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NUTRIENT REQUIREMENTS OF CHICKS FED "CARBOHYDRATE-FREE" DIETS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Nutrient requirements of chicks fed 'carbohydrate-free' diets" submitted by Su Hoon Looi, B.Sc., M.Sc., in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

ABSTRACT

Experiments were conducted to study the effect of fat on the chick's requirement for vitamin B_{12} when fat was substituted isocalorically for carbohydrate in a semipurified diet in which protein was supplied by isolated soybean protein and in which methionine was supplied in limited amounts.

Using growth as the criterion, the chick's requirement for vitamin B_{12} was shown to increase three-fold when hydrogenated fat (Crisco) was substituted isocalorically for glucose. Since further studies showed that the requirement for methionine was not increased when non-protein energy was supplied by hydrogenated fat, it was concluded that vitamin B_{12} is involved in fat metabolism. Support for this concept was the finding that in the presence of vitamin B_{12} the isocaloric substitution of hydrogenated fat for glucose permitted chicks to increase feed consumption, while in its absence feed consumption remained unchanged. Studies in which chicks were pair-fed showed that vitamin B_{12} increased growth of chicks fed either carbohydrate-containing or "carbohydrate-free" diets by stimulating appetite and not by increasing utilization of either protein or energy.

Results of experiments conducted to determine the role which vitamin B_{12} plays in fat metabolism showed that it was not involved in either fat digestion or absorption since studies showed that the absorbability of hydrogenated fat (Crisco) was not affected by supplementary vitamin B_{12} when

chicks were fed semipurified diets containing soybean protein. Studies conducted to determine whether vitamin B_{12} was involved in acetate metabolism showed that vitamin B_{12} deficiency did not alter levels of blood ketone bodies when non-protein energy was supplied by glucose, hydrogenated fat or tallow. These results suggest that deficiency of vitamin B_{12} does not affect oxidation of acetate through the citric acid cycle.

Although deficiency of vitamin B_{12} did not reduce absorbability of hydrogenated fat, studies showed a small but significant decrease in fat absorbability when chicks were fed a diet markedly deficient in methionine. The decreased absorbability of fat from diets deficient in methionine was found not to be caused by lack of choline or lack of pancreatic lipase. Since studies showed that the ability of chicks to absorb hydrogenated fat and tallow was also reduced when a lysine-deficient diet based on safflower meal was fed, it would appear that the reduced ability to absorb fat was not a specific effect of a deficiency of methionine or lysine but rather reflected protein deficiency.

In the course of these experiments, it was observed that the isocaloric substitution of hydrogenated fat for carbohydrate caused a relatively greater increase in growth when diets were fed which were deficient in lysine or methionine. These results suggested that fat might have an amino acid sparing effect; however, pair-feeding studies showed that fat stimulated growth of chicks fed diets deficient in lysine or methionine by increasing appetite and

not by increasing utilization. Since tallow did not have a similar growth stimulating effect, it would appear that the growth stimulating effect of fat when substituted isocalorically for carbohydrate is at least partially dependent on its fatty acid composition.

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INTRODUCTION

Recent studies have shown that the chick has the ability to utilize large quantities of fat when nutrient balance is maintained (Renner, 1964; Brambila and Hill, 1966). In these studies excesses of the B vitamins were added in order to avoid the possibility of deficiencies since information on the effect of high levels of dietary fat on nutrient requirements of chicks was limited.

In rats, the isocaloric substitution of fat for carbohydrate has been shown to decrease the requirement for thiamine (Evans and Lepkovsky, 1928) and to increase the requirement for riboflavin (Mannering _et al._, 1941; Mannering _et al._, 1944); however, the effect on other nutrients has not been studied.

In chicks, no studies have been reported on the effect on nutrient requirements of substituting fat isocalorically for carbohydrate. Studies (Fox _et al._, 1956; March and Biely, 1956a; March and Biely, 1956b and Siedler and Schweigert, 1953) have been conducted to determine the effect of fat on nutrient requirements when fat was substituted weight for weight for carbohydrate; however, these studies are difficult to interpret since chicks automatically reduce their consumption of these high fat diets due to their greater caloric density, thus reducing the intake of not only the nutrient under study but also others which may affect the requirement.

The following experiments were conducted to study the

effect of fat on the chick's requirement for vitamin B_{12} when fat was substituted isocalorically for carbohydrate. Subsequently, studies were conducted in an attempt to explain why higher levels of fat resulted in increased requirement for vitamin B_{12}.

PART I

VITAMIN B_{12} REQUIREMENT OF CHICKS FED "CARBOHYDRATE-FREE" DIETS

Literature Review

Shortly after vitamin B_{12} was isolated by Rickes *et al.* (1948) and Smith and Parker (1948), Ott *et al.* (1948) demonstrated that vitamin B_{12} was required by the growing chick. They reported that quantities of crystalline vitamin B_{12} as small as 6 μg per kilogram of diet stimulated growth of chicks fed purified diets low in the animal protein factor. Since that time many experiments have been conducted to determine the amount of vitamin B_{12} required by the chick for optimum growth. Recently, the Subcommittee on Poultry Nutrition of the National Research Council (1966) set the requirement of the chick for vitamin B_{12} at 9 μg per kilogram of diet for diets containing 20% protein and 2750 kcal of metabolizable energy per kilogram. The figure stated is tentative since the requirement of the chick for vitamin B_{12} has been shown to be affected by such factors as the vitamin B_{12} content of the dam's diet (Miller *et al.*, 1956) and by the levels of methionine (Briggs *et al.*, 1950; Patrick, 1950 and Fox *et al.*, 1957), choline (Schaefer *et al.*, 1949; Gillis and Norris, 1949) and folic acid in the diet (Schaefer *et al.*, 1950).

Studies also have been reported on the effect of two major dietary constituents, protein and fat, on the vitamin B_{12} requirement of the chick. In the case of protein, many investigators have found that a high level of protein in the

diet increases the growth response of chicks to vitamin B_{12} supplementation (Stokstad et al., 1949; Hill and Branion, 1952 and Spivey et al., 1954). An even greater growth response to vitamin B_{12} was observed by Hill and Branion (1952) when both extra fat and protein were added to an all-vegetable protein diet.

The effect of dietary fat on the vitamin B_{12} requirement is not clear. Bosshardt et al. (1950) reported a sparing action in mice, and McCollum and Chow (1950) a similar effect in rats; however, Erickson and O'Dell (1961) found that fat had no effect on the vitamin B_{12} requirement of the rat. In contrast, Schaefer (1949) obtained a marked growth stimulation of non-depleted chicks when vitamin B_{12} was added to a diet low in methionine and choline which contained 19% lard. Similarly, Spivey et al. (1954) and Fox et al. (1959) reported that a severe vitamin B_{12} deficiency could be produced in non-depleted chicks by the addition of fat to either a corn-soybean meal diet or a purified diet containing isolated soybean protein. Fox et al. (1956) showed that the vitamin B_{12} requirement of chicks from non-depleted hens is increased ten- to twenty-fold when 20% lard is substituted for an equivalent amount of corn in a corn-soybean meal diet. This, they suggest, may be due to a possible role of vitamin B_{12} in the utilization of dietary fat or possibly to an indirect effect mediated through some other nutrient since fat was not substituted isocalorically for corn. Support for the suggested role of vitamin B_{12} in fat metabolism has been provided by the finding of Moore and

Doran (1962) that vitamin B_{12} deficiency interferes with the utilization of triglycerides in the liver of chick embryos. Similarly, Williams *et al.* (1937) have found that the utilization of plasma triglycerides was impaired in patients suffering from pernicious anemia.

Recently Somers (1969), using clearance studies in sheep, reported that before signs of vitamin B_{12} deficieny were induced, the clearance rates of both acetate and propionate were so rapid, that within an hour, virtually all of the propionate and acetate injected had disappeared from the blood. During the stage of acute vitamin B_{12} deficieny, the clearance rates were so slow, that even after 4 hours, the levels remained markedly higher than the control, but after treatment with vitamin B_{12}, the clearance rates again increased rapidly and became normal when signs of deficiency disappeared. In human subjects, Cox and coworkers (1968) also found that 9 out of 12 patients suffering from pernicious anemia showed increased excretion of acetate, and after vitamin B_{12} therapy, most of these patients had returned to normal acetate excretion. These results indicate that vitamin B_{12} may play an important role in the utilization of acetate. The role which vitamin B_{12} plays in propionate metabolism has been established. Beck *et al.* (1957) and Lengyel *et al.* (1960) have shown that vitamin B_{12} is involved in the conversion of methylmalonyl CoA to succinyl CoA.

A relationship between vitamin B_{12} and ketone bodies has been suggested by Nath and Nath (1967a). They found that continued and prolonged injection of acetoacetate or

β-hydroxybutyrate into rats fed a normal laboratory stock diet caused depression of growth and marked depletion of vitamin B_{12} in blood and liver. They also observed a significant reduction in liver sulfhydryl content and an increase in liver fat. The growth depression was partially overcome, and normal vitamin B_{12}, sulfhydryl and lipid content resulted, after animals were treated with vitamin B_{12}. They also found that in rats suffering from severe diabetes accumulation of ketone bodies was accompanied by decreased body stores of vitamin B_{12} (Nath and Nath, 1969).

Biswas and Johnson (1964) observed that the activities of several sulfhydryl enzymes, including isocitrate dehydrogenase were depressed in liver preparations from vitamin B_{12}-deficient rats and chicks, and preincubation of the enzyme preparation from vitamin B_{12}-deficient animals with cysteine or mercaptoethanol essentially restored these sulfhydryl enzymes to normal activities. The activity of glutathione reductase was also found to be lowered; however, neither crystalline B_{12} nor its coenzyme was found to reactivate *in vitro* the depressed dehydrogenase activities. Thus, they proposed that vitamin B_{12} in the intact animal acts to maintain the sulfhydryl compounds, e.g. glutathione, in the reduced form by maintaining the activity of glutathione reductase. Hence, the effect of vitamin B_{12} deficiency on dehydrogenase activity appears to be secondary to its effect on glutathione reductase.

Vitamin B_{12} has also been found to affect the concentration of coenzyme A in chicks (Boxer *et al.*, 1953),

rats (Boxer and Shonk, 1955) and sheep (Smith et al., 1969). Arnstein (1955) postulated that the disulfide form of coenzyme A accumulated in vitamin B_{12} deficiency and would therefore be unavailable for the activation of the fatty acids. However, further studies of Boxer and Shonk (1955) showed that approximately 60% of the total coenzyme A was in the sulfhydryl form in livers of vitamin B_{12}-deficient rats. Thus, the accumulated coenzyme A in the deficient animal was due not only to oxidized but also to reduced coenzyme A. Since coenzyme A concentration in kidney was also increased to about the same extent as that of the liver, and the rate of degradation of coenzyme A was the same between the deficient and normal liver homogenates, they suggested that the accumulation of coenzyme A may be due to an increase in its synthesis during vitamin B_{12} deficiency. This suggestion is in contrast to the finding of Yacowitz et al. (1951) that vitamin B_{12} is involved in the incorporation of free pantothenate into coenzyme A in chick livers.

That the concentration of both total coenzyme A and methylmalonic acid were increased markedly in livers of vitamin B_{12}-deficient sheep was reported by Smith et al. (1969). Since the molar concentration of coenzyme A was calculated to be more than three times that of methylmalonic acid it was concluded that the increase in liver coenzyme A was not due solely to an increased level of methylmalonyl CoA. Whether the remainder of the accumulation was a result of reduced coenzyme A or other oxidized coenzyme A derivatives was not determined by these workers.

From experiments involving humans, rats and chick embryos, referred to above, evidence has accumulated in support of the involvement of vitamin B_{12} in the metabolism of triglycerides. In order to determine whether vitamin B_{12} is also involved in the utilization of triglycerides by growing chicks the following studies were conducted to compare the vitamin B_{12} requirement of chicks fed diets in which non-protein energy was supplied by either hydrogenated fat (Crisco) or carbohydrate.

Experiment 1, 2 and 3

Chicks have been reared successfully in this laboratory on a semipurified diet containing virtually no carbohydrate formulated by substituting either lard or corn oil isocalorically for glucose (Renner, 1964; Renner and Elcombe, 1964). By omitting vitamin B_{12} from the vitamin mixture and reducing the level of supplemental methionine a diet was devised which was deficient in one-carbon units but otherwise met NRC requirements (1966) for minerals and vitamins. Preliminary studies showed that the addition of vitamin B_{12} (100 μg/kg) to either the carbohydrate-containing diet or its "carbohydrate-free" counterpart caused a significant increase in rate of growth when the diet contained 0.1% added methionine. The object of the following studies was to compare the vitamin B_{12} requirement of chicks fed carbohydrate-containing and "carbohydrate-free" diets in which non-protein energy was supplied by glucose or an equicaloric amount of hydrogenated fat (Crisco).

Experimental

Three experiments were conducted in which duplicate groups of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks were fed "carbohydrate-free" or carbohydrate-containing diets in which non-protein energy was supplied by hydrogenated fat or glucose, respectively, and to which 0, 2.5, 5.0, 10.0, 50.0 or 100.0 μg vitamin B_{12} per kilogram diet was added.

The semipurified diets used in all experiments contained the constant dietary ingredients shown in Table 1. Carbohydrate-containing diets were formulated by the addition of glucose to the constant dietary ingredients. Their "carbohydrate-free" counterparts were formulated by replacing glucose isocalorically by hydrogenated fat using the values 3.64 and 8.59 kcal/g for the metabolizable energy of glucose and hydrogenated fat, respectively. Cellulose was added to improve the texture of the "carbohydrate-free" diets. Because the "carbohydrate-free" diets do not total to 100 their content of methionine and vitamin B_{12} will be referred to as the level present in the carbohydrate-containing diets from which they were derived. In experiments 1 and 2, the level of supplemental methionine was 0.1%, while in experiment 3 the level of supplemental methionine was 0.074%. All diets were formulated to contain 15.0 kcal/g protein.

For the first week the chicks were fed the "carbohydrate-free" diet containing 0.1% supplemental methionine and no added vitamin B_{12}. They were then assigned to the experimental groups on the basis of body weight,

Table 1

Composition of basal diets

Ingredients	Carbohydrate-containing	"Carbohydrate-free"
Variables	g	g
Glucose[1]	64.36	-
Hydrogenated fat[2]	-	27.27
Cellulose[3]	-	6.39
Constants		
Soybean protein[4]	25.03	25.03
DL-methionine[5]	0.10	0.10
L-cystine	0.25	0.25
Soybean oil	4.00	4.00
Limestone	1.49	1.49
Dicalcium phosphate	2.15	2.15
Sodium chloride	0.60	0.60
Mineral mixture[6]	1.26	1.26
Vitamin mixture[7]	0.43	0.43
Chromium oxide	0.30	0.30
Antioxidant[8]	0.025	0.025

[1] Cerelose.

