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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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(Received 2 January 1947)

In a foregoing paper (Fraenkel & Blewett, 1946b) 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, 1946a, 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

very improbable that the absence of the specific 'linoleic acid activity' of arachidonic acid was due to its becoming ineffective by oxidation during the course of the tests. In that case we would have expected toxic effects to become apparent, as has been the case in certain tests with linoleic acid (Fraenkel & Blewett, 1946b). It is also improbable

gallates, and there were indications that ascorbic acid had a similar action. On repeating tests with ascorbic acid we have now obtained a very striking acceleration of growth in its presence. It is evident from Fig. 2 that ascorbic acid does not act by itself but only in combination with fatty acids from which we may conclude that this effect is principally that of an antioxidant. Too great quantities of ascorbic acid are toxic. Addition of ascorbic acid alone, without the saponifiable fraction of wheat-germ oil, also has a toxic effect.

Fig. 1. The effect of arachidonic acid, α -tocopherol and the unsaponifiable fraction of wheat-germ oil on the growth of *Ephestia kuehniella*. — Normal moths emerge; --- no moths emerge.

that arachidonic acid would have shown a positive effect on the scales if it had been used in larger quantities. Linoleic acid produced an optimal effect with 4 mg./g. and a very noticeable effect with as little as 0.3 mg./g. There is a striking similarity between the effects of arachidonic acid and the highly unsaturated docosahexaenoic acid from cod-liver oil, which similarly did not improve the scale condition or emergence but accelerated growth very considerably (Fraenkel & Blewett, 1946b).

It is worth noting that α -tocopherol by itself on this occasion very considerably improved the rate of growth.

Fig. 2. The effect of ascorbic acid and the saponifiable fraction of wheat-germ oil on the growth and emergence of *Ephestia kuehniella*. — Normal moths emerge; --- no moths emerge.

The effect of ascorbic acid

We have formerly shown (Fraenkel & Blewett, 1946b) that the effects of α -tocopherol, i.e. promoting growth and stabilizing linoleic acid, can to some extent be reproduced by other antioxidants, e.g.

Synthesis of linoleic acid by insects

Judging from the importance of linoleic acid in the diet of *Ephestia*, one would expect that this species does not synthesize it, unless to an entirely insufficient degree. Insects which develop normally in the absence of linoleic acid may either not require it or synthesize it in sufficient quantity. To obtain evidence of the synthesis of linoleic acid, body fat from *Ephestia* and *Tenebrio*, grown in the presence and absence of linoleic acid, was tested for linoleic acid activity, using *Ephestia* itself as the test subject. Four different samples of oil were prepared, by extraction with light petroleum (Soxhlet), from *Ephestia* and *Tenebrio* larvae, grown on the following diets:

(1) *Ephestia* diet with linoleic acid: whole meal flour.

(2) *Ephestia* diet without linoleic acid: the artificial diet described above with the addition of 1% cod-liver oil, which as already shown by us (Fraenkel & Blewett, 1946b) has an excellent effect on growth and no effect on scales.

(3) *Tenebrio* diet with linoleic acid: whole meal flour with the addition of 10% yeast.

(4) *Tenebrio* diet without linoleic acid: as no. 2 but using ether-extracted yeast, and without cod-liver oil.

To record the states of linoleic acid deficiency which become apparent on certain diets, a classification of deficiencies has been used which is principally based on the appearance of scales on the wings. In this classification, which is fully explained elsewhere (Fraenkel & Blewett, 1946b), class 1 signifies a normal moth, class 8 failure to emerge from the pupa, while 2-7 indicate increasing tendency of the wings to lose their scales. The effect of the various insect oils described above on the degree of linoleic acid deficiency is shown in Table 1.

