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The Effect of Diet on Carnitine and Phospholipid Metabolism in Chicks

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Nutrition

FACULTY OF HOME ECONOMICS

EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Effect of Diet on Carnitine and Phospholipid Metabolism in Chicks submitted by Nancy Robblee in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

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ABSTRACT

An investigation was undertaken to study the effects of energy source, methionine, and vitamin B-12 on levels of muscle carnitine and liver phospholipids in chicks. Carbohydrate-containing and carbohydrate-free rations were formulated with and without supplemental methionine and with and without added vitamin B-12. The chicks were fed these rations for four weeks. The growth performance of the chicks was recorded, and muscle carnitine and liver phospholipids were analysed.

The addition of methionine to the diet resulted in improved growth regardless of the source of energy. In addition, there was a significant growth response when vitamin B-12 was added to the low methionine, carbohydrate-free rations.

The muscle carnitine levels were lower when the chicks were fed a high fat, carbohydrate-free ration as compared to those fed a carbohydrate-containing ration. The concentration of carnitine in the muscle was not affected by the amount of methionine or of vitamin B-12 in the ration.

Liver phospholipids were higher when carbohydrate-free rations were fed but the ratio of phosphatidylcholine: phosphatidylethanolamine did not change. The addition of methionine or of vitamin B-12 did not affect the concentration of phospholipids in the liver.

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Table of Contents

List of Tables

INTRODUCTION

Studies (Looi and Renner, 1974a) have shown that the isoenergetic substitution of fat for carbohydrate in the diet of the chick causes a three-fold increase in vitamin B-12 requirement. Only two functions of vitamin B-12 in animal metabolism have been well established. One of these is in the conversion of methylmalonyl CoA to succinyl CoA which requires a methylmalonyl CoA-B-12 dependent mutase. The other known function of vitamin B-12 is as a cofactor for the vitamin B-12 dependent methyl transferase reaction by which homocysteine is converted to methionine. Since S-adenosyl-methionine is involved in the synthesis of both carnitine and phosphatidylcholine, the question arose as to whether the increased requirement for vitamin B-12 when high fat diets were fed was due to increased metabolism of carnitine and/or phosphatidylcholine. The object of the following studies was to determine the effect of feeding vitamin B-12 deficient diets on tissue levels of carnitine and various phospholipids. In the course of these experiments the effect of source of nonprotein energy and level of dietary methionine on tissue levels of carnitine and phospholipids also was determined.

I. PART I

Effect of level of fat, methionine, and vitamin B-12 on growth and on tissue carnitine in the chick

A. LITERATURE REVIEW

Carnitine is a water soluble nutrient that is widely distributed in organisms. It is present in high concentrations in skeletal muscle (Ciman et al. 1978). Carnitine is required in order to transfer long chain fatty acids into the mitochondria where the enzymes for B-oxidation are located. The inner mitochondrial membrane is impermeable to long chain fatty acids but esters formed between fatty acids and carnitine can pass through this membrane (Fritz and Yue, 1963). Carnitine also may have a role in lipid synthesis. One theory is that carnitine is involved in transporting acetyl groups to the cytoplasm for fatty acid synthesis (Bressler and Katz, 1965) Another theory is that fatty acid synthesis may be stimulated by carnitine at the stage of malonyl CoA formation (Fritz and Hsu, 1967) Another function of carnitine may be in the utilization of ketone bodies since carnitine stimulates acetoacetate oxidation (Bressler and Katz, 1965)

Biosynthesis of Carnitine

The pathway of carnitine biosynthesis has been elucidated only recently. In 1971, Janphaichitr et al. showed that lysine is a precursor of carnitine in the rat. Further studies by Horne and Broquist (1973) using appropriately labelled lysine demonstrated that carnitine is synthesised from carbons 3,4,5,and 6 of lysine and from the 6-epsilon-nitogen group of lysine. Researchers also have shown that the three methyl groups are contributed by S-adenosyl-L-methionine to form trimethyllysine (Cox and Hoppel,1973). Thus, the two essential amino acids lysine and methionine are precursors of carnitine.

Only trimethyllysine which is bound to protein is used in carnitine formation (LaBadie et al.1976). The trimethyllysine is hydroxylated to form beta-hydroxytrimethyllysine. The enzyme involved in this reaction requires the presence of alpha-ketoglutarate, Fe^{++}, and ascorbic acid (Hulse et al.1978). In the next step, β-hydroxytrimethyllysine is split to yield glycine and gamma-trimethylaminobutyraldehyde. The last named compound is converted to gamma-trimethylaminobutyrate. In the last step in carnitine biosynthesis, gamma-trimethylaminobutyrate is converted to carnitine and the presence of alpha-ketoglutarate, Fe^{++}, and ascorbic acid again is required. Many tissues of the rat form gamma-trimethylaminobutyrate but the synthesis of carnitine

from gamma-trimethylaminobutyrate takes place primarily in the liver (Tanphaichitr and Broquist, 1974). Once carnitine is formed in the liver it is transported via the plasma to the tissues. Carnitine is secreted in the urine and a small amount may be metabolized to β-methylcholine (Khairallah and Wolf, 1967).

Dietary Factors Affecting Tissue Carnitine Levels

Level of Lysine

The effect of diet on the carnitine content of animals has been investigated. In rats fed diets deficient in lysine (Tanphaichitr and Broquist, 1973) the amount of carnitine per gram of skeletal muscle and heart muscle was found to be significantly lower than in rats fed the control diets. When rats were fed rice diets limiting in lysine and threonine (Tanphaichitr et al., 1976), growth was impaired and fatty livers developed. The effect of adding varying amounts of lysine, threonine, and/or carnitine was studied and it was found that carnitine reduced the lipid content of the liver only when it was added in combination with lysine or with lysine plus threonine. This probably reflects the requirement for protein for the transport of fat from the liver.

Level of Methionine

Khairallah and Wolf (1965) fed rats low protein diets containing limiting amounts of methionine. The addition of carnitine to these rations resulted in increased growth of the rats and in decreased lipid content of their livers. However, Tsai et al.(1974) could not demonstrate a methionine sparing effect of supplemental dietary carnitine. They fed diets low in methionine and cysteine to rats and found that the addition of dietary carnitine to the ration did not exert a growth promoting effect. They found that the metabolism of carnitine when high fat diets were fed was increased. However, the addition of carnitine to high fat, low methionine diets did not result in a growth response despite the increased demand for carnitine under these conditions. The authors concluded that only a small fraction of dietary methionine is required for carnitine synthesis.

Level of Choline

Tsai et al. (1975) found that carnitine metabolism is influenced by dietary choline. In rats fed choline deficient diets, the turnover time of carnitine is longer than in rats fed choline supplemented diets. In addition, the concentration of carnitine in the tissue was increased when choline was added to the ration. Carter and Frenkel (1978) measured carnitine levels in

choline deficient and choline supplemented rats. They also found that the levels of muscle carnitine were significantly lower in rats fed choline deficient diet. The decrease is thought to be due to a deficiency of methyl group donors in the rats fed choline deficient diets. In addition, it was found that the concentration of hepatic carnitine increased very rapidly after an injection of choline. The authors thought that choline must modify the transport of carnitine and favour its accumulation in the liver.

