

THE DIETETICS OF THE CATERPILLARS OF THREE *EPHESTIA* SPECIES, *E. KUEHNIELLA*, *E. ELUTELLA*, AND *E. CAUTELLA*, AND OF A CLOSELY RELATED SPECIES, *PLODIA INTERPUNCTELLA*

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(With Twelve Text-figures)

In the course of a previous analysis of the dietetics of several insects found on dried foods (Fraenkel & Blewett, 1943 *a*), it was discovered that the larva of *Ephestia kuehniella*, which normally feeds on flour, grew badly, unlike the larvae of several beetles, on artificial diets consisting of casein, glucose, cholesterol and salts and the moths failed to emerge. Later the investigation was extended to *E. elutella*, which is known to infest such divergent materials as grain, tobacco, cacao beans and dried fruit. *E. elutella* grew fairly well on artificial diets, but again the moths failed to emerge from the pupae. Here the work was held up until it was discovered that *Ephestia* required, for successful growth and metamorphosis, fat-soluble factors which can be supplied with wheat-germ oil (Fraenkel & Blewett, 1945). In the present paper, which mainly deals with the requirements for carbohydrates, sterols and vitamins of the B complex, most of the diets described contain wheat-germ oil. An analysis of the factors concerned in wheat-germ oil will be given in the following paper.

Most of the experiments to be described were carried out with *E. kuehniella* and *E. elutella*, but, in addition, a number of tests were performed with another *Ephestia* species (*cautella*), and a very closely allied species, *Plodia interpunctella* which lives under very similar conditions to the *Ephestia* species.

#### METHODS

All the tests were performed at 25° C. and 70% relative humidity. The methods employed are similar to those already described (Fraenkel & Blewett, 1943 *a, d*). As the caterpillars of *Ephestia* are considerably larger than the larvae of the beetles used in previous experiments, five larvae (instead of ten) were always grown together in a 2 × 1 in. tube which contained 3 g. of food (instead of 2). In view of the

great number of individual tests it was not found practicable to grow each caterpillar singly. Each test was performed with twenty larvae. When the larvae were fully grown and started wandering, a strip of corrugated paper, about 2 × 1 in., was inserted into each tube. Most larvae of the three *Ephestia* species, but not of *Plodia*, pupated in the folds of the corrugated paper. By holding the papers toward the light it was possible to distinguish the darker and shorter pupa from the larva, and this method had also the additional advantage that the dates of pupation could be marked on the papers. *Plodia* pupated inside the food and not in papers, which made it impossible to observe the date of pupation.

The efficiency of a diet for the three *Ephestia* species was estimated by the length of the larval stage and the number pupating and for *Plodia* by the larval plus pupal stage and the number of moths emerging. The duration of the pupal stage is independent of the duration of the larval stage, and lasts, at 25° C., about 10–12 days with the four species in question.

The method of preparing the diets has been slightly modified since the earlier descriptions (Fraenkel & Blewett, 1943 *a, b, d*). The salt mixture was not added as a suspension to each individual diet but was mixed into a large quantity of casein with some water and the mixture dried and ground. This ensured a more homogeneous distribution of salts. Another modification, introduced in the course of this investigation, consisted of regrinding the diets in a mortar after they had stood for one or more days at 25° C. and 70% R.H., and before larvae were added. This ensured a more homogeneous mixture and prevented caking.

All the curves show the total number of pupae or adults formed, plotted against time.

Most experiments to be described below are a modification of a diet which had proved adequate for

a number of insects (Fraenkel & Blewett, 1943 a). It consisted of

Casein, vitamin and fat free (E or A/E, Glaxo Laboratories)	50 parts
Glucose (or starch)	50 parts
Dried debittered brewers' yeast (Glaxo Laboratories)	5 parts
Cholesterol	1 part
McCullum's salt mixture	2 parts
Water	10 parts
(After mixing and equilibrating at 70% R.H. the water content of the diet is approximately 10-12%)	
Wheat-germ oil (added after the necessity for it had been discovered)	c. 1 part

different occasions may vary is given in Fig. 7, which also shows that otherwise the results are virtually identical.

THE BASIC FOOD REQUIREMENTS

*Ephestia elutella* grows about as fast on the artificial diet described above as on weatings (one of the offal fractions obtained in the milling of white flour) which so far has proved the best natural food for *E. elutella* (cf. Figs. 1, 6). The artificial diet, supplemented by wheat-germ oil, therefore constitutes, as far as we know at present, an optimal diet. Very little or no growth takes place if glucose is omitted. Growth is very slow when the amount of glucose is reduced from 50 to 20%. With 80% glucose growth

