwards at right angles, one being rather smaller than the other; the wider of the two has a fine line etched round it, to which the liquid in the tube may be adjusted, the U and the other capillary being completely filled.

The weight of the dry and empty tube is first ascertained, the tube is then filled with pure distilled water, and immersed in water at exactly  $15.55^{\circ}$  C. ( $60^{\circ}$  F.); when it is seen that no

further expansion or contraction takes place, the water should be adjusted to the line on the wider capillary by the cautious application of a piece of blotting-paper to the end of the narrow capillary; the tube is then wiped dry and weighed. The difference in the two weights gives the weight of the water contained in it. The tube is then filled with milk and immersed in water at  $15.55^{\circ}$  C. ( $60^{\circ}$  F.), and the milk similarly adjusted to the line; the weight of milk divided by the weight of water gives the specific gravity of the milk at  $\frac{15.55^{\circ}}{15.55^{\circ}}$ .

A specific gravity bottle is used in a similar manner; the liquid, after inserting the stopper and immersing the bottle in water at  $15.55^{\circ}$ , is adjusted to the line by drawing out the excess with a very fine tube.

A Sprengel tube of 10 to 20 c.c. capacity is the most suitable size; it is a disadvantage to use a larger one, as the time taken for the milk to assume the temperature of the surrounding water is so much increased that there is danger of a portion of the cream separating.

The advantages of a Sprengel tube over a specific gravity bottle are:—

(1) Greater surface for a given volume; and therefore the temperature is adjusted quicker.

(2) There is no stopper to fit; consequently, no error can be due to difference of position owing to inaccuracy of fit.

The Westphal balance (Fig. 3) consists of a balance of the "steel-yard" type, carrying a glass plummet at one end; it is so adjusted that the pointer is at zero when the plummet hangs in air, and is provided with a weight, which, when hooked on to the end, causes the pointer to be at zero when the plummet is immersed in water at  $15.55^{\circ}$  C. The beam is divided into ten parts, each indicated by a notch, and riders weighing the same,  $\frac{1}{10}$ ,  $\frac{1}{100}$ , and  $\frac{1}{1000}$  of the weight are provided.

To take the specific gravity, the plummet is immersed in milk at  $15.55^{\circ}$ , and riders are placed on the notches of the beam till the pointer is at zero. If the  $\frac{1}{10}$  rider is on notch 3, the specific gravity is 1.03; if, in addition, the  $\frac{1}{100}$  rider has to be placed on notch 2, the specific gravity is 1.032; and if, in addition, the  $\frac{1}{1000}$  rider has to be placed on notch 4 the specific gravity is 1.0324. If a rider is already on a notch, and it is desired to place another thereon, it may be hung on the turned up end of the rider already in position.

A rule may be given as follows:—Count 1 for the weight hung on the end, the first decimal from the notch on which the  $\frac{1}{10}$  rider is hung, the second decimal from the notch on which



the  $\frac{1}{10}$  rider is hung, the third decimal from the  $\frac{1}{100}$  rider, and the fourth decimal from the  $\frac{1}{1000}$  rider.

This method has the advantage of being somewhat more rapid than the use of the Sprengel tube, but is not quite so accurate, as the adjustment of riders and balance cannot practically be performed with very great accuracy.

In dairy work the lactometer is generally used. From a strictly scientific point of view, there are many objections to lactometers, but their practical convenience is so great that they are instruments of extreme value.

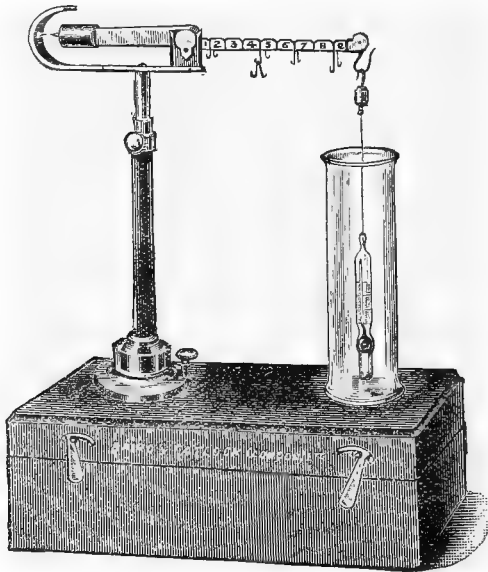


Fig. 3.—Westphal Balance.

The faults of lactometers are:—(1) They do not indicate true specific gravities, but the inverse of this—specific volumes; consequently, the scale is not divided into equal parts. The divergence from equality is, however, so small in a lactometer, which has only a limited range, as to render it practically admissible to treat the smaller divisions as equal.

(2) The exact point at which the level of the liquid cuts the stem of the lactometer cannot be ascertained, as, owing to surface energy, the liquid is attracted to a higher level round the stem of the lactometer than the surface of the liquid; moreover, the height to which the liquid is attracted varies with the nature

of the liquid. As milk has always the same composition within narrow limits, there is no practical difference in the height to which it is attracted round the stem; the eye soon becomes trained in making the proper allowance for this.

(3) Lactometers are only correct at the temperature at which they are graduated; at other temperatures their volume varies; no inconvenience on this account is felt in practice, as this is allowed for in the tables given for correcting the specific gravity to a temperature of  $15.55^{\circ}$  C. ( $60^{\circ}$  F.).

Practical instructions for the use of lactometers will be given later under the "Testing of Milk."

**Variation in Milk.**—The specific gravity at  $15.55^{\circ}$  C. ( $60^{\circ}$  F.) of the milk of individual cows varies from 1.0135 to 1.0397; when the mixed milk of a herd is tested it rarely falls outside the limits of 1.030 and 1.034. The average specific gravity of milk is 1.0322.

The specific gravity is dependent on two causes—the amount of solids not fat, which, being dissolved in water, raises the specific gravity; and the fat, which, being lighter than water, lowers it. By removing the fat (with a small proportion of other constituents) as cream the specific gravity of the milk is raised. By the addition of water the specific gravity is lowered. The specific gravity has been—and is—largely used as a test for the addition of water to milk; for the detection of large amounts of water to milk it has some value.

That it is a test of the roughest kind is shown by the following facts:—

(1) The variations in specific gravity are from 1.0135 to 1.0397—*i.e.*, nearly twice its bulk of water could be added to milk of the highest specific gravity to reduce it to the lowest. These, of course, are exceptional cases, and the specific gravity of the mixed milk of a herd is nearly always between 1.030 and 1.034. At least 10 per cent. of water could be added to milk of 1.034 specific gravity before it would be suspected by this test.

(2) A milk of 1.032 specific gravity, if the cream is all removed, would give a product of about 1.036 specific gravity; and an addition of rather more than 10 per cent. of water would bring the specific gravity back to 1.032.

(3) If to milk of 1.032 specific gravity sufficient cream be added to raise the percentage of fat 4 per cent., the specific gravity will be found to be about 1.028. The same result would be arrived at were the milk allowed to stand, and the upper portion removed.

As an absolute test the specific gravity is liable to be greatly misleading; as a preliminary test it is of the greatest importance, and should never be neglected.

As stated above, the specific gravity is raised by the solids

not fat, and lowered by the fat. This fact is not only true qualitatively, but also, as the following demonstration will show, quantitatively.

By our definition that specific gravity (S) is the weight (W) of the unit volume (V), we get the equation—

$$S = \frac{W}{V} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Let us suppose we have a mixture (having the specific gravity S) of two substances, A and B, of differing specific gravities  $S_A$  and  $S_B$ .

Let us suppose that the respective weights are A and B, and let  $A + B = 100$ .

Then by (1)

$$\text{Volume of A} = \frac{A}{S_A}, \text{ volume of B} = \frac{B}{S_B}, \text{ and volume of mixture} = \frac{100}{S}.$$

Then

$$\frac{100}{S} = \frac{A}{S_A} + \frac{B}{S_B} = \frac{A}{S_A} + \frac{100 - A}{S_B}.$$

$$\frac{100}{S} = \frac{A(S_B - S_A)}{S_A S_B} + \frac{100}{S_B}.$$

$$\frac{100}{S} - \frac{100}{S_B} = A \left( \frac{S_B - S_A}{S_A S_B} \right).$$

$$1 - \frac{S}{S_B} = AS \left( \frac{S_B - S_A}{100 S_A S_B} \right).$$

$$S = S_B + AS \left( \frac{S_A - S_B}{100 S_A} \right).$$

Now in the same way

$$S = S_A + BS \left( \frac{S_B - S_A}{100 S_B} \right).$$

As both  $S_A$  and  $S_B$  are constants, we may write

$$\left. \begin{array}{l} S = S_A + BS \times K_B \\ \text{and } S = S_B + AS \times K_A \end{array} \right\} K_B \text{ and } K_A \text{ being constants} \quad . \quad . \quad (2)$$

Now, as  $A + B = 100$ , A is the percentage by weight of this substance; and as  $100 \times S$  expresses the total number of grammes in 100 c.c. of the mixture, AS is the number of grammes of this substance in 100 c.c.

From the equations above given we can deduce the law that "if two substances of differing specific gravity be mixed, the specific gravity of the mixture will be equal to the specific gravity

of one of the substances *plus* the number of grammes of the other per 100 c.c. of the mixture multiplied by a constant factor.”

Regarding milk as a mixture of fat and a solution of solids not fat in water, we can say that the specific gravity of a milk is equal to the specific gravity of the solution of solids not fat *plus* the number of grammes of fat per 100 c.c. multiplied by a constant.

In the solution of solids not fat we have in 100 c.c. of it  $x$  grammes of solids not fat; let us assume that their density is  $y$ .

Then  $x$  grammes will occupy a volume  $\frac{x}{y}$ . Let the specific gravity of the solution be  $S$ . The 100 c.c. weigh  $100 S$  grammes, and the water in this weighs  $100 S - x$  grammes; it also measures  $100 - \frac{x}{y}$  c.c. Now, as the specific gravity of water is 1,

$$100 S - x = 100 - \frac{x}{y}$$

$$100 S = 100 + x - \frac{x}{y}$$

$$S = 1 + x \frac{y - 1}{100 y} \quad \dots \dots (3)$$

Now,  $\frac{y - 1}{100 y}$  is a constant, provided that  $y$  remains constant.

Putting the equation into words we find “ that the specific gravity of a solution of solids not fat is equal to 1 *plus* the number of grammes of solids not fat in 100 c.c. multiplied by a constant.” It is known, however, that the specific gravity of substances in solution is not quite constant, but varies slightly with dilution.

The following figures will show that in milk the law just enumerated holds good within the limits of experimental error. A poor skim milk was diluted with water, and the total solids and specific gravity at 15.55° estimated :—

Total Solids per Cent.	Specific Gravity.	Constant.
9.280	1.03544	0.003688
8.758	1.03343	0.003693
8.318	1.03170	0.003694
7.777	1.02950	0.003684
7.456	1.02829	0.003690
6.455	1.02439	0.003688

From the laws expressed by equations (2) and (3) we see that the specific gravity of an aqueous liquid containing a substance in solution or in admixture can be expressed equally as a direct

multiple of the number of grammes per 100 c.c. ; for if we suppose that the substance A is water,  $Sa$  will equal 1, and equation (2) can then be written—

$$S = 1 + BS \times K_B.$$

which is practically equation (3).

**Formulae for Calculations.**—In order to deduce a formula expressing the relation between specific gravity and percentage by weight of fat and solids not fat, let us call the specific gravity (for convenience)  $1 + S$ , the percentage by weight of fat  $F$ , and of solids not fat  $N$ .

Then the number of grammes of fat per 100 c.c. will be  $F \times (1 + S)$  and of solids not fat  $N \times (1 + S)$ .

The weight of the water in 100 c.c. is then

$$100 \times (1 + S) - N \times (1 + S) - F \times (1 + S) \text{ grammes ;}$$

and its volume  $100 \times (1 + S) - N \times (1 + S) - F \times (1 + S)$  c.c.

The volume of fat and solids not fat in 100 c.c. is therefore

$$100 - (100 \times (1 + S) - N \times (1 + S) - F \times (1 + S)) \text{ c.c.}$$

which equals

$$N \times (1 + S) + F \times (1 + S) - 100 S \quad (4)$$

Let us assume that the specific gravity of fat is  $f$  and of solids not fat  $n$ .

Then the volume of fat in 100 c.c. is  $\frac{F \times (1 + S)}{f}$  and of solids not fat  $\frac{N \times (1 + S)}{n}$  ; therefore

$$N \times (1 + S) + F \times (1 + S) - 100 S = \frac{F \times (1 + S)}{f} + \frac{N \times (1 + S)}{n}$$

$$\text{or } 100 S = N \times (1 + S) - \frac{N \times (1 + S)}{n} + F \times (1 + S) - \frac{F \times (1 + S)}{f}$$

$$\text{or } 100 S = N \times (1 + S) \left( \frac{n-1}{n} \right) + F \times (1 + S) \left( \frac{f-1}{f} \right).$$

Now, as  $n$  and  $f$  are constant, we may write for  $\left( \frac{n-1}{n} \right)$ ,  $a$  ; and for  $\left( \frac{f-1}{f} \right)$ ,  $b$ .

Then the equation stands

$$\frac{100 S}{1 + S} = a N + b F \quad \dots \quad (5)$$

It is usual, however, to estimate total solids (T) and fat in an analysis.

$$T = N + F, \text{ and, therefore, } N = T - F.$$

The equation (5) may be written

$$\frac{100 \cdot S}{1 + S} = a (T - F) + b F$$

or 
$$\frac{100 \cdot S}{1 + S} = a T + (b - a) F \dots \dots (6)$$

In expressing the specific gravity of milk it is usual to do so in lactometer degrees, which are the specific gravity multiplied by 1,000 *minus* 1,000.

Thus if the specific gravity be 1.032, the lactometer degrees are  $1.032 \times 1,000 - 1,000 = 32$ .

Let us express lactometer degrees by the symbol G.

Then  $G = 1,000 S$ , and, substituting this in (6), we get

$$\frac{G}{1 + S} = 10a T + 10 (b - a) F.$$

The specific gravity was expressed as  $1 + S$  for ease in calculation; it is better, however, to substitute the symbol D in the formula, which then stands as

$$\frac{G}{D} = 10a T + 10 (b - a) F$$

or 
$$T = \frac{1}{10a} \times \frac{G}{D} - \left( \frac{b - a}{a} \right) F \dots \dots (7)$$

As, by the definition above,  $a = \frac{n - 1}{n}$  and  $b = \frac{f - 1}{f}$ , we could calculate a formula, did we know the specific gravities of solids not fat and fat, but we do not know both of these. Fleishmann has determined the specific gravity of the fat of milk to be 0.9307 at  $15^\circ \text{C}$ , but it is impossible to determine the specific gravity of solids not fat in solution. Moreover, Fleischmann's determination of the specific gravity was made on fat in the solid state, and it is possible that in milk it may have a different specific gravity.

By transforming equation (7) into the form

$$1 = \frac{1}{10a} \times \frac{G}{TD} - \left( \frac{b - a}{a} \right) \times \frac{F}{T}$$

and making a large number of determinations of  $\frac{G}{D}$ , T, and F in different milks, we can form each pair of results into simultaneous equations and solve them. In this way we can get a large number of values for a and b, and, from the mean of these, we can calculate the specific gravities of fat and solids not fat respectively. This method is not wholly free from objection,

as, unless there is a considerable difference between the figures actually determined, the figures from which  $a$  and  $b$  are calculated are so small as to be greatly affected by experimental error; while, if the difference be large (as in the case of analyses of cream and skim milk), it is found that the experimental error is also increased. For this reason, and also for the reason that the specific gravity of fat and solids not fat are themselves liable to slight variations, it is necessary to deduce the formula from a great many determinations, which means much labour in calculating.

In order to ascertain the specific gravities of fat and solids not fat in milk, the author has calculated their value from over 200 analyses made by the most exact methods at his command, and finds the following figures:—

Specific gravity of fat, . . . . .	0.93
„ „ solids not fat, . . . . .	1.616

It is seen that the author's figure, calculated from actual analyses, agrees with Fleischmann's determination of the specific gravity of fat.

As the specific gravity of milk does not vary much, it will not make an appreciable error if, instead of  $\frac{G}{D}$ , the expression  $\frac{G}{1.0322}$  be used; this form of calculation is much easier.

The idea of deducting a relation between specific gravity, fat, and total solids appears to have arisen with Behrend and Morgen, who published a table. Shortly afterwards Clauznitzer and Mayer, and Hehner published formulæ, but as they were founded on inaccurate data, they are now abandoned.

Fleischmann and Morgen next published a formula in which the specific gravity of fat was assumed to be 0.94; this was corrected by Fleischmann after his determination of the specific gravity of fat as 0.93. His formula is

$$T = 0.2665 \frac{G}{D} + 1.2 F.$$

Hehner and the author deduced the formula

$$T = 0.254 G + 1.164 F.*$$

This is in the less scientifically correct, but more convenient form; as it was found that milk differing appreciably in specific gravity from 1.0322 did not give results which agreed well with the formula, various approximations have been made to this.

The author has calculated a formula which gives practically

\* In the original paper a slight correction for skim milks was included in the formula; this has now been abandoned.

the same results, but is more scientifically correct, and which does not require the application of approximations. This is

$$T = 0.262 \frac{G}{D} + 1.17 F.$$

As the previous formulæ were deduced from analyses to which objection could be taken, the author has deduced a new formula from the results of analyses made as exactly as possible.

$$T = 0.2625 \frac{G}{D} + 1.2 F.$$

This has been found to be expressed by the simpler formula  $T = \frac{G}{4} + \frac{6}{5} F + 0.14$  within very small limits if the specific gravity lies between 1.020 and 1.036.

The formula  $T = \frac{G}{4} + \frac{6}{5} F$  also approximates closely to that of Hohner and the author.

Other formulæ have been devised by J. C. Brown, Babcock, Leonard, and others.

Of the above formulæ, that of Fleischmann agrees best with the results when Soxhlet's method of fat estimation is used; that of Hohner and the author when the Society of Public Analysts' methods are employed; while if the methods mentioned later as most exact in the author's opinion be employed, the author's formula gives the most satisfactory results.

The fat calculated from the specific gravity and total solids almost invariably agrees within 0.2 per cent. with the determination made by the appropriate method.

**Milk Scale.**—In order to save calculation the author has devised a slide rule, known as the "milk scale" (Fig. 4), from which the percentage of fat can be read off directly from the specific gravity and percentage of total solids. On one side a scale is placed indicating total solids, 1 per cent. of total solids being represented by 1 inch; on the other side the fat is shown by a scale of 1.2 (1½ inches) to 1 per cent.; the slide carries the specific gravity scale, 1 being equal to 0.25 (¼) inch. The line indicating the specific

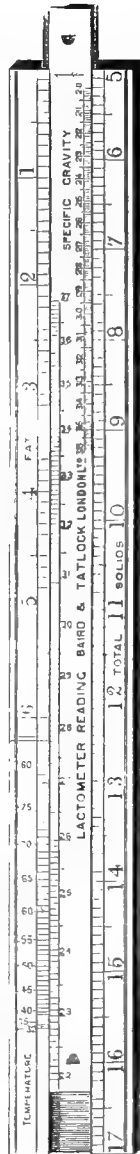


Fig. 4.  
Milk Scale.



gravity found is placed against the total solids estimated; an arrow placed 0.14 ( $\frac{1}{7}$ ) inch from the end of the specific gravity scale then gives the fat as calculated by the formula.

$$T = 0.25 G + 1.2 F + 0.14.$$

To facilitate reading, Cassal and Gerrans propose to add two sliding pointers, one on the total solids scale, and one on the specific gravity slide, which are first placed against the part of the scales corresponding to total solids and specific gravity found respectively, and the two pointers are then adjusted. This arrangement prevents the possibility of error in adjusting the slide.

The author has also employed a runner made out of a piece of brass bent round the milk scale, in which two holes are cut, leaving a narrow bar between them; this bar partially covers both the total solid and specific gravity scales, and has a fine line drawn upon it at right angles to the scales. By adjusting this line to the total solids, and the specific gravity to the line, the object sought by Cassal and Gerrans is attained. Stokes uses a strip of transparent celluloid on which a fine line is drawn. The idea of the runner is due to Lieutenant Mannheim of the French Artillery, who, in 1851, devised it for a logarithmic slide rule.

The scales are divided into tenths, hundredths being estimated by the eye; a decimal scale, or, better, a vernier, as suggested by Sykes, can be applied to the runner, rendering it easier to read the second place of decimals. This, however, is not necessary, the error due to unavoidable circumstances being greater than the error of computation of the hundredths.

Tables are given from which the fat calculated from different percentages of total solids and specific gravities can be read off.

By our definitions (p. 58)

$$a = \frac{n-1}{n} \text{ and } b = \frac{f-1}{f},$$

and from the values given above in the formula mentioned,  $a$  and  $b$  can be calculated.

Thus, taking the formula  $T = 0.2625 \frac{G}{D} + 1.2 F$ ,

$$0.2625 = \frac{1}{10a} \quad \text{and} \quad 1.2 = -\frac{b-a}{a} \text{ or } \frac{a-b}{a}.$$

$$\therefore a = 0.381 \text{ and } b = 0.0762;$$

$$\text{now, } n = \frac{1}{1-a} \quad \text{and} \quad f = \frac{1}{1-b};$$

and, therefore,  $n = 1.616$  specific gravity of solids not fat,

and  $f = 0.900$  ,, ,, fat.

From the mode of deducing equation (7) we see that  $a$  is the number of grammes that the weight of 100 c.c. of milk is greater than the weight of 100 c.c. of water, when 1 gramme per 100 c.c. of solids not fat is contained therein; the density being, by definition, the weight of 1 c.c.,  $\frac{a}{100}$  and  $\frac{b}{100}$  are respectively the difference in specific gravity due to 1 gramme per 100 c.c. of solids not fat and fat.

It is also apparent that we may calculate from any analysis the amount that 1 gramme of total solids per 100 c.c. has raised the specific gravity, and, from this, the specific gravity of the total solids.

Thus, using the same symbols as before

$$T = \frac{G}{D} \times \frac{1}{10x}, \quad \text{and} \quad t = \frac{1}{1-x}$$

( $t$  here representing the specific gravity of the total solids).

Thus, if a milk has a specific gravity of 1.032 and contains 12 per cent. of total solids,

$$12 = \frac{32}{1.032} \times \frac{1}{10x} \quad \text{and} \quad x = 0.2584$$

and

$$t = 1.348.$$

It is occasionally useful to calculate the specific gravity of the total solids of a milk, as the total solids of skimmed milk have a considerably higher specific gravity than those of whole milk.

**Specific Volume.**—By our definition of specific gravity, we write  $S = \frac{W}{V}$ ; we may also write,  $\frac{1}{S} = \frac{V}{W}$ ; or, in words,  $\frac{1}{S}$  expresses the volume of 1 gramme; this is called specific volume. The expression  $\frac{G}{D}$  is, therefore, a mode of indicating specific volumes; as  $G$  (degrees of specific gravity) is 1,000 times the specific gravity *minus* 1,000, so  $\frac{G}{D}$  (degrees of specific volume) is 1,000 *minus* 1,000 times the specific volume.

In Table VII. the values of degrees of specific volume for each half degree of specific gravity from 20 to 36 are given.

It is seen from the formulæ above, that 1 per cent. by weight lowers the specific volume to the same extent as 1 gramme per 100 c.c. raises the specific gravity—*i.e.*, specific volume, not specific gravity, varies directly as percentage by weight.

TABLE VII.—SPECIFIC GRAVITY AND VOLUME OF MILK.

Degrees of Specific Gravity.	Degrees of Specific Volume.	Degrees of Specific Gravity.	Degrees of Specific Volume.
20·0	19·6	28·0	27·2
20·5	20·1	28·5	27·7
21·0	20·6	29·0	28·2
21·5	21·0	29·5	28·7
22·0	21·5	30·0	29·1
22·5	22·0	30·5	29·6
23·0	22·5	31·0	30·1
23·5	23·0	31·5	30·5
24·0	23·4	32·0	31·0
24·5	23·9	32·5	31·5
25·0	24·4	33·0	31·9
25·5	24·9	33·5	32·4
26·0	25·4	34·0	32·9
26·5	25·8	34·5	33·4
27·0	26·3	35·0	33·8
27·5	26·8	35·5	34·3
		36·0	34·7

**Mode of Averaging Specific Gravities.**—It is not, therefore, correct in averaging milk analyses, where specific gravities and percentages by weight are expressed, to obtain the average specific gravity by adding the specific gravities together and dividing by the total number, but specific gravities must be first calculated to specific volumes, and these averaged, and the average specific gravity deduced from the average specific volume.

Thus, to average the following analyses :—

Specific gravity, . . .	1·022	Total solids, . . .	20·0
„ . . .	1·036	„ . . .	10·0

The average total solids is  $\frac{20 + 10}{2} = 15$ ; but the average specific gravity is not  $\frac{1·022 + 1·036}{2} = 1·029$ , but  $\frac{1}{\frac{0·9785 + 0·9653}{2}} = \frac{1}{0·9719} = 1·0289$ .

The error is, however, small if the specific gravities do not differ greatly, and may very frequently be neglected.

On the other hand, if, instead of averaging percentages of total solids by weight, we average the number of grammes per 100 c.c., we obtain correct results by averaging the specific gravities.

The following rules may be stated :—

(1) If equal volumes of different milks be mixed, the specific gravity of the mixture will be the mean of the specific gravities of the milks.

(2) If equal weights of different milks be mixed, the specific volume of the mixture will be the mean of the specific volumes of the milks.

Instead of taking the solids not fat as one substance, we may consider its constituents—lactose, protein, and mineral matter—separately.

(Calling the percentage of fat F, lactose L, protein P, and mineral matter A, we may write

$$\frac{G}{D} = 13b F + 10c L + 10d P + 10e A$$

by the same reasoning employed in deducing formula (5).

The author has deduced from the mean of many analyses the formula

$$\frac{G}{D} = - 0.761 F + 4 L + 2.5714 P + 8.46 A.$$

From these factors the following specific gravities are deduced, as previously shown :—

Specific gravity of fat,	0.93
" " lactose,	1.666
" " protein,	1.346
" " mineral matter,	5.5

Using these specific gravities, together with Vieth's estimate of the proportions of lactose, protein, and mineral matter—13 : 9 : 2—we can calculate what the specific gravity of solids not fat should be. As Vieth's proportions are by weight, we must transform specific gravities into specific volumes.

Specific volume of lactose	$= \frac{1}{1.666} = 0.6.$
" " protein	$= \frac{1}{1.346} = 0.743.$
" " mineral matter	$= \frac{1}{5.5} = 0.182.$

Then specific gravity of

$$\text{S.n.F.} = 13 \times 0.6 \div 24 + 9 \times 0.743 \div 24 + 2 \times 0.182 = 0.6188 = 1.616,$$

which agrees with that given above, 1.616.

It is a useful check on an analysis to calculate the specific gravity from the percentage of milk-sugar, protein, fat, and

mineral matter; this should agree within small limits with that found.

**The Alteration of Specific Gravity by Change of Temperature.**—Milk, like all other substances, alters in specific gravity by change of temperature.

Though it contains a large amount of water, it does not share the anomaly which this substance possesses of attaining its maximum specific gravity at 4° C. (39° F.). It decreases in specific gravity when heated from its freezing point — 0·55° C. (31° F.).

The following figures (Table VIII.) show the average apparent expansion of milk in glass —

TABLE VIII.—EXPANSION OF MILK.

Temperature in ° F.	Volume.	Temperature in ° F.	Volume.
31	1·00000	60	1·00229
35	1·00016	65	1·00298
40	1·00041	70	1·00372
45	1·00074	75	1·00451
50	1·00114	80	1·00549
55	1·00164		

The expansion is greater with rich milk than with poor milk; the above figures referring to milk having a specific gravity of 1·032 and containing 3·8 per cent. fat.

Table CXXXI. in the Appendix affords a means of correcting to 60° F. the specific gravity of milk when taken by a lactometer at any temperature from 33° F. to 85° F. The table gives specific gravities from 1·020 (20 degrees) to 1·036 (36 degrees) and is applicable to whole milk only. The portion from 45° F. to 75° F. is, with a few alterations, due to Vieth; this has been repeatedly checked by the author, and in a few places slightly changed in accordance with the results obtained. The other portion has been calculated by the author.

The table is used by looking up the degrees of specific gravity found (or the nearest whole degree) in a horizontal line, and the temperature in a vertical line; the figure at the intersection of the two lines is the specific gravity corrected to 60° F.

The specific gravity of separated milk may be corrected to 60° F. by the following table. As the expansion is practically uniform for the variations in quality met with, the table is given in a simpler form.

TABLE IX.—FOR CORRECTING SPECIFIC GRAVITY OF SEPARATED MILK TO 60° F.

Temp.	Take off.	Temp.	Add on.	Temp.	Add on.
° F.		° F.		° F.	
50	1.1	60	0.0	70	1.4
51	1.0	61	0.1	71	1.6
52	0.9	62	0.3	72	1.8
53	0.8	63	0.4	73	1.9
54	0.7	64	0.5	74	2.0
55	0.5	65	0.6	75	2.2
56	0.4	66	0.8	76	2.3
57	0.3	67	0.9	77	2.4
58	0.2	68	1.1	78	2.6
59	0.1	69	1.2	79	2.7

The author has devised a scale for correcting the specific gravity of milk to 60° F. It is usually engraved on the "milk-scale," and is used by adjusting the specific gravity found (on the slide) to the arrow at 60° F. The corrected specific gravity is found opposite the temperature at which the determination was made.

The corrected specific gravities obtained by the "milk-scale" agree generally within 0.1 of those taken from the table. At very low temperatures, however, there is sometimes a larger difference.

S. H. Collins has devised a milk scale in which the temperature correction for specific gravity is automatically made; the points denoting temperature and specific gravity observed are brought together, and on the other side of the scale the percentage of solids not fat corresponding to any percentage of fat is read off.

**The Rise of Specific Gravity of Milk on Standing.**—Milk drawn from the udder contains a large number of air bubbles, and its specific gravity cannot be taken; after the expiration of an hour or so these have disappeared, and a specific gravity determination is possible. It was first observed by Recknagel that the specific gravity taken after the expiration of one hour was lower than the specific gravity subsequently obtained. He found the rise in specific gravity to be regular, more rapid at low temperatures than high ones, and to amount on the average to 0.001. He attributed the change to an alteration in the volume of the casein.

Vieth completely confirmed Recknagel's observation, and found the average rise to be 0.0013; Bourcart also observed the phenomenon.

The author has studied Recknagel's phenomenon (as this

change in specific gravity has been called). In about 70 per cent. of his experiments the rise in specific gravity has been observed, varying from 0.0015 to 0.0003, and averaging 0.0006, while in 30 per cent. of the observations no rise in specific gravity was indicated.

The experiences of Babcock and Farrington agree with that of the author.

The author's experiments have confirmed the statement of Recknagel, that the rise is more rapid when the temperature is low than when high; the same ultimate specific gravity is attained whatever the temperature.

Recknagel's phenomenon appears to be unconnected with the milk-sugar, and Recknagel's explanation is not the correct one. It is difficult to reconcile the idea that it is enzymic, with the fact that the rise is more rapid at low than high temperatures.

The author's experiments on the change of density and specific heat of cream by heating made in conjunction with S. O. Richmond have shown that Recknagel's phenomenon is due largely to the increase of density of the fat on solidification.

Contrary to the author's former conjecture, there seem to be no particular periods of the year in which Recknagel's phenomenon is observed or not. Samples have been found at all seasons which show a marked change in specific gravity, while others examined almost simultaneously have shown no change.

It must be mentioned that Recknagel's phenomenon has been denied by some. Smetham attributes the change in specific gravity solely to the presence of air bubbles. The weight of evidence is, however, greatly against this view; it is inconceivable that air bubbles generated by milking a cow should be persistent for twelve hours, while if they are formed in the milk by other means, say by running through a separator, they disappear in one hour.

The final specific gravity is always taken as the true specific gravity of milk, and the term is so used in this volume.

**The Estimation of Total Solids.**—The total solids of milk are estimated by evaporating the water and weighing the residue.

**Wanklyn's Method.**—Wanklyn proposed to limit the time of drying to three hours at the temperature of boiling water; he weighed 5 grammes in a platinum basin, kept it for three hours on a briskly boiling water-bath, and, after cooling in a desiccator, weighed the residue. This method has now entirely fallen into disuse, as the residue thus obtained still contained a quantity of water, which could be driven off by further evaporation.

**Method of Society of Public Analysts.**—A very obvious modification of this is to continue the drying on a water-bath or

in an oven at 100° C. till the weight is constant. This method has been adopted by the Society of Public Analysts. It is not, however, possible to attain absolute constancy, as a decomposition due to heating takes place, and the weight continually slightly diminishes on further heating; for this reason the weight is usually considered constant when less than 1 milligramme per hour is lost on further drying. The method gives very good results, and duplicates agree closely; but it is doubtful whether the results represent accurately the true total solids of the milk. First, an absolutely constant weight is not attained; and, next, the residue is markedly brown, indicating decomposition; the point taken as constancy is really a point where it may be assumed that the bulk of the water is driven off, and comparatively little decomposition has taken place.

The author has found by taking smaller quantities of milk—about 1 gramme—and spreading this over a large surface during evaporation, that a nearly white residue is obtained, and constancy of weight can be attained. It appears probable that the decomposition to which the browning is due takes place during the heating of the milk before evaporation is concluded. In support of this view it may be noted that Cazeneuve and Haddon have observed that formic acid is produced by heating milk to the temperature of boiling water, and Johnstone has found that formic acid added to milk had an enormous influence on the results. The results obtained by the estimation of total solids by the evaporation of 1 gramme spread over a large surface, from which the water was very quickly driven off, were always slightly higher than when 5 grammes were used, when evaporation was very much slower.

In order to secure rapid evaporation, the milk has been spread over a large surface by the use of sea sand and other granular substances. Vieth has found that evaporation on sand gives practically the same results as direct evaporation in a basin.

**Babcock's Method.**—Babcock has used asbestos as a medium for spreading the milk over. The method as adopted by the Association of Official Agricultural Analysts (of America) is described elsewhere. The author has found Babcock's method most satisfactory, and finds it convenient to operate as follows:—Place about 3 grammes of fine asbestos fibres in a small platinum basin, and ignite strongly (preferably in a muffle). The asbestos should be soaked in hydrochloric acid, and thoroughly washed before use; when ignited and shaken with water containing a few drops of phenolphthalein no red colour should be produced. After weighing, add about 5 grammes of milk, and again weigh as quickly as possible to the nearest milligramme. Place the basin



for an hour or two on a water-bath, and dry in a water-oven till constant in weight.

The residue thus obtained shows no signs of browning, and a constant weight, which shows no appreciable change on prolonged drying, can be obtained. The "total solids" by this method are somewhat hygroscopic, and care must be exercised in weighing.

**Macfarlane's Method.**—Macfarlane uses "chrysotile" or Canadian asbestos for this purpose; this substance cannot be ignited, being a hydrated mineral, and on treatment with water affords a very sensible amount of soluble alkali; this causes a loss of weight owing to its action on the milk, and for this reason chrysotile is not so satisfactory as the Italian asbestos. The residue obtained by drying on "chrysotile" is very distinctly brown, and the results are much lower than those given by other methods.

**Adams' Method.**—Adams uses a paper coil, which is first dried at 100° C. and weighed, for the estimation of total solids. The results thus obtained are frequently low, owing probably to the presence of alkaline salts.

Duclaux has proposed the use of sponge, and Gannetner of wood fibre; but these substances have never come into general use.

**Drying.**—The author has found that by evaporation *in vacuo* over sulphuric acid good results are obtained if the milk be spread on blotting-paper or on asbestos; the result is slightly higher than when the drying is performed at 100° C.

In order to shorten the time of drying, Gerber and Radenhausen experimented with acetic acid and alcohol. They found that by coagulating the casein with these substances a skin no longer formed on heating, and the time of evaporation was materially shortened. For this reason the use of acetic acid, or alcohol, or a mixture of the two, has been largely adopted for the estimation of total solids. A much greater browning of the total solids takes place, and constant results are quite impossible of attainment when acetic acid or alcohol is used; by a somewhat close adherence to arbitrary conditions as to time of drying very fair results may, however, be obtained in this way in a short time, but the method cannot be recommended where accuracy is of importance. Revis proposes the use of acetone.

It may sometimes be of importance to estimate the water driven off, instead of deducing it from the difference between the percentage of total solids and 100. To accomplish this, about 4 grammes of asbestos should be placed in a U-tube, and, after drying by passing a current of dry air, this should be weighed. About 5 grammes of milk should be weighed in, and the U-tube

suspended in a beaker of water. This is connected with another weighed U-tube filled with pumice moistened with strong sulphuric acid, and provided with a bulb in which the bulk of the water can condense. A current of air (or preferably, hydrogen) dried by sulphuric acid should be passed through the tubes, and the water in the beaker boiled. After about three hours' heating, the sulphuric acid tube should be removed, and, after cooling, weighed. The increase of weight of the sulphuric acid tube will give the weight of the water in the milk taken.

It is not advisable to dry the total solids at temperatures exceeding 100° C., as the decomposition of the residue by heat is increased at higher temperatures.

**Drying Apparatus.**—For the drying of total solids the following conditions may be laid down for the drying apparatus:—

(1) The temperature must not exceed 100° C. (212° F.).

(2) The moisture must be removed as soon as it is converted into vapour.

The usual form of water-oven used consists of a water-jacketed metal box with a door to it; very little provision is made for the removal of the moisture, as no current of air is allowed to circulate through the whole of the interior.

Various forms of air-baths, with a regulator for maintaining a constant temperature of 100° C. (212° F.), have been proposed; of these, the best are those of Griffin and Adams. These do not give quite satisfactory results for milk analysis, because the temperature for which they are regulated is the temperature of the air in the bath, while the basins in which the milk is dried are heated to a somewhat higher temperature by conduction.

The following figures were obtained with a Griffin's air-bath; a porcelain capsule filled with mercury was placed on various shelves in the bath, and the temperature of the mercury noted. The air had a constant temperature of 100° C.:—

Temperature on bottom, . . . . .	136°
„ on cork on bottom, . . . . .	102°
„ on shelf, . . . . .	104°
„ in upper part, . . . . .	96°

Constancy of temperature cannot be depended upon in an air-bath; it is, therefore, preferable to use a water-oven. The author has devised a water-oven for milk analysis, which has given highly satisfactory results.

It consists of a jacketed copper box, opening only at the top, and closed with a movable lid; on the lid is a chimney about 1 foot high. The bottom portion of the jacket contains four 8-foot coils of thin copper tubing, which communicate with the exterior by four holes at the side of the bath, and with the interior

by four holes, one in each corner. The jacket is closed, except for one opening, in which a condenser is placed. About 1 inch from the bottom a sheet of copper is fixed, in which is a round hole of diameter equal to half the width of the interior.

The jacket is filled with distilled water, which is heated either by a steam coil or a gas flame; perforated copper shelves are used to support the basins, etc., containing the substances to be

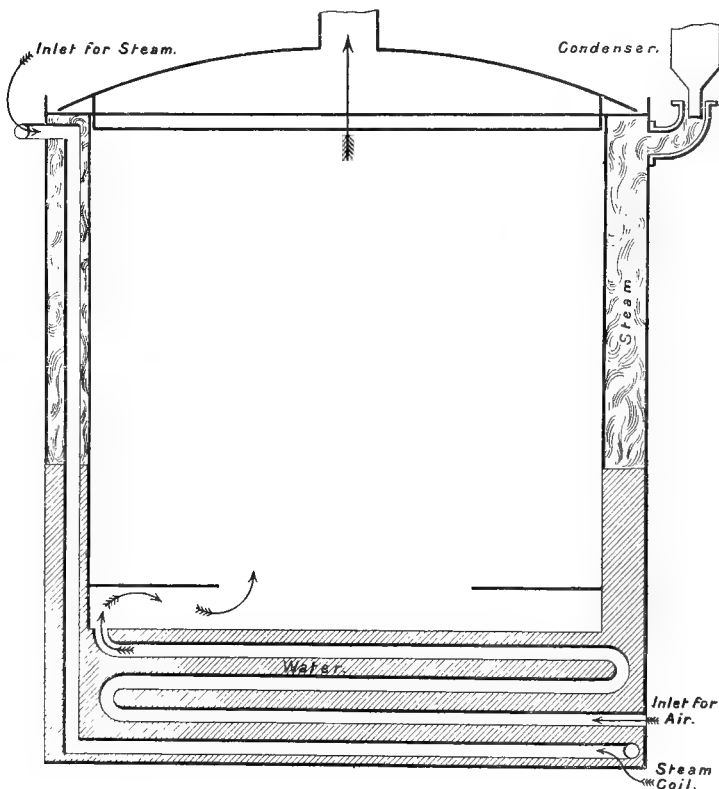


Fig. 5.—Diagrammatic View of Air-bath.

dried. The heating is chiefly done by conduction from the sides of the bath through the shelves; a current of hot air, approaching in temperature to that of boiling water, is always passing through the bath, and rapidly removes the vapour.

The condenser is supplied with cold water, and prevents loss of water. It conduces greatly to the efficiency of the bath to use distilled water, as no scale is produced on the coils and sides

of the oven. To prevent loss of heat the oven may be lagged with felt, asbestos, or kieselguhr.

The most efficient condenser has been found to be a spiral coil of tubing (preferably copper), which fits rather closely into a tube. The cold water enters at the top, and passes by a straight portion of the tubing to the lowest coil, whence it circulates upwards, and finds an exit at the top.

A diagrammatic figure of the bath will assist comprehension of the details (Fig. 5).

A very convenient water-bath for milk analysis is that devised by Vieth: the chief advantage of this lies in the lid, which, instead of merely having holes made in it in which the basins fit, has a copper ring fastened into each. This enables the basins to be taken off without contact between the fingers and the hot lid. This bath may be heated by a steam coil or a gas flame, and is conveniently supplied with water by a constant level apparatus.

If dry steam is available it is very convenient to use steam coils to heat the bath, and to condense the steam after it has passed the coils. The condensed steam can be used as distilled water, and is usually pure enough for all purposes. It is liable, however, to contain traces of copper, if this metal is employed in the construction of the coils, condenser, etc.: if the boilers prime to any extent, it will also contain impurities (salts, scale-preventing composition, etc.), derived from the boiler.

**Estimation of Ash.**—The residue of total solids serves excellently for the determination of the ash. By igniting over a small Bunsen flame, or an Argand burner, or in a muffle, a white ash can be obtained. The temperature must not be allowed to rise above a barely perceptible red heat, or distinct volatilisation of alkaline chlorides may occur. If the asbestos method of total solid estimation has been used, a somewhat higher temperature may be employed than if simple evaporation in platinum has been resorted to.

A more exact determination is obtained by evaporating a larger quantity of milk than is usually taken for total solid estimation—25 to 50 grammes—and gently igniting till thoroughly charred: the mass is extracted with hot water and filtered, the insoluble portion and the filter being (after washing) ignited at a red heat till white: this will give the insoluble ash.

By evaporating the filtrate and cautiously igniting at a low temperature, the soluble ash is obtained. The sum of the soluble and insoluble ash gives the total ash: the results obtained in this way are usually slightly higher (about 0.02 per cent.) than the ash obtained by ignition of the total solid residue.

If it be desired to examine the ash further, it is desirable to keep the insoluble ash separate from the soluble portion.

In the solution of the soluble ash the alkalinity may be determined by titration with  $\frac{N}{10}$  acid, methyl orange being used as an indicator; and the chlorine, by titration with standard nitrate of silver, using potassium chromate as indicator.

The insoluble ash is dissolved in a slight excess of dilute hydrochloric acid, and the solution (nearly neutralised with ammonia, if necessary) heated to boiling; a cold saturated solution of ammonium oxalate is dropped in slowly till the addition of a further drop gives no more precipitate. After standing at least two hours the precipitate is filtered off, washed, and ignited at a low temperature to convert the oxalate into carbonate; it is best to moisten the ignited precipitate with ammonium carbonate solution and re-ignite at a very low temperature. The precipitate, after weighing, is dissolved in dilute hydrochloric acid, keeping the bulk small; ammonia is added to alkaline reaction, and the small precipitate of calcium phosphate collected, ignited, and weighed. Its weight is subtracted from the previous weight, and the difference gives the weight of the calcium carbonate, which, multiplied by 0.4, gives the calcium, or by 0.56 the lime, contained in it; the weight of the calcium phosphate multiplied by 0.3871 gives the calcium, or by 0.5419 the lime, contained in it. The total calcium or lime is the sum of the two.

The filtrate is made strongly ammoniacal by the addition of 0.880 ammonia and allowed to stand twenty-four hours. The precipitated magnesium-ammonium phosphate is filtered off, washed with dilute ammonia, ignited, and the magnesium pyrophosphate weighed. Its weight multiplied by 0.21622 will give the magnesium, and by 0.36036 the magnesia contained in it.

To the filtrate from this, magnesia mixture is added. The precipitate of magnesium-ammonium phosphate is filtered off after twenty-four hours and treated as above.

From the total weight of the two quantities of magnesium pyrophosphate the phosphoric anhydride is calculated by multiplying by 0.63964; to this is added the phosphoric anhydride in the calcium phosphate calculated by multiplying the weight by 0.4581.

The above method has proved satisfactory in the author's hands, though it takes no account of the traces of iron present, which is precipitated with the calcium phosphate, or the magnesium-ammonium phosphate. If desired, this may be estimated by dissolving up the precipitate of calcium phosphate and the first magnesium-ammonium phosphate precipitate in dilute hydrochloric acid, and determining the iron colorimetrically as sulphide, ferrocyanide, or thio-cyanate.

To estimate alkalis, another portion of milk is ignited, as before, and the total ash dissolved in dilute hydrochloric acid and boiled; a few drops of barium chloride solution are added containing not more than 0.1 gramme of barium to 100 grammes of milk, and the boiling continued for some minutes. After some hours the precipitate of barium sulphate is filtered off, ignited, and weighed; its weight multiplied by 0.34335 will give the sulphuric anhydride in the milk. If an excess of barium chloride has been added, a little phosphoric acid, or ammonium phosphate, may now be dropped into the filtrate, though it is not necessary if the quantity of barium chloride given above has been employed. A quantity of ferric chloride solution sufficient to colour the solution brown is added, and the filtrate made alkaline with ammonia. The precipitate is well washed, and the filtrate evaporated and very cautiously ignited; the weight will give the alkaline chlorides. The residue is dissolved in water, and the solution should be quite clear; if it is not so, a little ammonium carbonate is added, the liquid evaporated to dryness, and the residue cautiously ignited; the residue is again taken up with water, the solution filtered and evaporated, and the residue cautiously ignited and weighed.

The chlorine in this may be titrated by standard silver nitrate, using potassium chromate as indicator. The potassium and sodium are calculated by the following formula:—

Let W = weight of alkaline chlorides  
and C = weight of chlorine therein.

$$\begin{aligned} \text{The weight of sodium} &= 2.997 C - 1.4254 W. \\ \text{,, ,, potassium} &= 2.4254 W - 3.997 C. \end{aligned}$$

The potassium may be directly estimated by evaporating the solution of alkaline chlorides with an excess of platinum tetrachloride solution almost to dryness; the pasty residue is treated with 80 per cent. alcohol containing about 5 per cent. of ether, and washed repeatedly with this; the alcohol is passed through a weighed filter or, preferably, a Gooch crucible, and the precipitate is finally transferred to this and washed with ether. It is then dried at 100° C. and weighed; the weight multiplied by 0.3056 will give the potassium chloride; this subtracted from the weight of the alkaline chlorides will give the sodium chloride.

The potassium chloride multiplied by 0.5241 will give potassium and by 0.6314 potash. The sodium chloride multiplied by 0.3932 will give sodium and by 0.5299 soda.

The above scheme of analysis has been worked out so as to use as little milk as possible, as the amount available is sometimes limited. Many obvious modifications are available and will readily suggest themselves to analysts; thus the chlorine

may be gravimetrically estimated, and, if the amount of milk be sufficient, the phosphoric acid may be separated from another portion by the molybdcic acid method. Such modifications will be found in works on inorganic analysis, and need not be described in detail.

If boric acid be present, it will be found to interfere with the results of the analysis, as a portion of this remains in the insoluble ash; this may be removed by evaporating the acid solution to dryness and repeatedly evaporating with small portions of methyl alcohol. It will also interfere with the estimation of alkalinity in the soluble ash, as the alkali shown by methyl orange will be due to borax; the chlorine is best estimated in this case gravimetrically as silver chloride.

Boric acid is detected by slightly acidifying the ash with hydrochloric acid and dipping a piece of turmeric paper into the solution; on drying, this will assume a pinkish-brown coloration, turned a very dark green—almost black—on moistening with a solution of sodium bicarbonate. Cribb and Arnaud prepare turmeric paper by boiling 2 grammes of turmeric and 2 grammes of tartaric acid with 80 per cent. alcohol till the latter is dissolved, and soaking strips of filter paper in this solution. It is very delicate, and should be kept in the dark. Another test is to moisten the ash with dilute sulphuric acid and add strong alcohol; if boric acid be present, the alcohol will burn with a greenish flame on applying a match.

Boric acid may also be detected in the milk direct, by acidifying with hydrochloric acid, and dipping the turmeric paper in the serum.

Another simple test for the presence of boric acid consists in putting about  $\frac{1}{2}$  oz. of milk in a glass, adding half its bulk of phenol-phthalein, and dilute caustic soda solution drop by drop, with constant stirring, till a faint permanent pink colour is produced. Some of the pink-coloured milk is poured into two test tubes. To one tube is added an equal bulk of water, and to the other an equal bulk of a neutral mixture of 1 part pure glycerol and 1 part water. In genuine milk both tubes remain pink, and the colours are practically identical, but in the presence of boric acid the water tube becomes darker in tint, and the glycerol tube much lighter—usually quite white.

If boric acid be present, 25 to 50 grammes of milk should be taken for estimation. After addition of about 0.2 gramme caustic soda, the milk is evaporated and thoroughly charred by ignition; the residue is extracted by dilute acetic acid, and well washed with as small a quantity of water as possible; the solution is filtered into a small flask, to which a condenser is fitted, and

distilled to dryness into about 10 c.c. of strong ammonia; eight successive portions of 10 c.c. each of methyl alcohol are added and distilled off.

About 1 gramme of lime is ignited in a capacious platinum basin in a muffle at the highest temperature attainable, and the basin and lime weighed. The ammoniacal distillate is now added and the liquid evaporated on the water-bath; the basin is again ignited in a muffle and weighed. The increase of weight represents the boric anhydride.

Hehner prefers the use of a measured quantity of sodium phosphate solution of known strength for fixing the boric acid instead of ammonia and lime. He distils directly into the sodium phosphate solution, evaporates and cautiously ignites. The weight of the residue of pyro-phosphate obtained from an equal measure of sodium phosphate solution is subtracted from the weight of the residue; the difference represents boric anhydride. It is necessary, however, to ignite very cautiously, as sodium phosphate is liable to spurt.

Thompson has shown that boric acid may be titrated with caustic alkali, using phenol-phthalein as indicator, provided at least 30 per cent. of glycerol be present. His directions are: 1 or 2 grammes of caustic soda are added to 100 c.c. of milk and the whole evaporated to dryness in a platinum dish. The residue is thoroughly charred, heated with 20 c.c. of water and hydrochloric acid added drop by drop till all but carbon is dissolved. The whole is transferred to a 100 c.c. flask, the bulk not being allowed to get above 50 to 60 c.c., and half a gramme of dry calcium chloride added. To this mixture a few drops of phenol-phthalein solution are added, then a 10 per cent. solution of caustic soda, till a permanent pink colour is perceptible, and, finally, 25 c.c. of lime water. In this way all the phosphoric acid is precipitated as calcium phosphate. The mixture is made up to 100 c.c., mixed, and filtered through a dry filter. To 50 c.c. of the filtrate (= 50 c.c. milk) normal sulphuric acid is added till the pink colour is gone, then a few drops of methyl orange, and the addition of acid continued until the yellow is just changed

to pink.  $\frac{N}{5}$  caustic soda solution is added till the liquid assumes a yellow tinge, excess of soda being avoided. At this stage all acids likely to be present exist as salts neutral to phenol-phthalein, except boric acid and a little carbonic acid, which last is expelled by a few minutes boiling. The solution is cooled, a little more phenol-phthalein added, and as much glycerol as will give at least 30 per cent. of that substance in the final solution, and titrated with  $\frac{N}{5}$  caustic soda till a permanent pink colour is produced.



Each c.c. of  $\frac{N}{5}$  caustic soda solution is equal to 0.0124 gramme crystallised boric acid or 0.0070 gramme boric anhydride.

Phosphoric acid can be separated from boric acid by precipitation as calcium phosphate, if not more than 0.2 per cent. of crystallised boric acid be present.

As excessive heating is apt to drive off boric acid, it is necessary to carry the charring so far only as will give a colourless solution.

This method tends to give rather low results, as a portion of the boric acid remains in the calcium precipitate, while, on the other hand, all the phosphoric acid may not be removed. Shrewsbury recommends that after charring the milk, and dissolving the ash in acid, a little phenol-phthalein solution be added, and then dilute caustic soda solution and calcium chloride solution alternately till a permanent pink is produced. The filtrate is then titrated with acid using methyl orange as indicator, and then using phenol-phthalein with alkali in the presence of glycerol. If more than 1.7 c.c.  $\frac{N}{10}$  alkali is used the

precipitate should be dissolved up and reprecipitated, the second filtrate titrated, and the results added to the first titration; if necessary, the process should be repeated.

Allen and Tankard have devised a method for the estimation of boric acid, which consists in evaporating the liquid to be tested to dryness with a few cubic centimetres of 10 per cent. calcium chloride solution; in the case of milk or cream it is advisable to add just sufficient alkali solution to neutralise it to phenol-phthalein.

To 10 to 25 c.c., add  $\frac{1}{3}$  of its bulk of 10 per cent. calcium chloride solution, and just sufficient alkali to neutralise to phenol-phthalein; evaporate to dryness: ignite at a gentle heat till thoroughly charred, boil the residue with 150 c.c. of distilled water, and filter the liquid. The filter is returned to the dish, and the residue ignited till white at a moderate temperature, and boiled with a further 150 c.c. of water. The liquid is allowed to stand overnight, and is filtered cold; the mixed liquids are evaporated to a volume of 25 to 30 c.c.: and after cooling neutralised with  $\frac{N}{10}$  acid, using methyl alcohol as indicator. An equal volume of glycerol is added, and a little phenol-phthalein, and the solution titrated with  $\frac{N}{10}$  caustic soda (free from carbonate). The volume of  $\frac{N}{10}$  caustic soda required by an equal volume of glycerol is subtracted from the amount used, and the remainder multiplied by 0.0062 will give the weight of  $H_3BO_3$ .

The author and Miller have found that it is quite unnecessary either to evaporate the milk, ignite it, or to use any indicator other than phenol-phthalein; the method is—to a measured or weighed quantity of milk (10 c.c. suffices) add half its bulk of a 0.5 per cent. solution of phenol-phthalein, and run in alkali till a pink colour appears, boil, and titrate back while still boiling with acid solution till white, and finally with  $\frac{N}{10}$  alkali till faintly pink. The colour, though faint, is quite distinct, and no attempt should be made to obtain a pronounced pink colour. Add 30 per cent. of glycerol, and continue the titration with  $\frac{N}{10}$  alkali without further heating; subtract, if necessary, the glycerol blank, and the alkali used for the final titration multiplied by 0.0062 gives the boric acid.

Cassal and Gerrans find that an intense magenta-red colour is produced on treating solutions containing boric acid with curcumin—or ordinary turmeric itself—and oxalic acid, and drying the mixture on the water-bath. The colour is different from that obtained by the application of the ordinary turmeric test for boric acid and the reaction is far more delicate, extremely minute quantities of boric acid being easily detected. The colour is practically permanent for several hours—not less than ten or twelve—and fades very gradually on long keeping. The colouring matter is readily soluble in alcohol and ether without alteration, but is destroyed by the addition of water in excess. On treatment with alkali an intense blue colour is obtained, which is different from that obtained on treating the “rose-red” colouring matter formed in the ordinary turmeric test, with alkali. In applying the test for the detection of free or combined boric acid in milk and other food products it is convenient as a rule to operate on an ash. The ash is treated with a few drops of (1) dilute hydrochloric acid, (2) saturated solution of oxalic acid, and (3) alcoholic solution of curcumin or turmeric, and the mixture dried on the water-bath and taken up with a little alcohol. In cases where the amount of boric acid is very small the substance, the ash of which is to be operated upon, should be made alkaline with solution of barium hydroxide prior to evaporation and incineration. Caustic potash and caustic soda and salts of potassium and sodium in large amounts interfere with the formation of the colouring matter.

They also apply this reaction for the quantitative estimation of boric acid.

In the case of milk, from 15 to 20 grammes are weighed out, transferred to 100 c.c. flask, and made up to 100 c.c. with water. Ten c.c. (or more, according to circumstances) are transferred

to a porcelain dish and mixed with 15 to 20 grammes of purified sand (obtained by igniting "silver sand," boiling this with 25 per cent. hydrochloric acid, and thoroughly washing and drying). The use of such a medium as sand is essential in order to secure intimate and complete contact between the reacting substances at the drying point—which is the point of reaction. The mixture is made alkaline with barium hydroxide, and evaporated to dryness. Two c.c. of a 1 per cent. alcoholic solution of curcumin are added, and the mixture again evaporated to dryness, the mass being stirred from time to time to ensure thorough incorporation. To the mixture is now added 1 c.c. of a solution containing 25 c.c. hydrochloric acid and 10 grammes of oxalic acid in 100 c.c. of water, and the mass is again dried. The same operations are carried out with 10 c.c. of a standard solution of boric acid [1 c.c. being equal to 0.1 milligramme of boron trioxide ( $B_2O_3$ )].

The colour having been obtained in both cases, the sand is extracted with ordinary alcohol.

The coloured solution obtained from the milk is diluted with alcohol or methylated spirit until the colour is of the same degree of intensity as that formed from the standard; and the amount of boric acid is arrived at by an obvious calculation.

The colours are compared in two tubes of the same internal sectional diameter (about 1 centimetre) placed vertically against a white porcelain plate.

On comparing the two solutions it will occasionally be found that a certain amount of orange tint is exhibited by one or the other, due to the presence of curcumin in slight excess. When this is observed the tints must be made the same by the cautious addition of solution of curcumin to the solution which does not show the orange tint.

The result of a number of experiments made with known amounts of boric acid and borates show that the process is reliable and accurate.

**Estimation of Citric Acid in Milk.**—The proteins are precipitated by acid mercuric nitrate (p. 90) and a measured volume of the clear filtrate exactly neutralised with dilute caustic soda solution, using phenol-phthalein as indicator. A white precipitate of mercury and calcium phosphate and citrate is thrown down, collected on a filter, and washed with water; it is removed from the filter, suspended in water, and a little dilute hydrochloric acid added; sulphuretted hydrogen is passed through to precipitate the mercury as mercuric sulphide. After filtration, the the solution is boiled to remove sulphuretted hydrogen, and, after the addition of a little calcium chloride, cooled. It is then carefully neutralised, phenolphthalein being used as indicator; the

precipitate of calcium phosphate is filtered off, and the solution boiled and concentrated to a small bulk; the calcium citrate is thus precipitated. This should be washed with boiling water, collected on a small filter and ignited. To the ignited residue an excess of standard hydrochloric acid is added and the excess titrated back with standard alkali, methyl orange being the best indicator. Each cubic centimetre of  $\frac{N}{10}$  hydrochloric acid used represents 0.0064 gramme of citric acid.

The result must be corrected for the volume of the fat and protein thrown down as directed under milk-sugar.

**Gowing - Scopes' Method.** - L. Gowing-Scopes has investigated the method originated by Denigès, which consists in oxidising citric acid to acetone dicarboxylic acid, and the conversion of this into an insoluble basic mercury salt. He finds that, to obtain exact results, strict attention must be paid to details of manipulation.

For the estimation of citric acid in milk, the clear filtrate obtained by adding acid mercuric nitrate to milk as for the estimation of milk sugar is used; 10 c.c. of this is exactly neutralised with alkali, using phenol-phthalein as indicator; a precipitate will form, but this will be redissolved on the addition of 10 c.c. of a reagent prepared by covering 51 grammes of mercuric nitrate and 51 grammes of manganese nitrate with 68 c.c. of strong nitric acid, and after the addition of 100 c.c. of water to dissolve the salts, making up the volume to 250 c.c. and filtering.

The solution is diluted to 200 c.c., and boiled under reflux for three hours, filtered through a weighed Gooch crucible, and the precipitate well washed with cold water; the deposit on the sides of the flask may be removed by adding 1 or 2 c.c. of 1 per cent. nitric acid, and rubbing with a rod; after drying for five hours the precipitate is weighed. The colour of the precipitate should be nearly white; if it is yellow, this is due to the presence of basic salts, and the result will be high.

The weight of the precipitate multiplied by 0.1667 will give the amount of citric acid, and in calculating the percentage in the milk, the corrections for the volume of the precipitated proteins and fat must be made as in Vietl's method of milk-sugar estimation.

The method given above departs slightly from Gowing-Scopes' original method, as, owing to the presence of mercury in the filtrate, slightly more mercuric salts are present than he prescribes, but his researches have shown that variations of the amount of mercury used have far less influence on the results than variations in the other ingredients of his reagent, and the small

excess of mercury present does not necessitate any appreciable variation of the factor.

**Estimation of Milk-Sugar.**—Milk-sugar is generally estimated indirectly, as it is not possible to isolate it quantitatively from milk in a state of purity. The following method may, however, be used to obtain an approximate determination of the milk-sugar :—

**By Alcohol.**—To 10 c.c. of milk add 20 c.c. of 90 per cent. alcohol, well mix and filter; of the filtrate take 10 or 15 c.c., evaporate to dryness on a water-bath and dry at 100° C. (212° F.) till the weight is constant. Ignite the residue and weigh the ash. The weight of the residue less the weight of the ash will give the weight of the milk-sugar. The volume of the aqueous portion must be calculated; on mixing alcohol and water a contraction takes place; this with the quantities given is 0.4 c.c.; the volume occupied by the protein is on the average 0.25 c.c.; the volume of the fat is obtained by multiplying the percentage by weight by 0.111.

The percentage of milk-sugar is obtained by the following formula :—

$$M = \frac{30 - (0.65 + 0.111 F)}{x} \times (R - A) \times 10 \times \frac{1}{D},$$

where

- x = number of c.c. taken for estimation of residue.
- D = specific gravity of milk.
- F = percentage of fat in milk.
- R = weight of residue.
- A = „ „ ash.
- M = percentage of milk-sugar.

This method has a tendency to yield results about 0.2 to 0.3 per cent. too high.

**By the Polariscopes (Fig. 6).**—The quickest method of milk-sugar estimation is by the polariscopes; before the milk can be polarised it is necessary to remove the fat and proteins completely which interfere either by making the solution too opaque for reading or by polarising to the left.

**Wiley's Method.**—The investigations of Wiley have shown that mercury compounds are the most efficient for this purpose, of which “acid mercuric nitrate” is the most convenient. This is prepared as follows :—Mercury is dissolved in twice its weight of nitric acid of specific gravity 1.42, and, after solution, an equal bulk of water is added.

Basic lead acetate has also been used to remove fat and protein, but Wiley has proved that the results are not accurate, owing to the incomplete removal of protein. Still more inaccurate is the use of acetic acid, followed by boiling, which has been recommended by Blyth.

**Wiley-Ewell Method.**—Wiley and Ewell give the following method as the best for estimating milk-sugar by the polariscope:—They used a Schmidt & Hænsch polarimeter, with which 200 millimetres of a solution of 32.91 grammes of milk-sugar in 100 c.c. read 100 divisions of the scale. They take 65.82 grammes of milk, add 10 c.c. of acid mercuric nitrate solution (in this case the solution of mercury in nitric acid is diluted with 5 volumes of water), and dilute to 100 c.c. A similar quantity of milk is taken, 10 c.c. of acid mercuric nitrate added and diluted to 200 c.c. Each of these solutions is well mixed, filtered, and polarised in a 400 mm. tube.

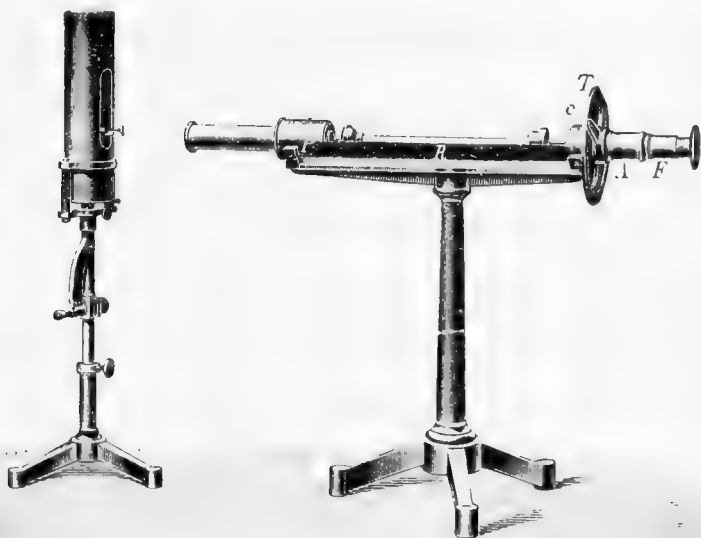


Fig. 6.—Polariscope.

Calling the reading of the solution obtained from 100 c.c.  $x$ , and that obtained from 200 c.c.  $y$ , the true percentage of milk-sugar is  $\frac{4y}{4(x-y)}$ .

The double dilution does away with any correction for the volume of the precipitated fat and protein. The rationale of the process lies in the fact that, while the percentage of milk-sugar and the volume of the precipitate are constant, the total volume varies.

Let  $m$  be the percentage of milk-sugar, and  $v$  the volume of precipitate ;

$$\text{then} \quad x = 4m \times \frac{100}{100-v} \quad \dots \quad (1)$$

$$\text{and} \quad y = 2m \times \frac{200}{200-v} \quad \dots \quad (2)$$

$$\begin{aligned} \text{Now} \quad \frac{xy}{4(x-y)} &= \frac{8m^2 \times \frac{100 \times 200}{(100-v)(200-v)}}{4\left(4m \times \frac{100}{100-v} - 2m \times \frac{200}{200-v}\right)} \\ &= \frac{8m^2 \times 100 \times 200}{4(4m \times (200-v) \times 100 - 2m \times (100-v) \times 200)} \\ &= \frac{8m^2 \times 100 \times 200}{4(4m \times 100 \times 200 - 2m \times 100 \times 200 - 4m \times 100v + 2m \times 200v)} \\ &= \frac{8m^2}{8m} - m. \end{aligned}$$

The volume may be calculated thus :—From (1) we see that

$$100-v = \frac{4m \times 100}{x}$$

$$\begin{aligned} \text{or} \quad v &= 100 - \frac{4m \times 100}{x} \\ &= 100 - \frac{4xy \times 100}{4x(x-y)} \\ &= 100 - \frac{y \times 100}{x-y} \\ &= \frac{100x - 200y}{x-y} \end{aligned}$$

which may be similarly deduced from (2).

This method not only allows of an estimation of milk-sugar to be made in milk without correction of any kind, but enables the volume of the precipitate of fat and protein to be calculated. The author, in conjunction with Boseley, has shown that the experimental error of Wiley and Ewell's method is, however, very appreciable ; and, though correct in principle, it is not so accurate in practice as originally claimed.

It is not necessary to adhere strictly to the volumes given ; by a modification of the formulæ the percentage can be calculated from any two dilutions, but the greatest delicacy of Wiley and Ewell's method—*i.e.*, the point at which the influence of unavoidable errors in reading is least—is obtained when the volume of water added to the more dilute solution is equal to the volume of the milk taken, less that of the fat and precipitated protein.

**Vieth Method.**—Vieth, when using the small Mitscherlich half-shadow polariscope made by Schmidt and Hænsch, prefers to add the stronger mercuric nitrate solution, described above, direct to the milk, and to polarise the resulting filtrate. He finds the volume of precipitated proteins from 100 c.c. of milk to amount on the average to 3 c.c., and, consequently, adds 3 c.c. of acid mercuric nitrate solution to allow for this. The method is carried out as follows:—Measure 50 c.c. of milk into a small flask, add 1.5 c.c. of acid mercuric nitrate, and well mix by shaking violently; pour the mixture on to a filter, and fill a polarimeter tube with the filtrate; polarise, and correct the reading for that obtained in a blank reading—*i.e.*, by reading a tube filled with water.

As the  $[\alpha]_D$  of milk-sugar is 52.5, the reading, if in angular degrees, can be converted into percentages of milk-sugar by the following formula—

$$m = \frac{100 \cdot 100}{52.5 \cdot l \cdot r}$$

Where  $m$  = number of grammes of milk-sugar per 100 c.c. of solution polarised.

$l$  = length of tube in millimetres.

$r$  = reading in angular degrees.

If a tube of 198.4 millimetres be used (these tubes are supplied with the instrument used by Vieth), the formula becomes

$$m = \frac{r}{1.042}$$

If the length of the tube be 200 millimetres, the formula is

$$m = \frac{r}{1.05}$$

The resulting figure representing milk-sugar in the solution polarised must be submitted to correction.

The volume of the liquid from which the fat and protein have been precipitated is the volume of the milk *plus* that of the mercuric nitrate *minus* that of the protein precipitate and fat.

As the volume of the mercuric nitrate was purposely made equal to that of the protein, both of these may be neglected, one compensating for the other.

Taking the volume of the milk as 100 c.c., the volume of fat in this will be the percentage by weight of fat multiplied by the specific gravity of the milk, divided by the specific gravity of the fat.

The milk-sugar may be either calculated as hydrated or anhydrous sugar, but it is usual to calculate it in milk analysis as anhydrous sugar.



The following formula expresses the percentage of anhydrous milk-sugar in the milk when a tube of 198.4 mm. is used :—

$$m^1 = \frac{r}{1.042} \times \frac{100}{100} \times \frac{Fd}{0.93} \times \frac{1}{d} \times 0.95,$$

$m^1$  = percentage of anhydrous milk-sugar by weight,

$r$  = reading,

$F$  = percentage of fat by weight,

$d$  = specific gravity of milk.

For the expression  $\frac{Fd}{0.93}$  it is usually exact enough to employ the expression  $F \times 1.11$ .

As an example, let us suppose that, using a 198.4 mm. tube

$$r = 5.5^{\circ}$$

$$F = 4.1$$

$$d = 1.032$$

$$m^1 = \frac{5.5}{1.042} \times 0.9545 \times \frac{1}{1.032} \times 0.95 = 4.58 \text{ per cent.}$$

Vieth states that when cream is analysed by this method, it is necessary to dilute with an equal bulk of water, the results being, of course, doubled.

**Richmond - Boseley Method.**—The author, in conjunction with Boseley, has shown that the calculation necessary in Vieth's method can be eliminated by adding to 100 c.c. of milk

(a) A quantity of water in c.c. equal to  $\frac{1}{16}$  degree of specific gravity.

(b) " " " " the fat  $\times 1.11$ .

(c) " " " " to reduce scale readings to percentages of milk-sugar.

(d) 3 c.c. of acid mercuric nitrate.

The percentage of milk-sugar can be read off directly in scale readings.

The values of  $c$  are :—For polariscopes reading angular degrees—

With 198.4 mm. tube, 10.0 c.c.

“ 200 “ 10.85 “

“ 500 “ 10.85 “ (divide readings by 2.5).

For the Laurent sugar scale ( $100^{\circ} = 21.67$  angular degrees)—

With 200 mm. tube, 2.33 c.c. (divide readings by 5).

“ 400 “ 2.33 “ ( “ “ 10).

“ 500 “ 2.33 “ ( “ “ 12.5)

For the Ventzke scale ( $100^{\circ} = 34.64$  angular degrees)—

With 200 mm. tube, 6.65 c.c. (divide readings by 3).

“ 400 “ 6.65 “ ( “ “ 6).

“ 500 “ 6.65 “ ( “ “ 7.5).

The author has recently shown that mercuric nitrate does not precipitate the whole of the proteins, and that a small further

precipitate is obtained by the addition of phospho-tungstic acid; the difference in the percentage of milk-sugar found after adding phospho-tungstic acid is, however, very small, and it is usually only in concentrated milks that it exceeds the experimental error. For exact estimations add to a measured volume of the mercuric filtrate  $\frac{1}{10}$  of a 10 per cent. phospho-tungstic acid solution and  $\frac{1}{10}$  of 1:1 sulphuric acid; filter, polarise, and multiply the readings by 1.1.

**Denigès' Method.** Denigès objects to the use of mercuric nitrate because it necessitates the use of a glass polarimeter tube, brass being attacked by the solution, and prefers the use of meta-phosphoric acid to precipitate the proteins. His method is as follows:—Prepare sodium meta-phosphate by carefully heating sodium-ammonium-hydrogen phosphate (microcosmic salt) in a platinum dish, till it is completely fused and no longer evolves gas. Pour on a cool plate, break up, and preserve in a stoppered bottle. Prepare a 5 per cent. aqueous solution by boiling 5.7 grammes of the finely powdered salt with 50 c.c. of water for five minutes, at the expiration of which time solution should be complete. Add immediately 50 c.c. of cold water, cool under a jet of water, and make up to 100 c.c. Twelve per cent. of the meta-phosphate is converted into ortho-phosphate by the boiling, and this is allowed for by taking 5.7 grammes instead of 5 grammes.

Add 25 c.c. of this freshly prepared solution to 10 c.c. of milk, then 60 c.c. of water, and 0.3 c.c. of acetic acid; make up to 100 c.c. and filter; after rejecting the first few drops, fill a polarisation tube with the filtrate. A 500 mm. tube is to be used, if possible, in preference to one of less length. It is hardly necessary to make any correction for the volume of the precipitate on account of the great dilution. As only 10 c.c. of milk are taken and diluted to 100 c.c., a very good polariscope must be used if accuracy is required. Unless glass polarisation tubes are unobtainable, the use of mercuric nitrate is preferable; an advantage of employing mercuric nitrate is that citric acid can be estimated in the same solution.

The proteins may also be precipitated by adding to milk an equal volume of a saturated solution of picric acid containing 1 per cent. of acetic acid.

**Fehling's Solution Method.**—Another method, which is largely employed for the estimation of milk-sugar, depends on the oxidation of the sugar by alkaline cupric solution, and the consequent reduction of the copper to the state of cuprous oxide.

The alkaline cupric solution cannot be applied direct to milk, as the proteins are somewhat attacked by the alkali.

The solution usually employed is Fehling's cupric tartrate

solution, which is prepared by dissolving 34·639 grammes of pure, crystallised copper sulphate in water, and diluting to 500 c.c.; 173 grammes of pure sodium-potassium tartrate (Rochelle salt), and 51 to 55 grammes of sodium hydroxide of good quality are also dissolved in water and made up to 500 c.c. Equal parts of these solutions are mixed (preferably at the time of making the test) to form Fehling's solution. It is convenient to use a 50 per cent. solution of caustic soda solution, which has been filtered clear through asbestos, for making the alkaline tartrate solution. The percentage of sodium hydroxide is estimated in this by titration, and such a quantity is weighed out as will give 51 grammes. Most of the impurities in ordinary caustic soda are insoluble in a 50 per cent. solution, so that this affords a ready means of purification.

Before estimating the milk-sugar in milk, the fat and protein must be removed; this may be accomplished by the following methods:—

(1) By diluting 10 c.c. of milk to about 100 c.c., adding 1·5 c.c. of 10 per cent. acetic acid solution and boiling; after cooling, the whole is made up to 100 c.c. (Citric acid may be substituted for acetic acid.)

(2) Add to 25 c.c. of milk about 200 c.c. of water and 10 c.c. of copper sulphate solution, as above: carefully neutralise with dilute caustic alkali solution, and make up to 250 c.c. This solution contains a small amount of copper.

(3) Carefully neutralise 10 c.c. of the filtrate from the milk which has been treated with acid mercuric nitrate with caustic alkali till exactly neutral to phenol-phthalein, filter, and pass sulphuretted hydrogen through the filtrate: filter, to separate the precipitated mercuric sulphides, and boil the filtrate to expel sulphuretted hydrogen. Make up to 100 c.c. (The mercury may be precipitated by phosphoric acid; add a small quantity of phosphoric acid or a soluble phosphate to the filtrate from mercuric nitrate; exactly neutralise, filter and wash the precipitate, and make up to 100 c.c.)

(4) Denigès' method, as above described.

Each of the methods of separating the fat and protein gives a solution, of which 50 c.c. contains the milk-sugar in 5 c.c. of milk, which, in a normal milk, represents about  $\frac{1}{2}$  gramme of milk-sugar.

The following modes of manipulation are among those in use:—

(1) *O'Sullivan's Method*.—Measure 50 c.c. of the filtrate into a beaker, and place this in a briskly-boiling water-bath; dilute a mixture of 30 c.c. of the copper solution, and 30 c.c. of the alkaline tartrate solution, with about twice its volume of water, and boil over a flame. When the milk-sugar solution has attained the temperature of the water-bath, pour into it the Fehling's solution, and keep on the water-bath for 13 to 15

minutes. Filter through a small filter, or, preferably, through a Gooch crucible, leaving the cuprous oxide as much as possible in the beaker; immediately the last drops of solution have been poured on the filter, pour boiling *well-boiled* water on the precipitate, and wash several times by decantation. The precipitate may also be separated and washed by centrifuging. Finally, transfer the precipitate to the filter, and wash well with boiling water. If a filter is used, transfer it to a small crucible, and ignite over a very small flame till the filter is thoroughly charred, and then gradually increase the flame till the highest available temperature is obtained. It is better to use a porcelain crucible than a platinum one, because platinum is permeable to reducing gases from the flame, and complete oxidation cannot be obtained. If a Gooch crucible is used it should be ignited in a muffle, and the cuprous oxide thus converted into cupric oxide. The weight of the cuprous oxide multiplied by 0.6024 will give the weight of hydrated milk-sugar. If a filter is used, the weight of the ash must, of course, be deducted; this should be obtained by igniting filters of the same kind, which have been treated in exactly the same manner as in the estimation of milk-sugar, using the same quantity of Fehling's solution, but omitting the milk-sugar. This is necessary, because the filter takes up mineral matter from the Fehling's solution. If this precaution is taken, filtration through paper is satisfactory.

The author finds it far more satisfactory, instead of employing an arbitrary factor, which is not of absolute exactitude, to weigh out a quantity of pure milk-sugar, approximating as nearly as possible to that contained in the solution to be tested, and to estimate the copper oxide obtained by treating it side by side with the actual estimation. The extra trouble is nominal, and the slight variations in the factor, due to dilution, etc., are thereby compensated.

(2) *Wein's Method*.—The same quantities of solutions are used, but the Fehling's solution, instead of being diluted with water, is mixed directly with the milk-sugar solution, placed over a naked flame, and raised as rapidly as possible to boiling: boiling is continued for exactly six minutes. The solution is filtered through a tube about 1 cm. wide, constricted at the end, and plugged with asbestos (Fig. 7). This tube is weighed, after having been gently ignited, and the filtration is hastened by means of a water pump; a small funnel is used to pour the solution into the tube. The precipitate of cuprous oxide is washed with boiling *well-boiled* water, and transferred to the tube. When the precipitate is well washed, the tube is sucked as dry as possible by the pump. It is then detached from the filter pump, and connected with a hydrogen apparatus, being clamped

in a horizontal position. A gentle current of hydrogen is passed through, and the tube cautiously heated by a small flame till all the cuprous oxide is reduced to metallic copper, and the tube is dry; it is allowed to cool while the hydrogen is passed slowly through, and weighed. The increase of weight gives the amount of copper reduced. Table X. should be used to calculate the amount of hydrated milk-sugar from the copper.

The table is used as follows:—Look up in the table the weight of copper (expressed in milligrammes) nearest to the weight obtained, and calculate from this, by a proportion sum, the corresponding weight of milk-sugar.

Thus, if the weight of copper is 334·1 milligrammes, take the weight of milk-sugar corresponding to 335 milligrammes.

$$335 = 251·6 \therefore 334·1 = 251·6 \times \frac{334·1}{335} = 250·9.$$

By taking a weight of milk-sugar as nearly as possible equal to that in the solution, and estimating the copper reduced by this, the calculation can be made in a similar manner from this.

The cuprous oxide may be reduced in a Gooch crucible by placing it in a muffle, and passing in a current of hydrogen.

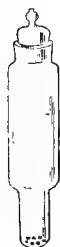


Fig. 7.  
Filter Tube for  
Milk-Sugar.

TABLE X.—FOR CALCULATING THE AMOUNT OF MILK-SUGAR FROM THE QUANTITIES OF COPPER REDUCED.

This Table is due to Wein.

Copper.	Milk-Sugar.	Copper.	Milk-Sugar.	Copper.	Milk-Sugar.
120	86·4	215	158·2	310	232·2
125	90·1	220	161·9	315	236·1
130	93·8	225	165·7	320	240·0
135	97·6	230	169·4	325	243·9
140	101·3	235	173·1	330	247·7
145	105·1	240	176·9	335	251·6
150	108·8	245	180·8	340	255·7
155	112·6	250	184·8	345	259·8
160	116·4	255	188·7	350	263·9
165	120·2	260	192·5	355	268·0
170	123·9	265	196·4	360	272·1
175	127·8	270	200·3	365	276·2
180	131·6	275	204·3	370	280·5
185	135·4	280	208·3	375	284·8
190	139·3	285	212·3	380	289·1
195	143·1	290	216·3	385	293·4
200	146·9	295	220·3	390	297·7
205	150·7	300	224·4	395	302·0
210	154·5	305	228·3	400	306·3

*Volumetric Method.*—Instead of weighing the copper reduced, the determination may be made volumetrically. The estimation is carried out as follows:—Place the solution obtained by removing the proteins by Methods 1, 3, or 4 (given above) in a burette graduated to  $\frac{1}{10}$  c.c. Place in a small flask 10 c.c. of Fehling's solution accurately measured (or 5 c.c. of each of the copper and alkaline tartrate solutions), diluted with 30 c.c. of water, and bring this to the boil by means of a small flame. Run in the sugar solution, adding 2 c.c. at a time, and boiling between each addition. When the blue colour of the liquid has nearly disappeared the sugar solution should be added in smaller amounts, but the titration should not be unduly prolonged. The end of the reaction is reached when, on removing the flame, and allowing the cuprous oxide to settle, the supernatant liquid appears colourless or faintly yellow when viewed against a white surface. To make sure that the copper is all reduced a few drops of the liquid may be filtered through a small filter into acetic acid, and potassium ferrocyanide added. If copper be still present, a brown coloration will be observed. It is advisable to repeat the titration, using 0.2 c.c. less of the milk-sugar solution, which may be all added at once, and the boiling continued for four minutes; a small excess of copper should be present, and this is reduced by small additions of the sugar solution. Should no copper be present, the experiment must be repeated, using a still smaller amount of liquid. Ten c.c. of Fehling's solution is reduced by 0.0676 gramme of hydrated milk-sugar; this quantity is, therefore, contained in the volume of sugar solution used for titration.

For example, 10.260 grammes of milk were, after removal of proteins, made up to 100 c.c. Ten c.c. of Fehling's solution required 13.0 and 13.1 c.c. of this in two experiments, mean 13.05 c.c.; therefore,

13.05 c.c. contain	0.0676 gramme milk-sugar.
and 100 c.c. contain	0.518            "
10.260 grammes milk contain	0.518            "
= 5.05 per cent. hydrated milk-sugar.	
= 4.80 per cent. anhydrous milk-sugar.	

It is advisable to titrate a solution of pure milk-sugar containing about 0.5 gramme per 100 c.c. to obtain the exact value of 10 c.c. of Fehling's solution.

**Ling and Rendle's Method.**—Ling and Rendle give the following method for the volumetric determination of reducing sugars:—

Fehling's solution is prepared thus:—

*Solution No. 1.*—69.278 grammes of crystallised copper sulphate are dissolved in water and made up to 1 litre.

*Solution No. 2.*—346 grammes of crystallised Rochelle salt are dissolved in hot water, mixed with 142 grammes of caustic soda, also dissolved in water, and, after cooling, made up to 1 litre.

Equal volumes of these two solutions are mixed for use as wanted, and the mixed solution should not be kept longer than a day.

For the estimation of milk-sugar 10 c.c. of the freshly mixed Fehling's solution is measured into a 200 c.c. flask and raised to boiling. The solution of milk from which the proteins have been removed (about 5 c.c. of milk per 100 c.c.) is then run into the boiling solution in small amounts, commencing with 5 c.c. After each addition the mixture is boiled, the solution being kept rotated. About a dozen drops of the indicator (see below) are placed on a porcelain or opal glass plate, and when it is judged that the precipitation of cuprous oxide is complete, a drop of the liquid is withdrawn, and brought into contact with a drop of the indicator. The test must be carried out rapidly, and it is essential to keep as far as possible an atmosphere of steam in the flask, to keep out atmospheric oxygen. When a red coloration is no longer produced, all the copper has been precipitated. A second or third titration should be made to establish the end point accurately. The Fehling's solution should be standardised on a solution containing 0.25 gramme pure sugar in 100 c.c.

The indicator is prepared by dissolving 1 gramme of ferrous ammonium sulphate and 1.5 grammes of ammonium thiocyanate in 2.5 c.c. of concentrated hydrochloric acid and 10 c.c. of water. The solution then has a brownish-red colour, which is removed by the addition of a trace of zinc dust. On keeping, a red colour develops, but may be again decolourised by the addition of a further quantity of zinc dust; the delicacy is, however, impaired after it has been reduced several times. When freshly prepared it is almost too delicate, and the indicator is most useful after it has been reduced twice.

**Pavy's Solution Method.**—Pavy's ammoniacal cupric solution may be substituted for Fehling's solution. This is prepared by mixing 120 c.c. of Fehling's solution, 400 c.c. of 12 per cent. caustic soda solution, and 300 c.c. of strong ammonia (specific gravity 0.880), and diluting the whole to a litre.

One hundred c.c. of this solution are placed in a small flask, which is closed by an india-rubber stopper with two holes; through one passes the nozzle of a Mohr's burette, and through the other a bent tube, which dips into a flask containing cold water to absorb the ammonia given off. Hydrogen or coal gas may be passed through the flask containing the Pavy solution.

The solution is brought to boiling, and the sugar solution run in gradually, till the blue colour of the liquid is destroyed, the boiling being maintained the whole time, and the sugar solution run in slowly towards the end.

As the reaction takes place somewhat slowly, boiling must be continued for a few minutes before it can be finally decided that the blue colour is permanent.

It is necessary to repeat the titration, adding a little less of the solution, as with Fehling's solution. This may be advantageously added all at once, and the boiling continued for five minutes. If the boiling be unduly prolonged, the ammonia may be boiled off, and cuprous oxide will then begin to deposit: in order to avoid this, Shenstone places a tapped funnel in the cork, by means of which an addition of strong ammonia can be made if necessary.

Stokes and Bodmer strongly recommend this method, and state that the reducing power of milk-sugar is 52 per cent. of that of glucose—*i.e.*, 100 c.c. of Pavy solution = 0.0961 gramme of milk-sugar.

It is advisable to standardise the Pavy's solution on a solution of pure milk-sugar containing 0.5 gramme per 100 c.c.

Helmer has shown that by varying the proportion of salts in solution, such as alkaline tartrates and carbonates, the accuracy of the results is affected; but by standardising the solution at the time of using with a solution of pure milk-sugar, the effect of any such variations is eliminated.

Allen has modified the procedure by placing a layer of petroleum over the Pavy solution, and dispensing with the cork. This enables an ordinary burette, or even pipette, to be used.

**Estimation of Cane Sugar in Milk.**—Cane sugar is sometimes added as an adulterant of milk, but the determination is more often required in the case of condensed milks.

An approximate estimation may be made by estimating the sugar by precipitation with alcohol, and the milk-sugar by Fehling or Pavy solution; the difference between the two will not be far from the cane sugar.

**Muter Method.**—Muter estimates as follows: 10 grammes of milk are evaporated to dryness on 4 grammes of hydrated calcium sulphate with frequent stirring, so that nothing sticks to the basin. The dry residue is powdered, placed in a *dried* filter, and extracted with ether in a Soxhlet apparatus. The residue, together with the filter, is transferred to a beaker, 20 c.c. of hot (not boiling) water added, and the whole well stirred; 30 c.c. of rectified spirit (60° overproof, sp. gr. 0.825) are then added, and the mixture allowed to cool, stirring occasionally. When cool, it is thrown on a filter and washed with



*proof* spirit till the filtrate measures 120 c.c. 60 c.c. are evaporated in a dish on the water-bath and the residue weighed; the residue is then ignited and the ash weighed, the weight of the residue less that of the ash being the total sugar. In the other 60 c.c., the milk-sugar is estimated by Fehling's or Pavy's solution; the difference between this and the total sugar is cane sugar. A deduction should be made from this of

0.5 per cent.	when the difference is 0.5 or under,
0.2	” ” ” ” 0.5 to 1.0,
0.1	” ” ” ” 1.0 to 1.5, and
none per cent.	if the difference exceeds 2.0.

This method is stated to give fair results with percentages of cane sugar exceeding a half per cent.

**Shenstone's Method.**—The cane sugar may also be estimated by determining the total polarisation of the sample as directed for milk-sugar, and by estimating the milk-sugar gravimetrically or volumetrically by Pavy's or Fehling's solution. The difference between the percentage of anhydrous milk-sugar found by reduction of copper and that deduced from the polarisation divided by 1.217 will give the percentage of cane sugar. This method yields excellent results with mixtures of fresh milk and cane sugar, and with many samples of sweetened condensed milks, but is apt to lead to figures below the truth, if the milk has been much heated, owing to the reduction in the rotatory power of milk-sugar under these conditions.

Grünhut and Rüber have shown that the estimation of milk-sugar and cane sugar in condensed milk by reduction with Fehling's solution does not give accurate results; the figures are always high for milk-sugar, and the cane sugar correspondingly low.

By polarisation before and after inversion, using Herzfeld's formula for cane sugar, they obtain satisfactory results.

**Stokes - Bodmer Method.**—Stokes and Bodmer prefer to estimate the milk-sugar by titration with Pavy's solution (Fehling's solution can be substituted for this), to invert the cane sugar by boiling with 2 per cent. of citric acid for ten minutes, and then to estimate the combined milk-sugar and resulting mixture of glucose and fructose by titration; the difference between the two figures will be due to the products of hydrolysis of cane sugar. In this case it is advisable to standardise the solution on a mixture of milk-sugar and inverted cane sugar in about the same proportions as found in the milk. The determinations may also be made gravimetrically.

Watts and Tempany have proved that in the presence of milk constituents cane sugar is not completely inverted by boiling

for 10 minutes with citric acid, and recommend that the time of heating should be continued for 40 minutes.

**By Invertase.**—The best method of estimating cane sugar depends on the hydrolysis of cane sugar by invertase, the enzyme of yeast. This is carried out as follows:—Estimate the rotation due to milk and cane sugar by polarisation of the solution obtained by precipitation with mercuric nitrate (100 c.c. of milk should be taken). 25 c.c. of the solution are placed in a flask, a drop or two of phenolphthalein added, and dilute caustic soda solution run in till neutral. This solution is filtered into a 50 c.c. flask, and the precipitate washed with water till the filtrate and washings measure about 45 c.c. 0.05 gramme of invertase, or 1 gramme of yeast, is added, together with a drop of acetic acid and a few drops of toluene, and the whole made up to 50 c.c. The flask is corked and allowed to remain at about 55° (131° F.) for five hours. A little alumina cream is added and the whole made up to 55 c.c., filtered, and polarised; the temperature at which the solution is polarised should be noted.

The reading should be multiplied by  $\frac{55}{25} = 2.2$ ; the reading due to cane sugar is found by the formula

$$R_s = \frac{100 (R_i - R_a)}{142.66 - \frac{t}{2}}$$

$R_s$  = rotation due to sucrose.

$R_i$  = " before inversion.

$R_a$  = " after inversion corrected by multiplying by 2.2.

$t$  = temperature in degrees Centigrade.

The percentage of cane sugar is calculated from the rotation deduced from this formula by the method given for milk-sugar, bearing in mind that the  $[\alpha]_D$  of cane sugar is 66.5 instead of 52.5, and that it does not require to be converted into anhydrous sugar.

Harrison has shown that when cane sugar is inverted by citric acid the polarimetric reading of the inverted cane sugar is  $-42.03 + 0.5t$  for each 100 that the cane sugar polarised to the right; also that inversion of mixtures of milk and cane sugar with citric acid fails to yield a clear solution. He proposes the following method:—About 75 grammes of condensed milk are weighed out and diluted to 250 c.c.; to 100 c.c. of this solution 3 c.c. of acid mercuric nitrate are added, the mixture well shaken and filtered, and the filtrate is polarised in a 200 mm. tube. 50 c.c. or more of the filtrate are introduced into a clean dry flask of about 100 c.c. capacity, and the whole weighed on a balance turning to 0.02 gramme. The solution is then placed in

a briskly boiling water-bath, and kept there for *exactly* seven minutes. The flask and its contents are then cooled, reweighed, and the amount of water lost by evaporation replaced, and the whole thoroughly mixed, filtered, and examined polarimetrically, the temperature ( $t$ ) at the time of polarisation being noted.

The reading after inversion is subtracted from that before inversion and divided by  $\frac{142.66 - 0.5t}{100}$  ( $t =$  degrees Centigrade), the result will be the reading due to cane sugar (C), and this, subtracted from the reading before inversion, will be the reading due to milk sugar (M).

From these readings the percentages of cane sugar and milk-sugar are calculated as follows:—

Let  $W =$  number of grammes of condensed milk per 100 c.c.,  
 $F =$  percentage of fat,  
 $P =$  percentage of proteins,

then percentage of cane sugar is—

$$C \times \frac{100 - \left( \frac{W \times F \times 1.08 + W \times P \times 0.84}{100} \right)}{100} \times \frac{1}{1.33} \times \frac{100}{W},$$

and the percentage of milk-sugar is—

$$M \times \frac{100 - \left( \frac{W \times F \times 1.08 + W \times P \times 0.84}{100} \right)}{100} \times \frac{1}{1.106} \times \frac{100}{W}.$$

He also, at the author's suggestion, has worked out the following method, which avoids the calculation; add to 100 c.c. of diluted condensed milk—

- (a) 10.25 c.c. of water,
- (b) a number of c.c. equal to  $W \times F \times 1.08$ ,
- (c) 3 c.c. of acid mercuric nitrate;

polarise, and calculate the reading due to cane sugar as before.

Then the difference between the reading before inversion and that due to cane sugar multiplied by  $\frac{100}{W}$  will give the percentage of milk-sugar, and the reading due to cane sugar multiplied by  $\frac{100}{1.2W}$  will give the percentage of cane sugar.

The fat may be estimated by a rapid method, as the deviation from the truth due to an error in the fat estimation of 0.5 per cent. is only 0.1 per cent. in the cane sugar; the possible error due to the use of the simplified method does not exceed  $\pm 0.2$  per cent. Richardson and Jaffé have proposed a somewhat

similar method, but invert and polarise at  $86^{\circ}$ , at which temperature inverted cane sugar reads  $0^{\circ}$ ; the method is, however, less convenient than that of Harrison.

The addition of phospho-tungstic acid to the acid mercuric nitrate filtrate (p. 95), gives slightly higher and more correct results, and should not be neglected with condensed milk.

**Other Methods.**—An approximation to the percentage of cane sugar can be obtained by determining the total polarisation, and deducing the milk-sugar by multiplying the ash by 6.5, or the aldehyde figure by 0.24. This method serves for controlling the preparation of condensed milk, but is of course of only approximate accuracy.

Another method, which gives fair approximate results, is to calculate the solids not fat (*a*) from the specific gravity and percentage of fat, (*b*) from the percentage of ash by multiplying by 12, or the aldehyde figure by 0.44. The cane sugar will be represented by  $a - b$ . If the original milk is available the ratio between the solids not fat and the aldehyde figure may be determined, and this figure substituted for 0.44.

**Cotton's Method of Detecting Cane Sugar.**—Cotton gives the following test for cane sugar in milk:—10 c.c. of the milk are mixed with 0.5 gramme of powdered ammonium molybdate and 10 c.c. of dilute hydrochloric acid (1 : 10). In a second tube 10 c.c. of milk of known purity, or 10 c.c. of a 6 per cent. solution of lactose, are similarly treated. The two tubes are placed in the water-bath and the temperature gradually raised. At about  $80^{\circ}$  C. the milk, if adulterated with saccharose, assumes an intense blue colour, whilst the genuine milk, or solution of lactose, remains practically unaltered. On boiling, these also turn blue, but to a less extent than the adulterated milk.

This test will detect 0.1 per cent. of cane sugar, but if this substance be added to milk, larger quantities are almost invariably added.

De Koninck modifies Cotton's test by adding 2 c.c. of a saturated solution of ammonium molybdate and 8 c.c. of acid (1 : 8) to 10 c.c. of milk. The test tube is gradually heated to  $80^{\circ}$  C. (not higher), and kept for five minutes at this temperature.

W. H. Anderson has recently recommended Cayaux's test for cane sugar; this consists in adding to 15 c.c. of milk 1 c.c. of hydrochloric acid and 0.1 gramme of resorcinol. On boiling a red colour is produced in the presence of cane sugar; 0.2 per cent. is detected by this test.

Leffmann and Gawalowsky later employ the reaction with sesamé oil and hydrochloric acid to test for cane sugar. 1 c.c. each of sesamé oil and hydrochloric acid are mixed with a little of the filtrate produced by adding strong hydrochloric acid to

milk, and the mixture actively shaken for a few moments; if a red colour is produced on standing for 30 minutes, the presence of cane sugar may be assumed.

**Detection and Estimation of Starch in Milk.**—Starch is occasionally added to milk as an adulterant, and can be detected by the blue colour given by iodine; the iodine test is best applied to the whey, as the proteins of milk somewhat interfere.

Starch cannot be estimated with any great exactitude, as it becomes partly converted into other bodies in milk, either by means of an enzyme or by micro-organisms.

To estimate it, the milk should be raised to boiling and cooled; the milk-sugar should be estimated by one of the gravimetric methods given above, preferably by that of Wein. 20 c.c. of milk should be diluted to about 95 c.c., 3 c.c. of 10 per cent. acetic acid added, and the whole warmed to about 80° C., cooled and made up to 100 c.c. 50 c.c. of the filtrate should be carefully neutralised, and warmed to 65° C. (150° F.); 2 c.c. of a diastase solution (containing the diastase from 2 grammes of malt) should be added, and the solution kept at 65° C. for two hours. At the expiration of that time it should be raised to boiling and evaporated on the water-bath to less than 25 c.c.; a little alumina cream should be added, if the solution be not clear, and the total made up to 25 c.c. This should be filtered and polarised. The copper reduced by 10 c.c. of this solution should be estimated by Wein's method.

The results should be calculated as follows:—Multiply the polarisation by 2·5; the result will be the polarisation due to milk-sugar, maltose, and dextrin; calculate, from this and the determination of milk-sugar, the polarisation due to maltose and dextrin.

Calculate the copper reduced from the 10 c.c. of diluted filtrate = 4 c.c. of milk by the table on p. 98, and subtract from this the milk-sugar present in this as calculated from the first estimation; the difference multiplied by 1·2 will represent maltose. The polarisation due to maltose can be calculated, using the value 137° for the  $[\alpha]_D$ , and the difference will be due to dextrin. From this the percentage of dextrin can be calculated, using the value for  $[\alpha]_D$  of 200°.

The percentage of dextrin *plus* that of the maltose divided by 1·056 will give that of the starch.

An approximate estimation may be made by subtracting from the solids not fat the ash multiplied by 12.

The estimation of starch is unsatisfactory, even if no other foreign carbohydrate be present; if other sugars have also been added, it is nearly impossible to estimate them.

**The Estimation of Fat.**—More attention has perhaps been

paid to the estimation of fat in milk than that of any other constituent. The methods are very numerous, and may conveniently be divided into three classes:—

(1) Gravimetric methods, in which the fat is separated from the milk by a suitable solvent, and weighed after evaporation of the solvent.

(2) Volumetric methods, in which the fat is separated from the milk by suitable means, and its volume measured.

(3) Indirect methods, in which the amount of fat is deduced from the determination of some physical property.

Of these methods the gravimetric methods are undoubtedly the most accurate, though they are all to a certain extent tedious and not capable of use by unskilled persons.

The solvent chiefly used for extracting the fat is ether, which is convenient on account of the low boiling point and heat of volatilisation, its great solvent power for fat, and its comparative great miscibility with water, which renders it unnecessary to have the milk solids in a state of absolute dryness.

Petroleum ether, chloroform, carbon disulphide, benzene, carbon tetrachloride, and amyl alcohol have also been used, but, though they give the same results, are not so convenient.

### (1) Gravimetric Methods.

The leading gravimetric methods are discussed in the following pages; on the whole the Gottlieb method is the best, though those due to Adams, Storch, Werner-Schmid, and Bell are little, if at all, inferior in accuracy.

**The Adams Method.**—Dr. M. A. Adams, Public Analyst for Kent, was led to devise this method from his observation that when milk was dropped on blotting-paper, it spread out to a much greater extent than was possible in a basin, flask, or even on a flat surface of glass; he was of opinion that extraction of fat by ether would be much more complete.

