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comments in biochemistry

Carnitine metabolism in human subjects I. Normal metabolism[1, 2]

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ABSTRACT Carnitine (vitamin B_T) is a compound which is involved with lipid metabolism. This article deals with the carnitine content of foods and diet, the absorption, transport, storage, and excretion of carnitine in humans. The metabolic functions and biosynthesis of carnitine are also reviewed. *Am. J. Clin. Nutr.* 31: 293-306, 1978.

Should carnitine (vitamin B_T) be considered an "essential" nutrient? That is, should carnitine be supplied preformed in the human diet? Most nutritionists would be emphatically negative to these questions. Carnitine (β-hydroxy-γ-trimethylamino butyrate) can be synthesized endogenously in mammals (1) hence no need apparently exists to supply this vitamin in food on a daily basis. Recent studies suggest however (2, 3) that for some individuals synthesis of body carnitine may be inadequate. Malnourished Egyptian farmers (4) had lower levels of serum carnitine than healthy control Egyptian subjects. In addition, a number of disease states including those with disrupted lipid metabolism have been shown to alter levels of carnitine in human biological fluids (5-7) and tissues. The question, therefore, arises as to whether carnitine needs are indeed met entirely by endogenous synthesis and what role, if any, dietary carnitine plays in both normal metabolism and in disease.

This review in three parts (Part I. Normal metabolism, Part II. Carnitine in body fluids, Part III. Metabolism in disease) assembles most of the data available to attempt to answer these questions. It will show, however, that there are large gaps in our knowledge and that more research on human carnitine metabolism is needed. Part I will show that we know little about the content of carnitine in foods or diets. Part II collates the data on "normal" levels of carnitine in human tissues and body fluids. Part III will show the number of pathological conditions under which carnitine metabolism appears to be altered and where knowledge of dietary carnitine intake is needed to evaluate experimental treatments. Carnitine is a quaternary amine, β-hydroxy-γ-trimethylamino butyric acid:

$$(CH_3)_3N^+ - CH_2\ CH\ CH_2\ COO^-$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad\ OH$$

Its existence was known in the early 1900's (8) but it was not until the late 40's and early 50's that Fraenkel and co-workers (9) showed that carnitine was an essential nutrient in the diet of the yellow meal worm *Tenebrio molitor*. They called it vitamin B_T for its water soluble properties with the T standing for Tenebrio. (In some commercial catalogs it is listed as vitamin B_7.) Although carnitine is acknowledged to be an important metabolite in mammalian tissues utilizing long-chain fatty acids as energy substrates, it has been considered by only a

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few to be a dietary essential for man or a vitamin in the true sense (1). Dietary and nutritional aspects of carnitine have not been widely studied in humans because assay methods have been imperfect, time-consuming, and expensive.

Carnitine assays

The first assay technique used was the bioassay using *Tenebrio molitor* (9). Growth or survival of larvae consuming the diet plus the test material was compared to control larvae consuming a diet plus a standard amount of carnitine. The quality of the standard may not have been as pure as it is presently. A diet which gave maximal survival up to 10 weeks and a weight of 60 mg was considered to contain 0.35 μg carnitine/gram (9). The problems of all bioassays are inherent in this method.

For development of some of the chemical methods see the review of Friedman and Fraenkel (9). The chemical method which became most widely used was that of Friedman (10). This involved esterification of carnitine and colorimetric determination of a bromophenol blue complex. However, the determination was non-specific and interference of the other quaternary amines in materials required preassay purification to remove these interfering substances.

The significant breakthrough was in the development of an enzymatic assay by Marquis and Fritz in 1964 (11). This method utilizes commercial acetyl carnitine transferase (E.C.2.3.1.7).

The reaction is:

$$\text{acetyl CoA} + \text{L-carnitine (from material)} \xrightleftharpoons[\text{Acetyl transferase}]{\text{carnitine}} \text{acetyl-L-carnitine} + \text{CoASH}$$

The CoASH produced is then measured spectrophometrically by reaction with DTNB (5,5-dithiobis-2-nitrobenzoic acid) as described in the original Marquis and Fritz method (11) or coupled to other enzyme reactions and their products measured as described by Pearson and Tubbs (12). The quantity of carnitine required in the test material is 5 to 10 nmoles. Cederblad and Lindstedt in 1972 (13) brought the quantity required down to the 10 to 20 picomole range by using 1-^{14}C-acetyl-CoA in place of acetyl CoA in the reaction above. The reaction mixture is passed through an anion exchange resin which traps the labeled acetyl CoA and passes through labeled acetyl carnitine which is counted in a liquid scintillation counter.

Since 1974, four additional methods have been published. One, published in 1974 from Brazil, is a new microbiological method using the yeast *Torulopsis bovina* (14). The second method developed in Israel is a gas chromatographic method (15) and the third published in 1976 from Dallas, Texas is a refinement for the Cederblad and Lindstedt method (16). Seccombe et al. (17) have "automated" the original Marquis and Fritz method. None of these four methods has been tested by other laboratories. The microbiological method gives higher serum values (14) for carnitine than the enzymatic (7) or enzymatic-radioisotopic methods (18), 2158 μg/dl versus 750 to 920 μg/dl, respectively. The gas chromatographic value of carnitine in seminal fluid (19) is much higher than determined enzymatically (20), 288 μg/ml versus 52 μg/ml, respectively.

The modified radiometric method proposed by McGarry and Foster (16) appears to be the most promising new technique. The problem noted with the Cederblad and Lindstedt method was that the enzyme reaction is reversible and standard curves tend to be non-linear. Bohmer et al. (5) used DTNB to trap the CoASH formed and hence partially prevent reversibility. However, linearity of the standard curves only was satisfactory if the ratio of acetyl-CoA:carnitine exceeded 5:1. The modification of McGarry and Foster (16) purports to overcome these problems of nonlinearity and reduce the amount of radioactive acetyl CoA needed. The main procedural step differences are *1*) use of tetrathionate, a CoASH oxidizing agent without inhibitory effect on the transferase and *2*) addition of the Dowex resin to the reaction mixture in an ice bath at three 10 min intervals rather than passage through a column, simplifying the steps. The supernatant of the final mixture is assayed for radioactivity. Hydrolysis

of acyl carnitines with KOH is performed in the reaction vessel prior to addition of 1-^{14}C-acetyl CoA and enzyme to determine esterified plus free carnitine, called by the authors "total" carnitine. Values of plasma carnitine were 902 μg/dl free and 1064 μg/dl "total" carnitine on one plasma sample (10 replicates). The free carnitine level is comparable to values found by Cederblad (18).

