The Discovery of a Vitamin Role for Carnitine: The First 50 Years

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The discovery of carnitine as an essential nutrient for one species of insect led rapidly to the elucidation of its central role in fat oxidation and its function in mitochondria. The history of this discovery illustrates how an unexpected result in one extremely narrow area of nutrition can lead to the opening up of an entire field in basic metabolism.

Carnitine belongs to a special class of nutrients termed "quasi-vitamins" or "conditionally-essential" nutrients (1). These nutrients include taurine, lipoic acid, choline, and carnitine. They are normally synthesized by the mammalian organism, but may be required under special conditions, such as during long-term parenteral nutrition, by hemodialysis patients, or by premature infants. Choline, for instance, appears to be essential for adult men (2). Carnitine is used as a drug in cases of carnitine deficiency syndrome and is available as a dietary supplement; it is advertised as an aid to weight loss and improved exercise performance.

As pointed out by Fraenkel and Friedman (3), the history of research into carnitine falls into 4 periods: first, the period of its simultaneous discovery as a constituent of vertebrate muscle, by Gulewitsch and Krimberg (4) and by Kutscher (5) in 1905; then, the period in which its chemical structure was established (6) (~1927); and next, the delineation of its major physiological function (1935–1965). Finally, the discoveries of its biosynthetic pathway, transport mechanisms, and primary and secondary carnitine deficiency and syndromes, occurred from 1961 to the present. Investigations of its metabolic role began in the 1940s, as a result of studies by Fraenkel (7) (**Fig. 1**) of the nutritional requirements of insects. What insects eat is not only of interest from a purely scientific viewpoint, but is, of course, of the greatest importance to agriculture in the search for ways to protect crops from insect damage.

Early work on insect nutrition, reviewed by Trager (8,9), established that the basic food needs of insects were proteins, carbohydrates, minerals, and accessory food factors. Fraenkel and Blewett (10), working at the Imperial College in London, beginning in 1943, set out to determine the vitamin and sterol requirements of insects. For their studies, they selected 6 different insect species that were pests found in flour, 5 flour beetles and 1 species of moth. They all thrived on a diet of whole-wheat flour. Each test was performed with 20 larvae. Insects were fed a diet of purified casein (41%), glucose (41%), McCollum's salt mixture (1%), cholesterol (1%), yeast or the equivalent of yeast extract (4%), and water (12%). The authors gave no explanation for using such high levels of protein beyond stating: "We do not know whether 15-20% casein would be sufficient…To be on the safe side, the quantity of protein was increased" (10). In ear-lier work, McCay (11) used insect diets containing between 15 and 30% casein. The total number of pupae formed from the larvae, or adults surviving, was plotted against time, resulting in curves of different steepness, according to the completeness of the diets (**Fig. 2**). Both the soluble and the insoluble fractions of yeast were found to be essential, as was cholesterol. Although whole-wheat flour does not contain cholesterol, earlier work by the authors (12) determined that the related steroid sitosterol, present in the wheat germ (13), was as effective as cholesterol for survival of the insects. Fat was not required. In later work (14), it was established that yeast could be replaced by a mixture of thiamin, riboflavin, nicotinic acid, biotin, and folic acid. The fat-soluble vitamins A, D, E, and K were not required. Omission of choline resulted in a somewhat reduced growth rate. One particular species of mealworm, *Tenebrio molitor,* required an additional substance for survival.

Figure 1 Gottfried S. Fraenkel (1901–1984).

