STUDIES ON THE DISTRIBUTION OF VITAMIN B_T (CARNITINE)

G. FRAENKEL

Department of Entomology, University of Illinois, Urbana, Illinois

The effect and isolation of a new vitamin of the B-complex, B_T, which is required by the mealworm and certain other beetle larvae, and its identification as carnitine has previously been reported (Fraenkel *et al.*, 1948; Fraenkel, 1951a, 1951b; Carter *et al.*, 1952). It was stated that B_T had a wide distribution in biological materials, that materials of animal origin were better sources than vegetable matter, and that the muscles of mammals constituted by far the richest known source. Carnitine was originally discovered in commercial meat extracts in which it occurs in amounts of one to two per cent, and was not known to be a general constituent of living systems. Heretofore, the only available method of assay was by isolation. Thus it was impossible to assay for carnitine in substances which contain it in quantities smaller than that found in muscle. With the development of a much more sensitive testing method, using growth and survival of *Tenebrio* as the criterion (Fraenkel, 1951a), it has now become possible to assay for carnitine in a great variety of biological materials.

The dietary need for carnitine has so far been demonstrated only for three beetle larvae, the yellow mealworm, *Tenebrio molitor,* the black mealworm, *Tenebrio obscurus,* and another related species, *Palorus ratzeburgi.* The present paper concerns the levels of carnitine in a great variety of sources, animal and vegetable, and also gives evidence of a synthesis of carnitine in organisms which can develop in the absence of carnitine. Throughout this paper the terms "carnitine" and "B_T" will be used almost synonymously; the possibility exists, however, that carnitine may not be the only naturally occurring substance with B_T activity. We are, therefore, assaying, strictly speaking, for B_T activity and not for carnitine.

Methods of Assay and Tabulation

The methods of assaying and expressing results have been previously described in detail (Fraenkel *et al.*, 1950; 1951a). Briefly, *Tenebrio* larvae grow slowly and begin to die after 3–4 weeks on a "synthetic" diet consisting of 20 parts casein, 80 parts glucose, one part cholesterol, two parts McCollum's salt mixture No. 185, 10 parts water and the following vitamins of the B-complex (expressed as μg./g. of the dry diet): thiamin 25, riboflavin 12.5, nicotinic acid 50, pyridoxin 12.5, pantothenic acid 25, choline chloride 500, inositol 250, folic acid 2.5 and biotin 0.25. When a good source of B_T or 0.35 μg./g. carnitine is added to the "basic" diet, the larvae grow at a steady rate and survive.

Table I shows the result of an experiment with graded doses of carnitine. It can be seen that survival is optimal at levels of 0.375 μg./g., while the weights still increase at levels of 0.75 and 1.50 μg. Growth and survival at the 50 μg. level are virtually identical with that at 1.5 μg. In all our determinations we have fol-

lowed a procedure whereby, in a series of graded doses, that diet which allows optimal survival and good, but not necessarily optimal growth, is assumed to contain 0.35 μg. carnitine/g. dry diet.

With levels of 0.35 μg. carnitine per gram, larvae reach an average weight of about 60 mg. after 9–10 weeks of growth at 29–30 ° C. and 60% relative humidity This level of carnitine is sufficient to maintain normal growth only up to this point. Subsequently, growth gradually slows down and the larvae may develop signs of a B_T deficiency. In order to reach a normal weight of 140 mg., after a period of 13 weeks, and to pupate and emerge as normal adults, a level of 1.5 μg. carnitine per gm. diet is required (unpublished data). To base assays on optimal growth and development to the adult stage, experiments would have to be continued for 25 weeks and would also require two to three times as much food. In order to economize in time and food consumption, the assays were all based on the 9 weeks-60 mg. criterion.

TABLE I

Numbers surviving and average weights of Tenebrio larvae on the basic diet and with the addition of yeast or graded doses of carnitine. Twenty larvae were used in each of the control diets and 60 larvae with each concentration of carnitine; at 30° C. and 60% relative humidity

Weeks	Control diets				μg. carnitine/g. of the dry diet									
	No addition		2% yeast		0.19		0.375		0.75		1.5		50	
	Nos.	Mg.	Nos.	Mg.	Nos.	Mg.	Nos.	Mg.	Nos.	Mg.	Nos.	Mg.	Nos.	Mg.
5	9	7.4	17	9.5	45	8.3	48	8.9	48	10.7	50	10.0	50	10.2
6	6	9.7	17	15.0	42	13.3	48	14.9	46	19.3	49	19.1	50	19.4
7	5	12.2	16	23.5	40	21.3	47	22.3	46	29.6	47	29.8	50	29.2
8	4	13.2	15	31.4	38	24.0	47	31.8	46	42.2	47	41.0	48	45.6
9	4	16.2	15	39.7	36	34.6	47	42.5	45	59.5	47	57.6	48	59.2
10	3	24.3	15	50.7	34	47.5	46	56.0	45	64.6	47	72.5	48	74.6