According to McGarry and Foster (16) plasma does not have to be deproteinized with perchloric acid prior to assay, both free and esterified carnitine recoveries were excellent in untreated plasma, the sample size range of 50 to 150 μl gives linear responses and choline does not interfere with the assay. However, they have tested the method only on plasma, not on tissues, urine or food. They suggested that any biological which contains significant amounts of acetyl CoA might dilute the radioisotopic acetyl CoA and hence invalidate free carnitine determination, but the hydrolysis with KOH used to liberate acyl carnitine should also hydrolyze acetyl CoA hence "total" carnitine measurements should be accurate by their method. Tissues containing carnitine acetyl transferase would require acid precipitation to remove the enzyme as well as other proteins.

Extraction procedures with trichloroacetic acid and perchloric acid of biologicals is presumed to extract free and short-chain acyl carnitines (mainly acetyl carnitine). If no alkaline hydrolysis is performed, the carnitine measured is called free carnitine. If KOH is used before assay, the carnitine so measured is called "total" carnitine as above (16) or total acid-soluble (12). Acid-insoluble carnitine or carnitine found when lipid solvent extractants are used is called variously "lipid bound" carnitine or (more often) acid-insoluble carnitine. This fraction is purported to contain long-chain acyl carnitine. In other words, when free carnitine, acetyl carnitine, and total acid-soluble carnitine content of a tissue is known the total minus free and acetyl carnitine is supposed to be other short-chain acyl carnitines, however, some C_{16} to C_{18} acyl carnitines could be present.

It will be apparent to the reader throughout this review that comparisons of carnitine values between studies are made difficult by lack of uniformity of assay procedures.

Dietary sources of carnitine

In general, carnitine is low in foods of plant origin and high in animal foods, but the data for carnitine in foods are scarce (less than 50 items) and unsatisfactory in many ways. The significant problems with these data are: *1*) old methodology—bioassays and the differences in extraction procedures; *2*) the form of carnitine reported; *3*) lack of knowledge of the effect of cooking losses; *4*) little confirmation of values when done in different laboratories; and, *5*) small number of replicates in the same laboratory.

Data have been assembled from the literature for almost 50 food items which are consumed by humans (Table 1). Avocado has the highest carnitine concentration among plant foods and sheep skeletal muscle the highest among animal foods, higher than beef muscle.

The nature of most of the experiments from which these data were compiled excluded consideration of the carnitine value of the foods to humans when cooked. For example, the data on beef and sheep were derived from the studies (21–23) where carnitine content of control animals was assayed for comparison to ketotic animals. Data from the control animals was used. Since carnitine is water-soluble, any cooking procedure using moist-heat methods would be likely to result in loss of free carnitine. High dry heat methods of cooking may or may not render carnitine and its acyl derivatives unavailable to the action of digestive enzymes.

There are no data where comparison of the bioassay and the newer enzymatic methods can be made in the same food item. In the Tenebrio bioassay, food (apparently) was not extracted in preparation for the test. Therefore, free short-chain and long-chain acyl carnitines would be measured presumably by this method. In food items listed in Table 1 where acid-insoluble (long-chain) acyl carnitines would be measured (21) it can be seen that this fraction is a very small amount of the "total" carnitine present. Therefore, in the studies where

TABLE 1
Carnitine content of foodstuffs

Food and description	Carnitine						Reference
	Free carnitine[a] A	Free plus hydrolyzed carnitine[b] B	"Lipid bound" carnitine[c] C	Bioassay (Tenebrio) D	Unknown E	"Total assayed" carnitine[d] F	
	mg/100g edible portion						
1. Avocado (mesocarp)	1.25					1.25	59
2. Alfalfa concentrate (spray dried)				2.00		2.00	8
3. Barley seed		0				0	59
4. Beef, muscle extract, concentrate, dry				4.68–29.20		4.68–29.20	8
5. Beef, tenderloin raw (psoas major)		59.80				59.80	22
6. Beef, shoulder, raw		67.40				67.40	22
7. Beef, rump, raw (gluteus median)		61.60				61.60	22
8. Beef, heart, raw		19.30				19.30	22
9. Beef, liver, raw		2.60				2.60	22
10. Beef, kidney, raw		1.80				1.80	22
11. Bread, Wasa, (Sweden)		0.24				0.24	18
12. Cabbage, head leaf		0				0	59
13. Casein, vitamin-free					1.50	1.50	24
14. Casein, acid-hydrolyzed					0.40	0.40	24
15. Castor Beans		0				0	59
16. Cauliflower		0.13				0.13	59
17. Corn seeds				0		0	8
18. Chicken, muscle, raw				4.55–9.10		4.55–9.10	8
19. Chicken, liver, raw				0.61		0.61	8
20. Egg, hen egg, before development				0		0	8
21. Egg, chicken, whole, raw and boiled		ND[e]				ND[e]	This lab.
22. Gruel (Findus) Sweden		11.70				11.70	18
Lamb, ram, sheep							
23. Ram, tenderloin, raw		162.80				162.80	22
24. Ram, rump, raw		168.50				168.50	22
25. Sheep, skeletal muscle, raw (biceps femoris)	159.73	208.98	0.28			209.26	21
26. Lamb, skeletal muscle, raw (biceps femoris)	58.16	77.44	0.53			77.97	21
27. Ram, heart, raw		59.50				59.50	22
28. Sheep, heart, raw	33.37	58.86	0.19			59.05	21
29. Ram, liver, raw		2.60				2.60	22
30. Sheep, liver, raw	1.20	2.17	<0.01			2.17	21
31. Lamb, liver, raw	1.39	2.48	0.16			2.64	21
32. Milk, cow's fluid	0.97	1.96[f]				1.96	23
33. Milk, cow's fluid		3.91				3.91	22
34. Milk, cow's fluid	0.47		0.06[g]			0.53	24
35. Milk, pig's, fluid		2.12				2.12	53

