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The Importance of Folic Acid and Unidentified Members of the Vitamin B Complex in the Nutrition of Certain Insects

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In our work on the nutrition of insects evidence has been accumulating that growth on purified diets containing all the known vitamins of the B complex in pure substance is inferior to that on similar diets which contain yeast or yeast extracts. This was particularly noticeable in the genus *Ephestia* (Lepidoptera) where the caterpillars always grew much more slowly in the absence of yeast or yeast fractions (Fraenkel & Blewett, 1946*a*). From the effect of adding fractions resulting from a charcoal treatment of yeast extract, it seemed probable that the missing factor might be 'folic acid' and this was finally established by tests with synthetic folic acid (Lederle). An even more striking case of the need for folic acid was found in the mealworm, *Tenebrio molitor*, the growth rate of which on certain diets was vastly increased in the presence of folic acid. In our earlier work with another beetle, *Tribolium confusum*, it seemed that a mixture of all the then known vitamins of the B complex would sustain growth equally as well as yeast or a yeast extract (Fraenkel & Blewett, 1943*b*). This result has been difficult to repeat on several occasions but it has now been established that a folic acid deficiency can also be demonstrated in *Tribolium* if the casein in the diet is sufficiently purified.

'Folic acid' is used throughout this paper to signify an unspecified preparation showing folic acid activity, while folic acid designates synthetic folic acid (Lederle) (pteroylglutamate, Angier, Boothe, Hutchings, Movat, Semb, Stokstad, Subbarow, Waller, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickels & Smith, 1946), which was formerly called *L. casei* factor. Some of the results have been briefly reported elsewhere (Fraenkel & Blewett, 1946*b*).

METHODS

The methods of breeding and of testing and preparing diets have been fully described (Fraenkel & Blewett, 1943*a*, *b*, 1946*a*). All tests were performed at 25° and 70% relative humidity. As a rule, 20 larvae were used in each test. All tests were started with newly hatched larvae and the criterion for the efficiency of a diet was either the length of the larval period (*Ephestia kuehniella, E. elutella, Tribolium confusum*), or the time taken until emergence of the adult (*Plodia interpunctella*), or growth expressed by the weight increase (*Tenebrio molitor*).

In most experiments the effect of the following three diets was compared:

(1) *Pure vitamins diet.* Casein (Glaxo Laboratories) 20, glucose anhydr. (British Drug Houses Ltd.) 80, McCollum's salt mixture (no. 185) 2, 'insoluble yeast' (yeast exhaustively extracted by boiling water) 2·5, cholesterol 1 and water 10 parts. To this mixture the following vitamins were added (expressed as μg./g. of dry diet): aneurin 25, riboflavin 12, nicotinic acid 25, pyridoxin 12, panthothenic acid 25, choline chloride 500, *i*-inositol 250. In tests with *Tribolium*, the diet consisted of 50 parts of casein and 50 parts of glucose. In tests with *Ephestia* and *Plodia*, 1 part of wheatgerm oil (Vitamins Ltd.) was added.

(2) *Yeast diet.* No. 1 with the addition of 1% dried brewer's yeast (Glaxo Laboratories).

(3) *Folic acid diet.* No. 1 with the addition of synthetic folic acid (Lederle).

The following preparations were also used:

(1) Yeast extract and yeast charcoal filtrate and eluate. Yeast extracts prepared from fresh brewers' yeast according to Chick & Roscoe (1930) or from dried brewers' yeast (Glaxo Laboratories) by a similar method, were added to the diets in quantities corresponding to 5% yeast. The charcoal treatment of yeast was carried out according to Hutchings, Bohonos & Peterson (1941) (adsorption at pH 3 with three successive portions of norite and elution of the norite adsorbates with ammoniacal ethanol).

(2) Grass juice (spray dried, Cerophyll Laboratories Inc.) was used in 10 % solution in hot water. Charcoal treatment was carried out as described for yeast extract.