[2] Crisco. Composition in g/100 g: $C_{14:0}$, 0.2; $C_{16:0}$, 14.0; $C_{18:0}$, 12.0; $C_{18:1}$, 44.0; $C_{18:2}$, 27.0; other polyunsaturated fatty acid, 1.8 (Bernfield *et al.*, 1962).

[3] Solka Floc S. W.-40-A, Brown Forest Products Limited, Montreal, Quebec.

[4] Promine, Central Soya, Chemurgy Division, Chicago 60639.

[5] Supplied at 0.1% level in experiments 1 and 2 and 0.074% in experiment 3.

[6] Supplied in milligrams per 100 grams ration: KH_2PO_4, 930; $MgSO_4$, 258; KI, 0.29; $FeSO_4 \cdot 7H_2O$, 28; $CuSO_4 \cdot 5H_2O$, 1.56; $ZnCO_3$, 11.50; $CoCl \cdot 6H_2O$, 0.17; $NaMoO_4 \cdot 2H_2O$, 0.83; Na_2SeO_3, 0.022; and $MnSO_4 \cdot H_2O$, 33.

[7] Supplied per 100 grams ration: thiamine, 1.0 mg; riboflavin, 1.0 mg; Ca pantothenate, 4.0 mg; biotin, 0.04 mg; pyridoxine, 2.0 mg; niacin, 8.0 mg; folic acid, 0.3 mg; menadione, 0.3 mg; vitamin A, 1000 IU; vitamin D_3, 150 ICU; vitamin E, 3.3 IU; chlortetracycline, 1.0 mg and choline chloride, 148 mg.

[8] Ethoxyquin.

equalizing both mean body weight and weight distribution among the groups, and fed the experimental diets to 29 days of age. The chicks were housed in electrically heated, thermostatically controlled battery brooders with raised wire-screen floors in a temperature-controlled laboratory. Feed and water were supplied ad libitum. Data on growth and feed consumption were obtained weekly and feed wastage was determined daily.

Results and Discussion

Data showing average weight gains and caloric efficiencies of chicks fed diets containing graded levels of vitamin B_{12} in which non-protein energy was supplied by either glucose or hydrogenated fat are summarized in Table 2. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the growth data obtained in the three experiments showed that chick growth was increased significantly by the addition of vitamin B_{12}. The results show that the addition of 10 μg of vitamin B_{12}/kg of diet caused a significant increase ($P < 0.05$) in growth of chicks fed the carbohydrate-containing diet with no further significant increase being observed on the addition of higher levels of vitamin B_{12}. In the case of chicks fed the "carbohydrate-free" diet in which non-protein energy was provided by hydrogenated fat, the addition of 2.5 μg of vitamin B_{12}/kg of diet caused a significant increase with a still further significant increase being observed on the addition of 50 μg vitamin B_{12}/kg of diet. Plots of weight

Table 2

Weight gain and caloric efficiency of chicks fed either carbohydrate-containing or "carbohydrate-free" diets with graded levels of vitamin B_{12}

Supplemental vitamin B_{12}	Exp. no.	Wt gain		Calories/g gain	
		Carbohydrate		Carbohydrate	
		Containing	"Free"	Containing	"Free"
µg/kg		g	g	kcal	kcal
0	1	258[1]	271	6.68	6.52
	2	283	291	6.54	6.17
	3	174	167	7.82	7.45
		238^{a}	243^{a}	7.01^{e}	6.71^{d}
2.5	1	284	298	6.24	6.09
	2	305	304	6.29	5.90
	3	170	222	7.38	6.84
		$253^{a,b}$	$275^{b,c,d}$	6.64^{d}	6.28^{c}
5.0	1	284	316	6.42	5.96
	2	291	310	6.47	5.86
	3	191	200	7.15	6.94
		$255^{a,b,c}$	$275^{b,c,d}$	6.68^{d}	$6.25^{b,c}$
10.0	1	313	304	6.14	6.01
	2	318	313	6.18	5.65
	3	213	234	6.84	6.54
		$281^{b,c,d}$	$284^{c,d}$	6.39^{c}	6.07^{b}
50.0	1	306	359	6.22	5.80
	2	340	369	5.87	5.52
	3	233	266	6.80	5.96
		293^{d}	$331^{e,f}$	6.30^{c}	5.76^{a}
100.0	1	332	337	6.18	5.62
	2	371	384	5.98	5.58
	3	215	290	6.99	6.28
		$306^{d,e}$	337^{f}	6.38^{c}	5.83^{a}

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[1] Values are averages of duplicate groups. Underlined values are averages of triplicate experiments. Values without a common letter in their superscript are significantly different ($P < 0.05$).

gains versus level of supplemental vitamin B_{12} expressed as $\log_{10}$ (10+ μg vitamin B_{12}/kg) showed that linear and plateau lines intersected at 9 μg/kg of carbohydrate-containing diet and at 27 μg/kg of "carbohydrate-free" diet. Thus, in these experiments the vitamin B_{12} requirement of chicks fed carbohydrate-containing and "carbohydrate-free" diets was 9 and 27 μg/kg of diet, respectively. The vitamin B_{12} requirement of chicks fed the carbohydrate-containing diet is in agreement with the finding of Ott et al. (1948) and Fox et al. (1956) and the requirement as stated by NRC (1966). The finding that the isocaloric substitution of fat for carbohydrate increased the vitamin B_{12} requirement implicates vitamin B_{12} in the utilization of fat.

Statistical analysis of the growth data obtained in the three experiments also showed that growth was affected significantly by both experiment ($P < 0.01$) and source of energy ($P < 0.01$). That chicks grew significantly slower in experiment 3 was to be expected since the amount of supplemental methionine was 0.074% rather than 0.1%. The finding that chicks fed the "carbohydrate-free" diets grew faster than chicks receiving the carbohydrate-containing diets without increasing caloric consumption suggests that fat has a methionine sparing action. Recently, Nakano and Ashida (1970) have shown that the protein sparing action of both fat and carbohydrate is closely related to their inhibiting effect on the induction of amino acid-degrading enzymes. They emphasized that the activity of all the hepatic amino acid-degrading enzymes in rats previously adapted to a high

fat diet was much less than that in rats previously fed a high carbohydrate diet. In parallel, the amount of urinary nitrogen output was found to be less in rats fed the high fat diet. Their results suggest that dietary fat improves protein retention through its inhibiting effect on the induction of hepatic amino acid-degrading enzymes. In the present study, chicks fed the "carbohydrate-free" diets should have been well adapted to the high fat diet, since the chick embryo develops on a medium containing only 1% carbohydrate, and after hatching, they were fed the "carbohydrate-free" diet until allotted to the experimental groups. Whether the greater growth of chicks receiving the "carbohydrate-free" diets was a reflection of a similar amino acid sparing action of fat as in the case of rats is unknown.

Similar statistical treatment of the data on caloric efficiency lends support to the finding that the isocaloric substitution of fat for carbohydrate increases the chick's requirement for vitamin B_{12}. The data (Table 2) show that the vitamin B_{12} requirement of chicks fed carbohydrate-containing and "carbohydrate-free" diets was 5.0 to 10 and 10 to 50 μg/kg, respectively, when caloric efficiency was used as the criterion of adequacy.

Experiment 4 and 5

These experiments were designed to determine whether vitamin B_{12} increased growth of chicks fed carbohydrate-containing and "carbohydrate-free" diets by increasing the utilization of protein and/or energy or by stimulating appetite.

Experimental

In the course of the preceding experiments (Exp. 1 and 2) duplicate groups of 10 male crossbred (Dominant White x White Plymouth Rock) chicks were pair-fed the carbohydrate-containing and "carbohydrate-free" diets containing supplemental vitamin B_{12} (100 μg/kg). Feed intake was limited to that consumed by chicks fed the vitamin B_{12}-deficient diet. The diets fed, method of allotment, housing and weighing were as in the foregoing experiments (Exp. 1 and 2). The pair-fed chicks received their daily allotment of feed once every 24 hours. The experimental diets were fed from 8-29 days of age. During the fourth week of the experiment, excreta were collected from each experimental group at 24 hour intervals for the determination of metabolizable energy. Chromium oxide was incorporated in each of the diets at a level of approximately 0.3% as an index substance in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. The methods of processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy and chromium oxide and computing metabolizable energy from these data have been described previously (Hill and Anderson, 1958; Hill _et al._, 1960).

At the termination of the experiment, the chicks were killed with chloroform. After cooling, the contents of the gastrointestinal tract were removed, the livers were weighed and the entire carcasses from each experimental group (minus the livers) were frozen, ground, mixed and

an aliquot dried by lyophilization. In order that tissue gains could be determined, a representative group of chicks was killed at the beginning of the experiment and prepared for analysis using the same procedure. Carcass samples were analyzed for protein, fat and moisture as described by Hill and Anderson (1958).

In experiment 4, liver fat was determined on pooled samples containing 10 livers. The samples were lyophilized, the moisture content was determined by loss in weight during lyophilization. The dried samples were then extracted for 16 hours with a 2:1 mixture of chloroform and methanol using a Goldfisch apparatus. The dried extracts were then reextracted with light petroleum (30^{o}-60^{o}). This method is similar to the method of Feigenbaum and Fisher (1963).

Results and Discussion

Summarized in Table 3 are data showing weight gains, caloric consumption and caloric efficiencies of chicks fed diets with and without supplemental vitamin B_{12} in which non-protein energy was supplied by either glucose or hydrogenated fat. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the combined data from the two experiments showed that when chicks were pair-fed, the addition of vitamin B_{12} to either the carbohydrate-containing or "carbohydrate-free" diets had no effect on growth or caloric efficiency. In comparison, chicks fed ad libitum the vitamin B_{12} supplemented diets consumed more feed, grew faster and utilized their

Table 3

Weight gain, caloric consumption and caloric efficiency of chicks fed diets with and without supplemental vitamin B_{12} in which non-protein energy was supplied by glucose or hydrogenated fat

Treatment		Wt gain		Calories consumed		Calories/g gain	
		Carbohydrate		Carbohydrate		Carbohydrate	
Supplemental vitamin B_{12}	Feeding regimen	Containing	"Free"	Containing	"Free"	Containing	"Free"
μg/kg		g	g	kcal	kcal	kcal	kcal
0	ad libitum	258[1]	271	1728	1767	6.68	6.52
		283	291	1849	1798	6.54	6.17
		270^{a}	281^{a}	1788^{a}	1782^{a}	6.61^{c}	$6.34^{b,c}$
100	pair-fed	265	282	1760	1776	6.63	6.29
		293	299	1872	1795	6.40	6.01
		279^{a}	290^{a}	1816^{a}	1786^{a}	6.52^{c}	6.15^{b}
100	ad libitum	332	337	2053	2146	6.18	5.62
		371	384	2221	1893	5.98	5.58
		352^{b}	360^{b}	2137^{c}	2020^{b}	6.08^{b}	5.60^{a}

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[1] Values are averages of duplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscript are signficantly different ($P < 0.05$).

feed more efficiently than chicks fed ad libitum the unsupplemented diet, irrespective of source of non-protein energy.

Data showing protein gained, fat gained, protein retained and caloric efficieny of chicks fed diets with and without supplemental vitamin B_{12} are summarized in Table 4. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that when chicks were pair-fed, supplementation with vitamin B_{12} did not alter body composition. The data show that chicks fed the vitamin B_{12}-deficient diets utilized both energy and protein as efficiently as chicks fed the vitamin B_{12}-supplemented diets. Thus, it can be concluded that vitamin B_{12} stimulated growth under ad libitum feeding by increasing appetite rather than by improving protein utilization or caloric efficiency. Previously, Chow and Barrows (1950) observed that vitamin B_{12} stimulated growth of rats, only when fed ad libitum. They found that vitamin B_{12} failed to stimulate growth when feed intake was restricted to 6-8 grams per day.

The reason that appetite is depressed when chicks are fed vitamin B_{12}-deficient diets is unknown. Blaxter (1964) generalizes that, in specific dietary deficiencies, animals reduce the load on the enzyme system affected by the deficiency. He states that the primary method that animals employ is reducing food intake but the enzyme system affected by the deficiency also may be avoided by using alternative metabolic pathways of dissimilation and

Table 4

Protein gained, fat gained, protein retained and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without vitamin B_{12}

Treatment		Protein gained		Fat gained		Protein retained[1]		Caloric efficiency[2]	
		Carbohydrate		Carbohydrate		Carbohydrate		Carbohydrate	
Supplemental vitamin B_{12}	Feeding regimen	Containing	"Free"	Containing	"Free"	Containing	"Free"	Containing	"Free"
$\mu g/kg$		g	g	g	g	%	%		
0	ad libitum	52.7^{3}	50.5	25.3	29.1	36.7	36.6	4.45	4.02
		53.6	51.8	25.4	33.4	41.6	39.6	4.24	3.45
		$\underline{53.2}^{a}$	$\underline{51.2}^{a}$	$\underline{25.4}^{a}$	$\underline{31.2}^{b,c}$	$\underline{39.2}^{a}$	$\underline{38.1}^{a}$	$\underline{4.34}^{b}$	$\underline{3.74}^{a}$
100	pair-fed	53.8	54.0	28.5	34.5	38.3	40.3	4.12	3.56
		55.9	53.8	25.4	34.8	43.3	40.9	4.10	3.30
		$\underline{54.8}^{a}$	$\underline{53.9}^{a}$	$\underline{27.0}^{a,b}$	$\underline{34.6}^{c}$	$\underline{40.9}^{a}$	$\underline{40.6}^{a}$	$\underline{4.11}^{b}$	$\underline{3.43}^{a}$

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[1] (Gain in carcass protein, g/protein consumed, g) x 100.

[2] Calories of metabolizable energy consumed/calories gained.

[3] Values are averages of duplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscript are significantly different ($P < 0.05$).

synthesis, some of which are not as efficient in the transfer of energy. Results of the experiments reported herein showed that chicks fed vitamin B_{12}-deficient diets reduced feed intake. If the chicks used alternative metabolic pathways, then these pathways must have been as efficient since no significant difference in caloric efficiency was observed on supplementation with vitamin B_{12} when feed intakes were equalized.