36. Milk, cow's, non-fat, dry		15.00		15.00[a]	This lab.
37. Orange juice		Presumed absent		0(?)	18
38. Peanut, seed, without skin	0.10			0.10	59
39. Peanut, embryo	0.76			0.76	59
40. Pork, liver		4.92		4.92	53
41. Rabbit, muscle, raw			21.0	21.00	8
42. Rabbit, liver, raw			11.1	11.1	8
43. Spinach, leaf, raw	0			0	59
44. Vegetable protein mixture (dry) (Soyameal, 50: rice, 30: pinto beans, 20)		ND		ND	This lab.
45. Wheat, germ		1.06		1.06	59
46. Wheat, seeds		0.35		0.35	59
47. Wheat, seeds			0.61–1.22	0.61–1.22	8
48. Yeast, torula, and brewers			1.60–3.29	1.60–3.29	8

[a] Carnitine measured without alkali treatment. [b] Carnitine measured after alkali treatment, extracted with water, acids (trichloroacetic acid and perchloric), "acid-soluble carnitine" methanol and water, and then treated with KOH. Presumed to contain free carnitine plus short chain acyl carnitines, including and mostly acetyl carnitine. [c] Carnitine measured after alkali treatment, fraction not extracted with acid, "acid-insoluble carnitine", "lipid bound", extracted with lipid solvents, chloroform presumed to be long chain acyl carnitine. [d] Columns B and C, where applicable, otherwise the value as found in the literature. Column F represents, therefore, the best estimate of the "total" carnitine present in the food. [e] Not detectable. [f] Known to be free and acetyl carnitine only. [g] Called "lipid bound" carnitine. [h] Equivalent reconstituted to fluid milk was calculated to be 1.54 mg/100 g fluid.

this fraction was not measured the total value for carnitine assayed (column F) may not be too inaccurate. There is, however, no reason to expect that the gut enzymes in the human cannot make long-chain acyl carnitine in food items available for absorption, therefore, for total accuracy this fraction should be assayed until its importance is refuted or confirmed.

The food items for which carnitine has been measured in two separate laboratories and found present is fluid cows milk. The data given in Table 1 illustrate some of the difficulties involved in interpretation of food carnitine data. Erfle's group (22) assert that only acetyl and free carnitine are present in milk. However, in their own two studies (22) and (23) total carnitine differed by a factor of 2. This could be due to different breeds of animals, different times of assay, the length of lactation or to difficulties of extrapolating from a graph (22). Wolf's group (24) measured lipid bound and total carnitine and free carnitine apparantly was calculated as total minus bound. His "total" value is very much lower than that of Erfle et al. (23), 0.53 versus 1.96 mg/100 g. In this laboratory, we measured dry skim milk powder for carnitine content and found 15 mg/100 g. If calculations for reconstituted fluid skim milk are made (28 g powder to 240 g water) the carnitine content of fluid skim milk would be 1.54 mg/100 g, a figure in line with that of Erfle but not Wolf. Fat removed in the process of skimming the whole milk would be expected to remove some short- and long-chain carnitine derivatives.

We could not detect carnitine in a vegetable protein mixture consisting of low fat soybean meal, white rice and pinto beans in the ratio of 50:30:20. (This mixture was developed to have the same amino acid composition as skim milk powder for some rat studies on carnitine and liver fat). The sensitivity of the method we used (11) is not as great as some of the newer modifications (13) which measure carnitine in the picomole range.

An interesting facet of the lower level of carnitine in plant foods in comparison to animal foods is that plant materials are also the most likely to be low in the essential

amino acids lysine and methionine. These two amino acids are the precursors of carnitine if one extrapolates from rat studies (25). Thus a pure vegetarian diet may be low not only in preformed carnitine but also may contain marginal levels of the amino acid precursors of carnitine. Mikhail and Mansour (4) suggested that the predominantly cereal diets of their Egyptian subjects may have contributed to the lower carnitine levels in serum.

Body and dietary requirements for carnitine

The daily requirements of the body for carnitine are unknown for all mammalian species including humans. Cederblad and Lindstedt (26) estimated that adult rats (160 g) consumed 113 μg of carnitine from the commerical diet and synthesized 486 μg daily with a total body pool of approximately 9100 μg. About 7% of the body pool was eliminated in urine daily, thus the dietary intake was only a fraction of the amount needed to replace urinary losses and most of the need was supplied by endogenous synthesis. (Some doubt exists as to the reliability of the above estimates, since a discrepancy occurred between kinetic data and actual urinary carnitine measured. Methyl-labeled ^{14}C carnitine was used in the experiments.)

In humans little attempt has been made to measure even "usual" dietary intake. Angelini et al. (27) suggest a range of 8 to 11 mg/day of oral intake. This estimate appears very low in view of the data on meat assembled in Table 1 but might be typical of a vegetarian diet. The lack of information may be due in part to lack of data on carnitine content in foods, but also may be due to dismissal of dietary intake as playing a significant role in overall carnitine metabolism since the body can synthesize it. Cederblad (18) fed one female a very limited diet containing 30.8 mg carnitine per day. This subject excreted 39 mg/day in urine, which indicates that endogenously synthesized carnitine was also being excreted. Such findings should not exclude dietary carnitine as playing a role in meeting the body's needs. An analogy which may be drawn is to the role played by dietary cholesterol in body cholesterol metabolism. The amount of dietary cholesterol consumed and absorbed may change the level of cholesterol synthesized in the liver. Similarly the amount of dietary carnitine absorbed may change the amount of carnitine synthesized in the liver.

Absorption

A survey of the literature has disclosed no studies, in either animals or humans, on the absorption of food carnitine. It is assumed that food carnitine is easily and completely absorbed as are other water soluble vitamins in food. The form of carnitine (free or esterified) which is absorbed is not known, nor has the mechanism of absorption across the mucosa been elucidated. The site of carnitine absorption in the gastrointestinal tract is not known.

Carnitine appears in foods as free carnitine, short-chain, and long-chain fatty acid esters of carnitine, the latter in smaller amounts than free or short-chain (predominantly acetyl) carnitine (see Table 1). Pancreatic juice contains esterases (cholesterol esterase, retinyl ester hydrolyase) and one of these enzymes or a separate enzyme may be capable of hydrolyzing carnitine esters if the free form is the molecule absorbed. If the mitochondrial membrane is assumed to be permeable to free carnitine, it is also likely that free carnitine easily passes the mucosal cell membrane. One report, however, suggests that free carnitine cannot penetrate the mitochondrial membrane (28), only carnitine esters. It is not clear if the difference between inner or outer mitochondrial membrane is considered in this report. In addition, Christiansen and Bremer (29) have shown that free carnitine is actively transported into isolated rat liver cells and it is also easily lost from the cells by washing techniques. The mechanism of mucosal absorption of carnitine needs to be investigated.