(3) A liver charcoal filtrate (El Sadr, Macrae & Work, 1940) originally prepared as a riboflavin-free source of B vitamins, and found to be free from folic acid.

(4) Synthetic folic acid (Lederle Laboratories), crystalline vitamin B_c (Parke, Davis and Co.), synthetic xanthopterin (Organon Laboratories).

Yeast and grass juice extracts were added to the diets in quantities which included half the normal quantity of water, and the pure vitamins were dissolved in the other half.

All the graphs concerned with *Ephestia*, *Plodia* or *Tribolium* show the total number of pupae or adults formed plotted against time. The results of tests with *Tenebrio* are expressed in weight curves.

RESULTS

Ephestia kuehniella, *E. elutella*, *Plodia interpunctella*

The caterpillars of these moths always grow much more slowly on diets which contain pure vitamins than in the presence of yeast or yeast extracts. This

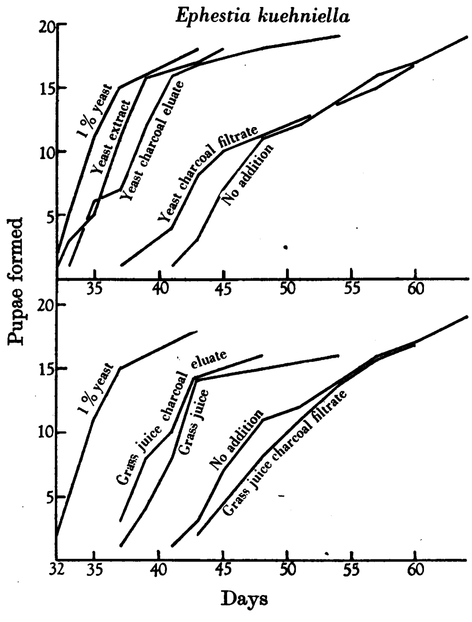

Fig. 1. Growth of *Ephestia kuehniella* on the pure vitamins diet with the addition of yeast, yeast extract, grass juice or the charcoal filtrate or eluate from yeast extract or grass juice.

is illustrated in Figs. 1–4 and also by Fraenkel & Blewett (1946*a*, Figs. 6–9). In a first attempt to identify the missing factor the effect of a preparation of grass juice (Cerophyll Laboratories Inc.) was investigated and it was found that 1 % of this added to the pure vitamins diet had a pronounced positive effect on the rate of growth (Fig. 1). This grass juice had been described as a rich source of 'folic acid'. An attempt was therefore made to fractionate grass juice and yeast extract by a charcoal treatment which consisted essentially of the first steps in the concentration of 'folic acid', as described by Hutchings *et al.* (1941).

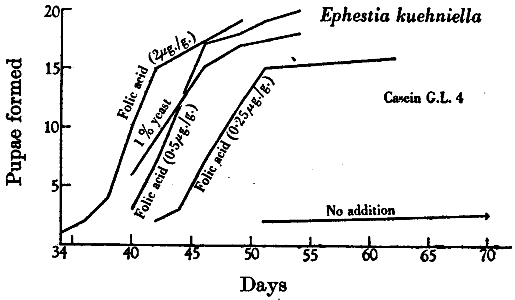

Fig. 2. Growth of *Ephestia kuehniella* on the pure vitamins diet with the addition of graded doses of synthetic folic acid.

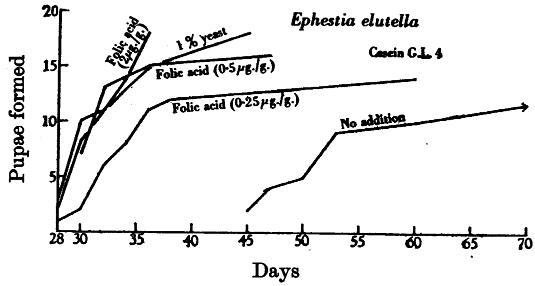

Fig. 3. Growth of *Ephestia elutella* on the pure vitamins diet with the addition of graded doses of synthetic folic acid.