As originally designed, the method was as follows:—Strips of white blotting-paper, "mill 428,"  $2\frac{1}{2}$  inches wide by 22 inches long, were coiled up loosely and held by having a brass ring slipped over them. These were dried at 100° C. to constant weight, the weighings being performed in a weighing bottle to prevent absorption of moisture from the atmosphere. Five c.c. of milk was pipetted out into a small beaker and the weight noted; one of the coils was dropped in and the milk absorbed as completely as possible by the blotting-paper. When absorption was complete, the coil was carefully removed and stood, dry end downwards, on a glass plate, the beaker being again weighed and the quantity of milk taken up by the coil found from the

difference of the two weights. The coil was transferred to a drying oven at  $100^{\circ}$  C., and left therein till it ceased to lose weight. The original method was thus available for the determination of total solids as well as of fat. The dry coil was placed in a Soxhlet extractor\* (Fig. 8), and the fat separated from the solids not fat by ether. The total extract, after evaporation of the ether and drying at  $100^{\circ}$  C., was regarded as fat.

Allen and Chattaway modified this method by rolling up a

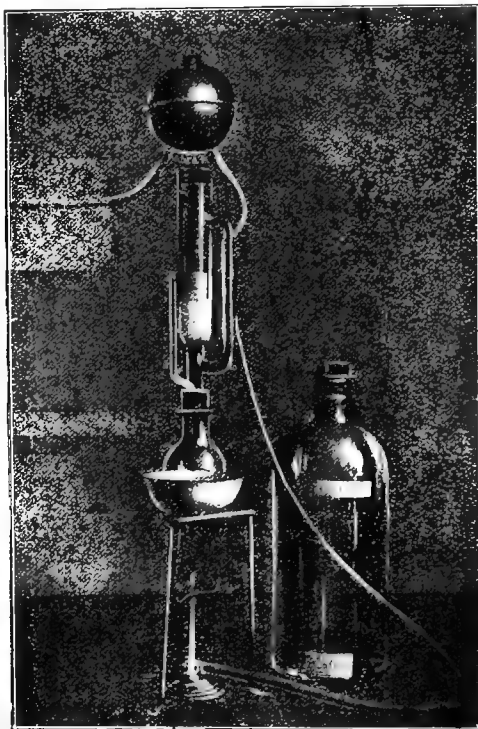


Fig. 8.—Soxhlet Extractor.

piece of string with the coil, to keep the layers of paper from touching each other; they also wrapped a piece of filter paper around it, in order that no milk might escape when a weighed quantity was poured thereon.

Thompson also modified it by hanging up a strip by one end

\* The Soxhlet extractor was really devised by Szombathy; it was described by Soxhlet, and due credit was given by him to the inventor. The apparatus is, however, always known by Soxhlet's name.

and running the milk on to it from a pipette, afterwards noting the weight of milk delivered by the pipette. He preferred to use filter paper instead of the blotting-paper recommended by Adams.

Vieth, immediately after the publication of the method, subjected it to an exhaustive test, and criticised it somewhat severely. He showed that blotting-paper contained matter soluble in ether, and that, as Adams had ignored this, the fat estimations made by Adams were too high; he also showed that the substance in filter paper soluble in ether was extracted with comparative slowness by this solvent. Faber later showed the same thing.

Notwithstanding these criticisms, the Milk Committee appointed by the Society of Public Analysts recommended its adoption by their members; it was indeed recommended that the papers should be previously extracted, but nothing was said of the difficulty of completely removing the matter soluble in ether, it being implied that twelve siphonings were sufficient to effect this. The recommendation of the Milk Committee was adopted at a General Meeting of the Society, and it thus became a quasi-official method. The use of this method for determining total solids was abandoned.

Notwithstanding the recommendation of the Milk Committee that the coils should be extracted previously to use, it became the general practice to omit this, and to use unextracted coils, making a deduction, from the weight of total extract, of the weight of the extract obtained from a coil when extracted alone for the same length of time.

The author showed that this last modification was not free from error; the matter soluble in ether was found to consist chiefly of a calcium salt of resinous acids, which was only of limited solubility in ether; the acids themselves were much more soluble, and when these were liberated by acids—even the small amount of acid found in milk—a greater extract was obtained in a given time. As the time usually allowed for extraction ( $1\frac{1}{2}$  hours) was not sufficient to remove the whole of the soluble matter from the blotting-paper—as much as ten hours being necessary—it followed that the matter extracted by ether from the coil was greater when a milk (containing small amounts of acid) was placed on a coil than when the coil was extracted alone. The difference was represented by the amount of resinous acids equivalent to the acidity of the milk, and was naturally not constant.

He found that alcohol completely extracted the coils—a fact also noted almost simultaneously by Soxhlet—but preferred the use of alcohol containing 10 per cent. of acetic acid. Ether containing acetic acid was also efficacious.



As the result of Soxhlet's researches, Schleicher and Schull placed a "fat-free" paper on the market, and this is very generally employed. Schleicher and Schull's "fat-free" paper gives a small ether extract consisting chiefly of loose fibres; this paper was at first remarkably free from extract, but later batches were found to contain quite an appreciable amount of extract. It is preferable for the analyst to extract his own papers for one to two hours with "acid alcohol."

Waller and Liebermann objected to the use of ether as a solvent for fat, on the ground that other substances contained in milk were soluble in this menstruum. The author has found, however, that, provided the coil is well dried previous to extraction, chloroform, benzene, and petroleum ether give the same results as anhydrous ether; ordinary ether, which contains small amounts of water and alcohol, gives, however, slightly higher results, especially if the coils are allowed merely to air-dry. The error introduced by the use of ordinary ether is small, and is very frequently neglected.

Attempts have been made to substitute other substances for the blotting-paper; Abraham, indeed, before Adams published his method, had used "Parker's fibre lint." Wiley, and also Johnstone, tried asbestos paper, but the results were not satisfactory.

The action of the blotting-paper appears to be slightly different from that supposed by Adams. Undoubtedly he was correct in supposing that the milk was spread out over a large surface; the author's experiments showed that when milk was filtered through blotting-paper the filtrate contained the solids not fat, but only a small amount of fat. This view was found by Vieth not to be entirely correct; he found that a portion of the casein was also removed from the milk by blotting-paper. When milk is spread on blotting-paper the portion which soaks in consists of the whole of the water, milk-sugar, and salts, and a considerable proportion of the proteins, together with a small amount of fat; the bulk of the fat, together with a proportion of the casein, is left on the surface, and is very easily extracted by the ether. If the fat globules have been broken up by a "homogeniser," the extraction is not complete.

The following mode of procedure is considered most correct by the author:—

Hang up a convenient number of strips of "fat-free" paper from clamps (letter-clips are very serviceable). Run on, from a pipette, 5 c.c. of each of the samples to be tested in a slow stream, spreading the milk well over the paper; the strip should be held by its free end, to be nearly horizontal. The weight of the milk delivered by the pipette should be noted, care being

taken that it is delivered into the weighing vessel at the same rate and in the same manner as it was run on to the paper. The papers are allowed to hang up till apparently dry; flies must not be permitted to settle on the surface of the paper, as they consume portions of the fat.

When the strips are dry enough to handle they should be rolled up in loose coils of a diameter such that they will easily go into the Soxhlet extractors ( $\frac{3}{4}$  to 1 inch), and these should be fastened either by a brass ring, a piece of cotton, or a small pin. A number, or other mark serving to identify the sample, should be placed on each, and a blank coil—*i.e.*, one containing no milk—should also be rolled up. The coils should be dried at 100° C. for about an hour.

A sufficient number of wide-necked flasks should be carefully cleaned, and allowed to stand for fifteen minutes inside the balance case. The lightest of these should be placed on the right-hand pan of the balance as a tare, and the others successively weighed against it, the weights required to produce equality being noted. The coils should be placed in the Soxhlet extractors, the flasks fitted, and a measured volume of dry ether, sufficient to fill the extractor well above the upper portion of the siphon, poured into each. The blank coil should be placed in a Soxhlet extractor, and extracted with the same volume of ether into the "tare." The Soxhlet extractors are connected to upright condensers, and the ether made to boil by partially immersing the flasks in water at 50° to 60° C. Extraction should be continued for five hours.

The ether should be distilled off, and the flasks placed, side downwards, in a drying oven at 100° C. for about twenty minutes, being rotated, and air being blown in every five minutes to remove the vapour of ether. This time is sufficient to dry them if dry ether has been used.

After cooling for fifteen minutes the flasks should be weighed, the "tare" being again placed on the right-hand pan of the balance, and the percentage of fat calculated from the increase of weight.

The "tare" is used to correct for the small quantity of "extract" obtained from the paper, and to neutralise the effect of any change of weight of the flasks due to handling.

The connection between the flasks and the extractors and between the extractors and condensers may be made by corks, provided they have been well extracted by ether.

The tare and the drying of the coils may be omitted, and ordinary ether used in place of dry ether without greatly affecting the results.

Dry ether is prepared by washing the commercial preparation

with water, shaking the washed ether with calcium chloride, and, after allowing it to stand over calcium chloride for a day or two, distilling.

Ether sufficiently pure for most purposes may be obtained by distilling (from a water-bath not exceeding 40° C. in temperature) the commercial product from a flask to which a fractionating column is fitted. The first fractions boiling below 34·3° C. and the last boiling above 34·8° C. should be rejected.

The following figures show the amount of difference that may be expected between the two modes of procedure :—

PERCENTAGE OF FAT.

Dry ether, etc., . . .	4·49	4·59	0·19	2·61	3·09	3·42	3·05	4·21
Ordinary ether, etc., . . .	4·58	4·61	0·28	2·68	3·13	3·45	3·05	4·34
Difference, . . .	0·09	0·02	0·09	0·07	0·04	0·03	..	0·13

The average difference is found to be 0·06.

**The Maceration Method** (often called the **Somerset House Method**).—Dr. James Bell, when Principal of the Inland Revenue Laboratory at Somerset House, on being appointed referee under the “Sale of Food and Drugs Act,” devised this method for the estimation of fat and solids not fat in milk, and it has since been perfected at the Government Laboratory.

The following details of procedure are those employed by the author and Miller, and are essentially those of the Government Laboratory :—About 10 grammes of milk are weighed into a basin of platinum or aluminium about 3 inches in diameter and a little over 1 inch high, with a flat bottom, provided with a flat-ended glass stirrer. Two drops of a 0·5 per cent. solution of phenolphthalein are added, and approximately  $\frac{N}{11}$  strontia solution run in till a faint pink colour appears. The contents are evaporated to a damp paste on the water-bath, when the basin is transferred to a hot plate, and the paste mixed with the stirrer ; at a certain point in the evaporation the paste comes away from the basin, and by careful manipulation both basin and stirrer can be obtained practically clean. On further evaporation and stirring, the paste begins to get into a condition in which it can be broken up and rubbed into pieces, and at this stage it is removed from the hot plate and about 20 c.c. of methylated ether (specific gravity 0·720) dried with calcium chloride are added. On gentle rubbing with the stirrer the solids begin to go to pieces ;

the stirrer and basin are now scraped with a knife or spatula to bring any small portions of solids adhering to the sides under the ether, and the solids are gently rubbed to a powder. The ether is decanted through a weighed filter 9 cm. in diameter, and the solids again treated with ether. The solids at this stage are in a condition in which they can be ground up to a fine powder; the ethereal solution is allowed to settle, and the ether decanted through the filter; without any further addition of ether the solids are now ground to a very fine powder. It is advisable to do this with only a very little ether in the basin, as it is then easy to see the larger portions which can be ground up one by one. A further addition of ether is made, and the solids further ground; the ether at this stage looks like whitewash, and the solids take some minutes to subside sufficiently to allow of decantation. After about six or eight treatments in this manner the solids are allowed to air-dry, the portions clinging to the stirrer and sides of the basin scraped down, and 5 c.c. of alcohol and a few drops of water are added; the solids are well mixed with the alcohol, and the basin is placed on the hot plate and evaporated till the paste begins to go to pieces, when the solids are again treated as before. A second treatment with alcohol and a further six to eight extractions with ether are given; the filter gradually becomes partially blocked with the finely-divided solids, but never to such an extent that filtration stops. The solids are air-dried, and then dried in the water-oven to constant weight (0.00128 gramme is subtracted for each c.c. of  $\frac{N}{10}$  strontia used). At the Government Laboratory the solids not fat are transferred to a weighing bottle, but the author and Miller omit this; although the solids not fat are hygroscopic, no appreciable error is due to this omission. The final weight is known from previous weighings to within a very small amount, and consequently the time of weighing is very short, and not more than a few tenths of a milligramme of hygroscopic moisture are taken up during the weighing. The top of the filter where fat collects is cut off and cut into pieces and thoroughly washed with ether; the knife or spatula is wiped on some of the pieces cut off to remove any particles of solids, and the filter containing the pieces cut off is placed in a weighing bottle and dried in the water-oven to constant weight. The ether is distilled, and the residue of fat weighed; the fat is extracted with petroleum ether, and the small residue insoluble in this solvent subtracted from the weight; this usually consists of phenolphthalein, and its weight may be neglected without appreciable error.

*Estimation of Fat in Fresh Milk.*—It has been shown by Thorpe, and by the author, in conjunction with Hehner and Bevan, and

later with Miller, that the results of estimation of fat by the maceration method agree with those obtained by other methods which are admitted to give substantially accurate results, and Simmonds has also obtained results which show that accurate determinations can be made on samples of homogenised milk.

*Estimation of Fat in Sour Milk.*—At the Government Laboratory it has been shown that the fat in the sour milk varies from 0·06 per cent. more to 0·15 per cent. less than in the fresh milk, and averages 0·05 per cent. less.

The author and Miller find that the results on eighteen samples of sour milk are in very fair agreement with those obtained when fresh by Gottlieb's method; the results varying from 0·10 per cent. above to 0·18 per cent. below, and averaging 0·03 per cent. below.

The difference between the results obtained on the fresh milk and those on the sour milk is partly due to the difficulty of completely redistributing the fat in the curdled milk. Examination of an apparently well-mixed sample of sour milk with a low-power lens shows the presence of quite large particles of cream, and no amount of whisking with a wire brush appears to reduce the milk to the same homogeneous condition easily obtained with fresh milk.

*Estimation of Solids not Fat in Fresh Milk.*—The author and Miller have made a number of comparisons of the solids not fat by the maceration method with those obtained by the Society of Public Analysts' method, and find that invariably the former are higher than the latter. The average difference was 0·20 per cent.

It appears from experiments by Miller and the author that the solids not fat obtained by the maceration method contain a portion of the sugar as hydrated sugar, but the amount of water of hydration, which averages about 0·1 per cent., is not sufficient to explain the difference between the results.

Another source of error in the maceration method is due to the presence of aldehydes in the ether; milk solids remove the aldehyde completely from ether, and this appears to be due to a condensation of the —COH group with the free amino groups of the protein. The solids not fat obtained by the maceration method are always more acid than the milk, and the aldehyde figure is less, the increase of acid and the decrease in the aldehyde figure being within the limits of experimental error identical. These figures afford data for the estimation of the increase of weight due to the condensation of the aldehyde, and assuming that it is acetaldehyde, the error is almost constantly 0·03 per cent. unless freshly-distilled aldehyde-free ether be used.

Even this addition does not explain the whole of the difference

between the maceration and Society of Public Analysts' methods, and the marked browning of the residue in the latter method suggests that the remainder of the difference is due to the results obtained by it being too low; this conclusion is strengthened by the fact that evaporation over a large surface, whereby browning of the residue is avoided, gives slightly higher results.

**The Storch Method.**—The essential point of this method consists in drying the milk on pumice (or other medium) and extracting with ether after finely grinding in a mortar.

As originally designed by Storch, 10 grammes of milk were dried at 100° C. on about an equal weight of pumice in pieces about the size of a small pea; the pumice was ground in a mortar to a very fine powder, which was then transferred to a conical tube, and ether allowed to percolate through it till no more fat was extracted, the ether being received in a tared flask. The pumice was removed and reground, and percolation again continued; and this treatment was repeated till no more fat was extracted. The method was somewhat tedious, though very exact.

**Kieselguhr Method.**—In order to avoid the troublesome grinding of a hard substance like pumice and to economise time, the author prefers to use kieselguhr in place of pumice; the method is performed as follows:—

About 3 or 4 grammes of ignited kieselguhr or fossil meal are placed in a porcelain basin, a cavity being made in the centre, and 10 grammes of milk allowed to flow in, care being taken that none is permitted to fall on the sides of the basin. The kieselguhr is dried on a gently-boiling water-bath, being stirred at frequent intervals as drying proceeds; after about an hour's drying the kieselguhr can be powdered in the basin with a small pestle. The powder is transferred to a wide test tube, with a hole at the bottom, containing a half-inch plug of cotton wool, which has previously been well extracted with ether, or to a Schleicher and Schull's thimble; both basin and pestle should be scraped, and the basin should be rinsed two or three times with kieselguhr, the pestle being used to grind up the rinsings with any portions adhering to the sides. The rinsings are added to the tube, and a circular piece of filter paper, of such size as to fit the tube, placed over the kieselguhr. The tube is placed in a Soxhlet extractor and extracted with ether for three hours; care must be taken that the top of the tube is well above the top of the siphon and that the ether is not distilled at such a rate that it fills and overflows the upper portion of the tube. After three hours' extraction the tube is removed from the extractor, the kieselguhr emptied out into the basin, and, after allowing the ether to evaporate, again powdered, and re-extracted

for another three hours; the quantity obtained in the second extraction is very small.

The ether is then evaporated and the fat dried at 100° C. in a tared flask; after weighing, the fat should be dissolved in a little petroleum ether, when any kieselguhr which may have run through will be detected; this should not be the case, if the plug of cotton wool was properly packed.

Other media, such as kaolin, plaster of Paris, etc., may be substituted for pumice or kieselguhr, and, so long as the essential point of the method, fine grinding, is adhered to, the results are independent of the medium. Kieselguhr is, however, the most convenient.

This method may be used with homogenised milk.

**The Werner-Schmid Method.**—This method differs from most others, in that the milk is not reduced to a solid state by evaporation previous to the extraction of the fat by ether. In order to render the casein, which hinders the extraction of the fat from milk, soluble, Werner-Schmid heated the milk with an equal bulk of hydrochloric acid till the fat floated in a nearly clear layer at the top, shook the resulting solution with ether, and drew off an aliquot portion of the ethereal layer.

Stokes has devoted much attention to this method, and has studied the effect of slight modifications.

Werner-Schmid's directions are:—Take a test tube of about 50 c.c. capacity, graduated in tenths of a c.c., and introduce 10 c.c. of milk; add 10 c.c. of hydrochloric acid, boil, with shaking, until the liquid turns dark-brown, and cool by placing the tube in cold water; add 30 c.c. of ether, shake round and let stand; then measure the volume of the ethereal solution, draw off 10 c.c. with a pipette, evaporate the ether, and dry the fat at 100° C.

This method has been thoroughly examined by Stokes, who prefers to draw off 20 c.c. of the ethereal solution.

T. E. Hill has also examined the method, and considers that the milk should be weighed, not measured. Hill notices that a fluffy-looking stratum is formed beneath the ether, and, following Stokes, adds three-fourths of this to the bulk of the ethereal layer for calculating purposes. He also points out that Werner-Schmid's method is not applicable to the determination of fat in milk to which cane sugar has been added, a conclusion confirmed later by Dyer and Roberts, who showed that by boiling cane sugar with hydrochloric acid a substance soluble in the water taken up by the ether was formed.

Stokes has pointed out that this can be got rid of by extracting the dried residue with dry ether, in which the caramel formed from the sugar is insoluble. Allen recommends petroleum ether.

The author prefers to add to the ethereal solution an equal bulk of petroleum ether, and wash with water containing just sufficient ammonia to neutralise the free acid.

Stokes later introduced a new form of tube, in which the middle portion is narrowed for greater accuracy of measurement of the ethereal layer (Fig. 9).

Yarrow points out that it is absolutely necessary when using Stokes' tubes to read the volume before and after pipetting off a known volume at the same temperature. He has observed that a tube which after pipetting showed 2.4 c.c. read, after 30 minutes and at a higher temperature, 3.4 c.c.—a very serious difference.

Allen proposes to draw off as much ether as possible, to add a further supply, to draw that off as completely as could be done, and to continue washing in this way till all the fat was separated from the aqueous layer.

Molinari and Stokes have both described forms of apparatus in which the ethereal solution can be completely removed from the aqueous layer without the necessity for pipetting it off.

Stokes apparatus has the advantage that the globules of ether and water during separation have but a short distance to travel, and the separation is complete in a much shorter time than in the longer tube, which was not devised for the purpose of complete extraction where rapidity of separation is important.

The author prefers to dilute the milk with an equal bulk of water before heating with hydrochloric acid, as there is then no tendency for the formation of a fluffy-looking layer at the point of junction. He finds that it is necessary to wait for ten minutes at least after the ether has apparently separated from the aqueous layer, in order to allow the fine globules of water to settle out of the ether.

Analysts who have used this method are generally agreed that it gives results practically identical with that of Adams; it is a question whether the drawing off of an aliquot portion of the ether is to be preferred to the extraction of the whole of the fat. In the first case, there is a tendency to be slightly low, owing to the fact that the ether, which dissolves in the aqueous portion, retains a minute proportion of fat; in the other, the tendency is to be somewhat high, as the water which dissolves in the ether dissolves a small amount of substances other than fat. In either case, however, the error introduced is very small and may usually be neglected.



Fig. 9.  
Stokes' Tube.



Stokes has devised a modification of this method, by which the total solid residue is treated with hydrochloric acid, and the fat and total solids estimated in one portion of milk.

This method is eminently adapted for the estimation of fat in sour milk. A weighed portion of the well-mixed milk should be placed in a graduated tube, and diluted with an equal bulk of water; a quantity of hydrochloric acid, slightly greater than the total volume of the diluted milk, should be added, and the whole boiled till clear. After cooling, ether should be added, the tube shaken, and the ether allowed to settle for ten minutes. Before taking out the stopper of the tube, it is an advantage to cool the upper portion of the tube to as low a temperature as possible, so

that any ether which may have collected round the stopper may be drawn inwards. An aliquot portion of the ether should be drawn off, and evaporated and the fat weighed. Owing to the presence of lactic acid in sour milk, which is soluble in ether, and gives a non-volatile lactone on heating, the results have a tendency to be slightly high. For this reason also, the whole of the fat should not be extracted by repeated shaking with ether, as a greater amount of lactic acid is thereby extracted; the ethereal layer may be, however, washed with water to remove this (see *ante*, p. 117).

Smetham has devised an extractor on a similar principle to Soxhlet's for extracting liquids with ether; the fat may be removed in this apparatus after boiling with hydrochloric acid.

**Gottlieb's Method.**—This method consists in adding to 10 c.c. of milk in a special tube devised by Farnsteiner (Fig. 10), 10 c.c. of alcohol, 1 c.c. of ammonia (sp. gr. 0.96), and 25 c.c. of ether, and mixing; on adding 25 c.c. of petroleum ether a layer of about 50 c.c. separates. In the original method it is recommended that an aliquot portion be evaporated and the fat

weighed, but the author finds that the layer is not homogeneous, and it is advisable to draw off as much as possible, and add further quantities of ether and petroleum ether to extract the whole of the fat. By mixing and allowing to separate several times a homogeneous layer is obtained, but this procedure renders the method a slow one.

Popp has shown that the strength of the ammonia used is immaterial, and that no saponification of the fat occurs.

The author finds the following modification to work well:—



Fig. 10.  
Farnsteiner  
Tube.

Place 5 c.c., or about 5 grammes, of milk in a tall narrow stoppered 50 c.c. cylinder, add 0.5 c.c. of ammonia (sp. gr. 0.96), and shake; add 5 c.c. of alcohol and shake, and if the solution contains lumps (as may happen with sour milk) warm in hot water till they all dissolve; add 12.5 c.c. of ether and shake well; finally add 12.5 c.c. of petroleum ether and again shake well; allow the tube to stand a few minutes and again shake. In about five minutes the ethereal layer is removed as completely as possible to an unweighed flask, and the residue shaken with three successive quantities of a mixture of equal parts of ether and petroleum ether (the recovered solvent serves very well for this purpose), which are transferred to the flask. The solvent is evaporated and recovered, and when only 2 or 3 c.c. are left in the flask (this is chiefly alcohol) it is placed in the water oven and dried to constant weight. After weighing, the fat is melted, and extracted from the flask by treatment with four successive quantities of about 5 c.c. each of petroleum ether, and the flask placed in the water oven for half-an-hour, and again weighed.

There is always a minute residue left after the petroleum ether treatment, which may be, however, neglected without great error.

Though the description is somewhat long, the method gives little trouble, and is expeditious. It is not wasteful, as the recovered mixed solvents can be used for many purposes.

Siegfeld has found from 0.0029 to 0.0036 per cent. of cholesterol, and from 0.0079 to 0.0166 per cent. of lecithin in milk: these are soluble in the solvents used in Gottlieb's method, and may form an appreciable portion of the fat in machine-skimmed milk.

**Richmond and Rosier's Method.**—Rosier and the author estimate fat in milk as follows:—9 c.c. of sulphuric acid (90 to 91 per cent.  $H_2SO_4$ ) are measured into a tube holding about 50 c.c., and constricted just above the point where 20 c.c. reach; 10 grammes of milk are weighed into this tube, care being taken to prevent the milk and acid mixing; 0.9 c.c. of amyl alcohol is added, the tube corked, and well shaken; after cooling to about 25° C., 20 c.c. of petroleum ether are added, and the tube well shaken. When separation is complete, the contents of the tube are again well mixed, and allowed to separate: a second re-mixture and separation is given, and the petroleum ether blown off into a tube containing 20 c.c. of water, with which it is shaken and allowed to separate. After separation from the water, the petroleum ether is blown off into a tared flask. Further portions of petroleum ether are added to the tube containing the acid liquid, blown off into the tube containing the water, and transferred to the flask.

To reduce the time necessary for separation, the tubes may be centrifuged.

That this method gives good results is due to a compensation of errors, as the fat is in this, as also in the Gerber method, somewhat attacked by the sulphuric acid.

**Babcock's Asbestos Method.**—Babcock has used asbestos as a medium for evaporation of the milk previous to extraction with ether. Originally it was contained in a glass tube and dried in a current of air, but he has modified it by the use of a perforated metal cylinder.

The following is the method as adopted by the Association of Official Agricultural Chemists (of America):—Provide a hollow cylinder of perforated sheet metal, 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disc of the same material. The perforations should be about 0.7 mm. in diameter and about 0.7 mm. apart. Fill loosely with 1.5 to 2.5 grammes of freshly-ignited woolly asbestos, free from fine and brittle material; cool in a desiccator and weigh. Introduce a weighed quantity of milk (3 to 5 grammes) and dry at 100° C. to constant weight for the determination of total solids. Extract with anhydrous ether until fat is removed, evaporate the ether, dry the fat at 100° C. and weigh. The fat may also be determined by difference, drying the extracted cylinders at 100° C.

This method has been studied by the Association and has been found to give the same results as the Adams method. It has the advantage that fat, solids not fat, and total solids are directly estimated.

Macfarlane has described a method essentially the same, but uses chrysotile or Canadian asbestos for the purpose; this being a hydrated mineral cannot be ignited. He uses a cup-shaped glass tube with a hole at the bottom, and operates with 10 grammes of milk.

The author finds that this method gives results for fat practically identical with those obtained by the Adams method, but the solids not fat and total solids are low. It is used to a considerable extent in Canada for official work.

**The Ritthausen Method.**—If milk be diluted with water, a solution of copper sulphate added, and the acid neutralised, the proteins are precipitated as copper salts; these carry down with them the whole of the fat. After washing, to remove milk-sugar, etc., and drying, the fat may be extracted with ether; or a little strong alcohol may be poured on the precipitate to remove water, after which ether will extract the fat; the ethereal and alcoholic solutions are evaporated together and the fat weighed.

The fat may be similarly extracted from the casein precipitated by the addition of acetic acid to the diluted milk.

The results agree well with the Adams method, except in the case of very highly skimmed milks, when there is a tendency to be low.

This method has the advantage that the fat can be determined on the same portion of milk used for the estimation of proteins and milk-sugar.

The following comparative figures will show the results which may be expected :—

Fat by Ritthausen, .	4.93	2.87	1.38	4.00	0.04
Fat by Adams, .	4.97	2.89	1.43	3.99	0.17

Harrison and Goodson, working in the author's laboratory, have shown that this method cannot be used for the estimation of fat in sterilised or condensed milks, nor is it available for homogenised milk.

**Other Methods of Gravimetric Fat Determination.**—The other methods for the estimation of fat in milk are very numerous ; a few of those which have been proposed may be briefly noticed.

Wanklyn's method consists in extracting the fat from the solids of milk dried *per se*. The totality of the fat is never obtained, as, owing to the hard, horny character of the residue, a considerable proportion of the fat is protected from the ether. This method attained considerable notoriety, owing to its semi-official adoption by the Society of Public Analysts more than twenty years ago, but has now fallen into almost complete disuse. It has been modified by stirring the residue during evaporation to obtain a more granular residue, and by evaporating in a conical flask to expose a large surface to the ether, but the results, even with these modifications, have been unsatisfactory.

Hoppe-Seyler and, later, Liebermann have proposed to shake the milk with potash (to dissolve the casein), and then to extract with ether, but the separation of the ether is so slow as to render this method impracticable.

Morse, Piggott, and Burton add the milk to anhydrous copper sulphate, which combines with the water, giving a dry residue at once ; they then extract with petroleum ether.

Baynes proposes to dry the milk on powdered glass, a method essentially the same as that of Storch. Sand has also been largely used in Germany ; but as it is very difficult to grind this up, its use is not to be recommended.

Marpmann has proposed the use of cotton-wool, Ganntter of wood-fibre, and Duclaux of sponge ; the principle of these methods is the same as that of Adams.

Fernandez-Krug and Hampe mix a measured volume of milk with a finely-divided mineral substance—usually 5 c.c. of milk with 7½ grammes of washed and dried kaolin—and add 5 grammes

of finely-powdered anhydrous sodium sulphate. The sodium sulphate absorbs the water contained in the milk, and, after well stirring, the residue is quite dry; this is transferred to a flask holding 100 c.c. and 25 c.c. of ether added; after shaking for five minutes, an aliquot portion is withdrawn by a pipette, over the point of which a piece of cotton-wool is wrapped, and the fat estimated by evaporation and weighing.

Froidevaux precipitates the casein and fat with a solution containing 35 grammes of calcium phosphate and 6 c.c. acetic acid per litre; 90 c.c. of this solution are mixed with 10 c.c. of milk, and the fat determined as in the Ritthausen process.

### (2) Volumetric Methods.

These, being suitable for use in the rapid testing of milk, will be more conveniently considered in the chapter on "The Testing of Milk."

### (3) Indirect Methods.

**Estimation of Cream.**—One of the earliest and simplest methods of estimating the fat in milk is to allow the milk to stand, and to measure the volume of cream thrown up. For this purpose a creamometer or cylindrical vessel, the upper portion of which is divided into spaces, each representing the  $\frac{1}{100}$ th part of the total volume up to the highest line, is employed. It is filled to the mark, and allowed to stand at rest for some time—six, eight, twelve, or twenty-four hours—and the volume of cream measured. A good milk should throw up about 10 per cent. of its cream in eight hours.

The method is of very slight value for a determination of the fat in milk, as comparatively slight variations in the conditions make enormous variations in the volume of cream. Thus, the author has found that a milk—freshly drawn and not cooled—containing 5.3 per cent. of fat threw up 25 per cent. of cream in six hours, while another milk with the same percentage of fat, which had been raised to the boiling point and cooled, only threw up 2 per cent. of cream in the same time. These, of course, are extreme instances, and it is found in a majority of cases that the percentage of cream thrown up in six to eight hours divided by 3 will give an approximation to the percentage of fat.

It has been proposed to modify this method by raising the cream by centrifugal force, and apparatus have been made to fit on to the spindle of cream separators; though more concordant results are thus obtained, these methods have not the accuracy of many of the volumetric methods, by which they have been superseded as practical methods.

Faber has, however, shown that very accurate results may be obtained by measuring the volume of cream thrown up from skim milk, after whirling in the lactocrite for one hour, and dividing by 3.

It has been proposed to deduce the percentage of fat in milk from the opacity of the sample. Various forms of "lactoscope" have been proposed which measure either the thickness of the layer of milk which obscures a given light, or the amount of light passing through a given layer of milk.

The accuracy of the lactoscope is approximate only, and the instrument has already been described.

The calculation of the fat from the specific gravity and total solids has already been described.

The author has tried an expansion method, which depends on the difference of the expansion by heat of fat and milk serum—that of the former being about three times that of the latter.

**Densimetric Method of Fat Estimation.**—By placing about 200 c.c. on a pleated filter and collecting such portions as run through in the first quarter of an hour, the milk serum—*i.e.*, a solution of the solids not fat in water—may be separated without much change in composition. A series of experiments by the author showed that the solids not fat in the filtered milk were, after correcting for the volume of the fat removed, on the average 0.12 per cent. lower than in the unfiltered milk.

Vieth gives the following experiment:—200 c.c. of milk were filtered; after one hour 50 c.c. of milk had run through; after four and a half hours more, 26 c.c. were collected; and 108 c.c. were poured out from the filter.

The results of analyses of these four milks were—

	Original Milk.	First Filtrate.	Second Filtrate.	Left on Filter.
	Per cent.	Per cent.	Per cent.	Per cent.
Total solids, . . .	13.42	9.14	8.14	13.95
Fat, . . . . .	4.43	0.06	0.03	4.80
Solids not fat, . . .	8.99	9.08	8.11	9.15
Protein, . . . . .	3.66	3.68	2.43	3.89
For every 100 parts of water there were present				
Solids not fat, . . .	10.38	9.99	8.83	10.63
Protein, . . . . .	4.23	4.05	2.65	4.52
The differences as compared with the original milk were				
Solids not fat, . . .	...	-0.39	-1.55	+0.25
Protein, . . . . .	...	-0.18	-1.58	+0.29

This experiment shows that a portion of the proteins is removed with the fat, and corroborates the author's conclusion that when the portion run through in the first fifteen minutes is taken, there is only a slight loss of solids not fat.

The author has found that if the specific gravity of the filtered milk less 1 be divided by 0.004, and the difference between the specific gravities of the milk before and after filtration be divided by 0.0008, the figures so obtained very fairly represent the solids not fat and fat respectively. The following figures (Table XI.) will show the agreement that may be expected:—

TABLE XI.—ESTIMATION OF MILK SOLIDS BY DENSIMETRIC METHOD.

Specific Gravity.			Solids not Fat.		Fat.	
Before Filtration.	After Filtration.	Differences.	Found.	Calc.	Found.	Calc.
1.0308	1.0340	0.0032	Per ct. 8.63	Per ct. 8.50	Per ct. 3.94	Per ct. 4.0
1.0318	1.0348	0.0030	8.71	8.70	3.35	3.7
1.0316	1.0345	0.0029	8.63	8.63	3.61	3.6
1.0298	1.0326	0.0028	8.18	8.15	3.80	3.5
1.0315	1.0331	0.0016	8.36	8.27	1.99	2.0

Great accuracy cannot be expected from this method, but it has the advantage of not requiring chemical apparatus. Determinations can be made with a small delicate lactometer, and an idea of the quality of a milk obtained in a short time.

**The Estimation of Proteins.**—Proteins may be either estimated collectively as total proteins, or separate determinations of casein and albumin can be performed.

**Total Proteins from Total Nitrogen.**—The determination of total proteins is generally performed either by making an estimation of total nitrogen in milk, and multiplying this by 6.38 ( $= \frac{100}{15.7}$ , as both casein and albumin contain this amount of nitrogen), or by precipitating the proteins as copper salts by Ritthausen's method.

**Kjeldahl's Method.**—To determine total nitrogen the method of Kjeldahl is the most convenient. Five grammes of milk are weighed into a round bottomed hard glass flask of about 150 c.c. capacity and 20 c.c. of pure sulphuric acid added. This is placed over a small flame and heated till it is thoroughly charred, the water being evaporated during this heating; the flame is now removed, and about 10 grammes of potassium

bisulphate added, and a small drop of mercury. A funnel or pear-shaped bulb with an elongated projection is placed in the neck of the flask, and heat applied, the flame being gradually increased as frothing ceases. In about half an hour the liquid becomes colourless; it is allowed to cool, diluted with water, and transferred to the distillation flask. This may conveniently be of copper or brass, and, for ease of manufacture, can be in the form of a bottle; the digestion flask is well washed with water, care being taken that any white crystals which form on cooling, and become yellow on dilution, are transferred to the distillation flask. A cork carrying a dropping funnel with stopcock, and a wide tube with one or more bulbs blown on it, which are loosely packed with asbestos, is fitted; one end of the tube is connected with a condenser, the other end of which is made to dip just below the surface of 50 c.c. of  $\frac{N}{10}$  sulphuric acid.

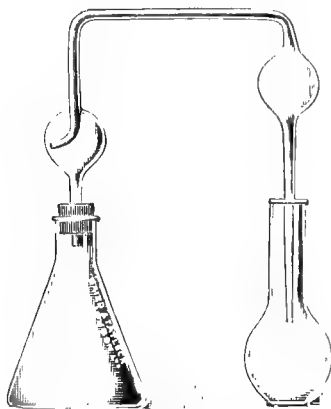


Fig. 11.—Kjeldahl Apparatus.

One hundred c.c. (more or less) of a 30 per cent. solution of caustic soda, followed by 10 c.c. of a 10 per cent. solution of potassium sulphide, or 1 gramme of sodium hypophosphite, are added through the dropping funnel, and the contents of the flask mixed by rotatory shaking; a flame is placed beneath the distillation flask, cold water run through the condenser, and the contents distilled till about 200 c.c. have passed over. The tap of the dropping funnel is opened, the flame removed, and the flask disconnected from the condenser; after washing the latter into the flask containing the distillate, it should be removed. The distillate is titrated with  $\frac{N}{10}$  alkali solution.



using litmus or cochineal as indicator. An amount of acid equivalent to the alkali used is subtracted from the amount of acid originally added; the difference represents the acid neutralised by the ammonia distilled. From this figure should be subtracted the figure obtained in a blank experiment—*i.e.*, an experiment performed without the addition of milk, but in other respects exactly the same. Each c.c. of  $\frac{N}{10}$  acid neutralised by the ammonia produced is equal to 0.0014 gramme of nitrogen. The percentage of nitrogen multiplied by 6.38 will give the percentage of total proteins.

Another form of apparatus gives equally good results if care be used; the caustic soda and sulphide solution are poured carefully down the side of the distilling flask, so that the alkaline solutions do not mix with the acid; the bulb tube rapidly inserted, and the contents of the flask mixed. No condenser is used, but the flask containing the standard acid may be placed in cold water (Fig. 11).

This method may be modified in many ways. The potassium bisulphate may be omitted, but the digestion then takes longer. Copper oxide or sulphate may be substituted for the mercury; if this be done, the potassium sulphide solution may be replaced by an equal bulk of Soxhlet's alkaline tartrate solution (see *Estimation of Milk-Sugar*).

The tube with two bulbs can be replaced by any other form of apparatus having for its object the prevention of splashing; for the copper flask a Jena or other glass flask, or even a tin bottle, may be used. If a tin flask is employed, it must be remembered that "rust" contains ammonia, and the tin must be boiled with strong soda solution before use; for this reason a tin flask cannot be recommended.

For the estimation of nitrogen the methods of Varrentrap and Will and of Dumas may be employed, but they are not so generally convenient. In large laboratories where many determinations are made the method of Dumas is perhaps more convenient than that of Kjeldahl; these methods will be found described in manuals devoted to analytical chemistry. The method of Kjeldahl is, however, the most generally employed in milk analysis.

**Ritthausen's method** is performed as follows:—Ten grammes of milk are diluted to about 100 c.c. and 5 c.c. of Soxhlet's copper sulphate solution (see *Estimation of Milk Sugar*) added; a solution of caustic soda (25 grammes per litre) is added, drop by drop, till the solution is nearly neutral. The precipitate settles rapidly; an excess of alkali must be avoided, as it prevents precipitation of the proteins. The precipitate is allowed to settle, and the supernatant liquid poured off through a tared filter, or Gooch

crucible. The precipitate is washed several times by decantation, and then transferred to the filter or crucible, the portions adhering to the beaker being removed by a "policeman." It is washed a few more times with water, and the filter or crucible allowed to drain.

The filter or crucible is washed once with strong alcohol, and then several times with ether, preferably in a Soxhlet extractor; it is then washed again with strong alcohol from a small wash bottle, using the jet to distribute the precipitate over the filter.

The filter or crucible and its contents and the tare or the crucible are dried in an air oven at a temperature of 130° C. and weighed; the filter or crucible and precipitate are incinerated in a porcelain capsule in a muffle, going up to as high a temperature as possible. The weight of the residue, *minus* that of the ash of the filter, is subtracted from the weight of the dried precipitate, the difference being the proteins.

The author and Boseley have obtained good results by neutralising the milk, using phenolphthalein as indicator, previous to the addition of the copper sulphate solution; the quantity of the latter may also be reduced to 2.5 c.c.

This method gives good results with all milk products, except whey; this is due to the fact that the copper salts of proteoses are not insoluble in water. There is a slight tendency for the results to be high, owing to copper hydroxide, which is always co-precipitated, not being entirely dehydrated at 130°; there is also a tendency to be low, because the phosphorus of the casein is converted into phosphoric acid on ignition, which swells the amount of ash. These two errors usually compensate each other to a greater or less extent.

The washing with alcohol and ether may be omitted, and the precipitate weighed as proteins and fat, the fat, estimated by other methods, being subtracted from the weight.

Bordas and Toutplain estimate proteins in milk by treating 10 c.c. with 20 c.c. of acetone, shaking the mixture to effect complete precipitation, and separating the precipitate in a centrifuge; the insoluble proteins are collected, washed with dilute and finally with pure acetone, dried, weighed, ignited, and the amount of ash deducted.

**Volumetric Determination of the Proteins in Milk.**—Denigès has worked out a process which depends on the estimation of the amount of mercury necessary to combine with the proteins; it is worked as follows:—

Twenty-five c.c. of the milk are mixed with 5 c.c. of a saturated solution of ammonium oxalate, 20 c.c. of  $\frac{N}{10}$  mercuric potassium

iodide (13.55 grammes mercuric chloride and 36 grammes potassium iodide per litre) and 2 c.c. of acetic acid, the volume made up to 200 c.c. and the liquid filtered. To 100 c.c. of the clear filtrate are added 10 c.c. of a solution of potassium cyanide equivalent to a decinormal solution of silver nitrate and 12 or 15 c.c. of ammonia. The liquid is then titrated with  $\frac{N}{10}$  silver nitrate solution until there is a permanent turbidity. The number of c.c. used =  $q$ .

A blank determination is made to determine the exact value of the silver nitrate solution. 10 c.c. of the cyanide solution, 12 to 15 c.c. of ammonia, and 10 c.c. of the mercuric-potassium iodide solution are mixed with 100 c.c. of water and titrated, as before, with the silver nitrate. The number of c.c. used (which with  $\frac{N}{10}$  silver nitrate should be 4.8 c.c.) =  $c$ . The percentage of proteins is calculated from the value  $q - c$  by means of Table XII.

TABLE XII.—FOR CALCULATING PERCENTAGE OF PROTEINS IN MILK.

$q - c$ .	Proteins.	$q - c$ .	Proteins.	$q - c$ .	Proteins.	$q - c$ .	Proteins.
c.c.	Per cent.	c.c.	Per cent.	c.c.	Per cent.	c.c.	Per cent.
0.0	0.0	1.2	1.0	2.4	2.225	3.6	3.9
0.1	0.10	1.3	1.1	2.5	2.35	3.7	4.05
0.2	0.17	1.4	1.2	2.6	2.475	3.8	4.275
0.3	0.25	1.5	1.3	2.7	2.6	3.9	4.5
0.4	0.30	1.6	1.4	2.8	2.7	4.0	4.7
0.5	0.375	1.7	1.5	2.9	2.8	4.1	4.9
0.6	0.45	1.8	1.6	3.0	2.925	4.2	5.15
0.7	0.55	1.9	1.7	3.1	3.075	4.3	5.4
0.8	0.65	2.0	1.8	3.2	3.2	4.4	5.72
0.9	0.715	2.1	1.9	3.3	3.35	4.5	6.0
1.0	0.8	2.2	2.0	3.4	3.5	4.6	6.25
1.1	0.9	2.3	2.1	3.5	3.7	..	..

Trillaut and Sauton base a method on the fact that formaldehyde renders the proteins of milk insoluble. Five c.c. of milk diluted with 25 c.c. of water are boiled for five minutes, then treated with 5 c.c. of 40 per cent. formaldehyde solution, boiled for two or three minutes longer, and allowed to stand for five minutes. The solution is shaken with 5 c.c. of a 1 per cent. solution of acetic acid, and the precipitate collected on a dried filter or Gooch crucible, washed with water, and subsequently extracted

with acetone to remove the fat. Finally, the precipitate is dried at 75° to 80° C., and weighed.

They have proved experimentally that the protein is completely separated, and that the weight is not increased appreciably by the condensation of the formaldehyde.

### Estimation of Casein and Albumin.

**Hoppe-Seyler's Method** consists in diluting 10 grammes of milk with about 100 c.c. of water and precipitating the casein by the addition of dilute acetic acid; carbon dioxide is passed into the solution in order to complete the precipitation. The precipitate is allowed to settle, and the liquid decanted through a tared filter and treated in exactly the same manner as the copper precipitate in Ritthausen's method. The casein, after drying, is ignited, and the weight of the ash, less the weight of the ash of the filter, subtracted from the total weight.

**Ritthausen** prefers to precipitate the casein at a temperature of 40° C., omitting the passage of the carbon dioxide. Van Slyke has compared these two methods, and finds that the results are identical; he gives the preference to that of Ritthausen, as being carried out with greater facility, and operates as follows:— Dilute 10 grammes of milk with 90 c.c. of water at 42 to 43° C., add 1.5 c.c. of 10 per cent. acetic acid solution, stirring well; after the expiration of five minutes, filter, and proceed as above described.

The author finds that it is an advantage to dilute 10 grammes of milk with 90 c.c. of a mixture of equal parts of saturated salt solution and water.

**Frenzel and Weyl** have proposed the use of sulphuric acid, but Van Slyke has found that either a slight deficiency or excess of acid causes inaccuracy. He, therefore, does not recommend the method.

Instead of weighing the casein, the nitrogen in the precipitate may be estimated by Kjeldahl's method, and multiplied by 6.38. In this case it is not necessary to dry the casein, but the precipitate with the filter may be dropped into a digestion flask, the acid added, and the method performed as directed for total nitrogen.

Maissen and Musso have proposed the use of rennet for precipitation of the casein, but this is not accurate.

As casein is not entirely insoluble in water, especially in the presence of acid, the results have a tendency to be low, especially if the nitrogen be estimated. On the other hand, it is difficult to wash the casein absolutely free from other milk solids (? calcium citrate): hence the weight of the "casein"

obtained by precipitation is thus raised. In practice, the two errors have a tendency to compensate one another.

Albumin is estimated by boiling the filtrate from the casein; it is preferable to use the filtrate from the solution containing salt solution, and this should be brought to a degree of acidity such that 100 c.c. require 2.2 c.c. N alkali for neutralisation; the filtrate is raised just to boiling over a small flame, and digested on the water-bath for fifteen minutes; the albumin separates in a pulverulent form. It is collected on a tared filter or, preferably, in a Gooch crucible, and dried at 100° C.; the nitrogen may be estimated by the Kjeldahl method, and multiplied by 6.38. The albumin is precipitated practically in a state of purity, and no correction for the ash need be made, but, owing to the precipitation not being complete, the results are slightly low; if the casein has not been completely precipitated, a portion may be found with the albumin.

A small quantity of so-called "lacto-protein" remains in solution after precipitation of the casein and albumin; this chiefly consists of the unprecipitated portions of casein and albumin. It may be estimated by precipitation as copper salt by Ritt-hausen's method, as described above; by precipitation by tannin and estimation of the nitrogen in the precipitate by Kjeldahl's method; or by evaporation of the solution and estimation of the nitrogen.

**Sebelein's method**, though more tedious, is preferable to the above; to 10 grammes of milk, 20 c.c. of a saturated solution of magnesium sulphate are added. Solid magnesium sulphate in the form of powder is then added in small quantities at a time till no more is dissolved. The solution is allowed to stand for twelve hours and filtered; the precipitate is washed four or five times with a saturated solution of magnesium sulphate, an operation which takes some time. The filter and its contents are dropped into a Kjeldahl digestion flask, 30 c.c. of sulphuric acid added, and the nitrogen estimated, as previously described; an increased volume of soda solution to neutralise the 30 c.c. of acid used must be employed. The nitrogen multiplied by 6.38 will give the weight of casein.

The magnesium sulphate must be free from sodium sulphate, commercial "Epsom salts" often containing this impurity. If distinct acidity be developed in the milk, this should be neutralised previous to the addition of magnesium sulphate.

The albumin is separated by diluting the filtrate and precipitating by the addition of tannin, or phospho-tungstic acid; the precipitate is collected on a filter, and the nitrogen therein estimated by Kjeldahl's method. The albumin may be less exactly estimated by boiling the filtrate after dilution and addition of a

small quantity of acetic acid ; it is collected on a tared filter and weighed as such.

In order to avoid the tedious washing with a saturated solution of magnesium sulphate, Leffmann and Beam take a larger quantity of milk (say 20 grammes), dilute with twice its bulk of saturated magnesium sulphate solution, add powdered magnesium sulphate till saturated, and make up to a definite volume with saturated magnesium sulphate solution in a graduated cylinder. The solution is allowed to stand and the lower clear portion is removed by a pipette ; this is filtered and an aliquot portion taken ; the albumin is estimated in this, as directed above. The casein is determined by subtracting the albumin nitrogen from the total nitrogen and multiplying the difference by 6.38.

Sodium chloride, to which a little calcium chloride has been added, can be substituted for magnesium sulphate ; the precipitate is less easy to treat, owing to the formation of hydrogen chloride on heating the precipitate with sulphuric acid.

**Estimation of Casein.—Lehmann's Method.**—Lehmann has devised a method for the estimation of casein in milk by means of unglazed porcelain plates ; the plate is wetted with water, and 5 grammes of milk diluted with 5 grammes of water placed in the centre. After about an hour and a half the serum is separated, and the casein, together with the fat, is removed with a spatula ; the last traces of casein are removed by setting the plate in water. The fat is removed by extraction with ether, the casein being ground up to extract the last traces ; the casein is dried at 100° C. on a weighed filter, and weighed ; from the weight is deducted the weight of the ash left on incineration.

The results are said to be very accurate. The casein is obtained in the state in which it exists in the milk.

**Schlossman's Method.**—Schlossman proposes to estimate casein by warming 10 c.c. of milk mixed with 3 to 5 parts of water to 40°, and adding 1 c.c. of a concentrated solution of alum. Should the flocculent precipitate not subside rapidly an additional 0.5 c.c. of alum solution may be added, since a slight excess (up to 1 c.c.) does not affect the results. The precipitate is allowed to stand for some minutes, and is then filtered. After having been washed with water and dried, the filter and its contents are extracted with ether in a Soxhlet extractor (an estimation of fat being thereby obtained) ; the nitrogen determined by Kjeldahl's method, and multiplied by 6.38, gives the weight of the casein.

**Richmond's Method.**—The albumin in milk, which has been raised to the boiling point, behaves with all methods as casein. An approximate estimation of real casein in milk, which has been

heated, can be made as follows:—Twenty-five grammes of milk are evaporated to dryness, ignited, and the phosphoric acid estimated in the ash, as directed on p. 82. Twenty-five c.c. of the filtrate, produced by adding 3 c.c. of acid mercuric nitrate to 100 c.c. of milk, are taken, evaporated down, and ignited; the phosphoric acid is estimated in the ash.

The casein is calculated from the following formula:—

$$\begin{aligned} \text{Let } P_1 &= P_2O_5 \text{ obtained from 25 grammes of milk,} \\ P_2 &= P_2O_5 \text{ obtained from 25 c.c. of filtrate,} \\ F &= \text{percentage of fat,} \\ \text{and } S &= \text{the specific gravity.} \end{aligned}$$

$$\text{Then casein} = \left( P_1 - P_2 \times \frac{100 - 1.11F}{S} \right) \times 205.2.$$

The coagulated albumin is deduced by subtracting this figure from the apparent percentage of casein estimated by one of the methods previously described.

**The Volumetric Determination of Casein in Milk.**—Van Slyke and Bosworth's method is to place 20 c.c. of milk in a 200 c.c. flask, add 100 c.c. of water, 1 c.c. of phenolphthalein solution, and titrate with  $\frac{N}{10}$  sodium hydroxide solution till a faint pink colour appears; raise the temperature to between 18° and 24° C., and add  $\frac{N}{10}$  acetic acid in 5 c.c. portions, shaking vigorously after each addition, till the casein separates in the form of white flakes; usually 25 c.c. is sufficient, but the addition of acid should be continued, 1 c.c. at a time, after 25 c.c. have been added, till on standing a short time the liquid appears quite clear. Water is now added to make up 200 c.c., the contents of the flask vigorously shaken, and poured upon a dry filter, and the whole of the filtrate collected. Of the filtrate, 100 c.c. are titrated with  $\frac{N}{10}$  sodium hydroxide solution till a faint pink colour appears, care being taken that the shade of pink is the same as that obtained before (a colour standard, see p. 133, may usefully be employed). A second titration may be made with 50 c.c. of the filtrate, the results being doubled. The percentage of casein is deducted by subtracting the number of c.c. of sodium hydroxide solution used per 100 c.c. of the filtrate from half the volume of the acetic acid added and multiplying by 1.0964.

The method works well with fresh milk, but milk that is sufficiently sour to coagulate on boiling does not give satisfactory results.

**Estimation of Curd.**—Lindet proposed to deduce the amount of curd from the difference of specific gravity of milk and the

they produced therefrom by rennet. The author has slightly modified his method to the following:—

Estimate the specific gravity and fat in the milk by any convenient method; to 100 c.c. of milk add 0.01 gramme of rennet powder, and keep at 42° C. till curdled; cut up the curd, and allow it to settle, and strain off the whey through muslin; cool the whey to 15.5° C., and estimate the specific gravity and fat as before.

Add the degrees of gravity and the percentage of fat in the milk, and subtract the sum of the degrees of gravity and the percentage of fat in the whey; the difference divided by 3.5 will give the percentage of dry curd available for cheese-making. This will, of course, be very much less than the pressed curd actually obtained, as this not only contains a considerable percentage of water, but also the bulk of the fat in the milk. Roughly speaking, the dry curd multiplied by 4 plus the difference in the percentage of fat in the milk and in the whey will give the curd actually obtained.

**Determination of Total Acidity—Lactic Acid.**—The acidity of milk is determined by titration with alkali, using phenolphthalein as indicator; the author prefers to place 10 c.c. in a small beaker, add 1.0 c.c. of phenolphthalein solution (0.5 gramme per 100 c.c. of 50 per cent. alcohol), and titrate with  $\frac{N}{10}$  baryta or strontia till a faint pink colour equal to that produced by the addition of 1 drop of a 0.01 per cent. solution of rosaniline acetate in 96 per cent. alcohol, is obtained. The procedure should not be varied.

Storch uses a solution of lime (lime-water), containing solid lime as a standard alkali solution; this remains constant in composition, and is almost exactly twentieth normal. The strength of the solution remains constant, as if any of the lime is removed by carbon dioxide, more is dissolved; its strength is but little affected by ordinary variations of temperature.

It is to be recommended for dairy use, as no precaution, except to have an excess of lime in the bottle, is necessary.

Generally speaking, about 17 c.c. to 20 c.c. of  $\frac{N}{10}$  alkali is required; each cubic centimetre of  $\frac{N}{10}$  alkali used per litre of milk is called 1° of acidity, hence a milk requiring 2 c.c. of  $\frac{N}{10}$  solution for 10 c.c. will have 20° of acidity.

McCreath has devised a convenient form of apparatus for the estimation of acidity (Fig. 12); he employs a caustic soda solution of such strength that each c.c. = 0.01 gramme of lactic acid. Ten c.c. of milk, after the addition of phenolphthalein solution,



is titrated with this, and the number of c.c. used divided by 10 will give the acidity in terms of lactic acid; this practice is, however, to be deprecated, as freshly-drawn milk has a very distinct acidity, though lactic acid is in all probability absent. The acidity of milk to phenolphthalein is due partly to the mono- and di-basic phosphates, and partly to the dissolved carbonic acid.

Soxhlet and Henkel have proposed the use of a  $\frac{N}{4}$  normal soda solution, and use a special apparatus (Fig. 13); they express the acidity as degrees; 1 Soxhlet-Henkel degree = the number

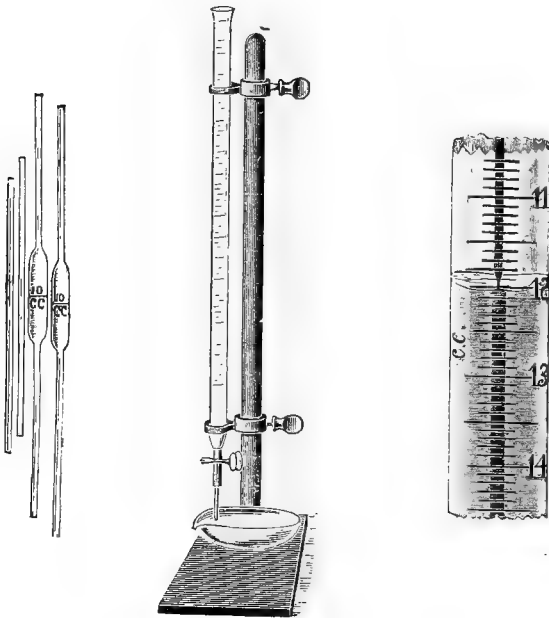


Fig. 12.—M'Creath Acidimeter.

of c.c. of  $\frac{N}{4}$  alkali used per 100 c.c. of milk; it is to be regretted that this "degree" has been introduced, as it is 2.5 times larger than the ordinary degree, and has caused confusion.

Instead of using phenolphthalein, delicate neutral litmus paper may be employed; milk is practically neutral to this. The acidity can be titrated with fair accuracy, though the end point of the titration is not well marked. There is more justification for calculating the acidity to litmus as lactic acid, as both

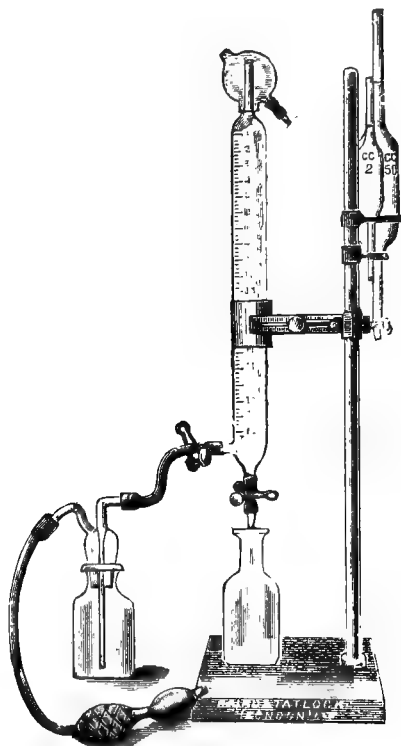


Fig. 13.—Soxhlet-Henkel Acidimeter.

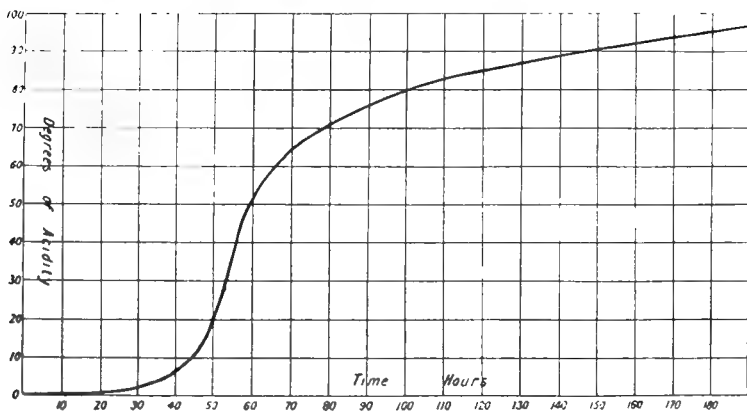


Fig. 14.—Curve of Development of Acidity.

the salts of milk and carbonic acid are not appreciably acid to litmus paper.

The following figures obtained by the author on sour milks will show the enormous difference in the two results; the figures have in both cases been calculated to lactic acid:—

	I.	II.	III.	IV.	V.
Acidity (to phenolphthalein),	1.24	1.89	1.82	1.52	1.32
„ (to litmus paper),	0.65	1.14	1.28	0.86	0.56

There is no good method for the quantitative determination of lactic acid; the results of the titration with litmus give approximate results. An approximation nearer to the truth may be made by distilling some of the milk into a little  $\frac{N}{10}$  alkali, titrating back the alkali with  $\frac{N}{10}$  acid, using litmus paper as indicator, and subtracting the volatile acidity from the total acidity to litmus; the non-volatile acidity is taken as lactic acid.

The curve on p. 135 (Fig. 14) shows the average rate of development of acidity in milk on standing at a temperature of 20° C.

**Determination of the Aldehyde Figure.**—Steinegger has devised a method which adds another datum to those usually obtained in the analysis of milk, and which serves as an indirect estimation of proteins. It may be combined with the acidity estimation. The method depends on the fact that when an amino-acid, which has been neutralised is treated with an excess of formaldehyde, it becomes acid, and requires the addition of a further quantity of alkali to again neutralise it.

Steinegger titrates the milk with  $\frac{N}{4}$  caustic soda solution till neutral to phenolphthalein, adds 6 per cent. of the total volume of 40 per cent. formaldehyde solution, and again titrates till neutral; the amount of alkali used, less the acidity of the formaldehyde solution added, is the aldehyde figure which he expressed in Soxhlet-Henkel degrees.

The author and Miller prefer the use of  $\frac{N}{11}$  strontia solution for titrating, and take 10 c.c. or 11 c.c. of milk, neutralise to phenolphthalein, add 2 c.c. of 40 per cent. formaldehyde solution, and again titrate till neutral, and subtract the acidity, previously determined, of 2 c.c. of formaldehyde solution. The acidity developed by the addition of formaldehyde calculated as degrees gives the aldehyde figure. The strontia aldehyde figure is about 1.1 times larger than that given with a  $\frac{N}{10}$

soda solution. This figure varies in normal milks from  $18.1^{\circ}$  to  $22.6^{\circ}$ , and averages  $19.9^{\circ}$ ; in fresh milks it is practically equal to the acidity.

The aldehyde figure obtained with strontia solution multiplied by 0.170 will give a close approximation to the percentage of proteins; it is not an absolutely exact measure of the proteins, as casein and albumin do not give the same aldehyde figure, and the relative proportion of these is liable to slight variations.

The acidity and aldehyde figure usually approximate in fresh milk to the same figure, it being only rarely that the difference amounts to more than  $2^{\circ}$ ; in certain abnormal samples, usually low in solids not fat, the acidity is much lower than the aldehyde figure, and in these the factor 0.170 for calculating proteins is not exact.

**Estimation of Lecithins.**—Bordas and de Raczkowski determine lecithins in milk thus:—100 c.c. of milk are shaken with 100 c.c. 95 per cent. alcohol, 100 c.c. of water and 10 drops of acetic acid. The precipitate is extracted with three successive quantities of 50 c.c. each of hot absolute alcohol. The mixed extracts are evaporated, and the residue taken up with a small quantity of a mixture of equal parts alcohol and ether; the ether is evaporated, and the residue saponified by potassium hydroxide, and the soap solution acidified with dilute nitric acid. The fatty acids are filtered off, and the filtrate evaporated to dryness, mixed with 10 c.c. of strong nitric acid, and completely oxidised by the addition of potassium permanganate little by little. A few drops of sodium nitrite solution (1 : 10) are added to dissolve the manganese hydroxide, and the nitrous acid expelled by boiling. The phosphoric acid is precipitated with ammonium molybdate and estimated as magnesium pyrophosphate. The weight of  $Mg_2P_2O_7$  multiplied by 1.5495 will give the glycerophosphoric acid, and by 7.27 the lecithins.

**The Analysis of Milk Products.**—For the analysis of milk products, the methods described above can generally be used. The following notes will show where it is advisable to depart from them or employ modifications.

**Homogenised Milk.**—The Adams or Ritthausen methods of fat estimation should not be used.

**Sterilised Milk.**—The fat cannot be estimated by the Ritthausen method.

**Cream.**—The methods given above may be employed in the analysis of cream. The following method of determining total solids, fat (by difference), solids not fat, and ash is convenient.

Four to five grammes are weighed in a wide platinum basin, which is placed in a water-oven, till the water has apparently evaporated and the solids not fat stick to the bottom of the

basin; when this occurs—after about an hour's drying—the basin is so inclined that the fat runs down to the side away from the solids not fat. Under these conditions drying is completed in about five hours. It is an advantage to add an equal volume of alcohol to the cream, and well mix before drying, as the time of drying is thereby shortened.

After weighing the total solids, the basin is replaced in the water-oven for a few minutes to melt the fat; 25 c.c. of amyl alcohol are poured on, the basin again placed in the water-oven for ten minutes, and the amyl alcohol solution of fat carefully decanted while still hot; with care, none of the solids not fat passes away with the amyl alcohol. This process is repeated eight times more, the basin being allowed to stand all night between the fourth and fifth treatments. After the last treatment, the amyl alcohol is drained off as far as possible, and the basin and its contents dried for three hours in the water-oven. The residue is weighed as solids not fat. This is now burnt over a low flame, and the residue weighed as ash. Ether or chloroform may be substituted for amyl alcohol, but the latter is cheaper and less volatile.

This method is not available for homogenised cream, and the fat should be estimated by the Gottlieb or Werner-Schmid methods.

An indirect estimation of the percentage of fat may be made from the total solids, and *vice versa*.

For this purpose it is assumed that the proportion of solids not fat to water in milk is constant, an assumption which causes no appreciable error in cream analysis. It is found that on the average 100 parts of water contain 10·2 parts of solids not fat (see p. 150); we may assume that the water in cream contains the same proportion of solids not fat, and estimate the fat by deducting the percentage of water multiplied by 0·102 from the total solids.

The following formula will express this relation:—

Let	T = total solids and F = fat,
then	F = 1·102 T - 10·2.

The following table (XIII.) may be used.

For the determination of milk-sugar by polarisation, the cream should be diluted with water; 50 grammes may be made up to 100 c.c., and 1 c.c. of acid mercuric nitrate added.

If total nitrogen is determined, the cream should be evaporated in a wide-mouthed flask, and the bulk of the fat extracted, as, otherwise, great charring of the fat takes place (if the cream be treated by the Kjeldahl method), and much carbon, difficult to dissolve, is produced.

TABLE XIII.—RATIO OF FAT TO TOTAL SOLIDS IN CREAM.

Total Solids.	Fat.	Solids not Fat.	Total Solids.	Fat.	Solids not Fat.
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
60	55.8	4.2	44	33.2	5.8
59	54.7	4.3	43	37.1	5.9
58	53.6	4.4	42	36.0	6.0
57	52.5	4.5	41	34.9	6.1
56	51.4	4.6	40	33.8	6.2
55	50.3	4.7	39	32.7	6.3
54	49.2	4.8	38	31.6	6.4
53	48.1	4.9	37	30.4	6.6
52	47.0	5.0	36	29.3	6.7
51	45.9	5.1	35	28.2	6.8
50	44.8	5.2	34	27.1	6.9
49	43.7	5.3	33	26.0	7.0
48	42.6	5.4	32	24.9	7.1
47	41.5	5.5	31	23.8	7.2
46	40.4	5.6	30	22.7	7.3
45	39.3	5.7	29	21.6	7.4

This Table is not applicable to clotted cream.

**Skim Milk.**—The methods of milk analysis may be applied; the fat is rather more difficult of extraction. If Ritthausen's method be used for protein determination, it is not necessary to extract the fat, but to dry and weigh the copper precipitate, and afterwards to subtract the percentage of fat found from the percentage of proteins *plus* fat.

**Condensed Milk.**—About 30 to 35 grammes of the well-mixed milk should be weighed into a 100 c.c. flask, diluted with 50 to 60 c.c. of water, and the solution raised to the boiling point; this is cooled, made up to 100 c.c., and the total weight taken. The diluted solution is analysed as a milk. The fat is rather more difficult to extract by Adams method than from ordinary milk, and longer extraction should be given: the Gottlieb method is the best; if cane sugar is present, the Werner-Schmid process must not be used for the estimation of the fat, as cane sugar yields a substance soluble in ether. The Ritthausen method must not be used. When soluble albumin is estimated, a fresh portion which has not been boiled is employed.

**Sour, Fermented, or Decomposed Milk**—*Preparation of Sample.*—The whole contents of the bottle are turned out into a beaker and whisked for a minute or two with a brush made of fine wire: the inside of the bottle is scraped all over with a wire, some of the milk is poured back, and the contents shaken: this is now emptied into the beaker and again whisked.

Many samples on mixing yield a portion of their fat in a churned

condition, which adheres to the wire brush; in these cases a separate estimation of the churned fat should be made.

The estimation of fat in sour milks has already been discussed under the various methods; milk-sugar and cane sugar, if present, are almost impossible to estimate, as they have usually undergone more or less hydrolysis, and are usually neglected or estimated by difference. Total acidity and aldehyde figure are estimated as described; it is, however, necessary to add a larger quantity of formaldehyde solution on account of the great dilution by the large volume of alkali required for neutralisation.

For the estimation of the specific gravity of curdled milk Weibull recommends the addition of ammonia, which dissolves the precipitated curd; the specific gravity of the mixture is taken, and the specific gravity of the milk is calculated from that of the mixture, and that of the ammonia. The method gives excellent results, but, as the estimation of the specific gravity of ammonia is an unpleasant operation, the author prefers to operate as follows:—To 100 c.c. of curdled milk add 5 c.c. of dilute ammonia (1 part ammonia, sp. gr. 0·885, to 4 parts of water), mix well, and take the specific gravity; to 100 c.c. of fresh milk add 5 c.c. of the same ammonia solution, and from the lowering of the specific gravity of the fresh milk, the correction to be made for the ammonia is deduced; this is usually 0·0026 or 0·0027, or 2·6 to 2·7 lactometer degrees.

de Koningh proposes the use of a solution of caustic soda of specific gravity 1·030 instead of the ammonia; he finds that on mixing 5 c.c. of this solution to 100 c.c. of milk that a lowering of the specific gravity by 0·0008 or 0·8 degree takes place. The author and Harrison find that with fresh milks the lowering is less, 0·0003 on the average, and increases with the acidity of the milk; the cause of this lowering is shown by their experiments to be due to the fact that the specific gravity of a solution of a sodium salt is always less than the sum of the specific gravities of equivalent solutions of caustic soda and an acid (less 1). The following procedure has given good results in the author's hands:—Add sufficient solution of caustic soda (sp. gr. 1·032) to render the mixture with the sour milk distinctly alkaline to phenolphthalein; determine the specific gravity of the mixture and the acidity of the sour milk (see p. 133). To the figure found for the specific gravity add the acidity in degrees multiplied by 0·000022, and subtract 0·0001; thus, if the specific gravity found be 1·0305, and the acidity be 70°, the corrected specific gravity is

$$1\cdot0305 + 70 \times 0\cdot000022 - 0\cdot0001 = 1\cdot0305 + 0\cdot00154 - 0\cdot0001 = 1\cdot0319.$$

*Estimation of Proteins.*—The proteins precipitated by the acid

developed in the milk are filtered off, and either weighed, or the nitrogen is determined in them; in the filtrate albumin is estimated as on p. 130; in the filtrate from this, albumoses are estimated by precipitation with tannin or phospho-tungstic acid, and determining the nitrogen in the precipitate.

The total nitrogen is estimated by Kjeldahl's method on a weighed portion.

Certain changes take place in the proteins in sour milk, and at the Government Laboratory to estimate ammonia 2 grammes of the milk are made up to 100 c.c. with distilled water, and filtered to a clear solution. Ten c.c. of the filtrate, increased to 50 c.c. by the addition of distilled water, are nesslerised against  $\text{NH}_4\text{Cl}$  solution, equivalent to 0.01 milligramme of  $\text{NH}_3$  in each c.c. As the Nessler colour produced in the presence of milk differs somewhat from that of pure saline ammonia, the blank experiment is carried out with the addition of 10 c.c. of the filtrate from 2 grammes of new milk slightly acidified, and diluted to the same extent as the sour milk. The quantity of test ammonia solution required varies from 0.5 to 4.0 c.c. In the case of a milk containing ammonia equal to 2.6 c.c. of the test solution, the ammonia is calculated as follows:—

$$0.01 \times 2.6 \times 500 = 0.013 \text{ per cent. ammonia.}$$

It is evident that any other degree of dilution may be conveniently adopted according to circumstances, or the proportion of ammonia which may be indicated in the milk.

*The Estimation of Alcohol.*—About 75 grammes are weighed into a 300-c.c. flask for the estimation of alcohol, and half neutralised with  $\frac{N}{2}$  soda solution; about half the volume is distilled and collected in a small flask, and neutralised with  $\frac{N}{2}$  soda—using litmus paper as indicator. From this 25 c.c. is distilled, and the density taken at 60° F. by a Sprengel tube. From the density the percentage of alcohol is calculated by Table XIV.

At the Government Laboratory the percentage of alcohol is deduced by multiplying the difference between 1 and the specific gravity of the distillate by  $1,000 \times 1.16$ , and this gives it as percentages of proof spirit: as proof spirit contains 49.5 per cent. alcohol by weight, it is evident that the factor  $1,000 \times 0.572$  will give the percentage of alcohol by weight.

The total acidity to litmus paper may be calculated as lactic acid: from this an amount equivalent to the volatile acids is subtracted.



TABLE XIV.—ALCOHOL TABLE (*Hehner*).

Specific Gravity at 60° F.	Alcohol per cent. by Weight.	Specific Gravity at 60° F.	Alcohol per cent. by Weight.	Specific Gravity at 60° F.	Alcohol per cent. by Weight.
0.9999	0.05	0.9969	1.75	0.9939	3.47
8	0.11	8	1.81	8	3.53
7	0.16	7	1.87	7	3.59
6	0.21	6	1.94	6	3.65
5	0.26	5	2.00	5	3.71
4	0.32	4	2.06	4	3.76
3	0.37	3	2.11	3	3.82
2	0.42	2	2.17	2	3.88
1	0.47	1	2.22	1	3.94
0	0.53	0	2.28	0	4.00
0.9989	0.58	0.9959	2.33	0.9929	4.06
8	0.63	8	2.39	8	4.12
7	0.68	7	2.44	7	4.19
6	0.74	6	2.50	6	4.25
5	0.79	5	2.56	5	4.31
4	0.84	4	2.61	4	4.37
3	0.89	3	2.67	3	4.44
2	0.95	2	2.72	2	4.50
1	1.00	1	2.78	1	4.56
0	1.06	0	2.83	0	4.62
0.9979	1.12	0.9949	2.89	0.9919	4.69
8	1.19	8	2.94	8	4.75
7	1.25	7	3.00	7	4.81
6	1.31	6	3.06	6	4.87
5	1.37	5	3.12	5	4.94
4	1.44	4	3.18	4	5.00
3	1.50	3	3.24	3	5.06
2	1.56	2	3.29	2	5.12
1	1.62	1	3.35	1	5.19
0	1.69	0	3.41	0	5.25

The method used at the Government Laboratory for the estimation of volatile acids is as follows:—

Ten grammes of milk are neutralised to the extent of one-half the total acidity with  $\frac{N}{10}$  NaOH, and a little phenolphthalein added. The mixture is then evaporated to dryness on a water-bath with frequent stirring, and, after treatment with 20 c.c. of boiling distilled water, so as to break up and thoroughly detach the milk solids from the capsule, a further addition of  $\frac{N}{10}$  NaOH is made, until the neutral point is reached. The difference between the original acidity of the milk and that of the evaporated portion is regarded as acetic acid. The number

of c.c. of soda shown, when multiplied by 0·06, will give the percentage of acetic acid.

*Example—*

Acidity of original milk . . .	=	11·6 c.c.	$\frac{N}{10}$	NaOH.
Acidity of evaporated portion . . .	=	9·2	,,	
		<hr style="width: 50px; margin: 0 auto;"/>		
Difference . . .	=	2·4	,,	
		<hr style="width: 50px; margin: 0 auto;"/>		

or,  $2·4 \times 0·006 \times 10 = 0·144$  per cent. of acetic acid.

The author and Miller have shown that this method is only accurate when the volatile acidity lies between 0·1 and 0·2 per cent. of acetic acid, as is the case in the majority of sour milks.

It sometimes happens that a considerable quantity of butyric acid is formed, and it is then preferable to employ the following modification of Duclaux's method :—

Add to the milk from which the alcohol had been distilled a quantity of acid exactly equal to the soda used for half neutralising, and distil this to a small bulk; water is then added in successive quantities of about 25 c.c., and distilled off till the distillate is practically neutral.

The mixed distillates neutralised to phenolphthalein are made up to 250 c.c., and an aliquot portion taken for fractional distillation; to this is added a quantity of N sulphuric acid, very slightly greater than is necessary to neutralise the soda used. Eight successive fractions, each about one-ninth of the total volume, are distilled, and separately titrated with  $\frac{N}{10}$  strontia; 25 c.c. of water is added to the residue, and a further 25 c.c. distilled and titrated, and, if desired, this treatment may be repeated. From the last titration the total quantity of volatile acid is obtained by extrapolation of the results; the total quantity is always slightly less than the total acidity of the first distillate, due, no doubt, to the presence of a little lactic acid. As much as possible of the first distillate is made up to a convenient bulk, after adding a quantity of N sulphuric acid in slight excess of that required to neutralise the soda, and exactly one-third is distilled; this is made up to a convenient bulk, and exactly one-third is again distilled. The last distillate is made up to 100 c.c., and eight fractions of about 11 c.c. each are distilled and separately titrated. The residue in the flask and condenser is washed out and titrated to obtain the total quantity.

For the calculation of the results the following formulæ are used :—

$$1-y=(1-x)^{2.1} \text{ for butyric acid, or } \log(1-y)=2.1 \log(1-x);$$

$$1-y=(1-x)^{1.18} \text{ for propionic acid, or } \log(1-y)=1.18 \log(1-x);$$

$$\text{and } 1-y=(1-x)^3 \text{ for acetic acid, or } 3 \log(1-y)=2 \log(1-x).$$

$$y = \text{quantity of acid distilled; total quantity} = 1.$$

$$x = \text{volume of liquid distilled; total quantity} = 1.$$

For each fraction calculate the proportion of each of the acids which would distil, and calculate the ratios of butyric to acetic, and butyric to propionic acid, which correspond to the proportion actually distilled. The ratios from first and last fractions are liable to be slightly erroneous, the first because a small amount of a very volatile acid, as carbonic, would appear in this fraction, and the last because experimental error is greatly magnified; but usually the other six ratios are practically constant for either butyric and acetic acids, or butyric and propionic acids.

The distillation of the third of a third is undertaken in order to decide definitely the composition of the mixture. From the formulæ above, the proportions which would distil, when one-third of one-third is collected, are :—

For butyric acid, . . . . .	0.3285
For propionic acid, . . . . .	0.1445
For acetic acid, . . . . .	0.061

Thus, a mixture of butyric and propionic acids in equal proportions should yield approximately a 2 : 1 mixture in the distillate, while a mixture of butyric and acetic in equal proportions would yield a 5 : 1 mixture. It is even possible to deduce the relative proportions of a mixture of the three acids.

An example, where the acids present are acetic and butyric, is given in Table XV.

The quantity distilled in  $\frac{1}{3}$  of  $\frac{1}{3}$  was 0.193.

The figure calculated for a mixture of 0.495 molecule of butyric to 0.505 molecule of acetic was 0.192.

The ratio of butyric acid in the second distillate should be 0.83.

*Corrections for Solids not Fat in Sour Milks.*—When it is desired to ascertain from the analysis of a sour milk the original composition of the sample, the following constituents should be determined; fat and solids not fat by the maceration method, alcohol, ammonia, acidity, and aldehyde figure of the milk, volatile acidity by the Government Laboratory method, and aldehyde figure of the neutralised solution obtained in this method. If the volatile acidity is high, the proportions of butyric, propionic,

TABLE XV.—ACETIC AND BUTYRIC ACIDS.

DISTILLATION OF ORIGINAL DISTILLATE.				
Volume Distilled.	Acid Distilled.	Calculated for—		Ratio.
		Butyric.	Acetic.	Butyric Total.
*0.108	0.141	0.213	0.073	0.49
0.221	0.2275	0.406	0.153	0.49
0.335	0.403	0.5755	0.238	0.49
0.440	0.515	0.708	0.324	0.495
0.559	0.6175	0.821	0.421	0.49
0.669	0.711	0.902	0.521	0.50
0.779	0.793	0.958	0.6345	0.49
0.887	0.8705	0.9915	0.780	0.48
DISTILLATION OF $\frac{1}{3}$ OF $\frac{1}{3}$ .				
0.113	0.190	0.2225	0.077	0.78
0.214	0.343	0.397	0.148	0.78
0.328	0.493	0.566	0.2325	0.77
0.437	0.619	0.701	0.318	0.785
0.547	0.726	0.8105	0.410	0.79
0.659	0.819	0.898	0.512	0.795
0.773	0.892	0.956	0.628	0.805
0.891	0.950	0.9905	0.782	0.805

\* The calculation is made as follows :—

$$\begin{aligned} \text{Log } (1 - 0.108) \times 2.1 &= \text{log } (1 - 0.213). \\ \text{Log } (1 - 0.108) \times 2 &= \text{log } (1 - 0.073) \times 3. \\ 0.213 - 0.073 &= 0.140. \\ 0.141 - 0.073 &= 0.068. \\ \frac{0.068}{0.140} &= 0.49. \end{aligned}$$

and acetic acids should be determined by Duclaux's method. The solids not fat should be mixed with 10 c.c. of hot water, and the acidity or alkalinity and aldehyde figure determined.

The following corrections should be made :—

#### *Alcohol Correction.*

1. For each 184 parts of alcohol add on 342 parts, or the difference between 1 and the specific gravity of the distillate multiplied by 977 and by the weight of the distillate (or volume in c.c.), and divided by the weight of milk taken.

*Volatile Acid Corrections.*

2. For each 60 parts of acetic acid add on 25·5 parts.
3. For each 88 parts of butyric acid add on 87·5 parts.
4. For each 74 parts of propionic acid add on 68·5 parts.

*Ammonia Correction.*

5. For each part of ammonia add on 5·2 parts.

*Lactic Acid Correction.*

6. For each part of lactic acid subtract 0·05 part.

*Aldehyde Correction.*

7. For each degree of difference between the aldehyde figure of the neutralised solution obtained in the volatile acid estimation and that of the solids not fat subtract 0·0026 per cent.

*Amino-Acid Correction.*

8. For each degree of aldehyde figure of the neutralised solution obtained in the volatile acid estimation above 20° subtract 0·0018 per cent. (This is rarely required.)

*Loss of Butyric Acid.*

9. Subtract the acidity of the solids not fat from the difference between the aldehyde figures of the volatile acid solution, and of the solids not fat; multiply the figure thus obtained by 0·0088, and add it to the solids not fat.

By this system of corrections the author and Miller have found that the solids not fat deduced from the analysis of sour milks has varied from 0·32 per cent. above the solids not fat in the original milk to 0·20 per cent. below, and to average 0·06 per cent. above. The determinations on the original milks were made by subtracting the fat by Gottlieb's method from the total solids by evaporation. With milks in which no very large amount of volatile acid was developed the figures were + 0·17, - 0·19, and + 0·07 respectively. At the Government Laboratory only corrections Nos. 1, 2, and 5, and, if necessary, a correction of 92 parts for each 88 parts of butyric acid are made. The author, however, believes that the additional corrections give more exact results, though the difference due to neglecting them is small. The whole system of corrections is based on a long investigation made at the Government Laboratory, which has

established that, if milk is adulterated with added water, the percentage added can be deduced from an analysis of the sour milk, and that the figure thus obtained does not differ by more than 3 per cent., and usually by much less from that estimated by the analysis of the fresh milk.

The author and Miller have submitted the Government Laboratory method to a critical examination, and generally endorse the above conclusions.

Table XVI. gives the results of the analysis of 18 samples of sour milk, corrected according to the foregoing scheme.

Carbonic acid can only be estimated in koumiss or kephir contained in a corked bottle. The worm of a champagne tap is carefully turned off to leave a perfectly smooth stem; the tap is also carefully reground to make sure that it fits.

A drying and absorbing apparatus is fitted up, consisting of (a) a U-tube containing pumice and sulphuric acid, (b) a U-tube containing soda lime immersed in a beaker of cold water, (c) a U-tube filled half with soda lime and half with calcium chloride. These are connected in the order named, and the end of (a) is connected by a short piece of india-rubber tubing to the champagne tap.

(b) and (c) are weighed, and the tap (closed) carefully forced through the cork of the bottle; the tap is slightly opened and the carbon dioxide allowed to slowly escape; when the escape of gas becomes slack the bottle may be slightly warmed, by placing it in warm water, and shaken to promote further escape. When no more gas comes off the tap is disconnected, a soda lime tube substituted, and a current of air drawn through the apparatus.

(b) and (c) are dried, cooled and again weighed; the increase represents the amount of carbon dioxide which has escaped from the bottle. The total contents of the bottle are now weighed and the percentage calculated.

There still remains a little carbon dioxide dissolved; this can be best estimated by titrating a weighed amount with  $\frac{N}{10}$  baryta water, using phenolphthalein as indicator; the difference between the acidity thus estimated and that estimated as previously described will represent, without great error, the carbon dioxide ( $1 \text{ c.c. } \frac{N}{10} \text{ alkali} = 0.0022 \text{ gramme CO}_2$ ). This should be added to the amount estimated by absorption.

**Buttermilk** and **whcy** are analysed by the methods given for milk; the total proteins of whcy cannot be determined by Ritthausen's method, and the total nitrogen must be estimated.

TABLE XVI.

No.	Fat (Original).	Fat (Sour).	Difference.	Solids not Fat (Original).	Solids not Fat (Sour).	Alcohol.	Correction.	Butyric Acid.	Propionic Acid.	Acetic Acid.	Volatile Acid (Correction).	Ammonia.	Correction.	Aldehyde Correction.	Loss of Butyric Acid.	Lactic Acid.	Correction.	Solids not Fat, Government Laboratory.	Solids not Fat, R. and M.	Age (Days).
1.	2.77	2.78	+0.01	8.29	8.00	0.165	0.32	..	..	..	..	0.014	0.08	-0.03	..	1.38	-0.07	8.40	8.30	246
2.	3.66	3.62	-0.04	8.95	8.90	0.021	0.04	..	..	0.136	0.06	0.009	0.05	-0.03	..	0.94	-0.05	9.05	8.97	31
3.	3.66	3.53	-0.13	8.95	9.03	0.021	0.04	..	..	0.133	0.06	0.009	0.05	-0.03	..	0.88	-0.05	9.18	9.10	31
4.	3.66	4.12	+0.09	8.83	8.68	0.050	0.10	..	..	0.170	0.07	0.009	0.05	-0.03	..	0.83	-0.04	8.90	8.83	46
5.	3.72	3.73	+0.01	8.77	8.85	0.071	0.14	..	..	0.116	0.05	0.002	0.01	-0.03	..	1.16	-0.06	9.05	8.96	49.
6.	4.06	4.02	-0.04	8.86	7.14	0.271	0.53	0.820	..	0.187	0.90	0.004	0.02	-0.03	0.11	0.25	-0.01	8.59	8.66	49.
7.	{ M 3.89 }	4.02	{ +0.10 +0.13 } 0.082	{ 8.74 9.04 }	8.72	0.082	0.16	..	..	0.171	0.07	0.001	..	-0.03	..	1.23	-0.06	8.95	8.86	37
8.	3.97	3.81	-0.16	8.77	7.74	0.180	0.35	0.385	0.360	..	0.66	trace	..	-0.03	0.10	0.37	-0.02	8.75	8.80	37
9.	3.84	3.92	+0.08	8.82	8.33	0.366	0.71	..	..	0.078	0.03	0.004	0.02	-0.02	..	0.93	-0.05	9.09	9.02	34
10.	{ M 3.86 }	3.88	{ +0.02 +0.02 }	{ 8.90 9.05 }	7.69	0.092	0.18	0.658	0.236	0.095	0.91	0.012	0.06	-0.02	0.06	0.29	-0.01	8.84	8.87	31
11.	3.61	3.64	+0.03	8.84	8.76	0.075	0.15	..	..	0.138	0.06	0.003	0.01	-0.01	..	0.95	-0.05	8.98	8.92	57
12.	3.82	3.90	+0.08	8.88	7.21	0.258	0.50	1.086	..	0.605	1.34	0.016	0.09	-0.01	0.10	0.66	-0.03	9.14	9.20	43
13.	3.71	3.53	-0.18	8.79	8.51	0.016	0.03	..	..	0.162	0.07	0.013	0.07	-0.03	..	1.01	-0.05	8.68	8.60	55
14.	3.48	3.36	-0.12	8.81	7.55	0.015	0.03	0.946	..	0.658	1.22	0.002	0.01	-0.03	0.11	0.91	-0.03	8.81	8.86	40
15.	3.57	3.44	-0.13	8.84	8.81	0.032	0.06	..	..	0.108	0.05	0.004	0.02	-0.02	..	0.91	-0.05	8.94	8.87	36
16.	2.63	2.54	-0.09	6.50	6.49	0.030	0.06	..	..	0.108	0.05	0.006	0.03	-0.01	..	0.88	-0.04	6.63	6.58	36
17.	3.63	3.57	-0.06	8.71	8.74	0.031	0.06	..	..	0.114	0.05	0.024	0.12	-0.01	..	0.97	-0.05	8.97	8.91	33
18.	2.70	2.69	-0.01	6.47	6.35	0.086	0.17	..	..	0.084	0.04	0.019	0.10	-0.01	..	0.90	-0.05	6.66	6.60	33

No. 2 and 3 were from the same bulk of milk and taken at the same time.

" 7 and 8 " " different times.

" 9 and 12 " " "

No. 16 was a mixture of 73.6 parts of 15 and 26.4 parts of water. " Water calculated from sour milk solids not fat 25.7 per cent.

No. 18 " 74.3 " 17 and 25.7

The determinations marked M were obtained on the fresh milk by the maceration method. " " " 25.9 "

**Milk Powders.**—The sample should be ground and well mixed to secure uniformity. *Moisture* is estimated by drying about 1 gramme in the water-bath.

**Fat** cannot be estimated by direct extraction, as the results are always low. The Gottlieb method is suitable, weighing out 0.6 to 0.7 gramme, making up with water to a weight of 5.15 grammes, and proceeding as described on p. 119. The Werner-Schmid method may also be used, and if there is no sugar except milk-sugar, the fat, after drying, should be dissolved in petroleum ether, and any residue weighed and subtracted from the total weight. In the presence of much of any other sugar, it is preferable to mix the ethereal solution with an equal bulk of petroleum ether, and shake out with water rendered slightly alkaline with ammonia before the solution is evaporated.

**Milk-sugar** may be easily and quickly estimated polarimetrically; 10 grammes of milk-powder are ground up in a mortar with sufficient hot water to make it into a paste, which is gradually thinned with hot water, and the solution made up to 100 c.c.; a little ammonia may be added if the milk-powder does not all go into solution. Unless this procedure is followed, incomplete extraction of the sugar may result. The usual method is then followed; it is necessary to use phosphotungstic acid to precipitate the last trace of proteins.

**Cane-sugar** may be conveniently estimated by the method described by Harrison (p. 103).

**Proteins** are calculated from the total nitrogen by Kjeldahl's method by the factor 6.38.

**Ash, Lime, and Phosphoric Acid** as usual.

**Acidity and Aldehyde figure** are estimated by grinding up about 1 to 2 grammes with hot water, and titrating with  $\frac{N}{11}$  strontia, using phenolphthalein as indicator.



## CHAPTER III.

NORMAL MILK : ITS ADULTERATIONS AND ALTERATIONS,  
AND THEIR DETECTIONS.

CONTENTS. — Chemical Composition of Milk — Colostrum — Limits and Standards of Milk — Adulterations of Milk — Preservatives — The Action of Heat on Milk — Sterilised Milk — Condensed Milk — The Action of Cold on Milk.

**The Chemical Composition of Milk—Average Composition.**—The milk of the cow has, on the average, the following composition (deduced from about 280,000 analyses made within the last seventeen years in the Laboratory of the Aylesbury Dairy Company, Limited) :—

Water, . . . . .	Per cent.	Albumin, . . . . .	Per cent.
Fat, . . . . .	87·35	Ash, . . . . .	0·40
Milk-sugar, . . . . .	3·74	Other constituents, . . . . .	0·75
Casein, . . . . .	4·70		0·06
	3·00		

It is essentially an aqueous solution of milk-sugar, albumin, and certain salts, holding in suspension globules of fat and, in a state of semi-solution, casein, together with mineral matter. Small quantities of other substances are also found, which have been referred to (Chap. I.).

When evaporated, a residue is left, which is known as the solids of milk ; these are empirically divided into fat and solids not fat. It was first pointed out by Wanklyn that the solids not fat in milk show comparatively small variations. Though this rule is by no means absolute, it is to a great extent borne out in practice, especially in dealing with the mixed milk of several cows.

**Limits and Variations.**—The following are the maximum and minimum percentages which have come under the author's notice ; the highest fat is recorded by Bannister, the highest and lowest solids not fat and the lowest fat were observed in the Aylesbury Dairy Company's Laboratory :—

		Fat.	Solids not Fat.
		Per cent.	Per cent.
Maximum, . . . . .		12·52	10·60
Minimum, . . . . .		1·04	4·90

**Variations of Fat with Solids not Fat.**—Speaking very generally, a high percentage of fat is accompanied by a high percentage of solids not fat. Vieth gives the following table:—

TABLE XVII.

Milk containing	Total Solids.		Fat.		Solids not Fat.	
	Per cent.	contains on the average	Per cent.	Per cent.	Per cent.	Per cent.
	12·5		3·80		8·70	
" "	12·7	" "	3·90		8·80	
" "	12·9	" "	4·05		8·85	
" "	13·1	" "	4·29		8·81	
" "	13·3	" "	4·34		8·96	

There are, however, very many exceptions to this rule.

**Variation of Proteins with Fat.**—Timpe has stated that the percentage of proteins can be calculated from the fat by the formula  $P = 2 + 0.35 F.$ , and gives 21 analyses of "normal milk" from single cows in support of the statement. The composition of these is:—

TABLE XVIII.

	Average.	Minimum.	Maximum.
	Per cent.	Per cent.	Per cent.
Total solids, . . . .	12·52	8·45	16·13
Fat, . . . . .	3·78	1·03	6·39
Sugar, . . . . .	4·70	4·41	5·00
Protein, . . . . .	3·32	2·37	4·26
Ash, . . . . .	0·72	0·62	0·78

When his series is extended the agreement practically disappears. The following table expresses the results of over 50 analyses in a condensed form:—

TABLE XIX.

Percentage of Fat.	Percentage of Proteins.			
	Extremes.	Mean.	Calculated.	Difference.
3·0-3·25	3·11-3·48	3·35	3·08	+ 0·27
3·25-3·50	3·22-3·60	3·38	3·19	+ 0·19
3·50-3·75	3·27-3·76	3·46	3·27	+ 0·19
3·75-4·00	3·30-3·63	3·40	3·36	+ 0·04
4·00-4·25	3·27-3·68	3·49	3·44	+ 0·05
4·25-4·50	3·45-3·55	3·50	3·60	- 0·10
Above 4·50	3·42-3·66	3·52	3·77	- 0·25

The maximum individual differences varied from + 0·46 to - 0·44, or nearly 0·5 per cent.

It is seen from the above table that there is a very slight tendency for the proteins to be higher when the fat is high, but the tendency is very much less than that indicated by Timpe's formula.

H. C. Sherman finds that a milk rich in fat is generally rich in protein, the excess of protein above the normal averaging one-third of the excess of fat.

**Variation of Constituents of Solids not Fat.**—Vieth gives the average proportion between milk-sugar, protein, and ash in milk as 13 : 9 : 2.

The author has found that this ratio is marvellously exact, the average being

Milk-sugar, 52·8 %	as against 54·2	calculated from Vieth's ratio.
Protein, 37·8	„ 37·5	„ „
Ash, 8·3	„ 8·3	„ „

In order to ascertain whether all the constituents of the solids not fat vary directly as the total percentage, or whether an excess or deficiency of solids not fat is due to excess or deficiency of any one constituent, the author has examined a large number of analyses of milk in which milk-sugar, protein, and ash were determined. On plotting out the average figures for solids not fat against each of the constituents, the figures for milk-sugar, protein, and ash lie each in a series of three straight lines. For each constituent the breaks occur between 8·8 per cent. and 8·9 per cent., and between 8·4 per cent. and 8·5 per cent., and are quite well defined. It is suggestive that the one figure is very near the average percentage, and the other is almost that adopted as the limit for normal milk, and the figures show that it would be difficult to dilute down a milk high in solids not fat without arousing a strong suspicion that it is watered. Table XX. gives the average figures deduced ; individual samples may show differences.

TABLE XX.

Solids not Fat.		Milk-sugar.	Protein.	Ash.
Range.	Average.			
about 10 per cent.	10·0	4·79	4·37	0·84
9·00-9·25	9·10	4·77	3·57	0·76
8·75-9·00	8·87	4·75	3·39	0·73
8·60-8·75	8·67	4·60	3·35	0·73
8·40-8·60	8·50	4·48	3·30	0·72
8·20-8·40	8·30	4·18	3·39	0·73
8·00-8·20	8·10	3·94	3·41	0·75

Any deficiency of solids not fat below 9.0 per cent. is chiefly due to a deficiency in the milk-sugar.

Any excess of solids not fat above 9.0 per cent. is chiefly due to an excess of protein.

The ash may be deduced with very fair accuracy from the protein by the formula  $A = 0.36 + 0.11 P$ .

H. C. Sherman supports this conclusion, but finds that the formula  $A = 0.38 + 0.10 P$  agrees rather better with his results.

Table XXI. will give the percentage of ash (calculated on the solids not fat) found in milks containing the percentages of solids not fat named.

TABLE XXI.—PERCENTAGE OF ASH IN MILK.

Solids not Fat.	No. of Samples.	Percentage of Ash on the Solids not Fat.	
		Limits.	Average.
10.5	1	...	8.1
9.7	1	...	8.1
9.6	1	...	8.1
9.5	1	...	8.4
9.4	2	8.1 to 8.5	8.3
9.3	2	8.0 ,, 8.6	8.3
9.2	12	8.0 ,, 8.6	8.3
9.1	33	7.9 ,, 9.1	8.3
9.0	43	7.9 ,, 8.8	8.25
8.9	53	7.9 ,, 8.7	8.3
8.8	36	8.0 ,, 8.9	8.25
8.7	15	7.9 ,, 8.0	8.3
8.6	11	8.0 ,, 8.6	8.3
8.5	8	8.1 ,, 8.7	8.4
8.4	10	8.3 ,, 9.5	8.6
8.3	5	8.5 ,, 8.9	8.7
8.2	7	8.6 ,, 9.1	8.9
8.1	7	8.8 ,, 9.4	9.0
8.0	4	8.8 ,, 10.0	9.2
7.7	1	...	9.1
	253		8.3

All the above samples were undoubtedly genuine.

**Abnormal Milk.**—Samples which differ greatly from the mean percentage of solids not fat almost invariably show a proportion of milk-sugar, protein, or ash very markedly varying from the average. The following analyses (Table XXII.) of abnormal milks will show this :—

TABLE XXII.—ANALYSES OF ABNORMAL MILKS.

No.	Water.	Fat.	Sugar.	Protein.	Ash.	Solids not Fat.	Analyst.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
1	89·00	4·90	1·91	3·35	0·86	6·10	Vieth.
2	85·20	7·80	3·13	3·32	0·76	6·60	„
3	85·3	9·4	..	..	0·78	4·9	„
4	83·3	10·5	..	..	0·76	6·2	„
5	86·14	3·62	4·66	4·58	0·82	10·24	„
6	..	3·14	2·59	3·77	0·88	..	Bodmer.
7	..	2·79	5·10		0·94	6·04	Lowe.
8	87·24	4·32	4·19	3·47	0·78	8·44	Lloyd.
9	89·34	2·60	3·23	3·98	0·85	8·06	Auther.
10	88·61	3·37	4·03	3·24	0·75	8·02	„
11	88·50	3·40	4·08	3·27	0·75	8·10	„
12	87·97	4·04	3·65	3·54	0·80	7·99	„
13	87·47	4·14	3·39	3·68	0·82	8·39	„
14	87·44	4·39	3·32	3·55	0·80	8·17	„
15	87·77	3·94	3·84	3·63	0·82	8·29	„
16	87·10	4·81	3·70	3·59	0·80	8·09	„
17	88·43	3·51	3·41	3·84	0·81	8·06	„
18	87·58	4·00	3·71	3·89	0·82	8·42	„
19	90·84	2·15	2·97	3·29	0·75	7·01	„
20	84·42	5·87	0·68	6·28	1·04	9·71	„

There appears to be good evidence of authentication in the case of all these samples; the table could easily be extended, but the author has chosen those milks which show a very marked departure from the average.

Composition of Milk of Different Breeds of Cattle.—Tables XXIII. and XXIV. give the number of samples which

are found to fall between the percentages named of fat and solids not fat respectively for the milk of single cows. Different breeds are kept separate.