It is not known if carnitine is absorbed by the portal route or via the lymphatic system (esters). In 1966 Strack et al. (30) suggested after the feeding "^{15}N-carnitine" to humans that exogenous carnitine did not mix with endogenous carnitine. It is not stated in the report whether L- or DL-carnitine was used

but it is most likely to be the latter. Tsai et al. (31) have shown that in rats the D-form of carnitine is rapidly excreted in urine but not as rapidly as L-carnitine. The use of a racemic mixture could have confounded interpretation of ^{15}N data. Crystalline L- and DL-carnitine are absorbed by humans. Engel's groups (3) have shown that in an 11-year-old boy with "systemic carnitine deficiency" plasma carnitine levels rose following administration of 500 mg DL-carnitine. The peak in plasma carnitine level was at 3 hr after administration but was still increased above fasting levels at 8 hr; their results are reproduced here:

Plasma carnitine after administration of DL-*carnitine in an 11-year-old boy with "systemic carnitine deficiency"*

Time	Plasma carnitine
hr	*μg/dl*
Fasting	314
1/2	377
1	454
2	515
3	595
8	484

However, results on the extent of absorption as measured by serum levels cannot be extrapolated to persons with "normal" carnitine metabolism as indicated by the work of Angelini et al. (27). A large therapeutic dose of L-carnitine (15 g) was given to a 10-year-old girl with carnitine deficiency and to "a voluntary control." In the voluntary control, serum carnitine rose to a peak within ½ hr, declined at 3 hr and was maintained at that level to 6 hr, whereas, in their patient, the serum carnitine peaked at ½ hr and declined to below fasting levels at 6 hr, unlike the above 11-year-old boy. The differences between patients could be due to the form of carnitine (L- or DL-), the size of the dose (15 versus 0.5 g) or the type of disease.

Transport

It is not known how carnitine is transported in blood from the gut to the tissues. Data on six patients undergoing dialysis (5) showed that plasma carnitine declined from 543 to 185 μg/dl, a decrease of 66%, predialysis to postdialysis. This indicates that carnitine is not tightly bound to a macromolecule in blood proteins since so much was removed so rapidly in the course of dialysis. For other water soluble vitamins, riboflavin, thiamin, niacin, the preponderance of the vitamin is found in its coenzyme form in the cellular constituents of blood. This would appear not to be the case with carnitine since acyl carnitines (which could be considered a coenzyme form) are usually found in lower concentrations in tissues than free carnitine.

In their data on exit of materials from isolated rat liver cells, Christiansen and Brewer (29) found that the preferential form of carnitine released by the liver was acetyl carnitine (in addition to free carnitine). These two forms, they suggest, are the means of transporting carnitine from the liver to peripheral tissues.

Storage

Data from animal studies show that carnitine is synthesized in the liver but stored in skeletal muscle (32). The highest concentrations of carnitine are found in rats in adrenal gland followed by heart, skeletal muscle, adipose tissue, and liver. Smaller concentrations are found in kidney and brain. This pattern of carnitine concentration seems to reflect the utilization of fatty acids by the tissues as energy sources. Those tissues, such as brain, which utilize mainly glucose have the least amount of carnitine.

In humans, skeletal muscle has about 40 times the concentration of carnitine found in blood (18). Since skeletal muscle mass is about 40% of the body weight, the major portion of all carnitine in the body is in the skeletal muscle. For a 70 kg man and a 58 kg woman, one may estimate the amount of carnitine present in this muscle store as 18 and 15 g for men and women, respectively (assuming 2600 μg carnitine per gram dry weight (33) and muscle to be 76% water and equal fractional muscle masses between men and women).

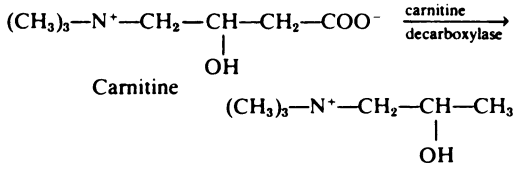
Vernon et al. (34) reported that spermatozoa have the highest level of the enzyme carnitine acetyl transferase and that the epididymis has the highest concentration of carnitine among rat organs. Levin et al. in 1976 (19) suggest that carnitine concentration in seminal fluid can be used as an index of epididymal function in tests on

infertile men. However, their carnitine levels measured by gas chromatography are much higher than findings of others (20).

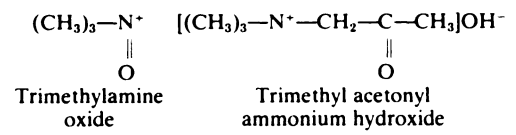
Excretion

Free carnitine is excreted in human urine. The amount excreted in urine from day to day is said to be extremely variable (35), however, in only one report was there an effort to control dietary intake (18). The latter report on a subject fed an extremely limited diet for 9 days shows less variation in urinary carnitine excretion than when an "ordinary diet" was consumed. Part of the apparent daily variation in urinary carnitine noted by researchers may be due to uncontrolled dietary intake of carnitine.

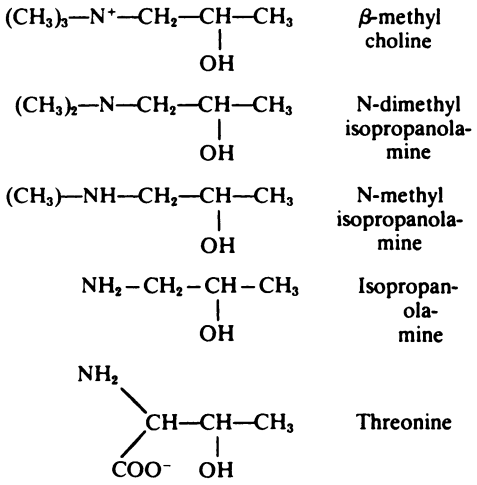
In 1967 Khairallah and Wolf (36) reported that carnitine was decarboxylated to β-methyl choline by an enzyme carnitine decarboxylase found in rat liver, kidney, muscle, and adrenals, with the largest amount in heart. From 2 to 20% of injected methyl-labeled carnitine was excreted as β-methyl choline in urine of rats depending on the dietary or physiological conditions used (in decreasing order these were: 9% amino acid diet, 30% corn oil diet, pregnancy, alloxan-diabetic rats, choline-deficient rats, 7-day starvation, cold adapted rats and a 9% casein diet). The enzyme catalyzes the following reaction:



Investigations of other urinary metabolites of carnitine have been done by Strack and coworkers (30). They found that in humans fed ^{15}N-carnitine the main excretory product was trimethylamine oxide. They have also detected small amounts of trimethyl acetonyl ammonium hydroxide in urine.