In assaying different materials for a particular substance, it is important to consider the form in which they exist in the diet. Only finely ground powders, such as flour, dried egg or dried milk, can be directly mixed into the diet in the dry state. In most cases, the materials have to be either homogenized in water, with the use of the Waring Blendor, or Potter-Elvehjem homogenizer, or else prepared as extracts. All such materials are then pipetted into the diets in the required amounts and dilutions. To avoid losses in the extraction process, it is always preferable to use homogenates. The Potter grinder homogenizes soft tissues well, and more resistant tissues can often be homogenized with the Waring Blendor. Wherever a tissue did not lend itself to homogenation suitable for pipetting, extracts had to be prepared. In such cases, tissues were first homogenized or ground in a mortar and then extracted with several portions of boiling water for periods of 30 minutes each, and the combined extracts concentrated to the desired volume. In some cases alcohol was added to aqueous extracts to make a concentration of 75 to 80%. The ensuing precipitates were removed by centrifugation and the alcohol evaporated off over a water bath.

In expressing results, a different procedure was followed with powdered materials or whole homogenates, than with extracts. In the former cases, amounts are expressed as percentages of the dry materials, added to the diet. With extracts, levels are often calculated as percentages of the dry, or in rare instances, wet materials from which the extracts were derived.

Experiments

1. *The B_T content of microorganisms (Table II)*

The effect of B_T was originally discovered in experiments with *Tenebrio* larvae which failed to grow on synthetic diets, but which grew well after two per cent yeast had been added. The B_T content of yeast seems to vary in different preparations. Two per cent dry brewers yeast in the diet, the level routinely used in our

Table II

The B_T content of several microorganisms and materials of vegetable origin

	Minimum amount to give full activity	Maximum amounts tested and found inactive	B_T μg./g. solids	Description of preparation
Microorganisms:				
Escherichia coli I[1]		5%	<1*	Grown on synthetic medium
Escherichia coli II[1]		5%	<1*	Grown on synthetic medium
Escherichia coli III[2]		5%	<1*	Grown on synthetic medium
Streptococcus hemolyticus[2]	1.75%		28	Medium not entirely synthetic
Torula yeast	1–2%		17.5–35	
Brewers yeast I	2–4%		8.8–17.5	Anheuser-Busch
Brewers yeast II	1%		35	Anheuser-Busch
Brewers yeast III	1%		35	Anheuser-Busch
Brewers yeast IV	1–2%		17.5–35	Nutritional Biochemicals
Neurospora[3]	1.25		28	Grown on synthetic medium
Tetrahymena geleii[4]		5%	<1*	Grown on synthetic medium
Vegetable matter:				
Wheat	2.5–5%		7–14	
Corn		10%	<0.5*	
Alfalfa concentrate[5]	1.8%		20	Spray-dried
Rye juice[5]		1.8%	<2*	Spray-dried
Oats juice[5]		1.8%	<2*	Spray-dried

Prepared by: [1] I. Gunsalus, [2] Sharp & Dohme, [3] E. L. Tatum, [4] G. W. Kidder, [5] Cerophyl Laboratories.

* Possibly none.

control experiments, usually ensures optimal survival, but the larvae may still be somewhat underweight (Table I). The B_T activity of three different strains of brewers yeast, obtained from Anheuser-Busch, varied between 1% and 4%. The B_T content of *Torula* yeast and of *Neurospora* was of the same order as that of brewers yeast.

Two of the microorganisms tested, *Escherichia coli* and *Tetrahymena geleii*, were entirely devoid of B_T activity when included in the diet in levels up to 5%. Full activity at this level would have indicated a carnitine content of 7 μg./g., and

there would have been an indication of activity had the carnitine content been only 1 μg./g. It would be difficult to test for such small quantities, because of the large proportion, 40% and more, of assay material, which would have to be included in the diet. Both these organisms were cultured in synthetic media which in all probability were free of B_T. We may, therefore, conclude that these two organisms neither require nor synthesize carnitine, or synthesize it in amounts below the sensitivity of our assay method. On the other hand, *Streptococcus hemolyticus* from a medium which was not entirely synthetic showed B_T activity at the 1.25% level.