Tables XXIII. and XXVI. are chiefly compiled from analyses by Vieth.

TABLE XXIII.—COMPOSITION OF MILK OF DIFFERENT BREEDS OF CATTLE (FAT).

Percentage of Fat.	Dairy Shorthorn.	Pedigree Shorthorn.	Kerry.	Jersey.	Red Polled.	Other Breeds.	Total.
Above 10	1	...	2	...	...	...	3
8 to 10	10	...	6	11	...	1	28
7 " 8	11	3	17	36	...	4	71
6 " 7	76	8	111	113	6	21	335
5 " 6	382	91	408	136	41	45	1103
4 " 5	1313	594	659	89	70	70	2795
3.5 " 4	625	362	182	14	31	43	1257
3.0 " 3.5	309	173	84	3	26	34	629
2.9	28	15	6	...	6	4	59
2.8	25	15	7	...	1	5	53
2.7	21	8	7	...	2	3	41
2.6	16	10	2	...	2	1	31
2.5	5	5	...	...	1	...	11
2.4	8	5	1	1	...	1	16
2.3	5	1	...	...	...	2	8
2.2	2	...	...	...	...	1	3
2.1	5	1	...	...	...	1	7
2.0	2	...	...	1	...	...	3
1.9	2	1	...	...	...	...	3
1.8	...	...	1	...	...	...	1
1.7	...	...	...	...	...	1	1
1.6	1	...	...	...	...	...	1
1.5	...	...	...	...	...	...	...
1.4	...	...	...	...	...	1	1
1.3	1	...	...	...	...	...	1
1.2	...	...	...	...	...	...	...
1.0	1	...	...	...	...	...	1

The analysis of the samples showing the highest and lowest fat was

Total solids, .	20.97	11.55
Fat, . . . . .	12.52	1.04
Ash, . . . . .	0.73	0.85
Solids not fat, . . . . .	8.23	10.51
Authority, . . . . .	Bannister.	Author

TABLE XXIV.—COMPOSITION OF MILK OF DIFFERENT BREEDS OF CATTLE (SOLIDS NOT FAT).

Percentage of Solids not Fat.	Dairy Shorthorn.	Pedigree Shorthorn.	Kerry.	Jersey.	Red Polled.	Other Breeds.	Total.
Above 10	21	...	6	15	2	7	51
9·5 to 10	112	37	88	91	16	47	391
9·0 ,, 9·5	972	390	744	200	69	114	2489
8·5 ,, 9·0	1491	734	594	91	76	59	3045
8·4	108	70	23	2	6	6	215
8·3	62	30	22	2	7	2	125
8·2	33	9	6	2	...	1	54
8·1	12	9	3	1	...	1	26
8·0	15	7	...	...	3	...	25
7·9	10	2	2	...	1	1	16
7·8	5	1	...	...	...	...	6
7·7	3	2	...	...	2	...	7
7·6	2	1	1	...	...	...	4
7·5	...	...	...	...	2	...	2
7·3	...	...	...	...	1	...	1
7·1	...	...	...	...	1	...	1
6·6	...	...	1	...	...	...	1
6·2	...	...	1	...	...	...	1
6·1	...	...	1	...	...	...	1
4·9	...	...	1	...	...	...	1

The samples yielding below 7 per cent. of solids not fat were all obtained from one cow.

The following are analyses on different dates of her milk:—

TABLE XXV.—VARIATIONS IN SOLIDS NOT FAT IN MILK FROM THE SAME COW.

	P.M. 9/vi/87.	A.M. 10/vi/87.	P.M. 11/vii/87.	A.M. 12/vii/87.	P.M. 13/vii/87.	A.M. 14/viii/87.	P.M. 11/xi/88.	A.M. 12/xi/88.
Total solids,	Per ct. 14·0	Per ct. 12·8	Per ct. 14·3	Per ct. 16·7	Per ct. 11·0	Per ct. 14·8	Per ct. 15·1	Per ct. 12·1
Fat, . . .	4·9	3·8	9·4	10·5	4·9	8·2	6·3	3·2
Milk-sugar, .	...	...	...	...	1·91	3·26	...	...
Protein, . .	...	...	...	...	3·35	3·32	...	...
Ash, . . . .	...	...	0·78	0·76	0·86	0·76	...	...
Solids not fat, .	9·1	9·0	4·9	6·2	6·1	6·6	8·8	8·9

Table XXVI. gives the maximum, minimum, and average percentages of total solids, fat, and solids not fat in the milk of cows of different breeds, obtained from analyses of the milk of cows kept on the Aylesbury Dairy Company's Estate at Horsham.

TABLE XXVI.—SOLIDS IN MILK OF COWS OF DIFFERENT BREEDS (*Vieth*).

Breed.	Total Solids.			Fat.			Solids not Fat.		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dairy Shorthorn,	p. ct. 18·7	p. ct. 10·2	p. ct. 12·90	p. ct. 10·2	p. ct. 1·3	p. ct. 4·03	p. ct. 10·6	p. ct. 7·6	p. ct. 8·87
Pedigree „	16·8	10·5	12·86	7·5	1·9	4·03	9·8	7·6	8·83
Jersey, . . .	19·9	11·0	14·89	9·8	2·0	5·66	10·4	8·1	9·23
Kerry, . . .	18·6	10·6	13·70	10·5	1·8	4·72	10·6	4·9	8·98
Red Polled, . . .	16·2	9·7	13·22	6·6	2·5	4·34	10·2	7·1	8·88
Sussex, . . .	17·4	11·5	14·18	7·6	2·9	4·87	10·3	8·4	9·31
Montgomery, . . .	16·1	10·2	12·61	6·5	1·4	3·59	10·0	7·9	9·02
Welsh, . . .	17·6	11·9	14·15	8·3	3·0	4·91	9·6	8·9	9·24

The figures below (Table XXVII.) were obtained at the New Jersey State Agricultural Experiment Station.

TABLE XXVII.—COMPOSITION OF MILK OF DIFFERENT BREEDS OF CATTLE.

Breed.	Total Solids.	Fat.	Milk-sugar.	Protein.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Ayrshire, . . .	12·70	3·68	4·84	3·48	0·69
Guernsey, . . .	14·48	5·02	4·80	3·92	0·75
Holstein, . . .	12·12	3·51	4·69	3·28	0·64
Jersey, . . .	14·34	4·78	4·85	3·96	0·75
Shorthorn, . . .	12·45	3·65	4·80	3·27	0·73

Fleischmann also gives the following figures :—

Breed.	Total Solids.	Fat.	Solids not Fat.
	Per cent.	Per cent.	Per cent.
Dutch, . . .	11·91	3·23	8·68
German, . . .	12·25	3·40	8·85

Bonnema gives for North Dutch or Frisian cows the following averages :—

Total Solids.	Fat.	Sugar.	Protein.	Ash.	Solids not Fat.
11·5	3·0	4·3	3·5	0·7	8·5

Liverseege gives, using analyses by James Bell, figures for the composition of the milk yielded by cows of different breeds; he notes that in some cases the number of samples is too small to be of much use.



TABLE XXVIII.—SOLIDS IN MILK OF DIFFERENT BREEDS OF CATTLE (*Bell*).

Breed.	Total Solids.	Fat.	Solids not Fat.
	Per cent.	Per cent.	Per cent.
Sussex, . . . . .	12·31	3·39	8·92
Welsh, . . . . .	13·55	4·40	9·15
Guernsey, . . . . .	14·46	5·16	9·30
Jersey, . . . . .	14·65	5·43	9·22
Kerry, . . . . .	13·54	4·67	8·87
North Devon, . . . . .	13·11	3·43	9·68
Dutch, . . . . .	12·40	3·75	8·65
Ayrshire, . . . . .	13·46	4·24	9·22
Shorthorn, . . . . .	12·78	3·92	8·86

**Variations of Fat in Different Churns.**—When milk is divided into portions, as is the case when it has to be transported by railway, considerable variations in fat are sometimes noticed. As examples, the following analyses may be quoted :—

TABLE XXIX.

	Series I.			Series II.		
	1·0345	1·0340	1·0320	1·0325	1·0320	1·0310
Specific gravity,	p. ct.	p. ct.	p. ct.	p. ct.	p. ct.	p. ct.
Total solids, . . . . .	11·28	11·66	14·16	11·22	12·42	13·42
Fat, . . . . .	2·10	2·50	5·10	2·60	3·70	4·80
Solids not fat, . . . . .	9·10	9·16	9·06	8·62	8·72	8·62

**Seasonal and Monthly Variations.**—Distinct variations according to season are found ; these will be shown by Table XXX., which gives the mean monthly averages of milk for the past seventeen years.

The year, roughly speaking, can be divided into four periods :—

(1) November, December, and January ; the milk is rich, both in fat and solids not fat.

(2) February, March, and April ; the solids not fat do not show appreciable diminution, but the fat becomes less in quality.

(3) May, June, July, and August ; the fat is low, though there is a tendency to rise at the end of the period. In July and August the solids not fat are below the average.

(4) September and October ; an improvement in quality both in fat and solids not fat is noticed.

These periods correspond approximately to the seasons ; winter milk is of very good quality, while summer milk is the poorest ; the spring and autumn are transition periods.

The quality varies in an inverse ratio to the quantity yielded.

In the analyses below (Table XXX.) the specific gravity has always been determined by a lactometer; the fat determinations were made by the Gerber method. The total solids have been calculated by the formula devised by the author.

TABLE XXX.—MEAN MONTHLY AVERAGES OF MILK.  
(1897-1913.)

Month.	Specific Gravity.	Total Solids.	Fat.	Solids not Fat.
		Per cent.	Per cent.	Per cent.
January, .	1.0323	12.75	3.78	8.97
February, .	1.0323	12.68	3.72	8.96
March, . .	1.0322	12.64	3.68	8.96
April, . . .	1.0322	12.56	3.65	8.91
May, . . . .	1.0323	12.52	3.57	8.95
June, . . . .	1.0322	12.43	3.52	8.91
July, . . . .	1.0317	12.40	3.63	8.77
August, . . .	1.0315	12.51	3.75	8.76
September, .	1.0318	12.69	3.84	8.85
October, . .	1.0321	12.86	3.92	8.94
November, .	1.0321	12.93	3.97	8.96
December, .	1.0322	12.86	3.90	8.96
Average, . .	1.0321	12.65	3.74	8.91

**Daily Variations.**—It has been found that the percentage of fat varies slightly according to the day of the week, as is shown by the following figures:—

Day,	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	Sun.
Per cent. fat,	3.70	3.78	3.75	3.75	3.75	3.73	3.74

It is seen that Monday's milk is the lowest in fat; this is probably due partly to a disturbance in the quality arising from the interval between milking on Sunday night and Monday morning not being identical with the usual interval, and partly to the influence of the Sunday holiday on the milkers, rendering them rather more careless about stripping the cows on Mondays than on other days.

**Morning and Evening Variations.**—In England it is the custom to milk cows twice a day; the quality is not the same at both meals, the evening milk being almost invariably richer in fat than the morning milk. In dairies where it is the custom to leave an interval of twelve hours between the milkings this is far less noticeable than in those where there is an interval of nine to ten hours between the morning and the evening meal, and fourteen to fifteen hours between the evening and the morning meal.

Table XXXI., giving the mean monthly average of morning and evening milk for the past seventeen years, will show the average difference.

The mean intervals of milking were 10·8 and 13·2 hours.

TABLE XXXI.—COMPOSITION OF MORNING AND EVENING MILK.  
(1897-1913.)

Month.	Morning Milk.				Evening Milk.			
	Specific Gravity.	Total Solids.	Fat.	Solids not Fat.	Specific Gravity.	Total Solids.	Fat.	Solids not Fat.
January, .	1·0324	12·58	3·62	8·96	1·0322	12·92	3·94	8·93
February, .	1·0325	12·52	3·56	8·96	1·0322	12·85	3·89	8·96
March, .	1·0324	12·46	3·51	8·95	1·0320	12·81	3·85	8·96
April, .	1·0323	12·40	3·48	8·92	1·0321	12·73	3·83	8·90
May, .	1·0326	12·27	3·33	8·94	1·0320	12·75	3·80	8·95
June, .	1·0325	12·21	3·29	8·92	1·0320	12·65	3·76	8·89
July, .	1·0320	12·24	3·46	8·78	1·0314	12·56	3·80	8·76
August, .	1·0318	12·31	3·55	8·76	1·0313	12·70	3·95	8·75
September, .	1·0320	12·49	3·63	8·86	1·0316	12·89	4·05	8·84
October, .	1·0322	12·67	3·72	8·95	1·0319	13·04	4·11	8·93
November, .	1·0323	12·77	3·80	8·97	1·0320	13·09	4·14	8·95
December, .	1·0324	12·72	3·75	8·97	1·0321	13·01	4·05	8·96
Average, .	1·0323	12·47	3·56	8·91	1·0319	12·83	3·93	8·90

**Variations on Partial Milking.**—The first portions drawn from the udder are known as “fore milk,” the last portions as “strippings”; it is not unusual to find more than 10 per cent. of fat in strippings. The quality of the milk first drawn from the udder is very different from the last portions. Boussingault has recorded the following analyses of milk drawn from a cow in portions :—

Portion	1	2	3	4	5	6
Total solids, .	Per cent. 10·47	Per cent. 10·75	Per cent. 10·85	Per cent. 11·23	Per cent. 11·63	Per cent. 12·67
Fat, . . . . .	1·70	1·76	2·10	2·54	3·14	4·08
Solids not fat, .	8·77	8·99	8·75	8·69	8·49	8·59

Heaton has also given a remarkable analysis of a partial milking; it contained only 0·26 per cent. of fat.

It is sometimes noticed that a cow, through restlessness or nervousness, holds back her milk, especially if the surroundings

are strange. Thus Dyer has recorded an analysis of milk obtained from a cow at an agricultural show which contained 1·85 per cent. of fat; the next day the milk was normal, containing 3·64 per cent. of fat. Many, if not all, of the very low fats recorded in Table XXIV. on p. 156 are due to this cause.

**Colostrum.**—The name “colostrum” is applied to the secretion of the udder before (and immediately after) parturition. Las-saigne pointed out that an albuminous liquid commenced to form sometimes two months before parturition. This secretion, according to Houdet, often appears under two forms—a brownish, viscous, honey-like product, and a lemon-yellow, non-viscous liquid; the two often co-exist in the same animal, the earlier milkings furnishing the first, and the later the second.

The viscous secretion is curdled by heat, and precipitated by acetic acid, mercuric chloride, and alcohol, but not curdled by rennet. The analysis is

	Per cent.
Water, . . . . .	63·14
Soluble protein, . . . . .	22·74
Colloidal „ . . . . .	14·12
Ash, . . . . .	trace

The non-viscous secretion contained more water and less soluble protein than the viscous secretion, and gave a barely appreciable precipitate with mercuric chloride and alcohol, but was coagulated by heat and acetic acid and unaffected by rennet.

One hundred cubic centimetres contained

Fat, . . . . .	0·15 gramme.
Sugar, . . . . .	0·80 „
Soluble proteins, . . . . .	1·38 „
Colloidal „ . . . . .	4·39 „
Calcium phosphate, . . . . .	0·11 „
Other salts, . . . . .	0·38 „
Total solids, . . . . .	7·21 „

The composition of the fluid secretion approaches more nearly to that of milk.

Four or five days before parturition the secretions are replaced by colostrum proper.

True colostrum is an opaque yellow liquid of pungent taste: sometimes blood is present, which shows its presence by a reddish colour.

It is curdled by heat, acetic acid, mercuric chloride, and rennet (though the action of this is not so rapid as with milk). It has a slimy, viscous appearance, and, if left to stand, has a tendency to separate into two layers.

The proteins probably consist of casein, albumin, nuclein, and

globulin, while lecithin, cholesterol, tyrosine, and urea are present. Proteoses and peptones have been found.

The sugars of colostrum consist of milk-sugar, dextrose, and, possibly, other sugars.

The fat differs from that of milk; the melting point is high (40° to 44° C.), and the amount of volatile acids low. Pizzi found that a few hours (3 to 6) before parturition the Reichert-Wollny figure of the fat was 4·4 to 4·7, and six hours after calving 6·2 to 6·3; a rapid increase was noticed, and in from three to six days a normal figure was reached.

The ash of colostrum has, according to Fleischmann, the following composition :—

	Per cent
Potash, . . . . .	7·23
Soda, . . . . .	5·72
Lime, . . . . .	34·85
Magnesia, . . . . .	2·06
Ferric oxide, . . . . .	0·52
Phosphoric anhydride, . . . . .	41·43
Sulphuric . . . . .	0·16
Chlorine, . . . . .	11·25
	<hr/>
	103·22
Less oxygen equivalent to chlorine, . . . . .	3·22
	<hr/>
	100·00

The best defined characteristic of colostrum is the presence of the "corps granuleux" of Donné, which consist of clusters of cells like bunches of grapes. These are from 0·005 to 0·025 millimetre in diameter, and are easily detected under the microscope. They do not disappear entirely from milk till three weeks after calving, according to Henle.

The specific gravity of colostrum is from 1·046 to 1·079 at 15° C. (59° F.), and averages 1·068.

Engling gives the following composition :—

	Per cent.	Per cent.	Per cent.
Water, . . . . .	76·60	to 67·43	average, 71·69
Fat, . . . . .	1·88	,, 4·68	,, 3·37
Casein (?), . . . . .	2·64	,, 7·14	,, 4·83
Albumin (?), . . . . .	11·18	,, 20·21	,, 15·85
Sugar, . . . . .	1·34	,, 3·83	,, 2·48
Ash, . . . . .	1·18	,, 2·31	,, 1·78

**Change of Colostrum to Normal Milk.**—The composition of colostrum changes rapidly after parturition. Houdet gives the following figures as illustrating the change :—

	Fat.	Sugar.	Soluble Proteins.	Colloidal Proteins.	Calcium Phosphate	Other Salts.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
Six days before calving,	0.50	2.35	0.47	17.43	0.44	0.36
Four " " "	3.01	3.17	0.45	12.08	0.47	0.40
Immediately after "	3.14	2.70	0.25	14.53	0.46	0.42

These figures show a sudden increase in the amount of proteins over that contained in the fluid secretion described above. Houdet ascribes this to a diversion to the udder of nutritive material which up to this time had been supplied to the fœtus. An increase of fat and a decrease of soluble proteins are also observed.

The colostrum from another cow was examined at intervals after parturition with the following results (Table XXXII.), which show the gradual transition into normal milk :—

TABLE XXXII.—CHANGE OF COLOSTRUM TO NORMAL MILK.

Date.	Fat.	Sugar.	Soluble Proteins	Colloidal Proteins.	Calcium Phosphate	Other Salts.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
Immediately after calving	5.69	3.30	0.51	14.05	0.51	0.54
1 day " "	4.48	4.05	0.93	5.21	0.43	0.43
2 days " "	5.70	4.32	1.98	3.52	0.43	0.45
3 " " "	7.40	4.26	2.41	3.45	0.43	0.40
4 " " "	3.20	4.44	0.56	5.20	0.40	0.30
6 " " "	4.20	4.64	1.19	4.02	0.38	0.29
8 " " "	4.10	4.96	0.48	3.56	0.40	0.30
14 " " "	3.85	5.03	0.58	3.74	0.35	0.36

Vaudin gives the following figures (Table XXXIII.) as showing the composition of colostrum :—

TABLE XXXIII.—COMPOSITION OF COLOSTRUM.

Date.	Fat.	Sugar.	Proteins.	Calcium Phosphate	Other Salts.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Evening before calving,	1.30	1.52	23.70	0.62	0.47
	6.32	2.17	14.91	0.63	0.46
Immediately after calving (4 cows),	3.84	2.37	20.10	0.66	0.39
	1.36	1.02	19.02	0.61	0.46
	2.42	2.86	17.68	0.87	0.34
Five days after calving,	5.18	4.07	4.35	0.38	0.49

Colostrum differs from milk in containing less sugar, a fat which is very poor in volatile acids, and a high amount of nitrogenous compounds, which differ from those of milk. The discrepancy between the results of Engling and Houdet is due to the methods for the separation of the nitrogenous compounds not being known.

Steinegger has shown that colostrum has a high aldehyde figure.

Milk containing colostrum is not used for dairy purposes ; at least four days should be allowed to elapse after parturition before the milk is employed for consumption. Miss E. G. Cook has, however, patented its use for the manufacture of milk for infants.

Generally speaking, the milk of newly-calved cows is poorer in fat than that of cows towards the end of their period of lactation. Kuhn's experiments have shown that the casein also increases as the period of lactation advances, while the milk-sugar decreases ; the mineral matter also increases towards the end of lactation. Most of the analyses on p. 154 which show a high percentage of proteins were obtained from cows which were getting dry.

The milk of cows in ill-health may have a very abnormal composition. Wynter Blyth has collated the information concerning these in his *Foods, their Composition and Analysis* (q. v.). They are, however, of interest from a pathological point of view, rather than of practical importance in dairying.

**Limits and Standards of Milk.**—The President of the Board of Agriculture and Fisheries has laid down, after enquiry had been made by a Departmental Committee on Milk Standards appointed by him, the limits of 3·0 per cent. of fat and 8·5 per cent. of solids not fat ; a presumption is raised, till the contrary is proved, that any milk yielding figures on analysis below these limits is not genuine, but has in the former case been deprived of a portion of its cream, and in the latter has been adulterated by water.

The figures are identical with the limits previously adopted, unofficially, by the Society of Public Analysts ; in practice the official adoption of the figures has resulted in a strengthening of the limits ; the wording of Clause 4 of the Sale of Food and Drugs Act, 1899, has transferred the onus of proof from the prosecution to the defence. These limits do not represent the absolute minima yet found, as will be readily seen by referring to the figures previously quoted, but are limits below which mixed milk of a herd of cows may be reasonably expected not to fall. Vieth, in discussing the question how far they could be applied to all milks, has written : " My object is by no means

to raise the cry that the standard adopted by the Society is too high; on the contrary; I think it is very judiciously fixed, but, in upholding the standard of purity, it should not be forgotten that the cows have never been asked for, nor have given their assent to it, and that they will at times produce milk below standard. A bad season for hay-making is, in my experience, almost invariably followed by a particularly low depression in the quality of the milk towards the end of the winter. Should the winter be of unusual severity and length, the depression will be still more marked. Long spells of cold and wet, as well as of heat and drought, during the time when cows are kept on pasture, also unfavourably influence the quality and, I may add, quantity of milk."

Table XXXIV. will show the probable number of samples per 100,000 examined which may be expected to be found between the percentages named.

TABLE XXXIV.—PERCENTAGE OF FAT AND SOLIDS NOT FAT IN MIXED MILK.

Percentage of Fat.	Number of Samples.	Percentage of Solids not Fat.	Number of Samples.
2.9 to 3.0	370	8.4 to 8.5	1892
2.8 " 2.9	209	8.3 " 8.4	242
2.7 " 2.8	87	8.2 " 8.3	27
2.6 " 2.7	37	8.1 " 8.2	22
2.5 " 2.6	16	8.0 " 8.1	8
Below 2.5	13	Below 8.0	2

Table XXXV., p. 166, shows the percentage of samples which have fallen below the Government Standard for fat in morning milk during May and June since 1900; it is practically only in these months that any serious number of low samples occurs.

In an extended series of analyses of milk, the author has found that the number of samples yielding any given percentage of fat is in agreement with that calculated by the usual methods from the theory of probabilities, provided that morning and evening milks are treated as separate series. This shows that standards can be calculated by actuarial methods from the actual results.

By a formula based on the theory of probabilities, the author has calculated standards for each month below which milk should not reasonably be expected to fall for both fat and solids not fat, and in the case of fat has confirmed them by taking the



TABLE XXXV.—FAT IN MORNING MILK ONLY.

	May.				June.			
	2·9 to 3·0	2·8 to 2·9	2·7 to 2·8	Below 2·7	2·9 to 3·0	2·8 to 2·9	2·7 to 2·8	Below 2·7
1900, .	4·1	0·9	0·4	..	3·0	2·0	0·4	0·2
1901, .	4·0	2·0	1·6	0·4	2·0	1·4	..	0·4
1902, .	2·2	2·0	0·8	..	4·7	1·5	0·4	..
1903, .	2·0	0·9	..	..	1·7	0·6	0·8	..
1904, .	2·7	1·8	0·9	0·5	3·6	3·2	0·8	0·2
1905, .	6·0	2·0	0·6	0·2	3·1	2·0	0·2	..
1906, .	3·0	0·8	0·4	0·2	5·5	1·7	0·2	..
1907, .	3·2	2·0	0·2	0·7	5·7	2·0	1·0	0·8
1908, .	0·6	0·5	0·2	..	2·3	0·4	0·2	..
1909, .	3·9	1·6	0·9	..	5·2	1·7	0·8	0·1
1910, .	3·0	2·1	0·8	0·5	3·0	1·2	1·0	0·1
1911, .	4·1	2·1	0·5	0·2	5·6	0·5	0·5	0·2
1912, .	4·6	4·2	1·6	0·4	3·9	0·9	0·7	0·5
1913, .	3·5	2·2	1·0	1·3	4·6	2·2	0·6	1·0
Mean, .	3·7	1·8	0·7	0·3	3·9	1·5	0·5	0·2

mean lowest percentage of fat in the milk sent out by the Aylesbury Dairy Company.

These are :—

TABLE XXXVI.

Month.	Calculated Standard.		Lowest Percentage of Fat in Milk sent out.
	Solids not Fat.	Fat.	
January, . . . . .	8·50	3·14	3·35
February, . . . . .	8·50	3·08	3·25
March, . . . . .	8·49	3·08	3·25
April, . . . . .	8·53	3·05	3·22
May, . . . . .	8·60	2·84	2·97
June, . . . . .	8·53	2·84	2·94
July, . . . . .	8·36	3·01	3·05
August, . . . . .	8·28	3·09	3·05
September, . . . . .	8·36	3·16	3·22
October, . . . . .	8·52	3·17	3·27
November, . . . . .	8·49	3·13	3·22
December, . . . . .	8·52	3·12	3·28

According to the author's experience the limits of 3·0 per cent. for fat is certainly reasonable for the mixed milk of a whole

herd, except perhaps in May and June; such milk very rarely, if ever, falls appreciably below this limit. It is far more frequent, especially during July, August, and September, for milk to contain less than 8·5 per cent. of solids not fat; in the majority of these cases, the author has found that at least 0·50 per cent. of total nitrogen and 0·70 per cent. of ash was present, and this experience has received much confirmation. Smetham and some American observers have, however, found that even these limits are somewhat too high for the milk of Dutch or Holstein-Frisian cows, and the author has also found some samples which do not conform to this rule. At the present time this breed of cows does not form a majority of English milch-cattle; on farms where they are kept other breeds yielding milk of higher quality are also milked.

**Multiple Standard.**—For all practical purposes the multiple standard of 8·5 per cent. of solids not fat, 4·5 per cent. of milk-sugar, 0·50 per cent. of total nitrogen, and 0·70 per cent. of ash may be adopted for the purpose of judging whether a milk is of genuine composition or not. The figure for the ash is, however, liable to be increased by the addition of mineral substances added to the milk; thus boric acid and borax, used as preservatives, and salt, added to mask the addition of water, would raise the ash; estimation of the boric acid, which is absent in genuine milk, or of the chlorine, which does not often exceed 0·10 per cent., will show additions of this nature. The amount of ash insoluble in hot water is also a useful figure; it amounts in milk to at least 0·50 per cent., and is very nearly equal to the total nitrogen.

A milk should never be pronounced as watered on the evidence of the solids not fat alone, unless this is well below 8·0 per cent.; a determination of the milk-sugar, total nitrogen, and ash should be made in addition; a judgment formed on the three determinations will be in all probability correct, and if the figures for at least two of them are above the limit, the milk is probably genuine.

**Variations of Fat in Milk on Standing.**—The fat globules of milk have a natural tendency to rise to the surface and to thus cause an unequal distribution of fat in different portions of the milk.

Table XXXVII. will give an idea of the rate at which appreciable change in the composition of the milk occurs; 12 gallons of well-mixed milk were placed in a churn with a tap at the bottom at 11·25 a.m., and a measure holding 7 quarts was drawn out every half-hour till 2·25 p.m.; each of these quantities was analysed, as also the residue left in the churn (5 quarts): the milk was undisturbed throughout.

TABLE XXXVII.—VARIATIONS IN COMPOSITION OF MILK ON STANDING (LARGE CHURN).

Time.	Specific Gravity.	Total Solids.	Fat.	Solids not Fat.
		Per cent.	Per cent.	Per cent.
11.25 a.m.	1.0324	12.69	3.71	8.98
11.55 „	1.0325	12.68	3.68	9.00
12.25 p.m.	1.0328	12.35	3.34	9.01
12.55 „	1.0331	12.13	3.10	9.03
1.25 „	1.0334	12.03	2.95	9.08
1.55 „	1.0334	11.97	2.90	9.07
2.25 „	1.0334	11.97	2.90	9.07
Residue.	1.0283	16.65	7.87	8.78

In another experiment two large churns, such as are used in restaurants, each holding 12 quarts, were stood side by side; every quarter of an hour 1 quart was drawn from the tap and the amount of fat in each portion was estimated (Table XXXVIII.). The residue was not analysed, but undoubtedly contained a high percentage of fat.

TABLE XXXVIII.—VARIATIONS IN COMPOSITION OF MILK ON STANDING (SMALL CHURNS).

Time.	No. I.	No. II.
Start.	3.72 % fat.	3.72 % fat.
15 min.	3.65 „	3.64 „
30 „	3.72 „	3.65 „
45 „	3.45 „	3.38 „
60 „	2.95 „	2.85 „
75 „	2.85 „	2.76 „
90 „	2.67 „	2.67 „
105 „	2.63 „	2.60 „
120 „	2.64 „	2.57 „
135 „	2.54 „	2.50 „

It is seen from the results of these experiments, which are typical of many, that milk left to stand remains approximately of the same composition for short periods only—not exceeding half an hour; by continually drawing off the bottom layer, samples are obtained which become poorer and poorer in fat till the upper layer of cream is reached.

A similar phenomenon is observed, if the milk is dipped from the top of a counter pan, as the following experiment will show:—

Three quarts of milk were placed in a pan; every half-hour

one pint was removed by dipping from the surface; and each portion was analysed (Table XXXIX.).

TABLE XXXIX.—VARIATIONS IN COMPOSITION OF MILK ON STANDING (PAN).

Time.	Percentage of Fat.
Start.	3.65
30 min.	3.75
60 "	4.40
90 "	4.15
120 "	3.75
Residue.	2.80

Here again it is seen that the milk does not remain practically constant in composition for more than half an hour.

**Court of Queen's Bench Decision.**—These figures were obtained under conditions which need never occur in practice; indeed, the decision of the Court of Queen's Bench in the case of *Dyke v. Gower* makes it necessary that they must not occur, as it has been decided that a vendor is bound to sell milk in its natural state; it is equally an offence against the law to sell milk which has been deprived of its cream by natural rising when the milk is undisturbed, and to sell that wilfully adulterated with skim milk.

**Practical Allowances for Fat Variation.**—It is, however, fortunately not a matter of extreme difficulty for a vendor to comply with the spirit of this judgment: for instance, in the sale of milk from a counter pan it is easy to stir the milk every half hour, or, what is preferable, before serving each customer. When milk is delivered in the streets the churn can be fitted with one of the numerous arrangements for automatically keeping the cream mixed; the action of these is, too, materially aided by the motion the milk receives from the movement of the cart or barrow when drawn along the streets. A steady current of about 1 foot per hour is enough to keep milk mixed, and, except in very hot weather, milk is not churned by gentle stirring. To ensure that the composition of the milk will not vary the minutest fraction perhaps demands more skill and attention than the average milk distributor possesses, but a practical compliance with the judgment mentioned above can and ought to be obtained. The author has calculated from a large number of results that the probable variation of fat in milk due to cream rising is only 0.11 per cent.

It cannot be too strongly insisted on that the calculated standards apply only to the mixed milk of a number of cows; the milk of a single cow may be below these figures to a serious extent. As this case is one which but rarely occurs—the sale of milk being almost entirely confined to the production of herds—it is not necessary to make any allowance for the greater variations of quality of the milk of individual cows.

**Appeal to the Cow.**—In cases of doubt it is advisable to resort to what is known as “appeal to the cow,” or the “stall or byre test.” This consists in having the cow—or cows—milked in the presence of a responsible witness who can certify to the absolute genuineness of the milk, which is analysed and compared with the suspected sample. It is desirable, if possible, that the milk of the morning and evening meals should both be examined. To make the test as fair as possible, the cows should be milked by their usual milkers at the same time of day as the previous sample, and under the same conditions; the test should be carried out at as early a date as convenient, and care should be taken that the meteorological conditions are nearly alike, as a poorer milk is yielded in warm, damp weather than if it is clear and frosty. The test should not be carried out on a Sunday or Monday, or on a public holiday or its morrow, unless the previous sample was taken on a similar day, as it has been shown that the irregularity in the time of milking—which occurs on such days—affects the quantity and quality of the milk; any serious divergence from the average quantity of milk yielded may be looked upon as throwing doubt on the reliability of the test. The witnesses must be specially careful in seeing that the cows are milked out, and that nothing occurs likely to disturb the equanimity of the cows, such as undue commotion or noise. If the milk is cooled, it is the duty of the witnesses to satisfy themselves that the refrigerator does not leak, as well as to see that all vessels into which milk is received are clean and dry.

This test, if properly carried out by competent witnesses, is very reliable; if the suspected sample were genuine, milk will be yielded of approximately the same composition at the appeal to the cow; everything, however, depends on the competency of the witnesses.

**Influence of Feeding and other Conditions on the Composition of Milk.**—If the food given to the cattle is sufficient both in quantity and ratio of constituents no appreciable variation in the composition of the milk is found on changing the food. The author has noticed that, if the food given makes the cows scour, the milk is likely to be low in fat, and the percentage of fat is raised by the addition of a more binding food—*e.g.*, cotton cake—to their ration.

TABLE XL.

	Mixed Milk.		Cow No. 5.	Cow No. 8	Cow No. 11.	Cow No. 13	Cow No. 17.		Cow No. 22.
	Churn I.	Churn II.					Fore Milk.	Strippings.	
Specific gravity, .	1-0311	1-0316	1-0320	1-0284	1-0337	1-0302	1-0314	1-0300	1-0330
Total solids,	10-19	10-69	10-56	8-96	9-99	11-10	9-69	11-07	10-54
Fat, .	1-87	2-08	1-90	1-47	1-21	2-98	1-36	2-93	1-63
Sugar, .	4-30	4-33	4-00	3-80	4-38	4-77	4-47	4-43	4-38
Protein, .	3-23	3-52	3-82	2-90	3-52	2-67	3-15	3-01	3-72
Ash, .	0-79	0-76	0-84	0-79	0-88	0-68	0-71	0-70	0-81
Solids not fat, .	8-32	8-61	8-66	7-49	8-78	8-12	8-33	8-14	8-91

The figures in Table XL. afford a striking illustration of the effect of food causing scouring on the composition of milk, and show how easy is the remedy.

A herd of 26 cows (Ayrshires) was turned out into a field of new grass at the end of April, and received no other food; the percentage of fat rapidly fell, and on May 7 the author saw all the cows milked, and the foregoing analyses of the milk were made.

The cows were very thin, though otherwise pronounced by a leading veterinary surgeon quite healthy, and suffered from profuse diarrhœa, the motions being quite liquid, and containing much undigested food. On this day the food was changed, and the quality of the milk steadily rose till within a week the percentage of fat was over 3·0 per cent., and remained above this figure. The only cow of the herd which had not been turned out to the new grass was No. 13, and it is seen that her milk was practically up to the standard in fat.

A too highly saccharine diet is not advisable, and may cause a disturbance in the composition of the milk. The author has examined the milk of three cows which had been fed on a ration containing much sugar; apparently the sugar had fermented in their stomachs, as the cows suffered from the effects of alcohol.

The analyses were :—

TABLE XLI.

	Cow No. 1.		Cow No. 2.		Cow No. 3.	
	Before.	After.	Before.	After.	Before.	After.
Sp. gr., . . .	1·0322	1·0243	1·0315	1·0304	1·0321	1·0284
Total solids, .	12·97	9·88	12·41	11·06	11·97	15·58
Fat, . . . . .	4·26	2·87	3·63	2·77	3·14	5·87
Sugar, . . . . .	5·00	2·15	4·72	4·05	4·72	0·68
Protein, . . . .	2·98	3·67	3·33	3·05	3·37	6·28
Ash, . . . . .	0·73	0·89	0·73	0·70	0·74	1·04
Solids not fat,	8·71	7·01	8·78	8·29	8·83	9·71

A. C. Abrahams has found that exposure to cold winds has the effect of causing milk low in fat to be produced.

It is seen that the following rules will go a long way towards preventing abnormally low fats.

- (i.) Do not let the cows scour.
- (ii.) Do not give too much saccharine food.
- (iii.) Do not expose the cows to very cold weather.

**Adulterations of Milk.**—The chief adulterations of milk are—

(1) The addition of water, which is sometimes masked by the use of a solid substance which is soluble.

(2) The addition of skim or separated milk, or the removal of cream.

**Calculation of Added Water.**—Watering is detected by the depression of the solids not fat, total nitrogen, and ash; if all three are below the limits given above, the milk may be condemned as watered. The amount of water added is best calculated from the solids not fat by the formula—

$$\text{Water} = 100 - \frac{S}{8.5} \times 100,$$

where S = solids not fat.

This formula will give the minimum percentage of water added. It is only correct if the original milk contained 8.5 per cent. of solids not fat.

The probable amount can be calculated by using the mean figure for solids not fat 8.9, instead of 8.5, in the above formula.

Another excellent method for calculating percentage of added water is to use the sum of the degrees of specific gravity and the fat as a datum.

$$\text{Water} = 100 - \frac{G + F}{34.5} \times 100,$$

where G = degrees of gravity, and F = the percentage of fat.

This will likewise give a minimum figure, and the probable amount can be obtained by substituting 36 for 34.5.

The latter formula has the advantage that it is applicable without correction to milk which contains an excess or deficiency of fat, while the percentage of solids not fat is affected to some extent by variations in the fat; Table XXXVII. will make this clear. The solids not fat vary from 8.78 to 9.08, a difference of 0.30 or 3.3 per cent. of the solids not fat; the sum of the degrees of specific gravity and fat only varies from 36.11 to 36.35, a difference of 0.24 or 0.7 per cent. of the sum.

Another formula in which the percentage of water is calculated from the aldehyde figure (A) is

$$\text{Water} = 100 - \frac{A}{20} \times 100.$$

As these formulæ fail with abnormal milks, the author has



proposed the two following formulæ, which hold good, not only with normal milks, but with abnormal milks in addition :—

$$(S - L) \frac{100}{100 - F} \text{ exceeds } 4.$$

$$G + F - 4L \text{ exceeds } 16.$$

S = solids not fat, L = milk-sugar, F = fat, and G = lactometer degrees.

**Adulterations by Cane Sugar, &c.**—Sometimes substances such as cane sugar, dextrin, or other carbohydrates or glycerine are added to mask the addition of water by raising the solids not fat; these will be detected by the sweet taste, the deficiency in total nitrogen and the ash. Cane sugar, dextrin, etc., can be detected by the discrepancy between the milk-sugar estimated by polarisation and that determined by Fehling's solution (see pp. 90 and 95). The detection and estimation of cane sugar is given on p. 101.

**Glycerine**, if added to any appreciable extent, will render the total solids sticky, and on analysing the sample the water, fat, milk-sugar, proteins, and ash will in the aggregate be seriously below 100 per cent. It can be detected, and approximately estimated, by evaporating 25 c.c. of milk to a pasty consistency, treating with a mixture of alcohol and ether, and following the procedure of the maceration method of analysis; the alcohol-ether extract is evaporated and the residue exhausted with a little water and this again evaporated. If glycerine be present, a residue having a sticky consistency when cold will be left; the weight of this, less that of the ash left on ignition, will approximately give the amount of glycerine.

**Starch** has also been used; this is detected by a blue coloration being obtained with a solution of iodine in potassium iodide (see p. 106).

Rennet is occasionally added to milk, and more especially to separated milk, with the idea that if mixed with warm milk it will cause curdling. Its presence may be inferred if the milk curdles on warming to 40° C., and the acidity is less than 25°; the whey on neutralising to an acidity of 12° will cause fresh milk to curdle at 40° C., and the amount of lime in the whey does not exceed 0.06 per cent.

**Brains and mammary tissue** are said to have been used; this is doubtful, but they would be shown at once by the large deposit obtained on centrifuging the milk.

Mineral adulterants have been employed. The use of chalk, which is popularly supposed to enter into the composition of adulterated milk, is probably hypothetical, as its insolubility would defeat the object of its use. Salt is detected in the ash

by an increase in the chlorides above 0·10 per cent. ; an estimation of sodium should also be made, as milk does not contain more than 0·05 per cent. ; **carbonate or bicarbonate of soda** is also detected by the increased alkalinity of the soluble ash: this does not exceed in genuine milk an amount equal to 0·025 per cent.  $\text{Na}_2\text{CO}_3$ ; an amount appreciably exceeding this is due to addition of alkali. The alkalinity of the ash should be estimated by titrating with  $\frac{\text{N}}{10}$  acid, using phenolphthalein as indicator; 1 c.c. of the acid is with this indicator equal to 0·0106 gramme of  $\text{Na}_2\text{CO}_3$ .

Other mineral additions, such as boric acid, borax, fluorides, etc., may be added as preservatives, and not to mask the addition of water; the methods of detecting these will be given later.

It has been alleged that **salts of ammonia** have been added to raise the total nitrogen. These would be detected by rendering alkaline with magnesium carbonate, distilling the milk, and testing the distillate with Nessler's reagent (an alkaline solution of mercuric chloride in potassium iodide).

**Calculation of Fat Abstracted.**—The detection of adulteration by removal of cream can only be effected with certainty by the estimation of fat; if this falls below 3·0 per cent., a presumption is raised that cream has been abstracted. From the table on p. 159 it is seen that the mean percentage of fat varies at different times of the year; a limit of 3·25 per cent. could be used from October to January with as much justification as a limit of 3·0 for the other months.

The percentage of cream abstracted is calculated by the formula

$$\text{Cream abstracted} = 100 - \frac{\text{F}}{3} \times 100,$$

where F = percentage of fat.

This formula gives a minimum percentage of fat abstracted. The figure thus calculated is almost always seriously below the truth; the probable amount can be calculated by substituting 3·74 for 3, or, better still, the monthly average figure given in Table XXX. on p. 159 for the month in which the analysis is made.

If "appeal to the cow" has been made, or if the mean composition of the milk was approximately or exactly known, the figure representing the actual composition should be substituted for 3.

The colour of the fat is of some aid in judging the amount of cream abstracted; if it is very yellow, the milk is very likely yielded by Jersey cows, and a high figure—*e.g.*, 4—may be substituted for 3.

The colour of the milk itself is no guide, as it is frequently artificially coloured to give it an appearance of richness. Annatto was the colouring-matter chiefly used, but this is now somewhat largely replaced by coal-tar colours, especially the sodium salt of di-methyl-amino-azo-benzene sulphonic acid or methyl orange. Artificial colouring-matters generally may be detected by precipitating the casein with acetic acid, washing well with water, and digesting with strong alcohol; the casein carries down the colouring-matter and gives it up to the alcohol; on evaporating this, and taking up with a little water, the colour can be detected. Annatto is unchanged by mineral acids, while many of the coal-tar colours turn pink.

The following method for the detection of colouring-matters in milk is based on a scheme devised by M. Wynter Blyth :—

*Preliminary Tests.*—(1) Allow a portion of the milk to stand in a cool place till the cream rises; if the skim milk is more highly coloured than the cream the milk is artificially coloured.

(2) Add a drop or two of hydrochloric acid to a little milk; a pink colour indicates the presence of an azo colour, of which the following, among others, may occur in milk :—

Aniline yellow. Amino-azo-benzene.

Butter-yellow. Chrysoidine. Di-methyl-amino-azo-benzene.

Acid yellow. Salts of amino-azo-benzene sulphonic acid.

Methyl-orange. Salts of di-methyl-amino-azo-benzene sulphonic acid.

Orange IV. Diphenylamine-yellow. Salts of di-phenylamine-azo-benzene sulphonic acid.

(3) Make the milk alkaline with sodium bicarbonate, and immerse a strip of filter paper therein for at least twelve hours. A reddish-yellow stain indicates annatto.

These tests may fail to show artificial colouring-matters, because (1) aniline-yellow and butter-yellow are soluble in fat, and may rise with the cream; (2) azo-compounds are reduced in stale milk to colourless compounds; and (3) a colour such as phosphine (di-amino-phenyl-acridine usually mixed with di-amino-toluy-acridine) or caramel has been used.

It is better, therefore, to use the general method :—Take 50 c.c. (or more) of milk, make just alkaline to litmus, and evaporate to a paste, and thoroughly extract the fat with ether. Evaporate the ethereal solution, and shake up the fat with a little hot distilled water, separate the water, and evaporate to dryness in a small porcelain dish. Pure milk gives no coloured residue; if the residue is coloured, this will be due to a reduction product of an azo colouring-matter, or to the unreduced colouring-matter. Next, extract the fat-free residue with absolute alcohol, filter the alcoholic extract, and evaporate to dryness in three or four

porcelain dishes. Unreduced colouring-matters will leave a coloured residue.

A pink colour is usually due to the presence of blood; this may be detected by warming the milk to 50° C., and separating it in a high speed centrifuge (Fig. 15); if blood is present a bright red deposit is seen at the bottom of the tube. The deposit may be examined microscopically, and it is usually found that the blood-corpuscles have become considerably disintegrated,

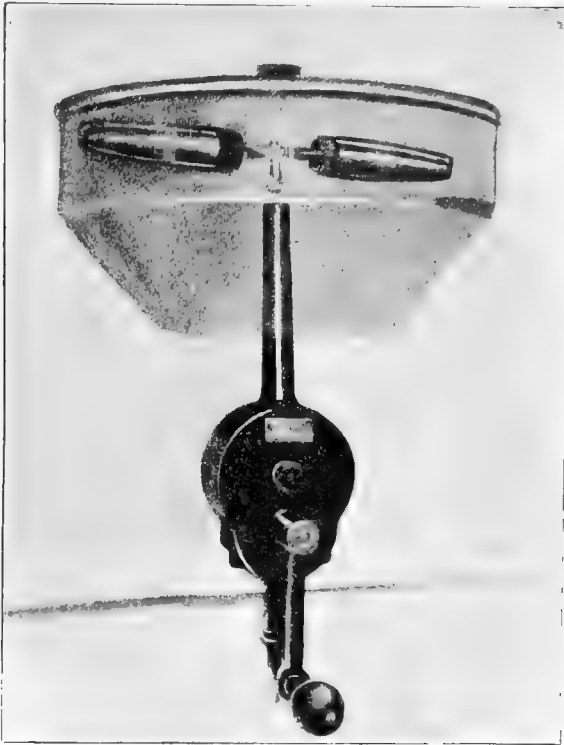


Fig. 15.—High-Speed Centrifuge.

and have the appearance shown in the plate (Fig. 16). As a confirmatory test the residue should be treated with a drop of acetic acid on a microscope slide, a cover glass placed over the mixture, and the acetic acid gently evaporated over a very small flame. When nearly dry, the slide should be examined with  $\frac{1}{8}$ -inch power, and the presence of brown rhomboid crystals of hæmin hydrochloride will indicate blood.

**Preservatives.**—In order to check the growth of micro-

organisms in milk, and thus make it keep for a longer time than it otherwise would, preservatives are frequently added. The most common additions for this purpose are boric acid and its sodium salt, borax; salicylic acid, either alone or mixed with borax and boric acid, and sometimes in alcohol or glycerol solution; fluorides, such as sodium fluoride or potassium acid fluoride; fluosilicates and fluoborates;  $\beta$ -naphthol and salts of the  $\beta$ -naphthol-sulphonic acids (abrastol), formaldehyde, and benzoic acid. Potassium nitrate has also been recommended, but it is a comparatively weak antiseptic, and is little, if at all, used. Hydrogen peroxide is also used.

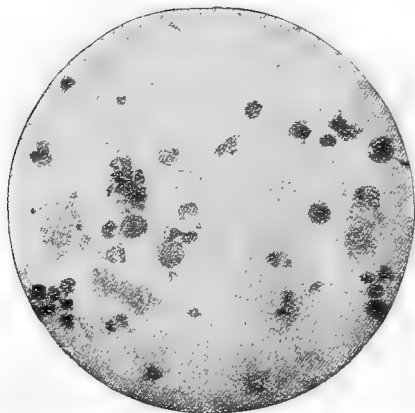


Fig. 16.—Blood in Milk.

**Effect of Preservatives.**—The effect of boric acid preservative and formaldehyde has been studied by the author and Harrison; taking milk which just curdles on boiling as the limit of fresh milk, the following are the extra times that the preservatives will keep milk fresh :—

TABLE XLII.

Temperature.	Boric Acid.		Formaldehyde.		
	0.05 per cent.	0.10 per cent.	0.0025 per cent.	0.005 per cent.	0.010 per cent.
60° F.,	26 hours.	40 hours.	8 hours.	35 hours.	63 hours.
70° F.,	6 "	14 "	5 "	17 "	41 "
80° F.,	3 "	9 "	4 "	13 "	31 "
90° F.,	2 "	8 "	2 "	11 "	26 "

Table XLII. shows how useless these preservatives in small amounts are, and an equal effect is produced by a few degrees lowering of temperature.

The author and Miller have investigated the action of other preservatives on milk; the results at 20° C. = 68° F. were:—

TABLE XLIII.

	0·2 per cent.	0·1 per cent.	0·05 per cent.	0·025 per cent.
Sodium benzoate,	16·7 hours.	8·9 hours.	4·1 hours.	1·3 hours.
Potassium "	15·6 "	9·4 "	3·9 "	1·0 "
$\beta$ -naphthol,	56·0 "	9·5 "	2·5 "	0·8 "
Salicylic acid,	21·5 "	15·5 "	7·0 "	4·2 "
Sodium sulphite,	15·5 "	5·5 "	-0·5 "	-2·0 "
Potassium meta-bisulphite,	134·0 "	43·0 "	21·0 "	5·5 "
	0·174 per cent.	0·10 per cent.	0·08 per cent.	0·044 per cent.
Boric acid,	30·5 hours.	20·5 hours.	17·5 hours.	8 hours.
				3·5 hours.

The following substances had no appreciable preservative effect:—Sodium fluoride, potassium acid fluoride, resorcinol, phloroglucinol, phthalic acid, abrastol, sodium  $\beta$ -naphthol-sulphonate, and cyllin.

**The Souring of Milk.**—Lactic acid is developed by the action of micro-organisms on the milk-sugar, and the acidity of the milk is a rough measure of this.

TABLE XLIV.—TEMPERATURE IN DEGREES FAHRENHEIT.

Time in Hours.	60°	70°	80°	90°
10, . . . . .	0·0	0·1	0·2	2·5
15, . . . . .	0·1	0·2	2·5	21·0
20, . . . . .	0·2	1·1	10·2	65·0
25, . . . . .	0·3	4·0	41·0	76·0
30, . . . . .	1·1	10·2	65·0	81·0
35, . . . . .	3·0	27·0	73·0	85·0
40, . . . . .	6·0	50·0	78·0	88·5
45, . . . . .	10·2	65·0	81·0	91·5
50, . . . . .	20·0	71·0	83·5	94·0
55, . . . . .	35·0	75·0	86·0	95·0
60, . . . . .	50·0	78·0	88·5	96·0
70, . . . . .	68·0	82·0	93·0	97·0
80, . . . . .	73·0	85·5	95·0	98·0
90, . . . . .	78·0	88·5	97·0	98·5
100, . . . . .	80·0	91·5	98·0	99·0

Table XLIV. gives the average amount of acidity above the normal acidity of milk developed in the times stated at various temperatures (see also Fig. 14, p. 135).

The rate of souring increases 1.5 times for a rise of 10° F. in temperature, or 2.075 times for a rise of 10° C.

When milk reaches an acidity of 13° above the normal it curdles on boiling, and at 65° it curdles spontaneously; the times taken to reach these points are:—

Acidity.	Time in Hours at			
	60° F.	70° F.	80° F.	90° F.
13°, . . . .	47	31	21	14
65°, . . . .	68	44	30	20

It is seen that lowering of temperature has an immense influence on the life of milk.

Boric acid preservatives are used in quantities varying from 0.01 up to 0.3 per cent.

The following figures show the amounts used:—

Up to	0.03 %	in 45 %	of samples containing boric preservative.
From 0.03 to 0.06	„	27.5	„ „ „
Above	0.06	„	27.5

In the bulk of the samples the quantity added was insufficient to have any really useful effect in hot weather.

Formaldehyde appears to be added in proportions varying from 0.002 to 0.005 per cent., and only the larger quantities would be really efficient as preservatives.

To sum up, it appears that to be of any real use in hot weather at least 0.1 per cent. of boric preservative, or 0.004 per cent. of formaldehyde is necessary, and even then the effect is only equal to that produced by cooling down the milk about 10° F.; the cost of cooling is approximately the same as the cost of preservatives, and so far as milk is concerned, there is absolutely no justification for the use of preservatives; the practice appears to be dying out.

**Objections.**—The practice of adding preservatives is by many considered highly reprehensible, while others are warmly in favour of this course. Evidence that any well-marked injurious effect follows the consumption of milk containing small amounts of preservatives is not forthcoming.

Wiley, as the result of an exhaustive experiment extending over many weeks, concludes that both boric acid and borax when continuously administered in small doses for a long period, or when given in large quantities for a short period, create disturbances of appetite, of digestion, and of health.

In certain patients medicinal doses of boric acid give rise to transient erythematous eruptions after relatively short periods, especially in cases of kidney disease, where the drug is not rapidly eliminated in the urine.

Tunncliffe and Rosenheim conclude that neither boric acid nor borax given for twelve days in any way affect the general health or well-being of children. On the other hand, the author has found a general consensus of opinion among medical men, who are specialists in infant feeding, that the presence of boric acid or its compounds tends to cause feeding troubles in young children.

Hehner, Weber, F. J. Allan, Cripps, Leffmann and Beam, Liebreich, Halliburton, Chittenden, Mayberry and Goldsmith, and Rideal and Foulerton have shown that neither boric acid nor borax have any inhibitory effect on rennet action, or on salivary, gastric, or pancreatic digestion, beyond that traceable to the acid radicle of boric acid or the alkali of borax.

They have, however, none of them ventured to claim that their experiments *in vitro* have more than a partial bearing on the question whether boric acid is injurious or not.

Salicylic acid is in rather a different category; it is a well-known drug, and, when taken in moderate quantity, has been proved to cause injurious symptoms; its use is forbidden in France as a preservative; it has an inhibitive effect on enzymes. Wiley has found that it tends to produce slight digestive disturbances. Formaldehyde is of considerable activity as a chemical agent, and combines with proteins to form compounds of a different nature.

It has been found by Tunncliffe and Rosenheim that formaldehyde, when given for fourteen days to children, diminished phosphorus and fat assimilation, and in a delicate child it had a chemically measurable deleterious effect on general assimilation combined with a slight intestinal irritant action.

Wiley was obliged to stop his experiments with formaldehyde on account of the alarming symptoms produced.

Rideal and Foulerton, Bliss and Novy, Pottevin, Halliburton, Freudenreich, Mayberry and Goldsmith, Loew, Wiegler and Merkel, and Cassal have experimented on the action of formaldehyde on artificial digestions, and all find some retardation of the time of digestion.

O. and C. W. Hehner have found that small amounts of fluorides have a very considerable effect in retarding artificial digestions.

To sum up, it seems that while healthy adults can take small doses of the preservatives usually employed in milk, there is evidence that young children are not unaffected. The practice



must, therefore, be considered undesirable. The author's experience has shown that in London, the use of preservatives in milk is entirely unnecessary; no difficulty has been found, even in summer, in delivering milk to customers in a fresh condition. Cream and butter are on a slightly different footing from milk. While the last is chiefly consumed for its food value, cream and butter are chiefly taken to improve the taste of other foods, and are consumed in comparatively small quantities; being, moreover, high in price, they may be considered as luxuries, and are expected to keep for a longer time than is naturally possible. It is readily seen that, under these circumstances, there is far more to be said in favour of the use of preservatives in cream and butter, than can be said when they are added to milk.

**Advantages.**—The advantages of using preservatives to the vendor are obvious; they enable a perishable article to be maintained in a marketable condition for a longer time than it would otherwise remain so. As change from the action of micro-organisms is not entirely stopped, the advantage to the purchaser is by no means so apparent, and there appears to be a well-founded public opinion against the use of preservatives.

A Departmental Committee of the Local Government Board, appointed to consider the question of preservatives and colouring-matters in food, reported in 1901, and made the following recommendations:—

(a) That the use of formaldehyde or formalin, or preparations thereof, in foods or drinks be absolutely prohibited, and that salicylic acid be not used in a greater proportion than 1 gramme per pint in liquid food, or 1 gramme per pound in solid food. Its presence in all cases to be declared.

(b) That the use of any preservative or colouring-matter whatever in milk offered for sale in the United Kingdom be constituted an offence under the Sale of Food and Drugs Acts.

(c) That the only preservative which it shall be lawful to use in cream be boric acid, or mixtures of boric acid and borax, and in amount not exceeding 0·25 per cent., expressed as boric acid. The amount of such preservative to be notified by a label upon the vessel.

(d) That the only preservative permitted to be used in butter and margarine be boric acid, or mixtures of boric acid and borax, to be used in proportions not exceeding 0·5 per cent., expressed as boric acid.

(e) That in the case of all dietetic preparations intended for the use of invalids or infants chemical preservatives of all kinds be prohibited.

The Local Government Board in 1906 issued a circular letter to Local Authorities drawing their attention to the report of the

Departmental Committee, and expressing the opinion that where preservatives were found prosecutions should be instituted under the Sale of Food and Drugs Acts, and suggesting 0·005 per cent. formaldehyde and 0·057 per cent. of boric acid as the points at which injury to health was caused.

In an appeal case at the Clerkenwell Sessions (Nov. 18, 1907), it was held that cream containing 0·313 per cent. of boric acid was injurious to the health of children, but not injurious to the health of adults, and further that cream is a food for infants. The fact that cream containing this amount of boric acid might be injurious to invalids was held not to affect the question whether it was injurious to health.

Under the Cream Regulations of the Local Government Board, the use of boric acid is permitted in cream containing over 35 per cent. of fat, provided that the quantity is notified on a label of prescribed form and size; 0·5 per cent. of boric acid is the quantity usually declared on these labels.

#### Detection of Preservatives.

The detection and estimation of boric acid have already been described (p. 84).

Some idea as to whether **boric acid** or **borax** has been added can be obtained by applying the turmeric test (1) to a solution of ash of milk in water, and (2) to a solution of the ash in dilute hydrochloric acid. If test (1) gives no reaction, while test (2) gives a strong reaction, borax has been added; if test (2) gives a reaction no stronger than that obtained by test (1), boric acid has been used; while if test (1) gives a reaction, while test (2) gives a stronger reaction, a mixture of the two is probable. These tests are far from absolute, owing to the difficulty of judging the strength of a reaction, and, further, owing to the fact that the ash of milk is usually feebly alkaline, which would cause some of the boric acid to be reckoned as borax. Occasionally, the ash of milk is acid, and some of the borax would then appear as boric acid. Nothing more than rough approximate results are claimed for this method.

Farrington has shown that when boric acid is added to milk its acidity to phenolphthalein is four times as great as its acidity in aqueous solution; if a milk is found to have a high acidity, say 40, and does not smell or taste sour or curdle on boiling, it is highly probable that boric acid is present.

**Salicylic acid** may be detected in the filtrate produced by adding mercuric nitrate to milk: if much salicylic acid be present this will acquire a red colour after some time, and when shaken

with a little amyl alcohol, the colour will pass to the amyl alcohol.

For the detection of benzoic and salicylic acids the milk is made alkaline with sodium carbonate and the casein precipitated by heating on a water bath with one-tenth of the volume of 10 per cent. calcium chloride solution; after cooling, the filtrate is neutralised and the proteins removed as in Ritthausen's method (p. 126). The filtrate from this is acidified and extracted with a mixture of ether and petroleum ether, and the solvent washed with water, and finally a small quantity of water and a drop or two of phenolphthalein added, and dilute caustic soda dropped in with constant shaking till the aqueous portion is pink. This should be removed, boiled to expel ether, and to a portion a drop of dilute ferric chloride solution is added, and a violet coloration is developed in the presence of salicylic acid, while benzoates give a buff precipitate insoluble in dilute acetic acid. To confirm the presence of salicylic acid a portion is tested with bromine water, a curdy yellowish precipitate is produced by salicylic acid, and the characteristic smell of halogen phenol derivatives developed; another portion is evaporated to dryness with strong nitric acid, and the residue taken up with a few drops of water; a yellow coloration is produced on adding ammonia if salicylic acid be present.

These reactions are not absolutely characteristic of salicylic acid, as phenol (carbolic acid) and other hydroxy-benzene derivatives behave in a similar manner.

Lintner gives the following test:—Boil with a few drops of 10 per cent. mercuric nitrate solution, add 2 or 3 drops of dilute sulphuric acid, and a very small quantity of 1 per cent. sodium nitrite solution, avoiding excess. A red colour indicates the presence of salicylic acid. This test will detect 1 part in 500,000.

Benzoic acid gives the reactions below; if salicylic acid is present a little bromine water is added, and a turbidity or precipitate will be produced; bromine water should be added till all the salicylic acid is precipitated, and the precipitate removed by filtration. The excess of bromine should be boiled off, and the following tests applied:—

(a) Add a few pieces of magnesium and hydrochloric acid till gas begins to be evolved; benzoates are reduced to benzaldehyde, which has a characteristic smell.

(b) Evaporate a little of the solution with soda-lime, and ignite in a current of inert gas (nitrogen formed by passing air through alkaline pyrogallol serves); benzoates are reduced to benzene (characteristic smell), which may be collected in a mixture of nitric and sulphuric acids, which form nitro-benzene

(another characteristic smell); this may be converted into aniline, diazotised and condensed with  $\beta$ -naphthol (red colour).

(c) Evaporate a little of the solution to dryness, add 2 c.c. of aniline and 0.02 gramme rosaniline hydrochloride, and boil for twenty minutes; a blue colour is produced if benzoates are present.

(d) Evaporate a little of the solution to dryness, add a little gallic acid and 1 c.c. sulphuric acid; if benzoates are present anthragallol is produced, which, on dilution and making alkaline, gives a red colour passing to brown.

$\beta$ -naphthol is best detected by taking advantage of its easy condensation with tetrazonium salts in faintly acid solution to form dark red compounds.

The author and Miller test as follows:—To 1 gramme of benzidine add 4 c.c. strong HCl and about 60 or 70 c.c. of water; keep this solution cool, and add little by little 1 gramme sodium nitrite dissolved in about 25 c.c. of water, cooling, and shaking well between each addition. When all the nitrite has been added nearly neutralise, using phenolphthalein as indicator. To a few c.c. of milk add a little of this solution; if  $\beta$ -naphthol is present a red colour will be produced. *Do not make alkaline*, as milk itself gives a brownish-red in alkaline solution.

As a confirmatory test, a diazotised solution of phenylhydrazine may be used, which gives a red colour in alkaline solution with  $\beta$ -naphthol, but no colour with milk.

If the milk is extracted with chloroform, and the chloroform heated with caustic potash for a few minutes, a deep blue colour indicates the presence of  $\beta$ -naphthol.

**Fluorides** are thus detected in the ash of milk. At least 25 c.c. of milk should be taken, and the ash treated in a platinum basin with a little strong sulphuric acid. Over the top of the basin a watch-glass coated with bees' wax, through which a few lines are scratched, is placed, and a piece of ice or some cold water is put into the concave depression. The basin is then gently warmed and the watch-glass exposed to the action of the fumes evolved for ten minutes. In the presence of fluorides it is seen that the glass has been etched, after removal of the wax. If a drop of water is placed on the wax, away from the lines scratched through it, a white film of silica will be formed on its surface if **fluosilicates** be present. If **fluoborates** be present, this drop of water will give a boric acid reaction; in the presence of fluoborates both a fluoride and a boric acid reaction are given by the ash of the milk.

O. and C. Hehner have pointed out that when there is much boric acid in relation to the fluoride present, the test for fluorides applied directly to the ash fails. The milk should be made

alkaline, ashed, and the ash dissolved in a little acid ; calcium chloride is added, and the solution made alkaline with ammonia ; the precipitate is collected, burnt, and extracted with acetic acid, and the test made on the insoluble portion.

**Formaldehyde**, which has been introduced of late years, is now frequently employed as a milk preservative.

It is generally added as a 1 per cent. solution in water, which is made by diluting the 40 per cent. solution known as "Formalin," "Formal," "Formol," or "Formine." A very large number of reactions for this substance have been worked out. The most easily applied test is that due to Hehner, which is best carried out as follows :—The milk is diluted with an equal volume of water, and a little 91 per cent. sulphuric acid run in so that it forms a layer at the bottom. In the presence of formaldehyde a violet-blue colour appears at the junction of the two liquids, and the colour is permanent for two or three days. This test will detect, easily, 1 part of formaldehyde in 200,000 of milk. Milk, in the absence of formaldehyde, gives a slight greenish tinge at the junction of the two liquids, and on standing a brownish colour is developed, not at the junction of the two liquids, but lower down in the acid.

Leonard and Smith's test for formaldehyde consists in heating a little milk with 3 to 5 times its volume of concentrated hydrochloric acid ; a fine violet colour is produced in the presence of formaldehyde (0·0001 per cent. to 0·1 per cent.). The presence of a trace of ferric chloride in the hydrochloric acid is essential.

These tests are not absolutely characteristic of formaldehyde, and are not given in the presence of large amounts of this body. It is a reaction of the tryptophane of the casein with formaldehyde, and certain other aldehydes—*e.g.*, vanillin—give similar colours. Leonard has pointed out that pure acids give no reaction, but the presence of an oxidising agent is necessary ; he found that a trace of ferric chloride gave the best results ; it is better to use commercial acid than a purer form, as the necessary oxidising agent is present.

As a confirmatory test, some of the milk may be curdled by dilute sulphuric acid and a little Schiff's reagent—a solution of rosaniline bleached by sulphurous acid—added to the filtrate in a test tube, which is corked and allowed to stand. In the presence of an aldehyde a violet-pink colour is produced after a short time. Excess of sulphurous acid must be avoided in preparing the reagent, or the test may fail with small amounts.

There are many confirmatory tests, which are best applied to the clear solution obtained by distilling the filtrate obtained by curdling the milk with sulphuric acid. Smith and Leonard have

shown that when milk containing formaldehyde is distilled, but a small fraction can be obtained in the distillate; if the milk be made alkaline, still less is obtained; but a very much larger proportion is obtained by distilling from an acid solution. This is due to the fact that formaldehyde condenses with the proteins of the milk; the more perfectly these are in a state of solution, the faster is the rate of combination. Combination is more rapid at high temperatures, but takes place at ordinary temperatures, and the total quantity added is never obtained; after a lapse of some time—several days—the formaldehyde disappears, and can no longer be detected.

If Schiff's test is applied to the distillate, it must be rendered faintly acid beforehand with hydrochloric acid; Hehner has shown that the distillate of milk gives a faint pink colour with Schiff's reagent after some time, but this disappears on the addition of a drop or two of sulphurous acid, while the colour due to the presence of formaldehyde does not. He ascribes this to oxidation, but as it is equally well prevented by a little hydrochloric acid, it appears that this explanation is not correct; it is probably due to traces of alkali dissolved from the glass.

The following tests are a selection from the many which have been devised:—

(1) To the distillate add one drop of a dilute aqueous solution of phenol, and pour in some strong sulphuric acid down the sides of the tube. In the presence of formaldehyde a bright crimson zone appears at the junction of the two liquids. This test, which is also due to Hehner, is as delicate as the test previously described, and has the further advantage that it is obtained by formaldehyde solutions of all strengths. If there is more than one part of formaldehyde per 100,000 a white turbidity is obtained in the solution above the sulphuric acid, while in strong solutions a white or pinkish curdy precipitate is obtained. Many hydroxy-derivatives of benzene, such as salicylic acid, resorcinol, and pyrogallol may be substituted for phenol. Quinol, however, gives not a red colour, but an orange-yellow one. Acetaldehyde gives an orange-yellow colour with phenol and sulphuric acid.

(2) Mix the distillate with strong sulphuric acid, and sprinkle a little morphine on the surface: a violet colour is produced in the presence of formaldehyde.

(3) To a decigramme of diphenylamine add 2 c.c. of strong hydrochloric acid, and pour some of the distillate into the warm solution. In the presence of formaldehyde, a white turbidity or precipitate is obtained, on further warming if necessary. The precipitate on prolonged boiling turns green. This test, like the last, is characteristic of formaldehyde, but is not of such

great delicacy as the former ones, and may not be obtained with milk containing only a small amount.

(4) Heat some of the milk for thirty minutes on the water bath with a little sulphuric acid and a drop of dimethylaniline; filter; render alkaline with caustic soda; and boil till the smell of dimethylaniline has disappeared. Filter; moisten the filter paper with acetic acid, and sprinkle lead peroxide on it. A blue colour is developed if formaldehyde is present.

(5) To the distillate add a 3 per cent. solution of aniline. Formaldehyde produces a white precipitate, which is dissolved on boiling, but is deposited again on cooling.

(6) To 5 c.c. of the distillate add 1.5 c.c. of a 2 per cent. solution of phenylhydrazine hydrochloride, 4 drops ferric chloride solution, and 12 drops sulphuric acid. A rose or dark red colour is produced in the presence of formaldehyde.

A preservative containing a nitrite in addition to formaldehyde has been put on the market; the nitrite masks the formaldehyde reactions, but Monier-Williams has pointed out that if this is destroyed by the addition of a little urea, the tests for formaldehyde may be obtained.

Hydrogen peroxide is employed as a preserving agent; Budde has patented a process which consists in adding hydrogen peroxide to milk, and heating to 50° to 55° C. to complete the liberation of the oxygen by the catalase of milk. It appears to act by liberating oxygen in the interior of the micro-organisms present, and thus bursting them.

If a milk is found to contain abundance of soluble albumin, and not to give the para-phenylene-diamine or ortol reactions (p. 195), it is probable that it has been treated by Budde's process.

Hydrogen peroxide may be detected in milk by adding to a small quantity of fresh milk a little ortol, and adding an equal bulk of the suspected milk. In the presence of hydrogen peroxide a red colour will be produced.

A preservative consisting of a solution of hydrogen peroxide in brine and pellets of potassium carbonate and citrate has been put on the market; sodium peroxide and perchlorate are also used.

M. Wynter Blyth has devised the following method for determining the presence of preservatives in milk:—

(1) Measure 10 c.c. of each milk into clean wide test tubes.

(2) Measure 10 c.c. of a sterile milk known to be free from preservatives into a test tube (these control tubes can be kept ready for use).

(3) Add to each milk 2 c.c. of a very strong slightly alkaline solution of litmus. If any tube is not the same shade of blue

as the control, add very carefully a  $\frac{N}{2}$  solution of caustic soda drop by drop till the correct shade is obtained.

(4) Plug all tubes with cotton wool, and heat them in a water bath kept at 80° C. for ten minutes.

(5) Cool the tubes, and add to each 0.5 c.c. of a solution containing 0.5 c.c. of sour milk per 100 c.c., shake well, and let the tubes stand for twenty-four hours at a temperature between 15° C. and 24° C., or until the control tube is white.

If preservatives are absent the milk will become white at the same time as the control; in the presence of preservatives the tubes will remain blue or pink.

If formaldehyde is found a quantitative estimation may be made by making up a series of tubes containing known amounts of formaldehyde, and keeping these and the tubes of the milks to be tested at a temperature of 37° C.; it is also advisable to dilute the milk 10 and 100 times, prepare tubes from the diluted milk, and keep these at 22° C. The controls kept at 37° may contain 0.005, 0.003, and 0.001 per cent. formaldehyde, and those kept at 22°, 0.001, 0.0008, 0.0005, and 0.0003 per cent.

By noting which of the control tubes is decolourised at the same time as the sample to be tested, a fairly accurate estimation of the amount of formaldehyde present may be made.

### Preservation of Milk Samples.

Where any special importance is attached to the analysis of any sample, it is an advantage to preserve the sample for reference and further corroborative analysis. Preservatives are added to effect this. The following substances have been used:—

**Alcohol.**—Allen has suggested adding to the milk to be kept twice its weight of alcohol; his experience and that of Hehner show that analytical data can be obtained on the preserved milk (making allowance for the alcohol added) which agree with the original sample. The objection to this method is that a large amount of a volatile substance is added, and a correction, the exactness of which depends on the amount of alcohol present, must be made. Milk-sugar and salts are also deposited after some time, and are difficult of complete redistribution.

**Chloroform.**—When added in the proportion of 1 c.c. to 100 c.c. of milk it keeps the milk well for a short time. It has the advantage of dissolving in the fat and keeping the cream in an easily miscible condition. As Babcock and Russell have shown, it does not stop enzymic action; hence changes in the proteins, due to this cause, proceed as if no chloroform had been



added. The correction to be applied is small. For keeping samples for a short period, say ten days, this method is good.

**Ether.**—This preservative is nearly as good as chloroform ; it is, however, not quite so good a preservative and is more volatile.

Collins recommends a mixture of ether and chloroform of specific gravity 1·032, as it does not affect the specific gravity of the milk. The author has, however, shown that ether and chloroform keep the fat in a liquid condition, and that the specific gravity is lowered by this cause. The estimation of the fat by the Gerber method is too high in the presence of chloroform.

**Terpenes, Thymol, Dichlorophenol, and Salicylic Acid.**—These keep the milk, but allow the cream to rise to the surface, where it sets in a firm layer and is not easily redistributed.

**Hydrofluoric Acid and Fluoboric Acid.**—The author has proved that these substances, when added to fresh samples in the proportion of  $\frac{1}{2}$  c.c. to 100 c.c. of milk, keep them in good condition, and, after a year, analysis gives the same figures as those previously found. They curdle the milk, however, so that the sample must be well shaken to bring the precipitated casein into a fine state of division ; a little of the bottle is dissolved and the ash is thereby slightly increased. The author has found this method to be one of the best.

**Formalin.**—The addition of formalin has many advantages. A very minute amount of the 40 per cent. solution need be added (2 drops per 100 c.c.), and no correction is necessary for so small a quantity.

Siegfeld finds that the presence of much formaldehyde in milk has a tendency to increase the amount of fat by the Gerber method. This may be obviated by adding 1 c.c. of hydrogen peroxide, or better, 0·5 of a 40 per cent. solution of hydroxylamine hydrochloride per 100 c.c. of milk, and correcting for increase of volume.

The formaldehyde, however, combines with the protein, and raises the apparent percentage of total solids and solids not fat. Bevan has also suggested that the milk-sugar is hydrolysed into dextrose and galactose, as he found the increase in total solids more than the total amount of formaldehyde added ; but this has been disproved by Höft.

**Potassium Bichromate, Mercuric Chloride, and Solid Antiseptics.**—These add considerably to the weight of the total solids and solids not fat, and cannot, therefore, be recommended. If fat only is to be determined they are efficient. Siegfeld does not consider that the analysis of samples preserved by potassium bichromate is trustworthy.

**Sterilisation** may be resorted to. Certain changes take place,

which do not usually interfere with the analysis. The cream rises and clots on the surface, and it is not easy to obtain an average sample.

**Cold Storage.**—Samples may be frozen and kept in a cold chamber, if one is available; they keep for an indefinite period thus, but require carefully remelting and remixing. This method, which is not always available, is superior to all others, and should be resorted to in those dairies which possess a freezing plant and cold storage room.

**The Action of Heat on Milk.**—When milk is heated the following changes occur:—At about 70° C. a change takes place in the albumin; it is not precipitated, but is converted into a form which is precipitated by acids, magnesium sulphate, and other precipitants of casein.

At about 80° C. certain organised principles, the nature of which is not fully known, undergo a change. The presence of these principles in an unchanged form is shown by the following reactions:—They cause an evolution of gas from hydrogen peroxide in the cold and give a blue colour with para-phenylene-diamine (para-di-amino-benzene), and hydrogen peroxide. Other substances may be substituted for the para-phenylene-diamine, but, according to Leffmann, this substance is the most characteristic. Rosier, working in the author's laboratory, has found that meta-phenylene-diamine gives very characteristic results, if a small quantity of amyl alcohol is added to dissolve and concentrate the light blue colouring-matter formed. Saul recommends "ortol," which is a mixture of quinol with ortho-methyl-amino-phenol.

Near 100° C. calcium salts in small amount are deposited, and, by keeping at this temperature for some time, slight oxidation sets in, with the production of traces of formic acid and a marked reduction of the rotatory power of the milk-sugar; a brown colour is produced at the same time. A deposition of salts, and perhaps, also of albumin takes place on the fat globules, which increases their mean density, causing them to rise slowly to the surface, when the milk is afterwards cooled; during the heating the fat globules are expanded and may somewhat coalesce.

If the surface of the milk is freely exposed to the air, a skin forms at temperatures exceeding 60° C. This has been stated to consist of casein, but has not the properties of this substance; it is partly of a protein character, and there is some reason to suppose that it is an oxidation product. It contains all the constituents of the milk in a concentrated form. The taste and smell of milk are changed by heating to above 70° C.

It is not known how far the action of heat on milk affects its digestive qualities. Milk which has been heated is curdled less

readily by rennet than fresh milk, but there are good grounds for the view that this is due to a change in the distribution of the calcium salts as well as possibly to a change in the casein. It has been claimed that sterilised or boiled milk is more easy of digestion than unboiled milk, but this, again, is possibly due to the fact that it is not curdled so easily in the stomach, and does not produce so firm a clot. There appears to be no evidence that healthy adults digest boiled milk either more or less readily than unboiled milk. In one respect boiled milk is less to be preferred than fresh milk. From the evidence adduced by Barlow, and since fully confirmed, it seems that children fed exclusively on sterilised milk have a scorbutic tendency. It has long been known that the absence of fresh food of any description is a predisposing cause of scurvy, but no substance has yet been identified as the agent which confers anti-scorbutic properties.

It is of considerable importance to be able to distinguish between fresh milk, on the one hand, and "pasteurised" or "sterilised" milk, on the other.

### Sterilised Milk.

Milk is a product which affords all the necessary nourishment for the growth of micro-organisms; these not only develop products which cause alteration of the milk—*e.g.*, lactic acid and proteolytic enzymes—but also are in some cases injurious to health.

They are destroyed by heat. Hence milk is frequently "sterilised" by heat, the object being to bring about the destruction of the micro-organisms.

Many processes are used. Pasteur originally recommended heating to 70° C. for a short time, a process which was sufficient to destroy all adult forms of pathogenic organisms and, practically, all others. The spores, however, were left untouched and retained their vitality; on cooling to the mean air temperature these developed into the adult forms and resumed their activity. To destroy the spores, a process of continued "pasteurisation" has been used. This consists of alternately heating to 70° C. for, say, twenty minutes; cooling to a lower temperature and keeping at this temperature for a sufficient length of time to allow the spores to develop; again heating to 70°; and repeating this process many times. By this process, which is very tedious, the taste and composition of the milk undergoes but little alteration.

It has been found that most spores can be killed by continued

exposure at higher temperatures. The temperature of boiling water is one much used, as it can be easily attained, but higher temperatures are sometimes resorted to by heating the milk under pressure; the higher the temperature, the shorter the time necessary to kill all microbial life. Another method adopted is to alternate successive short periods of heating to high temperatures with intervals during which the milk is kept at the ordinary temperature. Numerous modifications of these methods have formed the subjects of patents.

**Analytical Characters.**—As, practically, no milk sterilised by successive heating to a temperature not exceeding  $70^{\circ}$  C. is sold commercially, it will be sufficient to describe the methods for characterising milk which has been heated above the coagulating point of albumin.

The most marked characteristic distinguishing sterilised milk from new milk is the state in which the albumin exists. As previously stated, it is probable that albumin exists in milk in combination with a base; on heating milk, no coagulation of albumin takes place, but on acidifying, or saturating with magnesium sulphate, the albumin separates with the casein. The albumin appears to be changed from a soluble to a colloidal form. (Not more than 0.1 per cent. of albumin is found in sterilised milk in the soluble form.) The casein separates on acidifying in a more finely divided state.

If the milk has been heated to  $100^{\circ}$  C. or a higher temperature for any length of time, the rotatory power of the milk-sugar undergoes a serious reduction, the cupric reducing power not changing to any appreciable extent. The milk also assumes a slight brownish colour, due probably to the formation of a "caramelised" body of low rotatory power.

The cream rises with extreme slowness; in three hours, practically no cream is observed on the surface of the milk; and after six hours, the layer is only about one-tenth of that given by new milk. If sterilised milk be allowed to stand for twenty-four hours or more the bulk of the cream will rise to the surface, but the quantity will be less than that yielded by new milk; the cream will, however, contain a distinctly larger percentage of fat, about 40 per cent., as against less than 30 per cent. in the cream yielded by new milk.

The diminished yield of cream is a property shared also by milk which has been pasteurised by heating to about  $70^{\circ}$ , but the rate of rise of cream in pasteurised milk is fairly rapid; practically the same amounts are found in three hours as in six hours. The total quantity of cream from pasteurised milk is about half that of fresh milk.

The following figures, which, together with those in Table

XLIX., were obtained by Boseley and the author (Tables XLV., XLVI., XLVII.), will illustrate the above facts :—

TABLE XLV.—COMPOSITION OF STERILISED MILK.

Sterilised Milk allowed to stand for Six Hours.

No.	Fat in Milk.	Cream.	Fat in Cream.	Fat in Skim Milk.
	Per cent.	Per cent.	Per cent.	Per cent.
1	4·30	1·3	23·3	4·05
2	3·80	0·7	22·3	3·67
3	4·25	1·8	20·6	3·95
4	4·10	1·9	24·7	3·70
5	5·35	2·8	31·4	4·60
6	3·62	0·3	...	...

TABLE XLVI.—COMPOSITION OF STERILISED MILK.

Sterilised Milk allowed to stand for Twenty-four Hours.

No.	Fat in Milk.	Cream.	Fat in Cream.	Fat in Skim Milk.
	Per cent.	Per cent.	Per cent.	Per cent.
1	4·30	7·0	46·8	1·10
2	3·80	6·0	41·8	1·37
3	4·25	8·8	39·0	0·90
4	4·10	8·7	41·0	0·58
5	5·36	11·1	41·4	0·85
6	3·62	0·8	...	3·48

TABLE XLVII.—COMPOSITION OF NEW MILK.

New Milk allowed to stand for Six Hours.

No.	Fat in Milk.	Cream.	Fat in Cream.	Fat in Skim Milk.
	Per cent.	Per cent.	Per cent.	Per cent.
1	4·05	9·2	17·4	2·70
2	4·20	11·2	16·5	2·65
3	3·90	9·8	15·9	2·60
4	3·70	9·8	18·0	2·15
5	4·45	13·5	16·8	2·30

Table XLVII. showing the behaviour of new milk is produced here for the sake of comparison. The samples 1 to 5 are from the same cows which respectively yielded the samples with corresponding numbers in the tables illustrating the behaviour of sterilised milk.

Condensed unsweetened milk, which has been diluted to the original volume with water, has all the analytical characteristics of sterilised milk. It throws up its cream rather less readily even than sterilised milk. No. 6 in Tables XLV. and XLVI. was diluted condensed milk. This is in no way due to the fact that it has been condensed, but is owing to the sterilising process that it has undergone. There appears to be no good method of discriminating between condensed milk diluted with water and sterilised milk. If a water containing large amounts of nitrates has been used for diluting the condensed milk, a strong diphenylamine reaction will indicate the probability that water has been added; this test is not of a sufficiently absolute character to be relied on. This is to be regretted. The subject has been considered of sufficient importance by the British Dairy Farmers' Association to induce them to offer a gold medal for the discovery of such a method.

**Detection of Sterilised Milk in New Milk.**—To distinguish new milk on the one hand from milk which has been sterilised on the other, the following methods may be employed:—

(1) Place 100 c.c. of milk in a graduated cylinder (or fill a "creamometer") and allow it to stand for six hours at a temperature of 60° F. (15.5° C.); note the percentage of cream. If less than 2.5 per cent. of cream for each 1 per cent. of fat in the milk has risen to the surface, the milk may be considered suspicious. If the quantity of cream falls markedly below 2 per cent. for each 1 per cent. of fat, it is highly probable that sterilised milk is present.

(2) Estimate the albumin by the method of Hoppe-Seyler or, better, that of Sebelein. If less than 0.35 per cent. is found, sterilised milk may be considered to be present.

(3) Estimate the milk-sugar by the polariscope, and also gravimetrically in duplicate; if the difference between the two estimations be more than 0.2 per cent., it will be corroborative evidence of the presence of sterilised milk.

(4) To about 5 c.c. of milk add as much powdered paraphenylene-diamine as will lie on the point of a knife, and shake well; on the addition of a drop or two of a 10-volume solution of hydrogen peroxide fresh milk gives a blue coloration; "pasteurised" milk gives a similar reaction, not, however, so marked; while "sterilised" milk gives no coloration within ten minutes. A mixture of "sterilised" and fresh milk will give the characters of "pasteurised" milk.

The hydrochloride of meta-phenylene-diamine may be substituted with advantage for the para-compound. The coloration is paler, and not quite so quickly developed. By shaking with an equal volume of amyl alcohol the blue substance is dissolved in

the alcohol layer, and the test is thus rendered more reliable in the presence of substances which modify the tint (*e.g.*, formaldehyde).

Other substances, such as quinol or tincture of guaiacum, may be used ; of these the one recommended by Saul, and found most effective by the author, is "ortol," which is sold as a photographic developer. This gives a fine red colour with unboiled milk.

These tests show that the milk has been heated above 80° C., as the "peroxydase" which gives rise to the reactions is destroyed at this temperature.

If the milk has been heated with hydrogen peroxide, the tests with phenylene-diamine or ortol will fail, as the peroxydase is also destroyed if an excess of hydrogen peroxide is added to milk.

Another test is to add a solution of methylene blue containing formaldehyde and keep the milk out of contact with air ; fresh milk decolourises this, and boiled milk does not. This test appears not to depend on any constituent of the milk, but on the presence of enzymes (reductases) secreted by micro-organisms. Milk treated with hydrogen peroxide gives this test.

The rate at which methylene blue is decolourised is a rough index of bacterial contamination.

A cultivated palate may also detect a boiled taste ; this will certainly be noticed with "sterilised" milk.

It is doubtful whether a proportion of sterilised milk much below 30 per cent. could be detected with certainty when mixed with new milk.

The proportion of sterilised milk should be deduced from the percentage of soluble albumin by the following formula :—

$$\text{Percentage of sterilised milk} = \frac{0.4 - \text{percentage of soluble albumin}}{0.4} \times 100.$$

This is based on the supposition that new milk contains 0.4 per cent. of albumin, while in sterilised milk the albumin has been removed.

The estimation of albumin is the most reliable test. There are many causes which influence the rising of cream, such as the temperature to which the milk has been warmed or cooled ; the size of the fat globules, which varies with the stage of lactation ; and the acidity of the milk. The milk may also have been "homogenised" by the fat globules having been broken up, in which case practically no cream will rise. No quantitative deductions can be drawn from observations of the rate of the rising of cream. The fall in specific rotatory power of milk-sugar is by no means constant, as milks sterilised side by side may show very appreciable variations in this respect.

Tests other than the estimation of albumin must be considered as merely corroborative and of qualitative value only.

It must be remembered, however, that no sharp distinction can be drawn between milk which has been raised to a temperature over 70° C. for a short period, and which is naturally not sterilised in the true sense of the term, and the milk which has been heated for a sufficient length of time to destroy all microbial life. For this reason, a milk should not be reported as sterilised, solely on the result of a very low percentage of albumin, if neither the "creamometer" nor the para-phenylene-diamine nor "milk-sugar" tests give corroborative indications. It is probable that the milk, in this case, has been pasteurised slightly above 70° C.

The following figures (Table XLVIII.) by C. H. Stewart show the percentage of albumin found in milk raised to various temperatures:—

TABLE XLVIII.—PERCENTAGE OF ALBUMIN IN MILK AT VARIOUS TEMPERATURES.

Time of Heating.		Soluble Albumin in Fresh Milk.	Soluble Albumin in Heated Milk.
		Per cent	Per cent.
10 minutes at 60° C.,	. . .	0·423	0·418
30 " " "	. . .	0·435	0·427
10 " " 65° C.,	. . .	0·395	0·362
30 " " "	. . .	0·395	0·333
10 " " 70° C.,	. . .	0·422	0·269
30 " " "	. . .	0·421	0·253
10 " " 75° C.,	. . .	0·380	0·070
30 " " "	. . .	0·380	0·050
10 " " 80° C.,	. . .	0·375	none
30 " " "	. . .	0·375	none

The following analyses (Table XLIX.) will show to what extent the methods above described can be depended on:—

TABLE XLIX.—COMPARATIVE ANALYSES OF MIXED FRESH AND STERILISED MILKS.

No	Fat.	Cream.	Cream Fat.	Albumin.	Milk-Sugar.	Milk-Sugar Pol.	Percentage Sterilised.		
							Actual.	Calc. from Cream.	Calc. from Albumin.
1	3·86	7·2	1·87	0·30	4·85	4·65	33	29	25
2	4·10	9·6	2·34	0·34	4·79	4·68		Genuine	
3	3·90	7·5	1·92	0·29	4·79	4·64	28	26	27
4	3·70	4·3	1·16	0·16	4·75	4·57	56	61	60
5	4·10	7·4	1·81	0·26	...	...	30	31	35
6	4·00	7·5	1·88	0·27	...	...	30	28	32
7	3·75	7·9	2·11	0·35	...	...		Genuine	



### Condensed Milk.

For convenience of transport, milk is deprived of the bulk of its water by evaporation under diminished pressure in a vacuum apparatus fitted with a condenser, or by heating to a low temperature and exposing a large surface to evaporation; this is termed **condensed** or **evaporated milk**. It is made in two forms: sweetened condensed milk, which is a preparation of milk and cane sugar; and unsweetened condensed milk, which consists of milk evaporated *per se*.

The methods of manufacture are similar. In the manufacture of sweetened condensed milk  $1\frac{1}{4}$  lbs. of cane sugar are added to each gallon of milk, and the mixture heated to such a temperature that it will commence to boil at once on being admitted to the vacuum pan. It is allowed to flow in slowly, the pump being kept working the whole time, and no heat is applied till all the milk is in the pan. By this procedure the gases of the milk are drawn out, and on applying heat the milk boils without frothing over. By carefully regulating the supply of heat to the pan, and cold water to the condenser, the milk can be boiled at an even rapid rate till sufficiently concentrated, a point which can be easily told by an experienced operator. The whole operation is controlled by looking through a glass sight-hole let into the upper portion of the pan. The finished product has a density of about 1.28 and weighs one-third of the original milk; it only occupies three-elevenths of the original volume—*i.e.*, 1 gallon of milk is evaporated to  $2\frac{1}{4}$  pints.

Commercial glucose is sometimes substituted for a part or the whole of the cane sugar.

Machines employing heated discs or rollers which dip into the milk, and carry up thin layers on being rotated, or in which the milk is exposed in shallow trays, are also used to condense milk.

Milk may be also concentrated by freezing and removing the ice deposited.

**Composition of Sweetened Milk.**—The following analyses (Table L.) will show the composition of sweetened condensed milks.

The first three are condensed whole milks—*i.e.*, the milk has been evaporated without previous removal of the cream; the last four are condensed separated milk, the separated milk having been mixed with cane sugar and evaporated.

These milks are not usually sterilised, as the large amount of dissolved matter and the small amount of water renders them unsuitable for the development of micro-organisms; they keep for a long time without appreciable change.

TABLE L.—COMPOSITION OF SWEETENED MILK.

Authority.	Water.	Fat.	Milk-Sugar.	Cane Sugar.	Protein.	Ash.	Total.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
Author, . . . .	24·06	11·28	13·97	38·31	9·36	2·13	99·11
Pearmain & Moor,	26·10	10·84	14·68	36·93*	9·55	1·90	100·00
Fleischmann, .	25·69	10·98	16·29	32·37	12·33	2·34	100·00
Pearmain & Moor,	29·87	1·17	14·68	41·54*	10·74	2·00	100·00
Author, . . . .	29·05	1·28	14·91	40·07	10·63	2·33	98·27
„ . . . .	29·23	0·64	15·50	40·19	10·73	2·63	98·92
„ . . . .	28·43	0·36	16·88	39·27	11·73	2·58	99·25

\* By difference.

**Composition of Unsweetened Milk.**—Unsweetened condensed milks are prepared in a similar manner, except that the addition of sugar is omitted.

The composition of this product is shown by the following analyses (Table LI.) :—

TABLE LI.—COMPOSITION OF UNSWEETENED MILK.

Authority.	Water.	Fat.	Milk-Sugar.	Protein.	Ash.	Total.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Author, . . . .	63·47	10·22	12·98	10·30	2·07	99·04
„ . . . .	62·40	11·91	13·04	9·68	2·14	99·17
„ . . . .	63·07	10·86	13·38	9·80	2·21	99·32
Pearmain & Moor,	61·96	10·67	15·50	9·57	2·30	100·00
Aschmann, . . .	69·35	9·23	10·98	9·41	1·63	100·00

These milks have all been sterilised by heat. They have the analytical characters of sterilised milk.

Unsterilised condensed milk is also an article of commerce; Pearmain and Moor have found boric acid in a preparation of this kind, which was sold for diluting and mixing with whole milk.

It is noticed that the totals of analyses of condensed milk almost invariably add up distinctly below 100 per cent.; it is probable that the milk-sugar is underestimated. In condensed milk the layer of solution which is attracted round the fat globules by surface energy has probably a composition which is identical with the composition of the liquid in which the globules are suspended. When condensed milk is diluted with water it is doubtful whether the liquid in this layer is diluted by the water,

as it is held by a great force, and acts as though separated by a semi-permeable membrane, through which the dissolved solids must pass by osmose. As the milk is usually diluted with cold water, this process of osmose takes a considerable time, and the whole of the milk-sugar is not obtained in solution, but a portion is taken down by the fat globules, when they are removed previous to the estimation of the milk-sugar. The same cause can be assigned to the fact that the fat globules in diluted condensed milk rise with such extreme slowness; a dense layer round the globules increases its mean density, and makes this nearly approach the density of the serum.

**Dilution.**—The directions on the label of sweetened condensed milk are often somewhat misleading. For some purposes—*e.g.*, for infant feeding—the directions given are to dilute with five or even seven parts of water. Supposing that these dilutions are performed by volume, the composition will be as follows :—

TABLE LII.

	Condensed Whole Milk.		Condensed Skim Milk.	
	With 5 Volumes of Water.	With 7 Volumes of Water.	With 5 Volumes of Water.	With 7 Volumes of Water.
Fat, . . . .	2·02	1·51	0·21	0·16
Milk-sugar, . . .	2·57	1·93	3·20	2·40
Cane sugar, . . .	7·33	5·50	8·53	6·40
Protein, . . . .	1·83	1·37	2·24	1·68
Ash, . . . .	0·40	0·30	0·53	0·40

Compared with the average composition of human milk which is

Fat, . . . .	3·3	Protein, . . . .	1·5
Sugar, . . . .	6·8	Ash, . . . .	0·2

we see that there is a serious deficiency of fat, especially in the diluted condensed skim milk, and a great excess of total sugar.

**Food Value.**—Rübners has stated that as a food 2·43 parts of sugar are equal to 1 part of fat. Calculating the value of fat as sugar by this factor, we get the following values for the food value of fat and sugar :—

Human Milk.	Condensed Whole Milk.		Condensed Skim Milk.	
	With 5 Volumes.	With 7 Volumes.	With 5 Volumes.	With 7 Volumes.
14·82	14·61	10·96	12·34	9·23

Only in the case of the condensed whole milk diluted with

5 volumes of water does the food value approximate to that of human milk ; it is doubtful, however, whether fat can be replaced entirely by cane sugar, especially for young infants.

**Milk Powders.**—There are several methods of preparing milk powders ; in the Just-Hatmaker process the milk is evaporated on rollers heated by high-pressure steam above the temperature of boiling water, and the dried layer of milk is continuously taken off by a knife set at a suitable angle. Other processes employ evaporation on rollers or discs heated to a lower temperature *in vacuo*, and the milk is preferably previously concentrated in a vacuum pan to one-third of its bulk. The milk may also, after preliminary concentration, be dried in shallow trays *in vacuo*, or on bands of wire gauze or other material. Another excellent method is to atomise the milk by spraying, and to evaporate this in a current of hot air.

In order to ensure that the milk powder is soluble in water, a small amount of an alkali—sodium carbonate, sodium phosphate, or saccharate of lime—is sometimes added. To prevent a separation of the fat in large particles on remixing the milk powder, the milk is often homogenised before drying ; this has the further advantage that the fat has less tendency to become rancid.

The table below gives the analysis of seven samples :—

TABLE LIII.—COMPOSITION OF MILK POWDERS.

	1	2	3	4	5	6	7
Moisture, .	6.39	4.92	3.30	3.55	4.74	5.15	6.03
Fat, .	27.35	27.98	23.97	2.55	29.16	19.90	25.60
Milk-sugar, .	31.42	34.16	37.32	45.60	32.24	34.96	32.83
Cane sugar, .	..	1.25	1.53	2.80	..	..	2.00
Protein, .	27.48	24.59	26.38	35.45	26.66	31.10	23.84
Ash, .	6.00	6.24	6.19	7.89	5.63	7.11	6.44
Total, .	98.64	99.14	98.69	97.84	98.43	98.22	96.74
Water of hydration, .	1.65	1.80	1.96	2.40	1.70	1.84	1.73
Total, .	100.29	100.94	100.65	100.24	100.13	100.06	98.47
Change of temperature on mixing with water,	-0.2°	± 0.0	-0.2	-0.4	-0.2	..	-0.3°

It is noticed that none of the analyses add up to 100 per cent., but are considerably low; the milk-sugar has been calculated as anhydrous sugar, and here lies the reason for the deficiency.

On shaking the solid residue obtained by drying milk on the water-bath, in which the milk-sugar certainly exists as anhydrous sugar, with water a rise of temperature always takes place; anhydrous milk-sugar mixed with water always causes a rise of temperature, whilst hydrated milk-sugar causes a fall of  $0.55^{\circ}$  if more than can be at once dissolved is added. The milk powders examined, with one exception (No. 2), all caused a fall of temperature, and it is seen that the addition of the water of hydration to the total gives figures which are but slightly in excess of 100 per cent.; both the change of temperature and the slight excess over 100 per cent. indicate that the bulk of the milk-sugar, though not all, exists as hydrated sugar. Sample No. 2 differed in appearance from the others, being a heavy powder, instead of being light and flaky, and had doubtless been more dried, and probably contains a considerable proportion of anhydrous sugar; it is noticed that the addition of the water of hydration would make the total nearly 101 per cent. Sample No. 7 gives a low total, which is probably accounted for by the presence of invert sugar.

It will be noticed that samples 2, 3, 4, and 7 contain small quantities of cane sugar; this in sample 2 was admittedly added in the form of saccharate of lime, and was certainly so added, judging from the analytical figures, in No. 7.

In Table LIV. the composition of the original milks, on the assumption that they contain 9.0 per cent. of solids not fat, are given:—

TABLE LIV.—COMPOSITION OF ORIGINAL MILKS.

	1	2	3	4	5	6	7
Fat, . . .	3.79	3.88	3.09	0.26	4.07	2.45	3.65
Milk-sugar, .	4.36	4.73	4.87	4.62	4.50	4.30	4.68
Protein, . . .	3.81	3.41	3.40	3.58	3.71	3.82	3.40
Ash, . . . .	0.83	0.87	0.80	0.80	0.79	0.87	0.92
CaO, . . . .	0.19	0.21	0.17	0.17	0.17	0.19	0.27
P <sub>2</sub> O <sub>5</sub> , . . . .	0.23	0.24	0.23	0.23	0.23	0.29	0.24
Acidity, . . .	8.4°	13.2°	16.8°	16.5°	19.6°	..	11.4°

From this table it is seen that No. 4 is made from separated milk, and No. 6 from milk deprived of a portion of its cream.

The milk used to prepare No. 3 is only just above the Government standard. The normal percentages of lime and phosphoric anhydride in milk are 0·17 per cent. and 0·23 per cent. respectively, but vary somewhat with the protein, and the normal acidity is not far from 20°. From a consideration of the results, it would appear that Nos. 2 and 7 have received an addition of saccharate of lime; and No. 6 has received an addition of a phosphate. Nos. 3 and 4 contain cane sugar, but there is no evidence of the addition of saccharate of lime. No. 1 probably received an addition of sodium carbonate, as the lime is not high enough considering the high protein to indicate an addition of this substance, and No. 5 appears to have received no addition whatever.

Milk powders containing cane sugar are also made. Two samples examined by the author had the following composition :—

TABLE LV.

	Per cent.	Per cent.
Fat, . . . . .	15·2	13·5
Milk-sugar, . . . . .	21·7	21·3
Cane sugar, . . . . .	42·5	40·9
Protein, . . . . .	15·1	14·9
Ash, . . . . .	3·3	3·2

Both had a slightly rancid odour and taste. When dissolved in water some of the fat was not emulsified.