The writer has proposed (37) that carnitine may be decarboxylated to β-methyl choline, and that progressive demethylation of β-methyl choline could occur resulting in formation of N, N-dimethyl isopropanolamine, N-methyl isopropanolamine and isopropanolamine (the amine of threonine).



The scheme would conserve methyl groups in the body. The evidence supporting it is very slim. Smith and Jepson (38) injected uniformly labeled threonine into a rat and detected labeled N, N-dimethyl isopropanolamine, N-methyl isopropanolamine, and isopropanolamine as well as β-methyl choline in urine. This author is unconvinced that all the carnitine catabolic products in humans have been investigated.

Two studies (35, 39) have reported that free urinary carnitine is lower in women than in men. This has been associated with the lower muscle mass in women (35). DiMauro's group (6) also suggests that urinary excretion of carnitine is lower in children than in adults. Cederblad and Lindstedt (13) reported that more carnitine is excreted during periods of daily activity than during nocturnal sleep. These authors have suggested activity as the cause of this difference, but since carnitine is ingested in food taken during the waking hours, the difference cannot be attributed with confidence to variations in muscular activity. It is contended that the satisfactory interpretation of these and other measurements is impossible in the absence of adequate information about the dietary intake of carnitine.

Effect of diet on urinary carnitine

In our laboratory, we have suggestive evidence that diet can change urinary excretion of carnitine. (M. E. Mitchell, unpublished data).

The primary purpose of the trial was to see if we could measure urinary carnitine with the enzymatic method (11) in humans, i.e., a methodology study. The subject, a 37-year-old Oriental weighing 48 kg, 161 cm tall, consumed either a "meat diet" or a "vegetable diet" for 3 days each. Since fresh hen's egg was reported to contain no carnitine, boiled egg was included in the vegetable diet to try to increase the protein quantity and quality. This, however, was not achieved (Table 2). Although the average calculated kilocaloric intake differed only by 200 kcals on the diets, the vegetable diet contained 30 g less calculated protein and so there were less of the presumed precursors (lysine and methionine) in the diet. The subject was taking a multiple vitamin supplement containing thiamin, riboflavin, niacin, vitamin C, and iron prior to the test and continued to do so on both diets. The carnitine content of the diets was not determined. On the third day of consuming each diet, urine was collected for 24 hr. Total carnitine per 24 hr was 32.5 mg on the meat diet and 2.4 mg on the vegetable diet, a considerable difference (Table 3). Urine volume was almost the same on both diets.

The diets which were self-selected turned out in design to maximize the expected effects on urine carnitine. The meat diet was higher in protein and lysine and methionine and presumably carnitine and the vegetable diet was lower in these nutrients. This, however, is not the ideal situation to test the effects of preformed carnitine per se on urinary carnitine output since the presumed precursor concentrations were also unequal. We might infer from the work of Ansell et al. (40) that diets high in protein were concurrently high in carnitine. This very early work (1954) based on colorimetric methods showed that a protein-free diet (which was presumed to be carnitine-free) gave excretion values of 79 mg carnitine per day. A diet with 190 g protein increased urinary carnitine to 200 mg/day but was lowered to 150 mg/day when the protein was reduced to 153 g/day. It is

TABLE 2
Average daily intake[a] of nutrients from a 3-day diet designed to be high (meat diet) or low (vegetable diet) in carnitine

Dietary component	Recommended Dietary Allowances 1974	Meat diet[b]			Vegetable diet[c]		
		Food	Vitamin supplement	Total	Food	Vitamin supplement	Total
Energy, kcal	2000	1106		1106	913		913
Protein, g	46	74.2		74.2	37.8		37.8
Carbohydrate, g		124		124	130		130
Fat, g		34		34	32		32
Calcium, mg	800	614		614	299		299
Iron, mg	18	9.6	15.0	24.6	9.4	15.0	24.4
Vitamin A, IU	4000	4898		4898	5333		5333
Thiamin, mg	1.0	1.52	2.0	3.52	1.01	2.0	3.01
Riboflavin, mg	1.2	1.34	2.5	3.84	1.41	2.5	3.91
Niacin, mg	13	19.5	20.0	39.5	13.9	20.0	33.9
Vitamin C, mg	45	36.3	50.0	86.0	93.0	50.0	143.0
Sulfur amino acids, mg		2144		2144	1165		1165
Lysine, mg		4320		4320	2220		2220

[a] Subject, 37-year-old Oriental female 48 kg, 161 cm. [b] Total intake in 3 days: meat diet. milk powder, low fat, 100g; cheese, Monterey jack, 20 g; fish, raw, 20 g; salmon, 115 g; ground beef, 185g; pork, 175g; peanuts, roasted, 35g; rice crispy, 25 g; Taco shell, 75g; noodles, dry, 160 g; rice, boiled, 200 g; crackers, 15g; whole wheat bread, 60g; pineapple juice, 180 ml; orange, 120 g; carrots, 120 g; onion, 30 g. [c] Vegetable diet. Peanuts, roasted, 45g; peanut butter, 70g; kidney beans, boiled, 120 g; lima beans, fried, 60g; soybeans, boiled, 80g; green beans, 60g; corn chips, 20 g; noodles, dry, 160 g; whole wheat bread, 100 g; rice, cooked, 100 g; cabbage, raw, 460 g; carrots, 80 g; bananas, 220 g; peaches, 280 g; watermelon, 80 g; mushrooms, boiled 30 g; egg, 105 g.