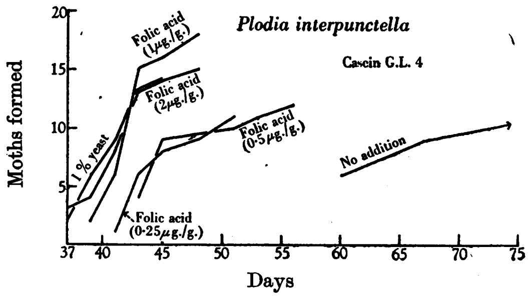

Fig. 4. Growth of *Plodia interpunctella* on the pure vitamins diet with the addition of graded doses of synthetic folic acid.

The results obtained with grass juice and yeast fractions were identical (see Fig. 1). Growth was greatly improved in the presence of the norite-eluate fractions while the filtrate fractions had little effect. This suggested that the factor which was missing in the pure vitamins diet might be 'folic acid'.

Highly potent concentrates of vitamin B_c (Parke, Davis and Co.) were later tested and found to have a positive effect on growth. Finally synthetic folic acid (Lederle Laboratories) was found to be as effective as yeast or yeast extract for *Ephestia kuehniella*, *E. elutella* and *Plodia interpunctella*. Figs. 2–4 give the result of tests with graded doses of folic acid on the development of these species. In each case growth was very slow in the absence of folic acid and the mortality high. With optimal quantities of folic acid growth was as good as on the control diets which contained 1% yeast. The minimum optimal quantity of folic acid for the two *Ephestia* species appeared to be 0·5 μg./g. and for *Plodia* 1 μg./g. of the dry diet.

Crystalline vitamin B_c (Parke, Davis and Co.) seemed to have an even better effect than synthetic folic acid (Lederle). Growth was optimal with as little as 0·25 μg./g., which was the smallest quantity tested (Table 1).

Table 1. *The effect of vitamin B_c and xanthopterin on the growth of* Ephestia kuehniella

Addition to diet	Larval period (days) Range	Larval period (days) Average	No. of pupae (out of 20)
No addition	109–121	112·5	4
1% yeast	35–73	58·3	12
Vitamin B_c 0·25 μg./g.	37–54	49·3	14
Vitamin B_c 0·5 μg./g.	37–60	52·2	15
Vitamin B_c 1·0 μg./g.	38–63	52·6	16
Vitamin B_c 2·0 μg./g.	37–54	47·5	13
Xanthopterin 10 μg./g.	57–76	68·4	5
Xanthopterin 100 μg./g.	44–120	98·0	8
Xanthopterin 1000 μg./g.	43–76	55·7	16

Table 2. *The effect of folic acid, liver charcoal filtrate and xanthopterin on the growth of* Ephestia kuehniella

Addition to diet	Larval period (days) Range	Larval period (days) Average	No. of pupae (out of 20)
No addition	61–95	78·4	5
1% yeast	44–61	50·9	17
Folic acid 2 μg./g.	38–54	44·0	16
Folic acid 2 μg./g. + liver extract	38–52	42·6	17
Xanthopterin 10 μg./g.	—	88	1
Xanthopterin 100 μg./g.	61–84	76·3	3
Xanthopterin 1000 μg./g.	50–95	64·7	15

Folic acid can be replaced by xanthopterin, but only when very large quantities of the latter are used. Even 1000 μg./g. of xanthopterin did not seem as effective as 2 μg./g. of synthetic folic acid or 0·25 μg. of B_c (Tables 1, 2). A positive effect with xanthopterin was obtained on two occasions, but not on a third.

Finally, the question arose whether any unspecified B vitamins were required by *Ephestia kuehniella* in addition to folic acid and the other known B vitamins. Addition to a folic acid diet of a liver charcoal filtrate which had proved to be essential for the development of *Tenebrio* had no further effect on the rate of growth of *Ephestia* (Table 2).