**The Action of Cold on Milk—Composition.**—When milk is exposed to a low temperature it partially freezes. Like other aqueous solutions the freezing point is below that of water, and is about  $-0\cdot55^{\circ}\text{C.}$  ( $31^{\circ}\text{F.}$ ), estimated in the Beckmann apparatus. The frozen portion has not the same composition as the milk from which it was prepared, but contains a larger quantity of water. Owing to the facts that milk has not a point of maximum density, and that it does not freeze as a homogeneous substance, ice never forms in a solid layer on the surface. The following analyses (Table LVI.) will show the composition of the ice and liquid portion respectively :—

TABLE LVI.—COMPOSITION OF THE SOLID AND LIQUID PORTIONS OF FROZEN MILK.

	Liquid Portion.	Melted Ice.
Percentage of Ice Formed, 1·2 per cent.		
Specific gravity, . . .	1·0320	1·0245
	Per cent.	Per cent.
Water, . . . . .	96·72	91·63
Fat, . . . . .	4·11	2·40
Protein, . . . . .	3·56	2·40
Sugar, . . . . .	4·87	3·05
Ash, . . . . .	0·74	0·52
Percentage of Ice Formed, 2 per cent.		
Specific gravity, . . .	1·0330	1·0190
	Per cent.	Per cent.
Water, . . . . .	87·10	91·83
Fat, . . . . .	3·87	2·56
Protein, . . . . .	3·21	2·28
Sugar, . . . . .	5·08	2·89
Ash, . . . . .	0·74	0·44
Percentage of Ice Formed, 2·25 per cent.		
Specific gravity, . . .	1·0330	1·0180
	Per cent.	Per cent.
Water, . . . . .	87·21	92·46
Fat, . . . . .	3·57	2·46
Protein, . . . . .	3·50	1·96
Sugar, . . . . .	4·98	2·72
Ash, . . . . .	0·74	0·40
Percentage of Ice Formed, 10 per cent.		
Specific gravity, . . .	1·0345	1·0090
	Per cent.	Per cent.
Water, . . . . .	85·62	96·23
Fat, . . . . .	4·73	1·23
Protein, . . . . .	3·90	0·91
Sugar, . . . . .	4·95	1·42
Ash, . . . . .	0·80	0·21

It is seen that no appreciable difference between the ratio of the sugar to the protein and the ash is found in the two series of analyses, showing that no separation of any constituent except water takes place during freezing.

It is seen that the greater the percentage of the ice separated, the more dilute is the melted ice; this is best seen by calculating the solids not fat (Table LVII.).

TABLE LVII.—COMPOSITION OF FROZEN MILK.

Percentage of ice, . . . . .	1·2	2·0	2·25	0·10
Solids not fat, . . . . .	6·17	5·61	5·08	2·64
Equal to percentage of water (approx.), . . . . .	30	38	43	70

As all these samples were taken from churns in which milk was brought up to London, the percentage of ice may be taken as roughly indicating the temperature below freezing point to which the milk was exposed, the time of exposure to the low temperature having been approximately the same in all cases. It appears that the lower the temperature to which milk is exposed the more dilute will be the ice after melting.

**Composition of Melted Frozen Milk.**—The difference in composition between frozen and unfrozen milk may have some importance, should samples be taken under the "Sale of Food and Drugs Act," in very cold weather; should an excessive proportion of ice be present in the portion sold to the inspector the sample may, though originally genuine, have the composition of watered milk.

Vieth has recorded an interesting experiment on the freezing of milk:—Two gallons of milk were exposed to a temperature of  $-10^{\circ}$  C. ( $14^{\circ}$  F.) for three hours; longer time than this did not render any more milk solid. Ice was formed on the bottom and sides of the vessel employed to contain the milk and a funnel-shaped cavity in the centre was filled with liquid. The ice was found to consist of two layers, one of cream, and the other of skim milk; these were separated as completely as possible and the liquid portion also poured off.

The results of analysis were:—

TABLE LVIII.—COMPOSITION OF FROZEN MILK (Vieth).

	Ice.		Liquid Portion.
	Cream.	Skim Milk.	
Proportion, . . . . .	8·8 per cent.	64·7 per cent.	26·5 per cent.
Specific gravity, . . . . .	1·0100	1·0275	1·0525
	Per cent.	Per cent.	Per cent.
Water, . . . . .	74·44	92·10	80·54
Fat, . . . . .	19·23	0·68	5·17
Protein, . . . . .	2·64	2·80	5·38
Milk-sugar, . . . . .	3·33	3·95	7·77
Ash, . . . . .	0·52	0·60	1·18
	100·16	100·13	100·04



Another experiment gave almost identical figures.

It is probable from these experiments that milk exposed to a temperature of  $-10^{\circ}$  C. will always yield a liquid portion having the composition given above. The figures also show that milk cannot be frozen in blocks, from which pieces can be cut off and melted for use, without modifying the composition to a serious extent.

The author has had the opportunity of examining three samples of milk which had been frozen for transport and remelted (Table LIX.).

The samples were taken under such conditions as would represent the retailing of the milk.

TABLE LIX.—COMPOSITION OF FROZEN MILK.

	I.	II.	III.
Specific gravity, . . .	1·0385	1·0270	1·0325
	Per cent.	Per cent.	Per cent.
Total solids, . . .	13·60	10·13	11·68
Fat, . . . . .	3·29	2·70	2·86
Ash, . . . . .	0·84	0·62	0·74
Solids not fat, . . .	10·31	7·43	8·82

No. I. has the composition of concentrated milk, No. II. of a watered milk, and No. III. of a slightly skimmed milk.

Attempts have been made to introduce frozen, or partially frozen milk, into the English market from Holland and other foreign countries. The above figures show what may be sometimes the composition of milk as retailed, unless extreme care be taken in melting the imported product.

## CHAPTER IV.

## THE CHEMICAL CONTROL OF THE DAIRY.

CONTENTS.—Duties of the Dairy Chemist—The Testing of Milk—Determination of Specific Gravity—Estimation of Total Solids—of Fat and Butter—The Control of Milk during Delivery—The Solution of Analytical Problems—Cause of Low and High Specific Gravity—of Sweet Taste—of “Poor” Milk—of unusual Taste and Smell—of Milk being Curdled—of Milk being Thick—Nature of Sediment—Skim Milk—Cream.

**Duties of the Dairy Chemist.**—The duties of the dairy chemist consist in the following :—

(1) To see that the milk at or from the farms is of good quality, containing a full percentage of cream, is not tampered with by the *employés*, and is in good condition.

(2) If milk is sold by retail, to see that the milk sent out is of good quality, and, by analysing samples taken without notice from the *employé* in charge of the milk, to ascertain that it is delivered in the same condition as it left the dairy.

(3) To see that cream separators, churns, etc., are worked in the most efficient manner, by examining the various products, and to obtain such figures representing their composition as will allow of accounts being kept of the manufacturing processes.

(4) To ensure that all products derived from milk are of good quality.

(5) To investigate specially products deemed unsatisfactory, and to elucidate the cause for dissatisfaction.

(6) To make chemical examinations of water supplies, disinfectants, etc., so as to ensure that sanitation is carried out by reliable means.

(7) To advise on chemical questions that may arise—*e.g.*, the suitability of metals for the construction of dairy apparatus, the examination of waters or boiler compositions for steam production, the analysis of feeding stuffs and fertilisers, etc.

A description of the duties of a dairy chemist must necessarily be somewhat hypothetical, as the conditions in different dairies differ exceedingly from each other. In large dairies the chemist is an official wholly responsible for his own department, and under the control of no one except the proprietor. It is not

advisable that he should have any direct control of the business, his functions being those of a scientific adviser; a good dairy chemist should have had a practical experience in dairying, so that he may be able to apply his scientific knowledge to the various points that may arise from time to time. In smaller dairies, the manager or other person may undertake the functions of chemist, and these must be arranged so as to interfere as little as possible with his other duties; this may introduce variations in the plan of work described, by curtailing it, but the general procedure will remain the same.

The main duties of the dairy chemist are to see that the milk received from the farms and supplied to the public is pure and contains its due proportion of cream; that the cream and butter are of good quality and of uniform composition; that the skimmed or separated milk is as poor as possible in fat; and that the water used is free from pollution; as also to elucidate the complaints of the customers by examining the product complained of.

**Samples and Sampling.**—The main difficulty in keeping a uniform quality of milk is due to the rising of cream whenever milk is allowed to be at rest. The attention of the chemist should be devoted to studying the conditions under which the milk is distributed in the dairy to which he is attached, and to discover how to prevent this separation of cream during distribution. For the proper performance of his duties he must be provided with samples of milk at all possible stages, from the entrance of the milk into the dairy till the final return of the small quantities of milk left after distribution; these samples should, if possible, be examined at once and before the milk has passed to the next stage of delivery. This is only practicable in large dairies where the chemist has no other functions. It is advisable that the persons employed in sampling the milk should be under the control of the chemist as far as this duty is concerned, as upon the proper sampling of the milk the whole value of his work depends. In certain cases where more value than usual is attached to the examination, the chemist should personally supervise, or even perform, the sampling.

The samples may be divided into two kinds, bulk-samples and samples taken during delivery. In the former case, the object to be attained is to take a sample in which the various constituents shall bear the same relative proportion in the sample as in the bulk. In the latter, the bulk from which the sample is taken will be of known composition, and the object of taking the sample is to test the person from whom it is taken; no attempt must then be made to take an average bulk sample, but the person giving the sample should furnish it in the same manner as he

would furnish milk for sale. The taking of the latter class of samples presents no difficulty; the only precaution to be observed is that the bottle into which the sample is poured is clean and dry, and that it has a well-fitting cork. The proper sampling of a large bulk of milk is by no means easy; the bulk to be sampled will be, in most cases, a churn, and the milk in these should be mixed with a stirrer consisting of an iron rod carrying a perforated tin plate; the stirring is performed by working the stirrer up and down.

The "milk thief," a trough with a small opening in it, is also employed. The milk to be sampled is made to pass along the trough, and a little trickles out of the hole into a convenient receptacle beneath. This method gives fairly representative samples and requires no labour.

Small quantities of milk may be mixed by pouring to and fro from one vessel to another.

The samples should then be taken by means of a dipper or, better, by a sampling tube. This consists of a long tube open at both ends, the lower being flat; a flat plate is attached to a rod which runs down the middle of the tube, so that by raising the rod the plate can be pressed against the bottom of the tube enabling it to contain liquid. To take the sample the tube is lowered slowly into the churn, the plate being kept away from the bottom to allow the milk to rise; when the milk has completely filled the tube the rod should be raised, and the tube withdrawn to prevent the exit of the milk; the sample can then be transferred to a convenient receptacle by placing it under the tube and depressing the rod.

Another method of sampling bulks of milk, which is scarcely less exact than the preceding, is to tip the contents of the churn into a strainer with sides, the slope of which causes the milk to be thrown from side to side at an angle of  $45^\circ$ . The milk finds an exit through the wire gauze at the sides of a circular well, which should dip into a small tank of such size that the milk rises to the top of the wire gauze; a hood at the back prevents spilling and facilitates mixing. The sample should be taken from the tank with a dipper or sampling tube. This method of sampling has the advantage of removing any particles of straw, dust of food, etc., that may be found in the milk. If the tank be provided with a tap the milk can then be run off.

A convenient method of taking a composite sample of all the churns received from one farm is by the use of a large tin pot provided with a spout, which extends from top to bottom, and which communicates with the pot throughout its whole length by a series of holes  $\frac{1}{16}$  inch diameter, about  $\frac{1}{4}$  inch apart. The samples from each churn are emptied into the pot,

and the composite sample is taken by pouring out from the spout.

**Composite Samples.**—It is sometimes convenient to test the milk from a particular source once a week, or at other intervals; in this case the specific gravities should be noted daily, and a measured portion, such as 11 c.c. or 1 oz., placed each day in a bottle containing a little solid potassium bichromate; the fat estimation is made on the mixed sample.

**Sample Cans.**—The most convenient receptacles for the samples from bulk are round cans about 2 inches in diameter and  $4\frac{1}{2}$  inches deep, or wide-mouthed glass bottles which can be closed by a disc or stopper. In large dairies, the milk is received from different farms, and it is convenient to stamp the name of the farm legibly on its own sample can; sample cans should be provided and marked for all the samples it is desired to take regularly, and the use of unmarked cans, cans marked with paper labels, and cans marked with another designation should be avoided, if possible.

The lids of these cans should fit well, and they should not be filled more than three-quarters full, to obviate as far as possible the risk of spilling in transit. The samples, after being taken, should be placed in a box or tray made to contain twenty-four cans, or any other convenient number, in which they can be transported to the laboratory. The trays should have a strong handle in the middle, and it is desirable that they be also furnished with a lid which can be locked or sealed, to prevent any tampering with the samples in transit. The duty of conveying the samples should be entrusted to one man, who should be made responsible for any spilling of the contents. A tray holding twenty-four cans is not too heavy to be carried steadily, and there is no reason why any of the milk should be spilt. If the sampling is performed at a distance from the laboratory, the use of bottles is more reliable; a case to contain these should be provided. It is, of course, necessary for the chemist to see that the sample cans are in good repair; any which leak, or have ill-fitting lids, should be replaced at once. The cans or bottles before being handed over to the samplers should be quite clean and dry; the cans should be washed with hot water and dried, and when not in use, kept with the lids open. If bottles are used a large stock should be kept, and they should be well washed with warm water and allowed to drain for a couple of days; this will be found to dry them sufficiently.

**Testing.**—The tests applied to milk should be of two kinds, simple tests done in the dairy, and a more extensive examination in the laboratory. The only test sufficiently convenient for use in the dairy is the estimation of the specific gravity.

This will be sufficient to detect any gross adulteration. The taking of the specific gravity of every churn should be invariably performed, as this indication alone will be sufficient to detect gross adulteration, and it will also give a rough indication of the quality of the milk. For this purpose it is most convenient to employ a thermo-lactometer, or lactometer and thermometer combined in one instrument; the specific gravity should be corrected to 60° F. by means of Table LXXXI. It is not generally necessary that the chemist should personally take these specific gravities. This may be left to a foreman or other intelligent person who has been trained to this work under the supervision of the chemist. The instructions given to the foreman should be, to pass all milk of a specific gravity lying between certain limits, unless special instructions to the contrary in certain cases are given; these limits may be from 1.034 to 1.031, 1.0305, or 1.030, according to circumstances; but in special cases higher or lower limits may be used. Should the specific gravity of any churn fall outside these limits, the foreman should be instructed to take samples with special care, and send them at once to the laboratory to be further tested; the milk in this case should be put on one side and not used until the report of the chemist has been received. As it is possible that a dispute, perhaps in a law court, may arise with respect to these special samples, the foreman should be instructed to seal both the sample, and the churn from which it was taken, so as to prevent any possibility of the milk being tampered with. This preliminary testing has been found to give satisfactory results in the author's experience. In all cases where adulteration of milk before arrival at the dairy has been definitely proved, the specific gravity test revealed that the milk was not of normal quality. As an example of a case in which it was necessary to depart from the usual instructions given to the foreman, the author has found, in the case of the milk received from one particular farm, that the milk had a specific gravity as low as 1.029. As the genuineness of this milk was proved beyond a doubt, the lower limit for the milk derived from this farm was fixed for a certain period at this figure. It will not be necessary to teach the foreman to read the lactometer closer than half a degree, as this is an approximation quite sufficient in practice.

Undoubtedly, the most perfect control over the milk would consist in the analysis of the milk in every churn before it is used, and the rejection of those churns in which the milk does not come up to the standard of purity adopted. This is, however, hardly practicable in a large dairy, as the milk must be dealt with and sent out as soon as it arrives, and the time for analysis is short. It has been found that the time required for

handling a churn of milk—*i.e.*, straining and transferring to the vessel in which it is sent away from the dairy—is about 35 seconds, and the quickest analysis, other than the taking of the specific gravity, occupies at least two minutes; so that to test each churn in this way would mean delaying the handling of the milk to an extent which is incompatible with the proper working of the dairy. The testing of the milk in the laboratory that has passed the specific gravity test must, therefore, be done after the milk has been disposed of. It has been found that it is not necessary in this case to test the milk in every churn, as the results are for guidance as to the quality of milk that may be expected from different sources, and one sample per day from each farm is sufficient for this; the morning and evening meal should be sampled alternatively. If adulteration is detected, or if the milk is of abnormal quality, the whole of the churns from that particular farm should be tested. It is also desirable to test the whole of the churns from each farm at intervals, say once a month, in order to see that the difference between them is not excessive. No strict rule can be laid down for the taking of samples; the chemist must use his discretion, based on experience, as to what samples he will have taken.

**Analysis of the Samples.**—The cans should be brought at once to the laboratory and the lid of the tray opened; the samples are then to be arranged in alphabetical or numerical order, or in any way that may be most convenient; a methodical system in this respect will be found to minimise any chance of error in dealing with large numbers of samples. The procedure must vary in different dairies; if the samples are very few, the samples taken at different times of the day may be left till a sufficient number have accumulated and all examined at once. In this case, it is necessary to preserve them in a cool place, especially in summer; but where the time can be found it is preferable to make two or three series of analyses a day. An examination of the lids of the cans should be made to see if any of the milk has been splashed upon them, and if it is not possible to obtain a new sample the portion adhering to the lid should be washed down into the can with some of the sample. The analysis should comprise the following data:—Specific gravity, fat, and total solids, and (by difference) solids not fat. The specific gravity should be estimated by means of a lactometer. The fat may be estimated by the Leffmann-Beam or similar methods, or may be calculated from the total solids and specific gravity. The total solids may be estimated by evaporation, or may be calculated from the fat and specific gravity. The choice of how the analysis is to be conducted will depend upon the time available. The most reliable mode of work is to estimate both

fat and total solids, but it requires a considerable amount of time; if this cannot be done, it is most satisfactory to estimate the fat and to calculate the total solids, as the fat is the most valuable constituent of the milk, and also because the accuracy of this estimation by the Leffmann-Beam methods is rather greater than the accuracy of the total solid determination. In some dairies payment is made according to the amount of fat in the milk; in this case, the fat estimation should certainly be made.

### The Testing of Milk.

In this chapter methods requiring less attention in detail than those previously described, and suitable for the use of persons who have not had a thorough analytical training, are described. The results obtained by these methods have not the rigid accuracy which can be obtained by the best gravimetric methods, but determinations approximating sufficiently nearly to the truth can be made, to enable them to be used in practical work.

#### Determination of Specific Gravity.

**Lactometers.**—This is invariably done in milk-testing by lactometers (see p. 214); the lactometers used in dairy work are of two kinds, the thermo-lactometer and the ordinary lactometer.

The thermo-lactometer (Fig. 17) consists of a stem on which is marked a double scale, one part reading the specific gravity, and the other the temperature on the enclosed thermometer; a cylindrical body; and two bulbs, the upper one being the bulb of the thermometer, and the lower one (containing mercury or shot) serving for the adjustment. By its means the temperature and specific gravity can be read off from the same instrument.

The ordinary lactometer consists of a stem carrying a scale on which the specific gravity is read; a cylindrical, or globular, body; and a bulb containing mercury or shot.

Soxhlet's lactometer (Fig. 18) contains a scale from  $25^{\circ}$  (1.025) to  $35^{\circ}$  (1.035) divided up into suitable divisions ( $\frac{1}{2}$  or  $\frac{1}{10}$ ).

Vieth's lactometer (Fig. 19) has a globular body; it requires a smaller bulk and depth of milk than Soxhlet's, and is suitable for taking the specific gravity in a half-pint can. The scale reads from  $25^{\circ}$  to  $35^{\circ}$ .

Quevenne's lactometer has a scale from  $15^{\circ}$  to  $40^{\circ}$ , and marked to show proportions of water added to milk and skim milk respectively. This auxiliary scale is useless.

Another form of lactometer, the name of whose inventor is



deservedly lost in oblivion, has a scale from 0 to 100, 0 being equal to a specific gravity of 1.000 (water), and 100 being equal



Fig. 17.—Thermo-lactometer.

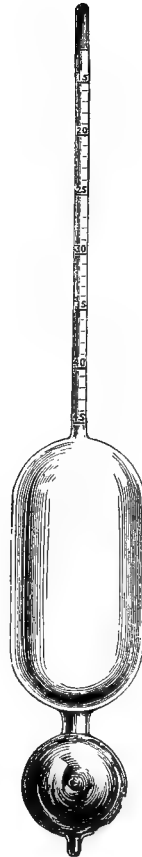


Fig. 18.—Soxhlet's Lactometer.

to a specific gravity of 1.029. It is of no practical use in milk testing.

Still another form is marked M at 1.029, and W at 1.00, the intermediate space being divided into quarters; this form is a mere toy.

An instrument has lately been put on the market, which consists of a glass tube in which is enclosed a bulb of specific gravity 1.029, which floats in milk above this specific gravity, but sinks

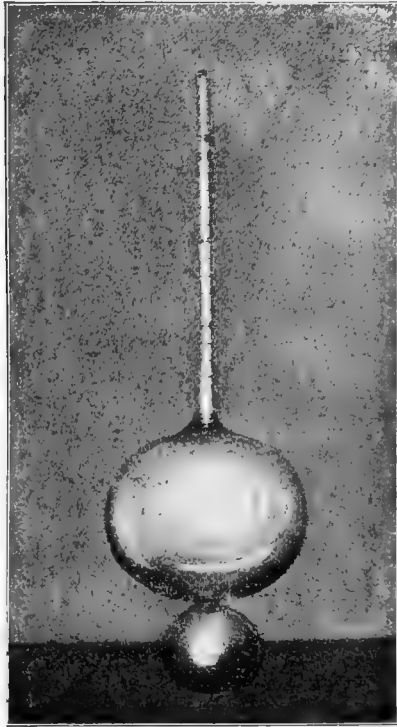


Fig. 19.—Vieth's Lactometer.

when the specific gravity is reduced below this figure by watering, by warming, or by excess of cream. It provides a harmless form of amusement, but is of no practical use.

The best lactometers for use in milk testing are the thermo-lactometer, Soxhlet's, and Vieth's.

The thermo-lactometer cannot be made very small nor very delicate on account of the enclosed thermometer, and requires a comparatively large bulk of milk; it is thus more suitable

for testing in the dairy than in the laboratory, where samples are often limited. It has, however, the advantage of not requiring a separate thermometer and a separate operation to determine the temperature.

Vieth's lactometer (Fig. 19) may be used in a can and, if the samples are received in cans, as is often the case in a dairy laboratory, no transference of the sample is necessary.

Soxhlet's lactometer has a wider scale, and may be conveniently used when greater accuracy is required.

Galaine's self-correcting lactometer has a metal ball completely filled with chloroform attached to the bottom, the object being to obviate the necessity of correction of the specific gravity for temperature; the expansion of the chloroform was supposed to compensate the expansion of the milk. Though excellent in theory, it has proved disappointing in practice.

Beam's lactometer is devised to obviate the difficulty of determining the exact point where the surface of the milk cuts the stem. It consists of a specially graduated lactometer and a float, and the reading is made by observing the point on the stem which corresponds with the uppermost portion of the glass tube of the float.

In use, it is essential that the stem remain dry. Beam's directions for use are:—The stem of the lactometer being dry the float is passed over it and allowed to rest on the bulb. The lactometer is then lifted by the point of the stem, and gradually let into the milk. If there is any doubt as to the instrument having found its proper level, the base of the jar may be held firmly to the table by one hand and the jar gently tapped with the other.

When removing the lactometer the float should be taken out first, in order to keep the tube dry and ready for another test.

The following directions are due to Vieth:—

**Use of Lactometer.**—In order to determine the specific gravity, the milk is poured into a vessel at least  $\frac{1}{4}$  inch greater in diameter than the widest part of the lactometer, and deep enough to allow the instrument to float. A cylindrical glass jar (Fig. 20), with foot, is the most suitable vessel for the purpose if Soxhlet's lactometer or the thermo-lactometer be used; Vieth's lactometer may be used in a can or tin cup. The lactometer is gradually lowered into the milk to the 25th degree, care being taken that the instrument is entirely wetted by the milk and that no air adheres to it. When released, the lactometer will move up and down, and after a little while become stationary. That degree of the scale which coincides with the surface of the milk is then noted. It will be observed that, where the milk touches the vessel and the stem of the lactometer,

the surface is not level, but, in consequence of the adhesion of the milk to the glass, forms a curve (Fig. 21). There is no difficulty, however, in ascertaining the extension of the curve sufficiently near, and this has to be allowed for in reading off the specific gravity. When using instruments of ordinary size, the curve will be found to extend to about one-half degree.

Lactometers indicate the exact specific gravity at a temperature of 60° F. It is, therefore, necessary, as soon as the position of the lactometer has been noted, to remove the instrument from the milk, immerse a thermometer, and ascertain the temperature.

If the temperature is found to be 60° F., the observed specific gravity is correct, but should the temperature of the milk be higher or lower than 60° F., the specific gravity must be corrected

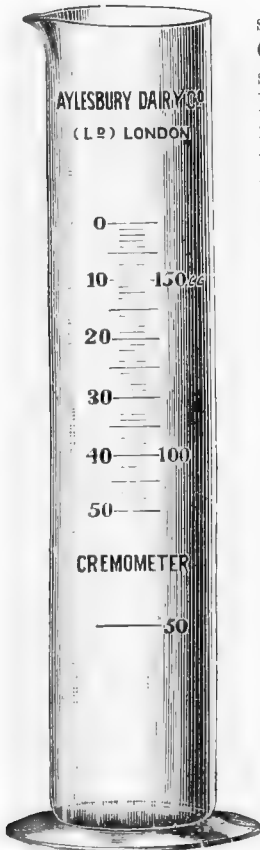


Fig. 20.—Glass Jar.



Fig. 21.—Lactometer in Milk.

by the aid of the Table in the Appendix, which is used as follows :—Find the temperature of the milk in the uppermost horizontal line, and the observed specific gravity in the first or last vertical column ; in the same line with the latter, and under the temperature, is given the corrected specific gravity. For example—Supposing the temperature to be 51° and the specific gravity

34°, the specific gravity corrected to 60° F. is  $32.9^\circ = 1.0329$ ; or if the temperature is 66° and the specific gravity 29°, the corrected specific gravity is  $29.8^\circ = 1.0298$ .

**Never take the specific gravity of a milk without also noting the temperature and correcting to 60° Fahrenheit.**

Instead of reading from the bottom of the curve and making a mental allowance, the lactometers may be read from the top of the curve and a definite figure (ascertained by a few carefully-conducted experiments) added on.

As soon as the specific gravity and temperature have been taken, the corrected specific gravity from the table should be entered in the book provided for the purpose of recording the results. It is not necessary to enter the specific gravity in full, but only the three significant figures; thus a specific gravity of 1.0325 may be entered simply as 325 or 32.5.

Though the determination of specific gravity has been described first, it is found when total solids are to be estimated that it is convenient in practice to proceed as soon as work is commenced with their estimation, as this is an operation which proceeds alone. Only in those cases where the sample is so small that the lactometer will not conveniently float, if the quantity necessary for total solid estimation has been removed, is it usually convenient to take the specific gravity first.

### Estimation of Total Solids.

A number of dishes or capsules, each marked with a separate number and previously weighed, and a pipette marked to discharge 5 grammes of milk of a specific gravity 1.032 at a temperature of 60° F., are necessary. The dishes should be, if possible, of platinum, and at least  $1\frac{1}{2}$  inches internal diameter, flat bottomed, and with a rim about  $\frac{1}{8}$  of an inch wide; this rim should be considerably wider at one side so as to form a lip on which the number should be legibly stamped. These dishes weigh about 12 grammes and cost £3 to £4 each. They may, however, be replaced by porcelain dishes of about  $2\frac{1}{2}$  inches diameter, glazed all over; these may be marked by scratching the number on the side with a new file, painting this over with a solution of platinum chloride, wiping off the excess, and igniting the capsule; the number will be marked in platinum.

The dishes must be previously weighed on a balance accurate to 1 milligramme, and the weights recorded on a table which should be kept in the balance case. It has been found in practice that, if the dishes be carefully cleaned, monthly weighings of the dishes are sufficient, the average loss in this period for dishes used daily having been found to be about 1 to 2 milligrammes.

The dishes should be arranged in a shallow tray—a photographic dish is suitable—according to their numbers from left to right, six or seven in a row, beginning at the bottom—*i.e.*, the side nearest the operator. This arrangement is chosen so that any milk accidentally dropped from the pipette will not fall into a dish which has been previously filled, but into an empty dish which can be easily wiped. The tray containing the dishes should be on the left of the tray in which the samples are placed. The samples should be arranged in order in rows, beginning at the left-hand bottom corner and going upwards—*i.e.*, away from the operator—and should, if in cans, have their lids turned back over the next sample can. The taking of samples for the estimation of total solids is much facilitated by having an assistant to stir the milk. The measurement of the quantities of milk for analysis is done as follows:—The assistant stirs No. 1 can, and when the cream has been mixed the chemist plunges the pipette into the milk and sucks it up till it enters the mouth; it is advisable to throw away this first quantity. The milk is again sucked up, the finger placed over the end of the pipette, and the milk allowed to run down to the mark, care being taken that air bubbles are not included in the portion measured. The pipette is held over dish No. 1 and the finger removed; the milk runs into the dish, and the drop adhering to the end is removed by touching it against the side of the dish; the last drops must not be removed by blowing. Meanwhile the assistant has closed can No. 1 and stirred can No. 2, from which 5 grammes are measured in a similar manner, without, however, washing out the pipette; this is transferred to dish No. 2, and the whole of the samples are taken similarly.

The next step is to enter the designation of the samples and the number of the dish into which each has been placed in a book provided for that purpose; the tray containing the dishes is then conveyed to the water-bath. The water-bath should be of copper, about 6 inches deep, and provided with a lid containing a suitable number of holes in which the dishes can rest: the number of these will vary with the number of samples to be daily examined; it is convenient to have a projecting collar about a quarter of an inch deep round each hole, as this facilitates the putting on and removal of the dishes, and each should be provided with a lid. If steam is laid on, the bath should be heated by means of a coil laid in the bottom, through which the steam circulates; the exit of this coil should be connected with a condenser, and the condensed steam serves to supply the laboratory with distilled water. If steam be not laid on, the bath must be supported on an iron support at such height as to allow of a burner being placed underneath; in either case an

arrangement for keeping the water level constant in the bath is necessary.

After the dishes have been for about half an hour on the bath, provided it has been boiling briskly, it will be noticed that a distended skin has formed on the surface of the milk; this must be broken with a needle mounted in a handle, care being exercised that no portion of the skin is brought away on the needle. The object of this is to prevent the milk drying in flakes, which may be blown away by draughts, and the estimation lost. Should the dishes be forgotten, or should any other reason prevent the stirring being done at the proper moment, a few drops of water may be added to the milk residue, which will have the effect of making the flakes settle down and adhere to the dish. When the dishes have been on the water-bath for three hours, they should be taken off, placed on a tray having two or three thicknesses of blotting-paper on the bottom to remove adhering drops of water, and transferred to a water-oven or air-bath. The important point about the water-oven is that it has an even draught passing through it; the form of air-bath devised by Dr. Adams of Maidstone is suitable, though the cover is a little troublesome to remove. The dishes are placed upon wire shelves one above the other, and it is convenient to have ten or twenty dishes on each shelf. If a good draught be maintained in the water-oven or air-bath, it is not necessary or advisable to keep this at a higher temperature than  $90^{\circ}$  to  $95^{\circ}$  C. After three hours drying in the air-bath, the dishes should be weighed.

**Weighing.**—Ten basins (or any number that can be conveniently placed in the balance case at one time) should be removed from the bath, placed on a tray and conveyed to a desiccator, and there allowed to cool for a few minutes. Platinum basins cool very much faster than porcelain, and much time is saved by their use; when cool, they should be weighed to the nearest milligramme, the weight entered in the book opposite to the number of the dish; the weight of the empty dish (from the table of weights) should be subtracted, and the weight of the milk residue will be the difference between the two weights; this also should be entered in the book. As 5 grammes of milk were taken, the residue, multiplied by 20, will give the percentage of total solids in the milk.

If the samples arrive in the laboratory in sample bottles and not in cans, a somewhat different mode of procedure must be adopted. A number of cylindrical tins without lids (of such a size as to hold the contents of a sample bottle) and a lactometer are necessary. The bottles and dishes are arranged in their proper order and entered in the book, as before. As many bottles as there are tins are shaken, to mix the cream, and emptied

into the tins; 5 grammes are taken from each and placed in the dishes, but before the milk is poured back into the bottle or otherwise emptied from the tin, the temperature and specific gravity should be taken; the remaining samples are then similarly treated in their proper order. The drying and weighing are performed as before.

**Rapid Methods.**—A pipette is used which delivers 2.5 grammes of milk, and the milk is run into flat-bottomed porcelain basins about 3 inches in diameter. The numbers are marked on the basins with copper paint, which paint, on ignition, forms an indelible blue mark. Before the basins are filled, Stokes recommends that two or three drops of a 10 per cent. solution of acetic acid in alcohol be sprinkled over each. The alcohol spreads itself over the surface of the milk, and the acid precipitates the casein. Revis adds 1 c.c. of acetone. Under these circumstances, drying proceeds very rapidly. The basins are placed on the water-bath till apparently dry, a matter of a few minutes only, and are further dried for about an hour in the water-oven or air-bath. They are then weighed as before. The difference between the weight of the basin with the residue and the weight of the basin alone, multiplied by 40, gives the percentage of total solids.

In the author's experience Stokes' method has a tendency to give results slightly above the truth, but according to Yarrow the difference does not exceed 0.08 per cent.; it has, however, the advantages of rapidity and of requiring very little attention.

### Estimation of Fat.

For the estimation of fat in a rapid manner, with an accuracy sufficient for milk control, a centrifugal method must be used. The Leffmann-Beam, and the acido-butyrometric methods, will be described in detail.

**The Leffmann-Beam Method, and Modifications.**—Leffmann and Beam, realising that the time of whirling necessary in Babcock's method, which consisted in treating the milk with an equal volume of strong sulphuric acid, and separating the fat by centrifuging, was a serious objection, experimented with a view to shortening this. They finally decided on the use of amyl alcohol as a means of assisting the fat to rise, and were thereby enabled to reduce the time of whirling.\* The method is usually employed in conjunction with the Beimling machine.

\* It was stated in the first edition that the same idea was independently worked out at the Vermont Experiment Station; but it appears that this was not correct, and was based on a misunderstanding.



The method has been subjected to a close investigation by the author, and is of considerable accuracy.

**The Beimling Machine.**—This consists of a cast-iron framework carrying a vertical spindle; on this is a small bevelled cog-wheel, which engages a larger bevelled cog-wheel on a horizontal spindle turned by means of a handle. In the larger machines a second spindle and set of cogs is introduced (Fig. 22).

On the top of the vertical spindle two, three, or six arms extending radially are fixed. To the ends of each of these are pivoted one or usually two cups, in which the bottles are placed.

When the handle is turned, the cogs cause the spindle and the arms carrying the cups to rotate. For one turn of the handle,

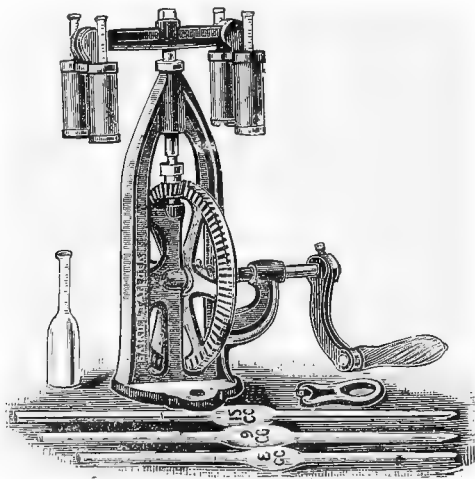


Fig. 22.—Leffmann-Beam Machine.

the vertical spindle turns eleven times. Centrifugal force causes the cups to assume a horizontal position when rotating, and they return to the vertical when the machine is at rest.

The bearings are all plain, which causes a considerable amount of friction; the centre of gravity of the rotating system is placed very high, which causes vibration, due to imperfect balancing, to be marked. The air resistance at high speeds is somewhat great.

These are serious faults, but are capable of improvement. The two-bottle Beimling machine is the only machine on the market, to the author's knowledge, in which the bottles assume a horizontal and radial position when rotating; in the larger sizes the

bottles are nearly, but not quite, radial. This position is advantageous, as it allows of the most rapid separation of the fat from the acid liquid.

**Apparatus.**—The test bottles consist of flat-bottomed flasks with a sloping upper portion terminating in a graduated neck. The bottles (English make) hold 29 c.c.; the necks are made of glass tube 5.96 mm. in internal diameter, and are so graduated that 80 divisions = 1.475 c.c. These dimensions are according to a specification laid down by the author, and differ slightly from those prescribed by Leffmann and Beam. The pipettes used are—

- 15 c.c. for milk,
- 9 c.c. for sulphuric acid,
- 3 c.c. for amyl alcohol mixture,
- 4.5 c.c. for cream, and
- 10.5 c.c. for water.

Automatic measuring apparatus and burettes may be also used for measuring the acid and amyl alcohol.

The author has devised a burette specially for the measurement of sulphuric acid and other corrosive liquids. It has been found, in practice, that ordinary burettes are liable to be filled to overflowing, and that considerable inconvenience is caused by spilling strong sulphuric acid.

An ordinary burette with a three-way tap is used, and to the tube, for filling from the bottom, a wider tube,  $\frac{1}{2}$  inch in diameter and 3 inches long, is fused. An india-rubber cork is inserted in this, and through it is passed a long glass tube bent as a syphon, which serves to convey the acid from a stock bottle above.

In the top of the burette an india-rubber cork is fixed, through which passes a tube going almost to the top of an air chamber of glass; to the bottom of the air chamber a glass tube of small bore passes upwards so far as just to enter into the stock bottle.

The illustration (Fig. 23) will make the construction clear.

The conditions necessary for satisfactory working are:—

1. The capacity of air chamber and tube leading to stock bottle must not be more than  $\frac{1}{5}$  of the capacity of the burette.
2. The bottom of the stock bottle must be well above the top of the tube leading into the air chamber.

The tube leading into the air chamber must be adjusted to the mark on the burette equal to its capacity.

The burette is used as follows:—The tap is turned so that the liquid enters and fills the burette. As it reaches the upper portion, it passes up the tube and overflows into the air chamber, from which it is forced up the tube leading to the stock bottle. When the liquid reaches a height corresponding to the level of the liquid in the stock bottle, the liquid ceases to run, and the

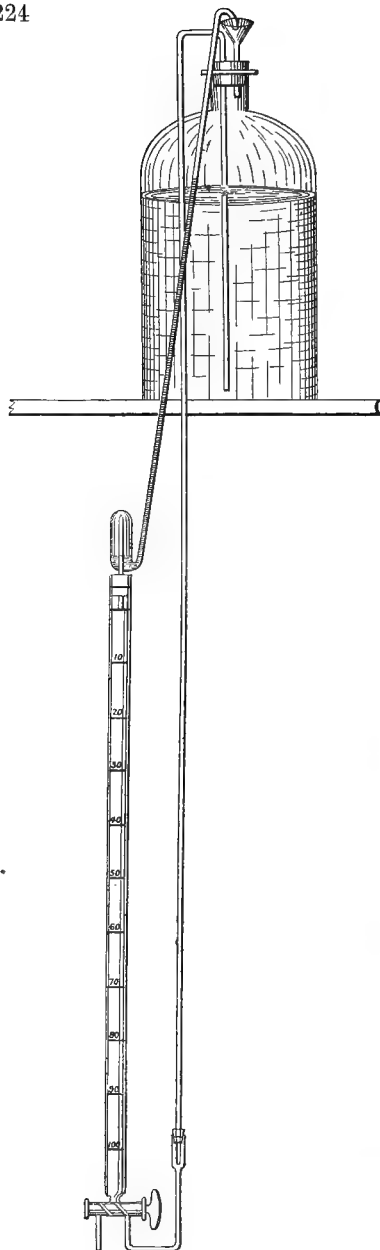


Fig 23 —Automatic Burette.

burette is automatically filled to the zero point. When the tap is turned the liquid runs out, air bubbling in from the stock bottle, and measured quantities may be taken. After the liquid has been run out as far as desired, the tap is turned, and the liquid enters the burette.

The liquid in the air chamber is forced back into the stock bottle, and the burette automatically fills itself.

The burette can be made of 9 c.c. capacity, but it is much quicker to employ a graduated burette of much larger capacity than any form of automatic measuring apparatus.

The advantages claimed for the burette are—

- (1) Automatic filling to zero point.
- (2) One turn of the tap only required to fill and to measure.
- (3) Impossibility of spilling corrosive liquids.
- (4) Saving of time, as the filling is done while other operations are conducted.

**Chemicals.** — Commercial sulphuric acid containing 96 per cent.  $H_2SO_4$ , which has a specific gravity of 1.842 at  $15.5^\circ C.$  ( $60^\circ F.$ ). Owing to the fact that strong sulphuric acid has a somewhat anomalous specific gravity, it is not advisable to test the specific gravity directly. The following test will give good

results:—Measure accurately 200 c.c. of acid into a large flask, and to it add cautiously 15 c.c. of water, cooling the flask by immersion in cold water. Take the specific gravity of this diluted acid, either with an accurate hydrometer or by other means. If the temperature be not exactly 15·5° (60° F.), add on 0·001 for each degree Centigrade above 15·5°, or 0·00056 for each degree Fahrenheit above 60° F. (or subtract for temperatures below).

The following table will give the strength of acid:—

Specific Gravity of Diluted Acid.	Per cent. $H_2SO_4$ .
1·8380, . . . . .	98 (94·20)
1·8349, . . . . .	97 (93·22)
1·8311, . . . . .	96 (92·24)
1·8268, . . . . .	95 (91·26)
1·8217, . . . . .	94 (90·28)

The figures in parentheses give the percentage of sulphuric acid in the diluted acid, the other figures referring to the percentage of acid before dilution.

Another method of determining the strength of acid is to weigh about 1 gramme of acid in a basin and, after diluting with water, to add an excess of strong ammonia. The solution is evaporated on the water-bath and, when nearly dry, a little more strong ammonia is added; it is then dried to constant weight at 100° C. (212° F.) and weighed. The weight divided by 1·347 will give the weight of sulphuric acid in the sample; this, divided by the weight taken and multiplied by 100, will give the percentage.

Purified amyl alcohol, free from petroleum, specific gravity 0·815 to 0·818 at 15·5° C. (60° F.), which completely dissolves to a clear liquid when mixed with an equal bulk of hydrochloric acid; this mixture must not become darker than sherry in three days.

Commercial hydrochloric acid.

The amyl alcohol is mixed with an equal bulk of hydrochloric acid for use; it is best not to keep this mixture longer than a few days.

**The Process—Testing of Milk, Skim Milk, Buttermilk, and Whey.**—Measure 15 c.c. each of the well-mixed samples into test bottles, holding the point of the pipette against the side of the neck, so that the liquid may run down, allowing room for the air to escape. Add 3 c.c. of the mixture of amyl alcohol and hydrochloric acid. Pour in, with care, 9 c.c. of sulphuric acid, so that it washes down any particles of milk on the neck of the bottle. Mix the contents of the bottle with a rotatory motion; a little practice is required to do this without the liquid boiling over, owing to the heat evolved on mixing

sulphuric acid with water, but when the way is once learned there is no difficulty in doing this. Fill up the bottles to the zero mark with a mixture of one part of sulphuric acid to two volumes of water, and place the bottles in the machine; rotate by turning the handle at 100 revolutions per minute for about one minute, when the fat will separate in a clear layer.

Read the fatty layer as follows:—Note the position of the lower surface of the fat (it is convenient to wait till it has fallen to one of the main graduation lines), then immediately note the position of the lowest point of the curve on the upper surface; the difference between the two will give the percentage of fat; repeat this once or twice; the results should be identical (Fig. 24).

Each small division is  $\frac{1}{10}$  per cent. fat; if, then, the fatty layer occupies thirty-six of these, the percentage is 3.6. Half or a quarter of a division may easily be read with practice.

Should the fatty layer have sunk below the lowest graduation, a little more diluted acid may be added, and the bottle whirled for a few seconds.

In very cold weather the fat may solidify in the neck; in such case it should be warmed slightly before reading; it is not otherwise necessary to warm the bottles before reading.

Skim milk and buttermilk should be whirled as soon as possible after mixing; in very hot weather, or if the bottles stand very long after the acid has been added, the fat may be of a dark colour.

It is advisable to compare the results given by this method with those given by the Gottlieb or other good gravimetric process whenever a new stock of sulphuric acid or amyl alcohol is used, and, if necessary, to work out a definite correction to be added to or subtracted from the results. With acid and alcohol corresponding to the specification above, no correction should be necessary.

The difference between the results by the Leffmann-Beam method and those by gravimetric analysis very rarely exceed 0.1 per cent. of fat.

**Testing of Cream.**—If the cream contains less than 32 per cent. of fat it can be measured direct by the 4.5 c.c. pipette; if it is thicker than this, it must be diluted with separated milk. Two beakers or tin pots are counterbalanced on a rough balance turning to 0.01 gramme; in one of them, about 25 grammes of cream are placed, and water is run into the other till the weights are equal. The cream and water are mixed together, and the



Fig. 24.  
Neck of bottle.

mixture can be measured. If the cream is sour a few drops of ammonia should be placed in the pot before the weights of water and cream are adjusted.

The measurement is performed as follows:—Fill the pipette with cream by sucking at the top, and close it with the finger; hold the pipette vertically, and allow the cream to run down till the upper surface is on a level with the mark; turn the pipette to a horizontal position and wipe the stem; then return it to the vertical and, holding the point over the neck of a test bottle, allow the cream to run out freely; after the quick succession of drops has ceased allow three more drops to run. Add 10·5 c.c. of water and proceed as in analysing milk.

Calculate the results from Table LX., using column 3 for undiluted cream and column 2 for diluted cream. This table should be checked by gravimetric analysis whenever a new pipette is used.

TABLE LX.—FOR ESTIMATING FAT IN CREAM.

Reading.	Diluted.	Undiluted.	Reading.	Diluted.	Undiluted.
8·5	63·8	32·0	6·7	49·8	25·0
8·4	63·0	31·6	6·6	49·0	24·6
8·3	62·2	31·2	6·5	48·2	24·2
8·2	61·4	30·8	6·4	47·4	23·8
8·1	60·6	30·4	6·3	46·6	23·4
8·0	59·9	30·0	6·2	45·9	23·1
7·9	59·1	29·6	6·1	45·1	22·7
7·8	58·3	29·2	6·0	44·4	22·3
7·7	57·6	28·9	5·9	43·6	21·9
7·6	56·8	28·5	5·8	42·8	21·5
7·5	56·0	28·1	5·7	42·1	21·1
7·4	55·3	27·7	5·6	41·4	20·8
7·3	54·5	27·3	5·5	40·6	20·4
7·2	53·7	26·9	5·4	39·8	20·0
7·1	52·9	26·5	5·3	39·1	19·6
7·0	52·1	26·1	5·2	38·3	19·2
6·9	51·4	25·8	5·1	37·5	18·8
6·8	50·6	25·4	5·0	36·7	18·5

**Testing of Sour Milk.**—Weigh in a small beaker about 15 grammes of the sample which has been previously well mixed by whisking with a brush formed of fine wires; pour as much as possible into a test bottle and re-weigh the beaker: the difference will give the weight of the milk taken: add sufficient water to make up to 15·25 grammes and proceed as in analysing milk.

Calculate as follows:—Multiply the reading by 15·25 and divide by the weight taken: the result will be the percentage of fat in the sour milk.

**Testing of Clotted Cream, Cheese, and Butter.**—Weigh the bottle and transfer to it about 1 to 1.5 gramme of butter, 2 grammes of clotted cream or 3 grammes of cheese, and weigh again. Butter should be melted in a closed vessel at a temperature of 40° C. (104° F.), and, after shaking, about 1½ c.c. sucked up in a tube which will just enter the neck of the bottle; the butter should be blown in as completely as possible. Clotted cream should be well mixed and sucked up in a tube in the same way as butter, and either blown or pushed in with a wire. Cheese should be cut up into small pieces, which can be dropped in. Add sufficient water to make up the weight to 15.25 grammes and proceed as in analysing milk. Cheese requires rather longer shaking than other products, but gives equally good results.

If desired, cream may be weighed instead of being measured.

The calculation is performed as for sour milk.

The above directions differ in some respects from those given by Leffmann and Beam. The author has had, however, some years practical experience of the methods described and is convinced of their accuracy. A stand for the bottles is to be recommended; this may conveniently be made of wire rings into which the bottles fit, with a flat plate for a bottom; the bottles can then be easily carried about.

To clean the bottles: empty while hot in a convenient receptacle, and wash twice thoroughly with hot water; if necessary, run a brush down the neck. They are conveniently washed in the stand. Never leave pipettes dirty.

**Failures and their Probable Causes.**—The only failures likely to happen are:—

1. Dark layer of fat.
2. Fluffy layer under the fat.

1. If the acid be too strong, or the temperature too high, or the mixture left too long before whirling, the fat may be dark. The remedy is obvious.

2. A fluffy layer under the fat is often caused by allowing the milk and acid to stand too long unmixed. It may sometimes be due to a bad quality of amyl alcohol.

Grit on the bottom of the bottles may cause fracture while in the machine. Fracture may also occur from too sudden a stoppage after the whirling is completed.

**Modifications of the Leffmann-Beam Method.**—The Leffmann-Beam method has been subjected to considerable modification; thus Paterson and, later, Gerber have used amyl alcohol alone without hydrochloric acid.

**Gerber's Acido-butyrometric Method.**—This is essentially the Leffmann-Beam method, the chemical principles of which

have been adopted. The use of hydrochloric acid as a solvent for the amyl alcohol has been, however, discarded, following Paterson.

Gerber employs a test bottle, which he terms an "acido-butyrometer," which differs from that employed by Leffmann and Beam; it is a modified form of Marchand's lacto-butyrometer, and, like this, is closed with a cork.

A definite strength of sulphuric acid is prescribed (90 to 91 per cent.), and rules for testing the acid and amyl alcohol used are laid down.

Gerber has shown considerable ingenuity in adapting the method of Marchand to that of Leffmann and Beam, and the method is reliable.

The following comparative statement will show the differences of detail between this and the Leffmann-Beam method:—

LEFFMANN-BEAM.	GERBER.
1. Test bottles are flasked-shaped.	Test bottles are butyrometer-shaped
2. 96 per cent. sulphuric acid is used.	90 to 91 per cent. sulphuric acid is used.
3. A mixture of amyl alcohol and hydrochloric acid employed.	Amyl alcohol alone employed
4. Fat read off cold.	Fat read off at 60° to 70° C.
5. Bottles are used open.	Bottles are stoppered.

There is no practical advantage in either method. The original Leffmann-Beam is somewhat more rapid, while the Gerber modification requires rather less skill. Both are equally accurate.

**I. The Tester with Catgut Action for Four and Eight Samples** (Gaertner and Hegershoff's Patent)—**Description.**—A steel spindle, running in two ball bearings, the upper with ten balls and the lower with seven, is supported in a well-stayed frame, which can be fixed to any table by means of a screw clamp. On top of the spindle is a boss, on which two discs with screw threads are fastened, which hold the disc-plate for the reception of four or eight samples. The cover is screwed on to the spindle by means of a loose milled-headed nut and the machine is ready for use. If the machine is destined for frequent use, it will be best to fix it to a strong bench and not to a movable table; to strengthen it further, two screws may be put in through the holes in the frame and the tester will then not be transportable.

The bearings can be adjusted by means of the brass collar in the upper one which is held in place by two screws: this should be so arranged that the spindle runs easily without play, and when this is found to be the case, the screws should be tightened to hold the collar in place. The bearings should be oiled with good machine oil, care being taken that the oil which runs down the spindle is wiped off.



To rotate the machine, put the metal end of the catgut into the hole in the spindle, wind the string around, by turning the disc-plate backwards till the handle is close to the spindle. Pull the handle with full strength, the whole weight of the body being brought to bear, and as the string unwinds the machine is rotated; when all the string is unwound the end comes out of the hole, and the machine rotates freely. If clean and well oiled it will run for ten minutes.

To stop the machine, take hold of the milled-headed nut of cover firmly and it will screw itself off; then press the edge of the under disc-plate gently with the finger till it stops. Do not stop it with a jerk.

**II. The "Excelsior" Gearing.**—This can be fitted to 8- or 24-sample machines. It consists of a hollow cylinder fixed to the frame carrying a hollow double pulley, inside which the spindle can rotate without touching. Round the lower portion of the pulley is coiled a spring, and in the opposite direction round the upper a strap is wound, which passes through an opening in the cylinder; on the strap is clamped a stop-plate, which serves a double purpose—(1) to prevent the spring from pulling the strap too far, and (2) to lift the pulley, which is capable of a slight vertical movement, when the strap is wound home. At the bottom of the pulley is a pawl, which when the stop-plate is pulled out, engages a ratchet wheel on the spindle and which is lifted with the pulley when the stop-plate is home, so that the pulley runs freely. The machine is rotated by pulling the handle on the end of the strap rapidly to and fro fifteen to twenty times, when a high velocity is obtained; the strap and stop-plate are then allowed to go home and the machine runs alone. If the speed slackens, it can be increased by a few further pulls. This gearing is only recommended for 24-sample machines.

**III. The "Rapid" Gearing.**—In this, a loose pulley surrounds the spindle; it runs on a separate bearing and is, when not in use, kept up by a spring. A strap passes round the loose pulley, and when this is pulled (in a slightly downward direction) the pulley is brought downwards; two bevelled teeth engage two similar teeth on the spindle and cause the machine to rotate.

When the strap is pulled back (in a slightly upward direction) the spring forces the pulley up and the machine rotates freely. By pulling the strap rapidly backwards and forwards, a high rate of speed can be obtained.

The 2-bottle machine differs in construction from the others in not having the disc-plates, which are replaced by two arms, carrying cups; these cups are larger than the cups used in the larger machine and have a cover; the test bottles fit completely into them and are surrounded by warm water.

The machine is fitted with the "Rapid" gearing, and has plain bearings; this causes continual driving to be necessary. The machines cannot be left to run alone.

None of the methods of driving the Gaertner-Hugershoff machine are satisfactory. The catgut requires a strong pull and is liable to soon wear out, if the metal end comes off; if it is required to rotate a second time, the machine must be stopped.

The "Excelsior" gearing has a weak point in the spring, which breaks and is difficult to repair; the strap also sometimes breaks, and cannot be replaced without some trouble.

The "Rapid" gearing makes an unpleasant noise, and a great deal of the power employed to drive the machine is wasted in friction.

It is far better to discard the methods of driving sold with the machine and to employ a yard of blind cord (of the best quality), one end of which is fixed into a wooden handle. This is given one or two complete turns round the spindle; the handle is held in the right hand and the loose end in the left. The cord is pulled with the right hand, just sufficient tension being kept on the end with the left to make it bite. At the end of the stroke, the left hand is brought up near the machine to loosen the cord round the spindle, otherwise there is danger of the cord winding up.

The cord is now pulled back with the left hand keeping it quite loose—*i.e.*, letting the right hand go back quite freely. The pulling with the right hand is repeated, and continued till the speed is high enough.

It is advisable to stop up the hole in the spindle, as it causes the cord to wear. Should the cord wear out and break, it can easily be replaced at an infinitesimal cost. This method of driving was worked out in the author's laboratory by Boseley and Rosier.

**The Lister Machine.**—This has practically the same form as the Gaertner-Hugershoff machine, but does not include the "Excelsior" or "Rapid" gearings, which are covered by patents. The frame is of different construction, and is S-shaped. Round the spindle a small brass pulley is fixed\* (in the 24-bottle machine a ratchet is added), and it is driven by a string wound round this by Boseley and Rosier's method, which, however, was independently applied by Lister.

The 2-bottle machine has the arms hinged, and clamped in place by a screw, instead of having them in one piece; it is more easily portable.

\* In practice it is better to take off this pulley, stop up the hole, and drive on the spindle direct. The machines have also been made without the pulley.

There are many other machines now on the market, which in general principle are much the same as the original Gaertner-



Fig. 25.—Gerber Tester.

Hugershoff centrifuges. Some of these are driven by a handle (Fig. 25), which, by means of a worm gearing, imparts a rapid

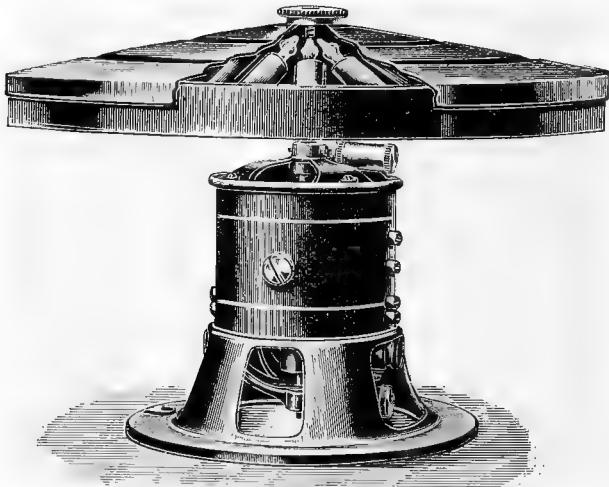


Fig. 26.—Gerber Tester, electrically driven.

motion to the spindle, and this method of driving finds favour with those who do not mind the extra expense.

Steam turbines, water turbines, and electro-motors (Fig. 26) are also largely used as the motive power, especially with large machines.

**Apparatus, etc.**—1. The acido-butyrometer is a glass vessel closed by an india-rubber cork, and with a graduated neck divided into ninety divisions; one division = 0.1 per cent. fat. Every tenth division is longer than the others, and the intermediate fifth divisions are also slightly lengthened to facilitate reading.

The neck may be either round, as in Gerber's original butyrometers, square section for extra strength, and flat for ease in reading (Figs. 27 and 28).

A special acido-butyrometer is made for skim milk; the upper portion of the neck is narrowed, and the divisions are much wider.

2. The acido-butyrometer for cheese, etc., is open at both ends, and the lower cork carries a small glass cup.

3. The author's butyrometer stand consists of a metal plate pierced with 4, 8, or 24 holes, and an india-rubber plate with 4, 8, or 24 corresponding holes so cut that the butyrometers are slid easily in and out and yet are retained when inserted.

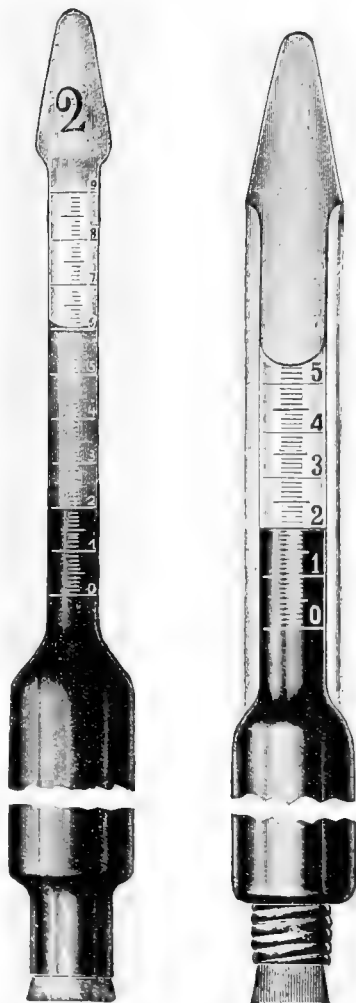
4. 11 c.c. pipettes for milk with, or without, automatic measurement.

4a. 3 c.c. pipettes for cream.

5. 1 c.c. pipette for amyl alcohol.

6. A water pipette, 10 c.c. in  $\frac{1}{10}$  c.c.

6a. A water pipette for cream, delivering 8.2 c.c.



Figs. 27 and 28.—Gerber Bottles.

7. 10 c.c. bulb pipette for acid. The bulbs prevent the acid from being drawn into the mouth.

8. Automatic measuring apparatus, or burettes, as an alternative means of measurement.

**Chemicals.**—Commercial sulphuric acid, specific gravity of 1.820 to 1.825 at 15° C. (59° F.) (contains 90 to 91 per cent.  $H_2SO_4$ ). The specific gravity may be taken with a hydrometer.

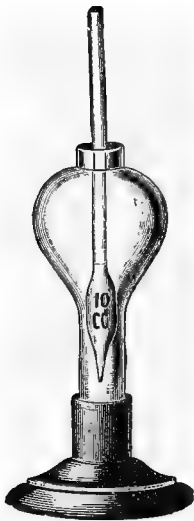


Fig. 29.—Aml Alcohol Measure for Gerber Test.

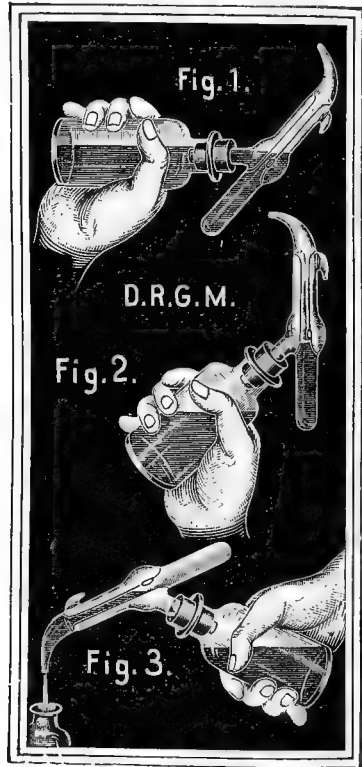


Fig. 30.—Acid Measure for Gerber Test.

Should the temperature not be exactly 15° C. (59° F.) the specific gravity may be corrected by adding on 0.001 for each degree Centigrade (or 0.00056 for each degree Fahrenheit above 59°) above 15°, and by subtracting 0.001 for each degree below 15°; thus, if the temperature be 20° and the specific gravity 1.818, the corrected specific gravity will be  $1.818 + 5 \times 0.001 = 1.823$ ;

and if the temperature be  $11^{\circ}$  and the specific gravity  $1.827$ , the corrected specific gravity will be  $1.827 - 4 \times 0.001 = 1.823$ .

Pure amyl alcohol—specific gravity,  $0.8165$  to  $0.818$  at  $15^{\circ}$  ( $59^{\circ}$  F.); boiling point,  $124^{\circ}$  to  $130^{\circ}$  C.—should give a clear solution with an equal volume of strong hydrochloric acid.

Amyl alcohol sometimes contains petroleum, due to the use of empty petroleum barrels as packages. A blank test with the Gerber method fails to reveal the presence of petroleum if the quantity is below 2 per cent., a quantity which gives an error of about 0.2 per cent. in the fat estimation.

It may be detected by running 10 c.c. of sulphuric acid, 10 c.c. of water, and 2 c.c. of amyl alcohol into a Gerber butyrometer, mixing and centrifuging; no layer should appear if the amyl alcohol be free from petroleum. If present an approximate estimation of the quantity may be made; the following results were obtained by the author and Goodson:—

TABLE LXI.

Percentage of Petroleum.	Reading.	Error with Whole Milk.	Error with Separated Milk.
4.8	0.67	+ 0.38	+ 0.37
2.4	0.25	+ 0.27	+ 0.16
1.2	0.11	+ 0.13	+ 0.07
0.6	trace	+ 0.09	+ 0.03
0.3	doubtful trace	+ 0.02	..
none	none	..	..

It is advisable to have a bottle of ammonia handy in case acid is spilt on the clothes; should this happen an application of a few drops of ammonia at once will prevent damage.

If strong acid is spilt on the skin, wash copiously with cold water without delay and the white swellings formed will soon disappear.

### Mode of Operation.

*Milk, Skim Milk, Whey and Buttermilk.*—Place a sufficient number of acido-butyrometers in the stand, open end upwards, and run 10 c.c. of acid into each. Well mix the samples to be tested and measure with the milk pipette, 11 c.c. of each into the bottles; add 1 c.c. of amyl alcohol.

Measuring liquids by means of pipettes is done as follows:—Hold the pipette near its upper end between the thumb and the middle finger of the right hand, insert the lower tapering

end into the liquid, and fill it by exhausting the air with the mouth, then remove the lips and quickly close the upper end by means of the first finger. The pipette is then removed from the liquid, and by raising the first finger slightly, so much of the contents are allowed to escape, drop by drop, until the lowest point of the curve forming the surface of the liquid coincides with the mark on the upper part of the instrument. The contents are then discharged, the pipette being allowed to run out.

Insert the corks, slightly damping them at the ends if necessary; place the hand over the corks and shake with an up and down motion until the curd is dissolved; invert the stand to allow the acid in the lower bulb to mix with the rest, and thoroughly mix the contents by inverting three or four times. Take the bottles out of the stand one by one and allow the contents to run into the larger portion, push up the cork, if necessary, so that the graduated neck is full, and place the bottles in the cups of the machine, screw on the cover and spin it for two or three minutes. Place a Bunsen burner, with a flame just high enough to touch the bottom of the disc-plate, underneath to prevent cooling, unless the machine is fitted with a steam turbine, or with one of Gerber's heaters, which will keep it at the necessary temperature. If the fat is not in a clear limpid layer in the neck, or if the upper portion is frothy, the rotation has not been sufficient and must be repeated. After taking out the bottles, Gerber directs that they be placed in the water-bath for about a minute, which must be kept at a temperature of from 60° to 70° C.; they are then ready for reading. The water-bath may, without sacrificing accuracy, be dispensed with if the disc is kept warm. It is an advantage to use a flame, as the corks have a tendency to come out in the bath, thereby spoiling the estimation.

*Reading the Fatty Layer.*—Hold the butyrometer up to the light, and by slight pressure on the cork adjust the bottom layer to one of the larger lines on the scale; count up the number of divisions between this and the lowest curved line at the top; each of the larger divisions is equal to 1 per cent. of fat, and the smaller  $\frac{1}{10}$  per cent. of fat; every fifth smaller division is also made somewhat longer to facilitate reading. In the illustration (p. 226) the percentage of fat shown is 3.6; observe that the lower layer is coincident with one of the longer lines, and that the lower curved line at the top is thirty-six smaller divisions above that.

All pipettes are graduated to run out; therefore the liquids must not be blown out.

Separated milks require to be whirled for a somewhat longer

time and at the highest attainable speed, and 0.05 per cent. must be added to the reading.

Condensed milks, both sweetened and unsweetened, may be tested by weighing about 20 to 25 grammes, making up to 100 c.c., and treating as a milk; a higher speed or longer whirling is, however, necessary to get up all the fat. The percentage of fat found must be multiplied by 100 and divided by the weight taken.

*Cream.*—Cream containing not more than 32 per cent. of fat can be measured with great accuracy. In the case of thin cream—*i.e.*, one with not more than 32 per cent. of fat—after the acid has been added, add 8.2 c.c. water, measure the cream with a 3 c.c. pipette, filling it up accurately to the mark while in a vertical position, turn the pipette in a nearly horizontal position, and wipe the stem perfectly dry; hold it over the bottle in a vertical position and, removing the finger from the top, let the cream run out freely; after the quick succession of drops has run out, allow three more drops to enter the bottle; add 1 c.c. of amyl alcohol, and then proceed as in analysing milk.

Calculate the results from Table LXII., column 2.

Crems containing more than 32 per cent. of fat must be diluted. Take two beakers or tin pots and counterbalance them on a rough balance turning to 0.01 gramme, pour about 25 grammes of cream into one and add separated milk or water to the other till the weights are equal, mix the cream and separated milk or water, and measure as before. Use column 3 for calculating the results.

TABLE LXII.—FOR CALCULATING FAT IN CREAM.

Degrees.	Undiluted.	Diluted.	Degrees.	Undiluted.	Diluted.
8.5	33.2	66.2	6.7	25.9	51.6
8.4	32.8	65.4	6.6	25.5	50.8
8.3	32.4	64.6	6.5	25.1	50.0
8.2	32.0	63.8	6.4	24.7	49.2
8.1	31.6	62.9	6.3	24.3	48.4
8.0	31.2	62.1	6.2	23.9	47.6
7.9	30.7	61.3	6.1	23.5	46.8
7.8	30.3	60.5	6.0	23.1	46.1
7.7	29.9	59.7	5.9	22.7	45.3
7.6	29.5	58.9	5.8	22.3	44.5
7.5	29.1	58.1	5.7	21.9	43.7
7.4	28.8	57.3	5.6	21.5	42.9
7.3	28.3	56.4	5.5	21.1	42.1
7.2	27.9	55.6	5.4	20.7	41.3
7.1	27.5	54.8	5.3	20.3	40.5
7.0	27.1	54.0	5.2	19.9	39.7
6.9	26.7	53.2	5.1	19.5	38.9
6.8	26.3	52.4	5.0	19.1	38.1



This table should be checked by a gravimetric method, and may require a slight correction added or subtracted, which may vary with each pipette.

The cream should be as near 15.5° C. (60° F.) as possible, but the error due to temperature is very small and is less than the errors of reading, etc.

The pipette does not deliver the same weight of a cream with 20 per cent. of fat as of one with 30 per cent. of fat; this has been allowed for in the table.

*Sour Milk.*—Well mix the sample by whisking for a few minutes with a brush made of fine wires; pour about 15 grammes into a small beaker and weigh; transfer from 10 to 11 grammes to the bottle and weigh again to get the weight added; add water to make up 11.22 grammes. Proceed as before.

$$\text{Calculate: Fat in sour milk} = \text{reading} \times \frac{11.22}{\text{wt. taken}}$$

An alternative method is to add to each 100 grammes 5 c.c. of strong ammonia and treat as a milk; increase the result by one-twentieth.

*Clotted Cream, Butter, Cheese, etc.*—Well mix the sample (in the case of butter it is advisable to melt it in a closed vessel at about 40° C. (104° F.) and to shake violently till solid). Place a few grammes in a small basin with a glass rod and weigh. After adding acid, transfer, with the rod, from 1 to 2 grammes (according to percentage of fat, 1 for butter, 1.5 for clotted cream, and 2 for cheese); add water to make up to 11.22 grammes and 1 c.c. of amyl alcohol, and proceed as before. Calculate as for sour milk.

*To Clean the Bottles.*—After reading, place the bottles in the stand, cork upwards; take out the corks and wash them several times with hot water. Do not use soda. Empty the bottles into a suitable vessel and fill them with hot water; empty this out completely and repeat twice; if not quite clean run a brush down them and wash again. Invert the stand and let the bottles drain. Dry the corks after use. Never leave pipettes dirty.

Keep the stopper in the sulphuric acid bottle when not in use. Gerber recommends a butyrometer with two openings for solid products; the lower cork carries a little glass cup of 1 c.c. capacity, and the product to be tested is weighed into this. The author has not found this advantageous.

*Cream and Clotted Cream.*—Well mix from 50 to 100 grammes of the sample to be tested, fill the cup with this, dry the outside and weigh. Place the cork carrying the cup in the butyrometer, add 6 c.c. of clear hot water through the upper opening, then 1 c.c. of amyl alcohol and 6.5 c.c. of acid, and shake well; add

6 c.c. more hot water, shake again and whirl in the machine. Read after one minute's standing in the water-bath.

*Butter*.—Melt about 10 to 20 grammes in a small closed bottle at 40° C. (104° F.) and shake violently till solid; fill the cup, and weigh. Add 12 c.c. of cold water, and 1 c.c. of amyl alcohol and 6.5 c.c. of acid. Shake well and proceed as before.

*Cheese*.—Mix 10 to 20 grammes in a mortar till of even consistency. Fill the cup and weigh; transfer the bulk of the cheese from the cup to the butyrometer, by inserting the cork and shaking gently; add 6 c.c. of hot water and 6.5 c.c. of acid and shake till the cheese is dissolved. Now add 7 c.c. of hot water and 5 drops of amyl alcohol (from the pipette), shake well and whirl in the machine. Stop the machine after about two to three minutes, take out the butyrometer, add a further 1 c.c. of amyl alcohol, and place for a minute in the water-bath at 60 to 70° (say 150° to 160° F.); whirl again and read after a minute's standing in the water-bath.

For *Skim Cheeses* whirl three times and add 8 c.c. of hot water instead of 7 c.c.

*Calculation of Results*.—The percentage of fat in the sample is found by dividing the number of degrees read off on the stem of the butyrometer by the weight taken, thus

$$\text{Butter wt. } 0.76 \text{ gramme. Reading } 62^\circ \text{ Fat} = \frac{62}{0.76} = 81.6 \text{ per cent.}$$

$$\text{Cream wt. } 0.90 \quad ,, \quad ,, \quad 49^\circ \quad ,, = \frac{49}{0.90} = 54.4 \quad ,,$$

$$\text{Cheese wt. } 0.68 \quad ,, \quad ,, \quad 22^\circ \quad ,, = \frac{22}{0.68} = 32.35 \quad ,,$$

*Water Estimation in Butter, Margarine, etc.*—A special form of butyrometer is used for this; it consists of an elongated bulb 5 c.c. in capacity, connected by a graduated tube with a vessel in which a cork carrying a cup of 3 c.c. capacity is inserted. The only reagent necessary is diluted sulphuric acid, made by diluting commercial sulphuric acid (sp. gr. 1.820 to 1.825) with an equal bulk of water; before use this should be cooled to the ordinary temperature, and decanted from any deposit of lead sulphate.

Five c.c. of diluted sulphuric acid are measured into the butyrometer, which is placed open in the machine, and whirled for about two minutes, in order to bring all the liquid into the bulb; the level of the acid (at as near 60° F. (15.5° C.) as possible) is read off on the graduated scale. About 2½ to 3 grammes of butter are weighed into the cup, and after the cork has been inserted, the butyrometer is stood in the water-bath at 60° to 70° C. (say 150° to 160° F.) to melt the butter; when this has

been accomplished, the whole is well shaken till the contents form a uniform emulsion; after standing for a minute in the water-bath, the butyrometer is placed in the machine, and whirled three times, warming in the water-bath for about two minutes between each; after the third whirling, it is cooled to as near 60° F. as possible, and the level of the aqueous liquid where it joins the fatty layer is read off. The difference between this reading and the level of the acid will give the percentage of water if exactly 3 grammes of butter have been taken; should any other weight have been taken it is necessary to multiply the result by 3 and divide by the weight taken; thus, in an experiment 2.780 grammes of butter were taken, the level of the acid was 2.5° and the level of the aqueous liquid 14.5°; the percentage of water indicated is, therefore,

$$\frac{(14.5 - 2.5) \times 3}{2.780} = \frac{12 \times 3}{2.780} = 12.95 \text{ per cent. water.}$$

For the convenience of weighing out the cream, butter, etc., in the cups, a balance of the steel-yard type can be obtained with the machine; it consists of a beam, with suitable supports, one end of which is longer than the other; from the shorter end, which also carries a pointer, a small wire cradle to support the cup is hung; the longer end is divided into 10 equal parts, each being indicated by a notch numbered 1 to 10; at the end of this is a fine screw carrying a counterpoise, which can be moved backwards or forwards by screwing round.

The weighing is accomplished by placing the cup in the cradle, and screwing the counterpoise backward or forwards, as required, till the pointer is at zero in the middle of the scale; the cup is now removed and filled with the product to be tested, and the riders are put on the various notches in the beam in succession till equilibrium is restored. The largest rider indicates grammes, the medium tenths of a gramme, and the smallest hundredths of a gramme.

Thus if the largest rider is in notch 2, the medium 7, and the smallest 8, the weight is 2.780.

If it be found that to restore equilibrium it is necessary to place the smallest rider intermediate between two notches, say between 2 and 3, the reading is taken as 0.025.

If it be found that two riders must be placed on the same notch to restore equilibrium, the smaller should be hung from the upturned end of the larger.

The use of this balance, though convenient when many samples are being tested, is not necessary, as the weighings may be made, but slightly less expeditiously, with an ordinary balance.

**Stokes' Modification.**—Stokes employs a modification of the

acido-butyrometer ; it is open at both ends, one being provided with an indiarubber washer kept in place by a screw cap, while the other can be closed with a cork. It is placed screw cap downwards : up to the zero mark of the graduations, the tube holds 1.5 c.c., and the neck is graduated to read percentages of fat ; the upper portion consists of two bulbs ; from the zero mark of the graduations up to a mark between the two bulbs the bottle holds 13.5 c.c., while from this mark to a mark above the second bulb the capacity is 15 c.c.

It may be used without a centrifugal machine as follows :— 1.5 c.c. of amyl alcohol are poured in to the zero mark, or, better, measured by means of a pipette provided with a rubber teat of about 1.5 c.c. capacity ; sulphuric acid is carefully poured in to the mark between the bulbs, and then the milk to be tested is poured in to the upper mark. An indiarubber cork is put into the upper opening, and the contents of the butyrometer well mixed by shaking and inversion. The tube is now stood, cork downwards, in hot water, the screw cap loosened, and left for an hour ; the fat will have collected in a layer in the graduated neck, and can be read off in the same manner as previously described.

This forms an extremely cheap method of estimating fat in milk, with an accuracy quite sufficient for most purposes, and can be recommended where only one or two samples per day are to be tested.

The apparatus can be used also for the more exact estimation of fat by measuring the milk, acid, and amyl alcohol by means of pipettes, or automatic measuring apparatus, and whirling in a centrifugal machine.

The Lister-Stokes machine is made to take these bottles ; it differs chiefly from the Lister-Gerber in the form of the disc. Instead of having a lid, the disc is made double and open in the centre, the butyrometers being slid into cardboard tubes in pockets, which are symmetrically arranged, radiating from the centre. This form of machine has the advantage of having the pockets comparatively large, and can be used for other purposes. The whole of the disc can be conveniently filled with hot water should it be desirable to prevent cooling during centrifugal separation.

**Alkaline Butyrometric Methods.**—Gerber has devised a method whereby the use of sulphuric acid is avoided, and this has found favour among those persons who have not had the advantage of a laboratory training.

The composition of the alkaline salt solution has not been made public ; the method is employed with the same apparatus as the acido-butyrometric method.

Eleven c.c. of the alkaline salt solution, 10 c.c. of milk, and 0.6 c.c. of isobutyl alcohol are placed in a butyrometer, which is closed with a stopper, and the contents mixed, and immersed in water at 45° for three minutes, and after shaking the tube is centrifuged, and the fat read off after again placing in water at 45°. These results are the same as those given by the acido-butyrometric method, but the accuracy is somewhat less. The amount of isobutyl alcohol added must not be varied.

Sichler's sinacid method is very similar; 10 c.c. of salt solution, containing 15 per cent. of trisodium phosphate and 1 per cent. of trisodium citrate, 10 c.c. of milk, and 1 c.c. of isobutyl alcohol are placed in a butyrometer, and well mixed. The mixture is heated to 75° to 90°, again well mixed, and centrifuged for one minute. The tube is placed in water at 70°, and the fatty layer read off. The isobutyl alcohol is usually coloured, and the colour passes into the fatty layer, facilitating reading.

#### **The Control of Milk during Delivery to Customers.**

A very important part of the work of the dairy chemist is the control of the men employed in delivering milk. It is evident that a man on a milk round, being under no supervision for a greater part of the time, has ample opportunities, should he be so disposed, to adulterate or "lengthen" the milk of which he has temporary charge. He may also, with the best intentions possible, unwittingly deteriorate the quality of the milk by allowing the cream to rise on the milk, and serving some customers with the richer portion, thereby leave a poorer quality for others. For the purpose of this control it is necessary to take three series of samples.

(1) Samples representative of the mixed bulk of milk that is placed in charge of the man.

(2) Samples taken in the streets in the course of delivery.

(3) Samples of the small quantities of milk returned.

The first and third samples should be taken by a foreman or other responsible person, preferably in the presence of the man; the foreman should be responsible for their conveyance to the laboratory.

The second series should be taken by an intelligent and responsible person, who should receive instructions to take samples at irregular intervals, and to avoid any semblance of rotation, in order that the man shall not be able to form an idea when he may expect a visit. He may be, with advantage, mounted on a bicycle if the area of delivery is large.

The first and third series may be conveniently taken in sample cans, while the second series may be taken in five-ounce bottles,

which have been found to hold sufficient milk for analysis, while not being too large to be easily carried. A case holding a dozen bottles should be provided for this purpose.

The testing of these samples may be performed largely with a lactometer; in fact, it is in this work that the usefulness of the lactometer is most appreciated.

The specific gravity of the samples of series (2) and (3) should be carefully compared with those of series (1), which will form a standard by which the others may be judged. In all cases the specific gravities must be corrected to 60° F. Three cases may occur.

1. The specific gravity of a sample of series (2) or (3) is equal to the specific gravity of the corresponding samples of series (1). In by far the greater number of cases this indicates that the composition of the samples is identical. It is possible, however, that the milk may have been skimmed, which would raise the specific gravity, and then slightly watered, which would bring the specific gravity back to the original degree. It is, however, excessively unlikely that a milk carrier could perform this feat with such accuracy as would be required, and an experienced observer would have his suspicions aroused by the thin appearance of the milk. It is also patent that a man adulterating milk for the sake of profit, or with malice, would not confine himself to one isolated occasion, but would do so habitually; and only a skilled scientist could habitually remove cream from milk, and reduce its specific gravity to the original degree by watering. For all practical purposes it may be taken that when the specific gravities agree the milk has not been tampered with.

2. The specific gravity of the sample of series (2) or (3) is higher than the specific gravity of the corresponding sample of series (1). This indicates that it contains less cream than the original.

3. The specific gravity of the sample of series (2) or (3) is lower than the specific gravity of the corresponding sample of series (1). This may be due to two causes—either the milk contains more cream than the original, or it has been watered. In some instances both causes may be responsible for the lowness of the specific gravity.

If the second or third cases have occurred, a further examination of the milk should be proceeded with. Either the fat or total solids should be estimated, and the solids not fat calculated; the corresponding sample of series (1) should be simultaneously examined. It is advisable that the samples—or a selected number of them—should be examined if it has been found that the specific gravities agree, as it affords a means of checking the accuracy of the specific gravity determinations,

and of detecting the somewhat hypothetical case of scientific skimming and watering.

A very important rule, which is of great use in the control work of the dairy chemist, may be enunciated as follows:—

The specific gravity of a milk in lactometer degrees added to the percentage of fat will remain constant, whether the cream has been diminished or increased. The sum of the two will be lowered by the addition of water. For instance, the original milk had a specific gravity of 1·0325 or 32·5°, and contained 4 per cent. of fat. The sum is, therefore, 36·5.

If a sample of (say) series (2) were found to have a specific gravity of 1·0315 or 31·5°, and contained 5·0 per cent. of fat, the sum would be still 36·5.

If the sample had a specific gravity of 1·0335 or 33·5°, it will be found that the fat amounted to only 3·0 per cent., and the sum would be 36·5.

If the sample, however, contained only 3·8 per cent. of fat, and had a specific gravity of 31·5°, the sum would be only 35·3; and it could then be concluded that the milk had been watered, and that it contained only  $\frac{35\cdot3}{36\cdot5} \times 100 = 97$  per cent. of the original milk, or, in other words, 3 per cent. of water had been added.

It has been found that this rule holds with remarkable accuracy for any percentage of fat between 0 and 10, and it is not very far out with even higher percentages of fat.

It must not be expected that the sum of the lactometer degrees and fat will always add up to the same identical figure, as there is a liability to error in both determinations; with care, however, the difference due to this cause should not exceed 0·5.

The value of the samples of series (3) lies in the fact that rising of cream is most easily detected by their percentages of fat being considerably different from that in series (1), as the total effect due to this cause is usually marked.

**The Solution of Analytical Problems.**—A dairy chemist is frequently called upon to solve the most diverse problems with regard to milk and its products. In the following paragraphs a few such problems are given, together with the analytical data from which the solution was deduced. They cover a fairly wide range, and may be taken as fairly representative of the questions a dairy chemist is called upon to elucidate. All are actual examples.

**PROBLEM I.**—To determine to what the low specific gravity of the milk is due.

*Example a.*—The analytical figures were :—

Specific gravity, . . . . .	1.0234
Total solids, . . . . .	9.82 per cent.
Fat, . . . . .	3.21 „
Ash, . . . . .	0.51 „
Solids not fat, . . . . .	6.61 „

From the extreme lowness of all the figures it was concluded that 26 per cent. of added water was present.

*Example b.*—The analytical figures were :—

Specific gravity, . . . . .	1.0300
Total solids, . . . . .	11.46 per cent.
Fat, . . . . .	3.33 „
Ash, . . . . .	0.68 „
Solids not fat, . . . . .	8.13 „

In this case it was concluded from the low solids not fat, and the correspondingly low ash, that a small amount (5 per cent.) of added water was present.

*Example c.*—Two samples of milk taken from two churns arriving at a station from a farm.

The analytical figures were :—

	No. 1.	No. 2.
Specific gravity, . . . . .	1.0234	1.0298
Total solids, . . . . .	9.19 per cent.	11.6 per cent.
Fat, . . . . .	2.67 „	3.30 „
Ash, . . . . .	0.55 „	0.69 „
Solids not fat, . . . . .	6.52 „	8.31 „

In this case analyses of milk from the same farm had been made for some time previous, and the solids not fat had not been found to fall below 8.6 per cent. It was concluded that No. 1 contained 24 per cent. and No. 2 3 per cent. of added water. The conclusion about No. 2 would not have been justified had there not been evidence of the normal composition of this milk.

*Example d.*—The analytical figures were :—

Specific gravity, . . . . .	1.0291
Total solids, . . . . .	12.78 per cent.
Fat, . . . . .	4.36 „
Ash, . . . . .	0.73 „
Solids not fat, . . . . .	8.42 „

Genuine authenticated samples from the same source had been found to contain as little as 8.28 per cent. of solids not fat. It was, therefore, concluded that this sample was genuine, but abnormally poor in solids not fat.



*Example e.*—The analytical figures were :—

Specific gravity, . . . . .	1.0292
Total solids, . . . . .	11.57 per cent.
Fat, . . . . .	3.51 "
Milk-sugar, . . . . .	3.41 "
Casein, } . . . . .	3.84 "
Albumin, }	
Ash, . . . . .	0.81 "
Solids not fat, . . . . .	8.06 "

It was concluded from the abnormally low proportion of milk-sugar, and high amount of casein and ash, that this sample was genuine, though of abnormal character.

*Example f.*—In this case the composition of the original milk was known.

The analytical figures were :—

	Sample Submitted.	Original Milk.
Specific gravity, . . . . .	1.0311	1.0325
Total solids, . . . . .	12.03 per cent.	12.46 per cent.
Fat, . . . . .	3.45 "	3.55 "
Solids not fat, . . . . .	8.58 "	8.91 "

The sample contained 3 per cent. of added water.

*Example g.*—The analytical figures were :—

Specific gravity, . . . . .	1.0287
Total solids, . . . . .	16.75 per cent.
Fat, . . . . .	8.10 "
Solids not fat, . . . . .	8.65 "

The low specific gravity was due to an excess of cream; this is shown by the high percentage of fat, and the comparatively high amount of solids not fat.

*Example h.*—The analytical figures were :—

Specific gravity, . . . . .	1.0245
Total solids, . . . . .	16.11 per cent.
Fat, . . . . .	8.21 "
Ash, . . . . .	0.65 "
Solids not fat, . . . . .	7.90 "

This sample contained a large excess of fat, but had also received an addition of 8 per cent. of water, shown by the low solids not fat and small amount of ash.

PROBLEM II.—To determine cause of high specific gravity of milk.

*Example a.*—The analytical figures were :—

Specific gravity, . . . . .	1.0346
Total solids, . . . . .	10.95 per cent.
Fat, . . . . .	1.80 "
Solids not fat, . . . . .	9.15 "

The low fat shows that this milk has been deprived of a portion (40 per cent.) of its cream.

*Example b.*—The analytical figures were :—

Specific gravity, . . . . .	1.0363
Total solids, . . . . .	13.86 per cent.
Fat, . . . . .	3.62 "
Milk-sugar, . . . . .	4.58 "
Protein, . . . . .	4.62 "
Ash, . . . . .	0.82 "
Solids not fat, . . . . .	10.24 "

From the abnormal ratio of protein to milk-sugar, it was concluded that this milk was genuine and of abnormal composition.

*Example c.*—The analytical figures were :—

Specific gravity, . . . . .	1.0614
Total solids, . . . . .	20.84 per cent.
Fat, . . . . .	4.73 "
Milk-sugar, . . . . .	8.86 "
Protein, . . . . .	6.02 "
of which Albumin, . . . . .	trace.
Ash, . . . . .	1.23 "

The practical absence of albumin showed that the milk had been boiled ; the high figures shown by milk-sugar, protein, and ash indicated that the milk had been concentrated, and that this was the cause of the high specific gravity.

PROBLEM III.—To determine cause of sweet taste of milk.

*Example a.*—The analytical figures were :—

Specific gravity, . . . . .	1.0352
Total solids, . . . . .	11.53 per cent.
Fat, . . . . .	1.96 "
Ash, . . . . .	0.80 "
Solids not fat, . . . . .	9.57 "

The normal ratio of ash to solids not fat and their excessive amount point to the milk having been concentrated.

This milk had also been deprived of a portion of its cream.

*Example b.*—The analytical figures were :—

Total solids, . . . . .	16.23 per cent.
Fat, . . . . .	2.05 "
Milk-sugar (polarised), . . . . .	15.83 "
" (gravimetric), . . . . .	3.12 "
Ash, . . . . .	0.59 "
Solids not fat, . . . . .	14.18 "

The extreme difference between the polarimetric and gravimetric figures for milk-sugar points to the presence of added sugar, probably cane sugar in aqueous solution. This sample may possibly have been a diluted condensed milk.

*Example c.*—The analytical figures were :—

Specific gravity, . . . . .	1.0293
Total solids, . . . . .	10.21 per cent.
Fat, . . . . .	2.43 "
Milk-sugar, . . . . .	5.12 "
Protein, . . . . .	2.25 "
Ash, . . . . .	0.41 "
Solids not fat, . . . . .	7.78 "

The low ash and protein point to the presence of 35 per cent. of added water; there has also been an addition of milk-sugar, probably to mask the addition of water.

PROBLEM IV.—To determine reason for milk being called "poor."

It is evident that either watering or abstraction of cream would cause the milk to appear poor; it is unnecessary to give further examples of this kind.

*Example a.*—The analytical figures were :—

Specific gravity, . . . . .	1.0326
Total solids, . . . . .	13.45 per cent.
Fat, . . . . .	4.30 "
Albumin, . . . . .	0.10 "
Ash, . . . . .	0.75 "
Solids not fat, . . . . .	9.15 "
Cream in six hours, . . . . .	1.3 "

This milk was normal in composition and contained a good percentage of cream. The low albumin and small amount of cream thrown up in six hours showed that it had been boiled. It was probably the slow rate of rising of cream, due to the milk having been raised to a high temperature, that caused a suspicion of "poorness."

*Example b.*—The analytical figures were :—

Specific gravity, . . . . .	1.0325
Total solids, . . . . .	12.70 per cent.
Fat, . . . . .	3.78 "
Solids not fat, . . . . .	8.92 "
Colour, . . . . .	quite white.

The fat was also seen to be nearly colourless.

The milk was of good quality, but of a very white colour, probably due to the cows having been fed on artificial food.

The "poorness" was here evidently judged by the colour. The widespread belief that absence of colour denotes poorness has led to the artificial colouring of milk.

*Example c.*—The analytical figures were :—

Specific gravity, . . . . .	1.0317
Total solids, . . . . .	14.11 per cent.
Fat, . . . . .	5.04 "
Ash, . . . . .	0.75 "
Solids not fat, . . . . .	9.07 "

This milk was unusually rich ; it is probable that it contained an excess of cream. It was the other portion of the milk (which naturally was deficient in cream) that was poor.

PROBLEM V.—To determine reason of unusual taste and smell.

*Example a.*—The smell was faint and like stale fish, and the taste soapy and unpleasant.

The following were the analytical figures :—

Specific gravity, . . . . .	1.0364
Total solids, . . . . .	11.56 per cent.
Fat, . . . . .	2.39 "
Ash, . . . . .	1.05 "
Solids not fat, . . . . .	9.17 "

The milk was alkaline and the ash titrated with phenolphthalein ; had an alkalinity equal to 0.33 per cent. of  $\text{Na}_2\text{CO}_3$ .

It was concluded that an addition of 0.3 per cent. of sodium carbonate had been added.

*Example b.*—The milk smelt of vinegar and curdled on warming.

The analytical figures were :—

Specific gravity, . . . . .	1.0329
Total solids, . . . . .	12.45 per cent.
Fat, . . . . .	3.50 "
Ash, . . . . .	0.74 "
Solids not fat, . . . . .	8.95 "
Acidity, . . . . .	50°

The milk was curdled by phosphoric acid ; 60 c.c. of the whey were distilled :—

The first 10 c.c. took	.	1.3 c.c.	$\frac{N}{10}$	alkali.
„ second „	.	1.8	„	„
„ third „	.	1.9	„	„

It was evident that the milk contained an acid somewhat less volatile than water ; this corresponds with acetic acid, and the whey distilled as a solution containing 0.11 per cent. of acetic acid, which is equivalent to 2 per cent. of vinegar.

*Example c.*—The milk had a faint burnt taste.

It contained 0.42 per cent. soluble albumin.

On centrifuging, a deposit was obtained which appeared to consist of proteid matter ; it was much browned. It was therefore concluded that the milk had been placed in a vessel, in which burnt milk had previously been kept.

*Example d.*—The milk tasted burnt.

The following analytical figures demonstrated that the milk had been boiled :—

Fat, . . . . .	3.72 per cent.
Cream in six hours, . . . . .	1.3 "
Soluble albumin, . . . . .	trace.

It was concluded that the milk had been burnt in boiling.

*Example e.*—The smell and taste were unpleasant, but could not be identified.

The following analytical figures were obtained :—

Specific gravity, . . . . .	1.0292
Total solids, . . . . .	13.22 per cent.
Fat, . . . . .	4.82 "
Milk-sugar, . . . . .	4.34 "
Protein, . . . . .	3.36 "
Ash, . . . . .	0.70 "
Solids not fat, . . . . .	8.40 "

The sediment obtained by centrifuging contained much mucus and cells from the udder.

It was concluded that the milk was the product of a cow in ill health.

It is evident that if dirty water has been added to milk, an evil smell and taste may occur; no further example need be given of this.

Turnips and other substances eaten by the cow or, what is more likely, handled by the milker, may communicate a taste to the milk. The action of certain organisms may have a similar effect. The author is unacquainted with chemical methods of identifying these causes.

PROBLEM VI.—To determine reasons for milk being sour or curdled.

*Example a.*—The acidity was 17.5°, and the cream in clots.

The analytical figures were :—

Specific gravity, . . . . .	1.0333
Total solids, . . . . .	12.73 per cent.
Fat, . . . . .	3.56 "
Ash, . . . . .	0.76 "
Solids not fat, . . . . .	9.17 "
Soluble albumin, . . . . .	none.
Taste, . . . . .	boiled.

The milk had been boiled and the cream allowed to rise and clot, giving the milk a curdled appearance.

*Example b.*—The milk was curdled, and the whey was analysed.

Acidity, . . . . .	43.1°
Total solids, . . . . .	6.25 per cent.
Fat, . . . . .	1.05 "
Ash, . . . . .	0.48 "
Solids not fat, . . . . .	5.20 "

From the low percentage of solids not fat and ash of the whey, it was concluded that about 15 per cent. of water had been added, and there was some probability that this water contained an acid. It may also have been watered milk which has gone sour.

*Example c.*—The milk was curdled and the whey analysed.

The analytical figures were :—

Acidity, . . . . .	35.2
Total solids, . . . . .	7.46 per cent.
Fat, . . . . .	0.88 "
Ash, . . . . .	0.60 "
Solids not fat, . . . . .	6.58 "

It was concluded from the normal percentage of solids not fat and ash of the whey, that nothing had been added and that the milk had become sour by natural causes.

*Example d.*—The milk was curdled and the whey analysed. It was slightly bitter and gave a pink biuret reaction.

The analytical figures were :—

Acidity, . . . . .	20.8
Total solids, . . . . .	11.13 per cent.
Fat, . . . . .	3.03 "
Ash, . . . . .	0.69 "
Solids not fat, . . . . .	8.10 "

The alkalinity of the ash to phenolphthalein was equal to 0.025 per cent.  $\text{Na}_2\text{CO}_3$ .

It was concluded that the milk had been peptonised, and was insufficiently alkaline.

*Example e.*—The milk was curdled and the whey analysed.

The following were the analytical figures :—

Acidity, . . . . .	26.2
Total solids, . . . . .	9.42 per cent.
Fat, . . . . .	1.27 "
Milk-sugar, . . . . .	5.53 "
Ash, . . . . .	0.60 "
Solids not fat, . . . . .	8.15 "

The milk was not bitter, but gave the biuret reaction.

It was concluded from the low acidity and the high milk-sugar that lactic fermentation was not the cause of curdling; from the biuret reaction being obtained, it was concluded that an enzyme was the cause.

Milk is often alleged to be sour because when used for making milk puddings and custards with eggs, a clear whey runs out. The curdling of the milk is due to the coagulation of the large amount of albumin of the egg on baking. The following is an analysis of a whey from rice pudding :—

Total solids,	.	.	.	.	17.67 per cent.
Fat,	.	.	.	.	0.82 "
Ash,	.	.	.	.	0.85 "
Solids not fat,	.	.	.	.	16.85 "

It was very sweet and contained much cane sugar.



Fig. 31.—Microscope.

PROBLEM VII.—To determine the reason for milk being thick.

*Example a.*—The analytical figures were :—

Specific gravity,	.	.	.	.	1.0262
Total solids,	.	.	.	.	17.92 per cent.
Fat,	.	.	.	.	9.36 "
Ash,	.	.	.	.	0.71 "
Solids not fat,	.	.	.	.	8.56 "

This sample contained an excess of cream, which made it appear thick.

*Example b.*—The sample gave a blue colour on adding iodine solution. It was thickened with starch (or flour).

*Example c.*—The milk was thick and, on dipping a glass rod into it and lifting it out, a stringy mass adhered to it. On putting the rod (which had been sterilised) into a bottle containing sterilised milk, the latter acquired the same property in twenty-four hours. The milk was “ropy.”

**PROBLEM VIII.**—To determine the nature of sediment.

In cases of this description, the milk should be placed in tubes and centrifuged; as much milk as possible must be decanted, distilled water added, and the tubes again centrifuged; this procedure should be repeated till the water is clear. The sediment is examined microscopically (Fig. 31).

Vegetable cells, if clear and sharply defined (Fig. 32), are usually due to the bark of hay and the dust of cake given to the cattle during feeding time. If indistinct and stained yellowish or brownish, these usually indicate cow-dung (Fig. 33).

Small hairs, cotton and woollen fibres usually show the presence of household dust.

Crystalline particles usually indicate road dust. In this case a little of the deposit is placed on a slide and warmed with dilute hydrochloric acid, which is evaporated nearly off; a drop of water is added and also a drop of a solution of potassium ferrocyanide. A blue colour, due to iron, is obtained from road dust. An estimation of the dirt may be made by allowing the milk to stand, and measuring the amount in a graduated tube (Fig. 34).

**Skim Milk.**—The term “skim milk” is applied to milk from which the bulk of the cream has been abstracted. Two ways of abstracting the cream are practised: (1) by allowing the milk to stand, taking advantage of the force of the earth’s gravity to separate the cream; (2) by employing centrifugal force to attain the same object.

**Distinction between Skimmed and Separated Milk.**—A distinction has been drawn between skim milk obtained by these two methods; that obtained by setting the milk being called “skimmed milk,” and that obtained by centrifugal force “separated milk.” The distinction is one of degree, not of kind, as, were it possible to keep milk without chemical change for an indefinite period, the same result would ultimately be obtained by either method.



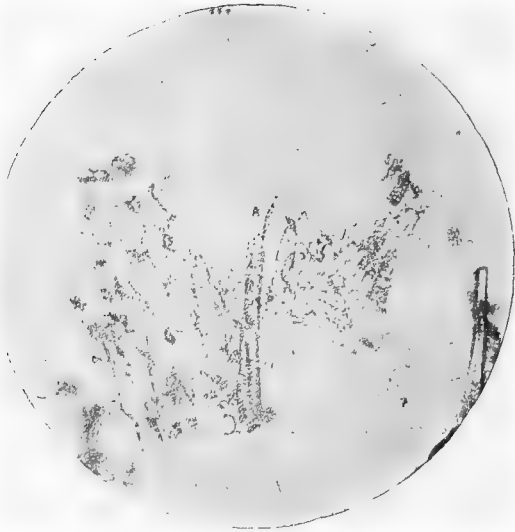


Fig. 32.—Dirt in Milk.

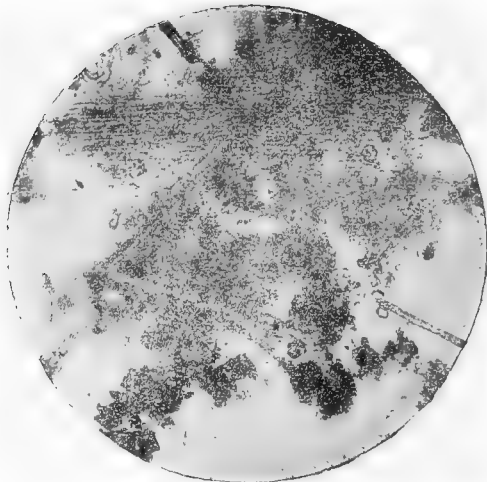


Fig. 33.—Dirt in Milk.

The following are the characteristics of skimmed and separated milks :—

## SKIMMED MILK

- Contains the solids not fat of the whole milk, partially changed by the action of micro-organisms.
- Contains usually more than 0·4 per cent. of fat.
- Contains a portion of the solid impurities of the milk.