2. The B_T content of vegetable matter (Table II)

It was previously shown (Fraenkel, 1951a) that coconut meal, peanut meal and cotton seed meal failed to show any B_T activity in concentrations up to 3% of the diet. The B_T content of all vegetable matter which has so far been tested was low. Of cereal seeds, wheat showed good activity in concentrations of 2.5 to 5%, but corn was inactive in amounts up to 10%. Of a few samples of juices which had been pressed out of young plants and spray-dried, alfalfa juice was active at a level of 1.8%, while rye and oat juice failed to show any activity at the same level.

3. B_T requirements and B_T contents of insects

A dietary requirement for carnitine has so far been demonstrated for three insect species only, *Tenebrio molitor, Tenebrio obscurus* and *Palorus ratzeburgi.* The larvae of at least 15 other species of insects have been successfully grown on basic diets, similar to that used for *Tenebrio,* in the absence of carnitine. The question then arose, whether carnitine was present in the tissues of insects grown in its absence. It would also be of interest to know to what extent the amount of carnitine present in the tissues of *Tenebrio* larvae corresponded to the levels which had been fed in the diet.

a. Carnitine content of Tenebrio larvae

1. *Larvae grown largely in the absence of carnitine.* These larvae had been grown for the first three weeks on the deficient basic diet, then for 10 days on a normal optimal diet (whole meal plus 5% yeast) and subsequently again on the synthetic deficient diet. They continued to grow for a period of about 7 weeks until they reached a weight of 40–60 mg., then stopped growing and ultimately died. Extracts from these deficient larvae, incorporated in diets in amounts corresponding up to 4% larval bodies, failed to show any B_T activity.

2. *Larvae grown on an optimal diet, consisting of wholemeal flour plus 5% yeast* (Table III). Extracts from these larvae, incorporated in diets in quantities corresponding to 1% of dry larval bodies, gave good growth and almost optimal survival. The result indicated that 2% would have ensured optimal survival.

One gram of wheat flour contains 7 μg. B_T (Table II) and the addition of 5% yeast (containing 17.5 B_T/g.) only insignificantly increases that amount. The growing *Tenebrio* larva eats food in amounts of about 4.5 times its ultimate dry body weight. Thus a nearly full-grown larva, which weighs about 100 mg. (40 mg. dry weight), has consumed 180 mg. of food, containing 1.3 μg. of carnitine. Therefore, 32.5 μg. carnitine are consumed per gram dry weight of larvae. If all this

carnitine were retained in the body of the larva, a diet with 2% dried larvae added as the sole source of carnitine would contain 0.65 μg. carnitine per gram. However, larvae grown on this particular diet only showed optimal activity. Since 0.35 μg. B_T is the minimal amount required for optimal activity, it may be assumed that the diet actually contained only 0.35 μg. carnitine per gram. Thus the actual carnitine content of the dried larvae would be 17.5 μg./g. The growing larvae therefore retained almost half the carnitine which they took up in the food.

3. *Larvae grown on graded levels of carnitine.* In one experiment, extracts from *Tenebrio* larvae, which had been grown on diets containing 0.37, 0.75 and 1.5 μg. B_T per gram, were incorporated in diets and tested in quantities correspond-

TABLE III

The B_T content of two beetle larvae, Dermestes vulpinus and Tenebrio molitor, grown in the presence and absence of B_T. Tested as aqueous extracts in their effect on growth and survival of Tenebrio larvae after 9 weeks

	Concentration in diet corresponding to	Grown in the			
		Presence of B_T		Absence of B_T	
		Nos.	Mg.	Nos.	Mg.
Dermestes vulpinus	0.125%	8	66.1	10	55.2
	0.25%	15	72.2	7	71.2
	0.5%	13	67.7	13	77.6
	1.0%	12	89.6	14	85.7
	Controls: no B_T	3	32.6		
	2% yeast	18	74.6		
Tenebrio molitor	0.125%			2	47.0
	0.25%	6	43.7	1	4.5
	0.5%	6	61.6	0	—
	1.0%	12	53.0	0	—
	Controls: no B_T	0	—		
	2% yeast	18	96.1		

Calculated B_T contents (μg./g. dry insect bodies)
grown in presence of B_T: *Tenebrio* 17.5; *Dermestes* 140
grown in absence of B_T: *Tenebrio* none; *Dermestes* 70–140

ing up to 4% of the diets. None of these diets showed B_T activity. It was calculated that even with 1.5 μg. in the diet, and even if all the carnitine had been retained in the larvae, only 0.27 μg. carnitine per gram could have been present in diets containing 4% of dried larval bodies.