Tenebrio molitor

In early tests with the meal worm, *Tenebrio molitor*, on the effect of charcoal filtrates and eluates from yeast extract, the results were very different from those already described for *Ephestia kuehniella*. Growth was poor in the presence of the eluate, and very much improved in the presence of the filtrate, though still short of growth on diets which contained yeast or unfractionated yeast extract (Fig. 5).

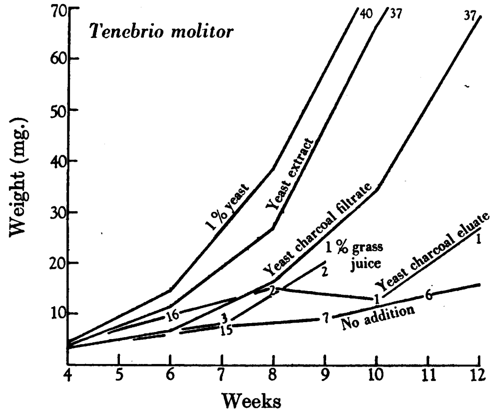

Fig. 5. Growth of *Tenebrio molitor* on the pure vitamins diet with the addition of yeast, yeast extract, a charcoal filtrate or eluate from yeast extract, or grass juice. The numbers on the curves indicate the numbers alive, out of 40 larvae.

Addition of a grass juice, which had a positive effect on *Ephestia* (Fig. 1) had none on *Tenebrio* (Fig. 5). A striking feature of these tests was the fact that the larvae died off quickly on diets which contained no addition, and still more quickly in the presence of the yeast eluate or grass juice, while they survived in the presence of yeast, yeast extract or yeast filtrate. From these results it was at first concluded that *Tenebrio* did not require folic acid, a conclusion which proved entirely erroneous.

In a subsequent test, yeast charcoal filtrate was replaced by a liver charcoal filtrate which had been treated with charcoal for the purpose of removing riboflavin (El Sadr *et al.* 1940) and which, from the nature of this treatment could also be expected to be free from 'folic acid' (Hutchings *et al.* 1941). Very little growth took place in the presence of the liver charcoal filtrate or of synthetic folic acid alone, but in the presence of both growth was as fast as with 1% yeast (Fig. 6).

The results of this test demonstrate that besides folic acid, *Tenebrio* also requires an unknown factor which is contained in the charcoal filtrate of yeast or liver extracts. Without this factor and without folic acid, growth is slow and the larvae die after 4–6 weeks. Without this survival factor, the presence of folic acid, either in pure substance or in a charcoal eluate or in grass juice, has the effect of increasing both the rate of growth and the mortality.

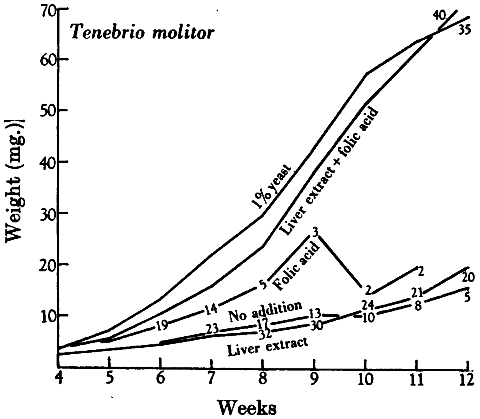

Fig. 6. Growth of *Tenebrio molitor* on the pure vitamins diet with the addition of yeast, liver charcoal filtrate and/or synthetic folic acid. The numbers on the curves indicate the numbers alive, out of 40 larvae.

It still remains to explain why on the former occasion to which Fig. 5 refers a yeast charcoal filtrate had such a good effect on growth in the absence of the eluate which, by analogy with the tests with *Ephestia*, would be expected to contain the bulk of the folic acid. The following explanations offer themselves: (1) *Tenebrio* might require significantly less folic acid than *Ephestia*. (2) The casein, which was used on that former occasion (Fig. 5) might have contained significant amounts of folic acid. (3) The fractionation of the yeast extract might not have been perfect and significant amounts of folic acid might have remained in the charcoal filtrate.