The effects of the oils from *Ephestia kuehniella* and *Tenebrio* grown on a diet with linoleic acid are similar. 9.3 mg./g. of *Tenebrio* oil supplies just sufficient linoleic acid for perfect wings and the same quantity of *Ephestia* oil is almost sufficient. With smaller quantities of oils the wings become progressively deficient in scales. With the oils from

insects grown on a diet without linoleic acid the difference between the two insects is very striking. Even with the largest quantities of *Ephestia* oil, the scale condition is about the same as in a diet which contains the unsaponifiable fraction of wheat-germ oil alone. The corresponding *Tenebrio* oil used as 9.3 mg./g. has an effect as good as if not better than 4.6 mg./g. *Tenebrio* oil from a linoleic acid-containing diet. From this we may conclude that hardly any synthesis of linoleic acid occurs in *Ephestia*, while there is considerable synthesis in *Tenebrio*.

grown on different diets by comparing their 'linoleic acid' effect with that of graded doses of wheat-germ oil, as determined in our former publication. The minimum optimal quantity was then found to be 4.3 mg. wheat-germ oil, containing about 55% of linoleic plus a small percentage of linolenic acid. The condition with 2.3 mg. wheat-germ oil corresponded roughly to the effect of 9.3 mg. *Ephestia* oil grown in the presence of linoleic acid and the effect of the corresponding *Tenebrio* oil was somewhat better. This would suggest a 'linoleic acid' content of the

Table 1. *The effect of various oils on the degree of linoleic acid deficiency in Ephestia kuehniella*

Additions to diet (per g.)	Classes of deficiencies (Fraenkel & Blewett, 1946b)								Total pupae from 20 larvae	Average pupation time (days)
	1	2	3	4	5	6	7	8		
No addition	—	—	—	—	—	—	—	11	11	77.8
Wheat-germ oil 8.6 mg.	18	—	—	—	—	—	—	—	18	46.9
Unsaponifiable fraction of wheat-germ oil	—	—	—	—	—	—	5	10	15	52.3
Fat from <i>Tenebrio</i> grown with linoleic acid	1.15 mg.	—	—	1	3	4	4	3	15	52.3
2.3 mg.	—	2	3	3	3	6	2	—	19	54.8
4.6 mg.	—	2	5	5	3	1	1	1	18	54.0
9.3 mg.	12	2	—	—	—	—	1	—	15	48.6
Fat from <i>Tenebrio</i> grown without linoleic acid	1.15 mg.	—	—	1	1	2	1	9	14	61.7
2.3 mg.	—	—	—	1	2	4	3	3	13	57.5
4.6 mg.	—	—	1	6	3	4	4	—	18	55.0
9.3 mg.	—	4	4	2	1	—	3	—	14	45.2
Fat from <i>Ephestia</i> grown with linoleic acid	1.15 mg.	—	—	—	2	4	1	8	15	54.1
2.3 mg.	—	—	1	—	3	7	4	3	18	52.2
4.6 mg.	—	2	4	5	3	1	2	—	17	48.5
9.3 mg.	5	7	3	—	—	—	—	—	16*	48.6
Fat from <i>Ephestia</i> grown without linoleic acid	1.15 mg.	—	—	—	—	1	—	16	17	59.9
2.3 mg.	—	—	—	—	—	1	2	15	18	60.6
4.6 mg.	—	—	—	—	—	—	5	9	14	51.4
9.3 mg.	—	—	—	—	—	2	3	10	15	53.1

* 1 damaged.

This difference in the linoleic acid content of oils from *Ephestia* and *Tenebrio* grown on a diet without linoleic acid is the more striking in view of the fact that the diet of *Ephestia* may have contained very small quantities of linoleic acid in the dried yeast, while the yeast in the *Tenebrio* diet was ether extracted.

In all tests, addition of the insect oils to the diet had a marked positive effect on the growth rate, which was of the same order as that induced by wheat-germ oil (Table 1, last column). This effect was most noticeable in the diets with the largest amount of oils. It had little relation to the linoleic acid content of the oils and was still marked with the smallest quantities of oil used. Oil from *Ephestia* grown in the absence of linoleic acid had a nearly maximum effect on growth and a minimum effect on the scales. In this it closely resembles cod-liver and halibut-liver oil (Fraenkel & Blewett, 1946b).

An attempt has been made to estimate the amounts of linoleic acid in the oils of our two insects

Ephestia oil of about 15%, and of the *Tenebrio* oil of possibly 20%. The effect of the *Tenebrio* oil, grown in the absence of linoleic acid, was about half that of the oil grown in the presence of linoleic acid. The corresponding *Ephestia* oil showed a minute linoleic acid activity, suggesting a content of less than 1%; too small to be measured with any degree of accuracy.