The data summarized in Table 4 also show that irrespective of whether the diet was adequate or deficient in vitamin B_{12} the substitution of hydrogenated fat isocalorically for carbohydrate increased the amount of energy deposited as fat and improved efficiency of the utilization of energy. These results are in agreement with those reported by Forbes _et al._ (1946) and Carew and Hill (1958) which showed that rats and chicks, respectively, utilized calories from fat more efficiently than calories from carbohydrate when fat was incorporated in the diet at a level of 20%. Recently, Baldwin (1970) calculated the theoretical efficiency of utilization of energy (100 x net energy/metabolizable energy) from glucose and triglyceride for fattening to be 89 and 96-97%, respectively. Blaxter (1962) summarized data on the efficiencies of utilization of metabolizable energy provided above maintenance for fattening in adult animals. Values for glucose were about 70% and for fat ranged from 80-86%. Although Baldwin's calculated efficiencies are higher than determined efficiencies, they do show that dietary fat can be

converted to body fat more efficiently than can dietary carbohydrate. Results of this experiment show that chicks like other animals utilize dietary fat more efficiently than dietary carbohydrate for fattening.

Relative liver weights and levels of liver fat in chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without supplemental vitamin B_{12} are summarized in Table 5. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that liver weight in proportion to body weight increased ($P < 0.05$) when glucose was replaced isocalorically by hydrogenated fat in the diet of vitamin B_{12}-deficient chicks fed ad libitum. When, however, the hydrogenated fat diet was supplemented with vitamin B_{12} and pair-fed, relative liver size decreased significantly ($P < 0.05$). Since level of liver fat was not altered by either source of energy or level of dietary vitamin B_{12}, it can be concluded that the increased liver size observed in vitamin B_{12}-deficient chicks when non-protein energy was supplied by fat was not due to the accumulation of fat. Previously, Moore and Doran (1962) observed that vitamin B_{12} deficiency did not affect level of total lipid in the liver of chick embryos, although lipid composition was altered. They observed increased triglyceride and decreased sterol esters in liver lipid of vitamin B_{12}-deficient chick embryos.

That vitamin B_{12} deficiency also results in an increase in relative liver size in rats was observed by

Table 5

Liver weight and level of liver fat in chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without supplemental vitamin B_{12}

Treatment			Exp. no.	Liver weight		Liver fat
Source of energy	Supplemental vitamin B_{12}	Feeding regimen				
				g/100 g body wt		% wet weight
Glucose	0	ad libitum	4	4.52^1		3.68^a
			5	4.98		-
					4.75^a	
	100	pair-fed	4	4.68		4.20^a
			5	4.84		-
					4.75^a	
Hydrogenated fat	0	ad libitum	4	5.08		4.22^a
			5	5.45		-
					5.26^b	
	100	pair-fed	4	4.40		4.63^a
			5	4.56		-
					4.48^a	

[1] Values are averages of duplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscript are significantly different ($P < 0.05$).

Hegsted _et al._ (1967) and György _et al._ (1967). In contrast to the chick, however, György _et al._ (1967) observed a marked accumulation of fat in the liver of rats fed diets high in fat and low in methionine, choline and vitamin B_{12}. The administration of vitamin B_{12}, choline or methionine alleviated the fatty infiltration of the liver.

Experiment 6

Results of experiments 1, 2 and 3 showed that the isocaloric substitution of hydrogenated fat for carbohydrate in the diet increased the requirement for vitamin B_{12}. Since vitamin B_{12} has recently been implicated in acetate metabolism in sheep (Somers, 1969) and humans (Cox _et al._, 1968), the question arose as to whether the increased requirement of chicks for vitamin B_{12} when hydrogenated fat was substituted isocalorically for glucose in the diet might be due to the fact that more acetate is produced in the oxidation of fat than in the oxidation of an equicaloric amount of carbohydrate. If in vitamin B_{12} deficiency oxidation of acetate is reduced then acetate, acetoacetate and/or β-hydroxybutyrate may accumulate in the bloodstream and this accumulation should be greater when non-protein calories are supplied by hydrogenated fat than when non-protein calories are supplied by glucose. Thus, the object of the following experiment was to compare levels of blood ketone bodies in chicks fed diets with and without vitamin B_{12} in which non-protein energy was supplied by either glucose, hydrogenated fat (Crisco) or beef tallow. In addition, the

effect of vitamin B_{12} on liver size was studied.

Experimental

The composition of the carbohydrate-containing diet used in this experiment was similar to that shown in Table 1 except that level of supplemental methionine was reduced to 0.074%. "Carbohydrate-free" diets in which non-protein energy was provided by hydrogenated fat (Crisco) or beef tallow were formulated by substituting hydrogenated fat or beef tallow isocalorically for glucose using the values 3.64, 8.59 and 6.78 (Renner and Hill, 1958) kcal/g for the metabolizable energy of glucose, hydrogenated fat and beef tallow, respectively. Cellulose was added to improve the texture of the "carbohydrate-free" diets in an amount to maintain their caloric density equal to their carbohydrate-containing counterpart. When diets were supplemented with vitamin B_{12} a level of 100 μg/kg was used.

Each diet was fed to duplicate groups of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks to 29 days of age. The feed intakes of chicks receiving diets containing supplemental vitamin B_{12} were restricted to that consumed by chicks fed comparable diets without supplementary vitamin B_{12}. Chicks that were pair-fed received their daily allotment of feed once every 24 hours. The method of allotment, housing and weighing were similar to those of experiment 1.

At 4 weeks of age, samples of blood were taken from representative chicks for the determination of blood ketone

bodies. Blood samples were obtained from the jugular vein of chicks using 1.5 mg sodium oxalate per ml blood to prevent coagulation. Protein-free blood filtrates were prepared using the procedure of Erwin et al. (1961). The blood filtrates were then frozen and stored at -24° until analyzed. Total blood ketone bodies were determined using a modification of the method of Bakker and White (1960). This modification consisted of heating the tubes in an oil bath at 110-120° for 10 minutes and for a further 30 minutes in an autoclave at the same temperature after mixing thoroughly with the potassium dichromate.

After the blood samples were collected, the chicks were killed using chloroform. After allowing the carcasses to cool, the livers were removed and weighed.

Results and Discussion

Liver weights and levels of blood ketone bodies in the chicks fed the diets with and without supplemental vitamin B_{12} in which non-protein calories were supplied by glucose, hydrogenated fat and tallow are summarized in Table 6. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on liver weight showed that liver weight was not affected significantly by source of energy or by level of vitamin B_{12}. The finding that in this experiment the isocaloric substitution of hydrogenated fat for glucose in the vitamin B_{12}-deficient diet did not increase relative liver size is contrary to the results of preceding experiments.

Table 6

Liver weight and blood ketone bodies in chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without vitamin B_{12}

Treatment			Liver weight	Blood ketone bodies[1]
Source of energy	Supplemental vitamin B_{12}	Feeding regimen		
	µg/kg		g/100 g body wt	mg/100 ml
Glucose	0	ad libitum	$3.34^{2,a}$	$4.7^{2,a}$
	100	pair-fed	3.54^{a}	4.7^{a}
Hydrogenated fat	0	ad libitum	3.48^{a}	$11.5^{b,c}$
	100	pair-fed	3.30^{a}	10.4^{b}
Beef tallow	0	ad libitum	3.58^{a}	$13.2^{b,c}$
	100	pair-fed	3.20^{a}	14.3^{c}

[1] Total ketone bodies as acetone.

[2] Values are averages of duplicate groups each containing 5 representative chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

Since in all three experiments (Exp. 4, 5 and 6) the substitution of hydrogenated fat for glucose did not alter feed consumption, no explanation can be suggested to account for the discrepancy.

Similar statistical treatment of the data on blood ketone bodies showed that addition of vitamin B_{12} to the diets did not alter blood level of ketone bodies significantly ($P > 0.05$), irrespective of whether non-protein calories were supplied by glucose, hydrogenated fat or tallow. These results suggest that vitamin B_{12}-deficient chicks were able to oxidize acetyl CoA just as completely as chicks pair-fed the vitamin B_{12}-supplemented diet and are in contrast to results reported for sheep and humans by Somers (1969) and Cox et al. (1968), respectively.

In this experiment source of non-protein energy affected level of blood ketone bodies. The data show that the isocaloric substitution of either hydrogenated fat or beef tallow for glucose increased levels of blood ketone bodies significantly ($P < 0.05$). In the case of vitamin B_{12}-deficient chicks, hydrogenated fat was as ketogenic as tallow; however, hydrogenated fat was less ketogenic than tallow ($P < 0.05$) when diets adequate in vitamin B_{12} were fed. The effect of degree of saturation of dietary fat on level of ketone bodies in chicks fed vitamin B_{12}-adequate diets ad libitum is unknown. Nath and Nath (1967b) have observed higher levels of blood ketone bodies in rats fed the more saturated fats. Recently, Dupont and Mathias (1969) presented evidence supporting the concept that

oxidation of unsaturated fatty acids results not only in the formation of acetyl CoA but also some propionyl CoA. Since propionyl CoA is glucogenic, this could account for unsaturated fatty acids being less ketogenic than saturated fatty acids. Further studies are required to determine whether fats vary in their ketogenicity for chicks. If propionyl CoA is produced in the oxidation of unsaturated fatty acids in chicks, it could contribute to the increased requirement for vitamin B_{12} observed when high fat diets are fed, since vitamin B_{12} is involved in the conversion of propionyl CoA to succinyl CoA.

Summarized in Table 7 are data showing average weight gains, caloric consumption and caloric efficiencies of chicks fed diets with and without supplemental vitamin B_{12}. Analysis of variance (Steel and Torrie, 1960) of the factorial arrangement of treatments indicated that the growth response to both vitamin B_{12} and source of energy was significant, the level of significance being $P < 0.025$ and $P < 0.01$, respectively. Application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on growth showed that under pair-feeding conditions, when non-protein energy was supplied by glucose or tallow the addition of vitamin B_{12} to the diets did not increase growth significantly ($P > 0.05$); however, when non-protein energy was supplied by hydrogenated fat, chicks pair-fed the vitamin B_{12}-supplemented diet grew significantly faster than chicks fed the unsupplemented diet suggesting that vitamin B_{12} increased the utilization of some dietary ingredient. This finding is

Table 7

Weight gain, feed consumption and feed efficiency of chicks fed diets with and without supplemental vitamin B_{12} in which non-protein energy was supplied by either glucose, hydrogenated fat or tallow

Treatment					
Source of energy	Supplemental vitamin B_{12}	Feeding regimen	Caloric Consumption	Wt gain	Caloric Efficiency
	$\mu g/kg$		kcal	g	kcal/g gain
Glucose	0	ad libitum	$1506^{1,b}$	$172^{a,b}$	8.78^{a}
	100	pair-fed	1496^{b}	$192^{b,c}$	7.82^{a}
Hydrogenated fat	0	ad libitum	1486^{b}	204^{c}	7.28^{a}
	100	pair-fed	1511^{b}	236^{d}	6.39^{a}
Beef tallow	0	ad libitum	1086^{a}	154^{a}	7.20^{a}
	100	pair-fed	1162^{a}	$170^{a,b}$	6.85^{a}

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[1] Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

in contrast to results of preceding experiments (Exp. 4 and 5) which showed that when the feed intake of chicks fed the vitamin B_{12}-supplemented diet was restricted to that of chicks fed the vitamin B_{12}-deficient diet weight gains were equal. The levels of supplemental methionine used in this experiment (Exp. 6) and the preceding experiment (Exp. 4 and 5) were 0.074 and 0.1%, respectively. Whether this difference in level of supplemental methionine accounts for the difference in results obtained is unknown.

The data also show that when chicks were fed ad libitum a diet in which non-protein calories were supplied by hydrogenated fat, they grew faster ($P < 0.05$) than chicks fed diets in which non-protein energy was supplied by glucose without increasing feed consumption. This finding is similar to the overall effect observed when hydrogenated fat was substituted isocalorically for glucose in experiments 1, 2 and 3 and suggests that hydrogenated fat has a methionine-sparing action. Since the isocaloric substitution of beef tallow for glucose failed to stimulate growth it would appear that the growth promoting property of a fat is dependent on its fatty acid composition.

Similar statistical treatment of the data on caloric efficiency showed that caloric efficiency was not affected significantly ($P > 0.05$) by either source of energy or vitamin B_{12}.

PART II

METHIONINE REQUIREMENT OF CHICKS FED "CARBOHYDRATE-FREE" DIETS

Literature Review

Methionine, an indispensable amino acid, is required by the chick for protein synthesis, as a source of -SH groups for cysteine synthesis and as a biological methylating agent. Recently, the Subcommittee on Poultry Nutrition of the National Research Council (1966) indicated that the minimum methionine requirement of chicks consuming a diet containing 20% protein, 0.35% cystine and 2750 kcal metabolizable energy per kilogram was 0.40%.

That the requirement for methionine is dependent on the criterion used to estimate adequacy has been shown by several workers. Thus, Almquist (1952) showed that chicks required approximately 0.1% more methionine for maximum feed efficiency than was required for maximum growth. Subsequently, Nelson _et al._ (1960) confirmed this observation and in 1961 Carew and Hill reported experimental evidence providing an explanation for this observation. They found that chicks fed a diet which was marginally deficient in methionine grew at the same rate but consumed more feed and deposited more fat and less protein than chicks fed a methionine adequate diet. As a consequence, they found that feed efficiency decreased but observed no difference in efficiency of energy utilization based on determination of metabolizable energy, productive energy, heat production and tissue gains.

Dietary factors known to affect the chick's requirement for methionine include level of choline, vitamin B_{12} and folic acid. That these nutrients affect methionine requirement is to be expected since they, like methionine, are involved in the metabolism of single carbon units. Thus, Almquist in 1952 stated that the minimum requirement for methionine should be estimated only under conditions of ample vitamin B_{12}, so that the metabolic load on methionine is diminished as far as possible.

Other dietary factors known to affect methionine requirement are level of dietary energy and protein. With the realization that chicks eat to meet their energy requirement, it soon became apparent that as the caloric density of the diet is increased, protein level must also be increased if growth rate is to be maintained. Subsequent studies conducted by Baldini and Rosenberg (1955) have extended this concept and showed that the methionine requirement of the chick, expressed in percent of diet, increases as the productive energy level of the diet increases. More recently, Nelson _et al._ (1960) showed that although the requirement for sulfur-containing amino acids was affected by both protein and energy it was a constant of the protein required to balance the energy content of the diet.

Whether the methionine requirement is affected by source of non-protein energy is unknown. High fat diets might tend to increase methionine requirement if the need for bile salts is increased, since taurine, which is required for synthesis of taurocholic acid, is derived from

cysteine which in turn can be synthesized from methionine. Furthermore, if the need for one carbon units increases as fat intake increases the requirement for methionine might increase. On the other hand, other factors may tend to decrease the requirement for methionine by increasing its utilization. In this regard, Pearson and Panzer (1949) observed that dietary corn oil tends to lower the urinary excretion of methionine and valine in growing rats. More recently Nakano and Ashida (1970) have shown that rats, prefed a diet in which non-protein energy was supplied by fat, excreted less urinary nitrogen when changed to a high protein diet than did rats prefed a high carbohydrate diet. They found the decreased excretion of urinary nitrogen to be due to retardation of the formation of amino acid degrading enzymes. These results suggest that fat may have a protein sparing action.