Level of Vitamin B-12

The effect of a vitamin B-12 deficiency on the levels of carnitine in the liver of the rat was studied by Burton and Frenkel (1975). The level of free carnitine in the liver of the vitamin B-12 deficient rats was not different from the controls. However, the concentration of carnitine in the muscle of the rat was not measured. Since the highest concentration of carnitine is found in muscle tissues, differences in carnitine concentration might have been observed if the levels in muscle had been measured rather than the levels in liver.

That carnitine levels might be reduced in vitamin B-12 deficiency is suggested by the finding that vitamin B-12 deficiency:

1. interferes with the utilization of triglycerides in the liver of chick embryos (Moore and Doran, 1962)
2. results in impaired utilization of plasma triglycerides (Williams et al, 1937)
3. results in increased acetate excretion in pernicious anaemia (Cox et al. 1968)
4. markedly reduces rates of clearance of acetate from blood of vitamin B-12 deficient sheep (Somers, 1969)
5. results in an accumulation of coenzyme A in livers of chicks (Cox et al., 1968), rats (Boxer et al., 1955) and sheep (Smith et al., 1969).

Level of Fat

The effect of feeding diets with varying amounts of lipid has been studied. Tsai et al. (1974) fed rats high fat diets and found that the level of carnitine in the muscle was decreased but the level in the liver was increased. Seccombe et al. (1978) studied the effect of feeding diets with varying lipid content to rats. Rats fed high fat diets had lower serum free carnitine levels than rats fed high carbohydrate diets. When diets containing long chain triglyceride were fed to the rats, the total carnitine content decreased significantly.

The carnitine concentration in the plasma and urine was measured in patients before and after a lipid load

(Konig et al., 1978); the excretion of carnitine in the urine was reduced and the level of carnitine in the blood was lowered. The decrease in the amount of carnitine excreted in the urine following an oral lipid load may indicate that there is limited free carnitine available for lipid utilization. The maximum rate of fatty acid metabolism following a lipid load might therefore be related to tissue carnitine concentrations.

Carnitine in Chick Nutrition

Only a few studies on carnitine in chick nutrition have been reported. Some studies have been done on chick embryos. Mehlman and Wolf (1962) observed an increase in the quantity of carnitine in the chick embryo between the twelfth and sixteenth day. Casillas and Newburgh (1969) measured the levels of carnitine and its derivatives in embryonic chick tissue. They found that the amount of carnitine acetyltransferase increased dramatically during the last week of embryonic development. This corresponds to a period of increased fat metabolism. They also reported that the yolk sac membrane contained high levels of the enzyme acetyl transferase. The authors suggested that the function of this enzyme may be to transfer acyl groups from the yolk sac to the embryo. Borum et al. (1978) compared the levels of carnitine in the thigh and breast muscle of chicks. They found that the red muscle of the chick contained five times

more carnitine than did the white muscle of the chick.

The following studies were conducted to determine the effect of dietary levels of fat, methionine, and vitamin B-12 on the levels of carnitine in the thigh muscle of chicks.

B. EXPERIMENTAL

Carbohydrate-free diets containing either 0 or 0.3 percent supplemental methionine were formulated with and without vitamin B-12 (50ug/Kg). Similarly, carbohydrate-containing diets containing either 0 or 0.3 percent supplemental methionine were mixed with and without vitamin B-12. The rations which did not contain supplemental methionine were deficient in this amino acid. The carbohydrate-free and the carbohydrate-containing diets were isocaloric. The composition of the methionine-deficient diets is shown in Table 1.

The chicks were fed a carbohydrate-free, vitamin B-12 deficient, methionine adequate diet until four days of age and then were assigned to experimental groups on the basis of body weight with the mean body weight and the weight distribution among the groups being equalized. The experimental diets were fed ad libitum to duplicate groups of ten male chicks from four to twenty-eight days of age. Water was supplied ad libitum. The chicks were housed in electrically heated, thermostatically controlled battery brooders with raised wire screen floors, in a temperature controlled laboratory. The chicks were weighed weekly and weekly feed consumption was recorded.

At four weeks of age, six chicks from each pen were killed by cervical dislocation and the thigh muscle was

Table 1. Composition of rations[1]

	Carbohydrate containing g	Carbohydrate-free g
Variables		
Glucose	64.645	0
Soybean oil[2]	4.00	30.67
Cellulose[3]	0	8.54
Constants		
Soybean protein[4] (88.8% protein)	25.03	25.03
L-cystine	0.25	0.25
Vitamin mix[5]	0.43	0.43
Antioxidant[6]	0.025	0.025
Choline chloride	0.296	0.296
Mineral mix[7]	1.315	1.315
Limestone	1.49	1.49
Dicalcium phosphate	2.15	2.15
NaCl	0.60	0.60

[1] Composition is expressed on a dry matter basis

[2] 8,820 kcal/kg

[3] Solka Floc , Brown Forest Products Limited, Montréal, Québec

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

[5] Supplied the following levels per 335 kcal of ration: Thiamine, 1.0 mg; riboflavin, 1.0 mg; calcium 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; retinyl palmitate, 1000 IU; cholcalciferol 150 ICU; α-tocopherol acetate, 3.3 IU.

[6] Contains 25% ethoxyquin, Monsanto Chemical Co., St. Louis, Mo.

[7] Supplied the following levels per 335 kcal of ration: KH_2PO_4, 930 mg; $MgSO_4$, 298 mg; KI 0.29 mg; $FeSO_4.7H_2O$, 40 mg; $CuSO_4.5H_2O$, 1.56 mg; $ZnCO_3$, 11.50 mg; $CaCl_2.6H_2O$, 0.17 mg; $Na_2MoO_4.2H_2O$, 0.830 mg; Na_2SeO_3, 0.022 mg; $MnSO_4.H_2O$, 33 mg.

removed immediately, immersed in liquid nitrogen, and stored at -50 °C. The livers were weighed, frozen at -20 °C., and then were freeze dried for further analysis. The dry matter content of the livers was calculated from the fresh and dry weights.

Records were kept on the growth performance of the chicks and measurements were made on the carnitine present in the thigh muscle.

The moisture content of the thigh muscle was determined by heating a small amount of the sample in a vacuum oven at 100 °C. for six hours or until no further change in weight was recorded.