TABLE 3
Carnitine excretion in urine on the third day of consuming a diet high or low in carnitine

Meal	Time	Urine collection	Urine volume	Carnitine concentrate	Total carnitine excreted
			ml	*μg/ml*	*mg*
Meat diet					
Breakfast	9 AM				
		11:20 AM	370	4.2	1.55
Lunch	12 noon				
		3:40 PM	200	27.6	5.52
Dinner	5 PM				
		6:30 PM	135	57.1	7.71
Snack	8 PM				
		10:40 PM	200	45.3	9.06
Snack	1 AM				
		1:40 AM	130	29.5	3.84
		9:40 AM	250	19.5	4.86
		Total	1385		32.54
Vegetable diet					
Breakfast	9:15 AM				
		10:10 AM	100	2.6	0.26
Lunch	12:05 PM				
		4:30 PM	170	4.7	0.80
		5:10 PM	130	1.5	0.20
Dinner	5:50 PM				
		9:20 PM	125	4.7	0.59
		6:40 AM	350	0.4	0.14
		8:50 AM	60	0.5	0.03
		Total	1105		2.37

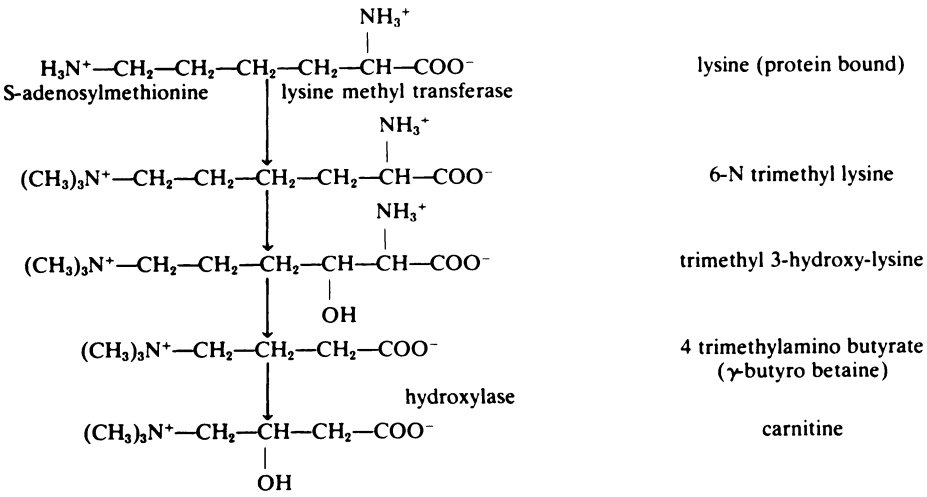
noted that the values in the last cited work are very much higher than data produced currently. The excretion of the 37-year-old Oriental subject in our laboratory on the meat diet, 33 mg/day, was comparable to the excretion of a 34-year-old Swedish subject on her "ordinary diet" 38.4 mg (18). The methodology we used, therefore, seemed adequate for determining urinary carnitine. It is emphasized that the diets used in this preliminary test were not planned to achieve a balance of the major vitamins and minerals according to the 1974 Recommended Dietary Allowances (41). The only criterion was to have animal products which might contain carnitine excluded from the vegetable diet and to select foods acceptable in taste and amount to the subject. The calculations, after the fact, revealed that the problem nutrient was calcium, 300 mg/day for the vegetable diet versus 600 mg/day for the meat diet (Table 2). Addition of cheese would have raised the calcium content of the vegetable diet but there were no data for carnitine content of cheese. Milk did contain carnitine so no dairy products were used on the vegetable diet.

Biosynthesis of carnitine

The mechanism of carnitine synthesis in humans is unknown. In 1961 Wolf and Berger (42) demonstrated, in rats, that the methyl groups of carnitine were derived from a common methyl group pool and ultimately from the essential amino acid methionine in the diet. Lindstedt and Lindstedt in 1960 to 1962 (43, 44), using rats, showed that the immediate precursor is γ-butyrobetaine which is hydroxylated in liver to form carnitine. The source of γ-butyrobetaine was not γ-amino butyric acid, however. The source of the carbon chain of carnitine was unknown in 1968 when the present writer proposed (37) a scheme for the synthesis of carnitine from threonine. This was based on support for the enzymatic steps from the literature and from the studies conducted by the writer which showed that carnitine as well as threonine and its amine, isopropanolamine, prevented liver fat accumulation in threonine-deficient rats.

The scheme was not tested. Working with *Neurospora crassa* and later with rats, Broquist and co-workers (32, 45) have shown that the carbon chain of carnitine is derived from another dietary essential amino acid lysine.

Hoppel (25, 46) suggests that in the rat, the sequence of liver synthesis is as follows:



The steps between 6N trimethyl lysine and 4-trimethylamino butyrate need confirmation. Butyrobetaine hydroxylase is found in liver but not found in kidney or muscle in mammals (47).

The synthetic pathway of carnitine does not appear to have been studied in higher species, such as primates (or man). It is very likely that carnitine is similarly synthesized from lysine and methionine in humans. (Trimethyl lysine is found in human plasma and urine (48)). From a nutrition standpoint it is important to note that lysine and methionine are two dietary essential amino acids and are often the most limiting amino acids (low lysine in cereals; low methionine in legumes) in the plant food products eaten by humans. Plant foods are also low in preformed carnitine as was shown.

Function of carnitine

The work of Fritz and associates (49–51) was instrumental in determining the function of carnitine. It is now established that carnitine plays an important role in lipid catabolism and energy production in mammals (9). Carnitine is part of the shuttle mechanism whereby long-chain fatty acids are made into acyl carnitine derivatives and transported across the mitochondrial membrane, which is impermeable to long-chain fatty acids per se and to their coenzyme A esters. Once across the membrane, the acyl carnitines are reconverted to their fatty acid CoA form and undergo β-oxidation to liberate energy. The enzyme carnitine palmityl transferase (E.C.2.3.1.21) responsible for the translocation exists in two forms, carnitine acyl (palmityl) transferase I and II. The transferase I is thought to exist on the cytoplasmic side of the inner mitochondrial membrane and its action is to form acyl carnitines:

$$\text{palmityl CoA + carnitine} \xrightarrow[\text{transferase I}]{\text{carnitine palmityl}} \text{palmityl carnitine + CoASH}$$

The palmityl carnitine crosses by an unknown mechanism to the matrix side of the inner mitochondrial membrane where the second form, transferase II, releases palmityl CoA and carnitine:

$$\text{palmityl carnitine + CoASH} \xrightarrow[\text{transferase II}]{\text{carnitine palmityl}} \text{palmityl CoA + carnitine}$$

Palmityl CoA is then available to the enzymes of β-oxidation. In fact, Bremer and coworkers (52) consider the transferase II

to be part of the β-oxidizing system in liver mitochondria.