Experiment 7

Results of experiments 1, 2 and 3 showed that the isocaloric substitution of fat for carbohydrate in the diet of the chick increased the requirement for vitamin B_{12}. Since vitamin B_{12} has a methionine sparing action, the question arose as to whether the observed increase was actually for vitamin B_{12} or whether it merely reflected an increased need for methionine. Thus, the following study was conducted to compare the methionine requirement of chicks fed diets in which non-protein energy was provided by either carbohydrate or fat.

Experimental

Carbohydrate-containing diets with and without supplemental vitamin B_{12} (100 μg/kg diet) and containing either 0, 0.1, 0.2 or 0.3% supplemental methionine were formulated from the carbohydrate-containing diet shown in Table 1, by either decreasing or increasing the level of supplemental methionine at the expense of glucose. Their "carbohydrate-free" counterparts were formulated by substituting hydrogenated fat (Crisco) isocalorically for glucose using the values 7.16 (Renner and Hill, 1958) and 3.64 kcal/g (Anderson et al., 1958) for the metabolizable energy content of hydrogenated fat and glucose, respectively. Cellulose was added to improve the texture of the "carbohydrate-free" diet in an amount to maintain the energy in a given volume equal to that of its high carbohydrate counterpart.

Each diet was fed to duplicate groups of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks to 29 days of age. The chicks were assigned at random at one day of age to each experimental group. They were maintained in electrically heated, thermostatically controlled battery brooders with raised wire-screen floors in a temperature controlled laboratory. Feed and water were supplied ad libitum. Data on growth and feed consumption were obtained weekly and feed wastage was determined daily. Fecal collections were made from selected groups on the twelfth, thirteenth and fourteenth day of life for determination of metabolizable energy. At 4 weeks of age, the

chicks were killed, the livers excised and weighed and the entire carcasses from each experimental group (minus the livers) were prepared for analysis as in experiments 4 and 5.

Results and Discussion

Summarized in Table 8 are data showing weight gains, caloric consumption and gross efficiency of feed utilization. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on growth showed that in the absence of vitamin B_{12}, the addition of 0.1, 0.2 and 0.3% methionine caused significant and progressive increases in rate of growth, irrespective of source of non-protein energy. In the presence of vitamin B_{12}, on the other hand, the addition of 0.1 and 0.2% methionine to carbohydrate-containing and "carbohydrate-free" diets caused significant and progressive increases in growth rate, with no additional growth response on the addition of 0.3% methionine. These results show the requirement of chicks for supplemental methionine when fed these carbohydrate-containing and "carbohydrate-free" diets to be at least 0.3% in the absence of vitamin B_{12} and from 0.1-0.2% in the presence of vitamin B_{12}. Assuming that the basal diet contained 0.63% sulfur-containing amino acids, these results show that the requirement for sulfur-containing amino acids of chicks fed a diet containing 24% protein was 0.73-0.83% when adequately supplemented with vitamin B_{12} and at least 0.93% in the absence of vitamin B_{12}. These results also show that vitamin B_{12} has a methionine-sparing action. Since source

Table 8

Effect of level of methionine and vitamin B_{12} on weight gain, caloric consumption and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets

Supplements		Wt gain		Caloric consumption		Caloric efficiency	
		Carbohydrate		Carbohydrate		Carbohydrate	
Vitamin B_{12}	Methionine	Containing	"Free"	Containing	"Free"	Containing	"Free"
µg/kg	%	g	g	kcal	kcal	kcal/g gain	
0	0	$68^{1,a}$	68^{a}	$628^{1,a}$	594^{a}	$9.19^{1,a}$	8.77^{a}
0	0.1	265^{d}	212^{c}	1748^{d}	1283^{c}	6.54^{c}	$6.20^{c,d}$
0	0.2	$354^{e,f,g}$	$358^{e,f,g}$	$1918^{d,e}$	$1922^{d,e}$	5.40^{e}	5.36^{e}
0	0.3	$398^{h,i}$	$419^{h,i,j}$	$2066^{e,f}$	2157^{f}	5.20^{e}	5.14^{e}
100	0	$94^{a,b}$	114^{b}	$750^{a,b}$	864^{b}	7.95^{b}	7.56^{b}
100	0.1	328^{e}	$341^{e,f}$	1796^{d}	$1850^{d,e}$	5.46^{e}	$5.67^{d,e}$
100	0.2	$375^{f,g}$	448^{j}	$1938^{d,e}$	2250^{f}	5.23^{e}	5.08^{e}
100	0.3	$391^{g,h,i}$	$425^{i,j}$	$2030^{e,f}$	2182^{f}	5.19^{e}	5.11^{e}

[1] Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

of non-protein energy did not affect methionine requirement, it can be concluded that the increased vitamin B_{12} requirement of chicks fed "carbohydrate-free" diets which was observed in experiments 1, 2 and 3 was not due to a methionine-sparing action.

Using caloric efficiency as the criterion to assess requirement, the data in Table 8 show that the amount of methionine required to produce optimal caloric efficiency is reduced by the addition of vitamin B_{12} to the diet but is unaffected by the source of non-protein energy. The results indicate that the requirement of chicks for supplemental methionine was not greater than 0.1% and 0.2%, respectively, when diets with and without supplemental vitamin B_{12} were fed. Comparison of these results with results obtained using growth as the criterion indicates that the amount of methionine required to promote optimal gain is greater than the amount required for optimal feed efficiency. This finding is in contrast to the results of Almquist (1952) and Nelson _et al._ (1960) who found that chicks require more methionine for maximum feed efficiency than for maximum growth.

Data on carcass composition summarized in Table 9 show that in this experiment chicks fed diets either severely or moderately deficient in methionine did not increase their fat deposition. This is in contrast to results reported by Carew and Hill (1961) and suggests that in this experiment chicks did not overeat to meet their methionine requirement. This experiment, however, differs from those of Carew and Hill (1961) in that the basal diet used was severely

Table 9

Carcass composition, caloric efficiency and protein retention of chicks fed carbohydrate-containing and "carbohydrate-free" diets with graded levels of methionine, with and without vitamin B_{12}

Supplements		Carcass fat		Carcass protein		Protein retained[1]		Caloric efficiency[2]	
		Carbohydrate		Carbohydrate		Carbohydrate		Carbohydrate	
Vitamin B_{12}	Methionine	Containing	"Free"	Containing	"Free"	Containing	"Free"	Containing	"Free"
μg/kg		% D.M.	% D.M.	% D.M.	% D.M.	%	%		
0	0	$19.7^{3,a}$	22.6^{b}	65.1^{h}	62.0^{g}	53.1^{a}	57.5^{a}	4.10^{a}	3.60^{b}
0	0.1	$35.0^{d,e}$	$36.2^{d,e}$	$55.4^{e,f}$	$53.3^{c,d,e}$	58.3^{a}	$66.0^{b,c}$	2.42^{d}	$2.17^{d,e}$
0	0.2	34.6^{d}	$42.2^{h,i}$	$54.6^{d,e,f}$	$48.8^{a,b}$	65.1^{b}	$66.9^{b,c}$	$2.18^{d,e}$	$1.88^{e,f}$
0	0.3	$35.9^{d,e}$	$40.8^{g,h}$	$53.9^{d,e,f}$	49.8^{b}	$67.5^{b,c}$	$74.4^{d,e}$	$1.98^{e,f}$	1.78^{f}
100	0	24.8^{b}	30.3^{c}	61.0^{g}	56.5^{f}	53.7^{a}	54.8^{a}	3.52^{b}	3.06^{c}
100	0.1	$37.5^{e,f}$	$42.4^{h,i}$	$52.7^{c,d}$	$49.2^{a,b}$	64.5^{b}	$67.4^{b,c}$	$2.04^{e,f}$	$1.86^{e,f}$
100	0.2	$36.1^{d,e}$	44.4^{i}	$54.9^{d,e,f}$	47.2^{a}	$71.7^{c,d}$	$69.0^{b,c,d}$	$1.94^{e,f}$	1.73^{f}
100	0.3	$35.9^{d,e}$	$39.2^{f,g}$	$53.8^{d,e}$	$51.1^{b,c}$	$69.3^{b,c,d}$	75.7^{e}	$2.02^{e,f}$	$1.91^{e,f}$

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[1] (Gain in carcass protein, g/protein consumed, g) x 100.

[2] Calories of metabolizable energy consumed/calorie gained.

[3] Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

deficient in methionine but was supplemented with cystine while the basal diet used by Carew and Hill (1961) was only moderately deficient in sulfur-containing amino acids and contained no added cystine. Whether these differences contributed to the differences in results obtained is unknown.

Also summarized in Table 9 are data showing the amount of protein retained by chicks fed carbohydrate-containing and "carbohydrate-free" diets containing varying levels of methionine with and without vitamin B_{12}. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that, in both the absence and presence of vitamin B_{12}, 0.2% supplemental methionine resulted in maximum nitrogen retention when chicks were fed carbohydrate-containing diets whereas 0.3% supplemental methionine caused a further increase in nitrogen retention when chicks were fed "carbohydrate-free" diets. These results suggest that the isocaloric substitution of fat for carbohydrate increases the methionine requirement for maximum protein retention; however, no firm conclusion can be reached since the diets were not isocaloric. The carbohydrate-containing and "carbohydrate-free" diets contained 15.2 and 18.7 kcal metabolizable energy/g protein, respectively. This difference was due to the hydrogenated fat having a metabolizable energy value of 8.59 rather than 7.16 kcal/g (Renner and Hill, 1958). Thus, whether the increased requirement for methionine for maximum nitrogen retention was due to the isocaloric substitution of fat for carbohydrate or to changes in calorie:protein ratio is unknown.

Experiment 8

Results of experiment 7 showed that the amount of methionine required to promote optimal growth of chicks was unaffected by source of non-protein energy. Since the calorie:protein ratio of the carbohydrate-containing and "carbohydrate-free" diets differed, the question arose as to whether comparable results would be obtained when calorie: protein ratios were similar. Thus, the following study was conducted to compare the methionine requirement of chicks fed carbohydrate-containing and "carbohydrate-free" diets.

Experimental

Carbohydrate-containing diets containing 0, 0.075, 0.150, 0.225 and 0.300% methionine were formulated from the basal diet shown in Table 10 by substituting methionine, weight for weight, for glucose. Their "carbohydrate-free" counterparts were formulated by substituting hydrogenated fat (Crisco), isocalorically, for glucose using the value 3.64 and 8.59 kcal/g for the metabolizable energy content of glucose and hydrogenated fat, respectively. The diets were formulated to contain 18.6 kcal metabolizable energy/g protein. This level is similar to the calorie:protein ratio of the "carbohydrate-free" diet used in experiment 7. The diets were not fortified with vitamin B_{12}.

Each diet was fed to duplicate lots of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks to 29 days of age. The method of allotment, feeding and housing were the same as in experiment 1. Fecal collections

Table 10

Composition of carbohydrate-containing basal diet

Ingredients	%
Soybean protein[1]	20.28
L-cystine	0.25
Soybean oil	4.00
Limestone	1.49
Dicalcium phosphate	2.15
Salt	0.60
Mineral mixture[2]	1.26
Vitamin mixture[3]	0.43
Antioxidant[4]	0.025
Chromium oxide	0.30
Glucose[5]	69.21

[1] See footnote 4, Table 1.

[2] See footnote 6, Table 1.

[3] See footnote 7, Table 1.

[4] Ethoxyquin.

[5] Cerelose.

were made at 24 hour intervals at 12, 13, 14 and 15 days of age and again at 26, 27 and 28 days of age for determination of metabolizable energy and fat absorbability. Data on growth and feed consumption were obtained weekly. Feed wastage was determined daily.

Results and Discussion

The results obtained for weight gain, feed consumption and gross efficiency of feed utilization are presented in Table 11. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the growth data showed that the addition of increasing levels of methionine to both the carbohydrate-containing and "carbohydrate-free" diets caused progressive and significant increases in growth up to a level of 0.225% supplemental methionine. Plots of weight gains versus levels of dietary methionine expressed as $\log_{10}$ (0.240+% added methionine) and calculation of the two lines which best fit these points using regression analysis showed that irrespective of source of non-protein energy these lines intersect at 0.20% supplemental methionine. These results confirmed the results of the preceding experiment, showing that the methionine requirement of chicks fed carbohydrate-containing and "carbohydrate-free" diets is similar when growth is used as the criterion.

Analysis of variance (Steel and Torrie, 1960) also showed that source of non-protein energy affected growth significantly ($P < 0.01$). The data show that chicks fed diets in which non-protein energy was supplied by fat grew significantly faster than chicks fed diets in which non-protein energy was supplied by carbohydrate even though caloric consumption was not increased. The combined results of experiments 1, 2 and 3 also showed that chicks grew significantly faster when non-protein energy was

Table 11

Effect of level of methionine on weight gain, caloric consumption and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets without vitamin B_{12}

Supplemental methionine	Wt gain		Caloric consumption		Caloric efficiency[1]	
	Carbohydrate		Carbohydrate		Carbohydrate	
	Containing	"Free"	Containing	"Free"	Containing	"Free"
%	g	g	kcal	kcal	kcal/g gain	
0	$74^{2,a}$	108^{b}	956^{a}	1008^{a}	13.00^{a}	9.37^{b}
0.075	240^{c}	253^{c}	2029^{c}	1829^{b}	$8.13^{b,c,d}$	$7.22^{c,d,e,f}$
0.150	292^{d}	360^{f}	$2389^{d,e}$	2352^{d}	$8.20^{b,c}$	$6.54^{d,e,f}$
0.225	318^{e}	394^{g}	2346^{d}	$2482^{d,e}$	$7.36^{c,d,e,f}$	$6.30^{e,f}$
0.300	329^{e}	396^{g}	2564^{e}	$2442^{d,e}$	$7.78^{c,d,e}$	6.18^{f}

[1] Calories of metabolizable energy consumed/g gain.

[2] Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

supplied by fat, however, no effect was observed in experiment 7. The failure of fat to promote more rapid growth in experiment 7 may have been due to the difference in the calorie:protein ratio between carbohydrate-containing and "carbohydrate-free" diets. The possibility exists that in experiment 7 chicks fed the "carbohydrate-free" diet containing 18.7 kcal/g protein were able to grow at the same rate as chicks fed the carbohydrate-containing diet having a calorie:protein ratio of 15.2 kcal/g protein because of the protein-sparing action of fat.