The amount of carnitine present in the thigh tissue was measured using a modification of the procedure of Pace et al. (1978). One modification was made because a report by Christiansen and Bremer (1978) indicated that Tris buffer interfered with the carnitine assay by acting as an acetyl acceptor for carnitine acetyl transferase. Hepes buffer was not found to have this disadvantage so this problem was overcome by replacing Tris buffer with Hepes buffer. In addition the levels of carnitine in the muscle of the chick are lower than in rats. Therefore, the specific activity of the labelled acetyl CoA was increased four-fold and the size of the sample containing total acid soluble carnitine and

long-chain acyl carnitine was doubled. The procedure also was modified in the hydrolysis of the perchloric acid extract; instead of 3.5M KOH, 0.9M KOH was used. This is approximately the same concentration as was used by McGarry and Foster (1976) in their radioisotopic assay for free and esterified carnitine.

Following the procedure of Pace et al. (1978), the tissue first was homogenized in perchloric acid using a polytron (Brinkmann Instruments). The perchloric acid extract was neutralized and analysed for free carnitine. The short-chain acyl carnitines were determined by taking an aliquot of neutralized extract, alkalinizing it in 0.9 M KOH, and incubating it for one hour at 40 °C. The analysis for long chain acyl carnitines was obtained by incubating the precipitate of the perchloric acid extraction in 0.9M KOH for two hours at 50 °C. Labelled acetyl CoA then was added to an aliquot of the solution containing carnitine and, after the addition of the enzyme carnitine acetyl transferase, the following reaction occurred:

$$\text{L-carnitine} + (1\text{-}^{14}\text{C})\text{-acetyl CoA} \rightleftharpoons (1\text{-}^{14}\text{C})\text{-acetyl carnitine} + \text{CoA}$$

The reaction was pushed in the forward direction by the addition of sodium tetrathionate which removes CoA. The reaction mixture was passed through an anion exchange column in order to separate the labelled carnitine from the labelled acetyl CoA. The amount of labelled carnitine was

measured using programme 3 of the Mark III Scintillation Counter. Each sample was counted for twenty minutes and each day a standard curve was prepared.

At the conclusion of the experiment, average values obtained for replicates were subjected to analysis of variance. Significance of differences were assessed by applying Duncans multiple range test (Steele and Torrie, 1960) at the 0.05 level of probability.

C. RESULTS AND DISCUSSION

Growth Performance of the Chicks

The effects of source of energy and levels of vitamin B-12 and methionine in the ration on growth and on feed conversion are summarized in Table 2. Weight gain and feed conversion were affected by the level of methionine in the ration. In the rations containing additional methionine, body weight at four weeks of age was significantly greater than on the low methionine rations irrespective of the source of energy. In addition, the feed required per unit of gain was significantly lower on the methionine supplemented rations. In the carbohydrate-free, low methionine ration, the addition of vitamin B-12 to the ration resulted in a significant improvement in weight gain and feed efficiency of the chicks. In contrast, the addition of vitamin B-12 to the carbohydrate-containing, low methionine diet did not stimulate growth significantly but did improve feed efficiency. In the carbohydrate-containing and in the carbohydrate-free diets that contained sufficient methionine, no effect on growth or feed efficiency was seen from the addition of vitamin B-12 to the ration. The finding that the addition of vitamin B-12 to the carbohydrate-free, low methionine rations resulted in increased rate of growth and improved feed efficiency is in agreement with the results of Looi and Renner (1974a) They showed that the isocaloric substitution of fat for carbohydrate increased the requirement for vitamin B-12.

Table 2. Growth and feed efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without methionine and vitamine B_{12}.

Treatment	Weight gain g		Feed efficiency g feed/g gain		Energetic efficiency kcal/g gain	
Carbohydrate-containing						
0 Methionine						
0 B_{12}	107		2.51		8.62	
	129		2.30		7.90	
		118[1,c]		2.41[a]		8.26[a]
+ B_{12}	131		2.12		7.27	
	142		2.20		7.53	
		136[c]		2.16[b]		7.40[b]
0.3 Methionine						
0 B_{12}	682		1.39		4.76	
	641		1.40		4.80	
		662[a]		1.40[d]		4.78[c]
+ B_{12}	719		1.41		4.85	
	671		1.50		5.16	
		696[a]		1.46[d]		5.00[c]
Carbohydrate-free						
0 Methionine						
0 B_{12}	165		1.59		7.96	
	146		1.68		8.38	
		156[c]		1.64[c]		8.17[a]
+ B_{12}	213		1.35		6.75	
	194		1.49		7.46	
		203[b]		1.42[d]		7.10[b]
0.3 Methionine						
0 B_{12}	683		0.99		4.95	
	667		0.97		4.84	
		675[a]		0.98[e]		4.90[c]
+ B_{12}	722		0.94		4.68	
	690		0.94		4.71	
		705[a]		0.94[e]		4.70[c]

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

When fat was substituted for carbohydrate in the diet, the chicks requirement for methionine did not change (Looi and Renner, 1974b) Therefore the increased requirement for vitamin B-12 when high fat diets are fed may be due to a requirement for vitamin B-12 in fat metabolism.

It was not possible to evoke a growth response from the addition of vitamin B-12 in chicks that were fed diets that contained adequate methionine. Many studies have been done on the relationships between methionine and vitamin B-12. In a vitamin B-12 deficiency, the metabolism of folate is altered but the adminstration of methionine appears to restore folate to normal (Gawthorne and Stokstad, 1971). Most of the diets that are used to study a vitamin B-12 deficiency also produce a partial methionine deficiency. In order to study a vitamin B-12 deficiency with normal amounts of dietary methionine, McGing and Scott (1980) induced a functional vitamin B-12 deficiency by exposing rats to nitrous oxide. Nitrous oxide inhibits the methylcobalamin dependent methionine synthetase and is thought to be an efficient method of producing a functional vitamin B-12 deficiency. The authors found that the decreased folate incorporation in rats made vitamin B-12 deficient could be restored to only one-third of normal by methionine supplementation.

Carnitine Content of Thigh Muscle

The moisture content of the thigh muscle and the levels of free carnitine, short-chain acyl carnitine, long-chain acyl carnitine , and total carnitine present in the thigh muscle of chicks from different dietary treatments are given in Tables 3 and 4. The average moisture content of the thigh muscle sample was 75.0%. The amount of short-chain acyl carnitine did not differ significantly with the different diets. Differences were found in the levels of free carnitine in the muscle of chicks fed carbohydrate-containing rations as compared to those fed carbohydrate-free rations. The chicks fed the carbohydrate-free rations had lower levels of free carnitine in the thigh muscle than the chicks fed carbohydrate-containing rations. The amount of free carnitine in the muscle of chicks fed carbohydrate-free, methionine supplemented rations was higher when vitamin B-12 was added to the ration.