## SEPARATED MILK

- Contains the solids not fat of the whole milk, practically unchanged.
- Contains usually less than 0·3 per cent. of fat.
- Is free from the solid impurities of the milk.

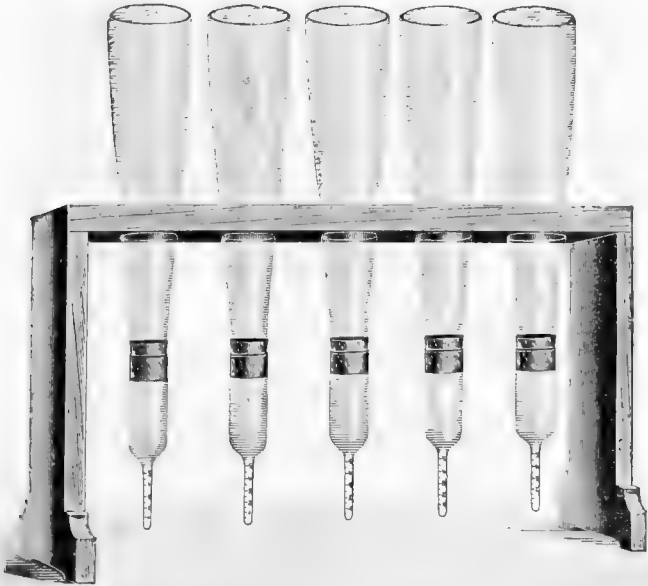


Fig. 34.—Dirt Estimation in Milk.

The term machine-skimmed milk is adopted in the Sale of Food and Drugs Act, 1899.

**Rising of Fat Globules.**—The globules of fat rise through the milk because they are lighter than the milk serum.

If we have globules of fat of radius  $r$ , the force impelling it to rise will be proportional to the weight of an equal bulk of milk

serum less the weight of the globule, or

$$\theta = k \cdot \frac{4}{3} \pi r^3 \cdot (d_s - d_f) \cdot g,$$

where  $k$  is a constant varying with the units adopted for  $r$ ,  $d_s$  and  $d_f$ .  
 $\pi$  is the ratio of the circumference of a circle to its diameter,  
 $r$  is the radius of the globule, and  
 $g$  is the acceleration due to gravitation.  
 $d_s$  and  $d_f$  are the specific gravities of the serum and fat.

The globule does not, however, rise freely; at the velocity at which the globules move the resistance is very nearly proportional to the square of the velocity.

We may then write the equation:—

Total force at any moment impelling the globule to rise is—

$$\frac{dv}{dt} = k \cdot \frac{4}{3} \pi r^3 \cdot (d_s - d_f) g - cv^2$$

by equating  $k \cdot \frac{4}{3} \pi \cdot (d_s - d_f) g$  to  $b^2$ , leaving only the variables, we may write this—

$$\frac{dv}{dt} = b^2 r^3 - cv^2.$$

Integrating this, we get—

$$v = \frac{br^{\frac{3}{2}} \cdot (e^{at} - 1)}{c(1 + e^{at})},$$

where  $a = 2br^{\frac{3}{2}}$ .

For large values of  $t$  the expression  $\frac{e^{at} - 1}{1 + e^{at}}$  will approach very nearly to 1, and the equation becomes very nearly equal to  $v = \frac{br^{\frac{3}{2}}}{c}$ , or, expanding this by substituting the value of  $b$ , we get—

$$v = \frac{\sqrt{k \cdot \frac{4}{3} \pi (d_s - d_f) g \cdot r^3}}{c}.$$

$c$  is the coefficient of viscosity of the serum. It is evident that equilibrium will be after a short time established when the resisting force is equal to the impelling force, and if the latter be constant the motion will be uniform.

The time taken by a globule to pass through a given layer of milk is, therefore, inversely proportional to the square root of the cube of the radius.

If the globule is acted on by centrifugal force, the expression  $\frac{4\pi^2 V^2 b}{3,600}$  must be substituted for  $g$ .

$V$  = velocity in revolutions per minute,

$b$  = distance of globule from the centre of revolution.

If submitted to centrifugal force, it is evident that the speed of a globule cannot be constant, as the centrifugal force tending to move it varies with the distance of the fat globule from the centre of revolution, and the equation for the motion of globules under these conditions is—

$$\frac{d^2s}{dt^2} = k \cdot \frac{4}{3} \pi r^3 (d_s - d_f) \frac{4\pi V^2 b}{3,600} - c \left( \frac{ds}{dt} \right)^2.$$

Solving this equation and integrating between the limits  $b$  and  $b_1$ , we get—

$$t = k_1 \frac{r^{\frac{1}{2}} \pi V}{30} \sqrt{\frac{2(d_s - d_l)}{c}} \log \frac{b}{b_1},$$

which expresses the time taken for a globule to pass from any point in the separator to any other point, provided the serum is at rest and the globule travels radially.

This is not the case in modern separators where the milk runs in continuously, and terms expressing the rate of flow of milk, and the shape of the separator, must be introduced. The resulting equations are so complex that it would serve no useful purpose to deduce a general equation.

Whatever the form of equation suited to any particular separator, the time taken by a globule to pass through a given space will always be proportional to the square root of the cube of the radius, and as the number of gallons per hour passed through the separator will be inversely proportional to the time, it follows that for each size of fat globule there will be a limit where its velocity against the stream of milk will be equal to the velocity of the stream itself, and all globules smaller than this will pass out with the separated milk. If we assume that the total weight of fat in globules of any size is equal to the total weight of fat in globules of any other size, it follows that the amount of fat in the separated milk is proportional to the cube root of the square of the number of gallons per hour. The coefficient of viscosity, and also the value of the factor  $(d_s - d_l)$ , vary with the temperature, and consequently the viscosity of the fat globules and the amount of fat in the separated milk.

The relative proportions of the cream and skim milk will also effect the percentage of fat in the separated milk, as not only is the rate at which milk travels towards the separated milk outlet effected, but any resistance to the exit of cream causes the fat globules to touch each other, and interferes with their free motion.

The author has, upon these considerations, worked out a formula to give the percentage of fat in the separated milk—

$$f = a \times b^{(40-t)^{38}} \times C^F \times \frac{m^{\frac{3}{2}}}{v^2},$$

where  $f$  = percentage of fat in separated milk,

$C$  = " " " cream,

$t$  = temperature in degrees Centigrade,

$m$  = number of gallons per hour,

$v$  = " " revolutions per minute.

$a$ ,  $b$ , and  $c$  are constants for each separator.

$b$  usually varies from 1.035 to 1.05.

$c$  " " from 1.00 to 1.05.

$c$  is appreciable, chiefly with separators in which the adjustment of the thickness of the cream is made at the cream outlet—e.g., in the Alpha separator, in which  $c$  has the value 1.04 to 1.05.

The following results were obtained with a separator, for which the following formula was applicable:—

$$f = 8,155 \times 1.046^{(40-t)^{38}} \times 1.0471^F \times \frac{m^{\frac{3}{2}}}{v^2}.$$

TABLE LXIII.

F	<i>t.</i>	<i>m.</i>	<i>v.</i>	<i>f.</i>	<i>f</i> calc.
Per cent.	Degrees.	Gallons.	Revolutions.	Per cent.	Per cent.
15.5	32	350	5 00	0.05	0.04
42.0	32	350	5600	0.12	0.13
51.0	32	350	5600	0.175	0.194
52.6	32	350	5600	0.210	0.207
56.3	32	350	5600	0.247	0.246
65.0	32	350	5600	0.330	0.369
60.4	38	350	5600	0.22	0.228
62.1	38	350	5600	0.25	0.247
51.0	32	240	5600	0.14	0.14
53.0	27	350	5600	0.30	0.27
42.0	32	325	5200	0.15	0.14
70.0	76	120	5600	0.07	0.07

The constant *a* depends on the following conditions :—

1. Size of drum and thickness of the layer of milk.
2. The specific gravity of the milk serum and of fat.
3. The units in which the variables are expressed.

The first condition is that which can be varied by a difference in type of separator.

The constant *b* chiefly depends on the viscosity (internal friction) of the milk serum ; also, to a slight degree, on the cubical expansion of milk serum and milk fat, and on the friction of the liquid against the drum.

The constant *c* depends on the viscosity of cream and on the friction of the cream against the sides of the outlet.

It is naturally advantageous for *a*, *b*, and *c* to be as low as possible.

To obtain *a* low, the drum should be of large capacity and the formation of currents in the milk should be prevented ; the discs placed inside the drum in separators of the Alpha type ensure the latter condition, and, therefore, decrease *a*.

To obtain *b* low, the exits, and especially the cream exit, should be as large as compatible with the proper working of the separator ; and the tubes, through which the skim milk and cream leave the drum, as short and as straight as possible.

To obtain *c* low, cooling of the cream inside the drum should be avoided, and the cream exit large. Separators in which the adjustment of the thickness of cream is performed at the cream exit have a large *c*.

It is more difficult to express by a definite formula the amount of fat in skim milk obtained by allowing milk to stand. Here we have not a definite space through which the globules of fat

must pass, as in the cream separator, where the layer of milk is always of constant thickness; the space is determined by the depth of the layer of milk set.

The formula

$$v = \frac{s}{t} = \frac{br^2}{c}$$

may be transformed into

$$t = \frac{k}{r^2}, \text{ where } k \text{ is a constant.}$$

Taking the diameter of the largest globules as 0.01 mm. and the smallest as 0.0016 mm., we calculate that the smallest globules will take about fifty times as long to pass through a given space as the largest; the author deduces from his experiments that the largest fat globules move at the rate of 15 mm. per hour. If we assume that the total weight of fat globules of any size, is equal to the total weight of fat globules of any other size, in an ordinary cream tube we may expect roughly the following figures:—

In	5	hours	about	35	per	cent.	of	total	fat	will	be	found	in	the	cream.
„	10	„	„	65	„	„	„	„	„	„	„	„	„	„	„
„	24	„	„	85	„	„	„	„	„	„	„	„	„	„	„

while from three to four days should elapse before the whole of the fat is found in the cream.

From the equation

$$v = \frac{\sqrt{k} \cdot \frac{4}{3} \pi (d_s - d_f) g \cdot r^2}{c},$$

it will be readily seen that if the density of the fat varies the time will be considerably affected. The density of solid fat at 60° F. (15.5° C.) is about 0.93; the density of liquid fat is about 0.92 at the same temperature; and, as has been shown by the author and S. O. Richmond, it is highly probable that the solidification of the fat is a process which takes time. The difference between the specific gravity of milk serum and milk fat is also accentuated at temperatures above 60° F.; it is probable that when milk is rapidly cooled, the fat globules do not so easily attain the lower temperature as the serum. It would appear, theoretically, that there is a considerable advantage in setting milk for cream immediately after milking, and that the fat globules will rise at a much more rapid rate than if the milk be cooled and kept for some time. The experiments of Babcock completely substantiate this view; he finds that delaying the setting for even a short time materially affects the percentage of fat in the skim milk.

**Composition of Skim Milk.**—Skim milk differs practically from whole milk in the percentage of fat. In milk from which the cream has been removed by skimming very wide variations are found in the percentage of fat; it varies from 0·4 per cent. to over 2 per cent. Much lower percentages are found in separated milk, and the limits, 0·05 per cent. to 0·3 per cent., are very rarely overstepped. By the removal of the fat the percentage of other solid constituents are slightly raised in amount; this is caused by the constituents which were contained in 100 parts being left in about  $96\frac{1}{2}$  parts, by the removal of  $3\frac{1}{2}$  parts of fat.

The following is the average composition of well-prepared separated milk:—

Water, . . . . .	90·48 per cent.
Fat, . . . . .	0·12 ”
Milk-sugar, . . . . .	4·88 ”
Casein, . . . . .	3·22 ”
Albumin, . . . . .	0·42 ”
Ash, . . . . .	0·78 ”

**Control of Separators**—The most important point in the control of separators is the estimation of the fat left in the separated milk. A separator leaving a proportion of fat appreciably higher than that deduced from the formula given above is working badly, and the cause should be at once investigated. It is important that the speed be properly maintained, that the milk be at the right temperature, and that the exit tubes be not clogged up; the chemist should make a practice of visiting the separators daily while they are running and of checking the speed and temperature of the milk. At least one sample of separated milk should be tested from each “run” of the separator; these samples should be taken from the skim outflow tube, at some period of the run, preferably not immediately after starting.

A further means of controlling the separators is to compare the total weight of the fat in the cream, separated milk, and the milk left in the drum after separating, with the total weight of the fat in the milk separated. This is done by weighing each product, multiplying the weight by the percentage of fat and dividing by 100. The total weight of fat in the cream and separated milk should be nearly equal to that in the milk, the difference representing loss in separating; the average loss should not amount to more than 2 per cent. of the total fat in the milk.

**Separator Slime.**—After running a separator a viscous substance is found on the inside of the drum. It is usually of a dirty white colour; but if the milk contains much solid impurity,

as happens most frequently in the winter, it may be distinctly brown.

This by no means consists, as is often considered, of dirt and cow-dung, though it naturally contains these impurities if present in the milk. Microscopical examination shows it to contain—

1. Inorganic impurities—*i.e.*, dust gathered during transport, and earthy matters due to uncleanness.

2. Vegetable matters derived from the dust of the food given to the cattle—*e.g.*, bark of hay, fine particles of cake, etc.; in many cases portions of leaves with stomata developed may be identified. Other portions of the vegetable matter have the cell walls considerably disintegrated; these have probably passed through the alimentary tract of the cow, and indicate the presence of cow-dung.

3. Substances derived from the cow; hairs are often found; much epithelium from the udder of the cow, and possibly also from the hands of the milkers; and empty sacs (gland cells), which form a very large portion of the slime. (If the cow was in ill-health, mucus, blood, and pus may be present.)

Micro-organisms are very numerous; should the cows be afflicted with tuberculosis of the udder, *Bacillus tuberculosis* may be found here.

The following composition is assigned to separator slime by the author and by Fleischmann, respectively:—

	Author. Per cent.	Fleischmann. Per cent.
Water, . . . . .	66.24	67.3
Fat, . . . . .	0.50	1.1
Casein (or analogous body), . . . . .	22	(approx.) 25.9
Milk-sugar, . . . . .	0.5	2.1
Other organic matter, . . . . .	7.75	
Ash, . . . . .	3.01	3.6

It is doubtful whether the substance returned as casein is wholly this body; it is undoubtedly a mixture of several proteins, including Storch's mucoïd protein.

The following is the composition of the ash:—

Total ash, . . . . .	3.01 per cent.
Soluble ash, . . . . .	0.166 "
Insoluble ash, . . . . .	2.844 "

consisting of

Silica, . . . . .	0.171 per cent.
Iron oxide and alumina, . . . . .	0.012 "
Lime, . . . . .	0.654 "
Magnesia, . . . . .	0.225 "
Alkalies, . . . . .	0.559 "
Phosphoric anhydride, . . . . .	1.233 "



There are 0.675 equivalent of lime and 0.325 equivalent of magnesia to 1.506 equivalents of phosphoric anhydride, showing that the insoluble ash consists chiefly of (Ca, Mg) (Na, K)  $\text{PO}_4$  like the insoluble ash of milk.

The quantity of separator slime amounts to about 0.04 part to 100 parts of milk separated, and varies within comparatively narrow limits—0.02 to 0.08—unless the milk is very dirty, when it may even reach 0.15; in a sample where the last figure was reached the slime was brown and very gritty.

It has been argued that the removal of the slime purifies the milk to such an extent that its keeping qualities are enhanced. This opinion is probably founded on observations of the number of microbes contained in the slime; but though a greater relative quantity are found than in the milk, the numbers left in the cream and separated milk are not appreciably diminished.

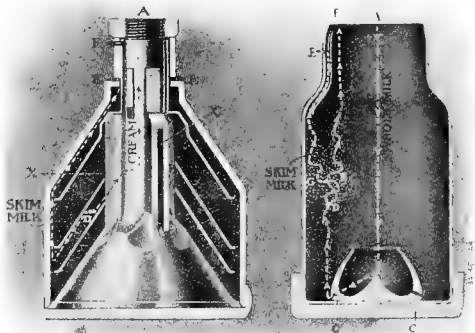


Fig. 35.—Diagrammatic Section of Separators.

Practice has, however, shown that a mixture of cream and separated milk in their original proportions keeps no better than the milk from which they were separated.

Micro-organisms are so small that their separation, unless carried with much larger solid particles, would be almost impossible under the conditions of the separation of cream; in addition, many of them have a density less than that of milk serum.

The author found

Top, .	. 197,000 colonies per c.c.	Growth on gelatine at 22° rapid, about 20 per cent. liquefied.
Middle, .	. 5,000	„
Bottom, .	. 194,000	„ Growth on gelatine slow, none liquefied.

Attempts have been made to remove the impurities in milk by filtration; straining through a fine wire sieve and through

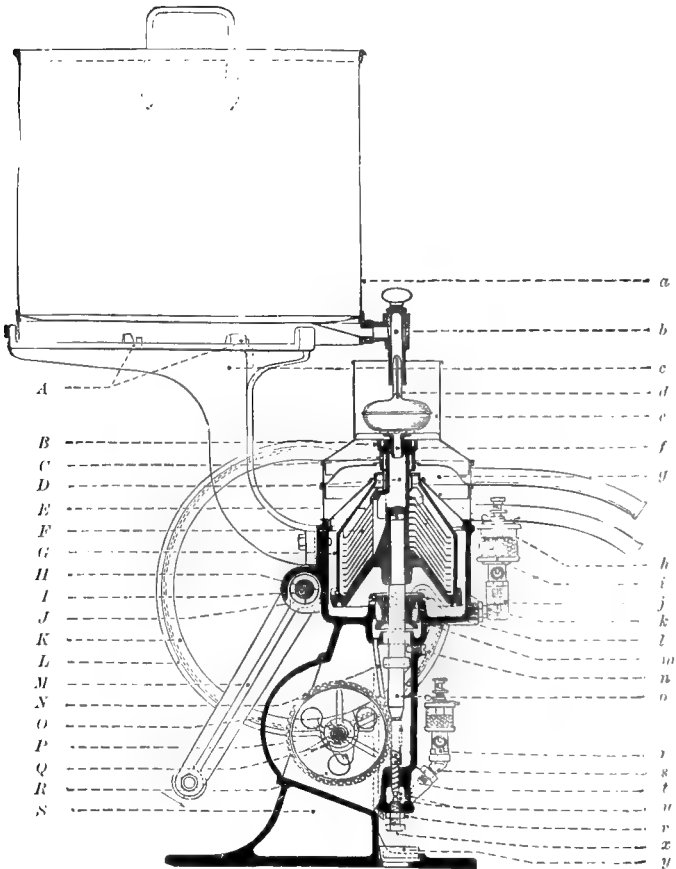


Fig. 36.—Section of Alfa-Laval Hand Separator.

- A*, Holding pins for bowl; *B*, bowl nut; *C*, cream screw; *D*, tubular shaft; *E*, top disc; *F*, alfa discs; *G*, bowl hood; *H*, bowl ring; *I*, driving wheel shaft; *J*, bushing for driving wheel shaft; *K*, driving wheel; *L*, guard ring; *M*, crank lever; *N*, oil tube for worm wheel; *O*, guard; *P*, worm wheel shaft; *Q*, bushing for worm wheel shaft; *R*, worm wheel; *S*, frame; *a*, supply can; *b*, milk faucet; *c*, bracket; *d*, float; *e*, regulating cover; *l*, regulating tube; *g*, cream cover; *h*, skim milk cover; *i*, lubricator for top-bearing; *j*, lubricator fixture; *k*, top-bearing brass; *l*, top-bearing bushing; *m*, top-bearing spring; *n*, stop screw; *o*, worm screw; *r*, lubricator for bottom screw; *s*, lubricator fixture; *t*, lower bushing; *u*, steel point; *v*, nut for bottom screw; *x*, bottom screw; *y*, waste oil catcher.

fine muslin or swandown is always practised in dairies; this removes the grosser impurities—*i.e.*, hairs, large vegetable fibres, etc.—but the quantity removed in this way does not exceed 0·0025 per cent. In Denmark and Germany, and in a few dairies in England, filtration through layers of gravel and sand is practised, though the method appears now to be dying out; this method, which adds considerably to the labour of handling the milk, owing to the necessity of washing the gravel and sand with caustic soda, followed by water, sterilising, and drying, fails to remove appreciably more from the milk than simple straining or upward filtration through muslin or swans down.

Another method which is considerably used is filtration through a thin layer of cotton wool; this method is fairly efficient, especially if practised as soon as possible after milking, and before the particles of dirt have had time to disintegrate and yield their soluble matters and micro-organisms to the milk; a special advantage of this method is that the cotton wool is very cheap, and it is impossible to wash it, and, therefore, it is thrown away and not used a second time.

The separator is also used as a cleaner for milk; for this purpose the separated milk and cream are either all made to come out of one outlet or they are mixed immediately after separation. This method is, of course, perfectly efficient in removing solid impurities, but it necessitates the milk being warmed and afterwards cooled, and makes the milk very frothy, and may even lead to incipient churning.

**Cream—Composition.**—The name **cream** is given to the layer which rises to the surface when milk is allowed to stand. This layer consists essentially of the fat globules, together with a proportion of the aqueous portion of milk (Fig. 37).

Qualitatively, it has the same composition as milk; quantitatively, it contains a higher proportion of fat, the other constituents being correspondingly depressed.

It is by many accepted as a fact that cream contains a larger proportion of solids not fat to water than the milk from which it was derived; and various explanations of this have been put forward. Thus a membrane round each fat globule has been alleged to exist by some (*e.g.*, Storch and Béchamp); others have considered that the proteins are concentrated in the aqueous layer formed round each globule by surface tension. The author's experiments have indicated that the ratio of solids not fat to water in cream is the same as that in milk, and Weibull and Smith and Leonard have confirmed this conclusion. It is true that in some cases a distinctly higher ratio has been found, but it has been noticed that in these cases ample opportunity for

evaporation of the water has been afforded, either by leaving the cream on the surface of the milk for some length of time in a dry atmosphere, or by pasteurising it, without any precautions to prevent evaporation; indeed, evidence of evaporation has been obtained by noting the quantity of cream before and after pasteurising. In cases where precautions have been taken to prevent evaporation, no evidence of a higher ratio has been obtained.

In the following analyses (Table LXIV.) the solids not fat have been calculated by dividing the percentage of water by 100 and multiplying by 10·2 (except in No. 2 where 10·0, and No. 9 where 10·4 has been used), this being the average ratio

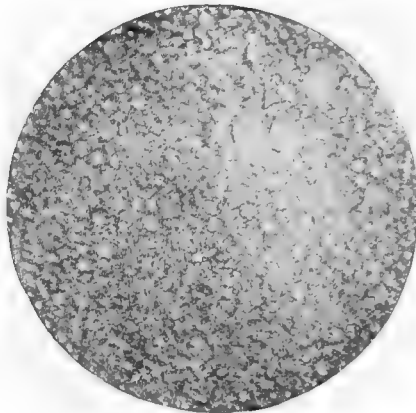


Fig. 37.—Cream.

in the milk from which these creams were prepared. The calculated ash is  $\frac{1}{12}$  of the calculated solids not fat:—

TABLE LXIV.—COMPOSITION OF CREAM.

No.	Total Solids.	Solids not Fat.	Solids not Fat, Calc.	Diff.	Ash.	Ash Calc.	Diff.
		Per cent.	Per cent.		Per cent.	Per cent.	
1.	32·50	6·83	6·90	—0·07	0·57	0·57	..
2.	37·59	6·14	6·24	—0·10	0·52	0·52	..
3.	50·92	5·02	5·01	—0·01	0·42	0·42	..
4.	55·05	4·65	4·59	—0·06	0·38	0·38	..
5.	55·18	4·77	4·57	—0·20	0·39	0·38	—0·01
6.	55·97	4·47	4·49	—0·02	0·38	0·37	—0·01
7.	56·37	4·40	4·45	—0·05	0·38	0·37	—0·01
8.	57·99	4·17	4·28	—0·09	0·41	0·36	—0·05
9.	68·18	3·30	3·31	—0·01	0·28	0·28	..

In cream No. 1 the proteins were also calculated, and found to be 2·60 per cent., while the figure calculated on the assumption that they are 37·8 per cent. of the solids not fat, as in milk, is 2·58 per cent.

The statement that cream contains a higher proportion of solids not fat to water than milk, though to some extent due to the evaporation of water which takes place, is probably also due to the methods of analysis employed. Thus it is known that when butter fat is heated in contact with air for some hours an increase of weight is noticed. As cream contains from 25 to 50 per cent. of fat, an apparent increment in the total solids of from 0·1 to 0·3 per cent. may be noticed. If the fat be estimated by a method which avoids a long heating, and the solids not fat deduced by difference, the increment will swell the amount of solids not fat. Many analyses of the fat in cream have been made by methods which do not completely extract the fat; the solids not fat are thus still further increased.

The following analyses show the composition of creams :—

	Thick Cream.	Thin Cream.
Water,	39·37 per cent.	63·94 per cent.
Fat, .	56·09 "	29·29 "
Sugar, .	2·29 "	3·47 "
Protein,	1·57 "	2·76 "
Ash,	0·38 "	0·54 "
	99·70	100·00 "

The following table will show the proportion of milk-sugar, protein, and ash to 100 parts of water contained in cream compared with those contained in milk and separated milk (see *Mean Composition* on pp. 150 and 260) :—

	Cream.	Milk.	Separated Milk.
	Per cent.	Per cent.	Per cent.
Milk-sugar, . . .	5·55	5·45	5·39
Protein, . . .	4·05	3·90	4·02
Ash, . . .	0·88	0·86	0·86

It is impossible to give an average composition of cream, as the variation of the fat is enormous; the author has obtained cream containing 9 per cent. of fat as a minimum, and 68 per cent., and even slightly more, as a maximum. As milk has been known to contain as much as 12 per cent. of fat (from Jersey cows), it follows that no sharp distinction between milk and cream can be drawn. Attempts have been made to fix a

standard for cream, but without success. Thus it has been proposed that any product containing less than 25 per cent. of fat should not be recognised as cream; the absurdity of this is shown by the fact that, while much of the cream sold in London contains between 40 and 50 per cent. of fat, being prepared by a separator, cream made by the Swartz process but rarely comes up to the standard proposed.

**Ash.**—It has been alleged that the ash of cream is practically free from chlorides; this, however, is not in the author's experience correct; the ash of cream differs in no respect from that of milk.

The following is the composition of the ash of cream according to Fleischmann:—

Potash, . . . . .	28·381	per cent.
Soda, . . . . .	8·679	..
Lime, . . . . .	23·411	..
Magnesia, . . . . .	3·340	..
Iron oxide, . . . . .	2·915	..
Phosphoric anhydride, . . . . .	21·735	..
Chlorine, . . . . .	14·895	..
	<hr/>	
	103·356	..
Less oxygen equivalent to chlorine,	3·356	..
	<hr/>	
	100·000	..

**Density.**—The percentage of fat varies inversely as the density of the cream.

A formula connecting the two can be deduced from the formula expressing the relation between specific gravity, fat, and total solids in milk.

$$T = 0.2625 \frac{G}{D} + 1.2 F.$$

Assuming that the solids not fat (S) are in the ratio to the water as 10·4 : 100,

$$\text{then} \quad \frac{100 - F}{10.615} = S$$

$$\text{and} \quad T = F + \frac{100 - F}{10.615} = \frac{100 + 9.615 F}{10.615},$$

substituting in the formula

$$\frac{100 + 9.615 F}{10.615} - 1.2 F = 0.2625 \frac{G}{D}$$

$$3.123 F = 100 - 2.786 \frac{G}{D}$$

$$F = 32.0 - 0.892 \frac{G}{D}.$$

It is impossible to test the specific gravity of a cream containing more than 30 per cent. of fat with a hydrometer direct; but if it is diluted with an equal weight of separated milk the hydrometer can be used as with a thinner cream.

To calculate the specific gravity of a thick cream from that of a mixture with an equal weight of separated milk, the following formula may be used:—

$$c = \frac{s \times m}{2s - m}.$$

$c$  = specific gravity of cream.  
 $s$  = " " of separated milk.  
 $m$  = " " of mixture.

Cream containing 25 per cent. of fat decreases in specific gravity 0.00027 or 0.27° for each 1° F. above 60° F.

Table LXV. shows the results obtained by calculating the fat from the specific gravity.

TABLE LXV.—CALCULATION OF FAT IN CREAM.

Specific Gravity.	Fat Estimated.	Fat Calculated.	
	Per cent.	Per cent.	
1.0035	29.2	28.9	
1.0057	27.3	26.9	
1.0070	26.2	25.8	
1.0085	24.8	24.5	
1.0090	24.0	24.1	
1.0110	22.4	22.3	
1.0125	21.4	21.1	
1.0130	20.8	20.6	
1.0210	13.3	13.7	

Specific Gravity of Separated Milk = $s$ .	Specific Gravity of Mixture = $m$ .	Fat Estimated.	Fat Calculated.
		Per cent.	Per cent.
1.0367	1.0053	55.5	54.7
1.0367	1.0041	57.7	56.3
1.0364	1.0081	49.8	49.0

The specific gravity of cream is affected by the state in which the fat globules exist; if they are in the solid state, the specific gravity will be very appreciably higher than if liquid. The formula given above assumes that they are solid; if, however, the cream has been separated at a temperature above the melting point of the fat, the globules are liquefied, and do not at once assume the solid state on cooling. For this reason the method

of calculating the fat from the specific gravity is liable to give at times very discordant results.

These figures show that, though the estimation of the specific gravity of a cream is scarcely exact enough to serve as a means of analysis, it is a useful corroborative figure. Considering the sources of error the agreement is very good, and serves as a further proof that cream does not contain a larger proportion of solids not fat to water than milk.

Vieth finds that cream containing 40 per cent. fat has, at a temperature of 175° F., a specific gravity of 0.960, and gives the following table :—

Cream containing	30 %.	40 %.	50 % Fat.
Gives at 185° F. a sp. gr., .	0.971	0.956	0.941
„ 175° „ „ .	0.975	0.960	0.945
„ 165° „ „ .	0.979	0.964	0.949

A sample of froth taken from the surface of cream running from a Burmeister and Wain separator had the following composition :—

Water,	. . . . .	48.41 per cent.
Fat,	. . . . .	45.44 „
Milk-sugar,	. . . . .	4.86 „
Protein,	. . . . .	1.89 „
Ash,	. . . . .	0.40 „

It does not differ in its chemical composition from cream somewhat concentrated by evaporation. The froth, for this reason, always contains more fat than the cream.

**Clotted Cream**, or cream prepared by the system practised in Devonshire and Cornwall, has been examined regularly in the Aylesbury Dairy Company's laboratory since 1886. The following are the average results, together with the maxima and minima found :—

	Water.	Fat.	Ash.	Solids not Fat.
	Per cent.	Per cent.	Per cent.	Per cent.
Average, .	34.26	58.16	0.60	7.52
Maximum, .	44.84	71.37	1.17	11.70
Minimum, .	21.08	44.29	0.42	5.03

It is seen that the ratio of solids not fat to water is very much higher in clotted cream than in milk, due to the evaporation which takes place from the surface during heating.



Roughly speaking, the ratio of solids not fat to water is double the average ratio in milk.

The ratio of ash to solids not fat is very nearly the same in clotted cream as in milk; it is, however, slightly lower. This is partly, if not entirely, due to the fact that on heating milk certain salts of calcium, probably chiefly citrate, are deposited, leaving a smaller proportion in the milk and also in the cream derived from it.

**The Thickness of Cream.**—The thickness is the factor by which cream is usually judged when used for direct consumption. This can be quantitatively estimated by the method generally employed for the determination of "viscosity"—*i.e.*, noting the time taken for a given volume of cream to flow through a tube of constant size. The viscosity of a liquid depends on the internal friction—*i.e.*, the friction of molecules passing each other; the viscosity or internal friction of cream is not quite of the same order as that of a homogeneous liquid; in the latter case, the molecules are of equal size (or nearly so), and very small in comparison with the diameter of the tube through which the liquid passes. The viscosity of cream depends on two factors—the internal friction of the very small molecules of the milk serum, and the friction between the comparatively large fat globules.

As the fat globules have an appreciable size compared to the size of the tube, we cannot expect the laws to be of the same kind as those governing the viscosity of a liquid composed of molecules of infinitely small size. The actual and relative size of the globules will also have considerable influence; thus if we have two creams identical in chemical composition, in one of which the relative size of fat globules is much larger than in the other, the "viscosities" will differ.

It is not possible to compare the thickness of cream by making a determination of the percentage of fat in a sample. It is possible, however, to make a comparison of cream which contain globules of relatively the same size. For instance, if cream be diluted with the separated milk, which is practically free from fat, the thickness can be deduced by making determinations of fat.

The law connecting thickness or viscosity and amount of fat is expressed by the following empirical formula—

$$V = 10^{xF_1^3},$$

where  $V$  = the viscosity,

$F_1$  = the volume of fat in 100 volumes of cream,

and  $x$  = a factor dependent on the units in which the viscosity is expressed, and on the relative size of fat globules.

The volume of fat in 100 volumes of cream may be calculated from the percentage of fat (by weight) by the following formula—

$$F_v = \frac{1.07527 F_w \times 100}{0.11 F_w + 96.5}$$

This is true at a temperature of 60° F. (15.5° C.), and may be used without appreciable error at other temperatures.

The following two series will illustrate the exactitude with which the formula agrees:—

TABLE LXVI.

## SERIES I.

Per cent. Fat by Weight.	Per cent. Fat by Volume.	Viscosity.	Calc. Viscosity.	Calc. Per cent. Fat by Volume.	Calc. Per cent. Fat by Weight.
62.9	65.4	151.6	157.6	65.3	62.8
60.3	62.9	88.0	90.3	62.8	60.2
57.7	60.3	55.2	52.7	60.6	58.0
52.6	55.3	21.2	21.4	55.3	52.6
SERIES II.					
61.4	63.95	170	165.8	64.05	61.5
53.7	56.4	31.5	33.3	56.1	53.4
46.0	48.7	9.8	9.6	48.9	45.2

The agreement is within the limits of experimental error.

Instead of the formula given above, which includes a calculation of the percentage by volume of fat, the following approximate formula may be used—

$$V = 10x.F^{2.7}$$

where F is the percentage of fat by weight. For small differences the results by the two formulæ agree sufficiently well.

A practical method for the dilution of cream to constant thickness may be founded upon the above formula. To take the viscosity of a cream, a 10 c.c. pipette with a fairly wide opening, marked with distinct lines both above and below the bulb, may be employed; it should be surrounded by a water-jacket made of glass to ensure a constant temperature; and the end should not project far beyond the jacket. Care must be taken that its position is always vertical during the test; this may usually be ensured by clamping the jacket firmly in position and fixing the pipette by rubber corks. The position should be tested by a plumb line, made of cotton, passing through the pipette.

The "viscosity" of the cream is represented by the number of

seconds that the cream in the pipette takes to flow from the mark above the bulb to that below, which can be determined with sufficient accuracy by any watch with a seconds hand, though it is preferable to use a stop watch. Care must be taken that the cream is free from lumps, or solid particles, and it may advantageously be filtered through muslin. The pipette should be clean and dry, and the cream should be allowed to remain in the pipette a few minutes before making the test, in order to ensure that its temperature is that of the jacket.

It will be found in practice that it is better to use water of the mean daily temperature in the jacket than water at any constant temperature, because a purchaser is not in the habit of reducing cream to a temperature of (say) 60° F. before passing judgment on its thickness.

At the same time that the viscosity is estimated a determination of the fat should be made by one of the methods recommended (pp. 138, 227, and 237).

The relation between viscosity and fat can be calculated by the formula given above, which may be expressed, for practical use, as

$$\log (\log V) = 3 \log \left( \frac{107.527 F}{0.11 F + 96.5} \right) + \log x,$$

$$\text{Or,} \quad \log (\log V) = 2.7 \log F + \log x.$$

A standard viscosity must be fixed, which evidently must be determined by each operator to suit his apparatus, and the conditions under which it is necessary to work; the value of  $\log x$  or  $\log x$ , having been found from the determination of viscosity and fat, the percentage of fat which will correspond to the standard viscosity can readily be calculated.

If  $a$  be the percentage of fat found in the cream, and  $b$  the percentage of fat which will be contained in the cream of standard viscosity, the cream may be reduced to standard viscosity by adding to each 100 parts  $100 \left( \frac{a-b}{b} \right)$  parts of separated milk, or  $100 \left( \frac{a-b}{b-f} \right)$  parts of milk containing  $f$  per cent. of fat. The figure 3.5 may generally be used for  $f$  without appreciable error.

As an example the following result may be taken :—

$$V = 170$$

$$\log v = 2.23045$$

$$\log (\log v) = \log 2.23045 = 0.34839$$

$$F = 61.4$$

$$\frac{107.527 F}{0.11 F + 96.5} = 63.95$$

$$\log 63.95 = 1.80584, \text{ and } 3 \log 63.95 = 5.41752$$

then

$$5.41751 = 0.34839 + 5.06913.$$

Now let the standard viscosity be 25.

$$\log 25 = 1.39794, \text{ and } \log 1.39794 = 0.14550$$

therefore,  $5.21463 = 0.14550 + 5.06913$

now  $5.21463 = 3 \times 1.73821 = 3 \log 54.7,$

and the percentage of fat in cream reduced to standard viscosity is 52.0 per cent. by weight, or 54.7 per cent. by volume.

Now  $a = 61.4,$  and  $b = 52.0$

then  $100 \left( \frac{a-b}{b-3.5} \right) = 19.4;$

that is, to each 100 parts of cream 19.4 parts of milk must be added to reduce it to standard thickness.

By the formula  $V = 10^{2.7F^{2.7}}$  the percentage of fat calculated in the cream of standard viscosity is 51.7, which is a figure sufficiently close for practical purposes.

**Artificial Thickening of Cream.**—Cream has been artificially thickened by the addition of various foreign substances; thus, gelatine, isinglass, and substances of like nature have been employed, but without great success, as the cream thus treated has an appearance markedly different from that of genuine cream. The following method, due to Stokes, may be applied to detect gelatine in cream:—To 10 grammes (approximately) of cream add 25 c.c. of water and 2 c.c. of Wiley's acid mercuric nitrate solution (p. 90), and shake well; filter through a dry filter. In the presence of much gelatine the filtrate cannot be obtained clear, and it is not essential that it should be so. On adding a saturated aqueous solution of picric acid a yellow precipitate is formed in the presence of gelatine; if the quantity of gelatine be but small, the precipitate does not form at once, but the solution becomes turbid, and precipitates after a lapse of some minutes. Starch, which has been gelatinised by heating, has also been used; this, of course, is readily detected by the characteristic blue coloration given with tincture of iodine. Of comparatively recent introduction is "viscogen," which is a solution of lime in cane-sugar syrup; the addition of a small amount of this substance has a remarkable effect in increasing the thickness of cream. It is sold under various fancy names.

Its presence may be detected by testing the cream by one of the methods (p. 105) for the detection of cane sugar; the amount of ash will be raised, and the ratio of lime to phosphoric acid in the ash will be higher than 17 : 23. It is usually added in quantities of about 0.5 per cent., and this amount increases the solids not fat by about 0.2 per cent. of cane sugar, the ash by about 0.04 per cent., and raises the ratio of lime to phosphoric acid to about 1 : 1.

As homogenised cream will not whip, it is not uncommon to add gelatine, agar, or gum tragacanth for the purpose of making

a fairly permanent foam when the cream is whipped. Of these, gum tragacanth added in the proportion of 0.1 per cent. is the most effective. A careful microscopic examination of the cream after the addition of a little iodine solution will reveal the presence of particles of gum tragacanth, in which starch granules can be detected. Agar, which has also been used as a thickening agent, gives Cayaux's resorcinol test for cane sugar, but not any of the other tests.

**Homogenised Milk.**—In the equations given on p. 256 the fat globules have been considered as being free from any condensed layer; this is not the case, as the surface energy of small globules condenses round them a layer of serum, which may, for physical considerations, be included in the globule; this will decrease the value of  $ds - df$ , retard, and in extreme cases stop, the rising of very small globules.

In the case of the globules of cows' milk the influence of the layer is sufficiently small, though not absolutely negligible, to be left out of consideration. When, however, the globules of fat are reduced to a diameter below that of the smallest naturally occurring globules it becomes of importance, and the rate of rising of cream is much less than that indicated by the formulæ.

By forcing milk, heated to such a temperature\* that surface energy is reduced to a minimum, while chemical change in the milk is prevented, under a high pressure through very small openings, the fat globules are reduced to a very small size. The condensed layer bears such a relation to the globule that the cream rises with extreme slowness, and practically speaking remains mixed with the milk. This process is termed homogenising. Owing to the fact that the condensed layer is held so firm by the great surface energy of small particles, it is impossible to churn milk or cream that has been homogenised; as the effective diameter of the globules is increased by the condensed layer, homogenised milk and, especially, cream are thicker for the same percentage of fat than fresh milk or cream. Surface energy varies considerably with temperature, and consequently the thickness of the layer; for this reason the thickness of homogenised cream varies more with temperature than the thickness of ordinary cream. Homogenised cream can neither be churned nor whipped.

\* The temperature should not exceed 60° C., as the mechanical work done in forcing the milk through small openings is partly converted into heat, which raises the temperature of the milk some degrees.

## CHAPTER V.

## BIOLOGICAL AND SANITARY MATTERS.

CONTENTS.—The Decomposition of Milk—Micro-organisms—Action on Milk—Pathogenic Organisms—Conveyance of Disease—Water Supply—Inspection of Source—Analysis of Water—Bacteriological Examination—Summary of Sanitary Precautions—Products formed from Milk by Micro-organisms.

**The Decomposition of Milk—Micro-organisms.**—The decomposition of milk is due to the action of micro-organisms. The description of their life-history, and the means of separating and identifying them belong to the science of Bacteriology. The following slight sketch will, however, be found of use to the dairy chemist :—

**Classification.**—Micro-organisms belong to the vegetable kingdom, and are classed among the fungi; they are divided into

*Schizomycetes* or fission-fungi.  
*Saccharomycetes* or yeasts.  
*Hyphomycetes* or moulds.

The *Schizomycetes* are again divided into families according to their shape and mode of growth :—

*Bacteria* ; short rod-like forms forming no spores.  
*Bacilli* ; rod-like forms forming spores.  
*Spirilla* ; curved rod-like forms.  
*Micrococci* ; round forms occurring singly.  
*Diplococci* ; " " " double.  
*Staphylococci* ; " " in bunches.  
*Streptococci* ; " " growing in chains.  
*Sarcinae* ; " " " in groups.  
*Leuconostoc* ; thread-like forms.  
*Clostrix* ; branching forms.

The distinction between these forms is by no means absolutely defined; thus many species forming spores with difficulty or only under certain conditions, which were formerly classed as *Bacteria*, are now called *Bacilli*. Some organisms grow as micrococci, streptococci, spirilla, and leuconostoc.

**Action on Milk.**

For the purpose of the dairy chemist micro-organisms may be classed according to their action on milk, as follows :—

- Those acting on milk-sugar (a) producing lactic acid ;  
 (b) " butyric acid ;  
 (c) " alcohol.
- Those acting on proteins (a) curdling milk without acidity and not dissolving the curd ;  
 (b) curdling milk without acidity and afterwards dissolving the curd ;  
 (c) peptonising the proteins without curdling the milk ;  
 (d) producing evil-smelling sulphur compounds.

Those producing coloured substances.

Those having no action on milk.

We may also place in another class those which are pathogenic.

Milk is a model food for micro-organisms, for it contains in an assimilable form all those compounds which are necessary for the sustenance of life.

It has been shown by experiment that it is possible, though not easy, to obtain milk which is quite free from micro-organisms. It is necessary, however, to reject the first portions drawn, as these contain micro-organisms which have found their way down the duct of the teat. The last portions are practically sterile, and it is highly probable the few organisms found are due to accidental contamination of the milk during its passage from the teat into the sterilised bottle into which it was drawn. Practically speaking, all the organisms found in milk fall in after milking; in certain diseases—*e.g.*, tuberculosis of the udder—the *Bacillus* of tuberculosis is not derived from external sources, but passes from the diseased tissue into the milk.

Generally speaking, micro-organisms only develop between the temperatures of 4° C. (39° F.) and 50° C. (122° F.); each organism has an *optimum* temperature—*i.e.*, one at which its development and action are most rapid; this varies from 12° C. (53·6° F.) to 40° C. (104° F.) in different species; the optimum temperature of pathogenic organisms and of most of those acting on milk is about blood-heat. Among other conditions which regulate their development are (1) the amount of acid present in the milk—thus most of the organisms which produce lactic acid are paralysed in their functions when more than about 1 per cent. has been produced; and (2) the presence or absence of oxygen. Some organisms can do without oxygen, and are called *anærobic*; others require it for their life processes, and are designated *ærobic*.

**Growth of Bacteria in Pasteurised and Unpasteurised Milk.**—L. A. Rogers has made experiments on the average acidity of raw and pasteurised milk, and the number of bacteria present at different times.

The following are his results :—

TABLE LXVII.

Average Increase in Acidity of Milk expressed as per cent. of Lactic Acid.				
Percentage of Lactic Acid after lapse of—	Treatment.			
	Raw Milk kept at 20° C.	Pasteurised Milk kept at 20° C.	Raw Milk kept at 10° C.	Pasteurised Milk kept at 10° C.
	Per cent.	Per cent.	Per cent.	Per cent.
0 hours,	0·131	0·134	0·136	0·134
6 "	0·168	0·134	0·152	0·129
12 "	0·254	0·127	0·167	0·131
24 "	0·449	0·130	0·177	0·129
48 "	0·696	0·251	0·233	0·129
72 "	0·711	0·271	0·388	0·130
96 "	..	0·359	0·497	0·132

Average Number of Bacteria per Cubic Centimetre in all Samples under each Treatment.				
Number of Bacteria after the lapse of -	Description and Treatment of Sample.			
	Raw Milk kept at 20° C.	Pasteurised Milk kept at 20° C.	Raw Milk kept at 10° C.	Pasteurised Milk kept at 10° C.
0 hours,	13,522,331	245	17,640,428	245
6 "	74,142,857	426	31,457,833	308
12 "	247,651,250	6,028	38,406,785	378
24 "	457,910,714	1,501,335	124,783,928	1,026
48 "	608,079,166	320,337,388	254,678,542	15,119
72 "	568,718,500	236,941,250	308,041,666	2,462,492
96 "	..	975,500,000	562,650,000	37,088,456

Average Number of Peptonising Bacteria per Cubic Centimetre of Milk.				
Number of Bacteria found after a lapse of—	Description and Treatment of Sample.			
	Raw Milk at 20° C.	Pasteurised Milk at 20° C.	Raw Milk at 10° C.	Pasteurised Milk at 10° C.
0 hours,	621,571	7	82,208	7
6 "	4,905,333	11	505,333	11
12 "	1,814,583	188	1,518,666	9
24 "	2,927,857	259,831	5,272,500	15
48 "	700,000	2,411,163	11,708,750	3,143
72 "	1,375,000	31,225,000	12,781,250	856,219
96 "	..	..	32,918,750	4,251,219



From these results he draws conclusions as under :—

*Milk held at 20° C. (68° F.).*—In the unheated milk the lactic bacteria increased rapidly, and the milk became acid in about twelve hours. The peptonising bacteria increased in six hours to about 5,000,000 per cubic centimetre, and then decreased slowly.

In the heated milk the peptonising bacteria increased rapidly after twelve hours, and the milk was usually curdled in forty-eight hours, with a disagreeable taste and odour. Occasionally lactic bacteria survived pasteurisation and multiplied rapidly after twenty-four hours, completely inhibiting the peptonising bacteria.

*Milk held at 10° C. (50° F.).*—In unheated milk the growth of the bacteria and the consequent curdling of the milk was much retarded. The average milk did not contain sufficient acid to affect the taste until it was over forty-eight hours old. The proportion of peptonising to lactic bacteria was greater than at the higher temperature, and the taste of the milk occasionally showed the influence of the former.

In the pasteurised milk the bacteria increased very slowly, and in nearly every case the milk was unchanged in taste and appearance ninety-six hours after pasteurisation. In only two of fourteen cases was there a marked increase of peptonising bacteria. The predominating bacteria were species having little or no effect on milk.

The lactic bacteria inhibited the development of the peptonising bacteria only when they had developed sufficient acid to render the milk unfit for use.

It seems probable that the acid had a distinct inhibitory action on the proteolytic enzymes of the peptonising bacteria.

**Distribution of Micro-organisms on Separating.**—When milk is separated by centrifugal force, both the cream and the separator slime contain a larger proportion of organisms than the original milk; this is partly due to the organisms being carried down mechanically, but partly also to an actual separation taking place, as the following experiment will show :—

A sample of separated milk was run for fifteen hours in a centrifugal machine at the rate of 1,000 revolutions per minute. Cultivations on gelatine were made from the top portion, the middle, and the bottom. The results were, after eight days at 22° :—

	Colonies per 10 <sup>000</sup> c.c.	Remarks.
Top, .	197	Growth rapid, about 20 per cent. liquefied.
Middle, .	5	
Bottom, .	194	Growth slow, none liquefied.

This appears to show that, while some micro-organisms have

a density greater than 1.036, others have a less density. The top cultivation was made from the portion immediately underneath the thin layer of cream, so that it is not probable that they were carried up by the cream.

**Lactic Fermentation.**—The most commonly observed effect of the action of micro-organisms is the souring of milk. This is due to a numerous class of organisms, chiefly bacteria and bacilli, which convert the milk-sugar into lactic acid. The chemical equation for this change usually given is



The change, however, never proceeds in this delightfully simple manner, certain quantities of carbon dioxide and often alcohol being always produced; by keeping up a free supply of oxygen a very large proportion of carbon dioxide can be obtained. Some lactic ferments give an amount of lactic acid agreeing approximately with the above equation; others produce notable amounts of alcohol and other products. Other organisms, again, produce very small quantities of lactic acid and large amounts of other substances. Hueppe has studied this class of organisms minutely and has described many species; few of these form spores and are destroyed with comparative ease by heat; generally speaking, their optimum temperature is about 35° C. (97° F.).

A number of organisms, grouped under the designation *Bacillus of Massol*, have been recently studied, which produce very large quantities of lactic acid (up to 3 per cent.). They do not ferment cane sugar. These all form chains of long rods, which stain often unequally by Gram.

**Butyric Fermentation.**—When this takes place the milk coagulates without the development of acidity, but the milk becomes alkaline; a bitter taste is acquired, the precipitated casein is redissolved, and butyric acid is formed; an unpleasant smell is usually developed. This fermentation, which does not readily occur if lactic acid is developed, appears to be also caused by many micro-organisms, which attack both milk-sugar and casein. When this fermentation takes place, the solid portion of the milk is reduced to a very much greater extent than by the lactic fermentation.

There is another butyric fermentation which takes place with development of a very high acidity, and in which the lactic acid produced by the lactic ferments is converted into butyric acid. Large quantities of gases—carbon dioxide and hydrogen—are produced, and other volatile acids, acetic and, more rarely, propionic, are produced at the same time. This fermentation does not develop till the milk has stood for some time, and appears

to be anærobie. Some organisms produce acetic or propionic acids as well as butyric.

**Alcoholic Fermentation.**—This does not readily occur in milk. As already mentioned, small quantities of alcohol are produced as bye-products by some organisms; ordinary yeasts, *Saccharomyces cerevisiæ*, etc., do not cause fermentation of milk-sugar, but one species of *Saccharomyces* is known which converts the bulk of the milk-sugar into alcohol; this is found in kephir grains, together with organisms producing lactic acid and others acting on the proteins.

**Curdling Organisms.**—These organisms act on the casein by the secretion of an enzyme, which resembles rennet in its action; these are usually bacilli, which readily form spores and are difficult to kill by heating.

**Organisms which Curdle without Acidity and Redissolve the Curd.**—This class is a very large one; the organisms act by the secretion of enzymes having proteolytic functions analogous to pepsin and trypsin. Many of the organisms producing butyric acid belong to this class; among the most noticeable of which are the hay- and potato-bacilli.

**Organisms which Peptonise the Milk without Curdling.**—This class is probably more numerous than has been described; they also act by the secretion of a proteolytic enzyme. It is rare to find milk which shows their characteristic behaviour, as there are generally other organisms present which curdle the milk. When cultivated in sterile milk, no action is at first apparent, but the milk gradually becomes more and more transparent till it assumes an appearance like a liquid jelly. The author has separated an organism of this class from “mazoum,” an Armenian preparation.

**Organisms acting on the Proteins with Production of Evil-smelling Sulphur Compounds.**—There is a class of strict anærobes which peptonise the milk with some curdling, and produce volatile sulphur compounds, including sulphuretted hydrogen. These form spores, which are very difficult to destroy, and are the most frequent cause of trouble in so-called sterilised milk, in which the sterilisation has not been efficient.

Golding and Feilmann have described a class of organisms that, in the presence of small quantities of copper, dissolved from an imperfectly tinned copper milk cooler, produced an objectionable flavour in milk.

**Chromogenic Organisms—Milk “out of Condition.”**—Several organisms have the property of producing coloured substances; these, and one or two other classes, are the chief causes of milk being “out of condition.”

*Blue Milk.*—Sometimes the formation of dark blue patches

on the surface of milk, having the appearance of a drop of blue-black ink which has fallen in, is noticed. This is due to an organism called *B. syncyanus* or *B. cyanogenus*; when cultivated alone a grey colour is produced, which turns an intense blue on the addition of acids; the blue colour is only noticed if the milk be sour with lactic acid.

*Red Milk*.—This is occasionally due to the action of micro-organisms; it is usual to ascribe the formation of red milk to *Micrococcus prodigiosus*, which forms an intense blood-red substance, but it is doubtful whether this organism is always the cause. The colour is often of a pinkish tinge, and is due to the pink yeast, *Micrococcus rosaceus*, *Bacillus lactis erythrogenes*, or *Sarcina rosea*. The organism causing a red colour varies according to the district, and only one organism is usually found in any district.

A red colour in milk may be due to the presence of madder in the food eaten by the cattle, but far more frequently arises from the presence of blood; this is produced by a diseased state of the udder, but far more frequently by some slight local damage, through a kick or a blow resulting in the breaking of a small blood-vessel in the udder.

*Yellow Milk*.—An organism which curdles milk and redissolves the curd to form a yellow liquid has been described as *Bacillus synxanthus*; there are probably several organisms which produce a yellow colour; all seem to have proteolytic functions. Yellow milk is very rare, though it is very common to see dirty vessels which have contained milk become quite yellow.

*Green milk*, *violet milk*, and *bitter milk* have been found to be produced by micro-organisms. Bitter principles may be derived from the food of the cattle, and some, though not all, of the butyric ferments give rise to a bitter taste. Peptones produced from casein may also be the cause of bitterness.

*Ropy Milk*.—Milk occasionally, instead of remaining liquid, becomes a thick slimy mass; if a glass rod is dipped into milk which has become ropy and withdrawn, a portion of the milk adheres and can be drawn out in long threads. Sometimes this action is confined to the cream on the surface, but with other organisms the whole milk becomes ropy.

The organisms which produce ropy milk do not grow well at a low temperature, and it frequently happens that milk at a dairy goes ropy in the summer, is free from this trouble in the winter, and becomes ropy again in the spring.

When milk is found to become coloured, or to be ropy, the dairy, and all vessels used for milk, should be submitted to a thorough disinfection, which will remove the cause. The *Bacillus of Massol* tends to produce a sour, ropy milk.

**Soapy Milk.**—After a few hours milk has been known to acquire a fishy odour, alkaline reaction, and soapy taste. Herz regards this as due to a disease of the cow, and has found that such samples have a high specific gravity; Weigmann has identified an organism, which he also found in the straw used as a litter, which gave a soapy taste to milk.

**Moulds.**—White mould (*Oidium lactis*) is very commonly found on sour milk; it forms a tough white skin on the surface, which is entirely formed by the hyphæ and mycelium of the mould. A brown mould, which penetrates down into the milk, is sometimes observed. Green moulds, *Penicillium glaucum*, and other species, also grow on milk, and are the colouring agents of some cheeses—*e.g.*, Roquefort and Gorgonzola. Camembert cheese is ripened by moulds.

**Pathogenic Organisms—Conveyance of Disease through Milk.**—If, as already mentioned, a cow is suffering from tuberculosis of the udder, the bacillus passes into the milk. It has been proved that the organism retains its toxic properties, and to this cause the bulk of cases of infantile tubercular intestinal disease can be traced. Tuberculosis is by no means an uncommon disease in cows. Evidence was given before the Royal Commission on Tuberculosis that in Copenhagen and Berlin, where all animals before being slaughtered are systematically examined by veterinary experts, the percentage of oxen and cows affected with tuberculosis was 17·7 and 15·1 per cent. respectively of the total number examined. In many herds the number exceeds this; on one farm as many as 80 per cent. of the cattle were affected.

In a large proportion of the cattle the disease did not affect the milk-producing organs, and in these the milk did not contain the tubercle bacillus; in a very noticeable proportion the milk was, however, affected. As there is no certainty that the disease may not spread to the udder, even though the bacillus be not detected in the milk, the presence of tuberculosis in a cow should always be taken as a sign of danger.

On the Continent and in America this subject has received much more attention than in this country, but now that the report of the Royal Commission on Tuberculosis is completed, it may be expected that legislation will follow, which will minimise this cause of infection.

An obvious means of preventing infection by tuberculosis is to remove the diseased cattle, and only use healthy cows as the source of milk supply. As the tubercle bacillus is comparatively easily destroyed by heat, pasteurisation of milk may be resorted to to destroy the organisms; keeping the milk for a quarter of an hour at 70° C. (162° F.) will practically remove the source of

infection. Another, but less satisfactory means, is to mix the milk with that of healthy cows and trust to Providence for the presence of sufficient lactic acid organisms to destroy the tubercle bacilli; even if they are not destroyed, they are sometimes so diluted that they have no toxic effect on healthy adults, though children and persons weakened by disease or predisposed by heredity to consumption may be affected.

Other diseases—pleuro-pneumonia, foot and mouth disease, and scarlatina (or an analogous skin disease)—may be derived from the cattle. These are much less common than tuberculosis and less insidious, as the symptoms can be detected with comparative ease in the cows. Practically speaking, tuberculosis is the only disease which needs to be guarded against by systematic veterinary inspection.

**Conveyance of Disease through Contamination of the Milk.**—The labours of the late Ernest Hart in collecting statistics have conclusively shown that typhoid, cholera, scarlet fever, and diphtheria can be conveyed through milk.

There are practically two causes: (1) the occurrence of the disease in the milkers and those handling the milk, and their families; and (2) the presence of the organisms to which the malady is due in water used for "cleansing" the utensils or for adulterating the milk.

The epidemics of scarlet fever and diphtheria which have been spread through milk have almost all been due to the milk being handled, shortly after milking, by those either affected with the disease, or living in the same house with sufferers. The remedy is, of course, obvious; a rule should be made in every dairy that all *employés* who feel unwell should absent themselves from their work, and pay an immediate visit to a medical man; if any members of their families be ill, medical advice should be similarly obtained; and if the disease be infectious, the *employé* must be at once suspended from duty, and not allowed to go near the dairy.

It is found in practice that this regulation can be carried out

- (1) By the employer providing for the services of a medical man.
- (2) By the payment of full wages to any *employé* who is suffering from infectious disease, and suspended from duty.
- (3) By a distinct understanding that the breaking of the regulation by an *employé* means instant dismissal without notice.

**Water-borne Diseases.**—Typhoid and cholera, which are essentially water-borne diseases, have, in the majority of cases investigated, been due to the use of contaminated water for the cleansing (*sic*) of dairy utensils; the small amount of water left on the sides of the vessel is sufficient, if the water contains virulent germs, to infect the milk; even more so does this occur if the

practice of washing out the dairy vessels with a little water after milking, and adding this to the milk, prevails. The precautions against this form of infection are also obvious, though more difficult to carry out in practice than those mentioned above.

### Water Supply.

A water supply which is not contaminated, nor liable to contamination, and a good system of sanitation are necessary. Before milk is supplied from a farm or dairy the water supply must be rigidly investigated. The investigation may be conveniently divided into three parts.

- (1) Inspection of source.
- (2) Chemical analysis.
- (3) Bacteriological examination.

### Inspection of Source.

The following sources of supply are almost always satisfactory :—

- (1) Deep artesian borings.
- (2) Deep wells passing through an impervious stratum—*e.g.*, clay.
- (3) Springs fed by an uninhabited watershed—*e.g.*, springs in the sides of hills.

Public water supplies, mountain rills, and wells sunk in open ground remote from habitations are very frequently—but by no means always—of a satisfactory nature.

On the other hand, shallow wells near dwellings, ponds, small brooks, and wells in pervious strata—*e.g.*, gravels—are usually unsatisfactory.

The following points must be considered to be highly unsatisfactory :—The proximity of privies, cowsheds, etc. ; the trend of the lands from habitations to the source ; faulty conditions of the sides of a well (otherwise satisfactory), which may allow surface drainage to enter ; and, except in the case of springs on the sides of uninhabited hills, a marked diminution of the supply after drought, and increase after rain.

It is advisable to ascertain the geological formation, and whether artificial fertilisers are much used in the vicinity ; if this is done to a large extent, some of the chemical evidence may be discounted.

### Chemical Analysis.

**Taking of Samples.**—At least half a gallon of water must be taken for the analysis ; a “ Winchester quart ” bottle is convenient for this purpose. The first portions—say, 10 to 20

gallons—should invariably be rejected, and the bottle should be rinsed with the water, filled nearly full, care being taken to avoid undue aëration, and despatched to the laboratory as quickly as possible.

The following data should be obtained :—

**Colour.**—This should be observed in a layer at least 12 inches in length; a yellowish-green tint is always suspicious, and points to sewage contamination; a brownish or brownish-yellow indicates vegetable products, not necessarily harmful, but usually undesirable. A nearly colourless water, with a faint blue or bluish-green tinge, is shown by most good waters.

**Smell.**—A small wide-necked bottle is half filled with the water, which is warmed to about 60° C. (140° F.); the water is shaken, the stopper removed, and the smell noted. Foul smells show badly polluted waters; a peculiar sweetish unpleasant odour is often given by waters containing sewage. Few waters are absolutely devoid of smell when tested thus; for instance, waters from the Oxford clay sometimes smell of petroleum, and a smell of pines is not uncommon in wooded districts.

**Analytical Figures—Total Solids.**—250 c.c. (or 100 c.c.) are evaporated in a weighed basin on the water-bath, and dried to constant weight at 150° C.

**Loss on Ignition.**—The residue is ignited over a very small flame; the smell of the vapours given off should be noted, as polluted waters often give an unpleasant smell. Much blackening indicates a large amount of organic matter; if nitrates are abundant, red nitrous fumes may be observed.

**Chlorine.**—100 c.c. of the water are placed in a white porcelain basin, 1 c.c. of a 1 per cent. solution of pure potassium chromate added, and silver nitrate (4·7887 grammes  $\text{AgNO}_3$  per litre) run in till a faint reddish colour is produced. The quantity of silver nitrate required to give a similar tint with 100 c.c. of distilled water is subtracted, and the difference represents milligrammes of chlorine, or parts of chlorine per 100,000 of water.

**Free and Albuminoid Ammonia.**—250 c.c. of water are placed in a stoppered Würtz flask, to the delivery tube of which a condenser is connected; the condenser must be a good one, and drawn out at the end, so that the diameter of the opening does not exceed 1 millimetre. If the water be distinctly alkaline to methyl orange, nothing need be added; but if not, a little freshly ignited sodium carbonate must be dropped in. A flame is placed under the flask, and about 125 c.c. of the water distilled and collected in a stoppered bottle. So soon as the flame is placed under the flask, about 250 c.c. of distilled water are placed in a flask and brought to the boil (or nearly so); the flask is removed to the bench, 10 grammes of caustic soda added, and,



when dissolution of this is complete, about half a gramme of potassium permanganate dropped in. This solution of alkaline permanganate is boiled, while the distillation is proceeding, at such a rate, that its bulk, when 125 c.c. have distilled from the flask, should be just about sufficient to make up the original volume.

The alkaline permanganate solution is added to the Würtz flask; and a further 125 c.c. are distilled off, and collected in a second stoppered bottle.

The first bottle contains the free (or saline) ammonia, and the second the albuminoid (or organic) ammonia.

The contents of the bottles are well mixed, and 50 c.c. of each are placed in a Nessler cylinder, 2 c.c. of Nessler solution added, and the tint in each of them matched by placing a known volume of standard ammonium chloride solution in a Nessler cylinder, making up to 50 c.c. with distilled water free from ammonia, adding 2 c.c. of Nessler solution. The waters must be allowed to stand for five or ten minutes before the final comparison is made, as the colour does not develop instantaneously.

After a little practice, it will be found easy to make an approximate match of tints at the first trial. It is not necessary to do this exactly. If the cylinder which contains the distillate is of approximately the same depth of shade as the standard, a little may be poured from the darker cylinder till the colours are matched; the positions of the cylinders should be several times reversed before finally deciding that they are equal, as a shadow may be cast on one cylinder more than the other and make it appear darker than it really is.

If the cylinder containing the distillate is the darker, and some of the solution has been poured from it, the calculation is performed as follows:—Let  $x$  = the weight of ammonia equal to the amount of standard ammonia solution taken,  $y$  = the amount of solution poured out, and  $z$  = the total amount in the cylinder; then the weight of ammonia in 50 c.c. of the distillate =  $x \times \frac{z}{z-y}$ , and the amount of ammonia obtained from 250 c.c. of water is found by multiplying by the total volume of the distillate, which should be measured, and dividing by 50.

If the cylinder containing the distillate is the lighter, and some of the solution has been poured from the standard, the calculation is slightly different:—Let  $x$  = the weight of ammonia equal to the amount of standard ammonia solution taken,  $y$  = the amount of standard solution poured out, and  $z$  = the total amount in the cylinder containing the standard solution; then the weight of ammonia in 50 c.c. of the distillate =  $x \times \frac{z-y}{z}$ .

It is very important that the 50 c.c. mark on each Nessler cylinder should be accurately the same height; unless this is so, the thickness of the layer will not be proportional to the volume.

**Nessler Solution.**—Dissolve 35 grammes of potassium iodide in 100 c.c. of water; next dissolve 17 grammes of mercuric chloride in 300 c.c. of water; reserve a little of the potassium iodide solution and add the mercuric chloride solution to the rest, till a permanent precipitate is formed; then add the remainder of the potassium iodide solution and cautiously drop in mercuric chloride solution, till a faint permanent precipitate is left. Dissolve 160 grammes of potassium hydroxide in water, cool it, and add this solution to the mercury potassium iodide solution and make up to 1 litre. The solution is more sensitive the lower the temperature at which it is prepared, and also if a little more mercuric chloride solution is added. The solution is left to settle and the clear portion decanted for use.

**Standard Ammonium Chloride Solution.**—Weigh out 0·3146 gramme of pure ammonium chloride, and dissolve in 100 c.c. of ammonia-free water; dilute 10 c.c. of this to 1 litre with ammonia-free water for use. 1 c.c. = 0·00001 gramme  $\text{NH}_3$ .

**Ammonia-free Water.**—Boil ordinary distilled water in a flask to half its bulk and cool in an atmosphere free from ammonia.

**Nitric Acid.**—Place about 0·01 gramme of diphenylamine in a porcelain basin, add 1 c.c. pure sulphuric acid, and mix; run two or three drops of the water down the sides of the basin, so that they will flow over the surface of the acid. In the presence of nitrates a blue colour will be developed. From the amount and depth of coloration produced a rough idea of the amount of nitric acid present can be formed, which will be useful in the quantitative estimation.

Measure 2 c.c. to 10 c.c. of the water by an exact pipette into a porcelain basin, according to the amount indicated by the diphenylamine test, add 1 c.c. of a 2 per cent. solution of sodium salicylate, and evaporate to dryness on the water-bath; measure also a known volume, usually 2 c.c., of the standard potassium nitrate solution into a porcelain basin, add 1 c.c. of a 2 per cent. solution of sodium salicylate, and evaporate to dryness. To each add 1 c.c. of sulphuric acid, and heat for five minutes on the water-bath. Dilute to about 20 c.c. with distilled water, make alkaline with caustic soda solution (30 per cent.), and dilute to 50 c.c. Compare the colours produced in Nessler cylinders and calculate in the same manner as directed under *Free and albuminoid ammonia*.

**Standard Potassium Nitrate Solution.**—Dissolve 1·85 grammes of pure potassium nitrate in 1 litre of water; dilute 30 c.c. to 1 litre for use. 1 c.c. = 0·00003 gramme  $\text{N}_2\text{O}_5$ .

If the chlorine be high, the method just described may give results below the truth, and the following method may be used :—

Place about 200 c.c. of water in a wide mouthed stoppered bottle with three pieces of copper-zinc couple. Leave for twenty-four hours in a warm place, or longer if a reaction for nitrites is obtained; then take 100 c.c. and distil off 50 c.c. of this, after making alkaline with sodium carbonate. Estimate the ammonia in the 50 c.c. (or an aliquot part, 5 or 10 c.c. diluted to 50 c.c. are often sufficient) in the manner previously directed. The ammonia found (less the free ammonia present) multiplied by 3·2 will give the nitric acid (as  $N_2O_5$ ).

**Preparation of Copper-Zinc Couple.**—Cut a number of pieces of sheet zinc  $4 \times 1$  inch, and immerse them successively in 2 per cent. caustic soda solution, distilled water, 2 per cent. sulphuric acid solution, and distilled water, keeping them for about two minutes in each solution and agitating them. Place them in 3 per cent. solution of crystallised copper sulphate till a firm black deposit is obtained; rinse them well in distilled water, without undue handling; and preserve in a stoppered bottle filled with strong alcohol.

**Nitrites.**—Dissolve about 0·05 gramme of meta-phenylenediamine in dilute sulphuric acid (10 c.c.); add 10 c.c. of water and allow the mixture to stand. A brownish-pink coloration is produced, if nitrites be present.

**Oxygen absorbed from Permanganate.**—Clean out a stoppered bottle with chromic acid and rinse well with distilled water. Take 250 c.c. of water, add 10 c.c. of dilute sulphuric acid and 10 c.c. of standard potassium permanganate solution, mix, and keep at a temperature of about  $80^\circ F.$  ( $27\cdot4^\circ C.$ ) for four hours. Add a crystal of potassium iodide and titrate with standard sodium thiosulphate solution till only a faint yellow colour remains; then add a little starch solution and continue the titration till the blue colour disappears. To 250 c.c. of distilled water add 10 c.c. of sulphuric acid and 10 c.c. of potassium permanganate; and titrate with standard sodium thiosulphate solution (2 grammes per litre)\*. The difference between the amounts of sodium thiosulphate solution used, divided by the amount of sodium thiosulphate used for the sulphuric acid, potassium permanganate and distilled water, and multiplied by 0·001 will give the weight of oxygen absorbed by 250 c.c. of water.

**Dilute Sulphuric Acid.**—Mix 100 c.c. of pure sulphuric acid cautiously with 300 c.c. of water, cool to  $80^\circ F.$ , and add

\* The addition of a little, say 0·05 gramme, of sodium salicylate will render this solution permanent.

so much potassium permanganate solution that a faint pink tinge remains after four hours.

**Standard Potassium Permanganate Solution.**—Dissolve 0.395 gramme of pure potassium permanganate in 1 litre of distilled water. 1 c.c. = 0.0001 gramme oxygen.

**Starch Solution.**—Make an emulsion of 0.5 gramme of starch in 2 c.c. of water and add this to 50 c.c. of boiling water. Boil for five minutes and cool.

**Phosphates.**—Dissolve the ignited residue from the total solid estimation in a little dilute nitric acid; evaporate the solution to dryness in a porcelain dish, and take up with 1 c.c. of dilute nitric acid, filter the solution, and wash the filter paper with very small amounts of water. Add to the filtrate, which should not exceed 2 or 3 c.c., an equal bulk of ammonium molybdate solution and warm to 60° C. (140° F.). A yellow coloration is called a "very faint trace" of phosphates, and a distinct precipitate a "very heavy trace."

**Ammonium Molybdate Solution.**—Mix 14 c.c. of strong ammonia (sp. gr. .880) with 28 c.c. of water, and add 10 grammes of molybdic acid and stir till all is dissolved. Add this solution, slowly and with constant stirring, to 125 c.c. of nitric acid (sp. gr. 1.2); stand the solution in a warm place for a few days and decant the clear solution for use. A slight deposit may form on keeping.

**Hardness.**—To 100 c.c. of the water add 5 drops of methyl-orange solution, and titrate with  $\frac{N}{20}$  hydrochloric acid solution till the tint is the same as that of 100 c.c. of distilled water to which five drops of methyl orange and 0.2 c.c. of  $\frac{N}{20}$  acid have been added. Subtract 0.2 c.c. from the reading, and the remainder multiplied by 2.5 will give the alkalinity or temporary hardness in parts per 100,000.

Transfer this solution to a porcelain dish, and boil down to half its bulk; pour it in to a 100 c.c. flask, rinsing the basin with well-boiled distilled water, and add a measured volume (10 c.c., 15 c.c., or 20 c.c.), according to the hardness, of a  $\frac{N}{10}$  solution of soda, half carbonate and half hydroxide; make up to near the mark with well-boiled distilled water, cork up, and cool; when cold make up to the mark, and allow it to stand at least one hour. Filter through a dry filter, and collect an aliquot portion (say 90 c.c.), and titrate with  $\frac{N}{20}$  hydrochloric acid till equal in tint to that of the distilled water to which methyl orange

and 0.2 c.c. of  $\frac{N}{20}$  acid have been added; calculate the number of c.c. used to the total volume (*i.e.*, if 90 c.c. have been taken, divide by 0.9), and subtract 0.2 c.c. Titrate in the same manner the volume of  $\frac{N}{10}$  soda added, and the difference between the two results multiplied by 2.5 will give the total hardness as parts of calcium carbonate per 100,000.

The amount of soda solution added should be such that at least half the quantity of acid is required to neutralise the filtrate.

The permanent hardness is obtained by subtracting the temporary hardness from the total hardness.

**Interpretation of Results of Water Analysis.**—Good waters contain, generally speaking:—

Total solids,		20 to 40 parts per 100,000.
Chlorine, .	.	1 to 2 .. ..
Free ammonia, .	not more than	0.001 .. ..
Albuminoid ammonia,	.. ..	0.010 .. ..
Nitric acid, .	.. ..	0 to 2 .. ..
Nitrites, .	.	none.

They absorb less than 0.1 part per 100,000 of oxygen, and are practically free from phosphates.

The total solids may be higher than the limits named, in chalk waters and in mineral waters.

A high chlorine content may be due to beds of rock salt—*e.g.*, in waters from the new red sandstone—or to admixture with salt derived from the sea (near the coast); it is, however, usually due to sewage.

Deep well waters often contain large amounts of free ammonia; and water which has passed through iron pipes may also contain free ammonia and nitrites.

A high albuminoid ammonia is usually very undesirable, though not conclusive of pollution by sewage; pools into which dead leaves fall may give rise to high albuminoid ammonia.

Nitric acid is a most reliable datum; any amount above 3 or 4 parts per 100,000 is certainly due to pollution.

The presence of nitrites is always unfavourable, except when the water has passed through iron pipes, and in chalk waters.

The amount of oxygen absorbed does not give much information as to whether a water is polluted with sewage; high figures are often due to vegetable matter. The proportion of oxygen absorbed to albuminoid ammonia is often a useful datum. Where vegetable contamination has taken place the oxygen absorbed is ten times (or more) the albuminoid ammonia; in polluted waters it is usually less.

The presence of phosphates is usually regarded as an unfavourable symptom; this may, however, be due to the use of artificial fertilisers; the nitric acid may be increased from this cause.

If waters known to be pure from the same district and from the same geological formation can be obtained, the water can be compared with them; any marked increase in the figures found must be regarded as evidence of pollution. By this means evidence of contamination is often obtained which would be difficult, or almost impossible, to acquire from chemical analysis alone.

It must be remembered in comparing waters with a "district standard" that in the autumn the figures for free and albuminoid ammonia, nitric acid, and oxygen absorbed usually are slightly higher than at other times of the year.

For further information on the subject works on "Water Analysis" must be consulted. It must be borne in mind that the judging of water supplies is not a subject that can be learnt from books entirely, but that prolonged experience is necessary to properly interpret the results obtained.

### Bacteriological Examinations.

A very simple examination is all that is usually necessary. A sample of the water for bacteriological examination must be taken in a sterilised bottle; a six-ounce stoppered bottle is plugged with cotton wool, and the stopper is wrapped in cotton wool and tied to the neck; the bottle is sterilised for three hours at a temperature of 150° C. (350° F.). The sample is best taken directly after the sample for analysis has been obtained; the plug of cotton wool is removed and the bottle filled with water without being rinsed; then the stopper is quickly removed from its cotton wool wrapping and inserted in the bottle. The examination must be commenced with as little delay as possible; and, if the sample has to be forwarded by post or rail, it should be packed in ice.

The examination usually consists in making a gelatine cultivation at 22° C. and a search for microbes of intestinal origin.

**Preparation of Nutrient Media**—*Nutrient Gelatine*.—120 grammes of gelatine (Coignet's Extra Gold Label) are dissolved in 1 litre of water on the water-bath; 5 grammes of Liebig's extract of meat and 10 grammes of peptone are added, and dissolved by further heating; the whites and shells of two eggs, stirred up together to make an intimate mixture are next added, and the heating on the water-bath continued till the liquid is

cleared. Five c.c. is titrated after dilution with 5 c.c. of water with  $\frac{N}{10}$  alkali solution, and from the figure thus obtained the quantity of a strong caustic soda solution necessary to reduce the acidity to 15 c.c., N acid per litre is calculated, and added. The liquid is now filtered, the filter being kept warm, and the clear filtrate is ready for use. Portions of 10 c.c. are placed in test tubes which have been previously plugged with cotton wool and sterilised by heating for half an hour at 150° C. (350° F.). The nutrient gelatine in these tubes is sterilised by heating to 100° C. (212° F.) in steam for fifteen minutes on four successive days, or by heating in an autoclave to 120° C. for fifteen minutes.

*M'Conkey's Bile-Salt Glucose Peptone Medium.*—Weigh out 20 grammes of Witte's peptone, and dissolve in about 300 c.c. of warm tap water, add 5 grammes sodium tauro-cholate, and stir well till dissolved, adding a little more water to wash down the sides of the vessel; a porcelain double saucepan, in the outside portion of which water is kept boiling, serves admirably for the preparation of media. Add 5 grammes of glucose and water to make altogether 900 c.c., and heat till the solution is clear; filter, and to the clear filtrate add 100 c.c. strong neutral litmus solution.

Prepare also some media of twice and some of three times the above strength.

Plug with cotton wool, and sterilise a number of test tubes, each containing a small 2-inch  $\times$   $\frac{1}{4}$ -inch tube (Durham tube); these tubes should be of different sizes, some of them holding about 180 c.c., others about 50 c.c., and the remainder about 30 c.c. The largest tubes should be marked at 150 c.c., and the medium-sized ones at 20 c.c. To the largest tubes add 50 c.c. of the triple strength medium, to the next size 10 c.c. of double strength medium, and to the others 10 c.c. of the ordinary medium. Sterilise these as directed for the gelatine.

*M'Conkey's Bile-Salt Lactose Peptone Neutral Red Agar.*—To 1 litre of tap water, 20 grammes Witte's peptone, 5 grammes of peptone, 5 grammes of lactose, 15 grammes of powdered agar are added, and dissolved by heating; the solution is then centrifuged in wide tubes till as clear as possible, care being taken that the temperature does not fall so low that the agar solidifies. Pour off the clear liquid, add sufficient of a strong solution of neutral red to colour the whole a deep red colour, place quantities of 10 c.c. in plugged test tubes, and sterilise.

Allow the agar to solidify so that a slanting surface is obtained.

*Peptone Water.*—This contains 1 per cent. Witte's peptone and 0.5 per cent. salt; the solution is heated till clear, filtered,

and quantities of about 1 c.c. placed in Durham tubes, which are plugged with cotton wool, and sterilised.

*Sugar Media.*—The sugars used are glucose, lactose, and cane sugar; other carbohydrates, such as dulcitol, adonitol, and inulin, may be also used, but the three sugars give a fairly good distinction between the organisms of intestinal origin.

These media are made up, containing 7.5 per cent. gelatine, 2 per cent. peptone, 1 per cent. Lemco, and 1 per cent. of the sugar; they are heated till clear, filtered, 1 c.c. of 5 per cent. potash solution added, and the media tinted blue with litmus. Quantities of about 1 c.c. are placed in Durham tubes, five of the small tubes being held together by an india-rubber band, and contained in a 3-inch  $\times$  1-inch plugged test tube. They are sterilised as usual.

*Milk Tubes.*—Plugged test tubes, each containing 10 c.c. of separated milk, are sterilised.

It is a convenience to plug tubes containing the various media with different coloured cotton wool; this saves labelling, and minimises the chance of error. The use of different coloured cotton wool also serves to distinguish different quantities of water taken.

Prepare also a number of test tubes, each containing 9 c.c. of distilled water; plug these with cotton wool; and sterilise. The tubes containing nutrient media and sterilised water must be covered with a rubber cap to prevent evaporation.

*Procedure.*—Sterilise a pipette delivering 1 c.c. by heating to 150° C. (350° F.); the pipette is best sterilised in a test tube plugged with cotton wool. As soon as this is cool, open the bottle containing the sample, and take out 1 c.c. Add this to one of the tubes containing sterilised water and replace the plug immediately. Take out another 1 c.c. and add this to a tube of nutrient gelatine, which should have been previously liquefied and allowed to cool to 27° C. (80° F.). Pour into a sterilised Petri dish.

With another sterilised 1 c.c. pipette add 1 c.c. of the mixture of water with sterilised water to a tube of nutrient gelatine, which has been previously liquefied by heating and cooled to about 27° C. (80° F.), and pour into a Petri dish.

The Petri dishes should be placed in an ice chest to solidify the gelatine. Place the gelatine cultivations in an incubator kept at about 22° C. (or 72° F.); after two and a-half days the number of colonies that have developed are counted.

If the water is suspected to be bad, smaller amounts of water may be taken, 1 c.c. of the diluted water being added to 9 c.c. of sterile water. If it is supposed that the water is good, the amounts taken may be increased. The quantities given will,



however, usually serve. Many of the colonies on the gelatine will be found to have liquefied the medium, and, if the counting is delayed, the liquid may run down and contaminate the other portions. The author has found that if the colonies are counted in two and a-half days, no practical inconvenience is found from this source.

**The Search for *Bacillus Coli Communis*.**—Add 100 c.c. of water to one of the tubes containing triple strength M'Conkey medium, 10 c.c. to one of the tubes containing double strength medium, and 1 c.c. of the diluted water to tubes containing the ordinary medium; mix well by shaking, and incubate (Fig. 38) at 40° C. (104° F.). Examine after one and two days. If acid and gas are not produced *B. coli communis* is absent; if acid and gas are produced there is presumptive evidence of this organism.

Dip a sterile iron wire slightly bent at one end, into each of the tubes showing acid and gas, and plunge the wire into a peptone water tube, stirring well. Rub the wire over the surface of M'Conkey agar, and incubate for twenty-four hours at 40° C. If red colonies are formed the evidence for presence of *B. coli communis* is strong.

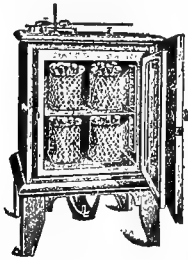


Fig. 38.

Bacteriological Incubator.

Inoculate five of the red colonies into peptone water tubes, and into tubes of the three sugar media. Place the sugar media in the incubator at 40° C. for three hours, and then for half an hour in an ice chest, and incubate at 22° C. (72° F.) for twenty-four hours. Incubate the peptone water tubes at 40° C. for twenty-four or

better, forty-eight hours.

*B. coli communis* produces indole in peptone water, and ferments both glucose and lactose with the formation of gas bubbles and acid, but does not ferment cane sugar: a variety of this organism, however, ferments cane sugar.

**Test for Indole.**—To the peptone water add a few drops of an alcoholic solution of *p*-dimethyl amino-benz-aldehyde, and a few drops of a saturated solution of potassium persulphate: warm slightly, and in the presence of indole a cherry-red colour is produced.

**The Search for *Bacillus Sporogenes Enteritidis*.**—Add a little alum to 500 c.c. of the water, and if a turbidity is not produced a few drops of ammonia; let the precipitate settle, and pour off the clear solution as completely as possible, and collect the precipitate by centrifuging. Add this to a tube of milk, pour a layer of melted vaseline on the surface, and heat to

80° C. (176° F.) for twenty minutes. Incubate at 40° for forty-eight hours; if *B. sporogenes enteritidis* is present, the milk is curdled, and the dry-looking curd is nearly blown out of the tube.

*Interpretation of Results.*—The presence of *B. coli communis* in 1 c.c. or less of the water, especially if accompanied by the presence of *B. sporogenes enteritidis*, is a very unfavourable sign, and points to sewage contamination. *B. coli communis* in 10 c.c. is very suspicious, whilst if this organism is present in 100 c.c., but not in less water, an investigation of the supply should be undertaken, but the water should not be condemned on this ground alone.

The presence of glucose fermenting organisms which do not prove to be *B. coli communis* is not a very favourable sign, but the other evidence should be carefully considered before condemning; they are often present in surface waters.

The colonies liquefying gelatine are usually those of putrefactive organisms, and any great proportion is undesirable. The number of organisms growing on gelatine varies greatly with the source of the water. Water from deep wells should be almost sterile and certainly should not give more than 100 colonies per c.c.; any number exceeding this may be taken as indicating contamination with surface water. Surface waters which are not contaminated may contain many more organisms, as many as 2,000 per c.c., and often a large number of these (25 per cent.) liquefy gelatine; such waters are generally found to contain organic matter derived from decaying leaves and other vegetable matter.

All waters giving evidence of organisms of intestinal origin, and a number of organisms running into many thousands on gelatine, may be condemned as unsatisfactory.

By the combined information from inspection of the source, chemical analysis and bacteriological examination, a usually reliable opinion can be made of the purity of the water; it is even more reliable, if the data are compared with those obtained on waters of known purity from the same district and of the same character.

For other methods of bacteriological examination and for the separation and identification of individual species, works on bacteriology must be consulted.

**Summary of Sanitary Precautions.**—The following recommendations were made by a commission held under the auspices of the *British Medical Journal*:—

1. That all milking be carried on in the open air, the animals and operators standing on a material which is capable of being thoroughly washed, such as a floor of concrete or cement. Such a floor could be easily laid down in any convenient place which

can be found. The site chosen should be removed from inhabited parts as far as possible, and should be provided with a plentiful [and pure] water supply.

2. That greater care be expended on the personal cleanliness of the cows. The only too familiar picture of the animal's hind-quarters, flanks, and sides being thickly plastered with mud and fæces is one that should be common no longer.

3. That the hands of the milker be thoroughly washed before the operation of milking is commenced, and that, after being once washed, they be not again employed in handling the cow otherwise than in the necessary operation of milking. Any such handling should be succeeded by another washing in fresh water before again commencing to milk.

4. That all milk-vendors' shops should be kept far cleaner than is often the case at present. That all milk-retailing shops should be compelled to provide proper storage accommodation, and that the counters, etc., should be tiled.

With reference to the last recommendation, it may be mentioned that the regulations of the London County Council provide that all places where milk is stored shall have an impervious floor, and the walls shall be tiled or cemented for a height of 6 feet from the floor.

The following additional recommendations are chiefly taken from the system adopted by the Aylesbury Dairy Company:—

1. The cows should be systematically examined by veterinary surgeons; and those in ill-health removed, and their milk not utilised.

2. The sanitary arrangements of the dairy and health of the *employés* should be systematically examined by a medical officer, who shall be empowered to insist on all unsatisfactory arrangements being instantly remedied, and, in default of this, should have the power to stop the milk supply.

3. To ensure the proper carrying out of these regulations, guarantees should be given that no financial loss should result from suspension of the milk supply owing to infectious disease, while very heavy penalties should be imposed for contravention of sanitary regulations.

4. All water used for cleansing dairy utensils should be previously boiled, to destroy disease germs if accidentally present; and, if possible, the vessels themselves should be steamed.

If the only available water supply be not above suspicion, an immunity from the consequences of its use may be attained by filtration through a Pasteur-Chamberland filter. This consists of one or more tubes of unglazed porcelain of a special quality, through which water will pass, but which keeps back micro-organisms. It has been found that in course of time that certain

micro-organisms will grow through the filter, but it appears to be firmly established that pathogenic germs are not among these. To secure efficient working, these filters should be frequently cleaned, and it is advisable to sterilise them by steaming from time to time. They have the advantage of being easily tested, as when efficient they will not, when wet, allow air under a pressure of 10 lbs. per square inch to pass; while, if defective, a passage is afforded at any place which will allow micro-organisms to traverse the filter.

**Products formed from Milk by the Action of Micro-Organisms.**

--Besides butter and cheese, in the manufacture of which micro-organisms play an important part, several preparations are made from milk; among these may be mentioned koumiss, kephir, and mazoum.

**Koumiss.**—This preparation was originally made from mare's milk by the Tartars. It is a product of combined alcoholic, lactic, and protein-hydrolytic fermentations on milk. Its production first from mare's milk is probably due to the fact that the sugar of this milk very easily undergoes alcoholic fermentation.

The following analyses of mare's milk koumiss are by Vieth:—

TABLE LXVIII.

	1 Day Old.	8 Days Old.	22 Days Old.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	91.43	92.12	92.07
Alcohol, . . . . .	2.67	2.93	2.98
Lactic acid, . . . . .	0.77	1.08	1.27
Sugar, . . . . .	1.63	0.50	0.23
Casein, . . . . .	0.77	0.85	0.83
Albumin, . . . . .	0.25	0.27	0.24
Proteoses, . . . . .	0.98	0.76	0.77
Fat, . . . . .	1.16	1.12	1.30
Ash, . . . . .	0.35	0.35	0.35

Koumiss is now very largely made from cow's milk by the selection of special organisms.

The following analyses of various kinds of koumiss have been made by Vieth on the preparations of the Aylesbury Dairy Company. The carbonic acid present has not been taken into account; it is, however, always present (except in the earlier stages) in sufficient amount to render the koumiss highly effervescent; hence, the preparation has been termed "milk champagne" on this account.

The term "casein" includes meta-caseins.

TABLE LXIX.

FULL KOUMISS.			
	1 Day Old.	8 Days Old.	22 Days Old.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	88.90	90.35	90.57
Alcohol, . . . . .	0.15	0.94	1.04
Fat, . . . . .	1.35	1.36	1.38
Casein, . . . . .	2.01	1.96	1.88
Albumin, . . . . .	0.30	0.23	0.20
Proteoses, . . . . .	0.34	0.53	0.77
Lactic acid, . . . . .	0.34	0.96	1.40
Sugar, . . . . .	6.03	3.10	2.18
Ash, soluble, . . . . .	0.17	0.23	0.23
„ insoluble, . . . . .	0.41	0.34	0.35
WHEY KOUMISS.			
Water, . . . . .	89.74	90.63	91.07
Alcohol, . . . . .	0.30	1.03	1.38
Fat, . . . . .	0.11	0.13	0.15
Casein, . . . . .	0.15	0.14	0.11
Albumin, . . . . .	0.39	0.36	0.32
Proteoses, . . . . .	0.44	0.49	0.58
Lactic acid, . . . . .	0.60	0.91	1.26
Sugar, . . . . .	7.48	5.52	4.34
Ash, soluble, . . . . .	0.37	0.37	0.37
„ insoluble, . . . . .	0.42	0.42	0.42
MEDIUM KOUMISS.			
Water, . . . . .	87.55	88.39	88.62
Alcohol, . . . . .	0.29	0.97	1.05
Fat, . . . . .	1.54	1.56	1.58
Casein, . . . . .	1.46	1.40	1.30
Albumin, . . . . .	0.43	0.25	0.14
Proteoses, . . . . .	0.48	0.76	0.97
Lactic acid, . . . . .	0.68	1.20	1.67
Sugar, . . . . .	6.80	4.70	3.90
Ash, soluble, . . . . .	0.28	0.32	0.33
„ insoluble, . . . . .	0.49	0.45	0.44
DIABETIC KOUMISS.			
Water, . . . . .	92.24	92.38	92.55
Alcohol, . . . . .	0.28	0.35	0.57
Fat, . . . . .	0.51	0.52	0.51
Casein, . . . . .	2.19	2.13	2.05
Albumin, . . . . .	0.30	0.25	0.18
Proteoses, . . . . .	0.36	0.48	0.65
Lactic acid, . . . . .	0.75	0.86	1.22
Sugar, . . . . .	2.78	2.42	1.64
Ash, soluble, . . . . .	0.22	0.24	0.26
„ insoluble, . . . . .	0.37	0.37	0.37

Wiley gives the following mean composition of koumiss prepared in America :—

Water, . . . . .	89.32 per cent.
Carbon dioxide, . . . . .	0.83 "
Alcohol, . . . . .	0.76 "
Lactic acid, . . . . .	0.47 "
Proteins, . . . . .	2.56 "
Fat, . . . . .	2.05 "
Sugar, . . . . .	4.38 "

Koumiss has the advantage of being a food and a stimulant at the same time; and is, for this reason, often prescribed by the medical faculty in cases of disease (*e.g.*, gastritis) when no other food can be retained.

**Kephir.**—This is a preparation of a similar nature to koumiss and is produced from milk by means of kephir grains.

The following is the composition of kephir according to various authorities :—

TABLE LXX.

	König (mean).	Hammarsten.	Vieth (an old sample).
	Per cent.	Per cent.	Per cent.
Water, . . . . .	91.21	88.915	90.09
Alcohol, . . . . .	0.75	0.720	0.64
Lactic acid, . . . . .	1.02	0.727	0.44
Fat, . . . . .	1.44	3.088	1.82
Sugar, . . . . .	2.41	2.685	1.87
Casein, . . . . .	2.83	2.904	2.90
Albumin, . . . . .	0.36	0.186	0.07
Proteoses, . . . . .	0.30	0.067	0.45
Ash, . . . . .	0.68	0.708	..

Kephir differs from koumiss chiefly in the comparatively small amount of proteoses it contains, showing that, although the alcoholic and lactic fermentations have taken place, the protein-hydrolytic fermentation is very weak.

Struve found in kephir grains :—

Water, . . . . .	11.21 per cent.
Fat, . . . . .	3.99 "
Protein, . . . . .	51.69 "

The author has examined a "kephir powder," which had the following composition :—

Water, . . . . .	2.29 per cent.
Milk-sugar, . . . . .	89.90 "
Other organic matter, . . . . .	7.42 "
Ash, . . . . .	1.39 "

It appeared to be a mixture of milk-sugar with pulverised kephir grains.

**Mazoum.**—This preparation, lately introduced from Armenia, where it has been made for centuries, has somewhat the appearance of clotted cream; on warming, it separates into a liquid whey and an insoluble curd.

The author has determined the following figures:—

Fat,	6.27	per cent.	} Curd.
Casein,	2.56	„	
Ash,	0.04	„	
Organic solids,	5.00	„	} Whey.
Ash,	0.77	„	
Water,	85.38	„	

There was no evidence of proteoses in the whey.

Mazoum appears to have been produced by the lactic fermentation of milk enriched with cream; the sample examined was very fresh and protein-hydrolytic fermentation was not appreciable.

An organism was separated from mazoum which gave colonies rapidly spreading on the surface of gelatine to 1 cm. or more in diameter, and which produced a slight putrid smell. This organism, which was a bacillus, slowly peptonised milk without curdling it, and finally transformed it into a semi-transparent liquid jelly.

**Bulgarian Sour Milk.**—This preparation is a thick gelatinous liquid of pleasant acid taste. It may contain as much as 2½ per cent. of lactic acid, and the proteins are hydrolised to a considerable extent. The milk used for its preparation is sometimes concentrated.

## CHAPTER VI.

## BUTTER.

CONTENTS.—Definition of Butter—Composition—Theory of Churning—The Proximate Analysis of Butter—The Analysis of Butter Fat—Preparation of the Fat for Analysis—Recapitulation of Properties—Estimation of Volatile Fatty Acids—Saponification Equivalent—Soluble and Insoluble Fatty Acids—Colour Tests for Adulterants—Behaviour of Butter Fat with Solvents—Iodine and Bromine Absorption—Heat Evolved by Sulphuric Acid—Physical Examination of Butter Fat—Microscopic Examination—Density—Refractive Index—Viscosity—Melting Point—Detection of Adulteration of Butter—Influence of Keeping on Butter—Buttermilk—Chemical Control of Churning Operations.

**Definition.**—Butter is the substance produced by churning milk or cream, during which process the fat globules coalesce to form granules; when freshly churned, butter has the appearance of a fine granular mass; but, after being worked, this assumes a structure homogeneous to the naked eye.

**Composition.**—Storch gives the following mean composition to butter:—

TABLE LXXI.—COMPOSITION OF BUTTER.

	From Fresh Cream.	From Ripened Cream.
	Per cent.	Per cent.
Fat, . . . . .	83.75	82.97
Water, . . . . .	13.03	13.78
Protein, . . . . .	0.64	0.84
Milk-sugar, . . . . .	0.35	0.39
Ash, . . . . .	0.14	0.16
Salt, . . . . .	2.09	1.86

He further argues that the milk-sugar must all belong to the buttermilk, which fills the spaces between the fatty portion; and, from the composition of the buttermilk, calculates the proportion of water, proteins, and ash belonging to this.



TABLE LXXII.—COMPOSITION OF BUTTER.

	From Fresh Cream.		From Ripened Cream	
	Per cent.		Per cent.	
Fat, . . . . .	83.75		82.97	
Buttermilk,	6.95		8.49	
Water,		6.31		7.74
Milk-sugar,		0.35		0.39
Protein,		0.23		0.29
Ash,		0.06		0.07
Mucoid substances,	7.21		6.68	
Water,		6.72		6.04
Protein,		0.41		0.55
Ash,		0.08		0.09
Salt,	2.09		1.86	

**Proportion of Solids not Fat to Water.**—Vieth has shown that in butter the proportion of solids not fat to water remains, so long as no water is added, the same as that in milk—*i.e.* 10 to 100; he gives the following average analyses:—

**Composition of Different Kinds.**

TABLE LXXIII.—COMPOSITION OF BUTTERS.

Designation.	Fat.	Water.	Curd.	Salt.	Curd Water $\times 100$ .
	Per cent.	Per cent.	Per cent.	Per cent.	
English, . . . . .	86.85	11.54	0.59	1.02	5
French, fresh, . . .	84.77	13.76	1.38	0.09	10
" salt,	84.34	12.05	1.60	2.01	13 *
German, salt,	85.24	12.24	1.17	1.35	10
Danish, ,,	83.41	13.42	1.30	1.87	10
Swedish, ,,	82.89	13.75	1.33	2.03	10

The following analyses by the author show the average composition of French fresh butter (giving the amount of preservative), and of Australian butter:—

Designation.	Fat.	Water.	Curd.	Salt.	Anhydrous Borax.	Anhydrous Boric Acid.	Commercial Preserva- tive. Per ct.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	
French, fresh,	83.92	14.33	1.36	...	0.21	0.18	= 0.65
Australian, salt,	84.50	12.70	1.21	1.57	...	...	...

Table LXXIV. will give the number of samples in which the water falls between the percentage named. The analyses were made by Vieth, Schnepel, Boseley, Livett, O'Shaughnessy, and the author in the Aylesbury Dairy Company's laboratory.

\* Contained boric acid.

TABLE LXXIV.—VARIATIONS OF WATER IN BUTTER.

Percentages of Water.	English Butters.		Foreign Butters.	
	No. of Samples.	Percentage.	No. of Samples.	Percentage.
7 to 8.	2	0·3	...	...
8 „ 9.	5	0·8	5	0·4
9 „ 10.	14	2·2	13	1·0
10 „ 11.	26	4·2	51	3·7
11 „ 12.	65	10·4	78	5·7
12 „ 13.	154	24·6	115	8·4
13 „ 14.	182	29·1	395	29·0
14 „ 15.	97	15·5	373	27·4
15 „ 16.	50	8·0	241	17·7
16 „ 17.	21	3·4	71	5·2
17 „ 18.	4	0·6	21	1·5
18 „ 19.	3	0·5	1	0·1
19 „ 20.	2	0·3	...	...
Total.	625		1,364	

The above table contains butters of all kinds—fresh, salt, preserved, unpreserved, fresh from churning, and samples which had been kept for various periods.

**Variations in Percentages of Water.**—The following table (LXXV.) is taken from a paper by Faber on "Water in Danish Butter":—

TABLE LXXV.—VARIATIONS OF WATER IN BUTTER (*Faber*).

Percentages of Water.	No. of Samples.		Percentage of Total.	
	Summer.	Winter.	Summer.	Winter.
9 to 10.	1	1	0·0	0·1
10 „ 11.	16	8	0·8	0·4
11 „ 12.	136	20	6·3	1·0
12 „ 13.	335	138	16·8	7·2
13 „ 14.	534	431	26·7	22·3
14 „ 15.	512	562	25·7	29·1
15 „ 16.	287	447	14·4	23·2
16 „ 17.	124	205	6·2	10·6
17 „ 18.	39	95	2·0	4·9
18 „ 19.	13	20	0·7	1·0
Above 19.	4	3	0·2	0·2
Total.	2,001	1,930	...	...
Average.	14·03%.	14·41%.	...	...

Table LXXVI. shows the effect of keeping on the percentage of water contained in the butter; fresh and salt butters, which were all prepared at the Aylesbury Dairy Company, are kept separate.