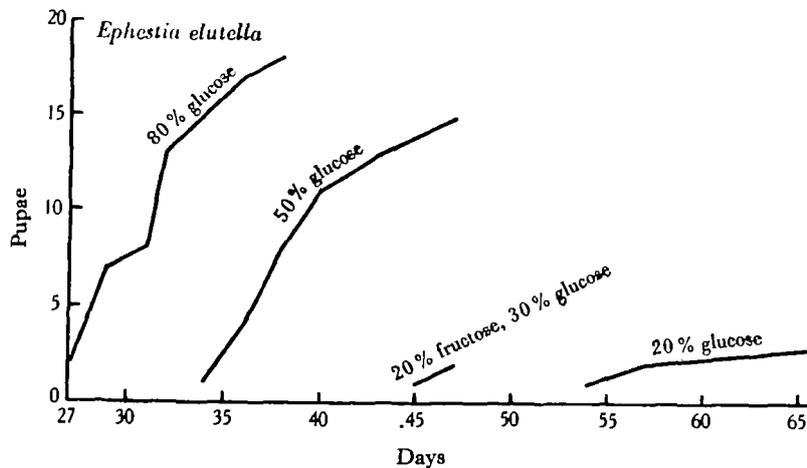


Fig. 1. Growth of *Ephestia elutella* on artificial diets containing varying amounts of glucose or fructose.

The ingredients of the dry diet add, therefore, up to 109 parts. For convenience we speak of percentages instead of parts, which introduces only a small error. In tests in which the amount of carbohydrates was modified, it was substituted by an equal amount of casein so that carbohydrates and casein together always constituted 100 parts. In earlier experiments 15 parts of water were added to the diets. As the dry ingredients were not water free the initial water content of the diets must have been well above 15%, and although the diets gradually lost water while standing at 70% R.H., on some occasions diets became mouldy. In later tests only 10 parts of water were added.

Most results depend on a relative comparison of the efficiency of different diets. The absolute results obtained on a particular diet on different occasions are not always identical, and it is therefore important that a comparison of diets be made in the same batch of larvae grown simultaneously. Most figures in this paper represent results obtained in one set of tests. An example of how widely results obtained on

is noticeably faster than with 50%, but on some occasions mortality was higher at 80 than at 50% (Fig. 1). In nearly all the tests with *E. elutella* described in this paper diets with 50% glucose were used. The results obtained in corresponding tests with *E. cautella* and *Plodia* were much the same (Fig. 2). Growth on an artificial diet with 50 parts glucose was about as good as on weatings. Without glucose there was no growth, and on 20% glucose it was much slower than on 50%. With 80% glucose growth was always faster, but the mortality was in some cases higher.

With *Ephestia kuehniella* (Fig. 3) a diet with 80% glucose became almost as efficient as wholemeal flour, which may be considered as an optimal diet for this species. With 50% glucose, growth was relatively much slower if compared with the other species, and hardly any growth took place with 20% glucose. 80% glucose was used for all tests described.

*E. kuehniella* grew equally well when starch (maize) was substituted for glucose and grew very

much better on 50% starch than 50% glucose (Fig. 3). The other three species, strangely, either failed to grow with starch or grew exceedingly slowly. This result was unexpected as all three species are known as pests on cereals. In order to account for the inability of *E. elutella* to grow on starch a rough comparison was made of the concentration of amylase in

of dried fruit is high, and much of it consists of fructose. The water content of dried fruit is also usually higher than that of our artificial diets, or of cereals. It was therefore considered possible that adding fructose to diets might improve their efficiency, either because of intrinsic nutritional properties of fructose, or because of the increase in water content which the

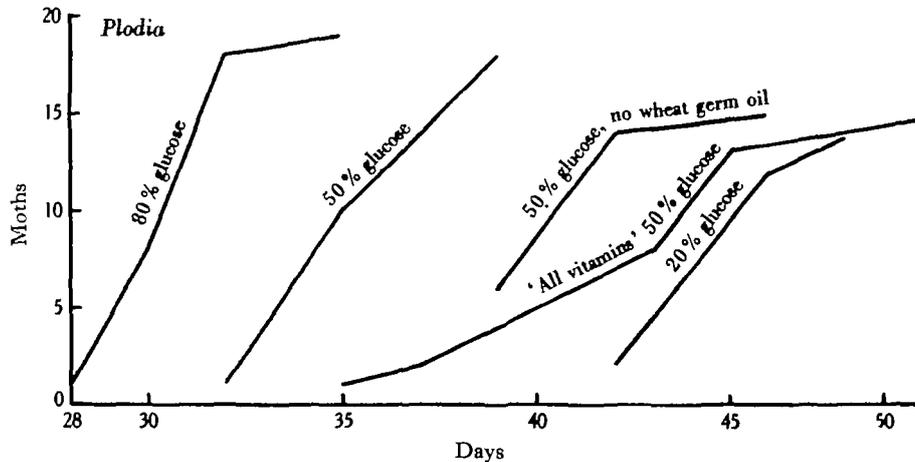


Fig. 2. Growth of *Plodia interpunctella* on artificial diets in the presence of varying amounts of glucose. All diets contain 5% yeast, except one ('all vitamins'), which contains a mixture of pure vitamins of the B complex and the water-insoluble fraction of yeast.