The amount of long-chain acyl carnitine (Table 4) did not differ significantly with the different diets. In the measurement of total carnitine, the thigh muscle of the chicks fed carbohydrate-free rations contained significantly less carnitine than did the thigh muscle of chicks fed carbohydrate-containing diets. This is in agreement with the results of Tsai et al. (1974) who found that high fat feeding decreased muscle carnitine levels in rats. Bohmer et al.(1966) studied the relative amounts of free carnitine,

Table 3. Levels of free and short-chain carnitine in thigh muscle of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Moisture content %	Nmol L-carnitine/g wet tissue Free		Short-chain	
Carbohydrate-containing					
0 Methionine					
0 B_{12}	76.0	323		102	
	76.0	317		181	
		76.0[1,a]	320[a]		142[a]
+ B_{12}	75.9	344		122	
	76.8	276		139	
		76.3[a]	310[a]		130[a]
0.3 Methionine					
0 B_{12}	74.8	378		93	
	75.6	309		134	
		75.2[ab]	344[a]		113[a]
+ B_{12}	74.2	317		82	
	75.4	352		43	
		74.8[ab]	334[a]		63[a]
Carbohydrate-free					
0 Methionine					
0 B_{12}	74.9	206		111	
	74.6	210		43	
		74.7[ab]	208[b]		77[a]
+ B_{12}	74.6	158		86	
	74.4	199		29	
		74.5[ab]	178[b]		59[a]
0.3 Methionine					
0 B_{12}	72.8	185		100	
	75.0	186		104	
		73.8[ab]	186[b]		102[a]
+ B_{12}	73.8	283		108	
	75.1	272		10	
		74.4[b]	278[a]		58[a]

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

Table 4. Levels of long-chain and total carnitine in thigh muscle of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Nmol L-carnitine/gram wet tissue			
	Long-chain		Total	
Carbohydrate-containing				
0 Methionine				
0 B_{12}	8		433	
	14		512	
		11[1,a]		473[a]
+ B_{12}	10		476	
	15		430	
		12[a]		453[a]
0.3 Methionine				
0 B_{12}	7		478	
	18		461	
		12[a]		470[a]
+ B_{12}	8		407	
	10		405	
		9[a]		406[a]
Carbohydrate-free				
0 Methionine				
0 B_{12}	13		330	
	12		265	
		12[a]		297[b]
+ B_{12}	17		261	
	13		241	
		15[a]		251[b]
0.3 Methionine				
0 B_{12}	17		302	
	13		303	
		15[a]		303[b]
+ B_{12}	30		421	
	13		295	
		22[a]		358[b]

[1] Values are averages of duplicate groups. Underlined column values without a common letter in their superscripts are significantly different ($P \leq 0.05$)

acetylcarnitine, and long-chain acyl carnitine in the heart, liver, and kidney of male rats and showed that the metabolism of carnitine was increased when high fat diets were fed.

The average amount of carnitine was 450 nmol/g wet tissue in chicks fed the carbohydrate-containing rations and was 302 nmol/g in chicks fed the carbohydrate-free rations. This is lower than the levels reported by Borum et al.(1978) who found 997 nmol/g wet weight of red muscle. In their analysis a Tris buffer was used which has been reported by Christiansen and Bremer (1978) to cause erratic high carnitine values. In addition, they did not specify the age of the chicks that they used nor the diet that the chicks had been fed so this may account for the differences that were found. The level of carnitine in the muscle of the chick are much lower than in the rat. Pace et al.(1978) reported 1616 nmol/g in the muscle of the rat.

The inclusion of methionine in the ration did not cause any appreciable change in the level of carnitine in the muscle. Methionine is required in the biosynthesis of carnitine. Methionine was shown to be a a precursor of carnitine by Bremer (1961) who injected methyl-labelled methionine into the rat and found it was incorporated into the methyl groups of carnitine. Khairallah and Wolf (1965) reported a methionine sparing action of carnitine. However,

Tsai et al.(1974) studied the methionine sparing action of carnitine and they concluded that the amount of methionine needed for carnitine biosynthesis is very small. They therefore felt that it would be difficult to demonstrate a growth promoting effect of carnitine. In this study the observation that the inclusion of methionine in the ration did not affect the level of carnitine in the muscle would indicate that the amount of methionine needed for carnitine biosynthesis is very small. The results of this study therefore would be in agreement with the results of Tsai et al.(1974).

The addition of vitamin B-12 to the ration did not affect the total amount of carnitine found in the muscle. Burton and Frenkel (1975) measured the carnitine level in the liver of vitamin B-12 deprived rats and found that the concentration of total carnitine and of propionyl carnitine was increased in a vitamin B-12 deficiency but that the levels of free carnitine were unchanged. The lack of effect in this study of a vitamin B-12 deficiency on muscle carnitine levels may be due to the fact that a very severe vitamin B-12 deficiency was not created.

II. PART II

The effect of dietary level of fat, methionine, and vitamin B-12 on liver lipids

A. LITERATURE REVIEW

Phospholipids synthesized in the liver participate in membrane formation within the organ or are transferred into bile or the lipoproteins of blood plasma (Coleman, 1973). The two most abundant phospholipids in animal tissues are phosphatidylcholine and phosphatidylethanolamine. Phosphatidylcholine is the major phospholipid of brain, liver, and most other tissues (Blusztajn et al. 1979).

Biosynthesis of Phospholipids

Many studies have been done on the biosynthesis of phospholipids. The phospholipid, phosphatidylcholine, may be synthesised in three ways. Kennedy and Weiss (1956) showed that choline is incorporated into phosphatidylcholine through CDP-choline. In another pathway, the methyl group of methionine is used for the conversion of phosphatidylethanolamine to phosphatidylcholine (Bremer et al. 1960). Phosphatidylcholine also may be formed through a base exchange reaction. Orlando et al. (1977) showed that choline may be incorporated into phospholipids by displacing bases present in the lipid. However, Sundler et al. (1972)

compared rates of choline incorporation into rat liver phosphatidylcholine through base exchange with that via CDP-choline and concluded that the base exchange pathway is not of quantitative significance.

Dietary Factors Affecting Phospholipid Biosynthesis

Level of Choline and Methionine

Many studies on the effect of different substances on the biosynthesis of phospholipids have been performed. Haines (1966) fed choline deficient diets to rats and found that the amount of phosphatidylcholine in the liver was reduced but the levels of phosphatidylethanolamine remained unchanged. Glenn and Austin (1971) showed that the methylation of phosphatidylethanolamine to phosphatidylcholine was increased in choline deficient rats. Sundler and Akesson (1975) studied the effects of choline and methionine on the synthesis of phospholipids in rat hepatocytes through the CDP-ester pathways and the methylation pathways. They found that, when methionine was present at saturating concentrations, the formation of phosphatidylcholine via methylation was approximately thirty percent of that through other pathways. They also noted that the synthesis of phosphatidylcholine by methylation was affected by the availability of methionine. Schneider and Vance (1978) fed rats choline deficient diets and

studied the effect of the diet on the activity of liver enzymes involved in phospholipid biosynthesis. In rats fed choline deficient diets they measured an increase in the activity of the methyltransferase responsible for the conversion of phosphatidylethanolamine to phosphatidylcholine.