TABLE LXXVI.—VARIATIONS OF WATER IN BUTTERS ON KEEPING.

Percentages of Water.	Percentages of the Total Number falling between the Limits Named.				
	Fresh Butters.		Salt Butters.		
	Less than 12 hours old.	24 to 48 hours old.	Less than 12 hours old.	24 to 48 hours old.	10 to 30 days old.
18 to 19,	...	...	1·3	...	...
17 „ 18,	...	...	2·5	...	...
16 „ 17,	1·7	...	15·0	3·8	...
15 „ 16,	10·3	10·0	22·5	5·1	3·6
14 „ 15,	31·3	15·0	25·0	12·6	...
13 „ 14,	32·8	35·0	25·0	34·1	10·7
12 „ 13,	20·7	40·0	7·5	38·0	28·6
11 „ 12,	3·4	...	1·3	5·1	42·9
10 „ 11,	...	...	...	1·3	10·7
9 „ 10,	...	...	...	...	3·6
Average percentage of water,	13·79	13·54	14·74	13·33	12·00

Taking butters from twenty-four to forty-eight hours old to represent commercial butter, it is seen that salt butter contains rather less water than fresh butter. The contrary is usually stated; but this is not according to the author's experience.

Fresh butter loses its water chiefly by evaporation, and it is seen that this loss is small: salt butter also loses water by brine running out. It will usually be noticed that salt butter looks wet on being cut, while fresh butter rarely has this appearance.

**Action of Salt.**—The action of salt, which is added both to give a flavour and as a preservative, seems to be as follows:—It first dissolves in the buttermilk left in the butter, and forms a strong solution, which curdles the buttermilk, giving an insoluble precipitate of protein matter and a clear whey. The salt solution has a smaller viscosity than the buttermilk; hence a smaller layer is condensed round the particles by surface energy, and there is liquid which is very loosely held in the butter; this gradually runs out, and gives rise to the wet appearance of salt butter. It is noticed that the liquid which runs out, or is squeezed

out, of salt butter is always clear and transparent, while the liquid squeezed out of fresh butter is usually milky.

By warming to a temperature near the melting point of the fat considerable quantities of water can be mixed with butter. In the preparation of "pickled" butter advantage is taken of this fact to add large amounts of salt by working in warm brine. Butter treated in this way does not lose its water easily, as an emulsion of fat and water is thus produced.

Storch has shown that by the action of certain micro-organisms such a condition (of the proteins?) is produced, that large amounts of water are retained and cannot be worked out. In this case an emulsion is produced, which contains large numbers of very minute water globules. These butters are designated "thick," and are rare in England.

**Theory of Churning.**—Several theories have been put forward to account for the phenomenon of churning. Thus, Fleischmann holds the view that the globules of fat in milk are in a superfused condition, and that churning is simply the phenomenon of solidification; with the recognition of the fact that the globules are solid at low temperatures this view is untenable. Soxhlet holds that churning consists in the rupture of a solid membrane, which he believes exists round the fat globules; as the existence of such a membrane is disproved, this view cannot be accepted. Storch attributes churning to the gradual rubbing off of a semi-solid membrane of "mucoid substance," and this hypothesis has much to recommend it; the whole of the evidence points to the existence of a layer, which is not solid, round the fat globules. As previously stated, the author cannot reconcile Storch's theory that this layer consists of "mucoid substance" with known facts; but it appears very highly probable that there is a layer, the composition of which is for the present purpose immaterial, round each fat globule. As it is improbable that this layer is elastic, the effect of the impact of one fat globule on another will be to squeeze out the layers between them, and bring the globules within the sphere of each other's attraction. In this way nuclei will be formed, which will, on continued churning, increase in size; as the nuclei get larger and larger, the resistance, owing to fluid friction on their surfaces, will gradually bear a smaller and smaller proportion to the force tending to bring them to the surface, and, at a given moment, the butter will "come." This theory is in accord with all the known facts. By microscopical examination of cream during churning the formation of nuclei of irregularly shaped masses of fat globules is noticed. As an irregular mass will occupy a greater apparent volume than a sphere, the transformation of spherical globules into irregular nuclei should be attended with

thickening of the cream, which is in accord with the facts; as the nuclei increase in size, the layer condensed by surface energy round them will rapidly become less, so that the cream will gradually decrease in thickness; this decrease in thickness of the cream should take place later than the increase mentioned above, which is also the case.

When the butter is taken from the churn it is in fine grains, which are the nuclei referred to. On working, the fat globules are brought still closer to each other, and the butter is formed into a nearly homogeneous mass; small amounts of liquid are, however, left distributed throughout the mass, and as these

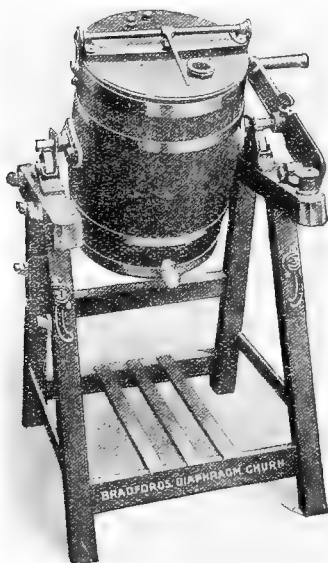


Fig. 39.—Churn.

liquid globules are very small and contained in a medium which, though solid, is still viscous, they are by surface energy transformed into spheres. The microscopical examination of butter shows a number of spherical globules of aqueous liquid in a nearly homogeneous medium consisting of fat; there are, however, many fat globules left, which, by careful examination with little light (best by dark stage illumination), can be made out. The whole of the globules usually seen, which are of all sizes, consist of aqueous liquid; in many cases where the globules are of sufficient size for the surface energy to become small, they cease to be spherical.

The reason that butter always does, and must, contain water is that the aqueous liquid present is finely divided, and assumes a spherical condition. It is impossible by pressure from the outside to remove small spheres from a homogeneous medium.

It appears certain, from the experiments of Storch on the density of butter, that the density of the fat is the same as that of butter fat in the solid state; it is, therefore, solid in butter. This view is nearly universally accepted.

With the recognition of the fact that butter is an approximately homogeneous fatty substance, the reason for its change

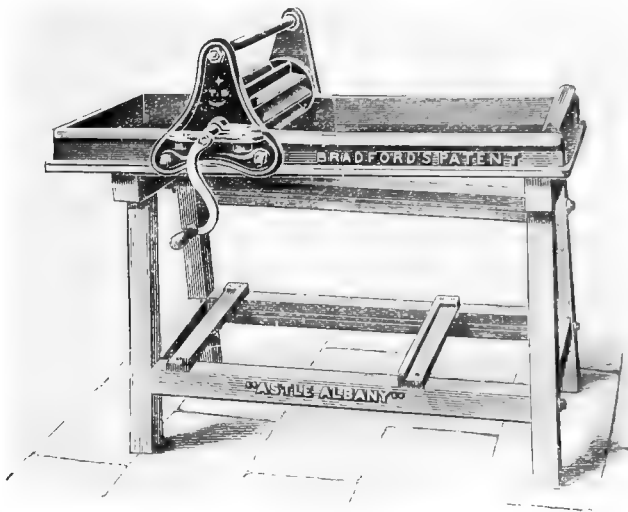


Fig. 40.—Butter Worker.

of consistency by alteration of temperature at once becomes apparent. To churn butter of the right consistency it is necessary that the fat in the cream shall be of that consistency. As pointed out by the author and S. O. Richmond, the fat in cream which has been warmed very slowly solidifies. If the cream has been kept at a high temperature, as in summer, it is necessary to churn at a lower temperature than if the cream has been kept at a low temperature, as the effect on the consistency of the fat of cream of cooling for a long time at a fairly low temperature is the same as that of cooling for a shorter time at a lower temperature.



**Water.**—The most important datum is the percentage of water. As the water is not always equally distributed throughout the mass of butter, especially in butters which have been salted, it is necessary to take precautions to obtain a fair sample—a matter of some difficulty. It is not advisable to use a scoop, as water is liable to be squeezed out while forcing it into the lump. Perhaps the fairest way of sampling is to cut the lump into halves, and to take a piece near (not at) one top corner, a second piece in the middle, and a third near the opposite bottom corner. The three pieces should be placed in a wide-mouthed stoppered bottle, melted at as low a temperature as possible, and violently shaken till the mass is nearly solid. If the analysis is to be commenced at once, suitable quantities may be poured out while the butter is still in a semi-liquid condition, and weighed as soon as possible. The water by this means is equally distributed throughout the sample, and a small quantity will be representative of the whole sample. In the case of well-made fresh butter the differences in the distribution of water is small, and a single sample taken from any part of the lump will represent with fair accuracy the whole bulk. Where extreme accuracy is not desired, the melting and shaking of samples of fresh butter may be omitted.

The water in butter may also be mixed by warming to such a temperature that the butter begins to lose its consistency, and stirring vigorously with a stout glass rod. The mixing of salt butter should not be omitted if accuracy is a desideratum.

The following methods are used for the determination of the water:—

1. About 10 grammes are weighed out into a small porcelain basin provided with a glass stirrer. This is placed over a very small flame, or on a sand-bath, and the butter carefully, but vigorously, stirred till all signs of frothing cease. The temperature must be so regulated that spirting is avoided, and that the "curd" does not become appreciably browned by the heat. The basin and its contents are, after cooling, weighed; the loss of weight indicates water.

2. A basin is filled with pumice, which is broken in pieces about the size of a small pea, washed, and ignited; 2 or 3 grammes of well mixed butter are weighed in, and the basin placed in a drying oven at 100° C. (212° F.), through which a good draught passes. At the expiration of an hour the basin is cooled and weighed, and then replaced in the oven for a further half hour; weighings are made at the expiration of succeeding half hours till the weight ceases to diminish. The lowest weight obtained is taken as that of the dry butter. The difference between this weight and that of the original butter is taken as water.



3. Four to five grammes of butter are weighed into a wide-mouthed flat-bottomed conical flask, which is placed in a water oven and shaken every ten minutes for the first half hour, after which it is shaken every half hour. At the expiration of four hours it is cooled, weighed, and returned to the bath for another hour; if there be any loss, the drying is continued till an hour's drying does not cause any diminution of weight.

4. From 2 to  $2\frac{1}{2}$  grammes of well-mixed butter are weighed into a flat-bottomed basin about  $2\frac{3}{4}$  inches diameter. This is placed in the water-oven till just melted, and 1 to  $1\frac{1}{2}$  c.c. of strong alcohol are added; the basin is replaced in the water-oven, and weighed after two hours. The loss represents water.

Of the four methods, the first is the most expeditious, and is nearly as accurate as the others; the second is the most accurate; the third is the most convenient if solids not fat and salt are also estimated; while the fourth is fairly accurate, rapid, and requires no attention. No one of the four methods has, however, any great advantage over the others.

**Solids not Fat and Salt.**—For the estimation of solids not fat and salt the residue from the determination of water is taken and melted at a low temperature. A solvent for the fat, of which ether is perhaps the best, though chloroform, amyl alcohol, and others may be also used, is poured on, the whole well mixed, and allowed to stand in a warm place till the solvent is quite clear. The solution is carefully decanted and a fresh portion of the solvent poured on the residue, and, when clear, poured off. Four or five successive treatments are sufficient to remove the whole of the fat. With a little practice the operation may be so performed that none of the solids not fat are poured away with the solvent. The residue is placed in the water-oven, and dried to constant weight; the weight represents solids not fat and salt.

**Salt.**—To estimate the salt the residue is treated with hot water and filtered, the filter together with the residue washed, and the filtrate, or an aliquot portion of it, is titrated with a standard silver nitrate solution, using potassium chromate as indicator. It is essential that the solution should be cold before titration, and the silver nitrate solution should be standardised on pure sodium chloride. The strength should not be deduced from the amount of silver nitrate present, as Hazen, and, later, W. G. Young, have pointed out that the amount of silver used is always greater than that theoretically required to combine with the chlorine. From the amount of silver nitrate solution used the weight of salt is readily calculated. It is convenient to make the silver nitrate solution of such strength that 1 c.c. = 0.005 gramme of sodium chloride.

**Solids not Fat.**—The weight of salt found by titration is subtracted from that of the residue left after the extraction of the fat, and the difference represents the solids not fat.

**Fat.**—The fat is best estimated by subtracting the total of the water, salt, and solids not fat from 100; though the solvent may be evaporated and the fat actually weighed, if desired.

**Curd.**—An estimation of the actual curd present can be made by submitting the residue, left after estimation of the fat, to Kjeldahl's process for the estimation of nitrogen (p. 124), and multiplying the nitrogen found by 6.38. The milk-sugar may be estimated in a portion of the solution used for the titration of the salt by one of the methods given for the determination of milk-sugar (p. 90). These determinations are rarely required.

Casein is estimated by extracting the solids not fat with dilute ammonia till no lumps are left, filtering the solution, and washing the residue; the filtrate is made acid with acetic acid, and the precipitated casein collected on a tared filter or Gooch crucible, and weighed as in the estimation of casein in milk (p. 129). The extraction of the fat, and ignition may be, however, omitted, as the fat has already been extracted, and the amount of ash is so small that it may be neglected without great error.

**Ash.**—In place of an estimation of the salt, an ash determination is often made, and the ash taken as salt. The results are, however, always slightly above those obtained by titration, as butter itself, to which no salt has been added, gives a small ash; preservatives, such as borax, will also swell the weight of the ash.

**Preservatives.**—The preservatives most largely used in butter consist of sodium borates; sulphites and nitrates have also been used, usually in conjunction with borates; fluorides are also employed; formalin has been recommended, but appears to be rarely used. These should be tested for in the aqueous portion which sinks to the bottom on melting the butter at a low temperature. The reaction with turmeric paper applied to the liquid direct will show the presence of free boric acid. If no reaction or a feeble one be obtained, a little of the liquid may be acidified with very dilute hydrochloric acid, and tested with turmeric paper. A pinkish-brown coloration, turned greenish-black by dilute alkali, will show the presence of boric acid in combination. It will usually be found, if the butter is preserved in this way, that a reaction is obtained from the liquid itself, and a much stronger one after acidifying. The presence of sulphites may almost always be detected by the smell of sulphurous acid developed on acidifying. Nitrates may be found by the diphenylamine test. Fluorides may be tested for as in milk. For the quantitative estimation of preservatives

50 grammes of butter should be placed in a stoppered cylinder, 50 c.c. of chloroform added, and the mixture gently warmed till perfect mixture takes place. A quantity of water, which will, with that present in the butter, make up 50 grammes, is added, and, after shaking, the cylinder is put aside to allow the aqueous portion to separate. Each cubic centimetre of the solution will contain the preservative in 1 gramme of butter.

For the estimation of boric acid Thompson's method is convenient (p. 85). As butter is practically free from phosphates, the process for their removal may be omitted, and the titration performed on an aliquot portion of the solution which has been made alkaline, evaporated to dryness, and ignited; the ash is extracted with hot water, and titrated first with acid till neutral to methyl-orange, and then with alkali in the presence of glycerol, till neutral to phenolphthalein, the result will be the total boric acid, free and combined.

It is, of course, obvious that any of the other methods for the estimation of boric acid (pp. 86 to 88) may be used in place of Thompson's method.

The author and Harrison have devised a rapid method for the estimation of boric acid in butter; 25 grammes of butter are weighed into a beaker, and just melted in the water-oven, 25 c.c. of water are added, and the contents of the beaker well mixed by stirring; the aqueous portion is allowed to settle; the contents are again mixed, and allowed to settle. 20 c.c. of the lower layer are withdrawn, and the boric acid estimated therein by the method of Miller and the author. The weight of boric acid multiplied by  $\frac{100 + W}{20}$  ( $W$  = percentage of water) will give the percentage of boric acid; the factor 5.65 may be used without great error.

The fat may be filtered, and used for the examination of its composition.

The boric preservative is usually expressed as boric acid,  $H_3BO_3$ .

The Preservatives Committee has recommended:—

(D) That the only preservative permitted to be used in butter and margarine be boric acid or mixtures of boric acid and borax, to be used in proportions not exceeding 0.5 per cent. expressed as boric acid.

An estimation of the total sulphurous acid may be made by distilling a portion of the liquid with dilute hydrochloric acid, passing the gas evolved into decinormal iodine solution, and titrating with sodium thiosulphate; 254 parts of iodine convert 64 parts of  $SO_2$  into sulphuric acid. The gas evolved may also be passed into bromine water, and the sulphuric acid formed

estimated as barium sulphate, of which 233·5 parts represent 64 parts of  $\text{SO}_2$ . The solution from which the sulphurous acid has been distilled may be advantageously evaporated to dryness after making alkaline and ignited, and the sulphuric acid estimated in this; the sulphuric acid present is probably due to the oxidation of the sulphite.

Nitrates may be estimated by one of the methods described under "Water Analysis." If much salt be present, the copper-zinc couple method should be employed.

Formalin cannot be estimated with any degree of exactitude, as it gradually enters into combination with the proteins present, and only the residue of uncombined formaldehyde, which gives no clue to the original amount, can be determined.

If adulteration is suspected, it may be of interest to examine microscopically the residue left after removal of the fat; adulterants, such as starch, mineral matters, etc., which it is alleged have been used, would be thus detected. This form of adulteration is of extreme rarity, but starch is sometimes added to ear-mark margarine.

**The Interpretation of Results.**—The President of the Board of Agriculture has laid down that any butter which contains more than 16 per cent. of water shall be presumed, till the contrary is proved, not to be genuine.

It is advisable always to calculate the solids not fat and salt, not only as percentages, but also as parts per 100 parts of water present. Table LXXVII. will show the characteristics of various classes of butter :—

TABLE LXXVII.

Class.	Per cent. Water.	Parts per 100 parts of Water.		Designation in North of England.
		Solids not Fat.	Salt.	
Fresh, unwashed,	12 to 16	8 to 12	none.	} Almost unknown.
" washed, .	12 ,, 16	3 ,, 10	none.	
Salt, unwashed,	10 ,, 16	8 ,, 12	5 to 25	} Mild.
" washed, .	10 ,, 16	3 ,, 10	5 ,, 25	
Pickled, . . .	May be high.	Rather low.	35 ,, 40	Salt.
Mixed with water,	" "	" "	Less than 25.	
Milk blended, .	22 to 26	8 to 12	varies.	

The figures given are not intended as absolute limits, but rather as indicating the composition of by far the greater number of samples met with. It is seen that the pickled butters contain

a very large amount of salt in proportion to the water present. This fact is of great use in distinguishing them from samples which have been purposely watered.

It is frequently stated, even by "experts," that salt butter contains more water than fresh. Unless the term "salt butter" is applied exclusively to pickled butter, this statement is contrary to fact, as it is found that if, after churning, the butter is divided into two parts, one being worked as fresh, and the other immediately salted, the percentage of water is almost identical in the two samples; after standing, the salt butter will be found to lose water by running out, while the fresh butter undergoes no such loss. It will be found that salt butter when placed on the market contains on the average less water than fresh butter.

A high percentage of water does not appear to have any effect on the keeping qualities of the butter; a large percentage of solids not fat or curd seems to be distinctly inimical to its good preservation.

Speaking broadly, butters containing about  $13\frac{1}{2}$  per cent. of water have the best flavour. When the limits of 12 per cent. on one hand, and 15 per cent. on the other, are passed, a distinct falling-off in quality is usually found. To this rule, however, exceptions are numerous.

During very hot weather, if the butter is very soft when taken out of the churn, there is a difficulty in working the water out to a sufficient extent; during very cold weather the butter may be so hard that it cannot be efficiently worked. In both these cases the water may somewhat exceed 16 per cent. An organism has been described which produces changes in the cream which prevent the water from being worked out, but it is fortunately not frequently met with.

**The Analysis of Butter Fat.**—Preparation of the Fat for Analysis. — A portion of the butter is placed in a beaker and melted by exposing to a temperature not exceeding  $50^{\circ}$  C. ( $122^{\circ}$  F.). The water, with a considerable amount of the other constituents, sinks to the bottom, leaving the fat (containing, however, particles of curd in suspension) as an upper layer. If the butter be genuine, fresh, and well made, the melted fat will usually appear transparent; while if it be mixed with butter substitutes, rancid, or churned at a high temperature, or if it has been melted and re-emulsified, the fat frequently has a turbid appearance.

The fat, with as little as possible of the other constituents, is poured upon a dry filter, which is kept at a temperature sufficient to prevent the fat from solidifying; the clear fat, separated from all the other constituents of butter, except a trace ( $0.2$  per cent.) of water and lactic acid, if present, is collected in a dry

vessel. It is sometimes of importance to prepare the butter free from water. This may be done by shaking it with a little calcium chloride (free from lime) and filtering again. Chataway proposes to remove the water by stirring in a number of pellets of filter-paper, which have been dried in the water-oven. The author has found that, as far as the proportions of the volatile acids, insoluble acids and saponification equivalent are concerned, the fat is entirely unaffected by this treatment, though certain properties—*e.g.*, rise of temperature with sulphuric acid—are slightly affected, owing to removal of the water.

After filtration, the fat is rapidly cooled, so as to prevent partial solidification and to ensure the homogeneous nature of the sample.

**Stokes' Fat-clearing Process.**—Stokes uses a tube open at both ends (Fig. 41), the smaller and lower of which is closed with a rubber plug. The lower divisions each represent 1 per cent. For butter it is used thus:—

The butter is put in it (as the tube stands immersed in boiling water) up to the 15 c.c. mark, and when melted the tube is transferred to a centrifuge (800 to 900 revolutions per minute).\* The casein and water are driven into the narrow end and read off. The result is taken as all water. (This is used to *sort out* butters which come for water determination, and is not absolutely correct if checked by gravimetric methods.) Into the hot fat a wad of cotton wool is placed, and is slowly forced down by a wire (see Fig. 41), so as to prevent any cloudy particle from oozing up. The fat thus obtained (above the pad) is perfectly clear, practically dry, and ready for use.

**Recapitulation of Properties.**—The following recapitulation of the essential differences between butter fat and other fats likely to be used as substitutes or for adulteration will serve to show the basis of the methods employed in the analysis of butter. Butter fat is characterised by the presence, in considerable amount, of glycerides of the fatty acids of low molecular weight. The lowest and most important is butyric acid, but the whole of the members of the series  $C_nH_{2n-1}COOH$ , in which  $n$  is an odd number from 3 to 17, are present in butter fat. A considerable amount of acids of the oleic series, of which not much is known, is also present; of this series, the lower members are certainly absent, and the unsaturated acids are of a higher



Fig. 41.  
Stokes' Butter  
Clearing Tube.

\* Gerber disc is quite sufficient.

mean molecular weight than the saturated acids: it is probable that oleic acid is the chief representative of the series, and, possibly, higher homologues occur. It is not known with certainty whether acids of other series occur in butter fat. The alcohol present is almost entirely glycerol.

The pioneer in butter analysis was Otto Hehner, who demonstrated in 1872 that upwards of 5 per cent. of the fatty acids were volatile, and that the quantity of insoluble fatty acids was very much less than that yielded by nearly all other fats. The bulk of the methods at present in use are the legitimate outcome of Hehner's work. Perhaps the only method which is not derived from the first investigation of Hehner is that of von Hübl, who showed that, by the action of an alcoholic solution of iodine and mercuric chloride, a quantitative addition of halogen could be made to unsaturated glycerides, but in the simplification of this method Hehner has had a large share.

#### Estimation of Volatile Fatty Acids.

**Reichert Process.**—Hehner and Angell, after showing that butter contained more butyric acid than was (then) generally supposed, attempted to estimate this by distillation, but finally relinquished the method on account of discordant results, due largely to the bumping of the liquid and the use of too strong an acid.

Reichert proposed to saponify 2·5 grammes of butter with caustic soda and alcohol, evaporate off the alcohol, add 50 c.c. of water and 20 c.c. dilute sulphuric acid, and to distil 50 c.c. in a weak current of air. This method, though Reichert himself calls it Hehner's method, is now known as the Reichert process. He showed that butters took a constant amount of deci-normal alkali for neutralisation, while fats and artificial butters took very small quantities (0·3 c.c.), and coco-nut oil took about 3 c.c.; he proposed 14·0 c.c. as the mean for genuine butters, and 13·0 c.c. as a limit; he showed also that mixtures of butter and margarine took quantities of  $\frac{N}{10}$  alkali equivalent to the amount of butter they contained.

Meissl proposed to saponify 5 grammes of butter-fat in a flask of about 200 c.c. capacity with 2 grammes of caustic potash and 50 c.c. of 70 per cent. alcohol, and to drive off the alcohol on the water-bath. The resulting soap is dissolved in 100 c.c. of water, and 40 c.c. of dilute sulphuric acid (1 to 10) are added and the solution distilled with a few small pieces of pumice; 110 c.c. are collected, filtered, and 100 c.c. titrated with deci-normal alkali. In common with Reichert and the earlier experimenters, he used litmus as an indicator, but the superiority of

phenolphthalein for this purpose soon became apparent to many.

To the number of cubic centimetres of  $\frac{N}{10}$  alkali used one-tenth is added.

**Reichert-Wollny Process.**—Wollny, in a now classic memoir, studied the errors of the Reichert-Meissl process; these are:—

- (1) Error due to the absorption of carbonic acid during the saponification (may amount to + 10 per cent.).
- (2) Error due to the formation of esters during saponification (may amount to - 8 per cent.).
- (3) Error due to the formation of esters during the distillation (may amount to - 5 per cent.).

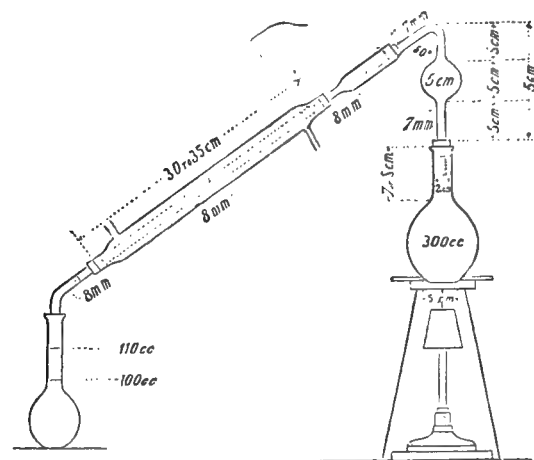


Fig. 42.—Reichert-Wollny Apparatus.

(4) Error due to the cohesion of the fatty acids during distillation (may in extreme cases amount to - 30 per cent.).

(5) Error due to the shape and size of the distilling vessel and to the time of distillation (may vary the results  $\pm 5$  per cent.).

To avoid these errors he lays down the following method of working:—

Five grammes of butter-fat are weighed into a round flask of about 300 c.c. capacity, with a neck 2 cm. wide and 7 to 8 cm. long; 2 c.c. of a 50 per cent. soda solution and 10 c.c. of 96 per cent. alcohol are added, and the flask heated for half an hour on the water-bath under a slanting inverted condenser; between the centre and the flask is a T piece, which is closed, the limb being turned upwards. At the expiration of half an hour the



limb of the T piece is opened and turned downwards, and the alcohol distilled off during a quarter of an hour; 100 c.c. of boiling water are added by the T piece, and the flask heated on the water-bath till the soap is dissolved. The solution is allowed to cool to  $50^{\circ}$  or  $60^{\circ}$ , 40 c.c. of dilute sulphuric acid (25 c.c. to a litre; 2 c.c. of soda solution should neutralise about 35 c.c. of this), and two pieces of pumice the size of peas are added. The flask is at once furnished with a cork carrying a tube 0.7 cm.



Fig. 43.—Caustic Soda Apparatus.

in diameter having, 5 cm. above the cork, a bulb 5 cm. in diameter; above this the tube is bent at an angle of  $120^{\circ}$ , and 5 cm. further on again at an angle of  $120^{\circ}$ ; this tube is joined to a condenser by an india-rubber tube. The flask is heated by a very small flame till the fatty acids are all melted, and the flame is then turned up and 110 c.c. distilled off in from twenty-eight to thirty-two minutes. The distillate is well mixed, and 100 c.c. are filtered off through a dry filter, 1 c.c. of a 0.05 per cent.

solution of phenolphthalein solution in 50 per cent. alcohol added, and the solution titrated with  $\frac{N}{10}$  baryta solution. To the figure thus obtained one-tenth is added, and the amount found by a blank experiment subtracted; the blank should not exceed 0.33 c.c.

In order to render this method more sensitive, if possible, for the detection of small quantities of butter in margarine, Hehner proposed the use of 5 c.c. only of alcohol, saponifying (almost instantaneously) in a closed flask, warming for five minutes with occasional shaking, and driving off the alcohol through a narrow tube in a cork, reduced pressure being applied towards the end, and the addition of 100 c.c. of water which has boiled at least half an hour. He finds the blank figure thus to be less than 0.1 c.c., and the same as that given by 100 c.c. of boiled water filtered through a dry filter; other fats and oils give less than 0.06 c.c., and no increase is observed in heating them on the water-bath with soda solution for two hours.

To facilitate the melting of the fatty acids, the author proposes lengthening the bulb tube, used by Wollny for distillation, above the bulb to 15 cm. and placing on it a small condenser, through which water is kept running during the melting of the acids, this being removed during distillation; the same results are obtained by the use of this apparatus as by Wollny's.

**The Polenske Process.**—For the detection of coco-nut oil in butter, Polenske has drawn up very careful directions for the carrying out of the Reichert-Wollny method, and his process includes a determination of the volatile insoluble acids in addition to the estimation of the volatile soluble acids. Alcohol must not be used for the saponification, and glycerol, which was first introduced by Leffmann and Beam, is employed: in fact, so far as the saponification is concerned the method is that of Leffmann and Beam.

Five grammes of butter fat are weighed into a 300 c.c. Jena glass flask (Fig. 44), and 20 grammes of glycerol are added, the weight of the glycerol being exact to 0.1 gramme; 2 c.c. of 50 per cent. caustic soda solution are added, and the saponification carried out by heating over a naked flame; at first a considerable amount of frothing takes place, and on continued heating the solution suddenly becomes clear, after which the flask should be set aside to cool slightly, and 100 c.c. of well-boiled distilled water added. A little ignited pumice, which has been powdered and sifted through muslin (Harris recommends that 0.1 gramme should be used), and 40 c.c. of sulphuric acid (25 c.c. per litre) added, and the flask immediately connected by a bulb tube to a condenser. The sulphuric acid solution should be of such

strength that 2 c.c. of caustic soda solution should neutralise about 35 c.c. The apparatus should have exactly the dimensions given in the figure, and the temperature of the cooling water should be such that the distillate enters the flask at about 20° C.

The flask is heated by a small flame till the fatty acids are just melted, and the flame then turned up to such a height that 110 c.c. of distillate are collected in from 19 to 21 minutes, when

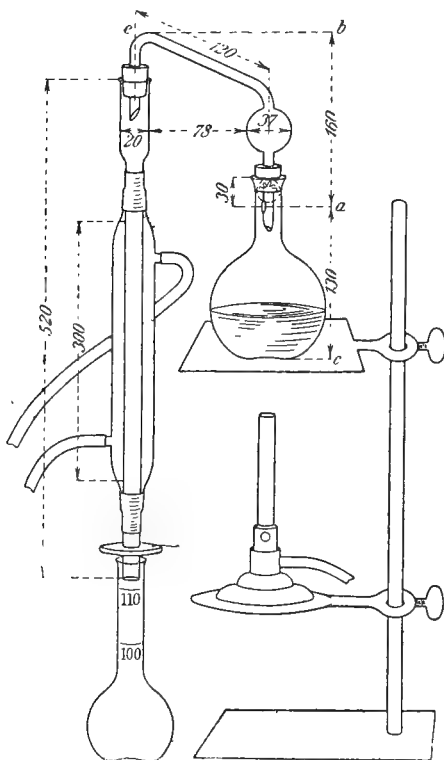


Fig. 44.—Polenske Apparatus.

the flame is immediately removed, and the flask replaced by a cylinder.

The flask is placed for ten minutes in water at 10° C., and after the physical condition of the insoluble fatty acids has been noted, the contents are well mixed, filtered, and 100 c.c. are titrated as in the Reichert-Wollny process.

The whole of the distillate is passed through the filter, and

the condenser is washed out with 18 c.c. of water, this being collected in the cylinder, poured into the flask, and used to wash the filter; the last 10 c.c. of filtrate should be neutralised by one drop of  $\frac{N}{10}$  alkali solution. The funnel is removed to the flask in which the distillate was collected, and three successive portions of neutral 90 per cent. alcohol (methylated spirit will serve) are poured through the condenser, cylinder, and filter, and the combined alcoholic filtrates titrated with  $\frac{N}{10}$  baryta solution; the number of cubic centimetres used gives the Polenske figure.

This method has been investigated by many observers, and it is found that the specification of the method is not quite sufficient to allow of absolutely concordant results being obtained. The author's investigations lead him to conclude that a very important factor, the temperature of the air around the flask, and the rate of conduction through the walls of the flask and bulb tube is left to chance, and he, therefore, proposes to support the flask on a piece of asbestos cardboard in which a hole 5 cm. in diameter is cut, and to surround the flask with a vessel in which water is boiled (see Fig. 45). Using this apparatus, it is found that with butters the length of time of distillation can be varied to a considerable extent without affecting the results, but with coco-nut oil the time given should be adhered to.

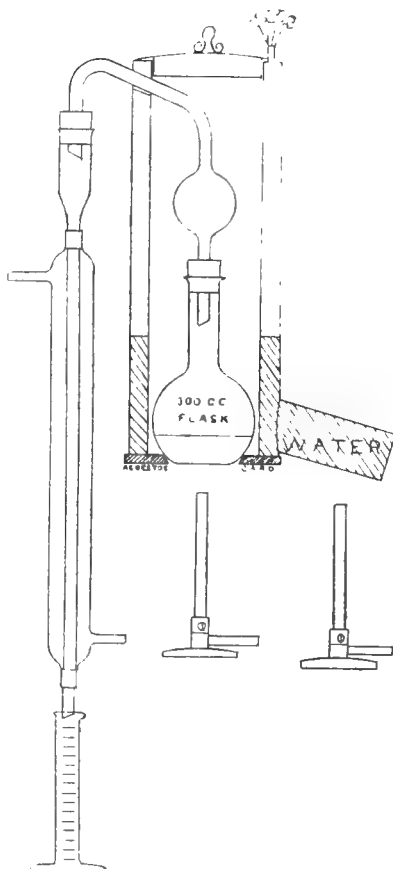


Fig. 45.—Steam Jacket.

Blank experiments should be performed; the figure for the soluble blank may be sometimes high (2 c.c.), without affecting the accuracy of the results.

The soluble acids obtained by the Polenske process are practically the same as those given by the Reichert-Wollny method.

The average figure for the soluble fatty acids is 28.4 c.c., but considerable variation is observed; in very rare cases figures slightly exceeding 40 have been obtained, while exceptional low figures of 14 have been observed as lower limits. These very high or very low figures are rare; low figures have usually been observed in cases where the butter is produced from the milk of cows near the end of lactation, especially if the animals have been exposed to inclement weather.

The average of the results of different observers shows that out of 100 samples

3	will probably yield Reichert-Wollny figures over 30 c.c.
86	„ „ „ „ between 26 and 30 c.c.
7	„ „ „ „ „ 25 and 26 c.c.
3	„ „ „ „ „ below 25 c.c.

All samples giving below 25 c.c. may be looked upon as suspicious, and should be further investigated.

The Polenske figure varies with the Reichert-Wollny figure, and the following table shows the relation:—

Reichert-Wollny Figure.	Polenske Figure.	
	Mean.	Maximum.
32	3.2	3.7
31	3.0	3.5
30	2.8	3.3
29	2.7	3.2
28	2.6	3.1
27	2.4	2.9
26	2.3	2.8
25	2.1	2.6
24	2.0	2.5
23	1.9	2.4
22	1.8	2.3
21	1.7	2.2

Coco-nut oil gives a Reichert-Wollny figure of 7 to 9 c.c., and a Polenske value of 16 to 18.5.

Should the Polenske figure exceed the maximum given in the table the quantity of coco-nut oil present may be deduced from the formula  $C = \frac{P - P'}{14.4} \times 100$ .

C = percentage of coco-nut oil.

P = Polenske figure.

P' = mean Polenske figure from the table for a figure equal to the Reichert-Wollny figure found + half the Polenske figure.

**Kirschner's Modification.**—To the 100 c.c. of the distillate of volatile fatty acids obtained in the Polenske process, which has been neutralised, 0.5 gramme of finely powdered silver sulphate is added, and after standing for an hour with occasional shaking, the liquid is filtered. 100 c.c. of the filtrate is placed in the distilling flask, 35 c.c. of water, which has been well boiled, and 10 c.c. of the sulphuric acid solution previously used added; a long piece of aluminium wire is placed in the flask, and 110 c.c. distilled as in the Polenske method; of the distillate 100 c.c. are titrated with  $\frac{N}{10}$  alkali, and after correction for the figure obtained in a blank experiment, the Kirschner figure is calculated by multiplying the number of c.c. of  $\frac{N}{10}$  alkali by 1.21 and by  $\frac{100+x}{100}$  (where  $x$  = the number of c.c. of alkali added to the first distillate for neutralisation).

The presence of coco-nut oil may be inferred if the Polenske figure is more than 1.0 c.c. higher than those given in the table below.

Kirschner figure.	Polenske figure.
26,	3.2
24,	2.6
22,	2.1
20,	1.6

The Kirschner method is of especial value in estimating the quantity of butter in mixtures containing small amounts when large quantities of other fats are present—*e.g.*, in margarine, which under the Margarine Act is not permitted to contain more than 10 per cent. of butter.

The percentage of butter may be calculated from the formula

$$B = K - \frac{(0.262 P^{0.63} + 0.09)}{0.242}$$

where B = percentage of butter fat,  
 K = Kirschner figure,  
 P = Polenske figure.

The following table will give the values of  $(0.262 P^{0.63} + 0.09)$  :—

P.	Value.	P.	Value.
0.5,	0.26	8,	1.06
1,	0.35	9,	1.13
2,	0.50	10,	1.21
3,	0.61	11,	1.28
4,	0.72	12,	1.34
5,	0.81	13,	1.41
6,	0.90	14,	1.47
7,	0.98	15,	1.53

A more simple formula, which gives nearly as good results, is

$$B = \frac{K - (0.1 P + 0.24)}{0.244}$$

Paal and Amberger distil the fatty acids from 2.5 grammes of butter in steam in a special apparatus, collect 200 c.c. of distillate in from 35 to 40 minutes, wash out the condenser by distilling 50 c.c. of neutral alcohol. The combined distillates are neutralised, and evaporated, made up to 50 c.c., and 2 to 4 c.c. of a 20 per cent. cadmium sulphate solution are added. The precipitate is collected on a Gooch crucible, washed with not more than 50 c.c. of water, dried, and weighed. The weight, calculated as milligrammes, gives the cadmium figure. The figure for butter usually lies between 70 and 90, and for coco-nut oil 441 to 470; higher results (100 or above) were obtained from the fat of milk yielded by cows fed on large quantities of coco-nut cake or beetroot leaves.

**Estimation of Saponification Equivalent**, or alkali necessary for complete saponification.

**Kœttstorfer's Method.**—Kœttstorfer proposed to utilise the fact that butter required a greater amount of alkali for its complete saponification than most other fats.

The method is performed as follows:—A standard alcoholic solution of sodium hydroxide is prepared by dissolving 25 c.c. of the 50 per cent. solution of caustic soda recommended by Wollny (p. 317) in 1 litre of strong alcohol; after a day's repose, during which a little salt settles out, the solution is clear and fit for use. This solution, which should be approximately semi-normal, is standardised against semi-normal hydrochloric acid. About 2 grammes of the fat are weighed out into a small flask, 25 c.c. of the alcoholic soda solution run in from a pipette, the flask connected with an inverted condenser, and the contents gently boiled for fifteen minutes. During the boiling the alcoholic soda solution is standardised; 25 c.c. of the solution are measured from the same pipette, which is allowed to drain for the same length of time as before, and titrated with semi-normal hydrochloric acid—a little phenolphthalein being added as indicator. The number of cubic centimetres of hydrochloric acid solution should be noted. It is advisable to perform this operation in duplicate. The flask containing the saponified fat is disconnected from the condenser, a few drops of phenolphthalein solution added, and the liquid titrated with semi-normal hydrochloric acid till the pink colour just disappears. The number of cubic centimetres used, subtracted from the number required by the 25 c.c. of soda solution alone, will give the equivalent of the

alkali required for saponification; this, multiplied by 0.02805, will give the weight as alkali calculated as potassium hydroxide, KOH; and the figure thus obtained multiplied by 1,000 and divided by the weight of fat taken will express the "potash absorption" as milligrammes of KOH per gramme of fat. It is also advisable to calculate the "saponification equivalent" (a term due to Allen), which is really an expression of the mean molecular weight. This is calculated from the number of cubic centimetres of normal acid, the definition of a normal solution being that it contains, or is equivalent to, in 1 litre a weight in grammes equal to the equivalent of a substance. It is, therefore, necessary to calculate the weight of fat which would be saponified by alkali equal to 1 litre of normal hydrochloric acid.

Let  $W$  = the weight of fat taken.

And  $V$  = the number of cubic centimetres of semi-normal hydrochloric acid equivalent to the alkali required for saponification. Then the saponification equivalent is expressed by

$$\frac{2,000 W}{V}$$

The relation between "potash absorption" ( $K$ ) and "saponification equivalent" ( $S$ ) is expressed by the formula

$$S = \frac{56,100}{K}$$

Instead of a pipette, the alcoholic alkali may be measured from a burette or automatic measuring apparatus, and the saponification may be conducted in a closed flask. An open flask or basin should not be used, as ethyl butyrate, an intermediate product of saponification (p. 43), is volatile; this would cause a low value to be obtained.

According to Kœttstorfer, the potash absorption varies from 221.5 to 233.0 in genuine butters, with an average of 227.0. His experience has been confirmed by numerous observers, and the limits have been extended 218 to 235. The saponification equivalent varies from 253.3 to 240.8, the average being 247.1.

Other oils and fats have a potash absorption of 190 to 199, with an average of about 195; or a saponification equivalent of 295.3 to 282.0, with a mean of 287.6.

Coco-nut and palm-nut oils yield, however, figures which are very different, 246.2 to 268.4.

**Estimation of the Baryta Value—Avé-Lallemant's Method.**—Two grammes of butter-fat are saponified as in the Kœttstorfer process, and the solution after neutralisation is evaporated to dryness, 10 c.c. of water is added, and the evaporation continued to remove the last traces of alcohol. The residue is



dissolved in boiling water, and the solution, which should measure about 180 c.c., poured into a 250 c.c. flask. The flask is placed on a boiling water bath, and 50 c.c. of  $\frac{N}{5}$  barium chloride solution are added, with constant shaking. After standing on the water-bath for fifteen minutes, the solution is cooled, and made up to 250 c.c. The solution is filtered, the first portions being poured back on to the filter till the solution runs through clear; the barium is estimated as sulphate in 200 c.c. of the clear filtrate. The strength of the barium chloride solution is estimated as sulphate in 10 c.c., and 25 c.c. of the alcoholic soda should be neutralised with hydrochloric acid, evaporated, and the residue taken up with water, and a little barium chloride added; any precipitate of barium sulphate due to impurities should be deducted from the amount of barium sulphate obtained from the 50 c.c. of barium chloride added.

The barium oxide found in 200 c.c. of the filtrate multiplied by 1.25 is deducted from the barium oxide added in 50 c.c. of solution (corrected, if necessary, for the blank), and the value calculated as milligrammes for 1 gramme of fat. This gives the barium oxide combined with the insoluble fatty acids. The potash absorption is calculated as milligrammes of barium oxide per gramme of fat by multiplying by 1.368, giving the total barium oxide, and the difference between the two values gives the barium oxide combined with the soluble fatty acids. To the last value 200 is added, and the value thus obtained is subtracted from the insoluble value. With butters the difference is always negative, varying from  $-23.8$  to  $-0.7$ , and averaging  $-9.6$ .

Other fats and oils give positive values; coco-nut oil giving a difference of 38.9 to 45.1, other oils and fats from 46.9 to 50.3. The soluble baryta value usually varies between 50 and 65 for butters, 54.1 to 57.6 for coco-nut oil, and is very small for other fats.

Fritzsche speaks well of this method, and shows that even butters low in Reichert-Wollny figures give normal results with the Avé-Lallemant process, and Bolton and Revis also strongly recommend it.

**Estimation of Soluble and Insoluble Fatty Acids — Hehner and Angell Method.**—The following method has been adopted by the American Association of Official Agricultural Chemists:—

*Reagents required.*—Deci-normal sodium hydroxide.

Alcoholic potash. Dissolve 40 grammes of good caustic potash, free from carbonates, in 1 litre of 95 per cent. redistilled alcohol. The solution must be clear.

Semi-normal hydrochloric acid accurately standardised.

*Indicator.*—One gramme of phenolphthalein in 100 c.c. of alcohol.

About 5 grammes of the sample are weighed into a saponification flask (250 to 300 c.c. capacity of hard, well annealed glass, capable of resisting the tension of alcohol vapour at 100° C.), 50 c.c. of the alcoholic potash solution added, and the flask stoppered and placed in the steam-bath until the fat is completely saponified. The operation may be facilitated by occasional agitation. The alcoholic solution is always measured with the same pipette, and uniformity further secured by allowing it to drain the same length of time (thirty seconds). Two or three blank experiments are conducted at the same time. In from five to thirty minutes, according to the nature of the fat, the liquid will appear perfectly homogeneous. Saponification being then complete, the flask is removed and cooled. When sufficiently cool, the stopper is removed and the contents of the flask rinsed with a little 95 per cent. alcohol into an Erlenmeyer flask of about 200 c.c. capacity, which is placed on the steam bath, together with the blanks, until the alcohol is evaporated. Titrate the blanks with semi-normal hydrochloric acid. Then run into each of the flasks containing the fatty acids 1 c.c. more of the hydrochloric acid than is required to neutralise the alkali in the blanks. The flask is then connected with a condensing tube, 3 feet long, made of small glass tubing, and heated on the steam-bath until the separated fatty acids form a clear stratum. The flask and contents are then cooled in ice-water.

The fatty acids having quite solidified, the liquid contents of the flask are poured through a dry filter into a litre flask, care being taken not to break the cake.

Between 200 and 300 c.c. of water are next brought into the flask, the cork with its condenser tube re-inserted, and the flask heated on the steam-bath until the cake of fatty acids is thoroughly melted. During the melting of the cake of fatty acids, the flask should occasionally be agitated with a revolving motion, but so that its contents are not made to touch the cork. When the fatty acids have again separated into an oily layer, the flask and its contents are cooled in ice-water and the liquid filtered through the same filter into the same litre flask. This treatment with hot water, followed by cooling and filtration of the wash water, is repeated three times, the washings being added to the first filtrate. The mixed washings and filtrates are next made up to 1 litre, aliquot parts titrated with the deci-normal sodium hydroxide, and the total acidity calculated. The number so obtained represents the volume of deci-normal sodium hydroxide neutralised by the soluble acids of the butter fat taken, *plus* that corresponding to the excess of the standard acid

used—viz., 1 c.c. The number is, therefore, to be diminished by 5, corresponding to the excess of 1 c.c. of semi-normal acid. This corrected volume, multiplied by 0·0088 gives the weight of (fatty acids calculated as) butyric acid in the amount of butter fat saponified.

The flask containing the cake of insoluble acids and the paper through which the soluble acids were filtered are allowed to drain and dry for twelve hours, when the cake, together with as much of the acids as can be removed from the filter paper, is transferred to a weighed glass dish. The funnel and filter are then set in an Erlenmeyer flask and the filter washed thoroughly with absolute alcohol. The flask is rinsed with the washings from the filter paper, then with pure alcohol, and these transferred to the glass dish, which is placed in the steam-bath. After the alcohol has evaporated, the residue is dried for two hours in an air-bath at 100° C., cooled in a desiccator, and weighed. It is heated in the air-bath for two hours more, cooled and weighed. If the two weighings are decidedly different, a further heating for two hours must be made. The residue is the total insoluble acids of the sample.

This method has been submitted to numerous modifications; Hager proposes to add a known weight of wax, picks out the lump, dries, and weighs it. Fleischmann and Vieth advise that the washing should be continued till at each succeeding washing the coloration produced by the addition of a few drops of litmus solution to a few cubic centimetres of the filtrate is not changed. Cassal has devised an ingenious flask, which has a tap at the bottom so that the liquid can be run off, leaving the fatty acids in the flask; washing can be thus much expedited, as hot water can be added, the fatty acids shaken up with the water, and the water run off.

The variation of insoluble fatty acids is from 85·5 per cent. (*Bell* and *Menozzi*) to 90·0 per cent. (*Reichardt*, *Cornwall*, and others) in genuine butters; the soluble fatty acids calculated as butyric vary from 7·0 per cent. to 4·0 per cent. Most other fats give about 95·5 per cent. of insoluble fatty acids and traces only of soluble fatty acids. Coco-nut and palm-nut oils are, however, exceptions to this, yielding from 82 to 85 per cent.

**Estimation of the Mean Combining Weight of the Insoluble Fatty Acids.**—The fatty acids are dissolved in alcohol, a little phenolphthalein added, and titrated with alcoholic alkali; when a pink colour is obtained a small excess is added (2 or 3 c.c.), the solution heated to boiling for ten minutes, and the excess titrated back, as in the Kœttstorfer process.

The mean combining weight of the fatty acids is calculated as the saponification equivalent. Direct titration of the fatty acids

does not yield correct results, owing to the formation of anhydrides on drying.

From the difference between the potash absorption, and the potash used for the insoluble fatty acids, an estimation of the soluble fatty acids can be obtained.

**Bömer's Phytosteryl Acetate Method for Detection of Vegetable Oils.**—While butter-fat and other animal fats contain cholesterol, vegetable oils are free from this alcohol, and contain phytosteryl. There is a difference in crystalline form and other properties between these alcohols, but the most striking and reliable difference is the melting point of the acetates.

Fifty grammes of butter fat are saponified with 100 c.c. of alcoholic caustic potash (200 grammes per litre), 200 c.c. of water are added, and the solution shaken out three times with ether, 500 c.c. being used for the first extraction, and 250 c.c. for the others. A larger amount of butter fat may be taken, and shaken out three times with an equal volume of warm alcohol, the alcohol evaporated, and the residue saponified with 20 c.c. of caustic potash, the solution diluted with 50 c.c. of water, and shaken out three times with 100 c.c. of ether; sufficient of the unsaponifiable alcohol is thus extracted for the test, and the manipulation is easier.

The ether is evaporated, and the residue again saponified with a little alcoholic potash solution, diluted with twice the volume of water, and shaken out with three or four successive quantities of ether. The ethereal solution is washed three times with 5 c.c. of water, filtered, and the ether evaporated.

The residue is transferred to a small basin (this may be accomplished by not quite distilling off the ether, and pouring the ethereal solution left into the basin), the solvent completely evaporated, and the residue treated with 2 or 3 c.c. of acetic anhydride, and covered with a watch-glass. The acetic anhydride is boiled for about a quarter of a minute, and the excess evaporated on the water-bath.

The acetate is dissolved in sufficient alcohol to prevent immediate crystallisation on cooling, and the solution left to crystallise; when about two-thirds of the alcohol has evaporated, the crystals are separated by filtration, washed with a very little 95 per cent. alcohol, redissolved in hot alcohol, and recrystallised; the recrystallisation is repeated five to seven times, and the melting point of the crystals determined after the third and subsequent recrystallisations.

Cholesteryl acetate melts at 115.4° C. (corrected), and phytosteryl acetate at 127°, if on continued recrystallisation the melting point rises above 117° the presence of phytosteryl acetate is certain.

Small quantities of paraffin wax are occasionally added, and this interferes somewhat with the test: if this is the case the alcohol should be crystallised from petroleum ether.

Although this method only detects vegetable oils, these are so largely used in the manufacture of margarine that this is a very reliable test.

### Specific Colour Tests for Adulterants.

**Baudouin's Test for Sesamé Oil.**—This test consisted, originally, in shaking the melted fat with a solution of cane sugar in hydrochloric acid. Villavecchia and de Fabris have modified this by using a solution of 2 grammes of furfuraldehyde in 100 c.c. of alcohol to replace the sugar; 10 c.c. of the melted fat are shaken thoroughly with 10 c.c. of hydrochloric acid and 0.1 c.c. of the reagent; a red coloration indicates the presence of sesamé oil. This reaction is very delicate, but is not entirely conclusive. Certain colouring matters—*e.g.*, turmeric and certain aromatic dyes—give a red coloration with hydrochloric acid alone, and, in the presence of these, sesamé oil cannot be detected, as the colour due to sesamé oil would be masked by that yielded by the dye. Furfuraldehyde and hydrochloric acid alone, after some time, yield a reddish colour; hence a slight pinkish tinge gradually appearing must not be taken to indicate sesamé oil. Spampani and Daddi have shown that the milk of goats fed with sesamé oil yields butter which gives this test. Hehner, Faber, and others were, however, unable to obtain it with butter prepared from the milk of cows fed on sesamé cake.

Sprink, Meyer, and Wagner modify this test, and extract 100 c.c. of butter fat twice with 20 to 30 c.c. of glacial acetic acid at 60° C., evaporate the acid, and test the residue by Baudouin's test.

To remove the colouring matters which give a red colour with hydrochloric acid they add 10 c.c. of alcohol and 5 c.c. of saturated baryta water to the residue, and evaporate. The residue is extracted several times with petroleum ether, which is evaporated, and the test performed on the residue. They claim that 0.1 per cent. of sesamé oil can thus be detected.

**Becchi's Test for Cottonseed Oil.**—This test was originally performed by heating the fat with a solution containing silver nitrate, alcohol, ether, nitric acid, amyl alcohol, and rape oil. The reagent has been frequently modified. Bevan prepares the reagent by boiling silver nitrate with amyl alcohol, and cooling the solution. Equal parts of this solution and of the fat are heated in a test tube on a boiling water-bath for ten minutes; a brown or black coloration indicates cottonseed oil. This test

is by no means conclusive of the presence of added cottonseed oil, as the milk of cows fed on large proportions of cotton cake yields butter which will give a brown coloration.

**Halphen's Test for Cottonseed Oil.**—One to 3 c.c. of fat are mixed with equal volumes of amyl alcohol, and carbon disulphide containing 1 per cent. of sulphur, and heated in a boiling brine bath for ten to fifteen minutes. A red or orange colour shows the presence of cottonseed oil. If no reaction is obtained a little more carbon disulphide should be added and the heating repeated.

**Wellmann's Test for Vegetable Oils.**—The reagent consists of a solution of sodium phospho-molybdate. It may be prepared by saturating 5 grammes of molybdic acid with sodium carbonate solution, adding 1 gramme of sodium phosphate, evaporating to dryness and fusing. The mass is dissolved in boiling water, and concentrated nitric acid (5 to 7 c.c.) is added till the yellow shade is permanent. The solution is then made up to 100 c.c.

One gramme of fat is dissolved in 5 c.c. of chloroform and shaken with 2 c.c. of the reagent for one minute. A green coloration (changing to blue on addition of ammonia) is formed in the aqueous layer when vegetable oils are present. Coconut oil is not, however, detected by this means.

### Behaviour of Butter Fat with Solvents.

**Critical Temperature of Solution.**—Crismer recommends that several drops of the melted and filtered fat be introduced into a small tube 10 millimetres in diameter and 100 to 120 millimetres long by means of a capillary pipette. An equal volume of alcohol is added and the tube sealed and fastened by a platinum wire to the bulb of a thermometer; it is then heated in a bath of sulphuric acid till the meniscus separating the two layers becomes a horizontal plane. At this point the thermometer is withdrawn from the bath, and turned sharply two or three times until the liquid becomes homogeneous, after which it is replaced and the temperature allowed to fall slowly, the thermometer and tube being constantly shaken. The temperature at which a marked turbidity is produced in the liquid is the critical temperature of dissolution. If absolute alcohol be employed an open tube may be used.

The alcohol used should have a specific gravity of 0.7967 at 15.5 C.; if the specific gravity differs 0.106° should be added or deducted for each 0.0001 below or above 0.7967.

When examining butter fat it is necessary to estimate also

the acidity by titrating 2 c.c. with  $\frac{N}{20}$  alkali and adding the figure thus obtained to the critical temperature.

Crismer has shown that the critical temperature varies with the percentage of insoluble fatty acids. Table LXXVIII. will show the variations.

TABLE LXXVIII.

Critical Temperature. Alcohol 0.8195 sp. gr.	Critical Temperature. Absolute Alcohol.	Insoluble Fatty Acids.
Below 100°	Below 54°	86 to 88
100° to 108°	54° to 62°	88 „ 90.5
108° „ 118°	62° „ 72°	90 „ 93.3
118° „ 124°	72° „ 78°	93 „ 95.5

Butter usually has a critical temperature of 53° to 57°, and in exceptional cases 59°.

The Reichert-Wollny figure may be calculated by the formula

$$\begin{aligned} \text{R-W} &= 129^\circ - \text{critical temperature (with alcohol of sp. gr. 0.8195).} \\ &= 83.5 - \text{ „ „ „ ( „ absolute alcohol).} \end{aligned}$$

**Valenta's Method—Solubility in Acetic Acid.**—Valenta showed that there was an enormous difference in the temperatures at which various fats and oils dissolved without turbidity in acetic acid. By the work of Allen and Hurst it was shown that the strength of acid made a considerable difference. Chattaway, Pearmain, and Moor have investigated the subject and recommend the following procedure for butters:—2.75 grammes of butter fat which has been previously dried (preferably by mixing with dried pellets of filter paper and filtering through a dried filter) are weighed into a test tube provided with a stopper; 3 c.c. accurately measured of acetic acid (containing 99.5 per cent.  $\text{C}_2\text{H}_4\text{O}_3$ ) are run into a tube, and this is placed in a beaker of water. The water is gradually heated and the tube shaken till the solution is clear; the water is then allowed to cool gradually, and the temperature at which a turbidity appears in the tube is measured by a thermometer held in close proximity. By slightly warming up and cooling down again, a second determination can be obtained.

Undue heating of the sample should be avoided, both in the preparation of the fat for analysis and during the performance of the test.

They give figures as follows :—

	Maximum.	Minimum.	Average.
Butter fat,	39·0° C.	29·0° C.	36·0° C.
Margarine,	97·0° C.	94·0° C.	95·0° C.

E. W. T. Jones prefers, instead of using an acid of estimated strength, to test it against a standard sample of butter, and to dilute the acid so that it gives a temperature of turbidity of 60°. Margarine then gives about 100°.

Hehner has found that this test depends almost entirely on the glycerides of the saturated fatty acids present, as these are almost completely deposited on allowing the acetic acid to cool.

### The Iodine and Bromine Absorption.

**Von Hübl's Method; Wijs' Modification.** This method depends on the fact that acids of the oleic, linolic, and linolenic series contain unsaturated bonds, and, under suitable conditions, combine with iodine and bromine.

For the iodine absorption, it has been shown that the presence of iodine chloride is necessary.

The process is worked as follows :—

*Reagents.*—13 grammes of iodine are dissolved in pure 99 per cent. acetic acid, and chlorine passed in till the strength of the solution is doubled: this point is sharply shown by a change of colour.

Deci-normal sodium thiosulphate solution. Dissolve 25 grammes of pure sodium thiosulphate solution and 1 gramme of salicylic acid in 1 litre of water. Allow this to stand a few days and filter. This solution is permanent and does not alter in strength. To standardise the solution, about 0·25 gramme of resublimed iodine is accurately weighed in a small stoppered flask, about 2 grammes of potassium iodide and 2 c.c. of water are added, and the flask gently shaken till the iodine is dissolved. The iodine solution is diluted with water, transferred to a larger flask, and titrated with the sodium thiosulphate solution till the yellow colour just disappears. This operation is repeated two or three times. The mean strength of the iodine deduced from these experiments is noted on the label of the bottle.

A 10 per cent. (approximate) solution of potassium iodide and a starch paste solution, made by pouring an emulsion of 1 gramme of starch in a little cold water into 200 c.c. of boiling water, and boiling for ten minutes: if a little mercuric iodide be added, this solution is permanent.

The process is performed as follows :—About 0·5 gramme of the fat is accurately weighed in a glass stoppered flask holding at least 100 c.c.: 10 c.c. of carbon tetrachloride are added, and



the flask gently rotated till the fat is dissolved; 20 c.c. of the iodine chloride solution is next added and the whole well mixed. The flask is now put aside for half an hour. At the same time one or more blanks—*i.e.*, flasks containing 10 c.c. of carbon tetrachloride and 20 c.c. of iodine chloride solution should be put aside with the tests.

After half an hour, 10 c.c. of potassium iodide solution are added to each flask, and the contents are washed out into a larger stoppered bottle with distilled water. The standard thio-sulphate solution is run in with continued shaking till only a faint yellow colour remains; a little starch paste is added, and the thiosulphate solution run in, drop by drop, till the blue colour disappears. The quantity of thiosulphate solution used for the flask in which the sample was placed subtracted from the mean of the blanks will give the equivalent of the iodine absorbed. This, multiplied by the strength of the solution, will give the weight of iodine. By multiplying this by 100 and dividing by the weight of fat, the percentage of iodine absorbed is obtained.

The following examples will make the mode of calculation clear:—

<i>Experiment 1.</i>	Weight of fat taken,	0.5006	gramme.		
	Titrated with	26.48	c.c. of thiosulphate solution.		
,, 2.	Weight of fat taken,	0.4991	gramme.		
	Titrated with	26.55	c.c. of thiosulphate solution.		
<i>Blank No. 1</i>	took	43.35	"    "		
,, 2	"	43.45	"    "		
	Mean,	43.40	"    "		

1 c.c. of the thiosulphate solution was equal to 0.001187 gramme of iodine.

Therefore 0.5006 gramme absorbed  $(43.40 - 26.48) \times 0.001187$  gramme of iodine.

= 0.2008 gramme or 40.11 per cent.

= 0.4991 gramme absorbed  $(43.40 - 26.55) \times 0.001187$  gramme of iodine.

= 0.2000 gramme or 40.07 per cent.

**Bromine Absorption.**—Instead of using the iodine chloride solution, a solution of bromine in chloroform, or, what is far preferable, carbon tetrachloride may be used.

Four c.c. of dry bromine (this is best dried by shaking bromine with anhydrous calcium chloride, decanting, and distilling from a small stoppered Würtz flask fitted with a good condenser) are dissolved in 1 litre of dry chloroform or carbon tetrachloride. The process is performed as above described, except that there is no need to wait before titrating; this may be performed at once. 20 c.c. of the bromine solution are substituted for the 20 c.c. of iodine chloride solution. It is advisable also to increase the amount of potassium iodide solution added to 20 c.c. or more.

**Gravimetric Method of H<sub>eh</sub>ner.**—H<sub>eh</sub>ner has proved that the bromine absorbed may be estimated gravimetrically. The fat is weighed in a small basin, dissolved in carbon tetrachloride, a solution of bromine in carbon tetrachloride added, and the excess of bromine and the carbon tetrachloride evaporated on a water-bath in a good draught cupboard. The residue is freed from the last traces of bromine by adding several successive portions of carbon tetrachloride and evaporating them, and, finally, by drying in an air-bath maintained at a temperature somewhat above 100° C. The increase in weight multiplied by  $\frac{127}{80} = 1.5875$  will give the iodine absorption.

**Thermometric Method.**—H<sub>eh</sub>ner and Mitchell have also devised a most ingenious means of rapidly and accurately calculating the iodine absorption, founded on the fact that when 1 molecule of bromine combines with 1 molecule of unsaturated fat a definite amount of heat is liberated.

One gramme of fat is weighed into a jacketed test tube about 1 inch in diameter and 6 inches long, from the jacket of which the air has been exhausted. 10 c.c. of chloroform are added, and the temperature noted; 1 c.c. of bromine is added, the mixture stirred with the thermometer, and the highest point to which the temperature rises is recorded. The difference between the initial and the highest temperatures multiplied by a factor will give the iodine absorption. The factor must be found empirically, as it varies slightly with each apparatus, thermometer, etc. It can be easily ascertained by submitting a few fats of known and varying iodine absorption to this test, and taking the mean relation between the difference of temperatures noticed and the iodine absorption. H<sub>eh</sub>ner and Mitchell found in their experiments that the temperatures multiplied by 5.5 gave the iodine absorption. The thermometer used should be a good one, capable of reading to  $\frac{1}{10}^{\circ}$  C.; the same thermometer and test tube should always be used. When the apparatus has been once standardised this method forms a rapid means of estimating the iodine absorption.

The bromine should be measured in a 1 c.c. pipette, having a bulb filled with soda lime in its upper portion; unless this is done, the fumes of the bromine are apt to prove very unpleasant.

The bromine thermal test for oils and fats is modified by Gill and Hatch by taking sublimed camphor as the standard substance, and dividing the rise with the fat by that with the camphor to a "specific temperature reaction." They find that this multiplied by 17.18 gives a figure very close to the iodine absorption.

### Heat Evolved by Hydrolysis by Sulphuric Acid.

**The Maumené Test.**—When fats are acted on by strong sulphuric acid a series of reactions takes place. The fat is first split up to fatty acids and glycerol, which combines with the sulphuric acid. The saturated fatty acids (stearic series) are not further affected, but the unsaturated fatty acids undergo sulphonation and other changes. Of these, the oleic series, which has only one saturated bond, is acted on to a less degree than the linolic and linolenic series, which contain two or three bonds respectively. Each of the actions which takes place evolves heat, and, by measuring the rise of temperatures which takes place, an index of the total amount of heat evolved is obtained.

**Modification by Thompson and Ballantyne.**—This test is due to Maumené, who measured the heat evolved on mixing 10 c.c. of sulphuric acid with 50 grammes of an oil or fat. His original method was faulty, in that he did not rigidly prescribe any strength of acid nor form of apparatus. Thompson and Ballantyne propose to compare the heat evolved on mixing 10 c.c. of sulphuric acid with 50 grammes of oil or fat with that evolved by mixing 10 c.c. of the same acid with 50 grammes of water in the same vessel. Taking the heat evolved by the water as 100, they term the figure obtained the "Specific Temperature Reaction" of the oil or fat. This method gives a very fair means of correcting for the differences of temperature observed when working with acids of differing strength and in different apparatus, and is convenient in practice.

The author has examined with some care the results obtained by the use of acids of different strengths. The following series will show that the effect of strength of acid can be corrected by a very simple calculation. These results were obtained with a pure olive oil.

TABLE LXXIX.

Strength of Acid.	Rise of Temperature.	Calculated for 100 per cent.
100·00 per cent. H <sub>2</sub> SO <sub>4</sub> , .	47·2°	47·2°
97·50 " " .	41·1°	46·6°
96·64 " " .	39·3°	46·6°
94·93 " " .	35·6°	46·6°
93·49 " " .	32·6°	46·8°
92·85 " " .	31·4°	46·9°
92·04 " " .	29·0°	46·3°
		Mean, 46·7°

The results calculated for 100 per cent. acid were obtained by the following formula :—

$$\text{Rise of temp.} \times \frac{21.5}{\text{per cent. H}_2\text{SO}_4 - 78.5} = \text{rise of temp. with 100 per cent. acid.}$$

**Richmond Modification.**—The author calculates the “Relative Molecular Maumené” figure by the following formula :—

$$\text{R.M.M.} = R \times \frac{21.5}{x - 78.5} \times \frac{20 + h}{20} \times \frac{19.5}{K};$$

where

R = observed rise of temperature.  
 x = percentage of H<sub>2</sub>SO<sub>4</sub> in acid.  
 h = heat capacity of apparatus.  
 K = potash absorption (per cent.).

25 grammes of oil were used and 5 c.c. of acid.

The method is performed as follows :—A beaker about 1½ inches in diameter and 3 inches deep is fitted, by means of a ring of cork, inside a slightly larger beaker; this is placed in a third still larger beaker, and the intermediate space packed with cotton wool. The heat capacity of this apparatus is next determined; about 10 grammes of water are placed in the innermost beaker and the temperature noted; about 25 grammes of water of higher temperature are added, and the final temperature noted. The heat equivalent of the apparatus is calculated by the formula—

$$h = \frac{y \times (b - c)}{c - a} - x;$$

where

h = heat capacity of apparatus.  
 x = weight of water placed in beaker.  
 y = weight of hot water added.  
 a = temperature of apparatus.  
 b = temperature of hot water.  
 c = final temperature after mixing.

The following experiments will show the nature of the value of *h* :—

<i>x</i> . grammes.	<i>a</i> .	<i>y</i> . grammes.	<i>b</i> .	<i>c</i> .	<i>h</i> . grammes.
10.0	16.0°	26.5	39.0°	31.2°	3.60
10.0	17.5°	23.5	50.5°	38.5°	3.43
0.0	20.0°	35.1	42.0°	40.0°	3.51

When the innermost beaker holds about double the volume of the oil and acid, its weight multiplied by 0.15 will give its heat capacity with considerable accuracy; the beaker used above weighed 23.2 grammes; this multiplied by 0.15 gives 3.48.

Twenty-five grammes of filtered and dried fat are weighed into the beaker, and the apparatus with thermometer, together

with the acid to be used in a small bottle, and a 5 c.c. pipette, are placed in an incubator kept at 30° C. for at least half an hour, and the temperature noted. 5 c.c. of acid are added, and the mixture well stirred with the thermometer, till the temperature ceases to rise. The difference between the initial temperature and that finally attained is taken as the rise of temperature, and the Relative Molecular Maumené figure is calculated from this.

The R.M.M. of butters varies from 33·0° to 34·5°, with a mean value of 34·0°. The ratio  $\frac{\text{R.M.M.} - 10}{\text{iodine absorbed}}$  has been about 0·633, varying from 0·615 to 0·649. Any increase in this ratio may be taken to indicate adulteration by vegetable oils.

This method is occasionally useful, but is rather troublesome, and cannot be well recommended, except as an additional test in cases of doubt. It is very important that the fat be well dried.

**The Physical Examination of Butter Fat.**—The most important physical properties are microscopic examination under polarised light, density, refractive index, viscosity, and behaviour on melting.

**Microscopic Examination under Polarised Light.**—This method is founded on the fact that when a crystalline substance is placed between two crossed Nicol prisms the light undergoes rotatory polarisation; the rays that would normally vibrate in the plane, which would cause total reflection, are caused to vibrate in a plane inclined to this, and the light consequently passes through the second Nicol prism. Substances which have no crystalline structure do not cause any interference with the plane of vibrations.

This method was first applied by Campbell Brown to detect adulteration of butter with foreign fat. The fat of milk when churned into butter is devoid of crystalline structure. The fats of which margarine is composed, having been melted and cooled, usually acquire a more or less pronounced crystalline form.

It has been studied by Taylor, Pizzi, and others, and is fairly reliable. The following are the sources of error:—The presence of salt, salicylic acid, and other crystalline substances added to butter as preservatives, or accidentally mixed with it, will cause the light to pass, and may be mistaken for crystalline fat; but a simple microscopical examination will usually reveal the nature of particles of this nature, and an experienced observer will rarely be misled. Butter which has been melted, re-emulsified, and re-churned will behave to this test as margarine, though no similar appearance is noticed in butter which has been kept just below the melting point for some length of time. Margarine which has been prepared by emulsifying the fat with skim milk

with a good emulsor, separating the cream, and churning this with ordinary cream, behaves as butter, and Pizzi has succeeded in adding 30 per cent. of foreign fat to butter in this way without being able to distinguish it. Finally, rancid butter, and butter which has been at once churned from pasteurised cream at a low temperature, may sometimes give an appearance resembling margarine. Butter prepared from clotted cream shows many crystalline particles (Fig. 46).

It is apparent that this test must be used with reservation, but it is without doubt of use as corroborative evidence in cases where other analytical data are not absolutely conclusive.

The method is carried out as follows:—The outer portions of a piece of butter are removed, and a piece about the size of a pin's head is transferred from the freshly exposed surface to a clean microscope slide. A cover glass is placed on the top, and

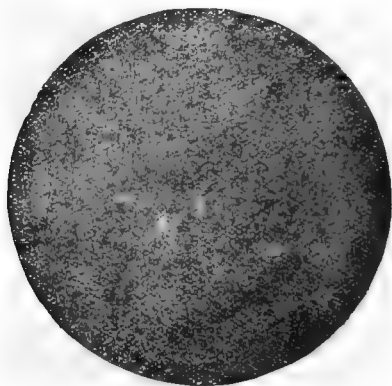


Fig. 46.—Butter under Polarised Light.

the butter spread out by gentle pressure on the upper surface of the cover. The slide is placed on the stage of a microscope fitted with crossed Nicol prisms, and examined with a 1-inch objective or higher power. To exclude light from the upper surface a blackened cardboard tube may be placed over the slide in such a manner that the objective dips into it, and the light falling on the upper portion of the slide is cut off. When pure butter is examined the field is uniformly dark, and only with the greatest difficulty can any structure be distinguished. When margarine is present certain portions of the field have a bright appearance, and indistinct crystalline forms can be made out. If any distinct and bright crystals are seen, the Nicol prisms should be turned parallel, and the slide examined in that spot in order to see whether salt or other crystalline matter is present; there is not

much difficulty in distinguishing this owing to its great refractive power. The slide should be moved about to examine all parts of it, as, in cases of small amounts of adulteration, the margarine is not equally distributed throughout, and two or more portions from different parts of the sample may be examined.

As a check, a selenite plate (a crystalline form of calcium sulphate, which possesses the property of rotatory dispersion to a large extent) is next placed under the slide, the microscope focussed, and the sample again examined. In this case the slide will be uniformly illuminated when the prisms are crossed, but will appear coloured; the colour depends on the thickness of the selenite and the position of the Nicol prisms, but when pure butter is examined the whole of the field appears of one colour. When margarine is under observation certain parts of the field are seen to be of a different colour.

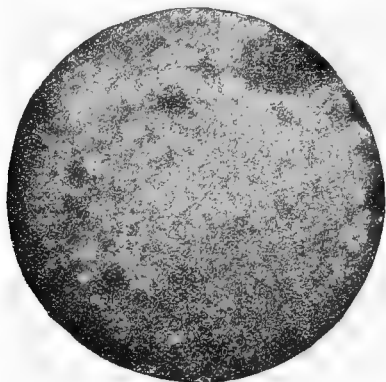


Fig. 47.—Margarine under Polarised Light.

This modification is, when used by persons of absolutely normal vision, quite as delicate as the examination without selenite, but it cannot be generally recommended, as the perception of colour is a sense in which many people—more than is commonly supposed—are somewhat deficient, though not absolutely colour blind. The usual colours which selenite plates are constructed to give—red and green—are those which are least easily distinguished by the majority of those who suffer from weak colour perception. It is advisable, therefore, never to omit the examination without a selenite plate.

It is, of course, essential to employ a good microscope, as any illumination of the slide, except by light which has passed through the polariser, will prevent the extinction of the field on crossing the Nicol prisms. Though it is impossible in practice

to secure an absolutely dark field, this can be done with a good instrument and a cardboard tube over the slide with a near approach to completeness. Any marked illumination of the field when the Nicol prisms are crossed will greatly impair the delicacy of the test.

**Microscopic Examination after Treatment with Solvents.**—A. Zega has suggested the following process:—The sample is melted and filtered into a test tube, which is kept for two minutes in a boiling water-bath. By means of a hot pipette 1 c.c. is measured into a 50 c.c. stoppered tube containing 20 c.c. of a mixture of 6 parts of ether, 4 of alcohol, and 1 of glacial acetic acid. The whole is well shaken, and allowed to cool in water at 15° or 18° C. Pure butter remains clear, and only gives a slight deposit after standing one or one and a half hours. Margarine shows a deposit in one or two minutes, and in ten minutes yields a copious precipitate. Mixtures of butter with 10 per cent. of margarine begin to separate in about fifteen minutes. As soon as a few solid particles have fallen, they are withdrawn and examined under the microscope. Genuine butter appears in long, very narrow crystalline rods, often pointed at the ends, sometimes bent, and usually joined together centrally into more or less symmetrical open stars. Margarine crystals consist of bundles of minute needles packed closely together into circles, sheaves, or dumb-bell-like masses.

Mercier digests 1 c.c. of melted fat with 30 c.c. of 90 per cent. alcohol for five minutes at 50° to 55° C., the fat and alcohol being well mixed; and after fifteen to twenty minutes' standing 20 c.c. of the alcoholic solution are withdrawn, cooled to 30° to 40° C., and filtered; the fat is then slowly allowed to crystallise, and the crystals filtered out and examined by the microscope. If coco-nut oil is present, little round bunches of crystals consisting of long needles are observed.

Hinks has also devised a process, which depends on the crystallisation of coco-nut oil from alcohol; 5 c.c. of butter fat are dissolved in 10 c.c. of ether in a test tube, which is then packed with ice. After half an hour, the clear ethereal solution is filtered through a pleated filter, the filtrate evaporated, and the residual fat boiled with three or four times its volume of alcohol (96 to 97 per cent. by volume—the strength is important). Complete solution takes place at the boiling point, and the liquid is allowed to cool to room temperature, and then placed in water at 5° C. for fifteen minutes. The alcoholic solution is rapidly filtered into a tube, which is kept at 0° C. for two or three hours. The flocculent deposit is examined by the microscope, using a magnification of 250 to 300 diameters, preferably on a cooled stage; butter deposits glycerides in round granular masses, but coco-



nut oil yields fine needle-shaped crystals; a mixture shows the granular butter spores, with numerous fine, almost feathery, crystals generally attached to the butter granules.

Five per cent. of coco-nut oil can be detected by this test.

### The Density of Butter Fat.

Butter fat, on account of the presence of glycerides of low molecular weight, has a greater density than the fats used for its adulteration. As it is more convenient and exact to take the density of a liquid than of a solid, the fat is almost invariably melted and the density determined at a temperature above its melting point.

The methods of estimating the density have already been discussed under the "specific gravity of milk" (p. 60), and (except that for butter a temperature considerably higher than that at which the density of milk is taken is employed) the same methods are employed.

**Expansion.**—Two questions arise: At what temperature shall the density of butter be taken? How shall the results be expressed? The experiments of Skalweit have indicated the most favourable temperature. He took the densities of butter and margarine at various temperatures from 35° C. to 100° C., using Koch's incubator to keep a constant temperature.

His figures are as follow:—

TABLE LXXX.

Temperature.	Butter.	Margarine.	Difference.
35° C.	0·9121	0·9017	0·0104
50° "	0·9017	0·8921	0·0096
60° "	0·8948	0·8857	0·0091
70° "	0·8879	0·8793	0·0086
80° "	0·8810	0·8729	0·0081
90° "	0·8741	0·8665	0·0076
100° "	0·8672	0·8601	0·0071

**Mode of Expressing Results.**—These figures clearly show that as the temperature rises the densities of butter and margarine tend to approach one another; the widest difference occurs at 35° C.; he, therefore, recommends that this temperature be adopted as the temperature at which the densities of butter should be determined.

In England a large number of determinations have been made by J. Bell, Allen, Muter, and others at a temperature of 100° F.

(37.8° C.), and this temperature is very near that found by Skalweit to give the largest difference.

In America the temperature of 40° C. is used to a considerable extent, and the author has taken a large number of densities at 39.5° C. (owing to the use of a thermometer which read 0.5° too high).

Estcourt proposed to use the temperature of boiling water (which he found to raise the butter fat to 97.8° C. [208° F.]), as being easily attained. Allen and others have warmly recommended this temperature, and find no difficulty in bringing the temperature up to 99° C.

There is a certain amount of confusion as to the manner in which densities are expressed. To ascertain the true density, the weight of a certain volume of fat should be divided by the weight of the same volume of water at the same temperature and multiplied by the density of water at that temperature. This is very rarely done, so that few published figures are true densities.

Muter gives the term "actual density" to the weight of a certain volume of fat divided by the weight of the same volume of water at the same temperature; densities expressed thus are usually denoted by the symbol  $D \frac{37.8^\circ}{37.8^\circ}$  for density at 37.8°, or  $D \frac{35^\circ}{35^\circ}$  for density at 35°, and the true density is often expressed as  $D \frac{37.8^\circ}{4^\circ}$  or  $D \frac{35^\circ}{4^\circ}$ .

It is usual when densities are taken at the temperature of boiling water to express them in a different way. The weight of a certain volume of fat is divided by the weight of water displaced by a piece of glass which occupies the same volume at the same temperature, when it is cooled down to 60° F. (15.5° C.). This mode of expression may be denoted by the formula  $D \frac{100^\circ}{15.5^\circ}$  in glass. Though apparently cumbersome this method of expressing results has certain advantages, as the instrument with which the densities are taken can be standardised at 60° F. (15.5° C.), and can then be used at any temperature without requiring to be restandardised. It must be remembered that, though the expansion of glass is very nearly constant, it is not quite so, and over a range of 85° C. appreciable differences may occur in the expansion of different instruments. If the glass be not well annealed, internal strains are set up, and these may be so accentuated at high temperatures as to cause distortion and change of volume. It will be readily seen that the method of taking the apparent density in glass at the temperature of

boiling water is liable to greater experimental error than determinations at lower temperatures, and, as the experiments of Skalweit have shown, that the effect of experimental error is magnified at 100° C., owing to there being a smaller difference between the densities of butter and margarine at this temperature than at lower ones. It is desirable not to adopt this method where accuracy is, as it always should be, a desideratum.

On the whole, it seems desirable to adopt 100° F. (37·8° C.) as the standard temperature at which determinations should be made, because it is sufficiently near Skalweit's minimum to give a large difference between butter and margarine, and because a large number of experiments on genuine butters have already been made at this temperature.

**Determination.**—The density of butter is best determined by the pycnometer. This is filled with distilled water, and the weight of the water which it holds at 37·8° determined. After drying, by placing in the water oven and drawing a current of air through it, it is filled with the fat and placed in water at 37·8° C. till the volume is constant; the temperature must be accurate to 0·1° C. if the result is required to be exact to the fourth place of decimals. The weight of fat divided by the weight of water will give the density at  $\frac{37\cdot8^\circ}{37\cdot8^\circ}$ .

The Westphal balance may be employed, the apparent density of water at 37·8° must be determined, and the density of fat indicated by the instrument divided by this to obtain the density at  $\frac{37\cdot8^\circ}{37\cdot8^\circ}$ .

The density is also sometimes determined by a hydrometer. If this instrument be used, it should be tested in fats of known density, and its indications thus controlled. A. Meyer states that the height of the meniscus depends somewhat on the barometric pressure, but the error due to this cause is not likely to exceed the experimental error of reading. Should the temperature not be exactly 37·8° C., a correction of 0·0007 for each degree may be added for temperatures above and subtracted for temperatures below, 37·8° C.

If it be desired to take apparent densities at  $\frac{100^\circ}{15\cdot5^\circ}$  in glass, the instruments should be standardised at 15·5°, and the density determined as above.

The author has used a bulb of specific gravity 0·865 at 15·5° for the purpose of determining rapidly an approximate density. A test tube is filled with the fat, the bulb dropped in, and the tube placed in boiling water. If the bulb floats at the top, the

density is above 0·865; and if it sinks, it is below. This has proved a fairly good rough test.

The limits observed for pure butter are :—

	Maximum.	Minimum.	Mean.
At $\frac{37.8^\circ}{37.8^\circ}$	0.9140	0.9094	0.9118
At $\frac{100^\circ}{15.5^\circ}$ (in glass)	0.8685	0.8650	0.8667

The fats usually employed as adulterants have a density at  $\frac{37.8^\circ}{37.8^\circ}$  of 0.901 to 0.905, mean 0.903; and at  $\frac{100^\circ}{15.5^\circ}$  (in glass) of 0.860 to 0.863, mean 0.861.

Certain oils have, however, a higher density; thus, at  $\frac{100^\circ}{15.5^\circ}$  (in glass)—

Palm-nut oil has a density of	0.873
Coco-nut oil,	0.874
Cotton-seed oil,	0.8725
Arachis oil,	0.863
Sesamé oil,	0.8675

Of these oils, palm-nut and coco-nut oils can be readily detected (see *Fat*), while the other oils cannot be used alone, but must be mixed with fats of less density to obtain the necessary consistency. With the reservation that the oils mentioned above would cause somewhat abnormal results, the determination of the density of butter is a very useful test, and, though not reliable as a single test, is of great use for corroborative purposes.

**Molecular Specific Volumes.**—A method of calculating which will sometimes be of use is to deduce the specific volume by dividing the density into 1, and to multiply the figure thus obtained by the potash absorption and to divide the result by 19.5.

The mean figure thus obtained for butter is 1.2766, and for margarine 1.1641 at 37.8° C. If the butter is adulterated with beef or other animal fat, the percentage of adulteration calculated with this figure will agree fairly well with that calculated from other determinations. If vegetable oils have been used, the percentage deduced thus is considerably more.

### Refractive Index.

**The Oleo-refractometer.**—When light passes from one medium to another it only passes in a straight line when it falls perpendicular to the surface separating the two media. If it passes through at an angle to the surface, it is bent or refracted.

and the ratio between the angle made by the path of the ray with the perpendicular to the surface in the first medium and the angle made by the path in the second medium with the perpendicular is a constant; the ratio of the sines of the two angles is known as the *index of refraction*. As the sine of an angle of  $90^\circ$  is 1, it is seen that the ratio between the sine of the angle at which light is first reflected and 1 is the index of refraction.

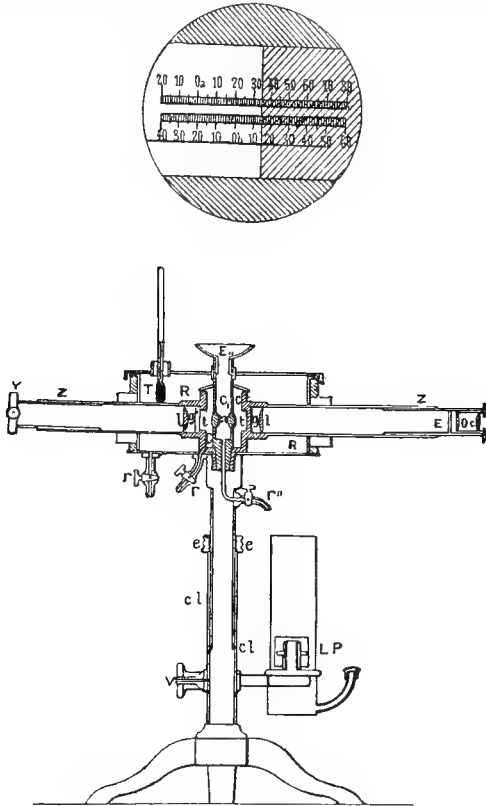


Fig. 48.—Oleo-refractometer.

tion; this angle is termed the *angle of total reflection*. As it is more convenient to measure this than to measure the two angles, and deduce the ratio of the sines, in practice the angle of total reflection is frequently measured.

Müller was the first to apply the determination of the refractive index to the analysis of butter. He allowed the butter to

solidify slowly, absorbed the liquid portion with filter paper, extracted this with ether, and examined it in Abbé's refractometer, an instrument which measures the angle of total reflection. Skalweit examined this method and showed that it was important to operate at a fixed temperature.

Owing to the difficulty of maintaining a fixed temperature in Abbé's refractometer, this method was not much used till special instruments were devised.

Amagat and Jean have devised an oleo-refractometer for determining the refractive index of oils and fats (Fig. 48); it consists of a collimator, a hollow prism with sides inclined at an angle of  $107^\circ$ , and a telescope furnished with an arbitrary glass scale placed in the focus of the eye-piece. In the collimator is placed a piece of opaque substance, which cuts off the light from one-half of the field. If the prism and the space outside between the collimator and the telescope are filled with the same liquid, there will be no refraction. If, however, the prism contains a different liquid, the refraction will be indicated (in arbitrary degrees) by the position of the junction between the light and dark halves of the field on the scale.

A standard oil (*huile type*)\* is supplied with the instrument, and the scale is so adjusted as to read zero when this is placed in the instrument. The oil or fat to be tested is placed in the hollow prism and the position of the dividing line read off on the scale. The temperature is kept constant by means of a jacket, and is usually  $45^\circ \text{C}$ .

Jean gives the following method for testing butter:—Melt from 25 to 30 grammes of the butter in a porcelain dish at a temperature not exceeding  $50^\circ \text{C}$ .; stir well with a pinch or two of gypsum, and allow to settle out at about the same temperature. Then decant the supernatant fat through a hot water funnel plugged with cotton wool, and pour (while warm) into the prism. Observe the deviation at  $45^\circ$ . Genuine butter gives a deviation of about  $30^\circ$  to the left, while margarine gives about  $15^\circ$ , and coco-nut oil about  $59^\circ$ . Lobry de Bruyn has shown that genuine butters may show a deviation of  $25^\circ$  to the left.

It is evident that the addition of a mixture of coco-nut oil and margarine would give a figure equal to that of butter. Muter has, however, shown that the figure given in the oleo-refractometer has a relation to the Reichert figure, which would be much disturbed by such a mixture.

Muter's relation is expressed by Table LXXXI.

\* This is usually translated as "typical oil"; the word "standard" is more nearly equivalent to the French "*type*," than is "typical."

TABLE LXXXI.

A deviation of	- 36°	is accompanied by a Reichert figure of	16·0.
"	- 35°	"	15·25.
"	- 34°	"	14·5.
"	- 33°	"	13·75.
"	- 32°	"	13 0.
"	- 31°	"	12·25.
"	- 30°	"	11·5.
"	- 29°	"	10·75.

Butyro-refractometer.—Zeiss' butyro-refractometer (Fig. 49)

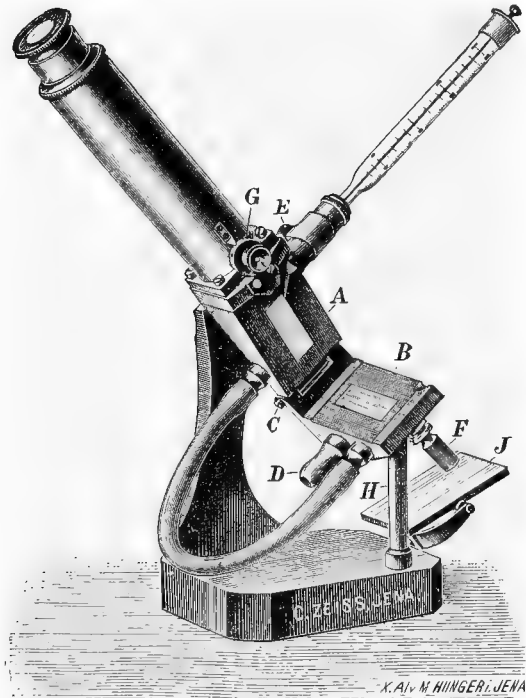


Fig. 49.—Butyro-refractometer.

measures the angle of total reflection and is a modification of the well-known Abbé refractometer. It consists of two prisms of glass, hinged so that they can be separated. The light enters at the bottom, passes through the prisms, and is viewed through a telescope having a fixed scale in the focus of the eye-piece. The prisms are provided with a jacket, through which water, the temperature of which is indicated by a thermometer, is passed.

A drop of the filtered fat is placed on the glass surface of the lower prism, spread evenly over it, and the prism closed; the reflector is adjusted so as to reflect clear daylight or lamplight through the prisms, and the refractive index in scale degrees is read off.

This instrument is extremely rapid, as a determination, including reading of the temperature and scale degrees, does not take more than a minute. After use, the instrument should be cleaned by rubbing off the fat with a duster, and polishing the prisms with a clean linen cloth slightly moistened with alcohol.

Scale divisions may be converted into refractive indices by Table LXXXII.

TABLE LXXXII.

Scale Division.	Refractive Indices for the D line.	Difference.
0	1.4220	...
10	1.4300	8.0
20	1.4377	7.7
30	1.4452	7.5
40	1.4524	7.2
50	1.4593	6.9
60	1.4659	6.6
70	1.4723	6.4
80	1.4783	6.0
90	1.4840	5.7
100	1.4895	5.5

There is a difference in the refractive index depending on the light used; this is corrected in the instrument by making the prisms of different kinds of glass, so that when used with butter ordinary white light behaves as if it were simple light. Other fats (and adulterated butters) may be tinged at the edge with blue or red. In this case it is not easy to read the dividing line accurately. The author is in the habit of using the sodium flame, obtained by heating sodium chloride in a Bunsen burner, as the source of light, and finds that absolutely sharp readings can thus always be obtained. The readings with butters do not differ, whether white light or sodium light be used.

The refractive index varies 0.55 scale degree for each 1° C., and can be corrected by means of this factor if the temperature differs from that adopted as normal.

For the correction of scale readings taken at any temperature to any other temperature or to that adopted as a standard, Leach and Lythgoe have devised a slide rule, but, except for small



differences of temperature, the author finds that the readings are not strictly correct.

A chart for the correction of butyro-refractometer readings for temperature may be constructed thus:—Select a sheet of squared paper at least 120 units by 200 units wide; at a point 34 units from the bottom, set out horizontally a series of points 5 units apart, and at a point 119 units from the bottom a similar series of points 7 units apart; join the corresponding pairs of points to form a series of temperature lines. The middle vertical line is selected as the standard temperature, say  $35^{\circ}$ , and each line to the right will represent a temperature  $1^{\circ}$  higher, and to the left  $1^{\circ}$  lower than the next preceding line.

From the bottom at a point 100 units from the standard temperature line to the left draw a line to the point which lies 20 units from the bottom and 100 units to the right of the standard temperature line; this will represent  $0^{\circ}$  on the scale of the butyrometer; draw parallel to this a series of lines 10 units apart measured vertically, and mark these  $10^{\circ}$ ,  $20^{\circ}$ , etc., of the refractometer scale. To use the chart, find the point of intersection of the lines corresponding to the observed temperature, and scale lines (differences between the lines can be estimated with sufficient accuracy by the eye), and the distance measured horizontally between this point and the vertical standard line will give the correction to be added if on the right, or subtracted if on the left; 10 units of distance equal one scale degree of correction.

This chart is easy to make, and still easier to use, and the author has found it to give very accurate results over a considerable range of temperature, not only for butter, but also for other oils and fats, and for the standard fluid.

The author has found that genuine butters vary from  $43.7^{\circ}$  (in a sample giving a Reichert value of 16.0 c.c.) to  $49.0^{\circ}$  (in a sample giving a Reichert value of 10.5 c.c.), and average  $46.0^{\circ}$  at a temperature of  $35^{\circ}$  C. The value  $47.0^{\circ}$  has been proposed as a practical limit.

The equivalent at other temperatures of this limit is as follows:—

Temperature.	Scale Division.	Temperature.	Scale Division.
$25^{\circ}$	$52.5^{\circ}$	$40^{\circ}$	$44.2^{\circ}$
$30^{\circ}$	$49.8^{\circ}$	$45^{\circ}$	$41.5^{\circ}$
$35^{\circ}$	$47.0^{\circ}$		

Some importance has been attached to the colour observed at the edge of the dividing line, and a blue colour has been alleged to be indicative of margarine. In the author's experience this property is valueless. Thus the sample giving a reading of  $43.7^{\circ}$  was tinged red, and that giving  $49.0^{\circ}$  was tinged blue, though both were authenticated as genuine butters.

Margarine has a value of about  $52^\circ$  at  $35^\circ$ , coco-nut oil of  $41^\circ$ , and cotton-seed oil of  $61^\circ$ .

The remarks made upon the oleo-refractometer apply equally well to the butyro-refractometer, except that the actual values are not identical.

The author's experience confirms that of Mansfeld, and shows that, while Muter's relation is, broadly speaking, correct, there are differences so large between the refractive index found and that calculated on the assumption that this property follows the Reichert value, that the rule cannot be depended upon. The refractive index is a property which is much more nearly related to the iodine absorption, or, in other words, to the unsaturated carbon atoms.

Though a very convenient test, it has but little value alone, unless the value is below the average,  $46^\circ$  at  $35^\circ$  C., but in the presence of coco-nut oil and margarine an adulterated butter may give a normal figure. When a butter is adulterated with vegetable oils—*e.g.*, cotton seed—its indications are of some value. It is also useful in detecting coco-nut oil, but its value is chiefly corroborative.

A standard fluid (*normal Flüssigkeit*) is supplied with the instrument, and the readings of the scale should be checked from time to time by its use. The point at which the dividing line should lie at  $35^\circ$  C. is marked in the instrument, and the scale should be brought to this point by means of a key just above the prisms.

**Viscosity.**—Killing proposes to take the viscosity of butter fat as a test by running it out of a pipette, marked above and below the bulb, and records the time taken for the melted fat to flow from one mark to another. The instrument must be graduated with butters and other fats of known purity.

He gives the following average times of flow:—

Butter, .	3 minutes 43½ seconds.
Margarine, .	4 „ 19 „
Lard, .	4 „ 28 „
Beef fat, .	4 „ 33 „

Wender uses an apparatus called a fluidimeter. This consists of a U-shaped capillary tube having at one end an enlargement holding 10 c.c., and at the other an enlargement holding 2 c.c.; the larger bulb is placed higher than the smaller; liquid, therefore, flows from it. A solution of the fat in chloroform is used; the upper bulb is filled with this, the solution allowed to flow into the lower bulb, and the time noted which it takes to pass from the lower mark to the upper one on the smaller bulb. The time taken for chloroform to flow is also noted, and this is taken as 100.

The viscosity of the fat is calculated by the following formula :—

$$\begin{aligned} \text{Let } V &= \text{viscosity of the fat,} \\ x &= \text{percentage of fat in chloroform solution by volume,} \\ \text{and } T &= \text{the time taken divided by the time taken by chloroform,} \\ \text{then } V &= \frac{100 T - 100 (100 - x)}{x}. \end{aligned}$$

The average time for chloroform to fill the lower bulb was 20·04 seconds.

Wender gives the following values as the mean figures at 20° C. (chloroform = 100) :—

Viscosity of pure butter,	.	.	344·3
„ „ margarine,	.	.	373·2

It does not appear that this test has any greater value than other physical determinations.

**Behaviour of Butter on Melting.**—When butter is melted at a temperature of about 60° C., the fat which flows from the aqueous portion is generally clear and transparent; when margarine is melted, the fat is almost always cloudy.

This has been used as a test for the purity of butter. It does not appear to depend on any property of the fat, but on the state in which the fat existed in milk, and the method of preparing the butter. Butters which have been overworked invariably melt in a cloudy manner.

Druot has devised an apparatus for observing the behaviour on melting. It consists of a number of cups stamped in tin plate, in which pieces of the samples to be tested, about 1½ grammes in weight, are placed. A piece of iron heated to about 60°, and of sufficient thickness to retain enough heat to melt the samples, is placed over the top, and left till the butters are melted. The appearance of the fat is observed, the polished surface of the tin plate materially aiding the observation.

This method can only be classed as a rough means of determining the purity of butter.

**Melting Point of the Fat.**—Formerly some importance was attached to the melting point of the fat; this, however, depends to some extent on the method employed in determining it. Butter melts at about 33° C.; and artificial butters are made up to melt at the same temperature.

Among other physical properties which have been proposed are the determination of the heat of combustion, which differs materially in butter and other fats, and the relative transparency to the X rays. These methods are not, however, practical analytical methods.

**Detection of Adulteration of Butter.**—The most useful and rapid preliminary test is examination with the butyro-refractometer. Any sample showing a refractive index of less than  $46^\circ$  at  $35^\circ$  C. is most probably genuine, but may, however, contain coco-nut oil. The Reichert-Wolny process should next be applied. Any sample requiring less than 20 c.c. for 5 grammes may be taken as adulterated; samples requiring more than 28 c.c. may be passed as genuine, though they cannot absolutely be certified as free from adulteration. Any sample taking a volume of  $\frac{N}{10}$  alkali between the limits given above must be

further examined. The Polenske and Avé-Lallemant methods should be employed, and if the results are suspicious the phytosteryl acetate test should be used. Baudouin's, Becchi's, Halphen's, and Wellmann's tests should be applied. A well-marked reaction with any or all of them will furnish strong presumptive evidence of the presence of margarine containing vegetable oils. The soluble and insoluble fatty acids, saponification equivalent, and especially the mean molecular weight of the insoluble fatty acids should be determined.

**Coco-nut oil** can be readily detected by the figures thus obtained. A high Polenske figure indicates this adulterant. The ratio between the Reichert-Wolny figure and the difference between the insoluble fatty acids and 95.5 is much depressed; in butter the ratio is about  $\frac{R-W}{95.5 - I} = 3.5$  ( $R-W =$

Reichert-Wolny figure, and  $I =$  Insoluble fatty acids); while coco-nut oil gives a value of approximately 0.75. The mean molecular weight of the insoluble fatty acids in butter is about 259, and varies but little from this figure, while the corresponding figure for coco-nut oil is about 200. The iodine absorption of coco-nut oil is also low, about 9 per cent.: while butter absorbs about 34 per cent. of iodine. Mercier's or Hink's microscopic methods should be used before the presence of coco-nut oil is certified.

It is far more difficult to detect other adulterants, if present in small quantities, unless vegetable oils are detected. Genuine butters which are below the average in the Reichert figure give high insoluble and low soluble fatty acids, a high iodine absorption, and a low percentage of potash absorbed. In the few samples that the author has examined the mean combining weight of the insoluble fatty acids has not been so high as would be expected. Thus the mean combining weight of the insoluble fatty acids is about 259, while the mean combining weight of the insoluble fatty acids of most adulterants is about 277. The

Valenta test is also useful, and the density may be used as a corroborative test.