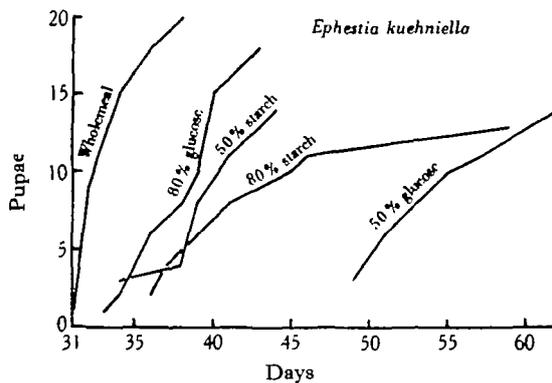


Fig. 3. Growth of *Ephestia kuehniella* in the presence of varying amounts of glucose or starch.

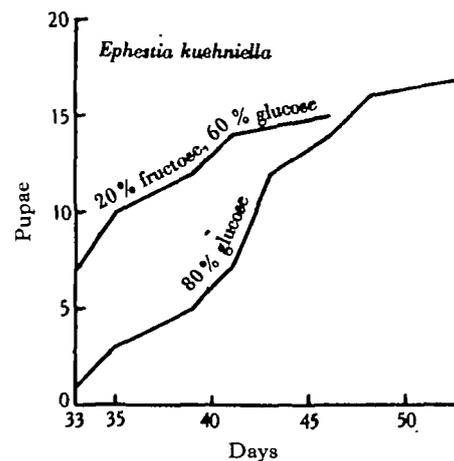


Fig. 4. Growth of *Ephestia kuehniella* in the presence of 80% glucose or 60% glucose plus 20% fructose.

the gut of *E. kuehniella* and *elutella*, using Cole's method of the 'first change' (Cole, 1933, Ex. 253). No difference was discovered in the rate of decoloration of a starch solution with enzyme suspensions from the guts of the two species.

*E. elutella*, *E. cautella* and *Plodia interpunctella* often occur as pests on dried fruit. The sugar content

highly hygroscopic fructose would induce in diets. With 30% fructose and 20% glucose, growth of these three species was usually inferior to that on 50% glucose, and in all cases the mortality was high (Fig. 1). With *Ephestia kuehniella* on a diet containing 60% glucose and 20% fructose growth was slightly faster than on one with 80% glucose (Fig. 4). It

appears, therefore, that the effect of fructose on the diet for reasons not yet elucidated, was detrimental, except for *E. kuehniella*.

Testing for the importance of cholesterol in the artificial diet is complicated by the fact that a sterol deficiency can only be produced in the absence of wheat-germ oil, which is important for successful emergence of adult moths in the three *Ephestia*

given instead of yeast (Fig. 5). The fact that some slow growth took place in the absence of cholesterol is almost certainly explained by the small amounts of ergosterol which are supplied to the diets with yeast. No growth took place on a diet which contained an aqueous yeast extract and no 'insoluble yeast' nor cholesterol, and which must have been virtually free of sterols.

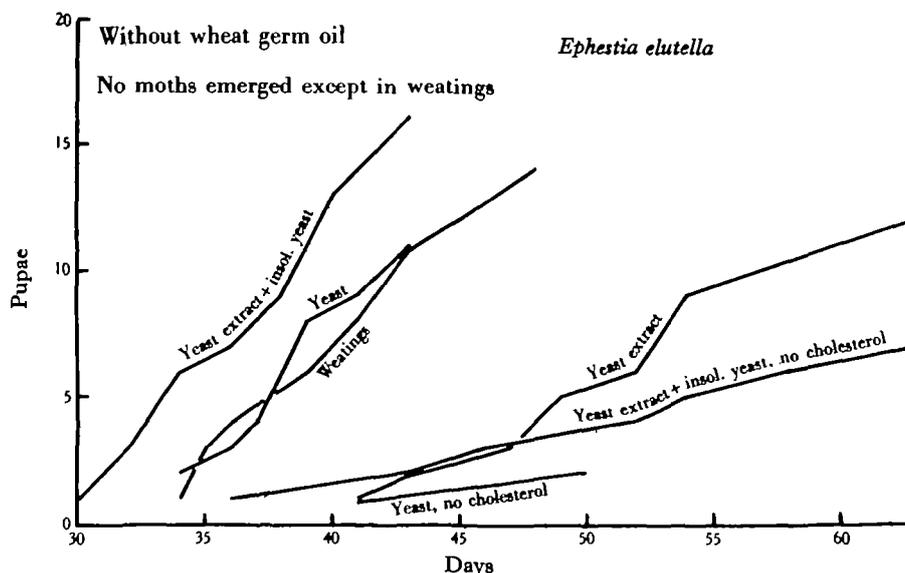


Fig. 5. Growth of *Ephestia elutella* on artificial diets with various combinations of yeast, yeast extract, insoluble yeast and cholesterol. Diets do not contain wheat-germ oil. No moths emerged except on weatings.