In a subsequent experiment the B_T content was determined of larvae which had been grown on synthetic diets containing 1.5 μg. and 50 μg. carnitine/g. respectively. Extracts from larvae, grown on 1.5 μg. carnitine per gram of food, gave an optimal response in quantities corresponding to 7.5% dry larval bodies; and extracts from larvae grown on 50 μg. carnitine/g. showed optimal activity in quantities corresponding to 0.3 to 0.6% larval bodies. Making use of these figures it is calculated that 7.5% of larval tissue, grown at a level of 1.5 μg. B_T/g., would

have provided 0.5 μg. carnitine/gm. diet, if all the carnitine fed to the larvae had been retained. Correspondingly, 0.3–0.6% of larvae raised on a diet with 50 μg./g. B_T could have provided a maximum of 0.35 to 0.7 μg. B_T/g. Since the diet at the levels stated only showed optimal activity which is assumed to correspond to a level of 0.35 μg. B_T/g., it is obvious that in this experiment almost all the carnitine which the larvae had eaten was retained (Table IV).

The B_T contents of larvae grown on diets containing 1.5 and 50 μg. B_T/g. were calculated as 5 μg. and 56 to 112 μg./g., respectively.

In a further experiment, larvae were raised on whole meal flour plus 5% yeast until they reached a body weight of 20 mg. live weight. One portion of these larvae was tested for B_T, and another transferred to a basic carnitine-free diet and tested when the average weight reached 105 mg. The 20-mg. larvae showed good activity at levels of 1.25–2.5% which correspond to a carnitine content of 14–28 μg./g. This is in good agreement with the results of a similar assay referred to

TABLE IV

Relations between B_T content of the food, the retention of B_T in Tenebrio molitor larvae and the B_T content of larvae grown on these foods

	Nature and B_T content of diets of *Tenebrio* larvae to be assayed for their B_T content		
	Whole meal flour + 5% yeast 7 μg. B_T/g.	Synthetic diet 1.5 μg. B_T/g.	Synthetic diet 50 μg. B_T/g.
Smallest quantity of *Tenebrio* tissue to give optimal B_T effect	2%	7.5%	0.3–0.6%
Amount B_T which would have been present in test diets if all B_T taken up had been retained	0.65 μg./g. diet	0.5 μg./g. diet	0.35–0.70 μg./g.
Amount B_T found by bio-assay	0.35 μg./g. diet	0.35 μg./g diet	0.35 μg./g. diet
Calculated B_T content of larvae	17.5 μg./g. tissue	5 μg./g. tissue	56–112 μg./g. tissue

above (Table IV). The other larvae, after having reached a weight of 105 gm., showed no carnitine activity in levels up to 7.5% of the diet. If all the carnitine which was present in the 20-mg. larvae had been retained, the 105-mg. larvae would have been expected to have 5 times less carnitine per unit weight, and would have shown carnitine activity at levels of 6–12%. The fact that no activity was shown at the 7.5% level indicates some loss of carnitine during growth from 20 to 105 mg.

All these tests with *Tenebrio* indicate clearly:

1. That no synthesis of carnitine takes place in *Tenebrio* larvae, and
2. that growing *Tenebrio* larvae retain 50% or more of the carnitine which they take up with the food.

b. The carnitine content of the larvae of Dermestes vulpinus

The larder beetle, *Dermestes vulpinus,* has been raised successfully on a diet consisting of 80 parts casein, 20 parts fructose, one part cholesterol, two parts

McCollum's salt mixture and the mixture of 8 vitamins, as described above (Fraenkel, 1953). A comparison of the carnitine contents of *Dermestes* grown on this diet in the absence of carnitine, or on a mixture of 95% fish meal and 5% yeast, which contains high amounts of carnitine (Table VIII), showed them to be virtually the same (Table III). In both cases *Dermestes* bodies gave good activity in the diet at levels of 0.25 to 0.5%, which corresponds to a carnitine content of 70 to 140 μg./g. Carnitine is therefore synthesized with great efficiency in the *Dermestes* larva.

c. The carnitine content of the larvae of the blowfly, Phormia regina

The larvae of *Phormia regina,* grown on a diet of raw liver, under nonaseptic conditions, showed full B_T activity when incorporated in the diet at a level of 0.062% which corresponds to the very high content of 560 μg. carnitine/gm. (dry). Larvae grown on a synthetic diet in the absence of carnitine under sterile conditions showed full activity in a *Tenebrio* diet in levels of 2%. This corresponds to a carnitine content of 17.5 μg./g. or about 30 times less than the amount found in larvae grown on liver. Carnitine is therefore also synthesized in the fly larva, but at a lower level than in *Dermestes.*

In another test, the carnitine content of fly larvae which had been grown on liver was followed from the fully grown larva through the pupal stage to the young adult. Fully grown larvae, two-, three-, and four-day old pupae and adults one day after emergence were tested. The carnitine content was the same in all stages, about 560 μg./g. dry weight. Since synthesis of carnitine in the fly larva is relatively slow, we may assume that the bulk of the carnitine, which has accumulated during larval development, is retained in the pupa and later incorporated in the tissues of the adult fly.