Statistical treatment of the data on caloric efficiency showed that supplementation with 0.075% methionine caused a significant increase in caloric efficiency in both carbohydrate-containing and "carbohydrate-free" diets with no further significant increase being observed on the addition of higher levels of methionine. These results show that, as in experiment 7, when caloric efficiency was used as the criterion the requirement of chicks for methionine was unaffected by source of non-protein energy.

PART III

ABSORBABILITY OF FAT BY CHICKS FED "CARBOHYDRATE-FREE" DIETS

Literature Review

Information on the major steps involved in the mammalian absorption of fat has recently been summarized diagrammatically by Isselbacher (1965) (Figure 1).

The diagram shows that both intraluminal and intramucosal processes are involved in fat absorption. The major intraluminal factors affecting fat absorption have been shown to include pancreatic lipase and bile salts. Studies have shown that deficiency of either in rats or dogs causes a marked reduction in absorption of triglycerides. Recent studies have shown that bile salts promote fat absorption by facilitating the formation of mixed micelles (Hofmann and Borgstrom, 1962), by facilitating the action of pancreatic lipase (Borgstrom, 1954) and by facilitating the reesterification of fatty acids in the intestinal mucosa (Kroebel and Ryan, 1963; Saunders and Dawson, 1963).

Intramucosal factors affecting fat absorption in mammals have been shown to include cell structure, enzyme levels and the presence of the required protein for chylomicron formation. The importance of normal cell structure in fat absorption is illustrated in studies conducted by Dawson and Isselbacher (1960) and Brice *et al.* (1965). They showed that patients with Whipples' disease or nontropical sprue whose mucosal cells were deranged

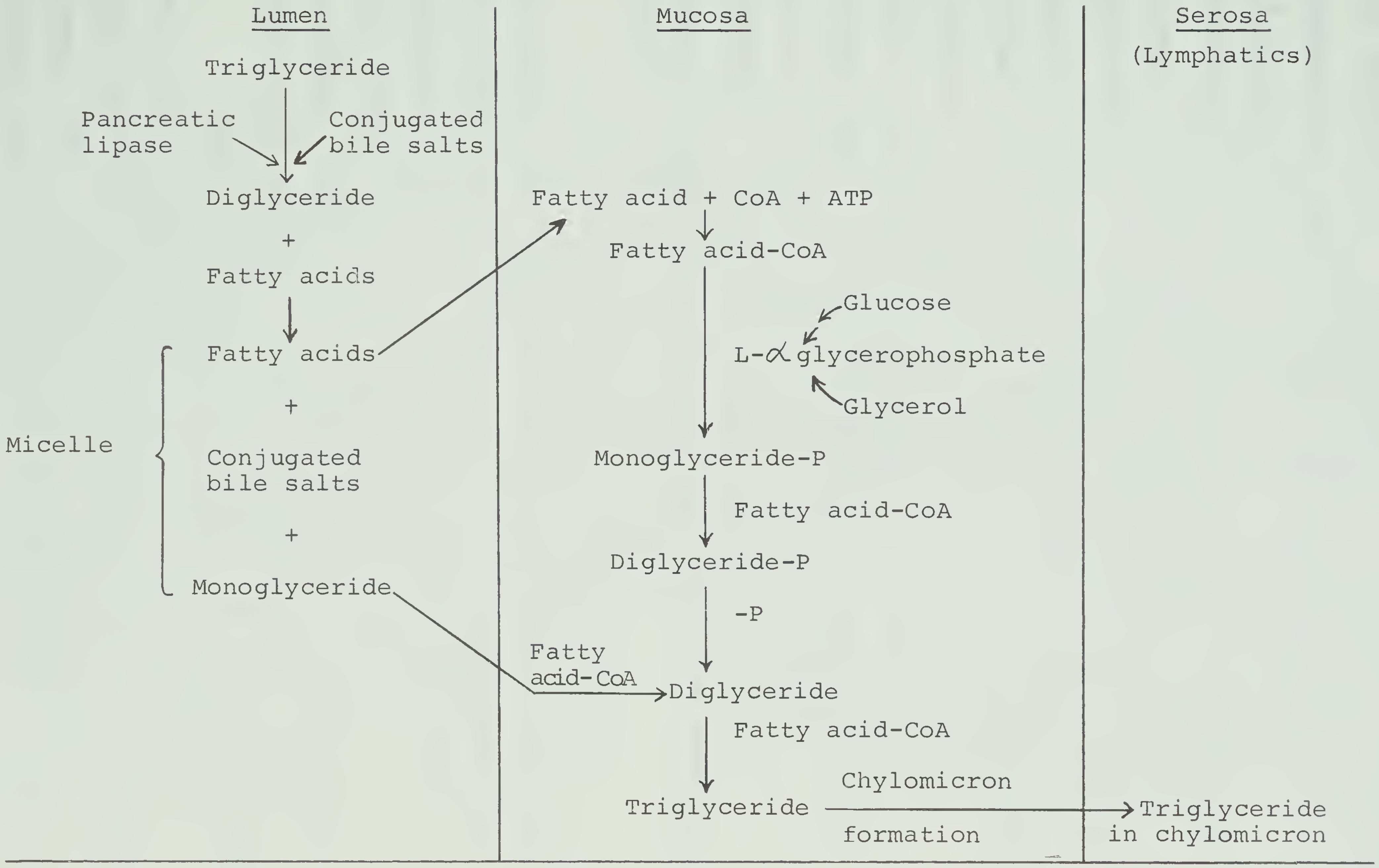

Figure 1. Major steps in the absorption of long-chain fatty acids by intestinal mucosa.

showed reduced rates of esterification of ^{14}C-palmitate into triglyceride. The importance of levels of intramucosal enzymes on fat absorption was shown by Isselbacher (1967) and Watson and Murray (1966). They showed that the decreased ability of adrenalectomized rats to absorb fat is due to decreased levels of enzymes required for the esterification of fatty acids and monoglyceride. Lack of protein for chylomicron formation whether caused by treatment of the rat with puromycin, acetoxycycloheximide (Isselbacher and Budz, 1963; Sabesin and Isselbacher, 1965) or ethionine (Hymans _et al._, 1966) resulted in reduced levels of plasma triglycerides, accumulation of neutral fat within mucosal cells and decreased fat absorption. In addition humans suffering from the genetic disorder abetalipoproteinemia also show decreased ability to absorb fat (Isselbacher, 1967).

In the case of chicks, the mechanism of fat absorption and intramucosal factors affecting it have not been studied as extensively as in rats. Information has accumulated using the chick as the experimental subject which favors a partial hydrolysis of fat prior to absorption (Renner, 1960; Renner and Hill, 1961a; Young, 1961; Renner and Hill, 1961b; Young and Garrett, 1963).

Studies have also shown that in the chick as in the rat, lack of bile markedly decreases the absorption of oleic acid and linoleic acid when fed in triglyceride form (Young, 1965). Hydrolysis of bile salts by enteric bacteria has been suggested by Donaldson (1967) as the

reason why conventional animals absorb less fat than "germ-free" animals. He proposed that hydrolysis of bile salts reduces the stability of the mixed micelles resulting in decreased fat absorption.

The role which pancreatic lipase plays in facilitating fat absorption in the chicken was demonstrated by Young (1965). Using pullets having ligated pancreatic ducts he found that the absorbability of triolein and oleic acid was 10 and 75%, respectively, when incorporated at levels of 10 and 20% in diets in which amino acids were furnished by enzymatically hydrolyzed casein and energy by glucose.

Information on the effect of intramucosal factors on the absorption of dietary fat in the chicken is limited. It is known that both the glyceride-3-phosphate and monoglyceride pathway of reesterification are operative in the chicken (Bickerstaffe and Annison, 1969). Studies have also shown that fatty acids pass from the mucosal cell into the circulatory system as triglycerides of very low density lipoprotein, however, chickens differ from mammals in that chylomicrons are absorbed directly into the portal rather than lymphatic system (Noyan et al., 1964).

The effects which dietary deficiencies have on fat digestion and absorption have not been studied extensively in either rats or chicks. Studies have shown that the absorbability of soybean fatty acids by chicks fed "carbohydrate- and glycerol-free" diets is high indicating that carbohydrate deficiency has little or no effect on fat absorption (Renner and Elcombe, 1964 and Brambila and Hill, 1966). Further

studies (Renner, unpublished) have shown that increasing the protein content of a high fat diet from 16% to 27% increased the absorbability of lard slightly but significantly ($P < 0.05$) during the first 4 weeks of life. Sibbald et al. (1961) observed that increasing the protein content of the diet from 24 to 34% significantly increased the metabolizable energy of fat. The effect appeared to be greater for tallow than for soybean oil but the interaction of protein and type of fat was not significant. Young et al. (1963) also observed that the absorbability of lard fatty acids was increased when the protein content of the diet was increased from 24 to 30%. In the case of the rat Carroll and Richards (1958) and Barnes et al., (1944) have reported that rats fed low protein diets absorb less fat than do rats fed high protein diets. They observed that protein level of the diet affected the absorbability of fats of low absorbability more than fats of high absorbability. The effect which methionine deficiency has on fat absorbability is unknown. Lyman and Wilcox (1963) have shown that omission of methionine from the diet of force-fed rats resulted in decreased activity of pancreatic lipase and protease but not amylase. This finding suggests that fat absorbability may decrease when methionine-deficient diets are fed.

In the case of the vitamins, Tidwell (1950) showed that choline given either orally or intraperitoneally enhanced the absorption of olive oil. In 1959 Fox et al. suggested that vitamin B_{12} and/or methionine may play a role in fat absorption. This suggestion arose as a result

of the observation that the addition of vitamin B_{12} to a diet high in fat but low in methionine and vitamin B_{12} caused a marked increase in caloric efficiency.

In order to study the effect of deficiencies of vitamin B_{12} and methionine on fat absorbability, samples of diets and excreta collected in experiment 7 were analyzed for fat and energy. Subsequently additional studies were conducted in an attempt to explain why methionine deficiency decreased fat absorbability.

Experiment 9

The object of this experiment was to determine the effect of deficiencies of vitamin B_{12} and/or methionine on the absorbability of fat.

Experimental

During the course of experiment 7, excreta were collected from chicks fed the carbohydrate-containing and "carbohydrate-free" diets with and without supplemental vitamin B_{12} and containing either 0 or 0.3% supplemental methionine. The excreta were collected at 24 hour intervals on 4 consecutive days during the second week of life and were maintained in the frozen state until processed. Chromium oxide was incorporated in all rations at a level of approximately 0.3% as an index substance in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. Two methods were used for determining fat utilization: (1) metabolizable energy determined by bomb calorimetry; and (2) absorbability

determined by analysis of diets and excreta for fat. The methods of processing excreta, conducting chemical analysis for moisture, nitrogen, combustible energy, fat and chromium oxide and computing metabolizable energy and fat absorbability from these data have been described previously (Hill and Anderson, 1958; Hill *et al.*, 1960 and Renner and Hill, 1960).

Results and Discussion

Summarized in Table 12 are data showing the effect of deficiencies of methionine and/or vitamin B_{12} on the metabolizable energy value of carbohydrate-containing and "carbohydrate-free" diets. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that the metabolizable energy value of the carbohydrate-containing diets was not affected significantly ($P > 0.05$) by dietary level of either methionine or vitamin B_{12}. Their metabolizable energy content varied from 3.57 to 3.69 kcal/g of diet. The finding that the metabolizable energy content of the carbohydrate-containing diets was not affected by the deficiency of methionine is at variance with the results of Baldini (1961) who claimed that a methionine-deficient carbohydrate diet contained more metabolizable energy than did the same diet supplemented with methionine. These findings are, however, in agreement with those of Carew and Hill (1961) and Shoji *et al.* (1966) who showed that metabolizable energy was not affected by either a marginal or severe methionine deficiency, respectively,

Table 12

Effect of deficiencies of methionine and/or vitamin B_{12} on the metabolizable energy value of carbohydrate-containing and "carbohydrate-free" diets

Supplements		Kcal metabolizable energy/g diet			
Methionine	Vitamin B_{12}	Carbohydrate-containing		"Carbohydrate-free"	
%	μg/kg				
0	0	3.596		5.148	
		3.542		5.002	
			$3.569^{1,a}$		5.075^{b}
0	100	3.655		5.164	
		3.717		5.034	
			3.686^{a}		5.099^{b}
0.3	0	3.635		5.382	
		3.628		5.387	
			3.632^{a}		5.384^{c}
0.3	100	3.675		5.523	
		3.647		5.611	
			3.661^{a}		5.567^{d}

[1] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

when carbohydrate-containing diets were fed.

In contrast, statistical analysis showed that the metabolizable energy content of methionine-deficient "carbohydrate-free" diets was increased when methionine was added irrespective of whether or not the diets were supplemented with vitamin B_{12}. The data also show that vitamin B_{12} increased the metabolizable energy content of the methionine supplemented "carbohydrate-free" diet but did not increase the metabolizable energy content of the methionine-deficient "carbohydrate-free" diet.

Data showing the utilization of fat by chicks fed "carbohydrate-free" diets as determined from metabolizable energy and absorbability are summarized in Table 13. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that the addition of vitamin B_{12} to either the methionine-deficient or methionine-supplemented diets had no effect on utilization of hydrogenated fat as determined from its metabolizable energy or absorbability. The data show, however, that utilization of hydrogenated fat is affected by level of dietary methionine. Methionine deficiency reduced absorbability of hydrogenated fat from 94.3 to 86.9% and utilization (ME/GE x 100) from 93.2 to 83.9%.

The reason why chicks fed methionine-deficient diets absorb less fat is unknown. Several reasons for the decreased fat absorbability can be proposed. First, it might be due to impaired transport of fat from the mucosa due to lack of protein. This is suggested by the finding

Table 13

Effect of deficiencies of methionine and/or vitamin B_{12}
on the utilization of hydrogenated fat
by chicks fed "carbohydrate-free" diets

Supplements		Utilization[1]		Absorbability	
Methionine	Vitamin B_{12}				
%	μg/kg	%		%	
0	0	87.3		88.4	
		83.7		86.1	
			$\underline{85.5}^{2,a}$		$\underline{87.2}^{a}$
0	100	83.9		88.0	
		80.3		85.2	
			$\underline{82.3}^{a}$		$\underline{86.6}^{a}$
0.3	0	91.4		95.2	
		91.5		94.9	
			$\underline{91.4}^{b}$		$\underline{95.0}^{b}$
0.3	100	93.8		92.9	
		96.0		94.3	
			$\underline{94.9}^{b}$		$\underline{93.6}^{b}$

---

[1] Metabolizable energy as a percentage of gross energy.