Table 1. Growth of *Ephestia kuehniella*, *E. elutella*, *E. cautella* and *Plodia interpunctella* on certain artificial diets

Diet contains	<i>E. kuehniella</i>	<i>E. elutella</i>	<i>E. cautella</i>	<i>Plodia</i>
Wholemeal flour	++++	++	±	±
80% glucose	++++	++++	++++	++++
50% glucose	++	++++	+++	+++
20% glucose	—	+	+	++
60% glucose, 20% fructose	++++	Not done	Not done	Not done
30% glucose, 20% fructose	Not done	++?	+++?	+++?
80% starch	++++	Not done	Not done	Not done
50% starch	++++	±	—	—
No carbohydrate	—	—	—	—
No cholesterol	—	—	—	—

species, and also affects the growth rate. The need of *E. kuehniella* for sterol in the diet has previously been demonstrated by us (Fraenkel & Blewett, 1943 a, c), using wholemeal flour extracted with chloroform. *E. elutella* on our basic diet, without wheat-germ oil, grew very much better in the presence of cholesterol than in its absence, and a corresponding difference in growth rate was also found when aqueous yeast extract and 'insoluble yeast' (yeast exhaustively extracted with water (Fraenkel & Blewett, 1943 a)) was

The results of this section are summarized in Table 1 using symbols which are self-explanatory.

### REQUIREMENTS OF VITAMINS OF THE B COMPLEX

#### (a) *Ephestia elutella*

After having ascertained the importance of the proximal constituents of the artificial diet described on

p. 163, the next step consisted in analysing the factors contained in yeast. Yeast was divided into a water-soluble and a water-insoluble fraction. The water-soluble extract was prepared from fresh brewers' (pitching) yeast according to Chick & Roscoe (1930), so that 1 c.c. of the extract corresponded roughly to 1 g. of the dry yeast. 15 parts of this extract were used in the diets and no further water was given. This quantity had been found previously to be adequate for optimal growth of other insects (Fraenkel & Blewett, 1943 a). It corresponds theoretically to 15 parts of dry yeast in the diet as against 5 parts in the original diet, but it is highly improbable that the water extract contains the whole of the water-soluble fraction of yeast. Water 'insoluble yeast' is the residue from the preparation of yeast extract which has been further treated to nine extractions with large quantities of boiling water for 1 hr. each.

In an earlier experiment (Fig. 5) where wheat-germ oil was not used growth was somewhat quicker with both yeast extract and insoluble yeast in the diet than with yeast, which may have been due to larger amounts of some soluble factor in the diet. With insoluble yeast alone there was no growth. With yeast extract alone growth became very much delayed. It was therefore clear that *E. elutella* required both soluble and insoluble factors from yeast.

In the following tests yeast extract was replaced by a mixture of the known constituents of the vitamin B complex, while 'insoluble yeast' was retained at first as a source of insoluble factors. The full diet, subsequently called 'all vitamins' diet, consisted of

	Parts		µg./g.
Casein	50	Thiamin (B <sub>1</sub> )	50
Glucose	50	Riboflavin	50
Cholesterol	1	Nicotinic acid	50
Salt mixture	2	Pyridoxin (B <sub>6</sub> )	50
Insoluble yeast	5	Pantothenic acid	50
Wheat-germ oil	½-1	p-Amino-benzoic acid	50
		Choline chloride	500
		Inositol	500

On this diet growth was relatively good, but noticeably slower than on a diet which contained whole yeast (Fig. 6). This indicated that it was lacking in some unknown factor(s). In a series of tests the importance of each factor of the B complex was then investigated by leaving vitamins out of the diet one at a time. No growth took place in the absence of thiamin, riboflavin, nicotinic acid, pyridoxin, or pantothenic acid. In the absence of choline growth proceeded at a considerably retarded rate, but most larvae ultimately pupated and emerged (Fig. 6). There was no indication that either inositol or p-amino-benzoic acid is of any importance for *E. elutella* (Fig. 7). Without them growth was in every respect as good as with them. It was considered possible that significant amounts of inositol might have been supplied in the diet with 'insoluble yeast', and therefore a further comparison was made of two diets which contained biotin but no 'insoluble yeast'.

Here again, the presence or absence of inositol made no difference to the efficiency of the diet (Fig. 8).

Leaving out insoluble yeast from the 'all vitamins' diet had the effect of slowing down growth and increasing mortality (Fig. 8). This deficiency was partly or entirely made good by the addition of biotin. The amounts of biotin used were relatively small, 0.1 µg./g., which is the amount found to be optimal in earlier experiments with another insect, *Tribolium* (Fraenkel & Blewett, 1943 b). In one test (Fig. 8) a diet with biotin was in every respect as good as one with insoluble yeast, while in another test (Fig. 6) the biotin diet was considerably inferior. The better effect was obtained with a sample of free biotin, while in the other test biotin methyl ester was used. It is not impossible that the difference in efficiency of the two samples was due to this difference in constitution. In earlier tests with *Tribolium* free biotin also appeared to be more efficient than the methyl ester.

#### (b) *Ephestia kuehniella*

The diet which was used for investigating the requirements of the members of the vitamin B complex was the same as that described above for *E. elutella* except that it contained 80 parts of glucose instead of 50. Here, as with *E. elutella*, a diet which contained eight known members of the B complex, and in addition insoluble yeast, was considerably inferior to a diet which contains yeast instead of a mixture of pure vitamins (Fig. 9). In the absence of either choline or inositol the diet appeared to become less efficient, and without p-amino-benzoic acid more efficient (Fig. 9). On these four diets, pupation started at almost exactly the same time, and it is possible that the apparent differences in efficiency are not significant. In the absence of either thiamin, nicotinic acid, pyridoxin or pantothenic acid, no growth took place. Without riboflavin, unlike the result obtained with *E. elutella*, sixteen larvae out of twenty eventually completed development. In these tests the quantities of vitamins used were arbitrary and almost certainly much in excess of the amounts required. Experiments designed with the view of determining the minimum optimal requirements were only undertaken with nicotinic acid. Graded responses were obtained to graded doses of nicotinic acid, with an optimum effect at 8 µg./g. of the diet and a threshold effect with 4 µg./g. (Fig. 10).