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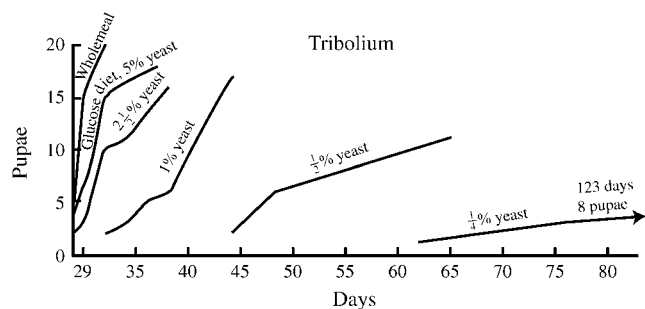
Figure 2 Growth of *Tribolium confusum* using a diet consisting of casein, glucose, cholesterol, salts, and water, with the addition of graded quantities of dried brewers' yeast. The total number of pupae formed is plotted against time [reproduced with permission from (10)].

Green leaves are the principal and usually the only food of >50% of known insect species. Fraenkel argued (15) that leaves must contain all of the nutrients required by insects. Yet insects are selective in the choice of plant on which they feed. To explain this selectivity, Fraenkel (7) considered 2 hypotheses. The first assumed that different plants have different chemical compositions and that a particular plant has a nutrient content to match the food requirement of a particular insect. Then "insects would be specific on those plants which meet their dietary needs" (7). The second hypothesis assumed that the basic dietary requirements are the same for all insects and that these requirements can be met by most green leaves. From this it would follow that the so-called "secondary plant substances" present in leaves, i.e., glucosides, saponins, tannins, alkaloids, essential oils, organic acids, and many others characteristic of different families of plants, have no nutritional value.

Fraenkel compared the dietary requirement of insects that he and others had determined, to the composition of insect foods. He found (16) as did others (17,18) that the larvae of several insect species thrived when fed a diet of casein or an amino acid mixture of the same composition as casein. When the amino acids tryptophan, leucine, isoleucine, histidine, lysine, valine, phenylalanine, threonine, arginine, or histidine were left out of the mixture, growth of the larvae was prevented and pupation time was greatly increased. Lemond and Bernard (19), studying the larvae of the flour beetle *Tribolium confusum*, concluded that "the amino acids known to be essential for growth in the rat are also essential of growth and pupation of the larvae of *T. confusum*" (**Table 1**).

TABLE 1 Essential amino acid composition

	Insect diet[1]	Cabbage	Spinach	Leaves[2]	Casein[3]
Arginine	2.4	7.3	2.7	4.0	4.2
Histidine	4.1	2.1	1.1	4.1	3.2
Lysine	7.0	3.9	3.0	5.8	8.5
Leucine	7.1	3.7	4.3	6.2	10.0
Isoleucine	4.7	3.1	2.4	4.4	7.5
Methionine	3.3	1.3	0.9	1.2	3.5
Phenylalanine	3.5	1.9	2.6	3.5	6.3
Threonine	4.1	2.8	2.4	3.8	4.5
Tryptophan	2.4	0.9	2.1	1.1	1.3
Valine	5.9	4.0	3.0	4.9	7.7

[1] The essential amino acid composition was that used by Lemonde and Bernard (19) and was the same as the rat diet of Rose et al. (20).
[2] The essential amino acid composition was adapted from Lyman and Kuiken (21).
[3] The essential amino acid composition was adapted from Block (22).

When we consider now the protein present in leaves, it becomes clear that leaves provided all of the amino acids required by insects. Fraenkel (7) suggested that their composition corresponded approximately to the amino acid composition of casein (Table 1), with some exceptions. He further showed that the amino acid composition of protein in the leaves of 14 species of forage plants, belonging to 3 different families, was similar and contained all of the amino acids required by insects in favorable proportions.

Fraenkel (7) summarized his conclusions as follows: *1*) a representative number of insect species requires the same nutrients in very similar proportions; *2*) green leaves contain protein, carbohydrate, minerals, and at least 8 water-soluble vitamins roughly in the proportions required as nutrients by insects; *3*) the composition of these nutrients is very similar in a wide variety of plants; and *4*) therefore, the secondary plant substances, in which plants differ greatly, cannot be regarded as insect nutrients. Most probably, they act as chemical sensory stimuli to determine which species of plants are selected by particular insect species, as earlier suggested by Delthier (23).