Level of Vitamin B-12

Vitamin B-12 is involved in the synthesis of methionine in the conversion of homocysteine to methionine. Therefore, in a vitamin B-12 deficiency, the amounts of methionine available for phosphatidylcholine synthesis may be reduced. In a study by Akesson et al. (1978) rats were raised on vitamin B-12 deficient diets for twelve to fifteen months. Analysis of the phospholipids in the liver showed that the proportion of phosphatidylethanolamine had increased and the proportion of phosphatidylcholine had decreased. Since the ratio of phosphatidylcholine:phosphatidylethanolamine normally is kept within narrow limits, any change in the ratio might affect the viscosity of the membrane (Esko et al. 1977). Akesson et al. (1979) bred rats from vitamin B-12 deprived dams. They then injected (^{14}C)-ethanolamine and found that the methylation of phosphatidylethanolamine to phosphatidylcholine was reduced in the vitamin B-12 deficient rats.

Kalamegham and Krishnaswamy (1975) studied the effect of a vitamin B-12 deficiency in chicks. They measured the amount of vitamin B-12 in the liver of the chick after the chicks had been raised on the diet for two months. They found that the liver stores of vitamin B-12 were significantly reduced in the chicks fed the vitamin B-12 deficient diets. They also measured the lipid content of myelin of the chicks and found a significant increase in total phospholipids and a concomitant decrease in total galactolipids. The altered molar ratio of these lipids may represent a relatively immature myelin.

Peifer and Lewis (1979) studied the fatty acid patterns of phospholipids in rats deprived of vitamin B-12. Analysis of the fatty acids showed an increase in the amount of 18:2 in both phosphatidylcholine and phosphatidylethanolamine in rats fed vitamin B-12 deficient diets. In addition, there was less 20:4 and 22:6 in the liver phosphatidylcholine of the vitamin B-12 deficient rats. These changes may be related to interference in the conversion of linoleate to arachidonic acid due to inhibition of synthesis of malonyl coA by methylmalonyl coA (Cardinale et al. 1970).

Level of fat

In a study by Artom and Sarzana (1938) the rate of formation of phospholipids in rats fed different diets was studied. When rats were given high fat diets, the rate of biosynthesis of phospholipids in the liver and the intestine was increased. Narayan et al. (1974) studied the effect on liver lipids of feeding high levels of corn oil to rats. Rats were fed a semi-purified diet containing 0, 10, and 40% corn oil. After the rats had been fed the diets for one, four, and ten weeks, there was no significant change in the level of phospholipids in the liver of the rats. Marion and Edwards (1962) studied the influence of protein level and added corn oil on the composition of liver lipids in chicks. They found that liver phospholipids were lower only when low protein, corn oil diets were fed.

Level of essential fatty acids

Yurkowski and Walker (1971) found that feeding essential fatty acid deficient diets resulted in changes in the phosphatidylcholine molecular species of the intestinal mucosa of rats. The arachidonic acid-containing species were partially replaced by 16:0-18:0 and 18:0-18:1. Van Golde et al. (1968) studied the effect of feeding corn-oil to essential fatty acid deficient rats on the molecular species of rat liver

lecithin. They observed a replacement of (1-palmitoyl-2-eicostrienoyl)- and (1-stearoyl2-eicosatrienoyl)-lecithin by (1-palmitoyl-2-arachidonoyl)- and (1-stearoyl-2-arachidonoyl)-lecithin, respectively. Wood (1975) measured phospholipid classes in the liver of rats fed chow diets and compared these to the levels in rats fed fat-free diets. The phospholipid class percentages were not affected by feeding a fat-free diet. The diets were fed to the rats for only five weeks so an essential fatty acid deficiency would not have developed.

Very few studies have been reported on the effect of nutrient intake on liver phospholipids in chicks. Thus the following studies were conducted to determine the effect of dietary levels of vitamin B-12, methionine, and fat on liver phospholipids of the chick.

B. EXPERIMENTAL

The livers from the chicks that were raised as described in Part I were analysed for their lipid content, fatty acid patterns, and phospholipid content.

The lipid content of the livers was determined using a Goldfisch apparatus and a mixture of chloroform:methanol (2:1) as the solvent (Feigenbaum and Fisher, 1963).

In the determination of fatty acids, the liver lipids were extracted with chloroform:methanol (2:1) and methylated using the method of Metcalfe et al. (1966). The pattern of the fatty acids was obtained using a Bendix 2500 Chromatograph provided with a flame ionization detector and a [redacted] by 3 mm glass column packed with 10% phenyl-50 cy[redacted] on 80/100 mesh chromosorb W. The peaks were qua[redacted]d using a digital integrator and the peaks were iden[redacted]ed by comparing retention data with that of known stand[redacted]s.

[redacted]or the analysis of the various lipid classes, lipids were extracted following the procedure of Atkinson et al. (1972). Th[redacted] lipid classes were determined using an Iatroscan analyser. The principle of operation of the Iatroscan Analyser is described by Sipos and Ackman (1978). One microlitre of extract from each sample was spotted in a narrow band on one end of each of ten chromorods coated

uniformly with silica gel Separation of the lipid classes was accomplished by placing the rods in a filter paper lined glass tank and developing them for fifty minutes in Solvent A (methylene chloride:chloroform:acetic acid:methanol (98: 8: 0.4: 0.15)(v/v/v/v)). After development in the solvent, the rods were dried in an oven for a few minutes and the various classes were detected using an Iatroscan TH-10 Analyser. The Iatroscanner works on the principle of flame ionization detection. When the chromorod is passed through the hydrogen flame the substances on the rod are ionized and the change in current between the burner and the collector electrode of the detector is measured. Since different molecules produce different numbers of ions, response factors were determined for each of the lipid classes. A standard solution containing known amounts of each of the lipid classes (cholesterol esters, triglycerides, free fatty acids, cholesterol, diglycerides, monoglycerides, and phospholipids) was spotted on the rods, developed in Solvent A, and the response was measured on the Iatroscan Analyser. The response factors were calculated by comparing the values obtained from the instrument with the known amounts of the compound that were contained in the solution.

Phospholipid classes were determined by burning off all of the lipid classes, following development in Solvent A, except those lipids which stayed at the origin (in this case the phospholipids and the monoglycerides). The rods then

were placed in Solvent B (chloroform:methanol:water (50:50:4.5)(v/v/v/v)) in order to separate the phospholipid components. After development in Solvent B for ninety minutes, the rods were dried in an oven and the proportions of the phospholipids were calculated following determination of the amounts present by using the Iatroscan Analyser.

Average values obtained for replicates were subjected to analysis of variance. Significance of differences was assessed by applying Duncans multiple range test (Steele and Torrie,1960) at the 0.05 level of probability.

C. RESULTS AND DISCUSSION

Liver Composition

The influence of the dietary treatments used on the composition of the livers is shown in Table 5. Values for wet weight of liver, milligrams of liver per gram body weight, and percentages of dry matter and of lipid in the livers of chicks from each treatment are presented.

There were large variations in the size of livers from chicks on different dietary treatments. The addition of methionine to the ration resulted in significantly larger livers in chicks fed both the high and low carbohydrate diets; however, when expressed in terms of body weight, the chicks receiving supplemental methionine had significantly smaller livers per unit body weight.