In summary we may therefore state that synthesis of carnitine takes place in two insect species (*Dermestes* and *Phormia*) which do not require it in the diet, while *Tenebrio,* which requires it in the diet, shows no signs of synthesis under any conditions.

4. The B_T content of eggs, chick embryos and chicks

The hen's egg had previously been shown to contain surprisingly little B_T. A test with dried egg powder suggested full activity at a level of 6% in the diet (Fraenkel, 1951a). Subsequently several tests with raw homogenized egg showed an even lower activity. Three per cent (on a dry weight basis) was entirely inactive and could hardly have shown optimal activity at less than a 12% level. It was then thought that the negative results obtained with raw egg white might have been caused by a toxic egg-white factor. This assumption proved to be incorrect. Extracts made from boiled egg were only a little more active than the corresponding amount of raw egg. Finally, pure carnitine, at levels of 1.5 μg./g. diet, was added to diets which contained raw or boiled egg in concentrations up to 3 per cent. Growth was uniformly optimal in all tests with added B_T. This showed conclusively that the negative results with raw egg could not have been due to the presence of a toxic factor.

Subsequently, it was found that the hatching chick contained demonstrable amounts of B_T. This suggested the synthesis of B_T in the growing chick embryo.

A test was then designed in which eggs (single comb White Leghorn) were incubated, and the embryos and remaining yolk and white portions analyzed for B_T after periods of 8, 12, 16 and 20 days, and in the one-day old unfed chick. The embryos were dissected out and, after removal of the membranes, homogenized in a Potter-Elvehjem homogenizer (8- and 12-day embryos) or the Waring Blendor (16- and 20-day embryos). The results are shown in Table V. All the embryos showed about the same carnitine activity, with optimal effects at levels of 0.5%. No B_T activity was shown in the remaining white and yolk fractions at levels up to 3%, in the 8-, 12- and 16-day egg, and a low activity, at a level of 2.3%, in the 20-day egg. The B_T activity of the whole one-day chick could not be measured accurately, but appeared to be of the same order as that of the embryos. However, tests with three tissues, brain, liver and muscle, showed B_T activities of an order similar to those encountered in several mammals (Table VI).

TABLE V

The B_T activity of egg and of chick embryo and other egg fractions after varying periods of incubation. Tested as whole homogenized preparations (with the exception of muscle—one-day chick) in their effect on growth and survival of Tenebrio larvae after 9 weeks

Period of incubation	Weight of embryo or chick	Material	Minimum amount to give full activity	Maximum amounts tested found inactive	Calculated B_T contents μg./g. dry tissue
0 days		Whole egg		3%	<3
8 days	0.9 g.	Yolk + white		2.8%	<3
		Embryo	0.35–0.7%		50–100
12 days	4 g.	Yolk + white		2.8%	<3
		Embryo	0.4–0.8%		44–88
16 days	14 g.	Yolk + white		3%	<3
		Embryo	0.5%		70
20 days	31 g.	Yolk + white	2.3%		15
		Embryo	0.4%		88
One-day chick	40 g.	Brain	0.5–1%		35–70
		Muscle extract	0.075%		466
		Liver	0.56%		70

If we assume a water content of the 20-day (31 g.) embryo of 80%, and an optimal B_T activity at the 0.5% level, this embryo would have contained 420 μg. carnitine and the egg less than 52.5 μg., assuming an egg of 50 g. (15 gm. dry weight) and optimal B_T activity with more than 10% egg in the diet. It is evident that nearly all the carnitine in the chick must have arisen from synthesis.

5. The B_T content of mammalian tissues, blood and urine

It has been previously shown that various organs of mammals are rich sources of B_T (Fraenkel, 1951a). These determinations were carried out with fat-free powders. Subsequently it was found that the mammalian skeletal muscle showed three to five times higher B_T activity than the best tissue preparations previously tested. More assays have now been carried out using homogenates and extracts from fresh organs from the dog, rat and rabbit (Table VI). With tissue homogenates, prepared with the aid of the Waring Blendor or Potter-Elvehjem homog-

enizer, the calculations in Table VI are based on the solid content of the homogenates. In the case of extracts, the water content of tissues from which the extracts were prepared was not determined at the time. For these calculations a water content of 70% was assumed, which introduces small errors.