**Margarine.**—It is advisable to calculate from the mean figures yielded by genuine butters and margarines the apparent percentage of margarine present. If the percentage thus calculated from the mean combining weight of the insoluble fatty acids, the Valenta value, and the density be less than that calculated from the other determinations and, at the same time, the iodine absorption and refractive index are slightly high, it is probable that the butter is genuine. If the contrary is the case, and the apparent percentages from all the methods give approximately the same value, it is probable that the butter is adulterated, especially if the Avé-Lallemant method shows adulteration. If, in addition, the colour tests for vegetable oils have given distinct reactions, the probability of adulteration is strengthened.

Though in the present state of science it is not possible definitely to certify many cases of small amounts of adulteration, for dairy control work the task is much simplified. The samples which must be regarded as suspicious can be reported as such, or even as adulterated, with a high degree of probability, and it will be frequently possible to trace such samples to their origin, by examining the fat of the milk of the cows which yielded the butter.

**Influence of Keeping on the Analytical Properties of Butter.**—When butter is kept and becomes rancid very pronounced changes take place in the composition of the fat. These may be classed under two heads—hydrolysis and oxidation. If butter fat be kept in the dark and out of contact with the air, it keeps indefinitely without change; but in the presence of light and air it becomes oxidised.

The general course of change may be roughly indicated thus—

- (1) The fat is partly hydrolysed into fatty acids and glycerol.
- (2) The glycerol is oxidised to fatty acids of low molecular weight
- (3) The unsaturated acids are oxidised, forming hydroxy-acids.

The general effect of these changes is—

The volatile and soluble acids are increased, the soluble in greater proportion than the volatile.

The insoluble acids are decreased.

The iodine absorption is lowered.

The density and refractive index are increased.

The potash absorption is increased.

If the butter has been kept in its natural state, the butter fat obtained on melting may have properties differing materially from those indicated above, owing to the solubility of some of the products in the water still left in the butter. The soluble and volatile acids in the filtered fat may be lowered from this cause, and the insoluble acids increased.

The change is not very rapid, and in the course of several weeks the changes are often not very pronounced.

Bell has recorded the following figures for the changes in the insoluble fatty acids; the butter in this case was kept for the times indicated:—

No. of weeks kept, . . .	12	7	7	6	8	6
Before keeping, per cent., .	87.30	87.80	85.50	87.40	87.72	87.65
After " " . . .	88.97	90.00	85.72	87.97	88.40	88.00

Vieth has made analyses showing the change in the insoluble fatty acids produced when butter fat is kept. In each case about a year had elapsed between the two analyses.

	Per cent.	Per cent.	Per cent.	Per cent.
Original insoluble fatty acids, .	87.43	88.33	87.61	87.72
Insoluble fatty acids, after keeping, .	85.07	85.97	84.41	83.82

The same observer has also examined old butter fat and old butter (kept for about ten years) which had not been previously analysed.

The old butter fat was divided into two portions—one, completely bleached, contained 83.52 per cent. of insoluble fatty acids; and the other, which still retained a trace of its natural colour, yielded 83.90 per cent.

The results with the old butter were as follows:—

Lower portion, . . .	89.28	per cent.	insoluble fatty acids.
" " washed, . . .	89.33	"	" "
Upper portion, . . .	90.94	"	" "

Allen and Moor have examined two samples of butter which had been kept for five and a half years. The following table gives their results:—

TABLE LXXXIII.

	B.			O.		
	Fresh.	Old.			Fresh.	Old.
		1	2	3		
Density $\frac{100}{15.5}$ (in glass),	0.8640	0.8634	0.8696	0.8730	0.8641	..
Reichert-Wollny, . . .	22.51	14.43	12.02	12.02	24.55	22.48
Potash absorption, . . .	221.6	219.9	225.5	228.8	220.9	233.3
Soluble fatty acids, per cent., . . .	4.44	3.82	5.66	5.80	4.68	5.89
Insoluble fatty acids, per cent., . . .	90.44	90.73	90.70	90.00	90.10	85.75
Iodine absorption, . . .	40.0*	30.01	27.17	25.08	?	25.57

\* This figure was determined by the author on a duplicate sample.

Clayton has analysed a butter which in 1879 gave in Hehner's hands 87.75 per cent. of insoluble fatty acids. His results were :—

TABLE LXXXIV.

	Melting Point.	Density $\frac{100^\circ}{15.5^\circ}$ (in glass).	Insoluble Fatty Acids.	Soluble Fatty Acids.	Reichert-Wollny.
January, 1895, October, 1897,	... 33° C.	0.8742 ...	Per cent. 85.72 ...	Per cent. ... 7.36	c.c. 22.36 ...
	Potash absorbed.	Iodine absorbed.	Maumené figure.	Rancidity.	
January, 1895, October, 1897,	Milligrms. ... 234.7	Per cent. 25.68 25.09	... 22° C.	... 100 grammes required 160.3 c.c. normal alkali.	

Besana has examined twenty samples after keeping for various periods of time; he estimated the Reichert-Wollny figure (Table LXXXV.).

TABLE LXXXV.—THE REICHERT-WOLLNY FIGURE OF BUTTERS.

No. of days between first and second test.	Reichert-Wollny Figure.		Difference.
	Fresh Butter.	Rancid Butter.	
173	27.70	27.42	- 0.28
171	27.28	26.98	- 0.30
170	27.50	27.28	- 0.22
169	27.51	27.64	+ 0.13
164	27.43	27.75	+ 0.32
162	28.49	28.30	- 0.19
161	27.90	27.65	- 0.25
160	27.54	27.40	- 0.14
157	27.72	27.31	- 0.41
157	28.49	27.97	- 0.52
134	29.15	29.40	+ 0.25
131	29.48	28.74	- 0.74
107	29.48	28.96	- 0.52
107	29.70	29.18	- 0.52
84	29.40	28.85	- 0.55
84	29.36	28.74	- 0.62
79	27.87	27.42	- 0.45
47	28.08	28.30	+ 0.22
46	27.86	27.75	- 0.11
46	28.85	28.96	+ 0.11

Vieth has examined a butter which had been kept more than ten years; the fat then yielded the following Reichert-Wollny figures—26·2, 25·6, 25·7, and 21·2 c.c. on different portions. He has also examined butter fat which had been kept for eighteen months.

The results were :—

Fresh, . . .	29·2 c.c.	29·9 c.c.
Old, . . .	30·4 c.c.	29·5 c.c. (determined by the author).

Another example of butter fat well protected from the light gave in July, 1888, from 31·6 to 32·1 c.c. of  $\frac{N}{10}$  alkali. In—

October, 1888,	it gave	31·8	c.c.
January, 1889,	„	32·1	„
May, 1889,	„	32·1	„
September, 1889,	„	31·8	„
December, 1889,	„	32·2	„
April, 1890,	„	32·0	„
July, 1893,	„	33·9	„

The last figure was determined by the author; the others by Vieth.

It is seen from the figures quoted above that the analysis of butter which has been kept for any length of time is a matter of considerable difficulty. Though in butter fat the volatile acids do not show any diminution, but rather an increase (due possibly to the oxidation of the glycerol), in butter the reverse is usually the case. It is by no means improbable that, besides the solubility of these in the water contained in the butter, a portion is destroyed by the action of micro-organisms. The most reliable datum would seem to be the determination of the volatile acids on the butter itself without separation of the fat, subsequent determination of the fat, and calculation of the Reichert figure on the actual fat present. The potash absorption does not appear to undergo much change.

The phytosteryl acetate test may be applied to rancid butters.

**Detection of Rancidity in Butter.**—The amount of free fatty acids in fresh butter does not exceed 5 c.c.  $\frac{N}{10}$  acid per 100 grammes, any higher figure indicates partial hydrolysis. Soltsien recommends that the fat be steam distilled, treated with alkali in excess, and again steam distilled. Wellmann's reagent is added to the distillate, and ammonia in excess; and a blue colouration in from half to one minute indicates rancidity.

**Buttermilk.**—**Definition.**—The term **buttermilk** is applied to the aqueous portion left after churning. It differs only slightly in composition from skim milk. As the cream used



for churning is usually slightly sour, the buttermilk contains appreciable amounts of lactic acid; it will also contain water or any other substance which has been added during churning. There is, in suspension, a considerable amount of Storch's mucoid protein, which may be removed by passing it through a cream separator, when it is deposited on the sides of the drum.

**Composition.**—The following composition of buttermilk from sweet cream is given by Storch:—

Water, . . . . .	89.74 per cent.
Fat, . . . . .	1.21 ”
Milk-sugar, . . . . .	4.98 ”
Protein, . . . . .	3.28 ”
Ash, . . . . .	0.79 ”

Buttermilk from ripened cream has the following composition:—

TABLE LXXXVI.

Authority, . . .	Storch.	Vieth.	Fleischmann.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	90.93	90.39	91.24
Fat, . . . . .	0.31	0.50	0.56
Milk-sugar, . . . . .	4.58	4.06	4.00
Lactic acid, . . . . .	(?)	0.80	
Protein, . . . . .	3.37	3.60	3.50
Ash, . . . . .	0.81	0.75	0.70

The author finds the following figures in buttermilks prepared in different ways:—

TABLE LXXXVII.

	Sour Cream.	Sweet Cream.	Milk.	Separated Milk.
Specific gravity, . . . . .	1.0314	1.0331	1.0329	1.0355
Water, . . . . .	91.61	90.98	91.13	90.77
Fat, . . . . .	0.50	0.35	0.70	0.10
Sugar, . . . . .	3.40	4.42	3.65	3.93
Lactic acid, . . . . .	0.50	0.01	0.76	0.56
Protein, . . . . .	3.30	3.51	3.28	3.65
Ash, . . . . .	0.65	0.73	0.68	0.79

**Variations of Fat.**—The author has found the amount of fat in buttermilk to vary from 0.15 per cent. to 5.60 per cent.; the last percentage is very unusual, and it is rare to find even

as much as 2.0 per cent., percentages higher than this denoting that the churning has been inefficiently carried out.

**Ash.**—The following composition is given by Fleischmann to the ash of buttermilk:—

TABLE LXXXVIII.

Potash, $K_2O$ , . . . . .	24.53	per cent.
Soda, $Na_2O$ , . . . . .	11.54	„
Lime, $CaO$ , . . . . .	19.73	„
Magnesia, $MgO$ , . . . . .	3.56	„
Phosphoric acid, $P_2O_5$ , . . . . .	29.89	„
Chlorine, $Cl$ , . . . . .	13.27	„
Iron oxide, &c., . . . . .	0.47	„
	<hr/>	
	102.99	„
Less oxygen = chlorine, . . . . .	2.99	„
	<hr/>	
	100.00	„

Buttermilk has usually a slightly acid flavour; it does not, however, taste quite like sour skim milk, but has a distinctive smell and flavour of its own; it is not known to what this is due.

On microscopic examination it is seen that the fat left is not entirely in globules; there exist many small nuclei consisting of two or more fat globules.

**Chemical Control of Churning Operations.**—The fat in the buttermilk from each churning should be estimated. Usually less than 1 per cent. of fat may be considered satisfactory, but if sweet cream is churned it is difficult always to keep within this limit. Any percentage of fat above 2 must be considered unsatisfactory, and the cause should be enquired into. This may be due to the use of cream which is too thick, mixtures of cream of different consistency and age, too high a temperature, or too rapid churning.

The fat in the cream to be churned should also be estimated. It has been found that cream containing from 25 to 30 per cent. of fat gives the most satisfactory results. If the cream contains more than 40 per cent. of fat, the buttermilk is very high in fat, and a larger percentage loss is obtained.

The weight of fat in the butter *plus* the weight of fat in the buttermilk should come within 2 per cent. of the weight of fat in the cream used. If a larger difference is found, a needless loss of fat is taking place, and the cause of this should be ascertained.

Table CXLI. (Appendix) gives the weight in pounds of butter which may be expected to be produced on churning cream varying in percentage of fat from 15 to 50.

An approximation to the amount of butter in pounds that may be obtained from milk by churning the separated cream can be obtained by subtracting 0·1 from the percentage of fat, multiplying the difference by  $\frac{7}{60}$ , and by the number of gallons of milk.

**Estimation of Water and Salt in Buttermilk.**—It sometimes happens that when churning both salt and water find their way in the buttermilk; when the buttermilk is to be sold, it is important to be able to estimate rapidly both the proportion of water and of salt.

It was found that chlorides could be titrated in milk with  $\frac{N}{10}$  silver nitrate solution, using potassium chromate as indicator, and that 10 c.c. of milk took on an average 3·45 c.c.  $\frac{N}{10}$  silver solution, with extremes of 3·35 c.c. and 3·6 c.c. in nine samples. It was further found that the number of c.c. of  $\frac{N}{10}$  silver solution for 10 c.c. of milk could be deduced with considerable accuracy by multiplying the aldehyde figure (obtained with  $\frac{N}{10}$  strontia) by 0·171, and subtracting this quantity from the quantity actually used; the remainder was a measure of the sodium chloride.

A series of determinations showed that 1 gramme of sodium chloride added to 100 c.c. of milk raised the density by 0·00735, and by multiplying the amount of salt found by this figure the increment of density due to the addition is deduced, and subtracting this from the density found, the density of the milk is obtained. From this last figure and the fat the solids not fat can be calculated, and from this the amount of added water roughly deduced.

The method is:—Estimate the specific gravity, fat, and aldehyde figure as usual; titrate 10 c.c. of buttermilk with  $\frac{N}{10}$  silver nitrate, using potassium chromate as indicator, till a reddish colour is produced. From the volume of silver nitrate used subtract the aldehyde figure  $\times$  0·171, and multiply the residue by 0·0585, the product is the percentage of salt; multiply this by 0·00735, and subtract the resulting figure from the specific gravity; the percentage of added water, if present, is calculated from the fat, and the corrected specific gravity in the same way as the extent of watering of milk is deduced (p. 173).

It is sometimes asserted that a certain amount of water (20 or 25 per cent.) is allowed to be added to milk or cream for churning purposes. This view, however, appears to be quite incorrect; the addition of "breaking" water does not appear to be recognised

by any statute, and if buttermilk is to be sold there is no reason why it should contain any added water. It is probable, however, that should a sample of buttermilk taken under the Sale of Food and Drugs Acts be found to contain a small percentage of added water, the Public Analyst would advise his authority that it is a custom to add water during churning, and a prosecution for the addition of small percentages is improbable.

**The Use of Starters in Butter-making.**—The acidity of the cream should be determined before churning, if ripened cream is used. An acidity of about  $60^{\circ}$  will generally yield good results.

In order to ensure that a good flavoured butter is produced, it is necessary that the proper organisms are present; this is best ensured by pasteurising the cream, and after cooling adding a starter, which has been found to produce a good flavour. The starter provides an enormous excess of lactic acid bacteria, which at the ripening temperature develop rapidly and overgrow any other organisms that may have found entrance.

A starter is prepared by sterilising 1 to 2 litres of milk, adding a tube of one of the preparations on the market, which are pure cultures of lactic acid bacteria, and keeping the milk at about  $70^{\circ}$  F. till thick.

A rough, but usually very successful, method is to allow a specimen of milk, which develops a clean acid taste on keeping to stand at about  $70^{\circ}$  F. till sour. The lactic acid organisms are very active at this temperature, and, as they tend to overgrow any others that may be present, a fairly pure culture is the result.

Starters may be kept going by adding to 1 to 2 litres of milk that has been sterilised a little of a previous starter, and keeping at  $70^{\circ}$  F.

## CHAPTER VII.

## OTHER MILK PRODUCTS.

CONTENTS.—Cheese—Rennet—Classification of Cheeses—Composition—The Ripening of Cheese—Analysis of Cheese—Adulteration of Cheese—Other Products derived from Milk.

## Cheese.

CHEESE is prepared by the action of rennet on milk; this separates it into whey and curd; the curd is finely divided, pressed to separate the whey and to consolidate it, and, usually, salted. From this, cheese is produced by ripening, which is due partly to the action of micro-organisms and fungi, partly (as Babcock and Russell have shown) to the action of an enzyme natural to milk.

**Action of Rennet.**—The action of rennet is to split up the casein into a dycaseose, the calcium compound of which is insoluble and which forms curd, and a soluble caseose; the insoluble curd carries down with it a large proportion of the fat.

**Composition of Curd and Whey.**—The following table will show the distribution of the various constituents of the milk when made into whey and curd :—

TABLE LXXXIX.

	Milk.	Whey.	Curd.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	87·30	80·80	6·50
Fat, . . . . .	3·75	0·30	3·45
Milk-sugar, . . . . .	4·70	4·40	0·30
Casein, . . . . .	3·00	0·40	2·60
Albumin, . . . . .	0·40	0·40	trace
Ash, . . . . .	0·75	0·60	0·15

The following is the composition of whey according to various authorities :—

TABLE XC.

	Fleischmann.	König (average).	Smetham.	Vieth (from skim milk).
	Per cent.	Per cent.	Per cent.	Per cent.
Water, . . . . .	93·15	93·38	93·33	93·00
Fat, . . . . .	0·35	0·32	0·24	0·09
Milk-sugar, . . . . .	4·90	4·79	5·06	5·45
Protein, . . . . .	1·00	0·86	0·88	0·92
Ash, . . . . .	0·60	0·65	0·49	0·54

The author has found the fat in whey to vary from 0·04 per cent. (from skim milk) to 1·35 per cent., and the solids not fat to lie between 6 and 7 per cent., averaging 6·6 per cent., which contain—

Milk-sugar, . . . . .	5·0 per cent.
Protein, . . . . .	1·0 „
Ash, . . . . .	0·6 „

On adding an acid to whey, a slight protein precipitate (which is difficult of filtration) is obtained. On heating the acid whey, a soft curd of little consistency is formed; this substance is a commercial article on the Continent.

The composition of the whey and the precipitated curd (after acidifying and boiling) are given :—

TABLE XCI.

	By Fleischmann.		By Bochiocchio.
	Whey.	Precipitate.	Precipitate.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	93·31	68·5	68·47
Fat, . . . . .	0·10	3·1	5·22
Milk-sugar, . . . . .	5·85	3·2	3·97
Lactic acid, . . . . .	...	0·8	...
Protein, . . . . .	0·27	22·1	18·72
Ash, . . . . .	0·47	2·3	3·62

On boiling whey without acidifying, a precipitate of a similar nature also occurs; this appears to consist of coagulated albumin.

Cheese is sometimes prepared by allowing the milk to become sour spontaneously, salting and pressing the curd, and allowing it to ripen. This variety of cheese is not considered of such good quality as rennet cheese.

Fleischmann gives the composition of the whey thus obtained as follows :—

Water, . . . . .	93·13 per cent.
Fat, . . . . .	0·12 „
Milk-sugar, . . . . .	4·38 „
Protein precipitated by acetic acid,	0·47 „
„ „ „ tannin,	0·59 „
Ash, . . . . .	0·82 „
Difference (lactic acid ?), . . . . .	0·49 „

Vieth has shown that whey prepared in this way undergoes alcoholic fermentation much more readily than rennet whey.

**Rennet.**—This substance is an enzyme produced in the stomachs of mammals; it occurs in the human stomach, and the curdling of milk when ingested is due to this; it is especially abundant in the young while still suckling.

**Preparation.**—It is usually prepared from the fourth stomach of the calf. The stomachs are dried and kept for some time; they are then cut up into small pieces and macerated in a 5 per cent. salt solution, usually containing boric acid, for some days; to the solution a further 5 per cent. of salt is added, and the liquid filtered; this forms extract of rennet. By adding more salt, the rennet is precipitated, and “rennet powder” is produced; this consists, essentially, of the ferment, together with other organic matter, and a considerable amount of salt.

**Properties.**—Rennet acts on casein only in neutral or acid solution, and its properties are destroyed by alkalies. Like all enzymes it has an optimum temperature at which it acts best; this has been found by Fleischmann to be 41° C. (105·8° F.). He gives the following table as showing the relative proportion of milk coagulated in a given time by the same quantity of rennet at different temperatures:—

TABLE XCII.

Temp. C.	Proportion.	Temp. C.	Proportion.	Temp. C.	Proportion.
20°	18	36°	89	44°	93
25°	44	37°	92	45°	89
30°	71	38°	94	46°	84
31°	74	39°	96	47°	78
32°	77	40°	98	48°	70
33°	80	41°	100	49°	60
34°	83	42°	99*	50°	50
35°	86	43°	96		

At the optimum temperature, and for several degrees on either side, the curd produced is very firm; at low temperatures, 15° C. to 20° C., the curd is quite soft and flocculent; and when the temperature is raised to 50°, the curd again becomes very soft.

By heating rennet to temperatures much above 60° C. (140° F.) it rapidly loses its properties, and it also loses strength by long keeping.

The action of rennet is affected by the acidity of the milk; the larger the amount of acid, the more rapid the action; the

\* Given as 98 in original, but from the experimental data it appears that 99 is more correct.

addition of water to milk causes it to be coagulated more slowly by rennet and the curd is less firm. By heating milk, the action of rennet is delayed, owing to the removal of some of the soluble calcium compounds. By the addition of soluble lime salts, the milk will be curdled by rennet in the usual manner.

Alkalies destroy the power of rennet to curdle milk; borax acts as an alkali, boric acid being inert to rennet.

**Testing of Rennet.**—It is important to know what the strength of rennet preparations are—*i.e.*, the amount of milk that will be curdled by 1 part in a definite time at a definite temperature. This may be estimated as follows:—5 c.c. of a rennet extract or 0.5 gramme of a rennet powder are made up to 100 c.c. with distilled water. After thorough mixing, 1 c.c. is measured out by means of a pipette and added to 100 c.c. of separated milk of acidity 20°, which has been brought to a temperature of 35° C.; the milk and rennet solution are immediately well stirred and the exact time at which the rennet was added noted. The milk should be contained in a beaker, which is placed in a water-bath kept at 35° C., and gently stirred with a thermometer till it is found, by the path becoming visible, that the milk has coagulated; the exact time which has elapsed from the addition of the rennet till the coagulation sets in is noted.

The strength of the rennet—*i.e.*, quantity of milk that will be coagulated by 1 part in forty minutes—is calculated by the following formula:—

Let  $x$  = quantity of milk coagulated.  
 $p$  = proportion between milk and rennet taken.  
 $t$  = the time.

Then  $x = \frac{40p}{t}$ .

The value of  $p$  is 2,000 when 5 c.c. was diluted to 100 c.c. and 1 c.c. taken, and 20,000 when 0.5 gramme was taken.

If the time taken is less than five minutes, or more than ten minutes, it is advisable to make another determination, using a smaller or larger proportion of rennet to milk.

**Classification of Cheeses.**—Cheeses may be divided into the following classes:—

1. **Soft Cheeses.**—These are obtained by coagulating the milk with rennet at a low temperature (below 30° C. or 86° F.). The period of coagulation lasts a long time. As representative of these cheeses the following kinds may be mentioned:—Gervais and Pommel made from cream; Brie, Camembert, Pont l'Évêque, and Bondon (or Neufchâtel) made in France; and Stracchino made in Italy.



2. **Hard Cheeses.**—These are obtained by coagulating at higher temperatures ( $30^{\circ}$  C. or  $86^{\circ}$  F. to  $35^{\circ}$  C. or  $95^{\circ}$  F.); they may be again divided as follows :—

- (1) Cheese made from milk and cream—Stilton.
- (2) Cheese made from whole milk—Cheddar, Cheshire, Dunlop, and Wensleydale made in England; Port de Salut made in France; Emmenthaler or Gruyère made in Switzerland, Edam in Holland, and Gorgonzola and Cacio Cavallo in Italy.
- (3) Cheese made from partially skimmed milk—Parmesan in Italy; Derby, Gloucester, Leicester, and, sometimes, Cheddar in England; Edam (usually made in this way) in Holland, and Gruyère in Switzerland.

Skim milk cheese and cheese made from skim milk enriched by margarine are also made.

A famous cheese, known as Roquefort, is prepared from sheep's milk; Besana has shown that many sorts of cheese may be made from sheep's milk.

Goat's milk is also employed in cheese manufacture, but these cheeses are not important articles of commerce.

In addition to rennet cheeses, cheese made from the curd precipitated by warming milk which has been allowed to become sour is also used. The only cheese thus made in England is cream cheese; frequently an acid is added to the cream instead of allowing lactic fermentation to take place. A Swiss cheese, Glarner, and the German caraway cheese come under this category; the latter is mixed with caraway seeds.

**Composition of Cheese.**—But little is known of the composition of cheese. Most of the analyses made have included only water, fat, ash, and total nitrogenous substances either by difference or by estimation of the nitrogen and multiplication of this by a factor. In very few cases has the separation of the nitrogenous matters been attempted, and it is doubtful whether, where this has been done, much real information as to the character of the products has been obtained. The chemical knowledge of cheese must be pronounced to be in a much less satisfactory condition than that of other milk products.

The following tables (XCIII. to XCVII.) will give the proximate composition of various cheeses; they will be useful as showing the most striking differences. Thus soft cheeses contain large amounts of water, and small percentages of fat and protein; cheeses made from whole milk contain an amount of fat at least equal to the protein, while skim milk cheeses contain usually less fat than protein; in cream cheeses the fat greatly exceeds the protein :—

TABLE XCIII.—CREAM CHEESES.

Authority.	Water.	Fat.	Protein.	Lactic Acid, &c.	Ash.
<b>1. English cream cheese made without rennet.</b>					
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Vieth, . . . . .	30·66	62·99	4·94	0·26	1·15
Smetham, . . . . .	20·56	80·03	2·99	0·57	0·83
Pearmain & Moor, . . . . .	57·6	39·3	19·0	..	3·4
Author, . . . . .	26·50	67·00	4·05	0·71	0·37
Vieth found the insoluble fatty acids of the fat to be—					
When fresh, . . . . .	. . . . .	. . . . .	. . . . .	87·31	per cent.
After 1 month, . . . . .	. . . . .	. . . . .	. . . . .	87·12	”
” 2 ” . . . . .	. . . . .	. . . . .	. . . . .	88·02	”
” 3 ” . . . . .	. . . . .	. . . . .	. . . . .	87·96	”
” 4 ” . . . . .	. . . . .	. . . . .	. . . . .	87·58	”
<b>2. Gervais cheese.</b>					
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Vieth, . . . . .	42·32	49·18	7·75	0·27	0·49
Stutzer, . . . . .	44·84	36·73	15·48	..	2·95
Author, . . . . .	41·0	49·5	4·1	1·12	0·5
<b>3. Pommel cheese.</b>					
Author, . . . . .	44·9	45·5	7·2	1·16	0·5

TABLE XCIV.—SOFT CHEESES.

Authority.	Water.	Fat.	Protein.	Lactic Acid, &c.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<b>1. Brie.</b>					
Duclaux, . . . . .	50·04	27·50	18·32	..	4·12
<b>2. Camembert.</b>					
Duclaux, . . . . .	45·24	30·31	19·75	..	4·70
Stutzer, . . . . .	50·90	27·30	18·66	..	3·14
Cameron & Aikman, . . . . .	51·30	21·50	19·00	..	4·70
Leffmann & Beam, . . . . .	51·30	21·00	18·90	..	4·70
Pearmain & Moor, . . . . .	45·65	22·25	23·10	..	4·25
Muter, . . . . .	48·78	21·35	19·71	0·36	9·80
<b>3. Neufchâtel (or Bondon).</b>					
Fleischmann, . . . . .	34·5	41·9	13·0	7·0	3·6
Pearmain & Moor, . . . . .	39·5	24·4	9·4	..	0·7
Muter, . . . . .	55·20	20·80	15·38	1·64	6·98
<b>4. Stracchino.</b>					
König (average), . . . . .	39·21	33·67	29·32	..	3·80

TABLE XCV.—HARD CHEESES.

Authority.	Water.	Fat.	Protein.	Lactic Acid, &c.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<b>1. Stilton</b> (made from milk and cream).					
Pearmain & Moor, .	20·30	44·00	23·70	...	2·75
Cameron & Aikman, .	35·20	33·97	24·12	...	3·56
Muter, . . . . .	28·60	30·70	35·60	1·08	4·02
König (average), .	32·07	34·55	26·21	3·32	3·85
<b>2. Cheddar.</b>					
Pearmain & Moor, .	27·19	30·76	29·20	...	4·66
„ „ . . . . .	33·90	29·05	27·37	...	(American) 4·05
Cameron & Aikman, .	27·20	32·05	36·60	...	(English) 4·15
„ „ . . . . .	28·09	22·52	45·75	...	(American) 3·64
Muter, . . . . .	29·70	30·70	35·00	0·90	(English) 3·70
„ . . . . .	33·40	26·60	34·17	1·53	(American) 4·30
König (average), .	33·89	33·00	27·56	1·90	(English) 3·65
<b>3. Cheshire.</b>					
Smetham, . . . . .	39·33	30·80	23·70	2·43	3·60
Pearmain & Moor, .	34·70	33·30	26·10	...	4·30
Leffmann & Beam, .	30·4	25·5	36·1	...	4·80
<b>4. Gruyère</b> (or <i>Emmenthal</i> ).					
Fleischmann, . . .	36·1	29·5	28·0	3·3	3·1
Duclaux, . . . . .	36·00	29·29	30·84	...	3·87
Stutzer, . . . . .	33·01	30·23	31·41	...	5·30
Pearmain & Moor, .	31·45	30·20	30·00	...	4·20
Cameron & Aikman, .	37·34	26·47	31·33	...	3·42
Muter, . . . . .	33·20	27·26	33·49	1·35	4·70
Leffmann & Beam, .	32·0	28·0	35·1	..	4·8
König (average), .	36·49	28·01	30·83	0·72	3·95
<b>5. Cacio Cavallo.</b>					
Sartori, . . . . .	19·76	36·71	34·12	3·70	5·60
Spica & De Blasi, .	23·67	25·49	29·25	17·35	4·24

TABLE XCVI.—SKIM MILK CHEESES.

Authority.	Water.	Fat.	Protein.	Lactic Acid, &c.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<b>1. Dutch.</b>					
Duclaux, . . . . .	37.31	24.41	32.50	..	5.69
Pearmain & Moor, . . . . .	32.90	17.78	30.80	..	6.40
König (average), . . . . .	37.35	24.61	32.40	..	5.65
Muter, . . . . .	42.72	16.30	28.27	1.35	11.36
<b>2. Gloucester.</b>					
Cameron & Aikman, . . . . .	28.62	23.67	43.54	..	4.17
Pearmain & Moor, . . . . .	35.25	25.80	30.05	..	4.80
Muter, . . . . .	37.20	22.80	33.64	1.80	4.56
<b>3. Grana.</b>					
Duclaux, . . . . .	32.56	21.75	42.27	..	5.07
König (average), . . . . .	31.33	23.90	35.34	4.17	5.26
<b>4. Parmesan.</b>					
Duclaux, . . . . .	30.09	26.04	38.42	..	5.45
König (average), . . . . .	31.80	19.52	41.19	1.18	6.31
Pearmain & Moor, . . . . .	32.5	17.1	43.6	..	6.2
Cameron & Aikman, . . . . .	27.56	15.95	44.08	..	5.72
<b>5. York.</b> (a soft cheese).					
Muter, . . . . .	63.64	15.14	18.50	1.80	0.92
Vieth, . . . . .	68.44	12.89	14.50	2.88	1.29
Author, . . . . .	70.5	10.8	13.8	0.85	1.1

Besides these cheeses, which are all made from cow's milk, the famous *Roquefort* cheese, made from sheep's milk, must be mentioned. Its composition is—

TABLE XCVII.

Authority.	Water.	Fat.	Protein.	Lactic Acid, &c.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
König (average), . . . . .	36.85	30.61	25.25	1.90	5.39
Pearmain & Moor, . . . . .	29.6	30.0	28.3	...	6.7
Leffmann & Beam, . . . . .	26.5	32.3	32.9	...	4.4
Muter, . . . . .	21.56	35.96	24.52	0.72	10.24

In the above analyses the figures under the term "proteins" include true proteins and their products of ripening, and, frequently, also such products as lactic acid.

**Proteins in Cheese.**—Besides the analyses given on pp. 367 to 369, the following, in which an attempt has been made to distinguish between the various constituents, may be noticed.

TABLE XCVIII.—DETAILED COMPOSITION OF CHEESES.

Kind of Cheese.	Water.	Fat.	Ash.	NaCl.	Protein.	Ammoniacal Nitrogen.	Amino-nitrogen.	Authority.
Cacio Cavallo,	Per cent. 19.76	Per cent. 36.71	Per cent. 2.34	Per cent. 3.26	Per cent. 34.12	Per cent. 0.0616	Per cent. 0.665	Sartori.
"	23.67	25.49	4.24	3.39	23.63	0.0973	0.987	Spica and De Blasi.
" made from sheep's milk and separated milk,	22.09	35.90	2.64	3.16	32.57	0.0503	0.609	Sartori.
Placentine,	29.07	24.74	4.22	5.04	23.71	0.0911	1.171	Spica and De Blasi.
Majorcan,	28.85	22.15	3.87	3.08	16.84	0.0826	1.045	Spica and De Blasi.
Skim cheese,	41.63	5.87	0.45	8.10	34.29	0.170	0.983	Carcano.
Grana (fresh),	48.37	13.24	3.71	..	31.88	Lactose. 1.50	Amino-com- pounds. 1.02	Sartori.

Kind of Cheese.	Water.	Fat.	Ash.	Casein.	Albumin.	Peptone.	Amino-com-pounds.	Ammonia.	Authority.
Stracchino (fresh),	Per cent. 55.02	Per cent. 24.51	Per cent. 2.43	Per cent. 14.26	Per cent. 1.28	Per cent. 0.74	Per cent. 1.54	Per cent. 0.05	Musso, Menozzi, & Bignamini.
" (ripe),	40.32	30.83	3.75	14.93	0.71	0.86	8.15	0.42	"
Emmenthal I.,	37.59	31.47	4.15	20.38	0.63	0.75	4.20	0.11	"
" II.,	41.22	26.53	4.58	21.90	0.35	0.74	3.79	0.11	"
Gruyère,	26.31	35.59	5.77	20.57	0.63	0.89	7.80	0.26	"
Gorgonzola,	33.37	37.47	3.38	5.66	0.87	1.74	17.52	0.79	"
Grana,	34.37	17.27	5.05	23.45	0.85	0.55	17.69	0.32	"

TABLE XCIII.---Continued.

Kind of Cheese.	Water.		Fat.		Ash.		Protein.		Products of Ripening.		Indigestible Protein.		Authority.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
Bellelay, . . . . .	37.58	30.05	3.48	24.33	1.37	0.22	1.37	0.22	1.37	0.22	0.15	0.85	Lindt & Muller.
Emmenthal, . . . . .	35.70	32.86	5.25	22.17	6.27	0.15	6.27	0.15	6.27	0.15	0.85	Schulze & Benecke.	
Glauczer, . . . . .	47.02	6.60	10.10	31.76	7.55		7.55		7.55			" "	

SOFT CHEESES (*Dacaux*).

Kind of Cheese.	Water.		Fat.		Insoluble Protein.		Soluble Protein.		Salt.		Ash.		"Caseone."		Ammonia.		Butyric Acid.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
Brie, . . . . .	50.04	27.50	12.90	5.42	3.22	0.90	5.72	0.157	0.233	0.094	0.90	5.72	0.157	0.233	0.094	0.094	
Camembert, . . . . .	45.24	30.31	13.96	5.79	3.69	1.01	7.98	0.067	0.142	0.070	1.01	7.98	0.067	0.142	0.070	0.070	
Port du Salut, . . . . .	47.72	24.96	19.92	3.50	1.73	2.12	4.27	0.002	0.535	0.235	2.12	4.27	0.002	0.535	0.235	0.235	
Crescenza, . . . . .	56.75	21.34	15.63	3.28	1.34	1.56	6.65	0.000	0.000	0.020	1.56	6.65	0.000	0.000	0.020	0.020	

HARD CHEESES (*Dacaux*).

Kind of Cheese.	Water.		Fat.		Insoluble Protein.		Soluble Protein.		Salt.		Ash.		"Caseone."		Ammonia.		Butyric Acid.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
Gorgonzola, . . . . .	40.75	31.88	12.00	11.96	2.42	1.99	10.11	0.020	0.036	0.125	1.99	10.11	0.020	0.036	0.125	0.125	
Dutch, . . . . .	37.31	24.41	22.09	10.41	2.84	2.85	8.44	0.001	0.270	0.212	2.85	8.44	0.001	0.270	0.212	0.212	
Gruyère, . . . . .	36.00	29.29	24.54	6.30	0.57	3.30	4.33	0.029	0.058	0.250	3.30	4.33	0.029	0.058	0.250	0.250	
Parmesan, . . . . .	30.09	26.04	23.70	14.72	1.76	3.69	15.80	0.003	0.250	0.180	3.69	15.80	0.003	0.250	0.180	0.180	
Grana di Reggion, . . . . .	32.56	21.75	25.56	16.71	1.65	3.42	18.50	0.002	0.150	0.200	3.42	18.50	0.002	0.150	0.200	0.200	

Steiben gives the following figures for Roquefort cheese :—

TABLE XCIX.

	Water.	Fat.	Ash.	Insoluble Protein.	Soluble Protein.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh, . . . . .	49·66	27·41	1·74	13·72	6·93
Month in cellar, . . . . .	36·93	31·23	4·78	5·02	20·77
Old, . . . . .	23·54	40·13	6·27	8·53	18·47

It is seen that comparatively few analyses are available, and that they are insufficient to allow any definite conclusions to be drawn as to the connection between chemical composition and degree of ripeness.

**Heavy Metals in Cheese.**—The presence of copper has been noted in cheese by Besana ; it is derived from the use of copper vessels.

Stoddart has found metallic lead in Canadian cheese ; its presence appeared to be accidental.

Allen and Cox have drawn attention to the use of sulphate of zinc in cheese manufacture ; this is called “cheese spice,” and is used to prevent the formation of gas in the cheese by fermentation.

**The Ripening of Cheese.**—The work of Freudenreich, Lloyd, Duclaux, Bochicchio, and Babcock and Russell has shed much light on the nature of ripening.

**Rôle of Micro-Organisms.**—When the curd is precipitated by rennet it carries down with it the bulk of the micro-organisms present in the milk. The first of these to develop are the lactic acid organisms, which increase rapidly and transform the milk-sugar into lactic acid. Freudenreich and Lloyd have both come to the conclusion that these organisms are the chief, if not the only, factor in the ripening of cheese. While it cannot be denied that they have some influence, it is hard to realise that lactic acid bacteria, which, when grown in sterilised milk, do not convert the proteins into albumoses and amino-compounds, should acquire this property in cheese. It appears to be an established fact, however, that the lactic acid organisms develop, rise to a maximum, and then gradually diminish. The acid they produce appears to be inimical to the growth of other organisms. The ripening of cheese goes on concurrently with the growth of the lactic acid organisms, and continues at an even rate while the organisms are decreasing. This shows that the ripening of cheese is not due to the direct action of micro-organisms.

**Rôle of Moulds.**—C. Thom has shown that for the ripening of Camembert cheese three organisms are necessary.

Lactic acid bacteria, which rapidly multiply, and produce acid, which inhibits the action of other bacteria.

A special mould, *Penicillium Camembertii*, which secretes an alkaline substance, and a peptonising enzyme; the latter diffuses into the curd, and produces the texture of the cheese.

*Oidium lactis*, which gives the cheese its characteristic flavour.

For Roquefort cheese the only organisms necessary are lactic acid bacteria and the *Penicillium Roqueforti*; the latter reduces the acidity, digests the curd, and produces the characteristic flavour.

The Roquefort *Penicillium* is also found on Stilton, Gorgonzola, and other cheeses. The common green mould, *Penicillium glaucum*, does not appear to play any part in cheese ripening.

**Rôle of Enzymes.**—Duclaux has recognised this, and attributes the ripening of cheese to enzymes (called by him “diastases”) secreted by various organisms to which he gives the name *Tyrothrix*; the enzyme would remain and be active after the organisms had died off.

Babcock and Russell have shown that milk itself contains a peptonising enzyme. By treating milk with a quantity of an antiseptic, such as chloroform, to check all microbial action, they found that a digestion of the proteins was still going on. They have isolated the enzyme from milk, and, finally, have prepared cheeses, which have been ripened under aseptic conditions. Though perfectly sterile, these cheeses show that the proteins are converted into albumoses, peptones, and amino-compounds in the same manner as in normal cheeses.

Babcock and Russell conclude that the action of the natural enzyme of milk is the chief factor in the manufacture of cheese, and consider that Freudenreich and Lloyd have been misled.

The true part played by micro-organisms in cheese is probably the production of compounds in small quantities which give the characteristic flavours to the cheese.

### The Analysis of Cheese.

A complete analysis of cheese includes determinations of the water, fat, ash, salt, proteins, primary products of ripening (as albumoses and peptones), secondary products of ripening (such as amino-compounds, ammonia, and nitrates), and lactic and fatty acids; also, when present, milk-sugar. Few, however, of these determinations can be made with accuracy, though results which are of great utility can readily be obtained. In addition to the determinations mentioned, the fat may be examined as to its genuineness, and the proteins as to their digestibility.



The earlier methods of cheese analysis consisted of the estimation of water, by drying at 100° C. (212° F.) to constant weight; of fat, by extracting with ether; of ash, by ignition; and of casein, by difference; this method has the advantage of simplicity, but gives no information as to the changes that have taken place during ripening.

The following method will give fair results, and is easy of execution:—

**Richmond's Method — Water, Fat, and Ash.**—Three to five grammes of cheese are weighed into a wide platinum dish and dried in the water-oven till the fat begins to run away from the cheese. The basin is then turned up, so that the fat collects at one side, and the drying continued for an hour or so. It frequently happens that no fat runs away from the cheese; in this case the turning up of the basin may be dispensed with. The basin is then removed from the oven, and treated several times with ether to remove the fat. The ethereal solution is collected in a flask, the ether evaporated, and the fat dried and weighed. The basin and its contents are replaced in the water-oven, and the residue dried to constant weight. The combined weight of the fat and residue subtracted from the original weight gives the water. It will be found that the removal of the fat facilitates drying, but it is difficult to remove the whole of the fat in this manner. The residue may now be incinerated at a low red heat, and the ash weighed; in this the salt may be estimated by solution in water, and titration with silver nitrate solution, using potassium chromate as indicator.

It is advisable to make a separate estimation of the fat. This may be done by Short's method, which consists in grinding up a few grammes of cheese with twice its weight of anhydrous copper sulphate, and extracting the mixture with ether in a Soxhlet extractor. Schleicher and Schull's thimbles are very convenient for holding the mixture. The Werner-Schmidt method is also applicable. To 2 or 3 grammes of cheese 5 c.c. of water and 10 c.c. of strong hydrochloric acid are added, and the whole boiled with constant shaking till all, except fat, is dissolved. The solution is cooled, about 25 c.c. of ether added, and the tube well shaken. After complete separation, as much as possible of the ether is drawn off, and a fresh portion added. After four or five repetitions of the same process, the extraction of fat is complete. The combined ether extracts are then evaporated, and the fat weighed.

M. Weibull uses the Gottlieb method as follows:—2 to 3 grammes of cheese are placed in a graduated tube, heated with 10 c.c. of ammonia to 75° C. with frequent shaking; if the cheese is not all dissolved by this treatment 10 c.c. of alcohol is added,

and the heating and shaking continued till solution is effected. The solution is cooled, 25 c.c. of ether are added, and the contents of the tube mixed, and 25 c.c. of petroleum ether poured in, the solutions mixed, and the fat estimated in the usual manner.

**Proteins and the Products of Ripening.**—About 10 grammes of cheese are well ground up in a mortar with ten successive portions of 20 c.c. each of hot water, the aqueous portions being poured off into a 250 c.c. flask. The grinding should be as thorough as possible, every lump of cheese being well crushed. After cooling, the solution should be made up to 250 c.c. and filtered.

Twenty-five c.c. of the filtrate should be evaporated in a platinum basin on the water-bath, and the residue dried in the water-oven to constant weight. This may be termed the "total soluble extract." The residue may be incinerated at a low red heat, and the ash of the soluble extract weighed.

Twenty-five c.c. of the filtrate are diluted to about 100 c.c., 5 c.c. of a solution of copper sulphate solution added (34.64 grammes to 500 c.c.), and caustic soda solution added, drop by drop, till the precipitate settles in the form of curd, and leaves the supernatant liquid quite clear. After standing for some time, the precipitate is collected on a Gooch crucible, washed with water, and dried at 120° C. After weighing, the crucible is ignited, and the residue of copper oxide and phosphate weighed. The difference between the two weights may be taken as "primary products of ripening." The difference between this figure and that of the "total soluble extract," less the ash, may be taken as "secondary products of ripening." The difference between the "total soluble extract," less ash of soluble extract, and the "solids not fat," less total ash, may be taken as proteins.

The above method will be found to be fairly rapid and to give an insight into the composition of the protein matter of the cheese. The separations between the different classes of protein substances are, however, arbitrary. Thus it is assumed that all the insoluble "solids not fat" consist of protein, and that all the products of ripening (and nothing else) are soluble. The distinction between primary and secondary products of ripening is based on the assumption that primary products are precipitated as basic copper compounds, while secondary products give soluble compounds under the conditions given above. In the present state of knowledge it is impossible to identify and separate all the products of ripening; therefore empirical methods which yield comparative results are necessary.

**Stutzer's Method.**—If it be desired to obtain further information, the method given above may be elaborated by some of the methods detailed below. Stutzer has published a study

of the methods of cheese analysis, of which the following is an abstract:—

**Ash and Mineral Matter.**—From 10 to 15 grammes of the cheese are burnt (preferably in a muffle) in a platinum basin. The weighed ash is dissolved in 250 c.c. of water and an aliquot portion used for the determination of chlorine (calculated to sodium chloride). The portion insoluble in water may also be dissolved in dilute hydrochloric acid and made up to 250 c.c.; in a mixture of equal aliquot portions of each of these solutions the calcium and phosphoric acid may be determined.

**Water.**—A weighed quantity of the cheese is mixed with washed, ignited, and sifted quartz sand. For most cheeses the proportion of 100 grammes to 400 grammes of sand is satisfactory, but with very rich cheeses 500 grammes of sand are taken. This sand mixture is used in all the estimations. For the determination of the water, an amount of the mixture corresponding to about 3 grammes of cheese is dried to constant weight in the water-oven.

**Fat.**—The dry residue from the water-determination is extracted for twenty-four hours with water-free ether, which has been dried over sodium.

**Nitrogen**—I. **Total Nitrogen.**—Ten grammes of the sand mixture are analysed by Kjeldahl's method (p. 124).

II. **Applicability of Copper Hydrate to the Precipitation of Albuminoids.**—Formerly, Stutzer employed copper hydrate to separate proteins and their primary cleavage products from secondary products (amino-compounds, etc.). He has since found that it only partially precipitates trypto-peptones (pancreas-peptone), and, extending his experiments to cheese, finds that there is sometimes a peptone present which is not completely precipitated.

III. **Phospho-tungstic Acid as a Precipitant.**—The conclusions of Bondzynski that phospho-tungstic acid is a suitable separating agent are confirmed. By its means the proteins and their primary cleavage products (albumoses and peptones) are separated from the secondary products (phenyl-amino-propionic acid, leucine, tyrosine, and other amino-compounds) and ammoniacal compounds, all of which Stutzer classes as worthless. The substances belonging to the first group may be further divided into—(a) Indigestible nitrogenous matters; (b) Albumoses and peptones soluble in boiling water; and (c) Proteins insoluble in boiling water.

IV. **Nitrogen in the Form of Ammoniacal Salts.**—An amount of the sand mixture corresponding to 5 grammes of cheese is mixed with 200 c.c. of water and the ammonia distilled, after the addition of barium carbonate. Magnesia and

magnesium carbonate cause a partial decomposition of the amides. The author prefers to operate on a portion of the hot-water extract.

**V. Nitrogen in the Form of Amino Acids.**—This is taken to be the nitrogen belonging to those compounds in the cheese which are not precipitated by phospho-tungstic acid, and which are not ammoniacal compounds. An amount of the sand mixture, corresponding to 5 grammes of cheese, is mixed with 150 c.c. of water, and well shaken for fifteen minutes in a closed vessel. After standing for fifteen hours at the ordinary temperature, 100 c.c. of dilute sulphuric acid (1 vol. : 3 vols. water) are added, and phospho-tungstic acid so long as a precipitate results. The liquid is filtered, the precipitate washed with dilute sulphuric acid until the filtrate amounts to 500 c.c., and the nitrogen is determined in 200 c.c. of this. By deducting from the amount that previously found as ammoniacal nitrogen, the nitrogen present in the form of amino acids is found.

**VI. Indigestible Nitrogenous Substances.**—The fresh mucous membrane of six pigs' stomachs is cut into small fragments and mixed with water and hydrochloric acid in a wide-necked flask in the proportion of 5 litres of water and 100 c.c. of 10 per cent. (by weight) hydrochloric acid to each stomach. At the same time, 2½ grammes of thymol dissolved in alcohol are added as a preservative. The mixture is left for twenty-four hours, with occasional shaking, and then filtered through flannel, coarse paper, and fine paper successively. If necessary, the amount of hydrochloric acid in the extract is brought to exactly 0.2 per cent. As thus prepared, the gastric juice remains unaltered for months.

Sand mixture, containing 5 grammes of cheese, is deprived of its fat by extraction with ether, mixed with 500 c.c. of gastric juice in a beaker, and the mixture warmed for forty-eight hours in a thermostat at 37 to 40 C. (99° to 104° F.). At intervals of about two hours, 5 c.c. of 10 per cent. hydrochloric acid are added, until the acidity of the whole reaches 1 per cent. The liquid is filtered through paper or asbestos, the residue washed with water, and the nitrogen in it determined.

**VII. Nitrogen in the Form of Albumose and Peptones.**—A weighed quantity of the sand mixture, containing 5 grammes of cheese, is extracted by boiling with successive portions (100 c.c.) of water, the liquid made up to 500 c.c. and filtered; and 200 c.c. of the clear filtrate (mixed with an equal volume of dilute sulphuric acid) are precipitated by phospho-tungstic acid. The precipitate is collected on a filter, washed, and the nitrogen estimated by Kjeldahl's method.

**Qualitative Test for Peptones.**—A portion of the hot-water extract is concentrated by evaporation, saturated with zinc sulphate, and filtered. Concentrated sodium hydroxide solution is added to the filtrate until the zinc hydroxide dissolves, and a few drops of a 1 per cent. solution of copper sulphate added; a violet-red colour (the biuret reaction) points to the presence of peptone. If desired, this may be estimated by evaporating 200 c.c. of the filtrate to 50 c.c., saturating with zinc sulphate, filtering, and washing with saturated zinc sulphate solution; the precipitate, which consists of albumoses, is treated by Kjeldahl's method. The nitrogen in the peptone is the difference between that in the albumoses and that in the precipitate formed by phospho-tungstic acid.

**VIII. Proteins.**—The nitrogen present in these substances, which are insoluble in boiling water, is obtained by subtracting from the total nitrogen the amounts found in IV., V., VI., and VII. It is not advisable to use the residue from VII. for this purpose, on account of the large amount of sand present.

**IX. Separation of the Proteins Digestible with Difficulty from those Readily Digestible.**—Cheese contains only small quantities of completely indigestible nitrogenous substances, and it is, therefore, useful to determine the comparative digestibility of the proteins. For this purpose, a process of "interrupted digestion" is employed. In order to obtain comparable results, care is taken to have constant (1) the amount of nitrogen in the form of insoluble, but digestible, proteins; (2) the amount of gastric juice; and (3) the acidity of the liquid, the temperature, and duration of digestion.

In each experiment so much of the sand mixture is taken as contains 0.15 gramme of nitrogen in the form of insoluble, but digestible, proteins, to which is added 150 c.c. of the gastric juice, with 343 c.c. of water and 7 c.c. of 10 per cent. hydrochloric acid. The acidity of the total liquid ( $\frac{1}{2}$  litre) is exactly 0.20 per cent., the temperature is maintained at 37° to 40° C. (99° to 104° F.), and the duration of the digestion is thirty to sixty minutes. The liquids are warmed to 40° C. (104° F.) before being measured and after mixing. At intervals of five minutes during the digestion the liquid is stirred with a glass rod; and at the conclusion the total liquid is placed in two large folded rapid filters, and the portion of the filtrate passing through in the first five minutes taken for the determination of the nitrogen. From the result a deduction must be made for the nitrogen contained in the gastric juice, and for the nitrogen in the cheese dissolving without the aid of the gastric juice (amino, ammoniacal, albumose, and peptone nitrogen).

TABLE C.—ANALYSES OF CHEESE (*Stutzer*).

	Camembert.	Swiss.	Gervais.
Water, . . . . .	Per cent. 50.90	Per cent. 33.01	Per cent. 44.84
Fat, . . . . .	27.30	30.28	36.73
Organic solids not fat, . . . . .	18.66	31.41	15.48
Ash, . . . . .	3.14	5.30	2.95
The ash contained—			
Calcium, . . . . .	0.03	1.56	0.14
Phosphoric acid, . . . . .	0.76	0.82	0.23
Sodium chloride, . . . . .	2.21	1.56	0.76
Total nitrogen, . . . . .	2.900	5.072	1.923
Nitrogen as ammonia, . . . . .	0.386	0.188	0.031
" amino-acids, . . . . .	1.117	0.459	0.099
" albumose and peptone, . . . . .	0.885	0.435	0.298
" indigestible matter, . . . . .	0.115	0.119	0.166
" digestible proteins, . . . . .	0.397	3.871	1.329
Percentage of proteins dissolved by gastric juice in 30 minutes, . . . . .	100	68	52
Percentage of proteins dissolved by gastric juice in 60 minutes, . . . . .	100	91	75
100 parts of nitrogen were present in the following forms :—			
As ammonia, . . . . .	13.0	3.7	1.6
" amino-acids, . . . . .	38.5	9.0	5.2
" albumose and peptone, . . . . .	30.5	8.6	15.5
" indigestible matter, . . . . .	4.0	2.4	8.6
" digestible proteins, . . . . .	14.0	76.3	69.1
The above analyses expressed according to the author's method give—			
Water, . . . . .	50.90	33.01	44.84
Fat, . . . . .	27.30	30.28	36.73
Ash, . . . . .	3.14	5.30	2.95
Proteins, . . . . .	3.27	25.46	12.73
Primary products of ripening, . . . . .	5.64	2.77	1.91
Secondary " " . . . . .	9.75	3.17	0.84

Table C. gives the results of the analysis of three varieties of cheese to illustrate the results of Stutzer's investigation. The nitrogen in the proteins multiplied by 6.38 will give, with fair accuracy, the amount of the proteins; the nitrogen of the albumoses and peptone multiplied by 6.38 will approximate nearly to the amount of primary products of ripening. The author has calculated from the nitrogen given in Stutzer's analysis the proteins, primary and secondary products of ripening, in

order to compare the method given above with that previously described.

For most practical purposes the author's method will give as much information as that of Stutzer, if the following facts are borne in mind:—(1) The ripening of a cheese is shown by the proportion of primary and especially secondary products; and (2) the digestibility of a cheese increases with its ripeness.

**Duclaux's Method.**—Duclaux has proposed the investigation of the fatty acids developed by ripening as a means of judging a cheese. The following are the methods used by him:—

**Water, Fat, Alcoholic and Aqueous Extracts.**—Twenty grammes of sand which has been previously dried, sifted, and ignited, are weighed out, and about seven-eighths are placed in an enamelled mortar; 2 to 3 grammes of cheese, accurately weighed, are ground up with the sand to form a homogeneous mass, which should become nearly pulverulent. The mixture is introduced into a small calcium chloride tube, fitted with a plug of asbestos to prevent loss, and the basin rinsed out with the remainder of the sand. The tube with its contents are weighed, and placed in a bath heated to 50° or 60°, and a current of dry air passed through for some hours. After cooling, the tube is weighed and the loss noted as water.

The fat is now extracted by carbon bisulphide (other solvents, such as ether or chloroform, may be used), the tube again dried and weighed, and the amount of fat deduced by difference.

The tube may be similarly exhausted by alcohol, hot or cold water, and the loss of weight noted after each extraction.

**Ash and Salt.**—A fresh portion of cheese is weighed out into a platinum basin, and ignited to obtain the ash; in this, the chlorine is titrated with standard silver nitrate, using potassium chromate as indicator.

**Proteins and Products of Ripening.**—About 10 grammes of cheese are weighed and intimately mixed in a mortar with about 10 c.c. of water; a very homogeneous paste is formed, and this is left for half an hour to ensure the perfect contact of the water with the solid matter. More water is added, little by little, the mixing in the mortar being continued till 100 c.c. have been added. The mixture is now filtered through a porous porcelain filter by means of reduced pressure; in several hours 60 to 70 c.c. can be obtained.

Ten c.c. are evaporated in a platinum basin, the residue dried at 100°, weighed, then ignited, and the ash weighed. The difference will give the organic matter; this is termed by Duclaux "caseone," and represents the products of ripening. The percentage may be calculated with approximate accuracy, by multiplying by 100 ÷ the weight of water in the amount of

cheese taken, and dividing by one-tenth of the weight of the cheese.

The remainder of the filtered liquid (50 c.c.) is brought, by the addition of water, to 150 c.c., and distilled into standard acid, to determine the free ammonia; this determination is not very exact as ammonia is gradually liberated as the distillation proceeds; hence it is usual to stop the distillation when 75 c.c. have distilled over. A little calcined magnesia suspended in 25 c.c. of water is next added, and about 50 c.c. are distilled into standard acid for the estimation of combined ammonia.

The residue in the distilling flask is rendered acid by the addition of a little sulphuric acid, and made up to 55 c.c.; 40 c.c. are distilled off, and the volatile acid received in standard alkali. The acid is calculated as butyric by multiplying the number of c.c. of  $\frac{N}{10}$  alkali used by the factor 0.00975 (this factor assumes that 90.2 per cent. of the total acid will be obtained under these conditions).

He gives the following analyses:—

TABLE CI.

	Curd Two Days Old.	Cantal Cheese in good condition.	Old Cantal Cheese.
	Per cent.	Per cent.	Per cent.
Water, . . . . .	40.7	44.4	36.26
Fat, . . . . .	30.1	23.9	34.70
Proteins (insoluble), . . . . .	20.0	13.7	} 11.09
" (soluble but not fil- trable), . . . . .	4.1	8.3	
" (filtrable), . . . . .	4.3	7.2	
Salt, . . . . .	0.8	2.5	2.23
Ammonia total, . . . . .	...	...	0.90
Volatile acids (as butyric), . . . . .	...	...	0.27

**Volatile Acids.**—The following proportions of volatile acids per 100 of fat are instructive:—

TABLE CII.

Fresh curd, . . . . .	0.04 per cent.
Curd five days old, . . . . .	0.55 "
" eight " . . . . .	2.33 "
Cheese from the same curd two months old, . . . . .	3.0 "
Cantal cheese, . . . . .	3.2 "
Fat from above rancid after one month, . . . . .	9.2 "
Salers cheese (bitter), . . . . .	8.8 "
" (taste good), . . . . .	2.0 "
Cheese five years old, . . . . .	71.2 "



**Van Slyke's Method for the Estimation of the Products of Ripening.**—25 grammes of cheese are well mixed in a mortar with an equal volume of quartz sand, and transferred to a flask; 100 c.c. of water at about 50° C. are added, and the mixture kept at 50° to 55° for half an hour with frequent shaking. The liquid is filtered through cotton wool into a 500 c.c. flask, and the residue is treated as before with four further successive quantities of 100 c.c. of water; after cooling the volume is made up to the mark.

*Water-soluble Nitrogen.*—This is estimated by the Kjeldahl method in 50 c.c. of the solution.

*Nitrogen as Paramuclein.*—Five c.c. of a 1 per cent. solution of hydrochloric acid are added to 100 c.c. of solution, and digested at 50° to 55° C. till the precipitate has completely separated. The precipitate is filtered, and washed, and the nitrogen estimated by the Kjeldahl method.

*Nitrogen as Coagulable Protein.*—Neutralise the preceding filtrate, heat to boiling, and estimate the nitrogen in the precipitate, if any; coagulable proteins rarely occur in cheese.

*Nitrogen as Caseoses.*—To the filtrate from the last determination add 1 c.c. of 50 per cent. sulphuric acid, saturate with zinc sulphate, and warm to 70° C. Cool the solution, filter, wash with a saturated solution of zinc sulphate, and estimate the nitrogen in the precipitate.

*Nitrogen as Amino-acids and Ammonia.*—To 100 c.c. of the aqueous solution of the cheese add 1 gramme of sodium chloride, and tannin solution till the precipitation is complete; filter and dilute the filtrate to 250 c.c., and Kjeldahl an aliquot portion. This will give the nitrogen as amino-acids and ammonia; the ammonia is estimated in another aliquot portion by making alkaline with a little magnesia, and distilling the ammonia into standard acid.

*Nitrogen as Peptone.*—The difference between the water soluble nitrogen and the sum of the other determinations will give the nitrogen as peptone.

*Nitrogen as Mono-calcium Caseinate.*—The portion of the cheese insoluble in water is treated with successive portions of 100 c.c. of a 5 per cent. sodium chloride solution, in a manner similar to the treatment with water, and the nitrogen as mono-calcium caseinate is estimated by the Kjeldahl method in an aliquot portion of the solution.

**Devarda's Method.**—Devarda recommends for the determination of water that about 10 grammes of finely-divided cheese should be dried *in vacuo* over sulphuric acid for twenty-four to thirty-six hours, and then for two to six hours at 100° C. until the weight becomes constant. In this way the bulk of

water is removed at the ordinary temperature, and, whilst the method is fairly quick, there is no material loss of organic matter, such as occurs with long-continued drying at 100° C. Complete drying *in vacuo* is too tedious and often impracticable.

The following examples show the accuracy of this process :—

TABLE CIII.

Name of Cheese.	Loss of Water per cent.				Water per cent.	
	24 hours <i>in vacuo</i> .	A second 24 hours <i>in vacuo</i> .	3 to 6 hrs. at 100°.	Total.	Dried at 100° C.	Dried <i>in vacuo</i> .
Romadur, . .	46·24	2·24	3·11	51·59	51·92	51·50
Limburger, . .	37·79	1·12	0·09	39·00	39·38	38·98
Gervais, . . .	47·89	...	1·36	49·25	49·36	49·10
Limburger, . . (air-dried).	11·10	...	2·17	13·27	13·46	...

**Adulteration of Cheese.**—The only forms of adulteration of any importance consist in the substitution of skim milk cheese for whole milk cheese, or milk cheese for cream cheese, and the addition of fat not derived from milk to skim milk before making it into cheese.

The former adulteration is detected by the estimation of fat and total nitrogen. In a whole milk cheese the ratio  $\frac{\text{fat}}{\text{total nitrogen}} \times 6\cdot38$  varies from 1 to 1·5; in a skim milk cheese it is usually less than 1.

Another method consists in calculation of the composition of the original milk (or cream); the author has found that the following calculation gives a very fair approximation to the composition of the milk or cream from which the cheese was prepared.

Multiply the percentage of proteins by 35·4, add the percentage of fat, and divide 100 times the percentage of fat by the figure thus obtained; to the resulting figure add 0·25, and the sum will be a close approximation to the percentage of fat in the milk or cream used for the preparation of the cheese. Owing to the natural variations of the ratio of fat to proteins in cheese, and of the loss of fat in the whey, no cheese should be certified by this method to be made from skimmed milk unless the calculated fat is less than 2·75 per cent. Similarly it is not advisable to condemn a cream cheese unless the calculated percentage of fat falls below 10 per cent.

The addition of foreign fat is detected by an examination of

the fat by the methods given under *Butter Fat*. The fat extracted during the process of analysis may be used. The cheese may be boiled with water to which a little alkali has been added, or shaken with boiling water, and then with an equal bulk of sulphuric acid 1·820 specific gravity (as recommended in the Gerber process). If the cheese has been extracted with water and ground up in a mortar, in most cases the bulk of the fat separates in the form of butter, and the fat can be readily separated from this.

Devarda recommends triturating 50 to 100 grammes of cheese with a little water in a mortar, mixing with 50 to 80 c.c. of water and 100 to 150 c.c. of ether in a stoppered flask and shaking with dilute potash till a red colour is shown with phenolphthalein. The ethereal layer is drawn off, the ether distilled, the fat dried at 100° C. and filtered (if necessary).

W. N. Yarrow digests the cheese with hydrochloric acid till the fat floats on the surface, and washes this with water till neutral.

It must be borne in mind that certain changes may take place in the fat, and that the limits of composition of butter fat do not apply quite so rigidly to cheese fat. As, however, any addition of fat is usually large relatively to the butter fat left in the cheese, there is not much difficulty in detecting its presence.

**Chemical Control of Cheese-making.**—The amount of cheese in pounds that may be expected to be obtained from 10 gallons of milk may be calculated by one of the following formulæ:—

$$(i.) \text{ lb. of cheese} = \{\text{Solids not fat} \times 0\cdot3 + (\text{fat} - 0\cdot23)\} \times \frac{100}{55}.$$

$$(ii.) \text{ lb. of cheese} = \{\text{aldehyde figure} \times 0\cdot135 + (\text{fat} - 0\cdot23)\} \times \frac{100}{55}.$$

$$(iii.) \text{ lb. of cheese} = \{\text{curd by Lindet's method} \times (\text{fat} - 0\cdot23)\} \times \frac{100}{55}.$$

These formulæ give the weight of green cheese assumed to contain 45 per cent. of water and salt, and apply fairly well to such cheeses as Cheddar, Cheshire, and Stilton.

The percentage of fat should be estimated in the whey, and this should not exceed 0·3; a higher figure shows that the curd has been cut too soon, or carelessly.

The acidity of the milk, the whey, and the curd should be determined; the milk before renneting should have an acidity of 22° to 24°, the whey should be drawn at about the same acidity, and the curd vatted when the acidity has reached about 100°. The acidity of the curd is best tested with a hot iron; a small piece of curd is placed on a hot iron, and immediately with-

drawn; when the curd pulls out in strings it is ready for vatting.

**The Fermentation Test for Milk.**—In order to ascertain the fitness of milk for making good cheese, the appearance of the curd on souring should be noted; the test is carried out by filling test tubes, which must be scrupulously clean, and preferably sterilised, with milk, and after covering them, keeping them for twelve hours at a temperature of 40° C. (104° F.), and examining the curd produced. A good milk will either not have curdled in the time, or will have produced a homogeneous curd, with the development of a clean acid smell. If there is much separation of whey, if the curd is granular, or especially if the curd contains many bubbles or is spongy, the milk will not make good cheese; a strong unpleasant smell is also very undesirable. All these conditions indicate the presence of undesirable organisms; to some extent the effects of these may be counteracted by the addition of a starter (a pure cultivation of lactic acid bacilli) before renneting, but a milk giving a very gassy curd in the fermentation test will not produce good cheese.

### Other Products Derived from Milk.

**Commercial Milk-sugar — Preparation.**—Where whey is a bye-product, in cheese making countries, it is treated for the manufacture of milk-sugar. This is done by allowing it to stand so that the cream present may rise to the surface, heating it, and removing the cream and a portion of the proteins. The whey is neutralised with lime, and a little alum added, which precipitates a further amount of proteins. The whey is then boiled down in vacuum pans, and the sugar allowed to crystallise out. Milk-sugar is purified by re-crystallisation from water or, where alcohol is cheap, by dissolving it in water and precipitating with alcohol.

The milk-sugar of commerce is usually in the form of fine powder, but it is also sold in crystals; it consists of essentially pure sugar, but may contain sensible amounts of lactic acid, ash, and, sometimes, proteins. Its chief uses are for the preparation of infants' food and the manufacture of penta-nitro-lactose, which forms a part of some high explosives.

**Examination of Commercial Milk-sugar.**—Add 6 or 7 grammes of the finely-powdered sugar to about 50 c.c. of distilled water; stir vigorously with a thermometer for ten seconds and allow the solution to settle for twenty seconds; read the fall in temperature on dissolution and rapidly filter the solution. When sufficient clear filtrate is obtained, fill a 200 mm. polariscope tube, and polarise as soon as possible. Take polarimetric

readings every minute till the specific rotatory power begins to diminish. If the temperature at which the solution is polarised is kept at 15° C., or below, there is no difficulty in obtaining several readings which are nearly constant, and the mean of these are taken as the *initial* rotation. Allow the tube to stand for twenty-four hours, and polarise again at the same temperature; this is the *normal* rotation. The initial rotation divided by the normal rotation will give the "birotation ratio." The amount of milk-sugar in 100 c.c. of this solution is estimated, either by drying 5 c.c. at 100° C., when a residue of anhydrous sugar will be left, or by deducing it from the normal rotation. This is done by dividing the reading in angular degrees by 1.106. The two figures should agree closely.

About 10 grammes of sugar are weighed out into a 100 c.c. flask and boiled with about 80 c.c. of water for a few minutes. The solution is cooled to 20°, made up to 100 c.c., and polarised in a 200 mm. tube. The reading in angular degrees multiplied by 100 and divided by the weight of sugar taken multiplied by 1.05 will give the percentage of milk-sugar in the sample.

Five grammes are weighed out in a platinum basin, ignited over a moderate flame and the ash weighed.

Ten grammes are dissolved in 100 c.c. of milk; this is brought to the boil; the milk should not be curdled. If it is, the acidity should be estimated by titrating 5 grammes dissolved in water with  $\frac{N}{10}$  alkali and calculated as lactic acid.

To a solution in water (10 per cent.) a little mercuric nitrate is added; the solution should not show more than the faintest turbidity.

Good commercial milk-sugar crystallised from water should give the following figures:—

Milk-sugar per cent.,	.	.	99.6 to 99.9.
Birotation ratio,	.	.	1.6.
Fall of temperature,	.	.	0.5° C.
Solubility at 15° C.,	.	.	7.0 grammes per 100 c.c. (anhydrous sugar), each 1° increase of temperature raises this figure about 0.1 gramme per 100 c.c.
Ash,	.	.	not more than 0.05 per cent.

Milk-sugars which have been precipitated with alcohol usually polarise slightly over 100 per cent.; have a birotation ratio below 1.6 and, usually, above 1.5; cause a slightly greater rise of temperature; and have a rather higher solubility in water.

**Detection of Adulteration.**—Milk-sugars which are adulterated with other sugars will show marked divergence from the

above figures. Cane-sugar can be detected by treating a solution with a little yeast and keeping at 55° C. for five hours; milk-sugar shows no change in specific rotatory power, while the presence of even 1 per cent. of cane-sugar will produce a marked alteration.

Dextrose is detected by an increase in the birotation ratio, by the solubility, and by a decrease in the fall of temperature.

Maltose and dextrin (present in commercial starch-sugar) are detected by a lowering of the birotation ratio, a great increase in the apparent percentage of milk-sugar, and in the solubility.

Mineral adulterants will be easily detected by the high percentage of ash.

The following are typical analyses :—

TABLE CIV.—ANALYSES OF SUGARS.

	Crystallised Sugar.	Precipitated Sugar.	Milk-sugar adul- terated with 3 per cent. Cane-sugar.
Polarisation, . per cent.,	99.8	100.3	{ 100.4 100.5
Birotation ratio, . . . .	1.603	1.546	1.585
Fall of temperature, . . .	0.4° C.	0.8° C.	0.55° C.
Solubility at 15°, per cent.	7.08	7.65	7.13
Polarised after treatment with yeast, . . . . .	99.8	100.3	95.2
Reaction with mercuric nitrate, . . . . .	none.	{ faint } { turbidity }	none.
Behaviour with milk, . . .	not curdled.	not curdled.	not curdled.
Ash, . . . . . per cent.,	0.025	0.048	0.032

**Junkets.**—This preparation is made by adding cane-sugar to milk and curdling by rennet at a low temperature. It is a sweetish gelatinous substance, and is usually eaten with nutmeg and cream.

**Casein.**—Many preparations of this protein are now on the market, and, besides being largely consumed as foods, they are employed in the arts for such purposes as sizing paper, as a mordant, and for clarifying wines.

Casein is prepared by precipitating the protein from separated milk by means of an acid; sulphuric or hydrochloric acids are generally employed, but sometimes acetic acid or the lactic acid of strongly acid whey is used. If a moderately pure protein is desired the precipitated protein is dissolved in a small quantity of alkali, and reprecipitated, but many of the preparations on

the market consist of once precipitated casein, which has not even been washed with water.

To prepare a casein soluble in water, a small quantity of alkali (sodium carbonate) is added to dissolve the protein, and the solution is dried on hot rollers, as a fine spray, or in thin layers, and the resulting solid ground. A casein nearly insoluble in water, but easily soluble in dilute alkali solutions, may be prepared by dissolving in ammonia, and evaporating the solution; practically all the ammonia passes off on drying. The curd produced by rennet may also be used, but it is more difficult to dissolve than that precipitated by acids. If the precipitated casein is directly dried a hard, horny mass is produced, and as, in the process of drying it is often overheated, it does not dissolve easily in dilute alkali solutions.

The following products of casein are commercial substances:—

**Plasmon, Tilia, etc.**—The sodium compounds of casein, containing the bulk of the salts of the milk.

**Sanatogen.**—Casein combined with 5 per cent. of sodium glycerophosphate.

**Lacto-Somatose.**—This consists of albumoses derived from casein by heating with superheated steam.

**Sanose.**—A mixture of 80 per cent. casein and 20 per cent. albumoses.

**Nutrose.**—The sodium compound of casein.

**Eucasin.**—The ammonia compound of casein.

**Argonin.**—The silver compound of casein.

**Lactoform** consists essentially of casein precipitated by metallic salts and subsequently hardened by formaldehyde. It is employed in place of horn, ivory, ebony, amber, etc. By painting walls twice with a 15 per cent. solution of casein and a 10 per cent. solution of zinc chloride, followed by an application of strong formaldehyde solution, they may be rendered damp-proof.

Casein treated with formaldehyde is also used in the preparation of photographic plates, and for artificial tortoiseshell, etc.

**Analysis of Casein Preparations.**—*Moisture* is estimated by drying in the water-oven.

*Ash* is determined by ignition at a temperature below red heat; a very pure casein should not be ignited in a platinum basin, as the phosphorus of the casein attacks the platinum; in the presence of sufficient base a phosphate is formed.

*Total Proteins* are deduced from the percentage of nitrogen by multiplying by 6.39.

*Fat* is estimated by the Werner-Schmid, or Gottlieb methods, as directed for dried milks (p. 149).

*Milk-sugar.*—25 grammes are dissolved in water, and, if

necessary, a little alkali, and made up to 500 c.c.; the bulk of the casein is precipitated by the addition of dilute hydrochloric acid, the volume of this being noted. An aliquot portion of the filtrate is neutralised, using phenolphthalein as indicator, and evaporated to less than 50 c.c.; 1 c.c. of acid mercuric nitrate is added, the volume made up to 50 c.c., and the solution filtered; to 40 c.c. of the filtrate, 2 c.c. of phospho-tungstic acid, and 2 c.c. of sulphuric acid (1:1) are added, the solution filtered, and the milk-sugar is estimated in the filtrate by polarisation.

*Casein* may be estimated by Sebelein's method (p. 130). The following determinations may be made on the filtrate obtained by adding acid mercuric nitrate (4 c.c. to 100 c.c.) to a 5 per cent. solution; calcium, magnesium, potash, soda, chlorides, sulphates, phosphates, citrates, acetates, and nitrogen in phospho-tungstic acid precipitate. These determinations will give a clue as to the mode of preparation of the casein.

The difference between the phosphoric acid in the ash and in the filtrate will give the organic phosphate.

If sodium glycerophosphate is present, the mineral phosphates must be estimated in the filtrate obtained by adding acid mercuric nitrate, by precipitating with ammonium molybdate in the cold, and the sum of the mineral phosphates and those derived from the glycerophosphates estimated in the ash of this filtrate; the difference between the  $P_2O_5$ , multiplied by 3.803 will give the percentage of sodium glycerophosphate.

The acidity and aldehyde figures are also useful determinations, as is the solubility in dilute alkali (if not water soluble).

**Milk wine** is produced by peptonising milk by means of special ferments or organisms, precipitating with acid, and fermenting, after the addition of sugar.

**Milk cocoa** is a mixture of cocoa with dried milk solids. A sample examined by the author had the following composition:—

Water.	Fat.	Ash.	Protein.	Starch.	Milk-Sugar.	Other Substances.
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
5.93	25.91	5.02	19.50	5.70	11.70	26.24.

**Milk chocolate** is a somewhat similar preparation, made by incorporating dried milk with cane-sugar and cocoa.



## CHAPTER VIII.

## THE MILK OF MAMMALS OTHER THAN THE COW.

CONTENTS.—Classification of Milks—Human Milk—The Milk of the Buffalo—the Gamoose—the Ewe—the Goat—the Mare—and the Ass—Milk as a Food and a Medicine—As a Food for Infants.

**Classification.**—Broadly speaking, the milk of all mammals may be divided into classes as under :—

(1) Milks forming hard curds with rennet. This class includes the milk of the ewe, buffalo, goat, and cow.

(2) Milks forming a very soft, or no, curd with rennet. Included in this class are human milk, and those of the ass, mare, and mule.

The composition of milk of all mammals, on the whole, resembles that of cow's milk—*i.e.*, they all contain fat in the form of globules, sugar, proteins, and mineral matter. Marked differences, however, occur in the composition of these bodies.

**Comparison of the Fat of Different Animals.**—(a) *Size of Globules.*—The following table (CV.) gives the results obtained by Pizzi :—

TABLE CV.—SIZE OF FAT GLOBULES IN MAMMALIAN MILKS.

Name of Mammal.	Relative Number of Globules of the Sizes Named.							
	0·0127 mm.	0·0109 mm.	0·0090 mm.	0·0072 mm.	0·0054 mm.	0·0033 mm.	0·0018 mm.	0·0009 mm.
Woman,	o	o	many	many	medium	few	v. few	v. few
Ewe, .	o	few	o	medium	medium	medium	few	”
Goat, .	v. few	v. few	few	few	”	”	medium	”
Cow, .	o	o	medium	many	”	”	v. few	”
Rabbit,*	many	many	few	few	v. few	v. few	v. few	v. few
Ass, .	o	v. few	v. few	many	medium	medium	v. many	”
Mare, .	o	”	medium	medium	few	many	many	”
Sow, .	o	o	v. few	v. few	v. few	medium	v. many	many
Bitch, .	o	o	many	many	medium	”	v. few	v. few
Cat, .	o	o	few	few	”	few	”	”
Mouse,*	many	many	”	”	”	v. few	”	”

\* The milk of the rabbit and mouse contained globules up to 0·0181 mm. in diameter.

(b) *Composition of Fat.*—The following table (CVI.) gives the comparative figures for the composition of the fat of various mammals :—

TABLE CVI.—PROPERTIES OF MAMMALIAN FATS.

Name of Mammal.	Melting Point.	Solidifying Point.	Reichert-Wolny Figure.	Insoluble Fatty Acids.	Zeiss Refractive Index at 33°.
Woman, .	32°	22·5°	1·42	Per cent.	51·9°
Ewe, .	29°	12°	26·7·32·89	...	...
Goat, .	30·5°	31°	26·1·28·6	...	...
Buffalo, .	38°	29°	25·4·39	...	...
Cow, .	...	...	20·0·34	86 to 90	{ 43·7°-49·0° mean 46·5°
Rabbit, .	...	...	16·06	..	...
Ass, .	...	...	13·09	..	...
Mare, .	...	...	11·22	...	...
Sow, .	25°	12°	1·65	...	...
Bitch, .	..	...	1·21	...	...
Cat, .	...	...	4·40	...	...
Mouse, .	...	...	2·97	...	...
Porpoise, .	A sample examined by Allen contained 5·06 per cent. of volatile acid, having a mean combining weight of 104·7, and agreeing with valeric acid (m.c.w. 102) in its properties.				

**Sugar.**—The author has proved that the sugar of the milk of goat, the ass, and the gamoose (in summer) contains milk-sugar.

With Pappel the author obtained analytical figures which showed that the sugar of the milk of the gamoose in winter differed from milk-sugar, and with Carter that the sugar of human milk did not correspond with milk-sugar. It is probable also that the sugar of mare's milk is not identical with milk-sugar.

Denigès and also Landolf state that all milks contain lactose, but that other sugars are present in addition.

**Proteins.**—But little is known of the proteins of milk. It appears probable that the curd-forming milks contain the same proteins as cow's milk. The milks which do not form curd may differ in their proteins, but it is possible that the different reaction with rennet is due to a deficiency of lime, or to an alkaline reaction.

The following milks have an alkaline reaction to litmus :—Human milk, the milk of the mare, ass, rabbit, sow, and cat.

**Composition of Milk.**—Table CVII. gives the mean composition of the milk of different mammals.

Of these milks those of the cow, goat, sheep, buffalo, mare, ass, and, in some countries (*e.g.*, Spain), sow are used for human

consumption, and, with the exception of that of the sow, are worthy of a more detailed notice. Human milk, the natural food of infants, will also be dwelt on.

TABLE CVII.—COMPOSITION OF MAMMALIAN MILK.

	Water.	Fat.	Sugar.	Casein.	Albumin.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Cow, . . . . .	87.32	3.75	4.75	3.00	0.40	0.75
Goat, . . . . .	86.04	4.63	4.22	3.49	0.86	0.76
Ewe, . . . . .	79.46	8.63	4.28	5.23	1.45	0.97
Buffalo, . . . . .	82.63	7.61	4.72	3.54	0.60	0.90
Woman, . . . . .	88.2	3.3	6.8	1.0	0.5	0.20
Mare, . . . . .	89.80	1.17	6.89	1.84		0.30
Ass, . . . . .	90.12	1.26	6.50	1.32	0.34	0.46
Mule, . . . . .	91.50	1.59	4.80	1.64		0.38
Bitch, . . . . .	75.44	9.57	3.09	6.10	5.05	0.73
Cat, . . . . .	81.63	3.33	4.91	3.12	5.96	0.58
Rabbit, . . . . .	69.50	10.45	1.95	15.54		2.56
Llama, . . . . .	86.55	3.15	5.60	3.00	0.90	0.80
Camel, . . . . .	86.57	3.07	5.59	4.00		0.77
Elephant, . . . . .	67.85	19.57	8.84	3.09		0.65
Sow, . . . . .	84.04	4.55	3.13	7.23		1.05
Porpoise, . . . . .	41.11	48.50	1.33	11.19		0.57
Whale, . . . . .	48.67	43.67	7.11			0.46

**Human Milk—Appearance.**—In appearance human milk has usually a chalky white, somewhat watery appearance; some specimens, usually those high in proteins, have a marked yellowish tint. A red coloration, due to blood, has been noticed by Carter and the author.

**Properties.**—The fat globules, according to Pizzi, vary in size from 0.009 mm. to 0.0009 mm. Carter and the author have observed also that they are, on the whole, smaller than those of cows' milk.

The taste is rarely, if ever, sweet, but rather saline. The reaction to litmus paper is almost always alkaline. The acidity is about 3.0°.

**Composition.**—Human milk appears to be more variable in its composition than that of the cow. This is probably due to the fact that, while the cow is forced to adopt regular habits and leads a life which is very regular, the many occupations and duties of woman do not permit of this.

Table CVIII. gives the mean composition of human milk according to recent observers.

TABLE CVIII.—MEAN COMPOSITION OF HUMAN MILK.

Observer.	Water.	Fat.	Sugar.	Proteins.	Ash
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Leeds,* . . . . .	86.69	4.16	6.95	2.02	0.22
Pfeiffer, . . . . .	88.22	3.11	6.30	1.94	0.19
Luff, . . . . .	88.51	2.41	6.39	2.35	0.34
Johanssen, . . . . .	..	3.21	4.67	1.10	..
Carter and Richmond, . . . . .	88.04	3.07	6.59	1.97	0.26
Lehmann, . . . . .	87.3	3.4	6.4	1.7	0.2
Camerer and Söldner, . . . . .	88.07	3.24	6.33	1.69	0.24
Szilasi, . . . . .	87.24	3.38	6.97	2.20	0.20
Backhaus, . . . . .	87.41	4.02	6.71	1.62	0.25
COLOSTRUM—					
Pfeiffer, . . . . .	85.75	2.38	3.39	8.60	0.37
Lajoux, 1st day, . . . . .	83.45	3.50	4.50	8.08	0.46
„ 2½ days, . . . . .	89.30	1.45	5.91	3.05	0.29

TABLE CIX.—VARIATIONS IN COMPOSITION OF HUMAN MILK DURING LACTATION.

	Water.	Fat.	Sugar.	Proteins.	Ash	Refractive Index of Fat at 35°.
	Per ct.	Per ct.	Per ct.	Per cent.	Per ct.	
Milk that did not agree ( <i>C. &amp; R.</i> )	88.11	2.95	6.28	2.36	0.31	54.3'
Normal Milk, 4 to 6 days „	88.01	2.97	6.47	2.25	0.30	53.2°
„ „ 7 to 14 „ „	88.21	3.06	6.62	1.85	0.26	51.5°
„ „ 15 to 29 „ „	87.74	3.42	6.95	1.67	0.22	51.4°
„ „ over 30 „ „	88.53	3.00	6.83	1.43	0.21	51.7°
First Month ( <i>Pfeiffer</i> ), . . . . .	88.18	2.74	5.77	2.98	0.24	..
Second „ „ . . . . .	88.22	3.37	6.33	2.04	0.18	..
Third „ „ . . . . .	88.90	2.71	6.43	1.99	0.18	..
Fourth „ „ . . . . .	87.56	3.91	6.89	1.77	0.15	..
Fifth, „ „ . . . . .	88.77	3.36	7.33	1.45	0.19	..
Sixth „ „ . . . . .	88.31	2.79	6.83	1.54	0.23	..
Seventh „ „ . . . . .	89.43	3.28	6.89	1.53	0.18	..
Eighth „ „ . . . . .	88.49	3.36	6.31	1.69	0.16	..
Ninth „ „ . . . . .	89.25	2.41	6.62	1.54	0.17	..
Tenth „ „ . . . . .	87.79	4.22	6.24	1.71	0.14	..
Eleventh „ „ . . . . .	87.92	3.59	6.66	1.47	0.14	..
Twelfth „ „ . . . . .	86.71	5.30	6.09	1.73	0.16	..
Thirteenth „ „ . . . . .	88.55	2.94	6.68	1.65	0.15	..

\* Leeds gives the average composition as :—

Water, . . . . .	86.733 per cent.	Protein, . . . . .	1.995 per cent.
Fat, . . . . .	4.131 „	Ash, . . . . .	0.201 „
Sugar, . . . . .	6.936 „		

His figures, however, do not agree with the average deduced from his analyses. There is internal evidence that his analyses are not so reliable as the other series, and comparatively little weight must be given to his series. He examined sixty-four samples derived from eighty women, some of his samples being the mixed milk of six women.

TABLE CIX.—*Continued.*

	Water.	Fat.	Sugar	Proteins.	Ash.
	Per cent	Per cent.	Per cent.	Per cent.	Per cent.
1st to 11th day (Leeds),	86.49	4.28	6.67	2.32	0.23
11th to 31st " "	86.60	3.90	7.06	2.01	0.20
31st to 91st " "	86.99	3.97	6.99	1.80	0.21
Over 91 days, "	86.51	4.44	7.09	1.76	0.22
4 to 10 days (Lajoux),	85.13	5.49	6.12	2.95	0.32
8 " 11 " (Camerer & Söldner),	87.99	2.92	6.39	2.38	0.27
20 " 40 " 1st series,	87.54	4.04	6.36	1.79	0.22
70 " 120 " "	88.33	3.29	6.66	1.49	0.18
170 or over, "	89.35	2.47	6.87	1.07	0.18
1 to 3 days (Camerer & Söldner),	87.35	2.92	4.96	3.25	0.41
5 " 6 " 2nd series,*	87.93	3.26	5.83	1.83	0.30
8 " 12 " "	87.88	3.11	6.16	1.72	0.28
20 " 40 " "	87.52	3.91	6.52	1.30	0.22
60 " 120 " "	88.21	3.31	6.81	1.10	0.19
170 or over, "	88.56	3.20	6.78	0.95	0.18

**Variation with Lactation.**—There exist several series of analyses in which the time which has elapsed since parturition has been noted. Of these, the results of Carter and the author represent normal milks, those which disagreed with the child in any way having been excluded. The Tables due to Pfeiffer, Leeds, and Camerer and Söldner contain all the analyses made by them without any eliminations (Table CIX.).

**Probable Mean Composition.**—From the above results the following probable composition may be deduced for normal human milk after lactation has become regular:—

Water,	.	.	.	.	88.2 per cent.
Fat,	.	.	.	.	3.3 "
Sugar,	.	.	.	.	6.8 "
Protein,	.	.	.	.	1.5 "
Ash,	.	.	.	.	0.2 "

**Variation of Constituents.**—The following maxima and minima have been found:—

	Per cent.	Per cent.
Fat, . . .	9.05 (Pfeiffer),	0.47 (C. and R.).
Sugar, . . .	8.89 (C. and R.),	4.22 (Pfeiffer).
Protein, . . .	5.56 (Pfeiffer),	0.85 (Leeds).
Ash, . . .	0.50 (C. and R.),	0.09 (Pfeiffer).

\* In Camerer and Söldner's second series the proteins are obtained by multiplying the nitrogen by 6.38.

**Composition Before and After Suckling.**—The average composition of 37 samples taken before and 37 samples after suckling was found by Carter and the author to be—

TABLE CX.

	Before Suckling.	After Suckling.
	Per cent.	Per cent.
Water, . . . . .	88.33	88.04
Fat, . . . . .	2.89	3.18
Sugar, . . . . .	6.51	6.53
Protein, . . . . .	1.99	1.99
Ash, . . . . .	0.28	0.26

In one case, where the secretion was excessive, the analyses before and after suckling were practically identical; in another, where a very deficient supply was given, the fat differed greatly.

TABLE CXI.

	Excessive Secretion.		Deficient Secretion.	
	Before Suckling.	After Suckling.	Before Suckling.	After Suckling.
	Per cent.	Per cent.	Per cent.	Per cent.
Water, . . . . .	87.40	87.36	90.59	87.65
Fat, . . . . .	3.12	3.12	0.98	4.07
Sugar, . . . . .	6.68	6.70	6.52	6.31
Protein, . . . . .	2.49	2.51	1.71	1.77
Ash, . . . . .	0.31	0.31	0.20	0.20

In 15 cases the fat was higher before suckling than after suckling, and in 21 it was lower, while in 1 case it was identical. The cases in which the fat was higher before suckling than after were generally when the mother was lying down, indicating that the separation of cream was largely mechanical.

**Analysis of Human Milk.**—As the quantity of the sample is often very limited, the Gerber-Ritthausen method for the analysis of human milk is useful; 5 c.c. are diluted with 100 c.c. of water, 3 c.c. of copper sulphate solution added, and caustic soda solution drop by drop till the precipitate settles readily; the precipitate is collected in a Gooch crucible washed with water, and dried in the water-oven. The fat is extracted by percolating with ether, and the crucible after several percolations being allowed to stand in a beaker containing ether overnight. The ether is evaporated and the fat weighed, and if very dark

green in colour, the fat should be extracted with dilute hydrochloric acid, and the amount of copper estimated and subtracted from the weight. The refractive index of the fat may be determined. The Gooch crucible is dried to constant weight, and ignited; the difference between the two weights gives the proteins. The milk-sugar may be estimated in the filtrate by Fehling's method.

The author has found that the aldehyde figure multiplied by 0.134 gives a close approximation to the proteins.

**Comparison with Cow's Milk.**—The following differences in composition of the various constituents from that of the cow have been noticed:—

*Fat.*—This is very low in volatile acids (see p. 391). It appears to contain free fatty acids; Leeds noted that many of the fats extracted from the copper-protein precipitate obtained by Ritthausen's process were tinted green; Carter and the author confirmed this, and found the following percentages of copper oxide (CuO) in the fats thus extracted:—

2.80      1.27      0.87      0.62      0.30

The copper could be easily removed by shaking with dilute hydrochloric acid, and the fat behaved to copper salts in every way as if it contained free fatty acids.

Carter and the author found the refractive index at 35° C. to vary from 58.4° (in a milk which upset the child, which finally died in convulsions) to 48.2° (in a milk on which the child throve remarkably well). The analyses of these two samples were:—

TABLE CXII.

Water	Fat.	Sugar.	Proteins.	Ash.	Refractive Index.
Per cent. 89.54	Per cent. 0.87	Per cent. 5.19	Per cent. 4.02	Per cent. 0.38	58.4°
87.10	3.95	7.11	1.64	0.20	48.2°

*Sugar.*—Carter and the author found that the sugar crystallised in rhomboid plates (not the wedge-shaped crystals of milk-sugar), and had a specific rotatory power of  $[\alpha]_D = 48.7^\circ$ .

The sugar was estimated by difference in the milk, as it was found that polarisation and estimation by Fehling's solution did not give satisfactory results. It was found that the gravimetric results were from 0.56 to 0.98 per cent. below the difference, and averaged 0.71 per cent. below, while the polarimetric results

were from 0·85 to 2·22 per cent. below the difference, and averaged 1·30 per cent. below.

It was noted that by precipitating the sugar crystallised from water with dilute alcohol an amorphous substance separated, soluble in water. The  $[\alpha]_D$  of the organic solids of the mother liquor from which the sugar was crystallised was 26·8°. These facts seem to indicate that more than one sugar was present.

*Proteins.*—The proteins differ from those of cow's milk by not giving a curd with rennet, and by giving a much finer precipitate with acids. By the addition of calcium phosphate they can be made to approach much more nearly in behaviour to those of cow's milk. The proteins of human milk are not precipitated by copper sulphate from a solution neutral to phenolphthalein, but require a further addition of alkali; the precipitate thus obtained yields a black ash, while the proteins of cow's milk precipitated from a neutral solution leave a green ash.

*Mineral Matter.*—Harrington and Kinnicutt give the following mean composition of the ash:—

TABLE CXIII.

	Per cent.
Uncombined carbon, . . . . .	0·71
Chlorine, Cl, . . . . .	20·11
Sulphurous acid, SO <sub>2</sub> , . . . . .	4·38*
Phosphoric acid, P <sub>2</sub> O <sub>5</sub> , . . . . .	10·73
Silica, SiO <sub>2</sub> , . . . . .	0·70
Carbonic acid, CO <sub>2</sub> , . . . . .	7·97
Iron oxide and alumina, (FeAl) <sub>2</sub> O <sub>3</sub> , . . . . .	0·40
Lime, . . . . .	15·69
Magnesia, . . . . .	1·92
Potash, . . . . .	29·84†
Soda, . . . . .	12·39†
	<hr/> 104·85‡
Less oxygen = chlorine, . . . . .	4·53
	<hr/> 100·31‡

The presence of citric acid has been established, and is about 0·1 per cent.

Béchamp describes a starch-hydrolysing enzyme. The author has established the presence of a proteolysing enzyme analogous to that described by Babcock and Russell in cow's milk.

**The Milk of the Buffalo.**—This has been examined in Europe by F. Strohmer, W. Fleischmann, A. Pizzi, and D'Alzac.

\* Given in original as sulphur, 2·19.

† Given in original as potassium, 24·77; sodium, 9·19; oxygen, 6·16.

‡ The total is given in original as 100·54.



**Composition**—They give the composition as—

TABLE CXIV.

	Strohmer.	Fleisch- mann	Pizzi.	D'Alzac.
Specific gravity, . . . . .	1.0319	1.0339	1.0332	..
Water, . . . . . per cent.	81.67	82.93	82.20	82.05
Fat, . . . . . "	9.02	7.46	7.95	7.98
Proteins, . . . . . "	3.99	4.59	4.13	5.18
Milk-sugar, . . . . . "	4.50	4.21	4.75	4.00
Ash, . . . . . "	0.77	0.81	0.97	0.79

Strohmer draws attention to the high percentage of fat and the musk-like smell. It is white in colour, and the butter prepared from it has only a slight yellowish tinge.

**Fat.**—The analytical figures for the butter fat are given by Strohmer as—

Melting point	Solidifying point.	Solidifying point of fatty acids	Potash absorbed.	Reichert-Wollny figure.
31.3°	19.8°	37.9°	222.4 milligrammes.	30.4 c.c.

and by Pizzi as melting point, 38.0°; solidifying point, 29.0°; Reichert-Wollny figure, 26.2.

**Milk of the Gamoose—Composition.**—The composition of the milk of the Egyptian gamoose or water-buffalo, which is the same as, or very closely allied to, that found in India, South Africa, Hungary, and Southern Europe, has been studied by the author and A. Pappel.

The average composition is—

TABLE CXV.

Specific gravity, . . . . .	1.0354		
Water, . . . . .	84.10 per cent.		
Fat, . . . . .	5.56	"	
Sugar, . . . . .	5.41	"	
Casein, . . . . .	3.26	"	containing nitrogen, 0.511
Albumin, . . . . .	0.60	"	" " 0.094
Salts, . . . . .	1.03	"	" ash, 0.85
Nitrogenous bases, . . . . .	0.09	"	" citric acid, 0.30
		"	" nitrogen, 0.035

A rise in specific gravity on standing was noticed, amounting to, in

6 hours, . . . . .	0.0006
24 hours, . . . . .	0.0007

One sample, however, showed a decrease.

The fat was found to vary from 7.35 to 5.15 per cent., and the solids not fat from 10.67 to 10.07 per cent.

The average percentage composition of the solids not fat was—

Sugar,	.	.	.	.	.	.	49.5	per cent.
Protein,	.	.	.	.	.	.	40.8	„
Ash,	.	.	.	.	.	.	8.2	„
Other substances,	.	.	.	.	.	.	1.5	„

Showing a ratio of sugar : proteins : ash = 6 : 5 : 1.

The following formula was found to be applicable to gamoose milk :— $T = 0.27 \frac{G}{D} + 1.191 F$ , indicating that the fat had a density of 0.934, and the solids not fat had a density of 1.589.

The formula

$$\frac{G}{D} = -0.761 F + 4 L + 2.5714 P + 8.46 A \text{ (see p. 73)}$$

was found to be applicable to the milk of the gamoose.

Two series of investigations into the constituents of the milk were made, one during the winter on milk yielded by a newly-calved gamoose, and the other during the summer on the milk of animals well on in their period of lactation. Notable differences were found.

*Fat.*—The fat gave the following figures on analysis :—

TABLE CXVI.

	Summer	Winter.
Potash absorption, . milligrammes	231.7	220.4
Insoluble fatty acids, . „	86.9	87.5
Mean combining weight, . . .	265.0	270.5
Iodine absorption, . . per cent.	35.1	41.8
Soluble fatty acids, . . „	6.99	6.09
(calc. as butyric),		
Reichert-Wollny figure, . . c.c.	34.7	25.4
Iodine absorption of fat, . per cent.	32.0	35.0

A winter sample of fat was found to contain 0.05 per cent. of sulphur and 0.01 per cent. of phosphorus.

J. N. Dutt finds the average Reichert-Wollny figure of buffalo “ghee” in India 34.5 c.c., with a minimum of 30 c.c.; the oleo-refractometer figure is  $-32^{\circ}$  to  $-35^{\circ}$ , and the melting point  $35^{\circ}$  to  $36^{\circ}$  C.

*Casein.*—The casein was prepared from the winter milk. The purest preparation contained 2.53 per cent. of moisture and 0.60

per cent. ash, the nitrogen calculated to the pure substance being 14·66 per cent. and the phosphorus 0·85 per cent. Other preparations, which were less pure, gave about 14·4 to 14·5 per cent. of nitrogen.

Though this casein was precipitated three times, and thoroughly washed with water each time, and finally with alcohol and ether, it was perhaps not pure, as a solution was made in a minimum of caustic soda, and equivalent amounts of sodium phosphate and calcium chloride added, and the nitrogen was estimated in this and in the precipitates obtained by (1) saturation with magnesium sulphate, (2) tannin, and (3) acetic acid at 40° C.

Calling the nitrogen in solution 100, the following amounts were obtained by

Magnesium sulphate,	. . . . .	95·2
Tannin,	. . . . .	96·6
Acetic acid,	. . . . .	94·7

*Albumin.*—The albumin contained 15·75 per cent. of nitrogen, and is probably identical with that of cow's milk.

Citric acid was identified by isolating it and determining the percentage of calcium in the calcium salt.

A yellow crystalline mercury compound was isolated from the winter milk in too small a quantity for identification, but was in all probability derived from a nitrogenous basic substance.

*Sugar.*—The sugar was prepared from the winter milk. Two preparations gave the following figures:—

Influence on density.	$[\alpha]_D$ .	Cupric reducing power.	Water of crystallisation.
3·94	48·66	73·6	4·77
3·94	49·10	73·9	4·82

Mucic acid was not obtained by oxidation with nitric acid. On treatment with acid the following figures were obtained:—

$[\alpha]_D$ .	Cupric reducing power.
53·74	101·0

figures approaching those given by dextrose.

This sugar evidently differed from milk-sugar, and was named "Tewfikose."

A second preparation was made from the summer milk; it was examined by A. R. Ling and the author, and yielded the following figures. (For the sake of comparison figures yielded by milk-sugar, prepared by the same method, are given.)

TABLE CXVII.

	Gamoose Sugar.	Milk-Sugar.
$[\alpha]_D$ , . . . . .	55.3°	55.1°
Cupric reducing power, . . . . .	79.7	79.5
Influence on density, . . . . .	3.92	3.92
Melting point, . . . . .	213° to 215°	214° to 220°
Water of crystallisation, . . . . . per cent.	5.03	5.00 (calc.)
Molecular weight (from freezing point), . . . . .	341.7	342 (calc.)
Carbon, . . . . . per cent.	39.75	39.60
Hydrogen, . . . . . ”	6.47	6.63
Mucic acid by oxidation with nitric acid, . . . . . ”	32	32

By heating with phenylhydrazine acetate two compounds were formed—one readily soluble in hot water (melting point, 197°), and the second almost insoluble in hot water, but soluble in hot dilute alcohol (melting point, 218° to 219°). These compounds agree with phenyllactosazone and its anhydride.