Acyl carnitine transferase is considered the rate limiting control enzyme of the β-oxidation pathway of fatty acid catabolism in rat liver (52), however, Bieber et al. (53) did not find that the transferase was rate limiting in liver mitochondria of 1-day-old piglets. According to McGarry et al. (54) an increase in the level of substrate (carnitine) for this enzyme results from an increase in circulating glucagon (with or without insulin deficiency). They suggest that the liver carnitine increases under the influence of glucagon by *1*) increased hepatic synthesis of carnitine, *2*) increased transfer of carnitine from muscle to liver, or, *3*) increased hepatic uptake from plasma in an analogous fashion to that of amino acids.

A more controversial role of carnitine has been suggested in lipid synthesis. One theory suggested that carnitine was involved in a shuttle for transporting acetyl groups (formed during β-oxidation of pyruvate metabolism) back to the cytoplasm for fatty acid synthesis (55). The enzyme acetyl carnitine transferase (E.C.2.3.1.7) is present in tissues and in fact in its isolated form is used in the current assays for carnitine. It can effect the transport of short-chain fatty acids into mitochondria. The reverse shuttle mechanism is thought, however, to be unimportant in contrast to the citrate shuttle for transfer of acetyl CoA to cytoplasm (56, 57). Another hypothesis is that carnitine and its derivatives stimulate fatty acids synthesis directly at the stage of malonyl CoA formation (58), at least in liver tissue.

A third role of carnitine is suggested by the finding (55) that carnitine stimulates acetoacetate oxidation; thus carnitine may play a role in ketone body utilization.

The main emphasis of current research, however, is on the role of carnitine in fatty acid oxidation. Where there are changes in fat catabolism with changes in physiological state (fasting, exercise, extensive burns, pregnancy, cold adaptation) or with disease (hyperthyroidism and hypothyroidism, diabetes, muscle fat storage diseases, atherosclerosis, etc.) carnitine metabolism will be studied with increasing frequency.

References

1. Goodhart, R. S. Carnitine (vitamin B_T) metabolism In: Modern Nutrition in Health and Disease edited by M. Wohl and R. Goodhart. Chapt. 5, Philadelphia: Lea & Febiger, 1973, pp. 264–266.
2. Engel, W. K., N. A. Vick, C. J. Glueck and R. I. Levy. A skeletal muscle disorder associated with intermittent symptoms and a possible defect of lipid metabolism. New Engl. J. Med. 282: 697, 1970.
3. Karpati, G., S. Carpenter, A. Engel, G. Watters, J. Allen, S. Rothman, G. Klassen and O. Mamer. The syndrome of systemic carnitine deficiency. Neurology 25: 16, 1975.
4. Mikhail, M., and M. Mansour. The relationship between serum carnitine levels and the nutritional status of patients with schistosomiasis. Clin. Chim. Acta 71: 207, 1976.
5. Bohmer, T., A. Rydning and H. E. Solberg. Carnitine levels in human serum in health and disease. Clin. Chim. Acta 57: 55, 1974.
6. Dimauro, S., and L. Rowland. Urinary excretion of carnitine in Duchenne muscular dystrophy. Arch. Neurol. 33:204, 1976.
7. Markesbery, W. R., M. P. McQuillen, P. G. Procopis, A. R. Harrison and A. G. Engel. Muscle carnitine deficiency. Arch. Neurol. 31: 320, 1974.
8. Fraenkel, G., and S. Friedman. Carnitine. In: Vitamins and Hormones, edited by R. Harris, G. Marrian, and K. Thimann, Vol. 15. New York: Academic Press, 1957, p. 73.
9. Friedman, S., and G. S. Fraenkel. Carnitine. In: The Vitamins, 2nd ed., edited by W. H. Sebrell and R. S. Harris. Vol. 5, New York: Academic Press, 1972, p. 329.
10. Friedman, S. Determination of carnitine in biological materials. Arch. Biochem. Biophys. 75: 24, 1958.
11. Marquis, N. R., and I. B. Fritz. Enzymological determination of free carnitine concentrations in rat tissues. J. Lipid Res. 5: 184, 1964.
12. Pearson, D. J., and P. K. Tubbs. Carnitine and derivatives in rat tissues. Biochem. J. 105: 953, 1967.
13. Cederblad, G., and S. Lindstedt. A method for the determination of carnitine in the picomole range. Clin. Chim. Acta 37:235, 1972.
14. Travassos, L. R., and C. O. Sales. Microbiological assay of carnitine. Anal. Biochem. 58: 485, 1974.
15. Lewin, L. M., A. Peshin and B. Sklarz. A gas chromatographic assay for carnitine. Anal. Biochem. 68: 531, 1975.
16. McGarry, J. D., and D. W. Foster. An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J. Lipid Res. 17:277, 1976.
17. Seccombe, D., P. Dodek, J. Frohlich, P. Hahn, J. Skala and D. Campbell. Automated method for L-carnitine determination. Clin. Chem. 22: 1589, 1976.
18. Cederblad, G. Plasma carnitine and body com-