Even in the presence of all the known vitamins of the B complex, the larvae of *E. kuehniella*, similar to those of *E. elutella*, grow considerably more slowly than on a diet with yeast (Figs. 9, 10). This indicates that important unknown factors are still missing. With insoluble yeast left out from the diet, growth becomes very much slower still, and only eleven larvae completed development. This deficiency was entirely, or almost entirely, remedied by adding biotin (Fig. 11). Biotin, therefore, is an essential growth factor for *E. kuehniella*.

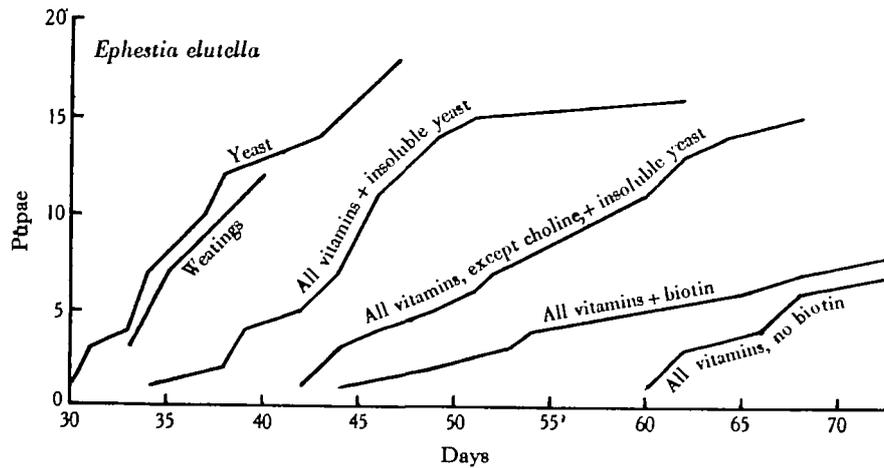


Fig. 6. Growth of *Ephestia elutella* on diets containing a mixture of the vitamins of the B complex and in the presence or absence of insoluble yeast, choline or biotin.

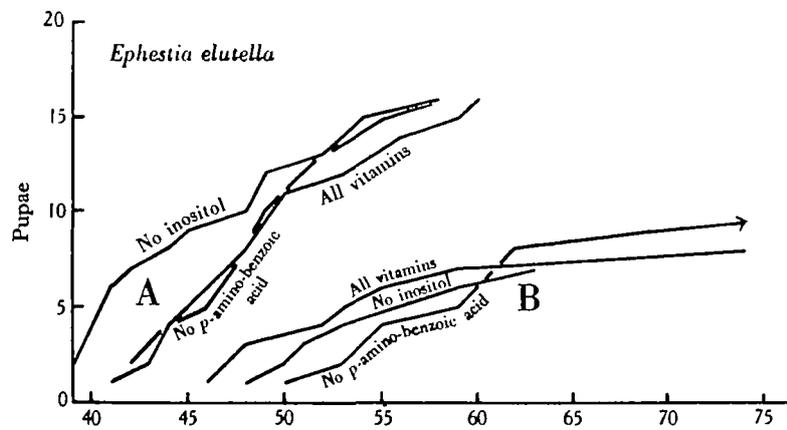


Fig. 7. Growth of *Ephestia elutella* on diets containing all the known factors of the vitamin B complex and in the presence or absence of inositol or *p*-amino-benzoic acid. A and B represent sets of tests carried out at different times.

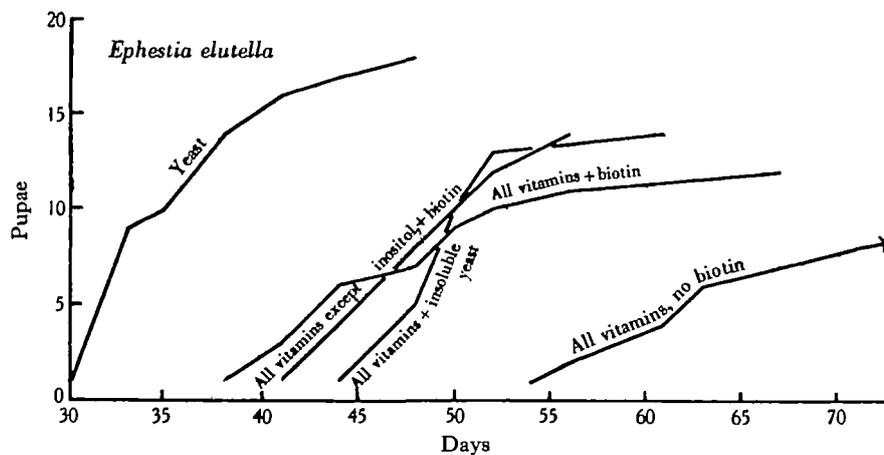


Fig. 8. Growth of *Ephestia elutella* on artificial diets in the presence or absence of insoluble yeast, biotin or inositol.