The acetyl derivative prepared by heating with acetic anhydride and anhydrous sodium acetate, and crystallising from alcohol melted at 75° to 80°. When crystallised from a mixture of 90 per cent. alcohol and chloroform it melted at 88° to 95°.

The solution in chloroform was nearly optically inactive; if anything, slightly lævo-rotatory.

A determination of acetic acid in the acetyl derivative gave 71.46 per cent. as against 70.79 calculated for an octacetyl derivative.

The properties of the acetyl derivative agree exactly with octacetyllactose.

The birotation ratio was found to be 1.6.

All the properties without exception agree with those of ordinary milk-sugar.

It appears that the sugar of the summer milk is “lactose” and not “tewfikose.”

*Winter and Summer Milk.*—There appears to be a distinct difference between the winter and summer milks, which may be summarised as follows:—

Winter.	Summer.
Fat low in volatile acids.	Fat high in volatile acids.
Contains “tewfikose.”	Contains “lactose.”

The author has tried to find evidence that the sugar called “tewfikose” was a product of the action of reagents used in the preparation of milk-sugar, but has been unable to do so, many preparations of milk-sugar having been made by the same method (including that from summer milk), always with the properties

of milk-sugar. Analyses of the milk used to prepare "tewfikose" only added up to 99.3 per cent., when the polarisations were calculated as milk-sugar, but gave a satisfactory approach to 100 when calculated as tewfikose.

There appears to be no reason to reject the view that "tewfikose" is a separate entity.

**The Milk of the Ewe.**—The following composition is given by different authorities:—

TABLE CXVIII.—COMPOSITION OF EWES' MILK.

Authority.	Water.	Fat.	Sugar.	Casein.	Albumin.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.		Per cent.
Pizzi, . . .	80.43	9.66	4.37	4.40		1.10
Besana, . . .	78.23	9.50	5.00	6.26		1.01
Vieth, . . .	81.3	6.8	4.8	6.3		0.8
Bell, . . .	75.2	11.3	3.6	8.8		1.1
Fleischmann, .	83.0	5.3	4.6	4.6	1.7	0.8
„ . . .	75.40	11.77	3.65	6.47	1.64	(average) 1.06
						Raden herd)
Piccardi, . . .	82.46	6.10	3.95	5.56	1.01	0.93
Hucho, . . .	83.10	6.23	4.46	5.39		0.88
Trillat and Forestier, 1902,	80.72	7.24	5.38	5.68		0.98
1903,	82.71	6.86	5.23	5.62		0.98
The composition of colostrum of ewe's milk is given by Voelcker as—						
	69.74	2.75	8.85	17.37		1.29

The following figures are given by Weiske and Kennepohl:—

TABLE CXIX.—COMPOSITION OF EWES' MILK.

Time Since Lambing.	Water.	Fat.	Sugar.	Casein.	Albumin.	Ash.	Non- protein Nitrogen.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
½ hour, . . .	47.03	25.04	1.54	4.96	18.56	1.19	0.28
7 hours, . . .	61.93	16.14	3.53	7.48	9.61	0.96	0.11
19 „ . . .	76.53	8.87	5.24	5.27	2.93	0.86	0.12
2 days, . . .	82.79	5.93	5.19	4.28	0.82	0.87	0.11
3 „ . . .	82.93	6.19	4.37	4.54	0.92	0.95	0.10
4 „ . . .	83.48	5.69	4.31	4.64	0.85	0.96	0.10
5 „ . . .	83.90	5.72	4.27	4.18	0.60	0.92	0.09
6 „ . . .	85.22	4.47	4.55	3.88	0.70	0.88	0.08
7 „ . . .	84.40	4.61	5.09	4.04	0.86	0.90	0.07
8 „ . . .	84.26	4.62	5.31	3.97	0.73	0.88	0.10
9 „ . . .	84.39	4.71	5.41	4.49	0.60	0.90	0.08

The specific gravity varies from 1·035 to 1·043.

Besana gives the following table for correcting the specific gravity to 15° C. :—

TABLE CXX.

Temp.	Correction.
5° to 10°, . . .	Subtract 1·25 + 0·20 (10 - t)°
11° „ 15°, . . .	„ .. 0·25 (15 - t)°
16° „ 20°, . . .	Add .. 0·30 (t - 15)°
21° „ 25°, . . .	„ 1·5 + 0·32 (t - 20)°
26° „ 30°, . . .	„ 3·1 + 0·35 (t - 25)°
31° „ 35°, . . .	„ 4·85 + 0·37 (t - 30)°

The fat globules vary in size, according to Besana, from 0·0047 mm. to 0·0309 mm. This is not in accord with Pizzi's observations (p. 390). Sheep's milk throws up no cream if left to rest, owing to its great viscosity. The cream may, however, be removed by a separator, or by dilution with an equal bulk of water.

The action of rennet does not differ from that with cow's milk, but the curd is firmer.

**The Milk of the Goat.**—The following is the mean composition given by various authorities :—

TABLE CXXI.—COMPOSITION OF GOAT'S MILK.

Authority.	Water.	Fat.	Sugar.	Ca-cin.	Albumin.	Ash.
König (average), . . .	Per ct. 85·71	Per ct. 4·78	Per ct. 4·46	Per ct. 3·20	Per ct. 1·09	Per ct. 0·76
Moser & Soxhlet, . . .	86·48	4·43	4·56	3·44	0·30	0·79
Fleischmann, . . .	85·5	4·8	4·0	3·8	1·2	0·7
Pizzi, . . .	86·75	5·35	3·60	3·64		0·66
Author, . . .	86·76	3·78	4·49	4·10		0·87
Piccardi, . . .	82·46	6·10	3·95	5·56	1·01	0·93
Steinegger, . . .	88·40	3·25	4·80	3·92		0·63

None of the constituents differ sufficiently from those of cow's milk to need detailed notice.

The fat is, however, very white, and the milk and butter have a smell of the goat.

**The Milk of the Mare.**—Most of our knowledge of mare's milk is due to Vieth, who carried out an extended series of observations on the stud of mares at the International Health Exhibition in London during 1884.

The following is an abstract of his results :—

TABLE CXXII.—COMPOSITION OF MARE'S MILK.

	Water.	Fat.	Sugar.	Protein.	Ash.
<b>Mixed milk.</b>					
Average, . . .	Per cent. 90·06	Per cent. 1·09	Per cent. 6·65	Per cent. 1·89	Per cent. 0·31
Maximum, . . .	90·41	1·44	6·82	2·11	0·34
Minimum, . . .	89·74	0·87	6·30	1·71	0·29
<b>Milk of individual mares.</b>					
Average, . . .	90·13	0·94	6·98	1·65	0·30
Maximum, . . .	90·46	1·18	7·21	1·76	0·36
Minimum, . . .	89·88	0·62	6·70	1·51	0·26
<b>Milk of mares specially fed.</b>					
Average, . . .	89·22	1·48	7·03	1·99	0·28
Maximum, . . .	89·88	2·14	7·28	2·20	0·32
Minimum, . . .	88·24	1·18	6·67	1·70	0·24
<b>Fleischmann gives the following composition :—</b>					
Average, . . .	90·7	1·2	5·7	2·0	0·4
Maximum, . . .	92·53	2·45	7·26	3·00	1·20
Minimum, . . .	89·05	0·12	4·20	1·33	0·28
<b>The following composition is also given :—</b>					
Authority.					
Landowsky, . . .	89·29	1·16	7·32	1·87	0·36
Biel, . . .	90·42	1·31	5·43	2·55	0·29
Camerer and Söldner,	90·58	1·14	5·87	2·05	0·36
<b>Vieth gives the following composition of samples of condensed mare's milk (containing cane-sugar 16 to 18 per cent.).</b>					
I., . . .	26·73	4·77	53·07	13·69	1·74
II., . . .	24·04	6·20	55·81	12·17	1·78
III., . . .	17·90	12·07	54·88	13·50	1·65
IV., . . .	18·80	10·08	54·09	15·23	1·80

Vieth describes the milk as of a chalky white colour, of sweet, and at the same time somewhat harsh taste, and of aromatic flavour. It had usually an alkaline reaction, the very few exceptions being neutral.

As this milk undergoes alcoholic fermentation very easily, while cow's milk does not, there is reason to suppose that the sugar is not identical with milk-sugar.

**The Milk of the Ass.**—The milk of the ass is considered by some authorities (*e.g.*, Tarnier) to approximate more in composition to human milk than that of any other animal; it is used to some extent for infant feeding.

The following is the composition given by various authorities :—

TABLE CXXIII.

Authority.	Water.	Fat.	Sugar.	Casein.	Albumin.	Ash.
Duclaux, .	Per cent. 90·70	Per cent. 1·00	Per cent. 6·54	Per cent. 0·99	Per cent. 0·34	Per cent. 0·43
Author, .	89·77	1·18	6·86	1·74		0·45
Schlossmann, .	88·85	0·36	4·94	0·98	0·33	0·31
König (mean),	90·12	1·37	6·19	0·79	1·06	0·47

Pizzi has shown that the fat is somewhat low in volatile acids (see p. 391).

The author has prepared the sugar and finds that it has a specific rotatory power  $[\alpha]_D = 52\cdot5'$  (for hydrated sugar), a birotation ratio of 1·6, and corresponds in every particular with milk-sugar.

The milk has a very feeble alkaline reaction; rennet produces a very soft curd after a long time, and acids give a finely divided precipitate. On boiling, it has a tendency to curdle and deposit flakes (coagulated albumin?). It has a white colour and a sweet taste.

The aldehyde figure multiplied by 0·154 gives a close approximation to the proteins.

**Milk as a Food and a Medicine.**—In considering the food value of milk, two points must be borne in mind; first, its value in repairing the waste of the tissues; and second, its value as a source of energy. As a food for infants it is required not only to repair waste of tissues, but to actually build them up.

**Composition of Constituents.**—The following table gives the percentage composition of the three main constituents of milk :—

TABLE CXXIV.

	Carbon.	Hydrogen.	Oxygen.	Nitrogen.
	Per cent.	Per cent.	Per cent.	Per cent.
Fat, . . . . .	75·63	11·87	12·50	...
Sugar, . . . . .	42·11	6·43	51·46	...
Protein, . . . . .	52·66	7·13	22·77	15·77

It is seen that fat is the richest in carbon and hydrogen, protein next, while sugar occupies the lowest place. Neither fat nor



sugar can replace proteins, as these compounds form the only source of nitrogen. Fat and sugar being composed of the same three elements may replace each other, but it is evident that in building up tissues containing high percentages of carbon and hydrogen, fat is a far more advantageous food than sugar. As a food for infants the value of milk largely depends on the fat present, and it is doubtful whether fat can be replaced by sugar without detriment to anabolic processes.

As a food for adults, where the tissues are ready formed, milk may be regarded chiefly as a source of energy. From this point of view fat may be replaced by the iso-dynamic quantity of milk-sugar.

**Heat of Combustion of Constituents.**—The following values for the heat of combustion of the constituents of milk are due to Strohmmer :—

TABLE CXXV.

Fats—Butter fat,	. . .	9,231·3	calories per gramme.
Other fats,	. . .	9,500	” ”
Sugar—Milk-sugar,	. . .	3,950	” ”
Cane-sugar,	. . .	3,955	” ”
Proteins—Casein,	. . .	5,858·3	” ”
Albumin,	. . .	5,735·2	” ”

These values assume that complete combustion takes place, and that carbon dioxide, water, and nitrogen are produced. In the case of fat and sugar it may be fairly assumed that an approach to complete combustion takes place in the human body, and that carbon dioxide and water are excreted. The nitrogen of proteins is not excreted as nitrogen, but as compounds, of which urea may be taken as the type.

Strohmmer calculates that 1 gramme of average protein yields 0·3428 gramme of urea, the heat of combustion of which is 2,537 calories per gramme; the heat of combustion of the urea from 1 gramme of proteins is, therefore, 869·7 calories, or, in round figures, 15 per cent. of the total heat of combustion. It is necessary, therefore, to deduct 15 per cent. of the heat of combustion of proteins in calculating iso-dynamic metabolic ratios.

In round figures, the following will be the calories per gramme developed in combustion of the three constituents in the human body :—

	Calories.
Fat,	. . . 9,230
Sugar,	. . . 3,950
Proteins,	. . . 4,970

These figures are in the ratio of 2·38 : 1 : 1·26.

The author proposes to calculate the ratio between the various constituents as follows :—

$$\begin{aligned} \text{Anabolic ratio} &= \text{fat} : \text{sugar} : \text{proteins.} \\ \text{Metabolic ratio} &= \frac{\text{fat} \times 2.38 + \text{sugar} + \text{proteins} \times 1.26}{\text{proteins}} \end{aligned}$$

Instead of the figures 2.38 and 1.26, the round figures 2.5 and 1.25 may be used without appreciable error. The author believes that the above ratios will give a truer idea of the proportionate value of different constituents than the usual nutritive ratio, which is  $\frac{\text{fat} \times 2.5 + \text{sugar}}{\text{protein}}$ .

**Food Value.**—We may now consider the food value of various milks.

The ratios for human milk are—

Anabolic ratio,	2.2 : 4.5 : 1
Metabolic ratio,	11.3

For cow's milk—

Anabolic ratio,	1.15 : 1.4 : 1
Metabolic ratio,	5.54

The marked difference of the two milks, due to the smaller amount of proteins in human milk, is very apparent.

It is assumed in calculating these ratios that the constituents are all digestible; this is approximately true with human milk. The same cannot be said of cow's milk, owing to a difference in the proteins; the action of rennet, one of the enzymes of the stomach, on cow's milk results in the formation of clots of curd, which are not readily digested. If the fat has been partially churned in the milk, this also is not perfectly digested.

Experiments have shown that children do not derive the most benefit from milk unless the anabolic ratio approximates to 2 : 4 : 1, and the constituents are in such a form that they are as finely divided as possible in the stomach.

**Milk as a Food for Infants—Artificial Human Milk.**—Many preparations of artificial human milk, or humanised milk, are made; they correspond in composition more or less exactly with human milk. The condition of the proteins necessary to produce a fine state of division in the stomach is attained—

- (1) By simple dilution with water, and addition of fat and sugar.
- (2) By removal of casein, and addition of fat and sugar.
- (3) By acting on the milk with a proteolytic enzyme—*i.e.*, peptonising it, and addition of fat and sugar.
- (4) By adding a preparation containing diastase and diluting it, and adding fat and sugar.
- (5) The fine division of the proteins is aided by the presence of a colloid, such as the small proportion of starch in barley water.

Various sugars are used, milk-sugar naturally being the most universally adopted; while cane-sugar, and maltose, and other carbohydrates, resulting from the diastatic conversion of starch are added.

The artificial feeding of children is to a large extent empirical. There is strong reason to believe that few of the constituents of cow's milk are identical with those of human milk, though closely analogous; yet it has been found that cow's milk suitably modified is an excellent food.

Again, it is found that human milk decreases in proteins as lactation advances. The best results have been obtained in artificial feeding by an exact reversal of this rule.

**Peptonised Milk.**—It is now conceded by the best authorities that the use of peptonised preparations is not an advantage, as, though the digestibility of the proteins is increased, it is at the expense of the development of the digestive organs.

The value of the milk in the treatment of disease lies in the fact that it is readily digestible, especially if diluted or modified so that the formation of hard curd in the stomach is prevented. As an example, it may be mentioned, that during the epidemic of typhoid at Maidstone in 1897, the Aylesbury Dairy Company sent many hundreds of bottles of humanised milk to the hospitals, which gave most satisfactory results, and provided a food which was readily retained and assimilated.

Peptonised milk is also used in cases of gastric disorders. Vieth gives the composition of this product as:—

TABLE CXXVI.

Water,	.	.	.	.	89.20	per cent.
Fat,	.	.	.	.	3.41	„
Sugar,	.	.	.	.	3.80	„
Casein,	.	.	.	.	0.96	„
Albumin,	.	.	.	.	0.07	„
Albumoses,	.	.	.	.	1.88	„
Ash,	.	.	.	.	0.68	„

**Diabetic Milk.**—In cases of diabetes, Ringer has recommended a solution of casein in a mixture of salts approximating to those present in milk as supplying protein nourishment; and Overend has used a diabetic milk in which the milk-sugar has been almost entirely replaced by lævulose with success.

The author found diabetic milk to have the following composition:—

TABLE CXXVII.

Water,	.	.	.	.	.	90.50 per cent.
Fat,	.	.	.	.	.	2.48 "
Lævulose,	.	.	.	.	.	4.41 "
Milk-sugar,	.	.	.	.	.	0.12 "
Protein,	.	.	.	.	.	2.44 "
Ash,	.	.	.	.	.	0.45 "

Koumiss is a remedial agent of great use in gastric disorders and many other diseases (see p. 297).

It owes its value to the fact that it is, first, a food of great digestibility; and, secondly, owing to the presence of alcohol, a stimulant. It is retained in cases where absolutely no other food can be given.

## CHAPTER IX.

## STANDARDISATION AND CALIBRATION OF APPARATUS.

**I. Weights.**—A good set of weights is a *sine quâ non* in a laboratory; they should consist of the following:—

100, 50, 20, 10, 10, 5, 2, 1, 1, 1, grammes, and  
 0·5, 0·2, 0·1, 0·1, 0·05, 0·02, 0·01, 0·01 gramme,  
 and some riders each 0·01 gramme.

Select one of the weights, preferably a 10 gramme, as a standard; mark one of the 10 grammes and one of the 1 gramme with a mark (') by means of a fine steel point; mark another 1 gramme with a mark ("); turn up one corner of a 0·1 gramme and of a 0·01 gramme. By this means the weights can all be distinguished from each other.

See that the balance is in adjustment by swinging it without any weights in the pans; if the pointer does not travel to an equal distance on both sides, alter the adjustment till this end is attained. After the adjustment, leave the balance for at least one hour and see if it is still in adjustment; if not, repeat the process, handling the beam, &c., as little as possible.

When the balance is in proper adjustment, place the 10-gramme weight on the right-hand pan, and the 10'-gramme weight on the left-hand pan; they should very nearly balance, and the pointer should swing nearly equally on both sides; if they do not balance, place the rider so that the balance is restored. The value of the 10'-gramme weight can now be obtained in terms of the 10-gramme weight, by adding the readings of the rider, if on the right arm, and subtracting, if on the left arm. Now reverse the weights, placing the 10-gramme weight on the left-hand pan, and the 10'-gramme weight on the right-hand pan, and repeat the weighing; the value of the 10'-gramme weight can be obtained in terms of the 10-gramme weight by adding the readings of the rider, if on the left arm, and subtracting, if on the right arm. Owing to minute differences in the lengths of the arms it is not unusual to find a difference between the two values.

The true value may be found by adding the two values together and dividing by 2. (It is more correct, theoretically, to multiply the two values and take the square root, but the values thus obtained are practically identical with the arithmetical mean.)

The total value of the  $5 + 2 + 1 + 1' + 1''$  weights are similarly obtained.

The value of the 20-gramme weight is obtained in a similar manner by weighing it against the  $10 + 10'$ ,  $10 + 5 + 2 + 1 + 1' + 1''$ , or the  $10' + 5 + 2 + 1 + 1' + 1''$  or, preferably, by weighing against all three series and taking the mean of the three values (which should not differ appreciably).

The value of the 50-gramme weight is obtained by weighing it against the  $20 + 10 + 10' + 5 + 2 + 1 + 1' + 1''$  weights.

The value of the 100-gramme weight is obtained by weighing it against the  $50 + 20 + 10 + 10' + 5 + 2 + 1 + 1' + 1''$  weights.

The 5-gramme weight is now taken, temporarily, as a standard, and the  $2 + 1 + 1' + 1''$  weights are weighed against that, and the value of the series obtained in terms of the 5-gramme weight.

The true value of the 5-gramme weight is obtained by the following formula :—

Let  $10 + x$  be the value of the series  $5 + 2 + 1 + 1' + 1''$ ,  
 $5 \times a + y$ , the value of the series  $2 + 1 + 1' + 1''$   
in terms of the 5-gramme weight,  
and  $5 \times a$  the true value of the 5-gramme weight ;  
then  $10 + x = 2 (5 \times a) + y$ ,  
or  $5 \times a = \frac{10 + x - y}{2}$ .

Now, temporarily assume that the 1-gramme weight is the standard, and ascertain the values of the  $1'$  and  $1''$  weights ; then ascertain the value of the 2-gramme weight by weighing it against  $1 + 1'$ ,  $1 + 1''$  or  $1' + 1''$  or, preferably, against all three.

The apparent values of the 2,  $1$ ,  $1'$ , and  $1''$  weights in terms of the 1-gramme weight will now be obtained.

The true values are obtained as follows : —

Let  $1 \times b$  be the true value of the 1-gramme weight,  
and  $2(1 \times b) + z$ ,  $1 \times b + w$ ,  $1 \times b + u$ , the values of the 2,  $1'$ , and  $1''$   
in terms of the 1-gramme weight ;  
then  $2(1 \times b) + z + 1 \times b + 1 \times b + w + 1 \times b + u = 5 \times a + y$ ,  
or  $1 \times b = \frac{5 \times a + y - z - w - u}{5}$ .

From the true value of the 1-gramme weight, the true values of the 2,  $1'$ , and  $1''$  weights are obtained.

The values of the fractions of a gramme are obtained by the same process as the values of the 5, 2,  $1$ ,  $1'$ , and  $1''$  weights.

TABLE CXXVIII.—VALUES OF WEIGHTS.

Weight.	True Value	Correction.
100	100.0031	+ 0.0031
50	50.0028	+ 0.0028
20	19.9992	- 0.0008
10	10.0000	...
$10'$	9.9991	- 0.0009
5	5.0002	+ 0.0002
2	2.0007	+ 0.0007
1	0.9989	- 0.0011
$1'$	0.9993	- 0.0007
$1''$	1.0001	+ 0.0001
0.5	0.4997	- 0.0003
0.2	0.2002	+ 0.0002
0.1	0.1001	+ 0.0001
$0.1'$	0.0998	- 0.0002
0.05	0.0497	- 0.0003
0.02	0.0200	- 0.0000
0.01	0.0097	- 0.0003
0.01'	0.0099	- 0.0001
Rider	0.0101	+ 0.0001

It is best to weigh the series 0·5, 0·2, 0·1, 0·1', 0·05, 0·02, 0·01, and 0·01' against the 1, 1', and 1'' weights, and take the mean of the three values so obtained.

When the weights have been standardised, a table should be drawn up in the above fashion (Table CXXVIII.).

**II. Burettes.**—Carefully clean out the burette with hot chromic acid mixture and rinse well with distilled water. Place it in a situation where sudden changes of temperature can be avoided, and fill it above the zero mark with distilled water; note the temperature of this, which should be as near as possible 60° F. (15·5° C.).

Weigh an empty weighing bottle provided with a stopper; cut two parallel slits about 2 inches long and three-eighths of an inch apart in a card (a visiting card answers admirably), and bend this so that the burette passes through the slits, the narrow strip being in front; adjust this so that the upper edge of the narrow strip is coincident with the graduation next below the zero mark. Now carefully run out the water so that the lower edge of the meniscus coincides with the zero mark, cork up the burette and leave it for a few minutes; after making sure that no alteration in level has occurred, adjust the card to the graduation next below the 5 c.c. mark, and run out slowly 5 c.c. into the weighing bottle. Weigh this and subtract the weight of the empty bottle; the difference will give the weight of water occupying the volume between 0 and 5.

After making sure that the level has not changed, adjust the card to the graduation next below the 10 c.c. mark and run out a further 5 c.c. into the weighing bottle; weigh again, and subtract the weight of the empty weighing bottle; the difference will give the weight of the water occupying the volume between 0 and 10.

Repeat this process till the lowest mark on the burette is reached.

The calibration of the burette should be repeated two or three times and the mean values tabulated.

With a finely-divided rule measure the lengths of the divisions 0 to 5, 0 to 10, &c.; multiply each of these lengths by the total weight of water and divide by the total length, to obtain figures commensurate with the weights of water.

Now plot out on squared paper two curves, one taking the scale readings as ordinates, and differences between scale readings and weights of water as abscissæ; the other taking scale readings as ordinates, and differences between scale readings and lengths of scale corrected as described above as abscissæ.

If both curves are nearly straight, it shows that the burette is made from a tube of uniform bore, and is correctly divided; if the two curves have a marked curvature, but coincide in form, it shows that the burette is made from a tube of uniform bore, but incorrectly divided; if the two curves do not coincide it shows that the tube is not uniform in bore.

Now, obtain the value of the weights of water contained in each 5 c.c., 0 to 5, 5 to 10, &c., by subtracting the weight contained in 0 to 5 from that contained in 0 to 10, &c., and the value of the lengths in a similar manner; divide one value by the other and plot out the values so obtained on squared paper, taking the mean scale readings (*i.e.*, for the volume 0 to 5 take 2·5) as ordinates, and the values obtained by the division as abscissæ. This will give the curve of irregularity of bore; if at any part of the curve it is noticed that the irregularity is very gross, the volume of each 1 c.c. should be obtained by weighing the water; if the curve is appreciably regular, it is evident that the errors of the burette must be due to incorrect division, and very careful measurements of the lengths of divisions intermediate between each 5 c.c. mark should be made; and if any very grave faults are found, the burette should be especially calibrated at that point. It is better, however, not to use a burette of this description.

Table CXXIX will give the figures obtained on a burette of fairly even bore, but badly divided.

TABLE CXXIX.—CALIBRATION OF BURETTE.

Scale.	Weights of Water.	Difference.	Length (inches).	Difference (corrected).
0 to 5	4.918	-0.082	1.752	-0.022
0 to 10	9.940	-0.060	3.550	+0.016
0 to 15	14.912	-0.083	5.329	+0.001
0 to 20	19.896	-0.104	7.1095	-0.010
0 to 25	24.880	-0.120	8.890	-0.023
0 to 30	29.908	-0.092	10.683	+0.000
0 to 35	34.849	-0.151	12.436	-0.087
0 to 40	39.817	-0.183	14.204	-0.135
0 to 45	44.817	-0.183	16.982	-0.153
0 to 50	49.877	-0.123	17.7775	-0.124

**III. Pipettes.**—Pipettes are used for measuring liquids by filling them to the mark and letting the liquid run out; the following points should be noticed:—

- The bottom of the meniscus should coincide with the mark.
- The pipette should be held vertically while it is running out.
- The liquid should always be allowed to run out in the same manner.

Perhaps the best manner of allowing the liquid to run out is to allow it to flow as fast as possible, and, when empty, to touch the surface of the liquid with the point and to withdraw it at once. It may, however, be allowed to run out slowly, or a definite number of drops may be permitted to run out after the main portion is delivered. Whatever method is adopted during graduation must be strictly adhered to in practice.

The graduation of pipettes is very simple; they are filled with water as near 60° F. (15.5° C.) as possible, the contents run into a weighing bottle and the water weighed.

The pipettes should be each etched with a number and the weight of water delivered tabulated for use.

Pipettes used exclusively for delivering known weights of milk should be graduated with milk of 1.032 specific gravity containing from 3.5 to 4.0 per cent. fat. In this case, the reading should be from the top of the meniscus, as the lower edge is invisible.

**IV. Flasks.**—Flasks of capacity sufficiently small to permit of being weighed when full, are filled with water as near 60° F. as possible, and weighed. Each should be marked with a number, and the weight of water contained by each tabulated.

Larger flasks (*e.g.*, litre flasks), if no balance sufficiently large is available, are graduated by the following method:—10 successive portions of a little less than 100 grammes of water at about 60° F. (15.5° C.) are weighed into the flask (best from a 100 c.c. flask). A beaker containing a little water, and a pipette are now weighed, and the litre flask filled to the mark by water from the pipette; the beaker, pipette, and remaining water are now weighed; the difference between the weights and the total weight of the ten portions added together will give the weight of water in the litre flask.

**V. Leffmann-Beam or Gerber Bottles.**—These can be graduated with sufficient accuracy by using each to make determinations of fat in several samples of milk, in which the fat has been carefully estimated by a good gravimetric method (*e.g.*, the Adams method). Those bottles which show a marked difference (*i.e.*, more than 0.1 per cent.) should be rejected.

The scale should also be measured with a finely-divided rule, and any bottles showing marked irregularities of graduation must be likewise rejected.



**VI. Lactometers.**—Lactometers are graduated by taking the specific gravity of several samples of milk which have had the density determined by a pycnometer; the range of specific gravities should be fairly wide; no lactometer showing differences of more than 0.0002 (0.2<sup>e</sup>) should be used, unless the differences are constant, when a constant correction may be applied.

**VII. Thermometers.**—One thermometer should be specially calibrated, and this will then serve as a standard of comparison for others. The calibration is divided into two parts.

(a) Calibration of scale.

(b) Determination of fixed points.

(a) *Calibration of Scale.*—By means of a finely-divided rule the distances between the marks on the scale (e.g., 0 to 10, 10 to 20, &c.; or 0 to 5, 5 to 10, &c.) are measured and tabulated.

The mercury is allowed to flow into the stem, and at a point, which should be as nearly as possible 10° from the end, the tip of a fine flame is carefully applied; by a gentle jerk a thread about 10° in length can be separated from the main portion, which is now allowed to flow back into the bulb. By gently tapping the tube, the thread is brought so that one end coincides with the zero mark, and the length of the thread is carefully measured; the thread is next brought to the 10° mark, and its length carefully measured again; and so on throughout the whole scale.

By dividing the lengths of the thread when it is between each pair of points (0 to 10, 10 to 20, &c.) by the distance between the same pair of points, the length of the thread in apparent degrees will be obtained; the average of these lengths will give the mean length of thread in mean degrees. By dividing the length of thread between each pair of points by the mean length, the value of a degree between each pair of points in terms of a mean degree will be obtained; and, on multiplying by ten, the distance between each pair of points in mean degrees will be obtained. The values in mean degrees of the scale from 0 to 10, 0 to 20, &c., should now be calculated, and also the lengths of scale between the same points. A curve of conicality can be plotted for the thermometer in the same way that a similar curve was plotted for a burette (q. v.).

(b) *Determination of fixed Points.*—A flask with a long neck is partially filled with water and placed over a flame; a shallow cork, with two holes, is fitted to the neck, and through one of the holes the thermometer is passed; in the other a short bent tube to take the steam away from the operator is placed. The water is boiled briskly, and the thermometer pushed in till only the top of the mercury is visible, and left in this position for several minutes. The exact point on the scale where the top of the mercury rests is now noted; the atmospheric pressure is read, and, from the table below, the *boiling point of water* is taken; the difference between this and 100° (or 212° if a Fahrenheit thermometer is used) is now added to (or subtracted from) the scale reading of the thermometer, and the value thus obtained noted as the true value of 100° C. (or 212° F.).

The thermometer is now removed, allowed to cool, and placed in melting ice; when the mercury is stationary, the position of the top of the mercury is noted as the *freezing point*.

The difference between the observed boiling and freezing points is taken, divided by 100 (or 180 if a Fahrenheit thermometer is used); the values in mean degrees of the scale from 0 to 10, 10 to 20, &c., are multiplied by the value thus obtained, and the corrected value tabulated. The differences between these and the nominal values of the scale are now plotted on squared paper, and will serve as a curve of correction of the instrument.

It is advisable to redetermine the boiling and freezing points from time to time, as they are liable to slight alteration.

Other thermometers may be standardised by comparison with this one.

## APPENDIX.

## USEFUL TABLES.

TABLE CXXX.—BOILING POINT OF WATER UNDER DIFFERENT PRESSURES (due to *Regnault*).

Boiling Point.	Pressure in Millimetres of Mercury.	Boiling Point.	Pressure in Millimetres of Mercury.
Centigrade.		Centigrade.	
98·5°	720·15	99·5°	746·50
98·6°	722·75	99·6°	749·18
98·7°	725·35	99·7°	751·87
98·8°	727·96	99·8°	754·57
98·9°	730·58	99·9°	757·27
99·0°	733·21	100·0°	760·00
99·1°	735·85	100·1°	762·73
99·2°	738·50	100·2°	765·46
99·3°	741·16	100·3°	768·20
99·4°	743·83	100·4°	771·95

TABLE CXXXIV.—FOR THE CALCULATION OF TOTAL SOLIDS FROM FAT AND SPECIFIC GRAVITY.

*(Richmond's Formula.)*

FOR USE IN THE DAIRY LABORATORY.

Specific Gravity.	FAT.															
	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	
30·0	10·65	10·75	10·90	11·00	11·15	11·25	11·35	11·50	11·60	11·75	11·85	11·95	12·10	12·20	12·35	
30·5	10·75	10·90	11·00	11·15	11·25	11·35	11·50	11·60	11·75	11·85	11·95	12·10	12·20	12·35	12·45	
31·0	10·90	11·00	11·15	11·25	11·40	11·50	11·60	11·75	11·85	12·00	12·10	12·20	12·35	12·45	12·60	
31·5	11·00	11·15	11·25	11·40	11·50	11·60	11·75	11·85	12·00	12·10	12·20	12·35	12·45	12·60	12·70	
32·0	11·15	11·25	11·40	11·50	11·60	11·75	11·85	12·00	12·10	12·20	12·35	12·45	12·60	12·70	12·80	
32·5	11·25	11·40	11·50	11·60	11·75	11·85	12·00	12·10	12·20	12·35	12·45	12·60	12·70	12·80	12·95	
33·0	11·40	11·50	11·65	11·75	11·85	12·00	12·10	12·25	12·35	12·45	12·60	12·70	12·85	12·95	13·05	
33·5	11·50	11·65	11·75	11·85	12·00	12·10	12·25	12·35	12·45	12·60	12·70	12·85	12·95	13·05	13·20	
34·0	11·65	11·75	11·85	12·00	12·10	12·25	12·35	12·45	12·60	12·70	12·85	12·95	13·05	13·20	13·30	
34·5	11·75	11·90	12·00	12·10	12·25	12·35	12·50	12·60	12·70	12·85	12·95	13·10	13·20	13·30	13·45	

TABLE CXXXI.—FOR CORRECTING THE SPECIFIC GRAVITY OF MILK

Degrees of Lactometer.	DEGREES OF THERMOMETER (Fahrenheit).																															
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62		
20	...	...	...	...	...	...	...	...	...	...	...	...	19.0	19.0	19.1	19.1	19.2	19.2	19.3	19.4	19.4	19.5	19.6	19.7	19.8	19.9	19.9	20.0	20.1	20.2		
21	...	...	...	...	...	...	...	...	...	...	...	...	19.9	20.0	20.0	20.1	20.2	20.2	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2		
22	...	...	...	...	...	...	...	...	...	...	...	...	20.9	21.0	21.0	21.1	21.2	21.2	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2		
23	...	...	...	...	...	...	...	...	...	...	...	...	21.9	22.0	22.0	22.1	22.2	22.2	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2		
24	...	...	...	...	...	...	...	...	...	...	...	...	22.9	23.0	23.0	23.1	23.2	23.2	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2		
25	...	...	...	...	...	...	...	...	...	...	...	...	23.9	23.9	24.0	24.0	24.1	24.1	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2		
26	...	...	...	...	...	...	...	...	...	...	...	...	24.8	24.9	24.9	25.0	25.1	25.1	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2		
27	...	...	...	...	...	...	...	...	...	...	...	...	25.8	25.9	25.9	26.0	26.1	26.1	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.2		
28	...	...	...	...	...	...	...	...	...	...	...	...	26.7	26.8	26.8	26.9	27.0	27.0	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.2		
29	...	...	...	...	...	...	...	...	...	...	...	...	27.7	27.8	27.8	27.9	28.0	28.0	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.2		
30	...	...	...	...	...	...	...	...	...	...	...	...	28.6	28.7	28.7	28.8	28.9	29.0	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3		
31	...	...	...	...	...	...	...	...	...	...	...	...	29.5	29.6	29.6	29.7	29.8	29.9	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.7	30.9	31.0	31.1	31.3		
32	29.7	29.7	29.8	29.8	29.9	29.9	30.0	30.0	30.1	30.2	30.3	30.4	30.4	30.5	30.5	30.6	30.7	30.9	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.1	32.3		
33	30.6	30.6	30.7	30.8	30.8	30.9	30.9	31.0	31.0	31.1	31.2	31.3	31.4	31.4	31.5	31.6	31.7	31.8	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.1	33.3		
34	31.5	31.5	31.6	31.6	31.7	31.8	31.8	31.9	32.0	32.0	32.1	32.2	32.3	32.4	32.5	32.6	32.7	32.8	32.9	33.0	33.1	33.3	33.4	33.5	33.6	33.7	33.9	34.0	34.1	34.3		
35	32.4	32.4	32.5	32.5	32.6	32.7	32.7	32.8	32.9	32.9	33.0	33.1	33.2	33.3	33.4	33.5	33.6	33.7	33.8	33.9	34.0	34.2	34.3	34.4	34.5	34.7	34.8	35.0	35.1	35.3		
36	33.3	33.4	33.4	33.5	33.6	33.6	33.7	33.8	33.8	33.9	34.0	34.1	34.2	34.3	34.4	34.5	34.6	34.7	34.8	34.9	35.0	35.1	35.3	35.4	35.5	35.7	35.8	36.0	...	...		



TABLE CXXXII.—FOR THE

Specific Gravity	Value of $\frac{D}{D_0}$																									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	
	0.12	0.24	0.36	0.48	0.60	0.72	0.84	0.96	1.08	1.20	1.32	1.44	1.56	1.68	1.80	1.92	2.04	2.16	2.28	2.40	2.52	2.64	2.76	2.88	3.00	
22.0	5.652	5.77	5.89	6.01	6.13	6.25	6.37	6.49	6.61	6.73	6.85	6.97	7.09	7.21	7.33	7.45	7.57	7.69	7.81	7.93	8.05	8.17	8.29	8.41	8.53	8.65
22.5	5.777	5.90	6.02	6.14	6.26	6.38	6.50	6.62	6.74	6.86	6.98	7.10	7.22	7.34	7.46	7.58	7.70	7.82	7.94	8.06	8.18	8.30	8.42	8.54	8.66	8.78
23.0	5.901	6.02	6.14	6.26	6.38	6.50	6.62	6.74	6.86	6.98	7.10	7.22	7.34	7.46	7.58	7.70	7.82	7.94	8.06	8.18	8.30	8.42	8.54	8.66	8.78	8.90
23.5	6.027	6.15	6.27	6.39	6.51	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.47	7.59	7.71	7.83	7.95	8.07	8.19	8.31	8.43	8.55	8.67	8.79	8.91	9.03
24.0	6.153	6.27	6.39	6.51	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.47	7.59	7.71	7.83	7.95	8.07	8.19	8.31	8.43	8.55	8.67	8.79	8.91	9.03	9.15
24.5	6.278	6.40	6.52	6.64	6.76	6.88	7.00	7.12	7.24	7.36	7.48	7.60	7.72	7.84	7.96	8.08	8.20	8.32	8.44	8.56	8.68	8.80	8.92	9.04	9.16	9.28
25.0	6.402	6.52	6.64	6.76	6.88	7.00	7.12	7.24	7.36	7.48	7.60	7.72	7.84	7.96	8.08	8.20	8.32	8.44	8.56	8.68	8.80	8.92	9.04	9.16	9.28	9.40
25.5	6.527	6.65	6.77	6.89	7.01	7.13	7.25	7.37	7.49	7.61	7.73	7.85	7.97	8.09	8.21	8.33	8.45	8.57	8.69	8.81	8.93	9.05	9.17	9.29	9.41	9.53
26.0	6.652	6.77	6.89	7.01	7.13	7.25	7.37	7.49	7.61	7.73	7.85	7.97	8.09	8.21	8.33	8.45	8.57	8.69	8.81	8.93	9.05	9.17	9.29	9.41	9.53	9.65
26.5	6.776	6.90	7.02	7.14	7.26	7.38	7.50	7.62	7.74	7.86	7.98	8.10	8.22	8.34	8.46	8.58	8.70	8.82	8.94	9.06	9.18	9.30	9.42	9.54	9.66	9.78
27.0	6.900	7.02	7.14	7.26	7.38	7.50	7.62	7.74	7.86	7.98	8.10	8.22	8.34	8.46	8.58	8.70	8.82	8.94	9.06	9.18	9.30	9.42	9.54	9.66	9.78	9.90
27.5	7.025	7.15	7.27	7.39	7.51	7.63	7.75	7.87	7.99	8.11	8.23	8.35	8.47	8.59	8.71	8.83	8.95	9.07	9.19	9.31	9.43	9.55	9.67	9.79	9.91	10.03
28.0	7.150	7.27	7.39	7.51	7.63	7.75	7.87	7.99	8.11	8.23	8.35	8.47	8.59	8.71	8.83	8.95	9.07	9.19	9.31	9.43	9.55	9.67	9.79	9.91	10.03	10.15
28.5	7.274	7.39	7.51	7.63	7.75	7.87	7.99	8.11	8.23	8.35	8.47	8.59	8.71	8.83	8.95	9.07	9.19	9.31	9.43	9.55	9.67	9.79	9.91	10.03	10.15	10.27
29.0	7.397	7.52	7.64	7.76	7.88	8.00	8.12	8.24	8.36	8.48	8.60	8.72	8.84	8.96	9.08	9.20	9.32	9.44	9.56	9.68	9.80	9.92	10.04	10.16	10.28	10.40
29.5	7.522	7.64	7.76	7.88	8.00	8.12	8.24	8.36	8.48	8.60	8.72	8.84	8.96	9.08	9.20	9.32	9.44	9.56	9.68	9.80	9.92	10.04	10.16	10.28	10.40	10.52
30.0	7.647	7.77	7.89	8.01	8.13	8.25	8.37	8.49	8.61	8.73	8.85	8.97	9.09	9.21	9.33	9.45	9.57	9.69	9.81	9.93	10.05	10.17	10.29	10.41	10.53	10.65
30.5	7.771	7.89	8.01	8.13	8.25	8.37	8.49	8.61	8.73	8.85	8.97	9.09	9.21	9.33	9.45	9.57	9.69	9.81	9.93	10.05	10.17	10.29	10.41	10.53	10.65	10.77
31.0	7.895	8.02	8.14	8.26	8.38	8.50	8.62	8.74	8.86	8.98	9.10	9.22	9.34	9.46	9.58	9.70	9.82	9.94	10.06	10.18	10.30	10.42	10.54	10.66	10.78	10.90
31.5	8.018	8.14	8.26	8.38	8.50	8.62	8.74	8.86	8.98	9.10	9.22	9.34	9.46	9.58	9.70	9.82	9.94	10.06	10.18	10.30	10.42	10.54	10.66	10.78	10.90	11.02
32.0	8.140	8.26	8.38	8.50	8.62	8.74	8.86	8.98	9.10	9.22	9.34	9.46	9.58	9.70	9.82	9.94	10.06	10.18	10.30	10.42	10.54	10.66	10.78	10.90	11.02	11.14
32.5	8.264	8.38	8.50	8.62	8.74	8.86	8.98	9.10	9.22	9.34	9.46	9.58	9.70	9.82	9.94	10.06	10.18	10.30	10.42	10.54	10.66	10.78	10.90	11.02	11.14	11.26
33.0	8.387	8.51	8.63	8.75	8.87	8.99	9.11	9.23	9.35	9.47	9.59	9.71	9.83	9.95	10.07	10.19	10.31	10.43	10.55	10.67	10.79	10.91	11.03	11.15	11.27	11.39
33.5	8.509	8.63	8.75	8.87	8.99	9.11	9.23	9.35	9.47	9.59	9.71	9.83	9.95	10.07	10.19	10.31	10.43	10.55	10.67	10.79	10.91	11.03	11.15	11.27	11.39	11.51
34.0	8.631	8.75	8.87	8.99	9.11	9.23	9.35	9.47	9.59	9.71	9.83	9.95	10.07	10.19	10.31	10.43	10.55	10.67	10.79	10.91	11.03	11.15	11.27	11.39	11.51	11.63
34.5	8.755	8.88	9.00	9.12	9.24	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76
35.0	8.878	9.00	9.12	9.24	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88
35.5	9.000	9.12	9.24	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88	12.00
36.0	9.122	9.24	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88	12.00	12.12
36.5	9.244	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88	12.00	12.12	12.24
37.0	9.366	9.49	9.61	9.73	9.85	9.97	10.09	10.21	10.33	10.45	10.57	10.69	10.81	10.93	11.05	11.17	11.29	11.41	11.53	11.65	11.77	11.89	12.01	12.13	12.25	12.37
37.5	9.488	9.61	9.73	9.85	9.97	10.09	10.21	10.33	10.45	10.57	10.69	10.81	10.93	11.05	11.17	11.29	11.41	11.53	11.65	11.77	11.89	12.01	12.13	12.25	12.37	12.49

## II.—FOR THE CALCULATION OF TOTAL SOLIDS FROM FAT AND SPECIFIC GRAVITY (*Richmond's Formula*).

FAT PER CENT.

2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3
2.64	2.76	2.88	3.00	3.12	3.24	3.36	3.48	3.60	3.72	3.84	3.96	4.08	4.20	4.32	4.44	4.56	4.68	4.80	4.92	5.04	5.16	5.28	5.40	5.52	5.64	5.76	5.88	6.00	6.12	6.24	6.36

TOTAL SOLIDS PER CENT.

8.20	8.44	8.53	8.65	8.77	8.89	9.01	9.13	9.25	9.37	9.49	9.61	9.73	9.85	9.97	10.09	10.21	10.33	10.45	10.57	10.69	10.81	10.93	11.05	11.17	11.29	11.41	11.53	11.65	11.77	11.89	12.01
8.42	8.54	8.66	8.78	8.90	9.02	9.14	9.26	9.38	9.50	9.62	9.74	9.86	9.98	10.10	10.22	10.34	10.46	10.58	10.70	10.82	10.94	11.06	11.18	11.30	11.42	11.54	11.66	11.78	11.90	12.02	
8.64	8.76	8.88	9.00	9.12	9.24	9.36	9.48	9.60	9.72	9.84	9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88	12.00	12.12	12.24	
8.86	8.98	9.10	9.22	9.34	9.46	9.58	9.70	9.82	9.94	10.06	10.18	10.30	10.42	10.54	10.66	10.78	10.90	11.02	11.14	11.26	11.38	11.50	11.62	11.74	11.86	11.98	12.10	12.22	12.34	12.46	
9.08	9.20	9.32	9.44	9.56	9.68	9.80	9.92	10.04	10.16	10.28	10.40	10.52	10.64	10.76	10.88	11.00	11.12	11.24	11.36	11.48	11.60	11.72	11.84	11.96	12.08	12.20	12.32	12.44	12.56	12.68	
9.30	9.42	9.54	9.66	9.78	9.90	10.02	10.14	10.26	10.38	10.50	10.62	10.74	10.86	10.98	11.10	11.22	11.34	11.46	11.58	11.70	11.82	11.94	12.06	12.18	12.30	12.42	12.54	12.66	12.78	12.90	
9.52	9.64	9.76	9.88	10.00	10.12	10.24	10.36	10.48	10.60	10.72	10.84	10.96	11.08	11.20	11.32	11.44	11.56	11.68	11.80	11.92	12.04	12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12	
9.74	9.86	9.98	10.10	10.22	10.34	10.46	10.58	10.70	10.82	10.94	11.06	11.18	11.30	11.42	11.54	11.66	11.78	11.90	12.02	12.14	12.26	12.38	12.50	12.62	12.74	12.86	12.98	13.10	13.22	13.34	
9.96	10.08	10.20	10.32	10.44	10.56	10.68	10.80	10.92	11.04	11.16	11.28	11.40	11.52	11.64	11.76	11.88	12.00	12.12	12.24	12.36	12.48	12.60	12.72	12.84	12.96	13.08	13.20	13.32	13.44	13.56	
10.18	10.30	10.42	10.54	10.66	10.78	10.90	11.02	11.14	11.26	11.38	11.50	11.62	11.74	11.86	11.98	12.10	12.22	12.34	12.46	12.58	12.70	12.82	12.94	13.06	13.18	13.30	13.42	13.54	13.66	13.78	
10.40	10.52	10.64	10.76	10.88	11.00	11.12	11.24	11.36	11.48	11.60	11.72	11.84	11.96	12.08	12.20	12.32	12.44	12.56	12.68	12.80	12.92	13.04	13.16	13.28	13.40	13.52	13.64	13.76	13.88	14.00	
10.62	10.74	10.86	10.98	11.10	11.22	11.34	11.46	11.58	11.70	11.82	11.94	12.06	12.18	12.30	12.42	12.54	12.66	12.78	12.90	13.02	13.14	13.26	13.38	13.50	13.62	13.74	13.86	13.98	14.10	14.22	
10.84	10.96	11.08	11.20	11.32	11.44	11.56	11.68	11.80	11.92	12.04	12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12	13.24	13.36	13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32	14.44	
11.06	11.18	11.30	11.42	11.54	11.66	11.78	11.90	12.02	12.14	12.26	12.38	12.50	12.62	12.74	12.86	12.98	13.10	13.22	13.34	13.46	13.58	13.70	13.82	13.94	14.06	14.18	14.30	14.42	14.54	14.66	
11.28	11.40	11.52	11.64	11.76	11.88	12.00	12.12	12.24	12.36	12.48	12.60	12.72	12.84	12.96	13.08	13.20	13.32	13.44	13.56	13.68	13.80	13.92	14.04	14.16	14.28	14.40	14.52	14.64	14.76	14.88	
11.50	11.62	11.74	11.86	11.98	12.10	12.22	12.34	12.46	12.58	12.70	12.82	12.94	13.06	13.18	13.30	13.42	13.54	13.66	13.78	13.90	14.02	14.14	14.26	14.38	14.50	14.62	14.74	14.86	14.98	15.10	
11.72	11.84	11.96	12.08	12.20	12.32	12.44	12.56	12.68	12.80	12.92	13.04	13.16	13.28	13.40	13.52	13.64	13.76	13.88	14.00	14.12	14.24	14.36	14.48	14.60	14.72	14.84	14.96	15.08	15.20	15.32	
11.94	12.06	12.18	12.30	12.42	12.54	12.66	12.78	12.90	13.02	13.14	13.26	13.38	13.50	13.62	13.74	13.86	13.98	14.10	14.22	14.34	14.46	14.58	14.70	14.82	14.94	15.06	15.18	15.30	15.42	15.54	
12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12	13.24	13.36	13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32	14.44	14.56	14.68	14.80	14.92	15.04	15.16	15.28	15.40	15.52	15.64	15.76	
12.38	12.50	12.62	12.74	12.86	12.98	13.10	13.22	13.34	13.46	13.58	13.70	13.82	13.94	14.06	14.18	14.30	14.42	14.54	14.66	14.78	14.90	15.02	15.14	15.26	15.38	15.50	15.62	15.74	15.86	15.98	
12.60	12.72	12.84	12.96	13.08	13.20	13.32	13.44	13.56	13.68	13.80	13.92	14.04	14.16	14.28	14.40	14.52	14.64	14.76	14.88	15.00	15.12	15.24	15.36	15.48	15.60	15.72	15.84	15.96	16.08	16.20	
12.82	12.94	13.06	13.18	13.30	13.42	13.54	13.66	13.78	13.90	14.02	14.14	14.26	14.38	14.50	14.62	14.74	14.86	14.98	15.10	15.22	15.34	15.46	15.58	15.70	15.82	15.94	16.06	16.18	16.30	16.42	
13.04	13.16	13.28	13.40	13.52	13.64	13.76	13.88	14.00	14.12	14.24	14.36	14.48	14.60	14.72	14.84	14.96	15.08	15.20	15.32	15.44	15.56	15.68	15.80	15.92	16.04	16.16	16.28	16.40	16.52	16.64	
13.26	13.38	13.50	13.62	13.74	13.86	13.98	14.10	14.22	14.34	14.46	14.58	14.70	14.82	14.94	15.06	15.18	15.30	15.42	15.54	15.66	15.78	15.90	16.02	16.14	16.26	16.38	16.50	16.62	16.74	16.86	
13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32	14.44	14.56	14.68	14.80	14.92	15.04	15.16	15.28	15.40	15.52	15.64	15.76	15.88	16.00	16.12	16.24	16.36	16.48	16.60	16.72	16.84	16.96	17.08	
13.70	13.82	13.94	14.06	14.18	14.30	14.42	14.54	14.66	14.78	14.90	15.02	15.14	15.26	15.38	15.50	15.62	15.74	15.86	15.98	16.10	16.22	16.34	16.46	16.58	16.70	16.82	16.94	17.06	17.18	17.30	
13.92	14.04	14.16	14.28	14.40	14.52	14.64	14.76	14.88	15.00	15.12	15.24	15.36	15.48	15.60	15.72	15.84	15.96	16.08	16.20	16.32	16.44	16.56	16.68	16.80	16.92	17.04	17.16	17.28	17.40	17.52	
14.14	14.26	14.38	14.50	14.62	14.74	14.86	14.98	15.10	15.22	15.34	15.46	15.58	15.70	15.82	15.94	16.06	16.18	16.30	16.42	16.54	16.66	16.78	16.90	17.02	17.14	17.26	17.38	17.50	17.62	17.74	
14.36	14.48	14.60	14.72	14.84	14.96	15.08	15.20	15.32	15.44	15.56	15.68	15.80	15.92	16.04	16.16	16.28	16.40	16.52	16.64	16.76	16.88	17.00	17.12	17.24	17.36	17.48	17.60	17.72	17.84	17.96	
14.58	14.70	14.82	14.94	15.06	15.18	15.30	15.42	15.54	15.66	15.78	15.90	16.02	16.14	16.26	16.38	16.50	16.62	16.74	16.86	16.98	17.10	17.22	17.34	17.46	17.58	17.70	17.82	17.94	18.06	18.18	
14.80	14.92	15.04	15.16	15.28	15.40	15.52	15.64	15.76	15.88	16.00	16.12	16.24	16.36	16.48	16.60	16.72	16.84	16.96	17.08	17.20	17.32	17.44	17.56	17.68	17.80	17.92	18.04	18.16	18.28	18.40	
15.02	15.14	15.26	15.38	15.50	15.62	15.74	15.86	15.98	16.10	16.22	16.34	16.46	16.58	16.70	16.82	16.94	17.06	17.18	17.30	17.42	17.54	17.66	17.78	17.90	18.02	18.14	18.26	18.38	18.50	18.62	
15.24	15.36	15.48	15.60	15.72	15.84	15.96	16.08	16.20	16.32	16.44	16.56	16.68	16.80	16.92	17.04	17.16	17.28	17.40	17.52	17.64	17.76	17.88	18.00	18.12	18.24	18.36	18.48	18.60	18.72	18.84	
15.46	15.58	15.70	15.82	15.94	16.06	16.18	16.30	16.42	16.54	16.66	16.78	16.90	17.02	17.14	17.26	17.38	17.50	17.62	17.74	17.86	17.98	18.10	18.22	18.34	18.46	18.58	18.70	18.82	18.94	19.06	
15.68	15.80	15.92	16.04	16.16	16.28	16.40	16.52	16.64	16.76	16.88	17.00	17.12	17.24	17.36	17.48	17.60	17.72	17.84	17.96	18.08	18.20	18.32	18.44	18.56	18.68	18.80	18.92	19.04	19.16	19.28	
15.90	16.02	16.14	16.26	16.38	16.50	16.62	16.74	16.86	16.98	17.10	17.22	17.34	17.46	17.58	17.70	17.82	17.94	18.06	18.18	18.30	18.42	18.54	18.66	18.78	18.90	19.02	19.14	19.26	19.38	19.50	
16.12	16.24	16.36	16.48	16.60	16.72	16.84	16.96	17.08	17.20	17.32	17.44	17.56	17.68	17.80	17.92	18.04	18.16	18.28	18.40	18.52	18										

5	5-1	5-2	5-3	5-4	5-5	5-6	5-7	5-8	5-9	5-0	6-1	6-2	6-3	6-4	6-5
0	5-12	5-24	5-36	5-48	5-60	5-72	5-84	5-96	7-08	7-20	7-32	7-44	7-56	7-68	7-80

55	11-77	11-89	12-01	12-13	12-25	12-37	12-49	12-61	12-73	12-85	12-97	13-09	13-21	13-33	13-45
75	11-90	12-02	12-14	12-26	12-38	12-50	12-62	12-74	12-86	12-98	13-10	13-22	13-34	13-46	13-58
90	12-02	12-14	12-26	12-38	12-50	12-62	12-74	12-86	12-98	13-10	13-22	13-34	13-46	13-58	14-10
93	12-15	12-27	12-39	12-51	12-63	12-75	12-87	12-99	13-11	13-23	13-35	13-47	13-59	14-11	14-23
5	12-27	12-39	12-51	12-63	12-75	12-87	12-99	13-11	13-23	13-35	13-47	13-59	14-11	14-23	14-35
8	12-40	12-52	12-64	12-76	12-88	13-00	13-12	13-24	13-36	13-48	13-60	13-72	13-84	13-96	14-08
6	12-52	12-64	12-76	12-88	13-00	13-12	13-24	13-36	13-48	13-60	13-72	13-84	13-96	14-08	14-20
3	12-65	12-77	12-89	13-01	13-13	13-25	13-37	13-49	13-61	13-73	13-85	13-97	14-09	14-21	14-33
5	12-77	12-89	13-01	13-13	13-25	13-37	13-49	13-61	13-73	13-85	13-97	14-09	14-21	14-33	14-45
8	12-90	13-02	13-14	13-26	13-38	13-50	13-62	13-74	13-86	13-98	14-10	14-22	14-34	14-46	14-58
0	13-02	13-14	13-26	13-38	13-50	13-62	13-74	13-86	13-98	14-10	14-22	14-34	14-46	14-58	15-10
3	13-15	13-27	13-39	13-51	13-63	13-75	13-87	13-99	14-11	14-23	14-35	14-47	14-59	15-11	15-23
5	13-27	13-39	13-51	13-63	13-75	13-87	13-99	14-11	14-23	14-35	14-47	14-59	15-11	15-23	15-35
7	13-39	13-51	13-63	13-75	13-87	13-99	14-11	14-23	14-35	14-47	14-59	15-11	15-23	15-35	15-47
0	13-52	13-64	13-76	13-88	14-00	14-12	14-24	14-36	14-48	14-60	14-72	14-84	14-96	15-08	15-20
2	13-64	13-76	13-88	14-00	14-12	14-24	14-36	14-48	14-60	14-72	14-84	14-96	15-08	15-20	15-32
5	13-77	13-89	14-01	14-13	14-25	14-37	14-49	14-61	14-73	14-85	14-97	15-09	15-21	15-33	15-45
7	13-89	14-01	14-13	14-25	14-37	14-49	14-61	14-73	14-85	14-97	15-09	15-21	15-33	15-45	15-57
0	14-02	14-14	14-26	14-38	14-50	14-62	14-74	14-86	14-98	15-10	15-22	15-34	15-46	15-58	16-10
2	14-14	14-26	14-38	14-50	14-62	14-74	14-86	14-98	15-10	15-22	15-34	15-46	15-58	16-10	16-22
4	14-26	14-38	14-50	14-62	14-74	14-86	14-98	15-10	15-22	15-34	15-46	15-58	16-10	16-22	16-34
6	14-38	14-50	14-62	14-74	14-86	14-98	15-10	15-22	15-34	15-46	15-58	16-10	16-22	16-34	16-46
9	14-51	14-63	14-75	14-87	14-99	15-11	15-23	15-35	15-47	15-59	15-71	15-83	15-95	16-07	16-19
1	14-63	14-75	14-87	14-99	15-11	15-23	15-35	15-47	15-59	15-71	15-83	15-95	16-07	16-19	16-31
3	14-75	14-87	14-99	15-11	15-23	15-35	15-47	15-59	15-71	15-83	15-95	16-07	16-19	16-31	16-43
5	14-88	15-00	15-12	15-24	15-36	15-48	15-60	15-72	15-84	15-96	16-08	16-20	16-32	16-44	16-56
7	15-00	15-12	15-24	15-36	15-48	15-60	15-72	15-84	15-96	16-08	16-20	16-32	16-44	16-56	17-08
9	15-12	15-24	15-36	15-48	15-60	15-72	15-84	15-96	16-08	16-20	16-32	16-44	16-56	17-08	17-20
1	15-24	15-36	15-48	15-60	15-72	15-84	15-96	16-08	16-20	16-32	16-44	16-56	16-68	16-80	16-92
3	15-36	15-48	15-60	15-72	15-84	15-96	16-08	16-20	16-32	16-44	16-56	16-68	16-80	16-92	17-04
5	15-49	15-61	15-73	15-85	15-97	16-09	16-21	16-33	16-45	16-57	16-69	16-81	16-93	17-05	17-17
7	15-61	15-73	15-85	15-97	16-09	16-21	16-33	16-45	16-57	16-69	16-81	16-93	17-05	17-17	17-29

DIFFERENCE TABLE.

a	T	F	T
0-1	0-03	0-01	0-01
0-2	0-05	0-02	0-02
0-3	0-08	0-03	0-03
0-4	0-10	0-04	0-05
...	...	0-05	0-06
...	...	0-06	0-07
...	...	0-07	0-08
...	...	0-08	0-10
...	...	0-09	0-11





—FOR THE CALCULATION OF TOTAL SOLIDS FROM FAT AND SPECIFIC GRAVITY (*Hehner and Richmond's Formulae*).

FAT PER CENT.

2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4
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TOTAL SOLIDS PER CENT.

0	5.77	5.89	6.01	6.12	6.24	6.36	6.47	6.59	6.70	6.82	6.94	7.05	7.17	7.29	7.40	7.52	7.64	7.75	7.87	7.98	8.10	8.22	8.33	8.45	8.57	8.68	8.80	8.92	9.03	9.15	9.27	9.38	9.50	9.62	9.74	9.85	9.97	10.09	10.21	10.33	10.45	10.56	10.68	10.79	10.91	11.03	11.14	11.26	11.38	11.49	11.61	11.73	11.84	11.96	12.07	12.19	12.31	12.42	12.54	12.66	12.77	12.89	13.01	13.12	13.24	13.36	13.47	13.59	13.71	13.82	13.94	14.06	14.18	14.29	14.41	14.53	14.65	14.77	14.89	15.01	15.12	15.24	15.36	15.48	15.60	15.72	15.84	15.96	16.08	16.20	16.32	16.44	16.56	16.68	16.80	16.92	17.04	17.16	17.28	17.40	17.52	17.64	17.76	17.88	18.00	18.12	18.24	18.36	18.48	18.60	18.72	18.84	18.96	19.08	19.20	19.32	19.44	19.56	19.68	19.80	19.92	20.04	20.16	20.28	20.40	20.52	20.64	20.76	20.88	21.00	21.12	21.24	21.36	21.48	21.60	21.72	21.84	21.96	22.08	22.20	22.32	22.44	22.56	22.68	22.80	22.92	23.04	23.16	23.28	23.40	23.52	23.64	23.76	23.88	24.00	24.12	24.24	24.36	24.48	24.60	24.72	24.84	24.96	25.08	25.20	25.32	25.44	25.56	25.68	25.80	25.92	26.04	26.16	26.28	26.40	26.52	26.64	26.76	26.88	27.00	27.12	27.24	27.36	27.48	27.60	27.72	27.84	27.96	28.08	28.20	28.32	28.44	28.56	28.68	28.80	28.92	29.04	29.16	29.28	29.40	29.52	29.64	29.76	29.88	30.00	30.12	30.24	30.36	30.48	30.60	30.72	30.84	30.96	31.08	31.20	31.32	31.44	31.56	31.68	31.80	31.92	32.04	32.16	32.28	32.40	32.52	32.64	32.76	32.88	33.00	33.12	33.24	33.36	33.48	33.60	33.72	33.84	33.96	34.08	34.20	34.32	34.44	34.56	34.68	34.80	34.92	35.04	35.16	35.28	35.40	35.52	35.64	35.76	35.88	36.00	36.12	36.24	36.36	36.48	36.60	36.72	36.84	36.96	37.08	37.20	37.32	37.44	37.56	37.68	37.80	37.92	38.04	38.16	38.28	38.40	38.52	38.64	38.76	38.88	39.00	39.12	39.24	39.36	39.48	39.60	39.72	39.84	39.96	40.08	40.20	40.32	40.44	40.56	40.68	40.80	40.92	41.04	41.16	41.28	41.40	41.52	41.64	41.76	41.88	42.00	42.12	42.24	42.36	42.48	42.60	42.72	42.84	42.96	43.08	43.20	43.32	43.44	43.56	43.68	43.80	43.92	44.04	44.16	44.28	44.40	44.52	44.64	44.76	44.88	45.00	45.12	45.24	45.36	45.48	45.60	45.72	45.84	45.96	46.08	46.20	46.32	46.44	46.56	46.68	46.80	46.92	47.04	47.16	47.28	47.40	47.52	47.64	47.76	47.88	48.00	48.12	48.24	48.36	48.48	48.60	48.72	48.84	48.96	49.08	49.20	49.32	49.44	49.56	49.68	49.80	49.92	50.04	50.16	50.28	50.40	50.52	50.64	50.76	50.88	51.00	51.12	51.24	51.36	51.48	51.60	51.72	51.84	51.96	52.08	52.20	52.32	52.44	52.56	52.68	52.80	52.92	53.04	53.16	53.28	53.40	53.52	53.64	53.76	53.88	54.00	54.12	54.24	54.36	54.48	54.60	54.72	54.84	54.96	55.08	55.20	55.32	55.44	55.56	55.68	55.80	55.92	56.04	56.16	56.28	56.40	56.52	56.64	56.76	56.88	57.00	57.12	57.24	57.36	57.48	57.60	57.72	57.84	57.96	58.08	58.20	58.32	58.44	58.56	58.68	58.80	58.92	59.04	59.16	59.28	59.40	59.52	59.64	59.76	59.88	60.00	60.12	60.24	60.36	60.48	60.60	60.72	60.84	60.96	61.08	61.20	61.32	61.44	61.56	61.68	61.80	61.92	62.04	62.16	62.28	62.40	62.52	62.64	62.76	62.88	63.00	63.12	63.24	63.36	63.48	63.60	63.72	63.84	63.96	64.08	64.20	64.32	64.44	64.56	64.68	64.80	64.92	65.04	65.16	65.28	65.40	65.52	65.64	65.76	65.88	66.00	66.12	66.24	66.36	66.48	66.60	66.72	66.84	66.96	67.08	67.20	67.32	67.44	67.56	67.68	67.80	67.92	68.04	68.16	68.28	68.40	68.52	68.64	68.76	68.88	69.00	69.12	69.24	69.36	69.48	69.60	69.72	69.84	69.96	70.08	70.20	70.32	70.44	70.56	70.68	70.80	70.92	71.04	71.16	71.28	71.40	71.52	71.64	71.76	71.88	72.00	72.12	72.24	72.36	72.48	72.60	72.72	72.84	72.96	73.08	73.20	73.32	73.44	73.56	73.68	73.80	73.92	74.04	74.16	74.28	74.40	74.52	74.64	74.76	74.88	75.00	75.12	75.24	75.36	75.48	75.60	75.72	75.84	75.96	76.08	76.20	76.32	76.44	76.56	76.68	76.80	76.92	77.04	77.16	77.28	77.40	77.52	77.64	77.76	77.88	78.00	78.12	78.24	78.36	78.48	78.60	78.72	78.84	78.96	79.08	79.20	79.32	79.44	79.56	79.68	79.80	79.92	80.04	80.16	80.28	80.40	80.52	80.64	80.76	80.88	81.00	81.12	81.24	81.36	81.48	81.60	81.72	81.84	81.96	82.08	82.20	82.32	82.44	82.56	82.68	82.80	82.92	83.04	83.16	83.28	83.40	83.52	83.64	83.76	83.88	84.00	84.12	84.24	84.36	84.48	84.60	84.72	84.84	84.96	85.08	85.20	85.32	85.44	85.56	85.68	85.80	85.92	86.04	86.16	86.28	86.40	86.52	86.64	86.76	86.88	87.00	87.12	87.24	87.36	87.48	87.60	87.72	87.84	87.96	88.08	88.20	88.32	88.44	88.56	88.68	88.80	88.92	89.04	89.16	89.28	89.40	89.52	89.64	89.76	89.88	90.00	90.12	90.24	90.36	90.48	90.60	90.72	90.84	90.96	91.08	91.20	91.32	91.44	91.56	91.68	91.80	91.92	92.04	92.16	92.28	92.40	92.52	92.64	92.76	92.88	93.00	93.12	93.24	93.36	93.48	93.60	93.72	93.84	93.96	94.08	94.20	94.32	94.44	94.56	94.68	94.80	94.92	95.04	95.16	95.28	95.40	95.52	95.64	95.76	95.88	96.00	96.12	96.24	96.36	96.48	96.60	96.72	96.84	96.96	97.08	97.20	97.32	97.44	97.56	97.68	97.80	97.92	98.04	98.16	98.28	98.40	98.52	98.64	98.76	98.88	99.00	99.12	99.24	99.36	99.48	99.60	99.72	99.84	99.96	100.08	100.20	100.32	100.44	100.56	100.68	100.80	100.92	101.04	101.16	101.28	101.40	101.52	101.64	101.76	101.88	102.00	102.12	102.24	102.36	102.48	102.60	102.72	102.84	102.96	103.08	103.20	103.32	103.44	103.56	103.68	103.80	103.92	104.04	104.16	104.28	104.40	104.52	104.64	104.76	104.88	105.00	105.12	105.24	105.36	105.48	105.60	105.72	105.84	105.96	106.08	106.20	106.32	106.44	106.56	106.68	106.80	106.92	107.04	107.16	107.28	107.40	107.52	107.64	107.76	107.88	108.00	108.12	108.24	108.36	108.48	108.60	108.72	108.84	108.96	109.08	109.20	109.32	109.44	109.56	109.68	109.80	109.92	110.04	110.16	110.28	110.40	110.52	110.64	110.76	110.88	111.00	111.12	111.24	111.36	111.48	111.60	111.72	111.84	111.96	112.08	112.20	112.32	112.44	112.56	112.68	112.80	112.92	113.04	113.16	113.28	113.40	113.52	113.64	113.76	113.88	114.00	114.12	114.24	114.36	114.48	114.60	114.72	114.84	114.96	115.08	115.20	115.32	115.44	115.56	115.68	115.80	115.92	116.04	116.16	116.28	116.40	116.52	116.64	116.76	116.88	117.00	117.12	117.24	117.36	117.48	117.60	117.72	117.84	117.96	118.08	118.20	118.32	118.44	118.56	118.68	118.80	118.92	119.04	119.16	119.28	119.40	119.52	119.64	119.76	119.88	120.00	120.12	120.24	120.36	120.48	120.60	120.72	120.84	120.96	121.08	121.20	121.32	121.44	121.56	121.68	121.80	121.92	122.04	122.16	122.28	122.40	122.52	122.64	122.76	122.88	123.00	123.12	123.24	123.36	123.48	123.60	123.72	123.84	123.96	124.08	124.20	124.32	124.44	124.56	124.68	124.80	124.92	125.04	125.16	125.28	125.40	125.52	125.64	125.76	125.88	126.00	126.12	126.24	126.36	126.48	126.60	126.72	126.84	126.96	127.08	127.20	127.32	127.44	127.56	127.68	127.80	127.92	128.04	128.16	128.28	128.40	128.52	128.64	128.76	128.88	129.00	129.12	129.24	129.36	129.48	129.60	129.72	129.84	129.96	130.08	130.20	130.32	130.44	130.56	130.68	130.80	130.92	131.04	131.16	131.28	131.40	131.52	131.64	131.76	131.88	132.00	132.12	132.24	132.36	132.48	132.60	132.72	132.84	132.96	133.08	133.20	133.32	133.44	133.56	133.68	133.80	133.92	134.04	134.16	134.28	134.40	134.52	134.64	134.76	134.88	135.00	135.12	135.24	135.36	135.48	135.60	135.72	135.84	135.96	136.08	136.20	136.32	136.44	136.56	136.68	136.80	136.92	137.04	137.16	137.28	137.40	137.52	137.64	137.76	137.88	138.00	138.12	138.24	138.36	138.48	138.60	138.72	138.84	138.96	139.08	139.20	139.32	139.44	139.56	139.68	139.80	139.92	140.04	140.16	140.28	140.40	140.52	140.64	140.76	140.88	141.00	141.12	141.24	141.36	141.48	141.60	141.72	141.84	141.96	142.08	142.20	142.32	142.44	142.56	142.68	142.80	142.92	143.04	143.16	143.28	143.40	143.52	143.64	143.76	143.88	144.00</
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4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5
11.67	11.68	11.80	11.92	12.03	12.15	12.27	12.38	12.50	12.61	12.73	12.85	12.96	13.08	13.20	13.31	13.43	13.55	13.66
11.69	11.81	11.93	12.04	12.16	12.28	12.39	12.51	12.63	12.74	12.86	12.97	13.09	13.21	13.32	13.44	13.56	13.67	13.79
11.82	11.94	12.05	12.17	12.29	12.40	12.52	12.64	12.75	12.87	12.98	13.10	13.22	13.33	13.45	13.57	13.68	13.80	13.92
11.95	12.06	12.18	12.30	12.41	12.53	12.65	12.76	12.88	13.00	13.11	13.23	13.34	13.46	13.58	13.69	13.81	13.93	14.04
12.07	12.19	12.31	12.42	12.54	12.66	12.77	12.89	13.01	13.12	13.24	13.36	13.47	13.59	13.70	13.82	13.94	14.05	14.17
12.20	12.32	12.43	12.55	12.67	12.78	12.90	13.02	13.13	13.25	13.37	13.48	13.60	13.72	13.83	13.95	14.06	14.18	14.30
12.33	12.45	12.56	12.68	12.79	12.91	13.03	13.14	13.26	13.38	13.49	13.61	13.73	13.84	13.96	14.07	14.19	14.31	14.42
12.46	12.57	12.69	12.81	12.92	13.04	13.15	13.27	13.39	13.50	13.62	13.74	13.85	13.97	14.09	14.20	14.32	14.43	14.55
12.58	12.70	12.82	12.93	13.05	13.16	13.28	13.40	13.51	13.63	13.75	13.86	13.98	14.10	14.21	14.33	14.45	14.56	14.68
12.71	12.83	12.94	13.06	13.18	13.29	13.41	13.52	13.64	13.76	13.87	13.99	14.11	14.22	14.34	14.46	14.57	14.69	14.81
12.84	12.95	13.07	13.19	13.30	13.42	13.54	13.65	13.77	13.88	14.00	14.12	14.23	14.35	14.47	14.58	14.70	14.82	14.93
12.96	13.08	13.20	13.31	13.43	13.55	13.66	13.78	13.90	14.01	14.13	14.24	14.36	14.48	14.59	14.71	14.83	14.94	15.06
13.09	13.21	13.32	13.44	13.56	13.67	13.79	13.91	14.02	14.14	14.25	14.37	14.49	14.60	14.72	14.84	14.96	15.07	15.19
13.22	13.33	13.45	13.57	13.68	13.80	13.92	14.03	14.15	14.27	14.38	14.50	14.61	14.73	14.85	14.96	15.08	15.20	15.31
13.34	13.46	13.58	13.69	13.81	13.93	14.04	14.16	14.28	14.39	14.51	14.63	14.74	14.86	14.97	15.09	15.21	15.32	15.44
13.47	13.59	13.70	13.82	13.94	14.05	14.17	14.29	14.40	14.52	14.64	14.75	14.87	14.99	15.10	15.22	15.33	15.45	15.57
13.60	13.72	13.83	13.95	14.06	14.18	14.30	14.41	14.53	14.65	14.76	14.88	15.00	15.11	15.23	15.34	15.46	15.58	15.69
13.73	13.84	13.96	14.08	14.19	14.31	14.42	14.54	14.66	14.77	14.89	15.01	15.12	15.24	15.36	15.47	15.58	15.70	15.82
13.85	13.97	14.09	14.20	14.32	14.43	14.55	14.67	14.78	14.90	15.02	15.13	15.25	15.37	15.48	15.60	15.72	15.83	15.95
13.98	14.10	14.21	14.33	14.45	14.56	14.68	14.79	14.91	15.03	15.14	15.26	15.38	15.49	15.61	15.73	15.84	15.96	16.08
14.11	14.22	14.34	14.46	14.57	14.69	14.81	14.92	15.04	15.15	15.27	15.39	15.50	15.62	15.74	15.85	15.97	16.09	16.20
14.23	14.35	14.47	14.58	14.70	14.82	14.93	15.05	15.17	15.28	15.40	15.51	15.63	15.75	15.86	15.98	16.10	16.21	16.33
14.36	14.48	14.59	14.71	14.83	14.94	15.06	15.18	15.29	15.41	15.52	15.64	15.76	15.87	15.99	16.11	16.22	16.34	16.46
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

DIFFERENCE TABLE

	T	V	T
...	...	0.01	0.01
0.1	0.03	0.02	0.02
0.2	0.05	0.03	0.03
0.3	0.06	0.04	0.05
0.4	0.10	0.05	0.06
...	...	0.06	0.07
...	...	0.07	0.08
...	...	0.08	0.09
...	...	0.09	0.10

TABLE CXXXIV.—(Continued).

Specific Gravity.	FAT.															
	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5
30.0	12.45	12.55	12.70	12.80	12.95	13.05	13.15	13.30	13.40	13.55	13.65	13.75	13.90	14.00	14.15	14.25
30.5	12.55	12.70	12.80	12.95	13.05	13.15	13.30	13.40	13.55	13.65	13.75	13.90	14.00	14.15	14.25	14.35
31.0	12.70	12.80	12.95	13.05	13.20	13.30	13.40	13.55	13.65	13.80	13.90	14.00	14.15	14.25	14.40	14.50
31.5	12.80	12.95	13.05	13.20	13.30	13.40	13.55	13.65	13.80	13.90	14.00	14.15	14.25	14.40	14.50	14.60
32.0	12.95	13.05	13.20	13.30	13.40	13.55	13.65	13.80	13.90	14.00	14.15	14.25	14.40	14.50	14.60	14.75
32.5	13.05	13.20	13.30	13.40	13.55	13.65	13.80	13.90	14.00	14.15	14.25	14.40	14.50	14.60	14.75	14.85
33.0	13.20	13.30	13.45	13.55	13.65	13.80	13.90	14.05	14.15	14.25	14.40	14.50	14.65	14.75	14.85	15.00
33.5	13.30	13.45	13.55	13.65	13.80	13.90	14.05	14.15	14.25	14.40	14.50	14.65	14.75	14.85	15.00	15.10
34.0	13.45	13.55	13.65	13.80	13.90	14.05	14.15	14.25	14.40	14.50	14.65	14.75	14.85	15.00	15.10	15.25
34.5	13.55	13.70	13.80	13.90	14.05	14.15	14.30	14.40	14.50	14.65	14.75	14.90	15.00	15.10	15.25	15.35

For 0.05 per cent. fat add on 0.05.

If the decimal in the specific gravity is 0.2 or 0.7 add on 0.05 to the nearest figure below

0.3 or 0.8 subtract 0.05 from nearest figure above.

0.1, 0.4, 0.6, or 0.9 take the nearest figure.





**TABLE CXXXVI.—FOR THE CALCULATION OF THE TOTAL SOLIDS OF SKIM MILK FROM THE FAT AND SPECIFIC GRAVITY.**

*For Use in the Dairy Laboratory.*

Specific Gravity.	FAT.								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
33·5	8·65	8·75	8·85	9·00	9·10	9·25	9·35	9·45	9·60
34·0	8·75	8·85	9·00	9·10	9·25	9·35	9·45	9·60	9·70
34·5	8·90	9·00	9·10	9·25	9·35	9·50	9·60	9·70	9·85
35·0	9·00	9·10	9·25	9·35	9·50	9·60	9·70	9·85	9·95
35·5	9·10	9·25	9·35	9·50	9·60	9·70	9·85	9·95	10·10
36·0	9·25	9·35	9·50	9·60	9·70	9·85	9·95	10·10	10·20
36·5	9·35	9·50	9·60	9·70	9·85	9·95	10·10	10·20	10·30
37·0	9·50	9·60	9·75	9·85	9·95	10·10	10·20	10·35	10·45
37·5	9·60	9·75	9·85	9·95	10·10	10·20	10·35	10·45	10·55

**TABLE CXXXVII.—FOR THE CONVERSION OF THERMOMETRIC SCALES.**

*For the Conversion of Degrees Fahrenheit into Degrees Centigrade.*

$$\text{Formula } C = (F - 32) \times \frac{5}{9}.$$

Degrees Fahrenheit.	Degrees Centigrade.	Degrees Fahrenheit.	Degrees Centigrade.	Degrees Fahrenheit.	Degrees Centigrade.
0	-17·78	51	10·56	78	25·56
5	-15·00	52	11·11	79	26·11
10	-12·22	53	11·67	80	26·67
15	-9·44	54	12·22	90	32·22
20	-6·67	55	12·78	100	37·78
25	-3·89	56	13·33	110	43·33
30	-1·11	57	13·89	120	48·89
31	-0·56	58	14·44	130	54·44
32	0	59	15·00	140	60·00
33	0·56	60	15·56	150	65·55
34	1·11	61	16·11	160	71·11
35	1·67	62	16·67	170	76·67
36	2·22	63	17·22	180	82·22
37	2·78	64	17·78	190	87·78
38	3·33	65	18·33	200	93·33
39	3·89	66	18·89	210	98·89
40	4·44	67	19·44	212	100·00
41	5·00	68	20·00	220	104·44
42	5·56	69	20·56	230	110·00
43	6·11	70	21·11	240	115·55
44	6·67	71	21·67	250	121·11
45	7·22	72	22·22	260	126·67
46	7·78	73	22·78	270	132·22
47	8·33	74	23·33	280	137·78
48	8·89	75	23·89	290	143·33
49	9·44	76	24·44	300	148·89
50	10·00	77	25·00		

TABLE CXXXVII.—(Continued).

*For the Conversion of Degrees Centigrade into Degrees Fahrenheit.*

$$\text{Formula } F = C \times \frac{9}{5} + 32.$$

Degrees Centigrade.	Degrees Fahrenheit.	Degrees Centigrade.	Degrees Fahrenheit.
-17.78	0	24	75.2
-15	5.00	25	77.0
-10	14.00	30	86.0
-5	23.00	35	95
0	32.00	37.78	100.0
1	33.8	40.0	104.0
2	35.6	45	113.0
3	37.4	50	122.0
4	39.2	55	131.0
5	41.0	60	140.0
6	42.8	65	149.0
7	44.6	70	158.0
8	46.4	75	167.0
9	48.2	80	176.0
10	50.0	85	185.0
11	51.8	90	194.0
12	53.6	95	203.0
13	55.4	100	212.0
14	57.2	105	221.0
15	59.0	110	230.0
15.56	60.0	115	239.0
16	60.8	120	248.0
17	62.6	125	257.0
18	64.4	130	266.0
19	66.2	135	275.0
20	68.0	140	284.0
21	69.8	145	293.0
22	71.6	150	302.0
23	73.4		

DIFFERENCE TABLE.

Degrees.	F into C.	C into F.
1	0.56	1.8
2	1.11	3.6
3	1.67	5.4
4	2.22	7.2
5	2.78	9.0
6	3.33	10.8
7	3.89	12.6
8	4.44	14.4
9	5.00	16.2
10	5.56	18.0



TABLE CXXXVIII.—WEIGHTS AND MEASURES.

*Linear Measure.*

1 inch . . . . .	= 0·0254 metre.
1 foot = 12 inches . . . . .	= 0·3048 „
1 yard = 3 feet = 36 inches . . . . .	= 0·9144 „
1 metre = 10 decimetres = 100 centimetres = 1000 millimetres.	
1 metre = 39·371 inches = 3·281 feet	= 1·094 yards.

*Square Measure.*

1 square inch . . . . .	= 0·000645 sq. metre.
1 square foot = 144 square inches . . . . .	= 0·0929 „
1 square yard = 9 square feet = 1296 sq. inches . . . . .	= 0·8361 „
1 sq. metre = 100 sq. decimetres = 10,000 sq. centimetres = 1,000,000 sq. millimetres.	
1 sq. metre = 1550·6 sq. inches = 10·764 sq. feet = 1·196 sq. yards.	

*Cubic Measure.*

1 cubic inch . . . . .	= 0·00001639 cub. metre.
1 cubic foot = 1728 cubic inches . . . . .	= 0·0283 „
1 cubic yard = 27 cubic feet = 46,656 cubic ins. . . . .	= 0·7645 „
1 cubic metre = 1000 cubic decimetres = 1,000,000 cubic centimetres = 1,000,000,000 cubic millimetres.	
1 cubic metre = 61,027 cub. ins. = 35·317 cubic ft. = 1·308 cubic yds.	

*Measures of Capacity.*

1 gill . . . . .	= 0·1420 litre.
1 pint = 4 gills . . . . .	= 0·5679 „
1 quart = 8 gills = 2 pints . . . . .	= 1·1359 litres.
1 gallon = 32 gills = 8 pints = 4 quarts . . . . .	= 4·5435 „
*1 barn gallon = 68 gills = 17 pints = 8½ quarts . . . . .	= 9·6548 „
1 litre = 1000 cubic centimetres = 1,000,000 cubic millimetres.	
1 litre = 7·043 gills = 1·7608 pints = 0·8804 quart = 0·2201 gallon = 0·1036 barn gallon.*	

*Avoirdupois Weight.*

1 grain . . . . .	= 0·0655 grammes.
1 drachm . . . . .	= 1·7718 „
1 ounce = 16 drachms . . . . .	= 28·349 „
1 pound = 256 drachms = 16 ounces . . . . .	= 453·59 „
1 quarter = 7168 drachms = 448 ounces = 28 lbs. . . . .	= 12,700·5 „
1 hundredweight = 28,672 drachms = 1792 ozs. = 112 lbs. = 4 quarters . . . . .	= 50·802 „
1 ton = 573,440 drachms = 35,840 ozs. = 2240 lbs. = 80 qrs. = 20 cwt. . . . .	= 1,016,047 „
1 metric ton = 1000 kilogrammes.	
1 kilogramme = 1000 grammes.	
1 gramme = 10 decigrammes = 100 centigrammes = 1000 milligrammes.	
1 gramme = 15·43 grains = 0·5644 drachm = 0·03527 oz. = 0·002205 lb. = 0·00001968 cwt. = 0·000000984 ton.	

\* The barn gallon is not a legal measure; all contracts made in barn gallons are null and void. It is, however, much used.

*Useful Data.*

The gallon weighs 10 lbs. (of distilled water at 62° F.).

The litre weighs 1000 grammes (of distilled water at 0° C.).

1 gallon =  $4\frac{1}{2}$  litres approximately.

1 barn gallon = 10 "

1 kilogramme =  $2\frac{1}{2}$  lbs. approximately.

1 hundredweight = 50 kilogrammes approximately.

1 cubic foot = 6.24 gallons.

*Note.*—The metre and litre compared with the standard English measures are those defined by the Act of 1878, and are not the true metre and litre. The difference is due to the fact that the English measures refer to a temperature of 62° F., and the metric measures to a temperature of 0° C. In the Weights and Measures Act of 1878 the difference of temperature has not been allowed for.

The following table shows the comparison between the two systems:—

	Metre.	Litre.	Kilogramme.
	Inches.	Gallon.	Lbs.
True values at 62° F., . . .	39.38203	0.22018	2.20462
Adopted in Act, . . . . .	39.37079	0.2200967	2.20462

TABLE CXXXIX.—BARN GALLONS AND IMPERIAL GALLONS.

*For the Conversion of Barn Gallons into Imperial Gallons.*

Barn Gallons.	Imperial Gallons.	Gallons.	Pints.
1	2.125	2	1
2	4.25	4	2
3	6.375	6	3
4	8.5	8	4
5	10.625	10	5
6	12.75	12	6
7	14.875	14	7
8	17.0	17	0
9	19.125	19	1
10	21.25	21	2

*For the Conversion of Imperial Gallons into Barn Gallons.*

Imperial Gallons.	Barn Gallons.	Imperial Gallons.	Barn Gallons.
1	0.47	10	4.70
2	0.94	11	5.17
3	1.41	12	5.64
4	1.88	13	6.12
5	2.35	14	6.59
6	2.82	15	7.06
7	3.29	16	7.53
8	3.76	17	8.00
9	4.23		

TABLE CXL.—TABLE OF WEIGHTS OF DAIRY PRODUCTS.

	Milk at Farm.		Milk at Dairy.		Skim Milk.		Butter Cream.		Thick Cream	
	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.
1 pint,	1	4½	1	4 <sup>5</sup> / <sub>8</sub>	1	4 <sup>3</sup> / <sub>4</sub>	1	4	1	3½
1 quart,	2	9	2	9½	2	9½	2	8	2	7
1 gallon,	10	4	10	5	10	6	10	0	9	12
2 gallons	20	8	20	10	20	12	20	0	19	8
3 "	30	12	30	15	31	2	30	0	29	4
4 "	41	0	41	4	41	8	40	0	39	0
5 "	51	4	51	9	51	14	50	0	48	12
6 "	61	8	61	14	62	4	60	0	58	8
7 "	71	12	72	3	72	10	70	0	68	4
8 "	82	0	82	8	83	0	80	0	78	0
9 "	92	4	92	13	93	6	90	0	87	12
10 "	102	8	103	2	103	12	100	0	97	8
11 "	112	12	113	7	114	2	110	0	107	4
12 "	123	0	123	12	124	8	120	0	117	0
13 "	133	4	134	1	134	14	130	0	126	12
14 "	143	8	144	6	145	4	140	0	136	8
15 "	153	12	154	11	155	10	150	0	146	4
16 "	164	0	165	0	166	0	160	0	156	0
17 "	174	4	175	5	176	6	170	0	165	12
18 "	184	8	185	10	186	12	180	0	175	8
19 "	194	12	195	15	197	2	190	0	185	4
20 "	205	0	206	4	207	8	200	0	195	0

## DIFFERENCE TABLE.

	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.	Lbs.	Ozs.
1 pint,	1	4½	1	4 <sup>5</sup> / <sub>8</sub>	1	4 <sup>3</sup> / <sub>4</sub>	1	4	1	3½
1 quart,	2	9	2	9½	2	9½	2	8	2	7
3 pints,	3	13½	3	13 <sup>7</sup> / <sub>8</sub>	3	14½	3	12	3	10½
2 quarts,	5	2	5	2½	5	3	5	0	4	14
5 pints,	6	6½	6	7 <sup>1</sup> / <sub>8</sub>	6	7 <sup>3</sup> / <sub>4</sub>	6	4	6	1½
3 quarts,	7	11	7	11½	7	12½	7	8	7	5
7 pints,	8	15½	9	0 <sup>3</sup> / <sub>8</sub>	9	1½	8	12	8	8½

*Note.*—The milk at farm is assumed to be warm and freshly milked.

The milk at dairy is assumed to be at the average temperature (60° F.) and a few hours old.

Skim milk is assumed to be at the average temperature (60° F.).

Butter cream is assumed to be at the average temperature (60° F.) and to contain 30 per cent. fat.

Thick cream is assumed to be at the average temperature (60° F.) and to contain 50 per cent. fat.

TABLE CXLI.—FOR THE CALCULATION OF THE WEIGHT OF BUTTER IN POUNDS OBTAINED BY CHURNING CREAM.

Percentage of Fat in Cream.	QUARTS OF CREAM CHURNED.																												
	1	2	3	4	5	6	7	8	9	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
15	0.44	0.87	1.31	1.74	2.18	2.61	3.05	3.48	3.92	4.35	8.7	13.1	17.4	21.8	26.1	30.5	34.8	39.2	43.5	47.9	52.2	56.6	60.9	65.3	69.6	74.0	78.3	82.7	87.0
16	0.47	0.93	1.40	1.86	2.33	2.79	3.26	3.72	4.19	4.65	9.3	14.0	18.6	23.3	27.9	32.6	37.2	41.9	46.5	51.2	55.8	60.5	65.1	69.8	74.4	79.1	83.7	88.3	92.9
17	0.50	0.99	1.49	1.98	2.48	2.98	3.47	3.97	4.46	4.96	9.9	14.9	19.8	24.8	29.8	34.7	39.7	44.6	49.6	54.5	59.5	64.5	69.4	74.4	79.4	84.3	89.3	94.2	99.2
18	0.53	1.05	1.58	2.10	2.63	3.16	3.68	4.21	4.73	5.26	10.5	15.8	21.0	26.3	31.6	36.8	42.1	47.3	52.6	57.9	63.1	68.4	73.6	78.9	84.2	89.4	94.7	99.9	105.2
19	0.56	1.11	1.67	2.23	2.79	3.34	3.90	4.46	5.01	5.57	11.1	16.7	22.3	27.9	33.4	39.0	44.6	50.1	55.7	61.3	66.8	72.4	78.0	83.6	89.1	94.7	100.3	105.8	111.4
20	0.59	1.17	1.76	2.35	2.94	3.52	4.11	4.70	5.28	5.87	11.7	17.6	23.5	29.4	35.2	41.1	47.0	52.8	58.7	64.6	70.4	76.3	82.2	88.1	93.9	99.8	105.7	111.5	117.4
21	0.62	1.23	1.85	2.46	3.08	3.70	4.31	4.93	5.54	6.16	12.3	18.5	24.6	30.8	37.0	43.1	49.3	55.4	61.6	67.8	73.9	80.1	86.2	92.4	98.5	104.7	110.9	117.0	123.2
22	0.65	1.29	1.94	2.58	3.23	3.87	4.52	5.16	5.81	6.45	12.9	19.4	25.8	32.3	38.7	45.2	51.6	58.1	64.5	71.0	77.4	83.9	90.3	96.8	103.2	109.7	116.1	122.6	129.0
23	0.68	1.35	2.03	2.70	3.38	4.05	4.73	5.40	6.08	6.75	13.5	20.3	27.0	33.8	40.5	47.3	54.0	60.8	67.5	74.3	81.0	87.8	94.5	101.3	108.0	114.8	121.5	128.3	135.0
24	0.70	1.41	2.11	2.82	3.52	4.22	4.93	5.63	6.34	7.04	14.1	21.1	28.2	35.2	42.2	49.3	56.3	63.4	70.4	77.4	84.5	91.5	98.6	105.6	112.6	119.7	126.7	133.8	140.8
25	0.73	1.47	2.20	2.93	3.67	4.40	5.13	5.86	6.60	7.33	14.7	22.0	29.3	36.7	44.0	51.3	58.6	66.0	73.3	80.6	88.0	95.3	102.6	110.0	117.3	124.6	131.9	139.2	146.5
26	0.76	1.52	2.29	3.05	3.81	4.57	5.33	6.10	6.86	7.62	15.2	22.9	30.5	38.1	45.7	53.3	61.0	68.6	76.2	83.8	91.4	99.1	106.7	114.3	121.9	129.5	137.1	144.7	152.3
27	0.79	1.58	2.37	3.16	3.96	4.75	5.54	6.33	7.12	7.91	15.8	23.7	31.6	39.6	47.5	55.4	63.3	71.2	79.1	87.0	94.9	102.8	110.7	118.7	126.6	134.5	142.4	150.3	158.2
28	0.82	1.64	2.46	3.28	4.10	4.91	5.73	6.55	7.37	8.19	16.4	24.6	32.8	41.0	49.1	57.3	65.5	73.7	81.9	90.1	98.3	106.5	114.7	122.9	131.0	139.2	147.4	155.6	163.8
29	0.85	1.70	2.54	3.38	4.24	5.09	5.94	6.78	7.63	8.48	17.0	25.4	33.9	42.4	50.9	59.4	67.8	76.3	84.8	93.3	101.8	110.2	118.7	127.2	135.7	144.2	152.6	161.1	169.6
30	0.88	1.75	2.63	3.51	4.38	5.26	6.14	7.02	7.90	8.77	17.5	26.3	35.1	43.8	52.6	61.4	70.2	78.9	87.7	96.5	105.2	114.0	122.8	131.5	140.3	149.1	157.9	166.6	175.4
31	0.91	1.81	2.72	3.62	4.53	5.43	6.34	7.24	8.15	9.05	18.1	27.2	36.2	45.3	54.3	63.4	72.4	81.5	90.5	99.6	108.6	117.7	126.7	135.8	144.8	153.9	162.9	172.0	181.0
32	0.92	1.87	2.80	3.74	4.67	5.60	6.54	7.47	8.41	9.34	18.7	28.0	37.4	46.7	56.0	65.4	74.7	84.1	93.4	102.7	112.1	121.4	130.8	140.1	149.4	158.8	168.1	177.5	186.8
33	0.96	1.92	2.89	3.85	4.81	5.77	6.73	7.70	8.66	9.62	19.2	28.9	38.5	48.1	57.7	67.3	77.0	86.6	96.2	105.8	115.4	125.1	134.7	144.3	153.9	163.5	173.2	182.8	192.4
34	0.99	1.98	2.97	3.96	4.95	5.95	6.94	7.93	8.92	9.91	19.8	29.7	39.6	49.6	59.5	69.4	79.3	89.2	99.1	109.0	118.9	128.8	138.7	148.7	158.6	168.5	178.4	188.3	198.2
35	1.02	2.04	3.06	4.08	5.10	6.11	7.13	8.15	9.17	10.19	20.4	30.6	40.8	51.0	61.1	71.3	81.5	91.7	101.9	112.1	122.3	132.5	142.7	152.9	163.0	173.2	183.4	193.6	203.8
36	1.05	2.09	3.14	4.18	5.23	6.28	7.32	8.37	9.41	10.46	20.9	31.4	41.8	52.3	62.8	73.2	83.7	94.1	104.6	115.1	125.6	136.0	146.4	156.9	167.4	177.8	188.3	198.7	209.2
37	1.07	2.15	3.22	4.30	5.37	6.44	7.52	8.59	9.67	10.74	21.5	32.2	43.0	53.7	64.4	75.2	85.9	96.7	107.4	118.1	128.9	139.6	150.4	161.1	171.8	182.6	193.3	204.1	214.8
38	1.10	2.20	3.30	4.40	5.51	6.61	7.71	8.81	9.91	11.01	22.0	33.0	44.0	55.1	66.1	77.1	88.1	99.1	110.1	121.1	132.1	143.1	154.1	165.2	176.2	187.2	198.2	209.2	220.2
39	1.13	2.26	3.39	4.52	5.65	6.77	7.90	9.03	10.16	11.29	22.6	33.9	45.2	56.5	67.7	79.0	90.3	101.6	112.9	124.2	135.5	146.8	158.1	169.4	180.6	191.9	203.2	214.5	225.8
40	1.16	2.31	3.47	4.62	5.78	6.94	8.09	9.25	10.40	11.56	23.1	34.7	46.2	57.8	69.4	80.9	92.5	104.0	115.6	127.2	138.7	150.3	161.8	173.3	184.8	196.3	207.8	219.3	230.8
41	1.18	2.37	3.55	4.73	5.92	7.10	8.28	9.46	10.65	11.83	23.7	35.5	47.3	59.2	71.0	82.8	94.6	106.5	118.3	130.1	142.0	153.8	165.6	177.5	189.3	201.1	212.9	224.8	236.6
42	1.21	2.42	3.63	4.84	6.05	7.25	8.46	9.67	10.88	12.09	24.2	36.3	48.4	60.5	72.5	84.6	96.7	108.8	120.9	133.0	145.1	157.2	169.3	181.4	193.4	205.5	217.6	229.7	241.8
43	1.24	2.47	3.71	4.94	6.18	7.42	8.65	9.89	11.12	12.36	24.7	37.1	49.4	61.8	74.2	86.5	98.9	111.2	123.6	136.0	148.3	160.7	173.0	185.4	197.8	210.1	222.5	234.8	247.2
44	1.26	2.52	3.79	5.05	6.31	7.57	8.83	10.10	11.36	12.62	25.2	37.9	50.5	63.1	75.7	88.3	101.0	113.6	126.2	138.8	151.4	164.1	176.7	189.3	201.9	214.5	227.2	239.8	252.4
45	1.29	2.58	3.87	5.16	6.45	7.73	9.02	10.31	11.60	12.89	25.8	38.7	51.6	64.5	77.3	90.2	103.1	116.0	128.9	141.8	154.7	167.6	180.5	193.4	206.2	219.1	232.0	244.9	257.8
46	1.32	2.63	3.95	5.26	6.58	7.89	9.21	10.52	11.84	13.15	26.3	39.5	52.6	65.8	78.9	92.1	105.2	118.4	131.5	144.7	157.8	171.0	184.1	197.3	210.4	223.6	236.7	249.9	263.0
47	1.34	2.68	4.02	5.36	6.70	8.04	9.38	10.72	12.06	13.40	26.8	40.2	53.6	67.0	80.4	93.8	107.2	120.6	134.0	147.4	160.8	174.2	187.6	201.0	214.4	227.8	241.2	254.6	268.0
48	1.36	2.73	4.09	5.46	6.82	8.18	9.55	10.91	12.28	13.64	27.3	40.9	54.6	68.2	81.8	95.5	109.1	122.8	136.4	150.0	163.7	177.3	191.0	204.6	218.2	231.9	245.5	259.2	272.8
49	1.39	2.77	4.16	5.55	6.94	8.32	9.71	11.10	12.48	13.87	27.7	41.6	55.5	69.4	83.2	97.1	111.0	124.8	138.7	152.6	166.4	180.3	194.2	208.1	221.9	235.8	249.7	263.5	277.4
50	1.41	2.82	4.23	5.64	7.05	8.46	9.87	11.28	12.69	14.10	28.2	42.3	56.4	70.5	84.6	98.7	112.8	126.9	141.0	155.1	169.2	183.3	197.4	211.5	225.6	239.7	253.8	267.9	282.0



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