(1) In a test on the folic acid requirements of *Tenebrio* it was found that 0·125 μg./g. gave an optimal response, against 0·5 μg./g. with *Ephestia*, i.e. an amount four times smaller (Table 3).

(2) This test on the folic acid requirements was carried out simultaneously with four different preparations of casein, three prepared by Glaxo Laboratories (G.L.) and one an American preparation (Labco). In the absence of folic acid and the presence of the liver charcoal filtrate by far the best growth was obtained on casein G.L. 3 which is the same as that used on the occasion to which Fig. 5 refers, when good growth occurred in the absence of the yeast charcoal eluate. Growth on casein G.L. 4 in the presence of liver charcoal filtrate was intermediate between G.L. 3 and 6 and was still inferior on Labco casein. In the presence of sufficient quantities of synthetic folic acid and liver charcoal filtrate growth was as fast as with 1 % yeast. There was also no significant difference between the three G.L. caseins on these diets, but on Labco casein growth was significantly slower. These results suggest that G.L. 3 casein contains more folic acid than the other casein preparations and that Labco casein contains very little, if any. Similar growth effects on these caseins were also obtained with another insect, *Tribolium confusum* (Fig. 7) on diets which contained no additional folic acid.

(3) The results of Table 3 show clearly that the liver charcoal filtrate must have been virtually free from folic acid, and exactly the same result has been more recently obtained with a yeast charcoal filtrate.

Further evidence is given in Table 3 to the effect that folic acid in the absence of liver extract has a pronounced effect on growth, while on most occasions only about 10 % of the larvae were alive after 10 weeks. Liver extract in the absence of folic acid always had a striking effect on survival, which however, was never as good as in the presence of additional folic acid except with casein G.L. 3 which, as

Table 3. *Growth and survival of* Tenebrio molitor *on artificial diets containing four different preparations of casein, and with the addition of graded doses of folic acid and/or liver charcoal filtrate*

(40 larvae were used in each test. Survival after 10 weeks is expressed as a percentage of the number of larvae which were alive after 5 weeks.)

	Casein G.L. 3		Casein G.L. 4		Casein G.L. 6		Labco casein	
Addition to diet	Average weight (mg.)	Survival (%)	Average weight (mg.)	Survival (%)	Average weight (mg.)	Survival (%)	Average weight (mg.)	Survival (%)
No addition	7·0	4·1	8·9	33·3	7·1	33·3	6·3	13·6
Folic acid (2 μg./g.)	55·5	10·0	29·5	9·0	34·2	33·3	50·0	12·5
Liver charcoal filtrate (l.c.f.)	31·8	88·8	15·0	69·2	8·4	66·6	5·4	38·4
l.c.f. + folic acid (0·06 μg./g.)	—	—	38·2	100	31·7	100	23·3	100
l.c.f. + folic acid (0·12 μg./g.)	—	—	59·5	72·2	44·9	96·1	43·6	100
l.c.f. + folic acid (0·25 μg./g.)	—	—	50·6	100	51·1	100	43·3	95·6
l.c.f. + folic acid (0·5 μg./g.)	—	—	54·2	84·6	46·2	100	34·6	100
l.c.f. + folic acid (1·0 μg./g.)	—	—	54·8	100	45·0	100	43·1	100
l.c.f. + folic acid (2·0 μg./g.)	50·3	100	53·1	100	45·5	81·8	36·2	96·4
1 % yeast	54·3	95·5	56·5	100	53·8	100	41·3	100

pointed out above, must have contained significant amounts of folic acid; survival was lowest on Labco casein which, as already suggested from the weight figures, must have contained significantly less folic acid (if any) than caseins G.L. 3 and 4.

In tests with *Tenebrio*, folic acid could not be replaced by xanthopterin, even in quantities up to 8000 times larger than effective quantities of folic acid (Table 4).