The results obtained from fresh dog tissues were of a similar order as those previously determined from tissue powders (Table VI). Brain and nerve contained relatively small amounts of B_T, and the B_T content of blood, on a dry weight basis, was also low, for an animal tissue. The highest concentrations of B_T were

TABLE VI

B_T (carnitine) content of various mammalian tissues, expressed as μg./g. dry tissues

Organism	Tissue	Minimal conc. for optimal activity %	B_T μg./g. dry tissue	Method
Dog I	Muscle, leg	0.03	1120	Homogenate
	Liver	0.12	280	Homogenate
	Brain	0.4	87	Homogenate
	Pancreas	0.33	105	Homogenate
	Small intestine	0.15	224	Homogenate
	Blood	1.0	35	Whole
Dog II	Muscle, leg (normal)	0.03	1120	Extract
	Muscle, leg (paralyzed)	0.03–0.06	560–1120	Extract
	Heart	0.06	560	Extract
	Bladder	0.06–0.12	280–560	Extract
	Nerve	0.25–0.5	70–140	Homogenate
Dog III	Liver	0.25	140	Homogenate
	Kidney	0.085	412	Homogenate
	Liver	0.5	70	Extract
	Liver, autolysed	0.25–0.5	70–140	Homogenate
	Liver, autolysed	0.5	70	Extract from autolysed liver
Rat	Muscle, leg, laboratory diet	0.03–0.06	560–1120	Homogenate
	Muscle, leg, B_T-free diet	0.05–0.1	350–700	Homogenate
	Liver, laboratory diet	0.17–0.34	100–200	Homogenate
	Liver, B_T-free diet	0.15–0.3	112–224	Homogenate
Rabbit	Muscle, leg	0.048	700	Homogenate
	Liver	0.09	370	Homogenate

again found in muscle, with the skeletal muscle highest, a smooth muscle (bladder) lowest, and the heart muscle intermediate. The B_T content of muscle and liver from rabbit and rat differed only slightly from those of the dog.

From the high concentration of B_T in skeletal muscle it was assumed that B_T may play some important function in the metabolism of muscle. In order to determine whether or not the concentration of B_T in the skeletal muscle was dependent on its functioning, one hind leg of a dog was paralyzed by severing the nerve leading to it, and corresponding portions of muscle from the paralyzed and normal leg were analyzed for their B_T content three weeks later. The carnitine

content of the paralyzed muscle was very slightly, and probably insignificantly, lower than that of the normal muscle (Table VI).

Another comparison was made between the B_T content of liver and muscles from rats fed on a normal laboratory diet, which contained carnitine, and others fed on a synthetic diet, which did not contain it. The results suggested that the carnitine contents of these two tissues were not dependent on the supply in the diet, and that therefore, in all probability, carnitine is synthesized in the rat.

In a further experiment the stability of carnitine to the action of tissue enzymes was investigated. Homogenates of dog liver were subjected to autolysis for three days at 30° C. in the presence of toluene. At the end of the period there was a strong smell of putrefaction in the samples. There was no change in the carnitine content as a result of autolysis and putrefaction.

Table VII contains data concerning the B_T content of urine and blood from human subjects. Here the B_T content is expressed as μg./ml. of urine or blood.

TABLE VII

B_T (carnitine) content of urine and blood from human subjects

Sex and age	B_T μg./ml.	Description
		Urine
♂50	132	24-hour sample
Same	132–264	3 hours after heavy meat meal
Same	28–56	20 hours on diet low in B_T
♀21	56	High protein diet
♂23	56	High protein diet
♀22	14–28	Diet high in vegetable and fruit
♂24	28–56	Diet high in vegetable and fruit
♀21	132	After 3 days of starvation
♂25	132–264	After 3 days of starvation
Students	28–56	Pooled sample from about 50 students
		Blood
	7–14	Pooled, whole blood
	7–14	Pooled, plasma

The B_T content of urine showed fairly large variations which seemed to be dependent on the B_T intake in the diet. The highest figures, 132–264 μg./ml., were found after a heavy meat meal and at the end of a starvation period of three days. This, in the case of starvation, may be due to breakdown of muscle substance and concomitant release of B_T. The lowest levels, 14–28 μg./ml., were obtained from persons who had been on a vegetable diet low in carnitine.

The B_T content of human blood was uniformly low in several determinations (7–14 μg./ml.) and the activity of whole blood and plasma was the same.