As mentioned above, in his investigation of the vitamin requirement of insects, Fraenkel found that 1 species, and 1 only, *Tenebrio molitor*, required an additional factor present in yeast or yeast extract, in addition to the usual B vitamins. Fraenkel et al. (24) then undertook an investigation of the additional requirements of *Tenebrio*. In his experiments, he grew 10–20 larvae for 12 wk in 2-oz (60-mL) bottles with 3 g food, each larva weighing initially ~0.5 mg. Under optimal conditions, they grew to ~60–100 mg. Their glucose or starch requirement was unexpectedly high (80%), in addition to purified casein (10%), cholesterol (1%), and salts (2%). The composition of this diet was much closer to the composition of flour than the diet used in their earlier experiments (10) (cf. whole-wheat flour: protein, 13%, carbohydrate, 71%). The vitamins thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, and folic acid were essential. The missing factor was obtained by extracting yeast or liver with 50% acetone:water, treating the extract with charcoal at pH 3, and filtering (25). The filtrate was designated "charcoal filtrate" and corresponded to ~0.6% of dried liver solids. For optimal growth of the larvae, 3 μg of the "charcoal filtrate" was added for each gram of food (**Fig. 3**). Tentatively, the "charcoal filtrate" factor was named vitamin B_T (for *Tenebrio*).

Vitamin B_T was found to be required exclusively by 7 members of the species of the insect family *Tenebrionidae*. Friedman and Fraenkel (26) concluded that because 4 genera belonging to 2 different subfamilies required this factor for survival, "the ability to synthesize it must have been lost early in the evolution of this family." Despite some disputed claims, there appears to be no record of any other species of insect, or indeed of any other organism, with an absolute requirement for carnitine, with the exception of 2 microorganisms: *Pediococcus soyae* and a carnitine-deficient mutant of yeast, *Torulopsis (Candida) bovina* (27).

In 1951, Fraenkel (28) found that although materials of vegetable origin, e.g., corn or wheat germ, contain some carnitine, it was abundant in animal sources such as liver, lung, plasma, and especially milk or whey. It could be concentrated 300-fold from whey by extraction with phenol.

In 1952, Carter et al. (29) purified the phenol extract further by chromatography on alumina and by Craig countercurrent distribution. A crystalline hydrochloride was thus obtained. The free base was fully active as vitamin B_T in the *Tenebrio* assay at a concentration of 0.37 μg/g food. Its empirical formula was
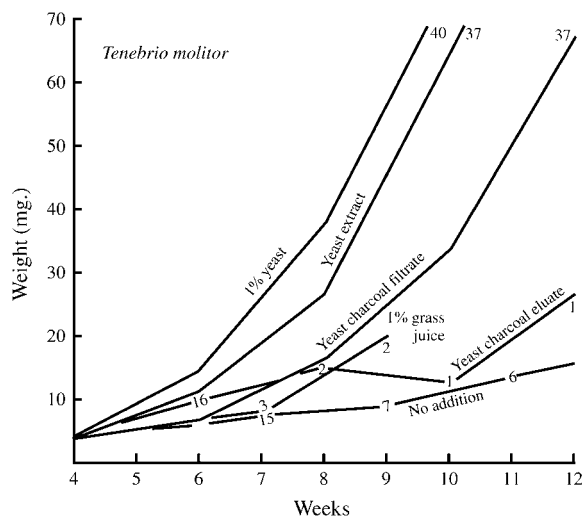
Figure 3 Growth of *Tenebrio molitor* using the pure vitamin diet with the addition of yeast, yeast extract, a charcoal filtrate or eluate from yeast extract, or grass juice. The numbers on the curves indicate the numbers alive, of 40 larvae. The charcoal filtrate, prepared as described in the text, contained the vitamin B_T [reproduced with permission from (39)].

determined to be $C_7H_{15}NO_3$. It yielded trimethylamine upon alkaline degradation and crotonobetaine when dehydrated by sulfuric acid. These properties, together with the empirical formula, led the investigators to the conclusion that vitamin B_T and carnitine were identical (**Fig. 4**). This conclusion was confirmed by testing the product of its chemical synthesis (30).