Table 4. *Growth and survival of* Tenebrio molitor *on the pure vitamins diet with the addition of liver charcoal filtrate and xanthopterin or folic acid*

(40 larvae were used in each test. Survival after 10 weeks is expressed as a percentage of the number of larvae which were alive after 5 weeks.)

Addition to diet	Average weight (mg.)	Survival (%)
No addition	8·9	33·3
Folic acid (2 μg./g.)	29·5	9·0
Liver charcoal filtrate (l.c.f.)	15·0	69·2
l.c.f. + xanthopterin (10 μg./g.)	11·7	77·2
l.c.f. + xanthopterin (100 μg./g.)	12·7	72·7
l.c.f. + xanthopterin (1000 μg./g.)	14·6	80·0
l.c.f. + folic acid (2 μg./g.)	53·1	100

Tribolium confusum

In our earlier work the conclusion was reached that the larvae of the flour beetle *Tribolium confusum* grew as well on a synthetic diet which contained all the then known B vitamins as the controls which received 5 % yeast in their diet (Fraenkel & Blewett, 1943*b*). From this it appeared that *Tribolium* did not require additional unknown B factors. This result has not been confirmed on later occasions. In comparing the results of such diets it became clear that the most obvious variable factor was the casein of which a number of different samples had been obtained in the course of time. This was in fact borne out in a test in which the effect of the pure vitamins diet, described on p. 469, was tested simultaneously with six different samples of casein. Of these caseins, five were obtained between 1939 and 1945 from Glaxo Laboratories, and one from British Drug Houses, and all the samples were of 'vitamin and fat free' quality. The result, given in Fig. 7, showed that the efficiency of these diets differed very widely. With three of the caseins, G.L. 2, G.L. 3 and B.D.H., growth on the pure vitamins diet was as good or nearly as good as on the control diet with 5 % yeast. G.L. 4 gave very much slower growth. The most striking difference between the efficiency of a yeast and a pure vitamins diet was obtained on a later occasion, when Labco casein was used (Fig. 8). Addition of synthetic folic acid to a pure vitamins diet, with either G.L. 4 or Labco casein made these diets as efficient as the yeast diet (Fig. 8) and exactly the same result was obtained with another species, *T. castaneum*, on a G.L. 4 casein diet. From this we may conclude that the differences in the efficiency of the diets revealed in Figs. 7 and 8 is due to the differences in folic acid

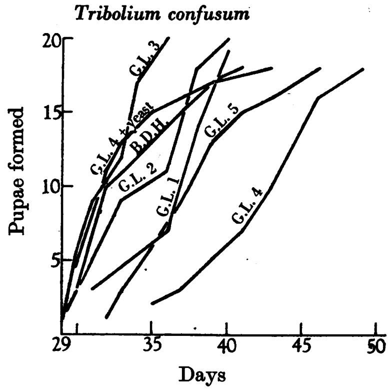

Fig. 7. Growth of *Tribolium confusum* on the pure vitamins diet using six different casein preparations. G.L. = Glaxo Laboratories; B.D.H. = British Drug Houses.

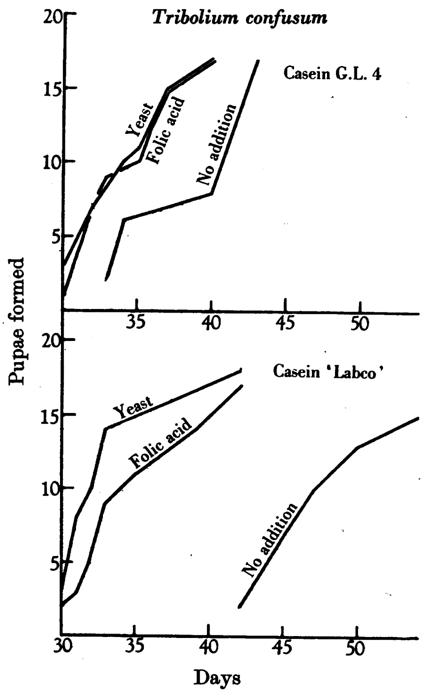

Fig. 8. Growth of *Tribolium confusum* on the pure vitamins diet with the addition of yeast or synthetic folic acid. G.L. = Glaxo Laboratories.

content of the caseins. G.L. 3 apparently contains the most folic acid, Labco the least and G.L. 4 an intermediate quantity as was concluded from the experiments with *Tenebrio*, already described.