Substitution of fat for carbohydrate in the diet when diets low in vitamin B-12 and methionine were fed resulted in enlargement of the liver, but did not affect the proportion of dry matter or of lipid in the liver. The addition of vitamin B-12 to the low methionine, carbohydrate-free diet resulted in a significant decrease in liver size in relation to body weight.

Percentage dry matter in the livers was affected by the addition of methionine to both the carbohydrate-containing and the carbohydrate-free diets. When all values were

Table 5. Composition of livers of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Wet weight g	Mg liver/ g body wt	Dry matter (DM) %	Lipid % DM basis
Carbohydrate-containing				
0 Methionine				
0 B_{12}	41.7	35.4	27.9	20.0
	55.3	41.7	28.2	22.7
	$\underline{48.5^{1,de}}$	$\underline{38.5^{b}}$	$\underline{28.1^{b}}$	$\underline{21.4^{a}}$
+ B_{12}	43.8	33.3	27.7	20.6
	45.9	34.2	27.8	20.9
	$\underline{44.8^{e}}$	$\underline{33.8^{bc}}$	$\underline{27.8^{b}}$	$\underline{20.7^{a}}$
0.3 Methionine				
0 B_{12}	116	25.2	30.4	23.3
	124	28.3	30.0	22.3
	$\underline{120^{b}}$	$\underline{26.7^{d}}$	$\underline{30.2^{a}}$	$\underline{22.8^{a}}$
+ B_{12}	146	30.0	30.6	23.3
	130	28.6	29.7	22.0
	$\underline{138^{a}}$	$\underline{29.3^{cd}}$	$\underline{30.1^{a}}$	$\underline{22.6^{a}}$
Carbohydrate-free				
0 Methionine				
0 B_{12}	60.2	46.3	27.6	19.1
	62.1	44.8	27.6	21.5
	$\underline{61.2^{cd}}$	$\underline{45.6^{a}}$	$\underline{27.6^{b}}$	$\underline{20.3^{a}}$
+ B_{12}	67.3	34.2	27.6	21.6
	70.5	39.6	28.2	24.9
	$\underline{68.9^{c}}$	$\underline{36.9^{b}}$	$\underline{27.9^{b}}$	$\underline{23.3^{a}}$
0.3 Methionine				
0 B_{12}	126	27.0	29.9	21.9
	121	26.3	29.9	21.3
	$\underline{123^{b}}$	$\underline{26.6^{d}}$	$\underline{29.9^{a}}$	$\underline{21.6^{a}}$
+ B_{12}	118	24.6	30.0	19.6
	118	24.3	30.1	20.3
	$\underline{118^{b}}$	$\underline{24.4^{d}}$	$\underline{30.0^{a}}$	$\underline{20.0^{a}}$

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

combined, the addition of methionine resulted in a significant increase from 27.8% to 30.1% dry matter in the livers of the chicks. Results show that neither source of nonprotein energy nor supplemental vitamin B-12 affect the dry matter content of liver. Analysis of the livers for lipid content revealed no significant difference between treatments.

Liver Fatty Acid Patterns

The saturated fatty acid compostion of the liver lipids is summarized in Table 6. The results show that the liver lipids of chicks fed a carbohydrate-free diet containing soybean oil were less saturated than the liver lipids of chicks fed a low fat, carbohydrate-containing diet. Results show that the liver lipids of chicks fed the carbohydrate-free diet also differed in the type of saturated fatty acid present. The lipids were characterized by being lower in palmitic acid and higher in stearic acid than the liver lipids of chicks fed the carbohydrate containing diet. The data also show that methionine supplementation significantly increased the palmitic acid but not the stearic acid content of liver lipids of chicks fed carbohydrate-containing diets but not carbohydrate-free diets. Analysis of variance (Steele and Torrie, 1960) of the stearic acid content of liver lipid showed that there was a significant methionine-vitamin-B-12 interaction. The data

Table 6. Saturated fatty acid (SFA) content of liver lipids of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Ration	Per cent of total fatty acids					
	SFA		16:0		18:0	
Carbohydrate-containing						
0 Methionine						
0 B_{12}	44.9		22.2		22.7	
	42.8		24.1		18.4	
		43.9[1,ab]		23.2[b]		20.6[b]
+ B_{12}	41.9		24.4		17.1	
	41.4		22.6		18.4	
		41.6[bc]		23.5[b]		17.8[b]
0.3 Methionine						
0 B_{12}	42.6		26.7		15.6	
	44.9		27.8		16.8	
		43.8[ab]		27.2[a]		16.2[b]
+ B_{12}	44.6		29.3		15.0	
	45.6		27.0		18.3	
		45.1[a]		28.2[a]		16.6[b]
Carbohydrate-free						
0 Methionine						
0 B_{12}	40.7		12.1		28.6	
	40.6		12.5		28.1	
		40.7[bc]		12.3[c]		28.4[a]
+ B_{12}	38.1		11.3		26.8	
	34.4		11.2		23.2	
		36.2[de]		11.2[c]		25.0[a]
0.3 Methionine						
0 B_{12}	35.5		11.2		24.4	
	35.3		10.1		25.1	
		35.4[e]		10.6[c]		24.8[a]
+ B_{12}	40.3		11.1		29.2	
	37.8		11.0		26.7	
		39.0[cd]		11.1[c]		27.9[a]

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

indicate that supplemental vitamin B-12 decreased the stearic acid content of liver lipid of chicks fed methionine deficient diets but increased the level of this acid when methionine adequate diets were fed. This interaction was more marked when nonprotein energy was supplied by soybean oil. A similar methionine-vitamin B-12 interaction which was more marked for carbohydrate-free diets was also observed for total saturated fatty acid content of liver lipids.

The effect of diet on monounsaturated fatty acid compostion is summarized in Table 7. The data show that the liver lipid of chicks fed carbohydrate-containing diets contained higher levels of monounsaturated fatty acids than the liver lipids of chicks fed carbohydrate-free diets. When chicks were fed the carbohydrate-containing diets, the addition of methionine to the diet resulted in livers with a higher percentage of palmitoleic acid than in the unsupplemented group. The additon of vitamin B-12 to the rations did not have any effect on the monounsaturated fatty acid content of the liverlipid.