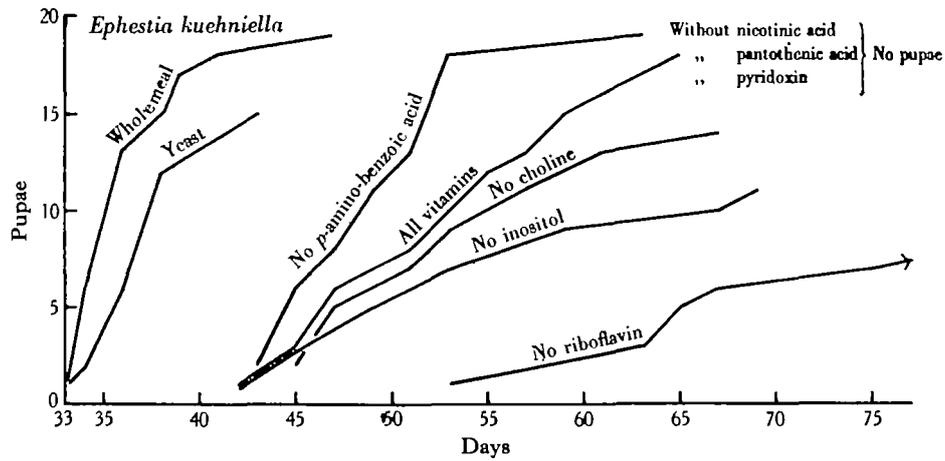


Fig. 9. Growth of *Ephestia kuehniella* on diets containing 'all vitamins' of the B complex, and on similar diets in the absence of *p*-amino-benzoic acid, choline, inositol or riboflavin. No growth took place in the absence of thiamin, nicotinic acid, pyridoxin or pantothenic acid.

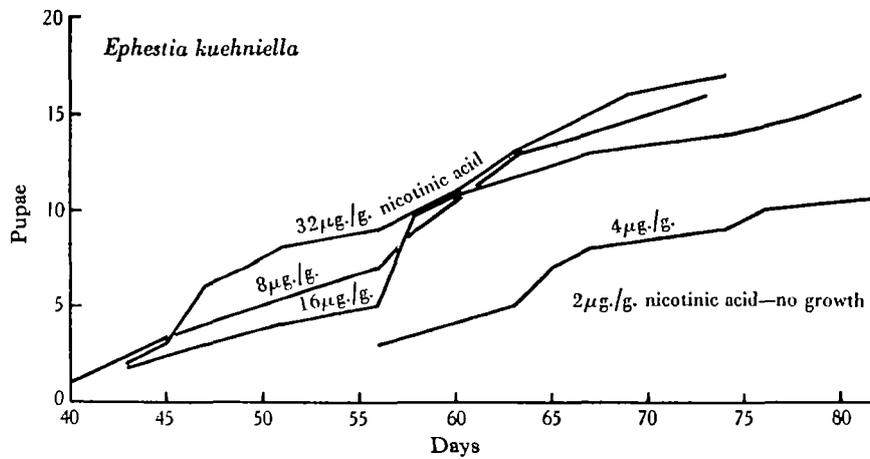


Fig. 10. Growth of *Ephestia kuehniella* on nicotinic acid-free diets, with the addition of graded doses of nicotinic acid.

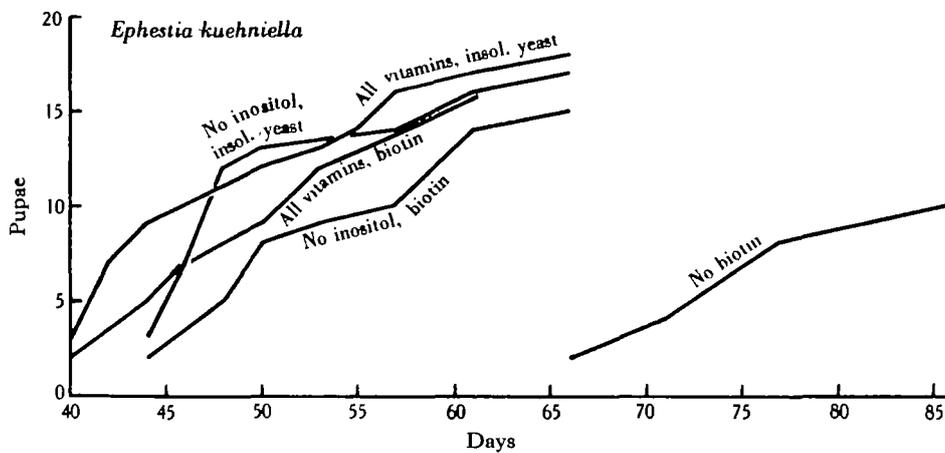


Fig. 11. Growth of *Ephestia kuehniella* on artificial diets and in the presence or absence of biotin or inositol.

view of the uncertain result about the requirements for inositol, obtained in earlier tests (Fig. 9), another series of tests was set up, in which the effect of omitting inositol was investigated in two different diets, one containing insoluble yeast and the other biotin. In both cases there was a slight delay in growth in the diets which did not contain inositol (Fig. 11), and this, together with our earlier result, makes it very probable that inositol is of some nutritional significance for *E. kuehniella*.