The minimum optimal requirement for folic acid of *T. confusum* was found to be 0·125–0·25 μg./g. of the dry diet. This result agrees well with the quantity of 0·15 μg./g. calculated by Grob, Reichstein & Rosenthal (1945) from tests with a very impure concentrate of folic acid. Cannon, Boutwell & Elvehjem (1945) have reported that the folic acid content of various casein preparations may vary between 0·19 and 0·03 μg./g. according to the casein and test organism used. The higher of these figures is of an order which ensures optimal growth of *Tribolium* and we have shown that this does occur on certain casein preparations.

From the results shown in Fig. 8 it appears that artificial diets which contain all the known B vitamins, including folic acid, are as good for *Tribolium* as control diets with 5 % yeast. This is not quite correct. In all the cases so far mentioned 2·5 % of the diets consisted of the water insoluble fraction of yeast which serves as a source of biotin. When 'insoluble yeast' is replaced by biotin, growth is noticeably delayed. The biotin diet becomes optimal with the addition of 1 % yeast, but is not improved by the addition of yeast extract which suggests that the missing factor is not easily soluble in water. The need of *Tribolium* for an unknown food factor, which is contained in the water-insoluble fraction of yeast has also been pointed out recently by Grob *et al.* (1945).

DISCUSSION

From the results presented it is clear that folic acid is an important growth factor for the six insects so far investigated, viz. *Tribolium confusum*, *T. castaneum*, *Ephestia kuehniella*, *E. elutella*, *Plodia interpunctella* and *Tenebrio molitor*. The minimum optimal quantities required were very similar in all cases: 0·125–0·25 μg./g. dry diet for *Tribolium*, 0·5 μg./g. for the two *Ephestia* species and 0·125 μg./g. for *Tenebrio*. For *Ephestia kuehniella*, vitamin B_c appeared even more efficient than folic acid, but it would be dangerous to draw conclusions from a single test, especially as crystalline vitamin B_c is now considered to be identical with synthetic folic acid. These quantities are very similar to those reported for the chick and stated by Luckey, Moore, Elvehjem & Hart (1946*a*) as 0·25–0·5 μg./g., and by Hutchings, Oleson & Stokstad (1946) as 0·5–1·0 μg./g. We have so far determined minimum optimal quantities of the chemically known vitamins for one insect only, *Tribolium confusum* (unpublished), and obtained the following values: thiamin 1 μg./g. diet, riboflavin 1–2 μg./g., nicotinic acid 8 μg./g., pyridoxin 1·0 μg./g., pantothenic acid 4 μg./g. and biotin 0·05 μg./g. Folic acid has therefore an optimal effect in quantities significantly smaller than any of the B vitamins except biotin.

From the evidence so far obtained by us it appears that, for *Ephestia kuehniella* and *Tribolium confusum*, a diet which contains all the known factors of the vitamin B complex, including folic acid and the water insoluble fraction of yeast, is not improved by the addition of liver or yeast extracts. *Tenebrio molitor*, on the other hand, fails to develop on diets which contain all the known members of the B complex, including folic acid and the water insoluble fraction of yeast. The missing substance, to which we shall in future refer as vitamin B_T (because it is necessary for *Tenebrio*) is contained in a charcoal filtrate prepared from yeast or liver extract. Similar results, showing the importance of at least another factor in liver or yeast, in addition to folic acid, have recently been reported for the guinea-pig (Woolley & Sprince, 1944), the mink (Schaeffer, Whitehair & Elvehjem, 1946), a monkey (Cooperman, Elvehjem, McCall & Ruegamer, 1946), and the chick (Luckey, Moor, Elvehjem & Hart, 1946*a*, *b* and Petering, Marvel, Glausier & Waddell, 1946) and similar conclusions are reached in the latest reports about the effect of folic acid in the treatment of certain nutritional anaemias (Wills, 1946; Watson & Castle, 1946). The possibility is not excluded that the missing factors reported in experiments with such widely different material may in the end prove identical. The characterization of the charcoal filtrate factor which is essential for the survival of *Tenebrio* will be the subject of a subsequent paper.