The polyunsaturated fatty acid content of the liver lipids is summarized in Table 8. The liver lipids of chicks fed carbohydrate-free diets contained higher levels of 18:2 and 20:4 than the liver lipids of chicks fed carbohydrate-containing diets. The data also show that the unsaturated fatty acid index of liver lipids of chicks

Table 7. Monounsaturated fatty acid content of liver lipid of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Percent of total fatty acids			
	16:1		18:1	
Carbohydrate-containing				
0 Methionine				
0 B_{12}	1.88		27.9	
	1.82		36.2	
		1.85[1,b]		32.0[a]
+ B_{12}	2.89		32.8	
	2.50		32.1	
		2.70[b]		32.4[a]
0.3 Methionine				
0 B_{12}	4.28		36.4	
	3.44		34.6	
		3.86[a]		35.2[a]
+ B_{12}	5.26		37.3	
	4.01		33.7	
		4.64[a]		35.5[a]
Carbohydrate-free				
0 Methionine				
0 B_{12}	0.03		8.82	
	ND[2]		11.6	
		0.02[c]		10.2[b]
+ B_{12}	ND		10.9	
	ND		12.8	
		ND[c]		11.9[b]
0.3 Methionine				
0 B_{12}	0.22		11.5	
	ND		10.4	
		ND[c]		11.0[b]
+ B_{12}	ND		10.9	
	ND		10.4	
		ND[c]		10.6[b]

[1] Underlined values are averages of duplicate groups. Underlined column values without a common letter in their superscripts are significantly different ($P \leq 0.05$)

[2] Not Detected

Table 8. Polyunsaturated fatty acid content of liver lipids of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Unsaturated index[1]	Percent of total fatty acids 18:2	20:3	20:4
Carbohydrate-containing				
0 Methionine				
0 B_{12}	96.0	16.0	1.26	7.65
	85.1	12.8	1.08	4.59
	90.6[2,b]	14.4[c]	1.17[b]	6.12[ab]
+ B_{12}	91.6	13.8	1.46	5.96
	94.5	14.4	1.57	6.58
	93.1[b]	14.1[c]	1.52[a]	6.27[ab]
0.3 Methionine				
0 B_{12}	81.6	10.9	1.04	4.01
	80.2	11.1	1.04	4.22
	80.9[c]	11.0[cd]	1.04[bc]	4.12[b]
+ B_{12}	72.9	8.64	0.54	2.86
	81.5	10.8	0.98	4.81
	77.2[c]	9.7[d]	0.76[c]	3.84[b]
Carbohydrate-free				
0 Methionine				
0 B_{12}	127	36.8	0.22	10.86
	122	38.5	0.21	8.24
	124[a]	37.6[b]	0.22[d]	9.55[a]
+ B_{12}	129	39.7	0.14	9.54
	126	43.4	0.12	6.50
	127[a]	41.6[a]	0.13[d]	8.02[a]
0.3 Methionine				
0 B_{12}	129	42.5	0.18	8.16
	130	44.0	0.005	8.01
	130[a]	43.3[a]	0.09[d]	8.08[a]
+ B_{12}	123	39.5	0	8.15
	129	41.3	0	9.10
	126[a]	40.4[ab]	0[d]	8.63[a]

[1] Sum of ((% fatty acid) X (number of unsaturated bonds))

[2] Underlined values are averages of duplicate groups. Underlined column values without a common letter in their superscript are significantly different ($P \leq 0.05$)

decreased when carbohydrate-containing diets were supplemented with methionine. Supplementation of carbohydrate-free diets with methionine had no effect on the unsaturated fatty acid index. Results also indicate that supplementation of the methionine deficient, carbohydrate-free diet with vitamin B-12 significantly increased the linoleic acid content of liver lipid.

Variations noted in the fatty acid composition of the liver lipids show that they are influenced by the diet fed. In chicks fed the carbohydrate-containing diets the levels of linoleic and arachidonic acids were lower than in chicks fed high fat diets reflecting the higher level of linoleic acid in soybean oil and the capacity of the chick to synthesize arachidonic acid from linoleic acid. The lower levels of essential fatty acids in chicks fed the carbohydrate-containing diets were partially offset by higher percentages of palmitoleic and oleic acid. However, the unsaturated fatty acid index still remained significantly lower when carbohydrate-containing diets were fed. The decrease in essential fatty acids in liver lipid observed on supplementation of the carbohydrate-containing diet with methionine may reflect the increased need of the chick for essential fatty acids for growth.

Liver Triglycerides

Analysis of variance (Steele and Torrie, 1960) of the data on triglyceride content of liver lipid (Table 9) shows that the source of energy but not the level of methionine or of vitamin B-12 affected the amount of triglyceride that was present. Results show that liver lipids of chicks fed the carbohydrate-free diets contained a lower proportion of triglyceride than did the livers of chicks fed the carbohydrate-containing diets.

Liver Phospholipids

Summarized in Table 9 are results showing the percent phospholipid in the liver lipid of chicks fed carbohydrate-containing and carbohydrate-free diets with and without supplemental methionine and vitamin B-12. Analysis of variance (Steele and Torrie, 1960) showed that source of energy but not level of methionine or vitamin B-12 affected the phospholipid content of liver lipid. Results show that chicks fed carbohydrate-free diets contained significantly more phospholipid when expressed as a percent of lipid and when expressed as a percent of wet liver weight. The increased level of liver phospholipid observed in chicks fed high fat carbohydrate-free diets may be due to increased formation of bile (a fluid which is rich in phospholipid (R.Coleman, 1973)), to increased synthesis of lipoproteins necessary for fat transport, or to increased membrane

Table 9. Triglycerides and phospholipids in livers of chicks fed carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B_{12}.

Treatment	Triglyceride, % Liver lipid		Phospholipid, % Liver lipid		Phospholipid, % Liver	
Carbohydrate-containing						
0 Methionine						
0 B_{12}	8.14		70.5		3.84	
	38.5		46.5		3.04	
		23.3[1,abc]		58.5[abcd]		3.44[b]
+ B_{12}	28.4		52.6		3.05	
	24.5		59.2		3.38	
		26.5[abc]		55.9[bcd]		3.22[b]
0.3 Methionine						
0 B_{12}	43.2		44.3		3.14	
	39.3		49.6		3.33	
		41.3[ab]		47.0[cd]		3.24[b]
+ B_{12}	49.0		40.1		2.84	
	36.3		50.6		3.29	
		42.7[a]		45.4[d]		3.07[b]
Carbohydrate-free						
0 Methionine						
0 B_{12}	4.47		79.5		4.28	
	10.4		75.4		4.39	
		7.4[c]		77.5[a]		4.34[a]
+ B_{12}	8.37		74.5		4.41	
	26.4		62.3		4.41	
		17.4[bc]		68.4[ab]		4.41[a]
0.3 Methionine						
0 B_{12}	18.2		66.5		4.44	
	19.2		64.4		4.02	
		18.7[abc]		65.4[abc]		4.23[a]
+ B_{12}	9.09		76.0		4.46	
	12.4		73.9		4.54	
		10.8[c]		75.0[a]		4.50[a]

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

formation.