(c) *Ephestia cautella* and *Plodia interpunctella*

On a diet containing all the known members of the vitamin B complex, and the insoluble fraction of yeast, these two species reacted very similarly to *Ephestia kuehniella* and *elutella*. Growth took place, but at a greatly reduced rate (Fig. 2). The requirements for single factors of the B complex have not yet been investigated, except for a test with biotin on *Plodia*. Here the result was very similar to that with *Ephestia kuehniella*. In the absence of biotin, hardly any growth took place, and only one out of twenty completed development while in the presence of biotin growth was slow and seven larvae completed development.

## DISCUSSION

The present results fully explain why on a former occasion (Fraenkel & Blewett, 1943 a) we did not succeed in growing *Ephestia kuehniella* on an artificial diet with glucose as the carbohydrate. This failure was mainly due to the absence of wheat-germ oil and partly to a suboptimal concentration of glucose (50 instead of 80 %).

The two *Ephestia* species *kuehniella* and *elutella*, in their requirements for vitamins of the B complex, closely resemble several other insects investigated by us which do not obtain additional vitamins from intracellular symbionts, viz. *Tribolium confusum*, *Ptinus tectus* (Fraenkel & Blewett, 1943 b), *Tenebrio molitor* and *Dermestes vulpinus* (unpublished). The five important members of the vitamin B group, thiamin, riboflavin, nicotinic acid, pyridoxin and pantothenic acid, are necessary for all these insects. *p*-Amino-benzoic acid is certainly not required and its inclusion is sometimes detrimental. With regard to inositol, we have now obtained evidence that it is of some nutritional significance for *Ephestia kuehniella*, an evidence which so far has been lacking for other insects. Choline chloride is required for *E. elutella*, *Ptinus*, *Dermestes* and *Tenebrio*, but possibly not for *Ephestia kuehniella* and *Tribolium*. *Ephestia kuehniella* and *Plodia interpunctella*, and, to a lesser degree, also *E. elutella* and *Tribolium* require biotin. On a diet which contains all the known members of the vitamin B complex, the three *Ephestia* species and *Plodia* grow relatively well, but the growth rate is far from being optimal. This indicates that other unknown components of the vitamin B complex are missing, though they are of lesser importance than the five known factors mentioned above.

An interesting difference between *Ephestia kuehniella* and *elutella* concerns the importance of riboflavin in the diet. *E. elutella* entirely fails to grow in the absence of riboflavin, while *E. kuehniella* grows satisfactorily, though at a rather slow rate. This perhaps partly explains why *E. kuehniella* grows so much better on flour of a low extraction than *E. elutella*. Grain is a more inferior source of riboflavin than of most of the other known factors of the B complex. Patent flour contains only very minute quantities of riboflavin (Table 2), and we have demonstrated that lack of riboflavin is the chief reason

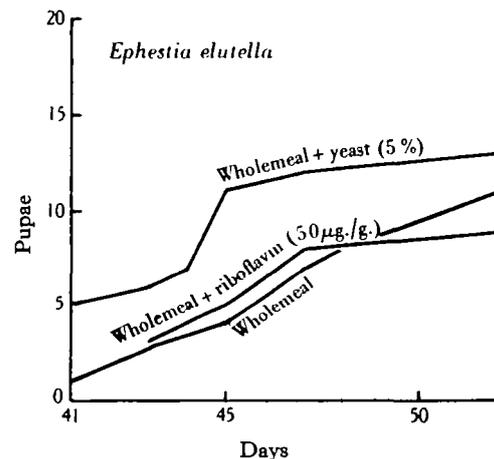


Fig. 12. Growth of *Ephestia elutella* on wholemeal flour, and with the addition of yeast or riboflavin.

for the slow growth of *Tribolium confusum* on it (Fraenkel & Blewett, 1941). *Ephestia kuehniella* also grows very slowly on patent flour (Fraenkel & Blewett, 1943 d), but the essential feature is that it grows at all. It is suggested here that *E. kuehniella* can grow on patent flour by virtue of being relatively insensitive to the lack of riboflavin, and this would account for its widespread occurrence in flour mills.

*E. elutella*, by way of contrast, does not grow on low-extraction flour. Even on wholemeal flour growth is relatively slow. It is better on middlings, a milling fraction rich in B vitamins, and also noticeably better on wholemeal flour to which 5% yeast has been added (Fig. 12). This suggests that a limiting factor for *E. elutella* on wholemeal flour is a lack of some vitamins of the B group. It was thought that this may perhaps be due to a shortage of riboflavin in wholemeal flour. Addition of riboflavin, however, did not improve growth on wholemeal flour (Fig. 12).