SUMMARY

1. Synthetic folic acid (Lederle) is an important growth factor for six insect species. In the absence of folic acid, growth is very slow (*Tribolium*, *Ephestia*) or ceases entirely (*Tenebrio*), and the mortality is high (*Ephestia*, *Tenebrio*).

2. For *Ephestia kuehniella*, crystalline vitamin B_c has an effect as good as, if not better than, synthetic folic acid.

3. Xanthopterin has a similar effect on *Ephestia*, but only in quantities approximately 1000 times larger than those used of folic acid. It has no effect on *Tenebrio*.

4. The minimum optimal requirements for folic acid of the insects investigated are between 0·125 and 0·5 μg./g. of dry diet.

5. *Tenebrio* requires in addition to folic acid and the other known B vitamins a factor, contained in a charcoal filtrate prepared from liver or yeast extract, which we have named vitamin B_T.

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Linoleic Acid and Arachidonic Acid in the Metabolism of Two Insects, *Ephestia kuehniella* (Lep.) and *Tenebrio molitor* (Col.)

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In a foregoing paper (Fraenkel & Blewett, 1946*b*) we have shown that the larvae of the moth *Ephestia kuehniella* and of two other *Ephestia* species grow badly on artificial diets in the absence of wheat-germ oil and that the effect of wheat-germ oil could be attributed principally to two substances: (1) linoleic acid, which is necessary for emergence and normal formation of the scales on the wings, and (2) vitamin E which is necessary for rapid growth and as an antioxidant for linoleic acid. The effect of linolenic acid was found to be similar to that of linoleic acid. Arachidonic acid had not been investigated at the time and we shall show in the present paper that unlike linoleic acid it has no positive effect on the scales and emergence. In our former paper we also showed that other antioxidants, viz. gallates and ascorbic acid can be substituted for vitamin E and we shall now give more data concerning the effect of ascorbic acid. Finally, we have now compared the linoleic acid contents of *Ephestia* larvae, grown in the presence and absence of linoleic acid, by observing the effects of their oils on the development of *Ephestia*, and similarly of the mealworm, *Tenebrio molitor*, which does not require linoleic acid in the diet. It has thus been established that synthesis of linoleic, or possibly linolenic, acid takes place in *Tenebrio* but not in *Ephestia*.

METHODS

The methods of preparing the diets, breeding and testing have already been fully described (Fraenkel & Blewett, 1946*a*, *b*). The fat-free diet used for *Ephestia* and *Tenebrio* consisted of

Casein (extracted, Glaxo Laboratories)	20 parts
Glucose (anhydrous)	80 parts
Yeast (dried brewer's, debittered, Glaxo Laboratories)	5 parts
Cholesterol	1 part
McCollum's salt mixture no. 185	2 parts
Water	10 parts

RESULTS

The effect of arachidonic acid

The tests with arachidonic acid were carried out in two quantities, 5 mg. and 1·7 mg./g. of the dry diet, and in combination with either α-tocopherol (1 mg./g.) or the unsaponifiable fraction of wheat-germ oil (5 mg./g.). There was no positive effect whatsoever on scales or emergence in any of the tests with arachidonic acid but growth was always accelerated in its presence (Fig. 1). This was especially striking in combination with vitamin E, which by itself had a lesser growth-promoting effect than the unsaponifiable fraction of wheat-germ oil. It is