6. The B_T activity of various extracts

In the preceding pages, the carnitine contents of diverse biological materials have been stated in terms of μg. per gram dry weight of that particular material. When assaying extracts, it is also of interest to know the carnitine activity in re-

lation to the solid content of the extracts. Table VIII contains data about the B_T activity of various extracts which were used in the course of this work. With commercial preparations, nothing is known about the procedures of preparation, but it is assumed that they were mainly hot water extracts. In our own preparations, the homogenized samples were extracted with two or three portions of water at 100° C. In some cases, the hot water extracts, after concentration in an evaporating dish, were treated with alcohol, to give a concentration of 75 to 80 per cent, and the ensuing precipitates removed.

Table VIII shows that meat extracts were by far the most active preparations, containing carnitine in amounts from one to almost three per cent. Whey and liver extracts, which were used in the isolation of carnitine (Carter *et al.*, 1952), contained 5 to 10 times less carnitine than muscle extracts. Fish extracts were

TABLE VIII

The B_T activity of various extracts. The activity is expressed as μg. solids per gram of the dry diet (A) and as μg. B_T per gram solids in the extracts (B)

	A Minimum amount to give optimum effect μg./g.	B B_T per gram solids μg./gm.	Method of preparation
Beef muscle extract (Wilson)	36–72	4860–9720	Aqueous extract from dried muscle powder
Meat juice (Wilson)	15–30	11,650–23,300	Commercial preparation, probably aqueous extract
Difco beef extract	12–24	14,600–29,200	Commercial preparation, for bacteriological use
Liver extract (Wilson)	125	2800	Aqueous extract, alcohol treated
Heart infusion	125–250	1400–2800	Commercial preparation
Whey extract	360	972	Aqueous extract from dried whey (Borden), alcohol-treated
Fish solubles	500	700	Commercial preparation
Fish meal extract	100	3500	Aqueous extract
Yeast (brewers) extract	2000	175	Aqueous extract, alcohol treated
Vitamin T concentrate	3400	103	Commercial preparation (Pharmazell GMBH)

of a similar order of activity. Brewers yeast, the material on which the B_T effect was originally discovered and which had been used in the early isolation work, proved a very inferior source. A standard preparation of vitamin T (Pharmazell GMBH, Raubling, Obb.) proved to be of the same order as yeast extract. (Vitamin T concentrate has the appearance, smell and taste of yeast extract, and, in all probability, is nothing but a crude yeast extract.)

DISCUSSION

Our investigations concerning distribution and requirements of Vitamin B_T (carnitine) have made it abundantly clear that B_T occurs widely, if not perhaps universally, in all living matter and that animals either require it in the diet or else synthesize it. We have demonstrated the presence of B_T in a great variety of

organisms, ranging from yeast to mammals, but have been unable to find it in a bacterium, *Escherichia coli,* a protozoon, *Tetrahymena geleii,* and in corn seeds. It would be rash to conclude from the available data whether or not it is a necessary constituent of all living matter. Our testing method, using growth and survival of an insect, *Tenebrio molitor,* is not sensitive enough to detect amounts below about one μg./gm. of the dry substance. Furthermore, the possibility must be kept in mind that it might occur in some organisms in a form in which it is not available for *Tenebrio.*

At this point we may ask why we would expect carnitine to occur in all living matter. We have seen that it occurs regularly in animals, and that those organisms which do not require it in the diet synthesize it. This, together with the fact that *Tenebrio* requires it in very small quantities, 0.37 to 0.75 μg./g. of the food, that is to say on the catalytical level, would indicate that it functions in a process of vital importance, at least for yeast, higher plants, insects and vertebrates. Processes of this description are known in the functioning of enzyme systems of the intermediary metabolism and are usually characteristic for all living matter.

On the other hand, it is difficult to reconcile the fact, that B_T is required by *Tenebrio* on the level of some of the most potent B vitamins, biotin and folic acid, but occurs in certain tissues, especially muscle, in quantities of one mg./g. This may suggest that it might be involved in two different functions, one in which it acts on the catalytical level, and one in which vastly larger quantities are involved.

Leclerq (1950) stated that the growth rate of *Tenebrio molitor* was greatly increased by the addition of a preparation of vitamin T (Goetsch, 1947) to the synthetic diet and considers the possibility that vitamin T and B_T could be identical. (The similarity in the designation of these two growth factors is entirely accidental.) We have found only weak B_T activity in a vitamin T preparation which entirely excludes this possibility. It has, however, since been shown that "vitamin T," a crude extract from Torula yeast, contains a multitude of factors (Goetsch, 1951; Wacker *et al.,* 1951).