The first indication of a connection between carnitine and fat metabolism was obtained by MacFarlane working in Fraenkel's laboratory (31). He divided a group of half-grown *Tenebrio* larvae into 2 subgroups: one was fed a synthetic diet lacking carnitine, and the other was not given any food. After some time, he analyzed both groups for protein, carbohydrate, and fat content and found that both subgroups were depleted in protein and carbohydrate. The starved larvae's fat content was also depleted to a low level, whereas the fat in the subgroup fed a carnitine-deficient diet had not declined significantly. This observation indicated an involvement of carnitine in fat oxidation. At the same time, Friedman and Fraenkel (32) showed that pigeon or sheep liver extracts contained an enzyme that reversibly acetylated carnitine in a mixture containing ATP, Mg^{++}, and CoA, whereas Fritz (33) demonstrated that added carnitine stimulated the oxidation of palmitic acid by muscle extracts.

Fatty acids serve as the principal energy source for the animal kingdom. The importance of carnitine in the energy economy apparently lies in its role in fatty acid oxidation. The extensive investigations of Fritz and his co-workers (34) established the metabolic function of carnitine (as L-carnitine) in facilitating the transport of fatty acids into mitochondria, the site of their oxidation. Separate enzymes reversibly catalyze the formation of short- and long-chain fatty acyl L-carnitine esters, which are transported across the mitochondrial membranes (35).
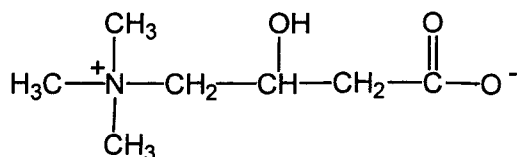

Figure 4 DL-Carnitine.

The primary metabolic function of carnitine in fatty acid oxidation currently established is as follows [reviewed in (36)]. The first step in long-chain fatty acid oxidation is the formation of long-chain acyl CoA by long-chain acyl CoA synthase located in the outer mitochondrial membrane facing the cytosol. There, long-chain carnitine acyltransferase I forms long-chain acylcarnitine by the reaction of acyl CoA with free carnitine, both residing in the cytosol. The acylcarnitine is then transported across the outer mitochondrial membrane into the mitochondrial matrix by the enzyme carnitine:acyl-carnitine translocase. In the matrix, the inner-mitochondrial carnitine acyltransferase II reconverts the acylcarnitine into carnitine and acyl CoA. The latter is then ready for oxidative breakdown. Thus, carnitine plays an essential role in the transfer of fatty acids for the purpose of their oxidation from the cytosol into the mitochondria. In addition to its role in the facilitation of long-chain fatty acid transport across the mitochondrial inner membrane, several other functions for carnitine have now been identified, such as in shuttling chain-shortened products produced by β-oxidation out of peroxisomes (37).

Knowledge of the metabolic functions of carnitine has found important applications in modern medicine. Carnitine deficiency disorders, particularly in children, were identified and described as a result of mutations [reviewed in (38)]. These can be primary, caused by a mutation in genes coding for carnitine transport and leading to a loss of carnitine in urine, or secondary, as consequences of mutations in genes for one of the enzymes of fatty acid oxidation. These metabolic errors cause blocking of acylcarnitine intermediates, resulting in decreased plasma levels of carnitine. The patients present with progressive cardiomyopathy and skeletal muscle weakness. The disorder can be cured by administration of pharmacologic doses of carnitine.

In looking back at the development of our knowledge of carnitine in metabolism, one can see how the discovery of the vitamin status of carnitine in one species of insect led to our understanding of the process whereby fat supplies energy to the organism.

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