position. Clin. Chim. Acta 67: 207, 1976.
19. LEWIN, L., R. BEER AND B. LUNENFELD. Epididymis and seminal vesicle as sources of carnitine in human seminal fluid: the clinical significance of the carnitine concentration in human seminal fluid. Fertil. Steril. 27: 9, 1976.
20. FRENKEL, G., R. N. PETERSON, J. E. DAVIS AND M. FREUND. Glycerylophosphorylcholine and carnitine in normal human semen and in postvasectomy semen: difference in concentrations. Fertil. Steril. 25: 84, 1974.
21. SNOSWELL, A. M., AND P. KOUNDAKJIAN. Relationships between carnitine and coenzyme A esters in tissues of normal and alloxan-diabetic sheep. Biochem. J. 127:133, 1972.
22. ERFLE, J. D., F. D. SAUER AND L. J. FISHER. Inter-relationships between milk carnitine and blood and milk components and tissue carnitine in normal and ketotic cows. J. Dairy Sci. 53: 486, 1970.
23. ERFLE, J. D., L. J. FISHER AND F. SAUER. Carnitine and acetylcarnitine in the milk of normal and ketotic cows. J. Dairy Sci. 53: 671, 1970.
24. ABDEL-KADER, M., AND G. WOLF. The distribution in carnitine and its possible function in corticosteroid biosynthesis. In: Recent Research on Carnitine, edited by G. Wolf, Cambridge: MIT Press, 1965, p. 147.
25. COX, R. A., AND C. L. HOPPEL. Carnitine and trimethylaminobutyrate synthesis in rat tissues. Biochem. J. 142: 699, 1974.
26. CEDERBLAD, G., AND S. LINDSTEDT. Metabolism of labeled carnitine in the rat. Arch. Biochem. Biophys. 175: 173, 1976.
27. ANGELINI, C., S. LÜCKE AND F. CANTARUTTI. Carnitine deficiency of skeletal muscle: report of a treated case. Neurology 26: 633, 1976.
28. BRESSLER, R., C. CORREDOR AND K. BRENDEL. Hypoglycin and hypoglycin-like compounds. Pharmacol. Rev. 21: 105, 1969.
29. CHRISTIANSEN, R., AND J. BREMER. Active transport of butyrobetaine and carnitine into isolated liver cells. Biochim. Biophys. Acta 448: 562, 1976.
30. GRUNER, E., H. AURICH AND E. STRACK. Uber das vorkommen von trimethylacetonylammoniumhydroxyd im menschlichen harn. Hoppe-Seylers Zeitschr. F. Physiol. Chem. 343: 240, 1966.
31. TSAI, A. C., D. R. ROMSOS AND G. A. LEVEILLE. Determination of carnitine turnover in choline-deficient and cold-exposed rats. J. Nutr. 105: 201, 1975.
32. TANPHAICHITR, V., AND H. P. BROQUIST. Site of carnitine biosynthesis in the rat. J. Nutr. 104: 1669, 1974.
33. CEDERBLAD, G., S. LINDSTEDT AND K. LUNDHOLM. Concentration of carnitine in human muscle tissue. Clin. Chim. Acta 53: 311, 1974.
34. VERNON, R. G., V. L. W. GO AND I. B. FRITZ. Testicular carnitine acetyltransferase (CAT); an enzyme marker for spermatocyte formation. Federation Proc. 30: 3, 1971. (abstr. 438).
35. CEDERBLAD, G., AND S. LINDSTEDT. Excretion of L-carnitine in man. Clin. Chim. Acta 33: 117, 1971.
36. KHAIRALLAH, E. A., AND G. WOLF. Carnitine decarboxylase. J. Biol. Chem. 242: 32, 1967.
37. MITCHELL, M. E. The role of threonine in the reduction of liver fat. Ph.D. thesis. Cornell University, 1968.
38. SMITH, A. D., AND J. B. JEPSON. The identification in urine of l-amino-propan-2-ol metabolically derived from threonine. Biochem J. 89: 41P, 1963.
39. MAEBASHI, M., N. KAWAMURA AND K. YOSHINAGA. Urinary excretion of carnitine in progressive muscular dystrophy. Nature 249: 173, 1974.
40. ANSELL, B., P. K. BHATTACHARYYA, D. RIX AND R. M. KARK. Metabolism of carnitine in man. Clin. Res. Proc. 2: 79, 1954.
41. Recommended Dietary Allowances, 8th ed. National Academy of Sciences, National Research Council, 1974.
42. WOLF, G., AND C. R. A. BERGER. Studies on the biosynthesis and turnover of carnitine. Arch. Biochem. Biophys. 9: 360, 1961.
43. LINDSTEDT. G., AND S. LINDSTEDT. On the biosynthesis and degradation of carnitine. Biochem. Biophys. Res. Commun. 6: 319, 1961.
44. LINDSTEDT, G., AND S. LINDSTEDT. On the hydroxylation of gamma-butyrobetaine to carnitine in vitro. Biochem. Biophys. Res. Commun. 7: 394, 1962.
45. TANPHAICHITR, V., M. S. ZAKLAMA AND H. P. BROQUIST. Dietary lysine and carnitine: relation to growth and fatty liver in rats. Federation Proc. 32: 884, 1973.
46. HOPPEL, C. L., R. NOVAK AND R. COX. 6-N-trimethyllysine metabolism and carnitine biosynthesis. Federation Proc. 35: 1478, 1976. (abstr. 624).
47. DIMAURO, S., C. SCOTT, A. S. PENN AND L. P. ROWLAND. Serum carnitine. Arch. Neurol. 28: 186, 1973.
48. COX, R. A., AND C. L. HOPPEL. Biosynthesis of carnitine and 4-N-trimethylaminobutyrate from 6-N-trimethyl lysine. Biochem J. 136: 1083, 1973.
49. FRITZ, I. B., AND B. MCEWEN. Effects of carnitine on fatty acid oxidation by muscle. Science 129: 334, 1959.
50. FRITZ, I. B., S. K. SCHULTZ AND P. A. SRERE. Properties of partially purified carnitine acetyltransferase. J. Biol. Chem. 238: 2509, 1963.
51. DELISLE, G., AND I. B. FRITZ. Interrelations between hepatic fatty acid oxidation and gluconeogenesis: a possible regulatory role of carnitine palmityltransferase. Proc. Nat. Acad. Sci. 58: 790, 1967.
52. LUMENG, L., J. BREMER AND E. DAVIS. Suppression of mitochondrial oxidation of (-) palmityl carnitine by the malate-aspartate and α-glycerophosphate shuttles. J. Biol. Chem. 251: 277, 1976.
53. BIEBER, L. L., A. K. MARKWELL, M. BLAIR AND T. A. HELMARATH. Studies on the development of carnitine palmityltransferase and fatty oxidation in liver mitochondria of neonatal pigs. Biochem. Biophys. Acta 326: 155, 1973.
54. MCGARRY, J., C. ROBLES-VALDES AND D. FOSTER. Role of carnitine in hepatic ketogenesis.

Proc. Nat. Acad. Sci. 72:4385, 1975.
55. BRESSLER, R., AND R. I. KATZ. The role of carnitine in acetoacetate production and fatty acid synthesis. In: Recent Research in Carnitine: its Role in Lipid Metabolism, edited by G. Wolf. Cambridge: MIT Press, 1965, p. 65.
56. LOWENSTEIN, J. M. A comparison of acetyl group utilization from various precursors. In: Recent Research in Carnitine: its Role in Lipid Metabolism, edited by G. Wolf. Cambridge: MIT Press, 1965, p. 97.
57. LEHNINGER, A. L. Biochemistry. New York: Worth Publishers, Inc., 1970, p. 515.
58. FRITZ, I. B., AND M. P. HSU. Studies on the control of fatty acid synthesis. J. Biol. Chem. 242: 865, 1967.
59. PANTER, R. A., AND J. B. MUDD. Carnitine levels in some higher plants. FEBS lett. 5: 169, 1969.