This investigation was supported in part by research grants from the National Institutes of Health, Public Health Service, and the Graduate College Research funds of the University of Illinois. I gratefully acknowledge the aid of Dr. F. R. Steggerda and Dr. H. M. Scott, University of Illinois, in certain phases of the work; and that of Drs. I. Gunsalus, University of Illinois, E. L. Tatum, Stanford University, and G. K. Kidder, Amherst College; also Cerophyl Laboratories, Sharpe and Dohme, Anheuser-Busch and the Wilson Company for supplying materials and preparations for testing. It is also a pleasure to acknowledge the careful assistance of Mrs. E. Nilson.

Summary

1. The procedure in the bio-assay of B_T (carnitine) with the use of *Tenebrio molitor* as the subject has been described. The minimum requirements of carnitine to give optimal survival and nearly optimal growth of the *Tenebrio* larva are 0.35 μg. per gram of the dry diet. For optimal growth 1.5 μg. per gram are required.

2. Yeast and Neurospora contain about 35 μg. carnitine/g., and wheat 7–14 μg./g. No evidence of the presence of carnitine could be obtained from *Escherichia coli, Tetrahymena geleii* and corn.

3. Synthesis of carnitine takes place in two insect species (*Dermestes* and *Phormia*) which do not require it in the diet, while *Tenebrio,* which requires it in the diet, shows no sign of synthesis under any conditions. The growing *Tenebrio* larva retains more than 50% of the carnitine which it takes up in the diet.

4. Little carnitine was found in the hen's egg. It is synthesized in sizeable quantities in the growing chick embryo.

5. In mammalian tissues, by far the largest quantities of carnitine, about 1000 μg./g. of dry tissue, occur in the skeletal muscle. Corresponding figures for other tissues were: heart 560, bladder 280–560, kidney 412, liver 140–280, small intestine 224, brain 87, nerve 70–140 and blood 35 μg./g. dry tissue.

6. In dog muscle which had been paralyzed for several weeks, the carnitine content was not significantly changed. Liver after autolysis had the same carnitine content as before.

7. The carnitine content of pooled samples of human urine was 28–56 μg./ml. In different individuals levels as low as 14–28 and as high as 132–264 μg./ml. were found. This variation depended on the carnitine content of the diet, with the highest figures after a heavy meat meal or a three-day period of starvation, and the lowest after a vegetable diet. Blood contains fairly uniform levels of 7–14 μg./ml.

8. Different commercial preparations of meat extracts had carnitine contents of one to three per cent (dry weight).

9. Since carnitine occurs universally, or almost universally in biological material, and an organism either synthesizes it, or else requires it as a "vitamin," it is considered to be of vital importance in the metabolism of most or all forms of life.

LITERATURE CITED

Carter, H. E., P. K. Bhattacharyya, K. R. Weidman and G. Fraenkel, 1952. Chemical studies on vitamin Bt. Isolation and characterization as carnitine. *Arch. Biochem. Biophys.*, **38**: 405–416.

Fraenkel, G., 1951a. Effect and distribution of vitamin Bt. *Arch. Biochem. Biophys.*, **34**: 457–468.

Fraenkel, G., 1951b. Isolation procedures and certain properties of vitamin Bt. *Arch. Biochem. Biophys.*, **34**: 468–477.

Fraenkel, G., 1952. The nutritional requirements of insects for known and unknown vitamins. *Trans. IX. Intern. Congr. Entomology,* **1**: 277–280.

Fraenkel, G., M. Blewett and M. Coles, 1948. Bt, a new vitamin of the B-group and its relation to the folic acid group, and other anti-anaemia factors. *Nature,* **161**: 981–983.

Fraenkel, G., M. Blewett and M. Coles, 1950. The nutrition of the mealworm, *Tenebrio molitor. Physiol. Zool.*, **23**: 92–108.

Goetsch, W., 1947. Vitamin T, ein neuartiger Wirkstoff. *Österr. zool. Zeitschr.*, **1**: 49–57.

Goetsch, W., 1951. Ergebnisse und Probleme aus dem Gebiet neuer Wirkstoffe. *Österr. zool. Zeitschr.*, **3**: 140–174.

Leclerq, J., 1950. La vitamin T, facteur de croissance des larves de *Tenebrio molitor. Arch. Intern. Physiol.*, **57**: 350–352.

Wacker, A., H. Dellweg and E. Rowold, 1951. Biochemical studies with vitamin T. *Klin. Wochenschr.*, **29**: 780–783. (Quoted from *Chem. Abstr.*, **46**: 5155, 1952.)