In Table 10 the percent phosphatidylcholine and the percent phosphatidylethanolamine in the liver of chicks from the different dietary treatments are summarized. Analysis of variance (Steele and Torrie, 1960) shows that the source of nonprotein energy but not the level of methionine or vitamin B-12 affected the level of phosphatidyl choline and phosphatidylethanolamine in liver lipid and in liver. Results show that the livers of chicks fed carbohydrate-free diets contained significantly more phosphatidylcholine and phosphatidylethanolamine when expressed as a percent of lipid and when expressed as a percent of liver on a wet weight basis than did the livers of chicks fed carbohydrate-containing diets; however, the ratio between phosphatidylcholine and phosphatidylethanolamine was not affected by the source of nonprotein energy or by the dietary level of methionine or vitamin B-12. The ratio of phosphatidylcholine and phosphatidylethanolamine normally varies within narrow limits (Akesson et al., 1978). It is thought that the viscosity of membranes may change when the ratio of phosphatidylcholine:phosphatidylethanolamine is altered (Esko et al., 1977).

The effect of a deficiency of one carbon units on liver phospholipids has been studied in rats (Lyman et al., 1975). They found that livers of rats fed low methionine, choline

Table 10. [redacted]dylcholine and phosphatidylethanolamine in livers of chicks [redacted]ohydrate-containing and carbohydrate-free diets with and [redacted]methionine and vitamin B_{12}.

Trea	Phosphatidylcholine, %			Phosphatidylethanolamine, %		
	Liver lipid		Liver	Liver lipid		Liver
Carb[redacted]ydra[redacted]containing						
0 Meth[redacted]nine						
[redacted]12	37.2		2.02	16.3		0.89
	22.4		1.47	11.6		0.76
		$29.8^{1,ab}$	1.74^{b}		14.0^{b}	0.82^{b}
[redacted] B_{12}	25.6		1.48	12.5		0.73
	29.2		1.67	12.6		0.73
		27.4^{ab}	1.57^{b}		12.6^{b}	0.73^{b}
0.3 Me[redacted]onine						
[redacted]B_{12}	21.5		1.52	12.6		0.90
	23.0		1.54	13.4		0.90
		22.3^{b}	1.53^{b}		13.0^{b}	0.90^{b}
+ B_{12}	17.8		1.27	9.30		0.66
	24.8		1.61	13.4		0.87
		21.3^{b}	1.44^{b}		11.4^{b}	0.76^{b}
Carbohydrat[redacted]						
0 Meth[redacted]						
0 [redacted]B_{12}	43.9		2.36	21.3		1.15
	39.2		2.28	21.8		1.27
		41.5^{a}	2.32^{a}		21.6^{a}	1.21^{a}
+[redacted]B_{12}	42.4		2.52	19.0		1.12
	28.4		2.01	18.0		1.27
		35.4^{ab}	2.27^{a}		18.5^{a}	1.20^{a}
0.3 Methionine						
0 B_{12}	34.5		2.31	18.6		1.24
	33.8		2.11	20.0		1.24
		34.2^{ab}	2.21^{a}		19.2^{a}	1.24^{a}
+ B_{12}	42.4		2.49	19.3		1.13
	37.8		2.33	19.2		1.18
		40.1^{a}	2.41^{a}		19.3^{a}	1.16^{a}

[1] Underlined values are averages of duplicate groups. Underlined column values without the same letter in their superscript are significantly different $P \leq 0.05$

deficient diets had a lower concentration of phospholipid characterized by significantly less phosphatidylcholine than did the liver of rats fed diets supplemented with choline and/or methionine. Lyman et al. (1975) showed that supplementation of the diet with both choline and methionine was no more effective than with either alone. Thus, failure of methionine to increase the level of phospholipid and of phosphatidylcholine in the liver of chicks fed diets low in methionine but adequate in choline is similar to results obtained by Lyman et al. (1975) using rats as the experimental animal.

The lack of effect of supplemental vitamin B-12 on liver phospholipids of chicks fed methionine deficient diets is similar to results reported by Fehling et al.(1978) when rats were fed vitamin B-12 deficient diets for six months. When a more severe vitamin B-12 deficiency was produced in rats by feeding vitamin B-12 deficient diets for twelve to fifteen months, Akesson et al.(1978) found liver phospholipids contained a higher proportion of phosphatidyl-ethanolamine and a lower proportion of phosphatidyl-choline,indicating impaired methylation. These results suggest that vitamin B-12 deficiency in chicks, if it were severe enough, might impair phosphatidylcholine synthesis.

III. GENERAL DISCUSSION

Results of this experiment show that the complete equicaloric substitution of fat for carbohydrate in the diet causes a decrease in the amount of total carnitine and of free carnitine in the muscle of chicks. Previously, Looi (1974) using similar diets, observed that the level of liver carnitine increased when high fat carbohydrate-free diets were fed. Since the liver is the major site of carnitine synthesis (Tanphaichitr and Broquist, 1974) these results suggest that the requirement for carnitine is increased when fat is substituted for carbohydrate in the diet.

Results also show that chicks fed high fat diets have higher levels of phospholipids in their livers than chicks fed comparable diets rich in carbohydrates. Elevated levels of liver phospholipids may indicate an increased need of chicks fed high fat diets for phospholipid for formation of bile a fluid rich in phospholipid and/or for synthesis of lipoproteins which are necessary for fat transport.

If the synthesis of carnitine and of phospholipids (i.e. phosphatidylcholine) is increased when chicks are fed high fat, carbohydrate-free diets, the need for S-adenosyl methionine to supply methyl groups for the synthesis of carnitine and phospholipids also would increase. This could

contribute to the increased requirement for vitamin B-12 when fat is substituted for carbohydrate in the diet (Looi and Renner, 1974), since vitamin B-12 is involved as a cofactor in the conversion of homocysteine to methionine. The possibility that the fibre added to improve the texture of the high fat, carbohydrate-free diets may also contribute to the increased requirement of the chick for vitamin B-12 is suggested by the finding of Cullen and Oace (1978) that fibre may bind vitamin B-12 and make it unavailable. The finding that supplementation of high fat, low carbohydrate diets with methionine or vitamin B-12 significantly increased growth but had no significant effect on the level of muscle carnitine or of liver phosphatidylcholine suggests that the maintenance of muscle carnitine and liver phosphatidylcholine takes priority over the requirement of these nutrients for growth.

To clarify the role which vitamin B-12 plays in lipid metabolism in chicks, the foregoing experiment should be repeated using chicks obtained from vitamin B-12 depleted hens.

IV. SUMMARY

An experiment was conducted to study the effects in chicks of feeding carbohydrate-containing and carbohydrate-free diets with and without methionine and vitamin B-12 on growth, on muscle carnitine levels, and on liver phospholipid concentrations. A summary of the results is given below:

1. Growth was improved when methionine was added to the ration regardless of the source of energy. The addition of vitamin B-12 to the low methionine, carbohydrate-free rations resulted in a significant growth response.

2. Chicks fed carbohydrate-free diets had higher muscle carnitine levels than did chicks fed carbohydrate-containing rations.

3. The concentration of liver phospholipids was higher when carbohydrate-free rations were fed. The ratio of phosphatidylcholine:phosphatidylethanolamine did not change regardless of the ration fed.

4. The addition of methionine or of vitamin B-12 did not significantly alter the concentration of muscle carnitine or of liver phospholipids.

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