[2] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

that incorporation of ethionine (a methionine antagonist) in the diet of the female rat inhibits protein synthesis and interferes with the intestinal transport of long chain fatty acids (Hymans et al., 1966). Secondly, it might be due to impaired chylomicron formation due to lack of choline for phospholipid synthesis. This is suggested by the finding of March and Biely (1956b) that the choline requirement of chicks is increased by the addition of 6-12% tallow or herring oil to a corn-soybean meal diet. Thirdly, it might be due to impaired digestion caused by decreased secretion of pancreatic lipase. This is suggested by the finding of Lyman and Wilcox (1963) that omission of methionine from the diet of force-fed rats depressed activity of pancreatic lipase. Finally, the possibility also exists that synthesis of triglyceride in the intestinal mucosa due to lack of coenzyme A or glutathione may impede fat transport and reduce fat absorption. This is suggested by the finding of Clark and Hübscher (1960) that coenzyme A and reduced glutathione are required for resynthesis of triglyceride in the intestinal mucosa and that methionine deficiency in rats results in reduced levels of hepatic coenzyme A (Kataoka, 1966) and reduced glutathione (Kano et al., 1968), respectively. Whether one or more of these factors contributes to the decreased ability of methionine-deficient chicks to absorb fat forms the basis of the following experiments.

Experiment 10

The object of this experiment was to determine whether the decreased absorption of fat observed when chicks were fed a methionine-deficient diet could be alleviated by increasing the choline content of the diet.

Experimental

Diets containing 0.150, 0.225 and 0.300% supplemental choline chloride and 2 levels of supplemental methionine (0 and 0.3%) were formulated from the carbohydrate-containing basal diet (Table 1) by adjusting the levels of methionine and choline. All adjustments were made at the expense of glucose. All diets were supplemented with 100 μg vitamin B_{12} per kilogram diet. "Carbohydrate-free" diets were formulated from their carbohydrate-containing counterparts by replacing glucose isocalorically by hydrogenated fat (Crisco). Cellulose was added to improve the texture of the "carbohydrate-free" diets in an amount to maintain the energy in a given volume equal to that of their high carbohydrate counterparts.

Each diet was fed to duplicate lots of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks to 29 days of age. The methods of allotment, feeding and housing were the same as in experiment 1. The chicks were weighed at weekly intervals, feed consumption was determined weekly and feed wastage was determined daily.

Excreta were collected at 24 hour intervals at 12, 13, 14 and 15 days of age and again at 26, 27 and 28 days

of age. Each 3 or 4 day collection was maintained in the frozen state until processed. Chromium oxide was incorporated in all rations as an index substance in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. The methods for processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy and fat and for computing metabolizable energy and fat absorbability from these data have been described previously (Hill and Anderson, 1958; Hill *et al.*, 1960 and Renner and Hill, 1960).

Results and Discussion

Summarized in Table 14 are data showing the utilization of fat by chicks fed "carbohydrate-free" diets containing graded levels of choline with and without supplemental methionine. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that increasing the level of dietary choline chloride from 0.15 to 0.30% did not increase the utilization of fat as determined by its metabolizable energy or absorbability at either 2 or 4 weeks of age. These results indicate that the decreased ability of chicks to absorb fat when fed a methionine-deficient diet is not due to lack of choline. The data also show that chicks fed the methionine-deficient diets absorbed significantly less fat than chicks fed the methionine-supplemented diet when either fat absorbability or metabolizable energy as a percentage gross energy was

Table 14

Effect of supplemental choline on the absorbability of dietary fat in chicks fed "carbohydrate-free" diets with and without supplemental methionine

Supplements		Absorbability		Utilization[1]	
Methionine	Choline chloride	2 wks	4 wks	2 wks	4 wks
%	%	%	%	%	%
0	0.150	89.6[2]	93.0	84.7	89.5
0	0.225	91.8	92.6	89.6	91.1
0	0.300	91.5	90.3	90.7	88.9
		$91.0^{3,a}$	$92.0^{a,b}$	88.4^{a}	$89.8^{a,b}$
0.3	0.150	95.5	96.4	95.2	96.1
0.3	0.225	95.4	95.4	92.9	94.0
0.3	0.300	93.1	96.2	87.4	91.5
		$94.3^{b,c}$	96.0^{c}	$91.8^{b,c}$	93.9^{c}

[1] Metabolizable energy as a percentage of gross energy.

[2] Values are averages of duplicate groups.

[3] Underlined values are averages for chicks receiving either 0 or 0.3% supplemental methionine irrespective of level of choline chloride. Values without a common letter in their superscript are significantly different ($P < 0.05$).

used as the criterion. These results confirm those obtained in experiment 7 although the differences are not as marked.

In the course of this experiment data were also collected on growth, energy consumption and caloric efficiency of chicks fed "carbohydrate-free" diets containing graded levels of choline chloride with and without supplemental methionine (Table 15). Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that increasing the level of supplemental choline in the diet to 0.300% did not alter growth, caloric intake or caloric efficiency of chicks irrespective of level of dietary methionine. These results indicate that 0.15% supplemental choline is sufficient to meet the choline requirement of chicks fed a diet high in fat and low in methionine. March and Biely (1956b) concluded that the choline requirement of chicks is increased when high levels of fat are added to a diet low in methionine. They found the requirement of chicks fed diets containing 12% tallow to be between 0.13-0.24%. The failure of chicks in the experiment reported herein to show an increased requirement for choline may be due to the fact that the calorie:choline ratio was maintained constant while in the experiment of March and Biely it was permitted to increase.

Table 15

Effect of supplemental choline on weight gain, caloric consumption and caloric efficiency of chicks fed "carbohydrate-free" diets with and without supplemental methionine

Supplemental choline chloride	Wt gain		Caloric consumption		Caloric efficiency	
	Supplemental methionine		Supplemental methionine		Supplemental methionine	
	0%	0.3%	0%	0.3%	0%	0.3%
%	g	g	kcal	kcal	kcal/ g gain	
0.150	$148^{1,a}$	509^{b}	1169^{a}	2912^{b}	7.92^{a}	5.72^{b}
0.225	138^{a}	538^{b}	1147^{a}	2995^{b}	8.28^{a}	5.56^{b}
0.300	170^{a}	503^{b}	1330^{a}	2790^{b}	7.82^{a}	5.54^{b}

[1] Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscript are significantly different ($P < 0.05$).

Experiment 11

Since Lyman and Wilcox (1963) found that omission of methionine from the diet of force-fed rats depressed the activity of pancreatic lipase, the question arose as to whether the decreased absorbability of fat observed when chicks were fed a methionine-deficient diet was due to decreased secretion of this enzyme. It was hypothesized that if the decreased ability of chicks to absorb fat is due to lack of pancreatic lipase then methionine deficiency should reduce the absorbability of an intact fat but not a hydrolyzed fat. Thus, comparisons were made of the absorbability of soybean oil and soybean fatty acids when chicks were fed a methionine-deficient diet. For comparative purposes the absorbability of soybean oil and soybean fatty acids was also determined when chicks were fed a diet adequate in methionine.

Experimental

Duplicate groups of 10 male day-old crossbred (Dominant White x White Plymouth Rock) chicks were fed diets containing two levels of added methionine (0 and 0.3%) in which non-protein energy was supplied by glucose, soybean oil or soybean fatty acids. All diets were supplemented with 100μg vitamin B_{12}/kg diet. The composition of the carbohydrate-containing diet containing no added methionine is given in Table 10. The diet containing 0.3% added methionine was formulated by adding methionine at the expense of glucose. "Carbohydrate-free"

diets were formulated from their carbohydrate-containing counterparts by replacing glucose isocalorically by soybean oil or soybean fatty acids plus 0.105 g glycerol/g soybean fatty acids using the values 3.64, 9.21 and 8.65 kcal/g for the metabolizable energy values of glucose, soybean oil and soybean fatty acids, respectively.

The diets were fed from 1-29 days of age. The methods of allotment, feeding and housing of chicks were the same as in experiment 1. The procedure used for determination of fat absorbability is described in experiment 9.

Results and Discussion

Summarized in Table 16 are data showing the extent to which chicks absorbed soybean oil and soybean fatty acids when fed diets with and without supplemental methionine. Analysis of variance (Steel and Torrie, 1960) showed that the interaction of fat x methionine x age was significant ($P < 0.01$). The data show that in this experiment level of dietary methionine had no effect on the absorbability of soybean oil at either 2 or 4 weeks of age but did affect absorbability of soybean fatty acids, decreasing absorbability at 2 weeks of age and increasing absorbability at 4 weeks of age. These results do not support the hypothesis that the decreased ability of chicks to absorb fat when fed methionine-deficient diets is due to lack of pancreatic lipase. The reason why level of dietary methionine did not affect absorbability of soybean oil is unknown. Others,

Table 16

Effect of methionine deficiency on the absorbability of soybean oil and soybean fatty acids by chicks fed "carbohydrate-free" diets

Level of methionine	Absorbability							
	Soybean oil				Soybean fatty acids			
	2 wks		4 wks		2 wks		4 wks	
%	%		%		%		%	
0	96.6 96.7	$96.6^{1,c}$	97.5 96.3	96.9^{c}	93.2 92.7	93.0^{b}	91.6 89.1	90.4^{a}
0.3	97.8 98.2	98.0^{c}	97.6 97.3	97.4^{c}	89.1 90.1	89.6^{a}	93.3 92.5	92.9^{b}

[1] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

however, have observed that in the case of protein deficiency the absorbability of fat of low absorbability is affected more than fats of high absorbability (Barnes et al., 1944).

Summarized in Table 17 are data showing weight gains, caloric consumption and caloric efficiencies of chicks fed diets with and without supplemental methionine in which non-protein energy was supplied by glucose, soybean oil and soybean fatty acids plus glycerol. The data show that the substitution of either soybean oil or soybean fatty acids for glucose in the methionine-deficient diet permitted chicks to increase caloric consumption by 50% and weight gain by 50% with no significant increase in caloric efficiency. In contrast, the isocaloric substitution of soybean oil for glucose in the methionine-supplemented diet

Table 17

Effect of source of energy on weight gain, caloric consumption and caloric efficiency of chicks fed diets with and without supplemental methionine

Source of non-protein energy	Weight gain				Caloric consumption				Caloric efficiency			
	Supplemental methionine				Supplemental methionine				Supplemental methionine			
	0%		0.3%		0%		0.3%		0%		0.3%	
	g		g		kcal		kcal		kcal/g gain			
Glucose	90		369		830		2222		9.21		6.02	
	93		383		811		2466		8.71		6.43	
		$\underline{92}^{1,a}$		$\underline{376}^{d}$		$\underline{820}^{a}$		$\underline{2344}^{d}$		$\underline{8.96}^{c}$		$\underline{6.22}^{b}$
Soybean oil	156		461		1325		2526		8.50		5.48	
	135		447		1126		2536		8.35		5.68	
		$\underline{145}^{b}$		$\underline{454}^{e}$		$\underline{1226}^{b}$		$\underline{2531}^{d}$		$\underline{8.42}^{c}$		$\underline{5.58}^{a}$
Soybean F A + 0.105 g glycerol/g F A	147		331		1253		1989		8.52		6.01	
	145		347		1257		2008		8.67		5.79	
		$\underline{146}^{b}$		$\underline{339}^{c}$		$\underline{1255}^{b}$		$\underline{1998}^{c}$		$\underline{8.60}^{c}$		$\underline{5.90}^{a,b}$

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[1] Underlined values are averages of duplicate groups; individual replicate values are in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

did not increase caloric consumption, significantly, but did increase weight gain ($P < 0.05$) and efficiency of utilization of energy ($P < 0.05$). The data suggest that soybean oil has a methionine-sparing action, an effect previously observed with hydrogenated fat (Crisco).

Experiment 12 and 13

This experiment was conducted to determine, whether the effect of methionine on fat absorption was specific for methionine or whether deficiency of another amino acid such as lysine would also decrease fat absorbability.

Experimental

Two experiments were conducted to determine the effect of lysine deficiency on absorbability of hydrogenated fat (Crisco) and beef tallow. The composition of the lysine-deficient carbohydrate-containing diet is shown in Table 18. A diet containing 0.8% supplemental L-lysine was formulated from this diet by substituting lysine, weight for weight, for starch. Diets containing either hydrogenated fat or beef tallow were formulated from these diets by substituting hydrogenated fat or tallow isocalorically for corn starch using the values 4.12, 8.59 and 5.78 kcal/g for the metabolizable energy content of starch, hydrogenated fat and beef tallow, respectively. Cellulose was added to improve the texture of the high fat diets. Because the high fat diets do not total to 100, their content of supplemental lysine will be referred to as the level present in the carbohydrate-containing diet from which they were

Table 18

Composition of lysine-deficient diet

Ingredients	Grams
Variable	
Corn starch	44.87
Constants	
Safflower meal[1]	45.65
DL-methionine	0.07
Soybean oil	4.00
Limestone	0.79
Dicalcium phosphate	2.15
Sodium chloride	0.60
Mineral mixture[2]	1.26
Vitamin mixture[3]	0.28
Chromium oxide	0.30
Antioxidant[4]	0.025
Vitamin B_{12}	100 μg/kg

[1] Pacific Vegetable Oil Corporation, World Trade Center, San Francisco 11, California. Contained not less than 42% crude protein, 0.50% crude fat, 15% crude fiber and 8% ash.

[2] See footnote 6, Table 1.

[3] See footnote 7, Table 1.

[4] Ethoxyquin.

derived. All diets were formulated to contain 15.4 kcal/g protein.

The diets were fed to triplicate groups of 10 male crossbred (Dominant White x White Plymouth Rock) chicks in experiment 11 and to duplicate groups in experiment 12. The diets were fed from 8-29 days of age. During the pre-experimental period chicks were fed the carbohydrate-containing diet supplemented with 0.8% L-lysine. The methods of allotment, housing, feeding and weighing have been described previously (Exp. 1). Excreta were collected during the second and fourth week of life so that fat absorbability could be determined. The methods for determining fat, chromium and moisture and for computing fat absorbability from these data have been described previously (Exp. 9).