There is, however, another factor which limits the growth of *E. elutella*, *E. cautella* and *Plodia* on flour. These three species, which grew very well on an artificial diet containing glucose, unexpectedly grew hardly at all on the same diet where glucose was replaced by starch. *Ephestia kuehniella*, on the other

hand, grows about equally well on starch or glucose. This result was somewhat surprising, because all four species are known as pests on grain and cereal products. According to several authorities (e.g. Cotton & Good, 1937), *Plodia interpunctella* is a major pest and *Ephestia cautella* and *elutella* are minor pests on grain and milled cereal products of all kinds. In feeding tests with *Plodia* and *Ephestia cautella* on flours of different extraction the results were similar to those previously reported for *E. elutella* (Fraenkel & Blewett, 1943 d). There was no growth on the low-extraction flours (40 and 72%), but even on wholemeal flour hardly any growth took place. On whole grain, *Plodia* and *Ephestia cautella*, in our experiments, also grew slowly and only very few specimens completed development. Like *E. elutella* these two species feed exclusively on the embryo, leaving the endosperm untouched, while *E. kuehniella* feeds on both embryo and endosperm. It is generally believed that pure wheat germ (as distinct from commercial germ, which as a milling product is always contami-

amounts of them. Growth on most of these substances is slow, and we would not expect them to contain necessarily optimal amounts of all vitamins. Figures for thiamin, riboflavin and nicotinic acid of some of these foods have only very recently become known, and a comparison with corresponding figures for wholemeal flour shows (Table 2) that the requirements of *E. elutella* of some B vitamins must be very low. Nothing is known about vitamins in cured tobacco, but, judging from the food requirements of *E. elutella*, it must be assumed to contain substantial quantities of carbohydrates and of vitamins of the B complex.

#### SUMMARY

1. The larvae of four species of moths, *Ephestia kuehniella*, *E. elutella*, *E. cautella* and *Plodia interpunctella*, have been grown successfully on artificial diets, consisting of casein, glucose, yeast, cholesterol, a salt mixture, wheat-germ oil and water.

Table 2. *Composition of certain dried foods*

	Carbo- hydrates %	Thiamin mg./100 g.	Riboflavin mg./100 g.	Nicotinic acid mg./100 g.	Source
Cocoa	31	0.09	0.45	1.5	N.R.C. (1944)
Ground nuts	24	0.4	0.13	16	N.R.C.
Dried prunes	71	0.09	0.1	1.5	N.R.C.
Raisins	71	0.14	0.1	0.5	N.R.C.
Wholemeal flour	72	0.47	0.11	4.1	N.R.C.
Patent flour	75	0.07	0.03	0.6	Cheldelin & Williams (1942)

nated with endosperm) contains no starch. The sugar content of germ has been given as about 10% (Booth, Carter, Jones & Moran, 1941), but there is reason to believe that this is an underestimate. (Whole wheat contains only 1-1% sugar.) It therefore seems feasible that on high-extraction milling fractions (such as middlings, weatings, germ, etc.) *E. elutella*, *E. cautella* and *Plodia* may successfully feed by picking out such particles of germ or perhaps bran which are low in starch and high in sugar and vitamins of the B complex. It is interesting to note that the effect of wholemeal flour on our four species is very much what we would expect from the result on artificial diets. For *Ephestia kuehniella*, which grows very well on starch diets, wholemeal flour is an optimal diet. *E. elutella*, which grows very slowly on starch diets, also grows very slowly on wholemeal flour though most larvae ultimately pupate. *E. cautella* and *Plodia* fail to grow on either diet (Table 1).

*Ephestia elutella* is also known as a pest on such divergent substances as cacao beans, dried fruit, nuts and tobacco, and as this species does not grow in the absence of most of the vitamins of the B complex it must be assumed that these foods contain sufficient

2. The requirements for carbohydrates are high. Growth of *Ephestia kuehniella* is optimal with 80% glucose in the diet. The other three species also grow fastest with 80% glucose, but survival is usually better with 50% glucose. With smaller quantities of glucose growth becomes slower, and little or no growth takes place with 20% glucose or less.

3. Cholesterol is an essential constituent of the diet for all four species.

4. On diets which contain the known factors of the vitamin B complex in pure substance in place of yeast, all four species grow relatively well, but the growth rate becomes noticeably slower. This indicates the presence of further important growth factors in yeast.

5. Of the known factors of the vitamin B complex, the following are indispensable for *E. kuehniella* and *elutella*: thiamin, nicotinic acid, pyridoxin and pantothenic acid. Riboflavin is also indispensable for *E. elutella*, while in its absence *E. kuehniella* grows very slowly. Choline chloride greatly improves growth of *E. elutella*, inositol has some beneficial effect on that of *E. kuehniella*, and *p*-amino-benzoic acid does not seem to be necessary for either species. Biotin is an

important growth factor for *E. kuehniella* and *Plodia*, and to a somewhat lesser degree, also improves growth of *Ephestia elutella*.

6. Diets in which glucose is replaced by starch are excellent for *E. kuehniella*, but unsuitable for the other three species.

7. These findings are discussed in connexion with

the natural occurrence and the type of feeding of the four species on flour, whole grain and other dried foods.

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