These results do not answer directly the question whether linoleic acid is of importance in the metabolism of such insects as do not require it as a constituent of their food, but its formation in appreciable quantities in the bodies of insects of the latter class would suggest that it served some particular purpose in their metabolism.

SUMMARY

1. Arachidonic acid, unlike linoleic acid, has no positive effect on the development of the wing of *Ephestia*, but it has a positive effect on growth of the

larva. In both respects it resembles the effect of docosahexaenoic acid from cod-liver oil.

2. Ascorbic acid, in combination with the saponifiable fraction of wheat-germ oil, has a positive growth effect which is attributed to an antioxidant action.

3. The linoleic acid content of various insect oils has been estimated by testing their effect on the wings of *Ephestia*. Oils from *Tenebrio* and *Ephestia* grown on a diet containing linoleic acid were

assessed to contain 20 and 15% linoleic acid, and from a diet without linoleic acid, 10 and less than 1% respectively. It is concluded that linoleic acid is synthesized by *Tenebrio* but not by *Ephestia*.

We gratefully acknowledge a grant by the Medical Research Council for the study of insect nutrition, of which this investigation is a part. We are also indebted to Prof. G. O. Burr and Dr R. T. Holman (Minneapolis) for a sample of methyl arachidonate.

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Further Observations on the Non-fermentability of *d*-Fructopyranose by Yeast

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(Received 21 February 1947)

In recent years we have investigated the fermentation rates of the various *d*-fructose, *d*-glucose and *d*-mannose modifications at low concentration (0.005M) under conditions (0°, pH 4.5) which reduce the rate of mutarotation to a minimum. On solution in water β -*d*-fructopyranose is reversibly converted to β -*d*-fructofuranose, the keto form contributing only a minute proportion to the equilibrium mixture

(Purves & Hudson, 1934; Isbell & Pigman, 1938; Bednarczyk & Marchlewski, 1938). The proportion of fructofuranose increases with temperature. The mutarotation of *d*-glucose, on the other hand, consists almost exclusively in an α -pyranose $\rightleftharpoons$ β -pyranose change.

The results obtained by this technique (for review, see Gottschalk, 1947b) seemed to substantiate the hypothesis, first proposed by Hopkins (1931), that only the furanose form of *d*-fructose is fermentable by yeast, whereas the pyranose modification is unfermentable. This interpretation implies that the concentration of *d*-fructofuranose, and not the total fructose concentration, has to be considered when the fermentation rate is referred to substrate concentration.

As an alternative interpretation of the slower fermentation rate, as compared with *d*-glucose, of *d*-fructose in low concentration at 30° and at 0° (cf. nos. 1 and 3 of the summary at the end of section 'Discussion') the following explanation may be offered: A lower concentration of *d*-fructose inside the cell resulting in a slower fermentation rate may be due to a higher energy barrier encountered by *d*-fructopyranose at the interface suspending fluid-cell membrane. Thus it was recently shown (Gottschalk, 1947a) that at 10° *d*-mannopyranose in low concentration (0.026M) is fermented at only half the rate of *d*-glucose by living yeast, whereas equal rates of CO₂ production are observed with yeast extract as fermenting agent under otherwise similar conditions. In view of this observation it seemed desirable to test the correctness of the alternative interpretation of the abnormal behaviour of yeast towards *d*-fructose in low concentration. If, by resisting the penetration of *d*-fructose more than that of *d*-glucose, the membrane of the yeast cell causes the slower fermentation of low concentrations of fructose compared with glucose, then the fermentation rates both of *d*-fructose and *d*-glucose may be expected to vary in the same way with yeast concentration. The present paper is concerned with the effect of yeast concentration on the fermentation rate of *d*-fructose and of *d*-glucose at low concentrations at 25°.

MATERIALS AND METHODS

The experiments were performed with baker's yeast (Effront), stored for 24 hr. at 1–2° after having been compressed. The yeast (2.0 g.) was suspended in 0.1M-KH₂PO₄