Results and Discussion

Data showing the effect of a moderate lysine deficiency on the absorbability of hydrogenated fat and tallow are summarized in Table 19. Analysis of variance (Bancroft, 1968) showed that fat absorbability was affected significantly ($P < 0.01$) by level of lysine, age, type of fat and experiment. Statistical analysis also showed that the interaction of level of dietary lysine and age was significant ($P < 0.05$). Application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that at 2 weeks of age the absorbability of hydrogenated fat was decreased by the addition of 0.8% lysine to the diet; while the absorbability of beef tallow remained unchanged. The data

Table 19

Effect of level of dietary lysine on the absorbability of hydrogenated fat and beef tallow

Type of fat	Supplemental lysine	Exp. no.	Absorbability			
			2 wks		4 wks	
	%		%		%	
Hydrogenated fat	0	12	81.6^{1}		87.6	
		13	66.8^{2}		77.6	
				$\underline{74.2}^{3,c,d}$		$\underline{82.6}^{e}$
Hydrogenated fat	0.8	12	77.6		90.9	
		13	56.4		85.6	
				$\underline{67.0}^{b}$		$\underline{88.3}^{f}$
Beef tallow	0	12	64.5		77.7	
		13	45.9		63.0	
				$\underline{55.2}^{a}$		$\underline{70.4}^{b,c}$
Beef tallow	0.8	12	64.1		85.7	
		13	43.1		73.3	
				$\underline{53.6}^{a}$		$\underline{79.5}^{d,e}$

[1] Values are averages of three replicate groups.

[2] Values are averages of duplicate groups.

[3] Underlined values are averages of the two experiments. Values without a common letter in their superscript are significantly different ($P < 0.05$).

show that as chicks aged, their ability to absorb dietary fat increased and that at 4 weeks of age the addition of lysine to a lysine-deficient diet increased the absorbability of both hydrogenated fat and tallow. These results indicate that lysine deficiency decreases absorbability of at least some fats at 4 weeks of age. Since this effect was previously observed in a methionine deficiency, the possibility exists that the deficiency is actually one of protein, lack of which could interfere either with the integrity of the intestinal mucosa or with transport of fat from the mucosal cell to the circulation.

The finding that the ability of the chicks to absorb beef tallow increased with age is in agreement with the finding of Renner and Hill (1960). The reason why hydrogenated fat was absorbed to a greater extent by chicks at 4 weeks of age than at 2 weeks of age when fed with safflower meal but not when fed with isolated soybean protein (Exp. 9) is not apparent.

In the course of the foregoing experiments, data were also collected on growth, caloric consumption and caloric efficiency of chicks fed diets with or without supplemental lysine in which non-protein energy was supplied by starch, hydrogenated fat or tallow (Table 20). Analysis of variance (Bancroft, 1968) and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that the isocaloric substitution of hydrogenated fat for starch increased caloric consumption, weight gain and caloric efficiency only when lysine-deficient diets were

Table 20

Effect of source of non-protein energy on weight gain, caloric consumption and caloric efficiency of chicks fed diets with and without supplemental lysine

Source of energy	Exp. no.	Wt gain		Caloric consumption		Caloric efficiency	
		Supplemental lysine		Supplemental lysine		Supplemental lysine	
		0%	0.8%	0%	0.8%	0%	0.8%
		g	g	kcal	kcal	kcal/g gain	
Starch	12	176^{1}	350	1288	2075	7.33	5.70
	13	126^{2}	356	1129	2131	8.98	5.98
		$\underline{151}^{3,a}$	$\underline{353}^{d}$	$\underline{1208}^{a}$	$\underline{2103}^{d}$	$\underline{8.15}^{c,d}$	$\underline{5.84}^{a}$
Hydrogenated fat	12	235	347	1549	1707	6.58	4.92
	13	168	315	1406	1879	8.38	5.96
		$\underline{202}^{b}$	$\underline{331}^{c}$	$\underline{1478}^{b}$	$\underline{1793}^{c}$	$\underline{7.48}^{b}$	$\underline{5.44}^{a}$
Beef tallow	12	143	230	1069	1337	7.56	5.81
	13	140	180	1412	1671	9.78	9.46
		$\underline{142}^{a}$	$\underline{205}^{b}$	$\underline{1240}^{a}$	$\underline{1504}^{b}$	$\underline{8.67}^{d}$	$\underline{7.64}^{b,c}$

[1] Values are averages of triplicate groups.

[2] Values are averages of duplicate groups.

[3] Underlined values are the averages of the 2 experiments. Values without a common letter in their superscript are significantly different ($P < 0.05$).

fed. The data show that when diets adequate in lysine were fed the isocaloric substitution of hydrogenated fat for starch decreased caloric consumption and decreased weight gain but had no significant effect on caloric efficiency. These results suggest that when lysine-deficient diets are fed, hydrogenated fat has a lysine-sparing action. A similar methionine-sparing action was observed in experiment 8. The failure of hydrogenated fat to increase growth of chicks fed diets in which protein was supplied by safflower meal plus lysine is in contrast to the increase observed when hydrogenated fat was substituted for glucose in diets in which protein was supplied by isolated soybean protein plus methionine. Whether some component in safflower meal interacts with dietary fat is unknown.

The possibility also exists that if fat does act by reducing the activity of the amino acid degrading enzymes, then one might expect fat to have a greater effect when the protein content of the diet is low. In this regard it should be noted that the calorie:protein ratios of the safflower meal and isolated soybean meal diets were 15.4 and 18.7 kcal/g protein, respectively. Furthermore, if dietary fat does reduce activity of amino acid degrading enzymes then lysine if added in excess in a diet in which non-protein energy is supplied by fat would be more toxic than in one in which non-protein energy is supplied by carbohydrate. Calculations indicate that lysine may have been added in excess. The calorie:lysine ratio for the lysine-supplemented diets was 204 kcal/g lysine.

Calculations from the requirements as stated by the Subcommittee on Poultry Nutrition of the National Research Council (1966) indicate the calorie:lysine ratio should be 250 kcal/g lysine. Whether lysine was present in sufficient quantities to depress growth in the presence of fat is unknown.

The effect on chicks of the isocaloric substitution of tallow for starch is in contrast to the effect observed when hydrogenated fat was substituted isocalorically for starch. The results show that the isocaloric substitution of tallow for starch failed to stimulate caloric consumption and growth when lysine-deficient diets were fed and markedly decreased caloric consumption and growth when diets adequate in lysine were fed. In a preceding experiment (Exp. 6) beef tallow was also observed to differ from hydrogenated fat (Crisco) in that it did not stimulate growth when substituted isocalorically for glucose in a diet deficient in both vitamin B_{12} and methionine. These results suggest that the growth response to fat is dependent on its fatty acid composition.

PART IV

UTILIZATION OF NUTRIENTS BY CHICKS FED HIGH FAT DIETS

Literature Review

Fats, particularly vegetable oils, added to nutritonally complete diets have been reported to stimulate growth and have been shown to improve efficiency of feed utilization in chicks (Hill *et al.*, 1958; Dam *et al.*, 1959; Rand *et al.*, 1958 and Carew *et al.*, 1964). The possibility that the growth stimulating effect may be related to the higher caloric density of diets containing added fat has been suggested in studies conducted by Baldini and Rosenberg (1957) and Begin (1961). However, Carew *et al.* (1963) reported that improvement in chick growth in response to dietary additions of corn oil or degummed soybean oil can also occur independently of changes in caloric density of the diet. They found that chicks fed diets containing soybean oil or corn oil consumed more metabolizable energy and grew faster than chicks fed comparable diets low in fat, regardless of alteration in caloric density. Carew and Hill (1964) also reported that the isocaloric substitution of corn oil for dietary carbohydrate increased metabolic efficiency of energy utilization by chicks, measured as total gain in tissue calories per unit of metabolizable energy intake, even when the intakes of all nutrients including energy were maintained constant. This effect of fat they suggested may be mediated through the heat increment component. A similar phenomenon in rats

had been observed by Forbes and Swift (1944). They claimed that fats decreased the heat increment of the dietary mixture, resulting in more efficient utilization of metabolizable energy.

It has also been reported that in some, but not all studies, dietary fat stimulates tissue protein gains. Thus, Carew et al. (1964) observed that soybean oil and lightly hydrogenated olive oil significantly increased protein gains, whereas corn oil decreased gains in tissue protein (Carew and Hill, 1964). Rand et al. (1958) observed that when chicks were fed isonitrogenous, isocaloric diets the substitution of fat calories for carbohydrate calories resulted in improved weight gains, greater protein and energy utilization and protein retention. Support for this contention has also been obtained from studies of other species by Forbes et al. (1946). They showed that on a constant intake of protein and energy, the digestibility and retention of protein as well as the growth rate was improved when the fat level of the rat's diet was increased from 2 to 30% on an isocaloric basis. Furthermore, Salmon (1947) reported that in rats, the sparing effect of cystine on the utilization of casein is exerted only when a high level of fat (30% lard) is added to the diet without changing the ratio of protein and non-protein calories. He also observed that the protein required per gram of gain was significantly less in the animals receiving diets containing 30% fat than in those receiving comparable low-fat diets. Pearson and Panzer (1949) showed that when the

protein intakes of rats were equalized the growth stimulating effect of substituting 8% corn oil or lard isocalorically for sucrose disappeared. They observed however that the incorporation of either corn oil or lard in the diet of growing rats reduced fecal excretion of lysine, methionine and valine and urinary excretion of valine and methionine. These results suggest that fat enhances the utilization of at least some amino acids. Recently, Katorski et al. (1965) claimed that in mice which had previously become adapted to a high-fat diet, fat showed a greater nitrogen sparing action than it did in mice which were not adapted to the high-fat diet. These findings tend to indicate that dietary fats improve growth by increasing the utilization of amino acids.

In this regard, it should be noted that several investigators have observed that the caloric needs of man (Rose et al., 1954), chicks (Leveille and Fisher, 1958) and hens (Fisher and Johnson, 1956) are increased when nitrogen in the diet is supplied by amino acids, rather than protein. Since in these experiments increased caloric needs were met by increasing the fat content of the diet, the question arises as to whether the need was merely for calories or whether fat had a greater amino acid-sparing effect than carbohydrate. Recently, Nakano and Ashida (1970) have observed that the activity of such hepatic amino acid-degrading enzymes as threonine dehydratase, tryptophan pyrollase and arginase was much lower in rats previously fed a high-fat diet than in rats previously fed a high

carbohydrate diet. In parallel, urinary nitrogen output was found to be less in rats fed the high-fat diet than in those fed the high-carbohydrate diet. In their experiment, the high-fat diet was "carbohydrate-free". Whether similar effects would be observed when non-protein calories were supplied by a mixture of fat and carbohydrate is unknown.

Munro (1951) observed in reviewing the literature that in the case of man and the dog, the substitution of fat for carbohydrate resulted in deterioration in nitrogen balance, whereas in the case of the rat the effect on nitrogen balance was variable. Subsequently, Thomson and Munro (1955) showed that when fat was exchanged isocalorically for carbohydrate in the diet of rats, urinary nitrogen output increased for a few days and then returned to its former level. Thus, it can be seen that the effect of high levels of dietary fat on the utilization of nutrients in the diet is difficult to judge on the basis of the data available, and more studies are required before any firm conclusion can be made.

The following experiments were conducted to determine whether fat stimulates growth by increasing utilization of the most limiting amino acid or by increasing appetite.

Experiment 14

Previous studies (exp. 12 and 13) have shown that the isocaloric substitution of fat for carbohydrate increased growth of chicks fed lysine-deficient diets but

decreased growth when diets adequate in lysine were fed. This experiment was designed to determine whether the isocaloric substitution of fat for carbohydrate in a lysine-deficient diet increased growth by increasing utilization of the most limiting amino acid or whether it increased growth by increasing appetite.

Experimental

In experiment 13 duplicate lots of chicks were fed ad libitum diets with and without supplemental lysine in which non-protein energy was supplied by either starch or hydrogenated fat (Crisco). In this concurrent experiment, duplicate lots of chicks were pair-fed. In the case of lysine-deficient chicks, the caloric intake of chicks fed the hydrogenated fat diet was restricted to that of chicks fed the starch-containing diet. In the case of chicks fed diets containing supplemental lysine, the caloric intake of chicks fed the starch-containing diet was restricted to that of chicks fed the hydrogenated fat diet. Chicks that were pair-fed received their daily allotment of feed once every 24 hours. The diets fed, the method of allotment, housing, feeding and weighing of the chicks were the same as in experiments 12 and 13.

Results and Discussion

Data showing average weight gains, caloric consumption and caloric efficiencies of chicks pair-fed high carbohydrate and high fat diets with and without supplemental lysine are summarized in Table 21. Also

Table 21

Effect of source of non-protein energy on weight gain, caloric consumption and caloric efficiency of chicks fed diets with and without supplemental lysine

Treatment			Caloric consumption		Wt gain		Caloric efficiency	
Supplemental lysine	Energy source	Feeding regimen						
%			kcal		g		kcal/g gain	
0	Starch	ad libitum	1278		145		8.78	
			981		107		9.19	
				$1129^{1,a}$		126^{a}		8.98^{b}
0	Hydrogenated fat	ad libitum	1284		149		8.64	
			1529		188		8.13	
				1406^{b}		168^{b}		8.33^{b}
0	Hydrogenated fat	pair-fed	1127		120		9.37	
			1120		136		8.28	
				1124^{a}		128^{a}		8.82^{b}
0.8	Starch	ad libitum	2117		352		6.01	
			2147		360		5.96	
				2132^{d}		356^{d}		5.98^{a}
0.8	Hydrogenated fat	ad libitum	1858		318		5.84	
			1900		313		6.08	
				1879^{c}		316^{c}		5.96^{a}
0.8	Starch	pair-fed	1877		292		6.43	
			1877		300		6.26	
				1877^{c}		296^{c}		6.34^{a}

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[1] Underlined values are averages of duplicate groups. Individual replicate values are given in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

included are data obtained from comparable groups fed the same diets ad libitum. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that when chicks were pair-fed, source of energy had no effect on growth or caloric efficiency, irrespective of the lysine content of the diet. These results indicate that when chicks have free access to a lysine-deficient diet the substitution of hydrogenated fat for starch stimulates growth by increasing appetite and not by increasing utilization of the most limiting amino acid, lysine. The question has arisen as to whether during periods when feed is not available, chicks being pair-fed catabolized tissue protein thus offsetting the beneficial effects of fat on protein utilization. In this regard, it should be noted that in previous studies (Renner and Elcombe, 1967), increased utilization of protein by chicks fed a glucose supplemented soybean fatty acid diet was observed when their intake was restricted to that of chicks fed the unsupplemented soybean fatty acid diet. In that experiment (Renner and Elcombe, 1967), the caloric intake of chicks that were pair-fed was restricted by 52%, while in this experiment caloric intake was restricted by 25%. A small but significant increase in protein utilization was also observed when the feed intake of chicks fed a "carbohydrate-free" diet in which non-protein energy was provided by soybean oil was restricted to that of chicks fed a diet in which non-protein energy was provided by soybean fatty acids (Renner and Elcombe, 1967).

In this case feed intake of chicks fed the soybean oil diet was restricted by 35%. Thus, it seems unlikely that periods without feed are the explanation for the failure of fat to promote greater growth than starch when chicks were pair-fed lysine-deficient diets.

Experiment 15

Combined results of experiments 1, 2 and 3 have shown that the isocaloric substitution of fat for carbohydrate in diets containing less than adequate amounts of methionine increased rate of growth. In subsequent experiments (exp. 8 and 11) the isocaloric substitution of fat for carbohydrate in the diet was shown to have a relatively greater growth stimulating effect when the diet was deficient in methionine than when methionine was present in more adequate amounts. This would be the expected observation, if fat increased the utilization of methionine, since a small increase in utilization would have a more marked effect on chicks fed a deficient diet than on those fed a diet which was more adequate. Thus, the object of this experiment was to determine through pair-feeding studies whether the isocaloric substitution of fat for carbohydrate increased growth by increasing utilization of the most limiting amino acid, methionine, or whether it increased growth by increasing appetite.

Experimental

The composition of the methionine-deficient carbohydrate-containing diet used is shown in Table 10.

A diet containing 0.3% supplemental methionine was formulated from this diet by substituting methionine weight for weight for glucose. "Carbohydrate-free" counterparts were formulated by substituting hydrogenated fat (Crisco) isocalorically for glucose using the value 3.64 and 8.59 kcal/g for the metabolizable energy content of glucose and hydrogenated fat, respectively. All diets were supplemented with vitamin B_{12} (100 μg per kg diet).

Each diet was fed to duplicate groups of 10 male crossbred (Dominant White x White Plymouth Rock) chicks from 1-29 days of age. Feed and water were supplied ad libitum. In addition, two duplicate groups of chicks were pair-fed "carbohydrate-free" diets with and without supplemental methionine, their feed intakes being limited to that consumed by chicks fed the carbohydrate-containing counterpart. Chicks being pair-fed received their daily allotment of feed once every 24 hours. The method of allotment of chicks, housing and weighing were as in experiment 1. Data on growth and feed consumption were obtained weekly and feed wastage was determined daily.

Results and Discussion

Summarized in Table 22 are data showing weight gains, caloric consumption and caloric efficiencies of chicks fed ad libitum carbohydrate-containing and "carbohydrate-free" diets with and without supplemental methionine. Similar data on chicks pair-fed the "carbohydrate-free" diets are also included. Analysis of

Table 22

Effect of source of non-protein energy on weight gain, caloric intake and caloric efficiency of chicks fed diets with and without supplemental methionine

Treatment			Caloric consumption		Wt gain		Caloric efficiency	
Supplemental methionine	Energy source	Feeding regimen						
%			kcal		g		kcal/g gain	
0	Glucose	ad libitum	830 811	$\underline{820}^{1,a}$	90 93	$\underline{92}^{a}$	9.21 8.71	$\underline{8.96}^{e,f}$
0	Hydrogenated fat	ad libitum	990 1136	$\underline{1063}^{b}$	130 142	$\underline{136}^{b}$	7.60 8.03	$\underline{7.82}^{c}$
0	Hydrogenated fat	pair-fed	857 841	$\underline{849}^{a}$	91 92	$\underline{92}^{a}$	9.44 9.18	$\underline{9.31}^{f}$
0.3	Glucose	ad libitum	2222 2466	$\underline{2344}^{c}$	369 383	$\underline{376}^{c}$	6.02 6.43	$\underline{6.22}^{b}$
0.3	Hydrogenated fat	ad libitum	2626 2561	$\underline{2594}^{d}$	464 433	$\underline{449}^{e}$	5.66 5.92	$\underline{5.79}^{a,b}$
0.3	Hydrogenated fat	pair-fed	2352 2364	$\underline{2358}^{c}$	397 417	$\underline{407}^{d}$	5.93 5.66	$\underline{5.79}^{a,b}$

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[1] Underlined values are averages of duplicate groups. Individual replicate values are given in the respective left columns. Values without a common letter in their superscript are significantly different ($P < 0.05$).

variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that when chicks were allowed free access to either the methionine-deficient or adequate diet, the isocaloric substitution of fat for carbohydrate in the diet caused a significant increase in feed consumption and growth. Calculations indicate that caloric intake and growth were increased 30% and 49%, respectively, when fat was substituted isocalorically for carbohydrate in the methionine-deficient diet, while caloric intake and growth were increased 11% and 24%, respectively, when fat was substituted isocalorically for carbohydrate in the methionine-supplemented diet.

The data show however that when the feed intakes of chicks fed the high fat diets were restricted to that of chicks fed the carbohydrate-containing diets, the growth response disappeared in chicks fed the methionine-deficient diet but a small but significant growth response was still maintained in chicks fed the methionine-supplemented diet. If the isocaloric substitution of fat for carbohydrate was increasing growth of chicks fed ad libitum by increasing utilization of methionine, then the growth response should be maintained under pair-feeding conditions, as long as methionine is not present in excess. Thus, results of this experiment indicate that when chicks are fed ad libitum the isocaloric substitution of fat for carbohydrate increased growth of chicks fed a methionine-deficient diet by increasing appetite.

The failure of chicks pair-fed the methionine-

deficient diet to grow faster when fat was substituted isocalorically for glucose is in contrast to the behavior of chicks in experiment 8. In experiment 8, the isocaloric substitution of fat for carbohydrate increased growth of chicks fed diets containing 0 and 0.3% supplemental methionine 46 and 20%, respectively, without increasing feed consumption. These experiments differ in that in this experiment all diets were supplemented with vitamin B_{12}, while in experiment 8 the diets were deficient in vitamin B_{12}. However, in other studies (Renner and Elcombe, 1964) the isocaloric substitution of fat for carbohydrate in diets containing 15.4 kcal/g protein and 0.8% supplemental methionine had the overall effect of significantly increasing growth without increasing caloric consumption, even though vitamin B_{12} was present in adequate amounts. It should be noted however that the overall increase was only 3% and in only 1 of the 5 experiments was the difference great enough to be significant.

Results of this experiment and others conducted in this laboratory (Renner and Elcombe, 1964) indicate that the chicks' response to the isocaloric substitution of fat for carbohydrate is variable and may be related to the level of dietary protein, level of vitamin B_{12} and/or amino acid balance. In this experiment, the increase in appetite observed when fat was substituted isocalorically for glucose in diets either deficient or adequate in methionine may be due at least in part to a decrease in heat increment. Carew and Hill (1964) and Forbes and Swift

(1944) reported that fat significantly reduced the heat increment, resulting in improved utilization of metabolizable energy for growth of chicks and rats, respectively. If the heat increment contributes to the regulation of feed intake in chicks as has been suggested for rats (Strominger and Brobeck, 1953), then reduction in heat increment in response to dietary fat may result in increased caloric consumption. On the other hand, the increase in appetite observed when fat was substituted for glucose in the methionine-supplemented diet may also be due to increased protein retention resulting from decreased levels of amino acid-degrading enzymes (Nakano and Ashida, 1970). This would not only increase amino acids available for protein synthesis and thus growth but might also decrease the heat increment if utilization of the most limiting amino acid was increased.

GENERAL DISCUSSION

The finding that the chick's requirement for vitamin B_{12} but not methionine is increased when hydrogenated fat (Crisco) is substituted isocalorically for glucose in the diet implicates vitamin B_{12} in fat metabolism. Additional support for this concept is the finding that in the presence of vitamin B_{12} the isocaloric substitution of hydrogenated fat (Crisco) for glucose permits chicks to increase feed consumption (Exp. 7 and 15) while in its absence or at levels less than 50 μg/kg diet the isocaloric substitution of fat for carbohydrate fails to stimulate feed consumption (Exp. 6, 7 and 8).

Results of experiments conducted to determine the role which vitamin B_{12} plays in fat metabolism showed that it was not involved in either fat digestion or absorption since studies showed that the absorbability of hydrogenated fat (Crisco) was not affected by supplementary vitamin B_{12} when chicks were fed semipurified diets containing soybean protein.

Studies conducted to determine whether vitamin B_{12} was involved in acetate metabolism showed that levels of blood ketone bodies were increased when non-protein energy was supplied by hydrogenated fat (Crisco) or tallow rather than glucose, however, the increase was similar irrespective of the presence or absence of vitamin B_{12}. These results suggest that deficiency of vitamin B_{12} does not affect oxidation of acetate through the citric acid

cycle. Since Somers (1969) reported that the rate of clearance of acetate declines in vitamin B_{12}-deficient sheep and Cox et al. (1968) have observed increased acetate excretion in humans suffering from pernicious anemia, further studies should be conducted to determine blood levels of acetate in chicks fed diets deficient in vitamin B_{12}.

The question of why vitamin B_{12} requirement is increased when fat is substituted isocalorically for glucose in the diet of chicks remains unanswered. Recently, Dupont and Mathias (1969) have presented evidence supporting the formation of propionyl CoA in rats during the oxidation of unsaturated fatty acids. If propionyl CoA is produced in chicks during the oxidation of unsaturated fatty acids, it could contribute to the increased requirement for vitamin B_{12}, since a vitamin B_{12}-dependent mutase is required to convert methylmalonyl CoA, an intermediate formed from propionyl CoA, to succinyl CoA. Thus, studies should be conducted to determine whether propionyl CoA is formed during oxidation of unsaturated fatty acids in chicks and whether production of propionyl CoA contributes to the increased requirement for vitamin B_{12} when fat is substituted isocalorically for carbohydrate.

Although deficiency of vitamin B_{12} did not reduce absorbability of hydrogenated fat (Crisco) studies showed a small but significant decrease in fat absorbability when chicks were fed a diet markedly deficient in methionine. Further studies showed that the decreased absorbability was not due to lack of choline or to pancreatic lipase.

Since studies showed that the ability of chicks to absorb hydrogenated fat (Crisco) and tallow was also reduced by a lysine deficiency at 4 weeks of age, it would appear that the reduced ability to absorb fat is not a specific effect of a deficiency of methionine or lysine but reflects protein deficiency. Deficiency of protein may reduce the amount of very low density lipoprotein which is required for transport of fat from the mucosa (Noyan *et al.*, 1964), thus permitting fat to accumulate in the intestinal mucosa and reducing fat absorption. That a dietary deficiency of protein does result in reduced fat absorbability in rats and chicks has been shown by Barnes *et al.* (1944) and Renner (unpublished).

In the course of these experiments it was observed that the isocaloric substitution of hydrogenated fat (Crisco) for carbohydrate in either methionine-deficient or lysine-deficient diets caused a marked increase in rate of growth. A smaller but still significant increase was observed when fat was substituted isocalorically for carbohydrate in a methionine-supplemented diet but a similar substitution in the lysine-supplemented diet resulted in growth depression. These results suggest that hydrogenated fat has an amino acid sparing effect. Since further studies showed that the effects on growth were lost when the intakes of chicks fed the high fat diets were restricted to that of chicks fed the high carbohydrate diets, it can be concluded that at least in the lysine- or methionine-deficient diets, fat stimulates growth by increasing appetite and not by sparing amino acids. If

the heat increment contributes to the regulation of feed intake in chicks as has been suggested for rats (Strominger and Brobeck, 1953), then the reduction in heat increment in chicks when fat is substituted isocalorically for glucose (Carew and Hill, 1964) may account for their increased caloric consumption.

The failure of tallow to stimulate growth when substituted isocalorically for carbohydrate in either lysine-deficient, lysine-adequate or methionine-deficient diets indicates that this growth stimulating property of fat is dependent on its fatty acid composition. In this regard Murray et al. (1958) observed that a certain proportion of saturated fatty acids is needed in dietary fat to produce maximum gain. They suggest the proportion to be in the neighborhood of 30% of total fatty acids but state it is at least more than 10% and less than 70% of the total fatty acids. In support of the observation of Murray et al. (1958) is the finding that lauric acid, myristic acid and methyl palmitate depressed growth when incorporated in the diet at a level of 20% (Renner, 1960). Her finding that palmitic acid does not depress growth when incorporated in the diet at a similar level may be due to the fact that only 5% was absorbed. In contrast 65, 25 and 40% of the lauric acid, myristic acid and methyl palmitate were absorbed.

SUMMARY

1. Using growth as the criterion, the chick's requirement for vitamin B_{12} was shown to be increased three-fold when hydrogenated fat (Crisco) was substituted isocalorically for glucose in a semipurified diet containing limited amounts of methionine.
2. On the basis of growth data, the chick's requirement for methionine was shown to remain unchanged when hydrogenated fat (Crisco) was substituted isocalorically for glucose in a semipurified diet, irrespective of the vitamin B_{12} status of the chick.
3. The finding that the chick's requirement for vitamin B_{12} but not methionine was increased when hydrogenated fat (Crisco) was substituted isocalorically for glucose in the diet implicates vitamin B_{12} in fat metabolism.
4. Pair-feeding studies showed that vitamin B_{12} increased growth of chicks fed either carbohydrate-containing or "carbohydrate-free" diets by stimulating appetite and not by increasing utilization of either protein or energy.
5. Vitamin B_{12} deficiency did not result in increased levels of blood ketone bodies in chicks fed diets in which non-protein energy was supplied by glucose, hydrogenated fat (Crisco) or beef tallow. These results suggest that vitamin B_{12} deficiency in the chick does not interfere with acetate metabolism.

6. Results of absorbability studies showed that vitamin B_{12} is not involved in either fat digestion or absorption since studies showed that the absorbability of hydrogenated fat (Crisco) remained constant irrespective of the vitamin B_{12} status of the chick.
7. Chicks fed a "carbohydrate-free" diet, markedly deficient in methionine, showed reduced ability to absorb hydrogenated fat (Crisco). Studies showed that the decreased absorbability was not due to lack of choline or to pancreatic lipase. Furthermore, since chicks fed lysine-deficient diets containing 21 and 27 parts of hydrogenated fat and beef tallow, respectively showed reduced ability to absorb the fats at 4 weeks of age, it would appear that the reduced ability to absorb fat is not a specific effect of lysine or methionine but reflects protein deficiency.
8. The substitution of hydrogenated fat isocalorically for carbohydrate was shown to have a relatively greater growth stimulating effect when the diet was deficient in amino acids (lysine and methionine) than when diets were supplemented with lysine or methionine. However, studies involving pair-feeding showed that the growth stimulating effect of hydrogenated fat was due to appetite stimulation and not to increased utilization of amino acids.
9. Unlike hydrogenated fat (Crisco), beef tallow failed to stimulate growth when substituted isocalorically for glucose in diets deficient in methionine or for starch

in diets deficient or adequate with respect to lysine. These results suggest that the growth stimulating effect of a fat is dependent on its fatty acid composition.

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