THE DIETETICS OF THE CLOTHES MOTH, *TINEOLA BISSELLIELLA* HUM.

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(With Seven Text-figures)

Much attention has been paid in the past to the peculiar mechanism of keratin digestion which enables the clothes moth to infest woollen materials (Schulz, 1925; Linderstrøm-Lang & Duspiva, 1935; Duspiva, 1936), but little is known about its general food requirements. It is known that the larvae will not grow on clean wool (Titschak, 1922; Crowell & McCay, 1937) and that damage to clothing is generally restricted to parts soiled by dirt and sweat. On the other hand, *Tineola* has been bred in the laboratory on various foods in the absence of keratin, and there are no indications that keratin is a necessary constituent of the food (Titschak, 1922). A study of some aspects of the nutrition of *Tineola* has been made by Crowell & McCay (1937) and will be discussed below.

METHODS

The methods employed are similar to those described in earlier publications (Fraenkel & Blewett, 1943*a, c*). Ten larvae were always grown together in a 2 × 1 in. specimen tube containing 2 g. of food, and each test was done in duplicate. All the tests were performed at 25° and 70% relative humidity. They were started with the first stage larva and concluded with the emergence of the adult moth. *Tineola* as a rule pupates inside the food and therefore pupation cannot be observed. The pupal period is most probably constant and independent of the nature of the larval food, and it lasts, at 25° and 70% R.H., about 10 days.

General food requirements

Tineola grows well on an artificial food mixture composed of

Casein (A/E, Glaxo Lab.)	50 parts
Glucose	50 ,,
Yeast (brewers', debittered, Glaxo Lab.)	5 ,,
Cholesterol	1 part
McCollum's salt mixture	2 parts
Water	10 ,,

On this food, called 'glucose diet' in Table 1, at 25° and 70% R.H., adult moths start to emerge after about 33 days, and most adults emerge between the 33rd and 39th day. As the pupal stage lasts about 10 days, the larval period is about 25 days. This growth rate, as will be shown later, compares very favourably with the speed of growth on other, more natural, food substances and is considerably faster than that reported for larvae on woollen materials. Glucose is not an essential constituent of this diet; if the same amount of casein is substituted for it, the food remains in every respect as efficient (Table 1).

Table 1. *Emergence of* Tineola bissellíella *on various diets. Total number of moths formed by the* 31*st*, 33*rd*, *etc., day. Twenty larvae in each experiment*

Days ...	31	33	35	37	39	41	43	45
Glucose diet*	—	3	11	11	18	18	19	—
Same, plus wheat-germ oil	1	4	8	8	14	14	15	—
Diet without carbohydrates	—	2	6	6	11	14	17	—
Glucose diet plus wool fibres	—	—	8	16	16	16	18	20
Glucose diet with ether-extracted yeast	—	1	8	10	15	17	18	—
'All vitamins' diet†	—	—	6	8	9	15	19	—
'All vitamins' diet plus yeast fat	—	—	3	9	9	10	16	17

* Composition given on this page.
† Composition given on p. 157.

If glucose is replaced by starch (maize), growth is retarded by about 10 days (Fig. 4). It is therefore evident that a carbohydrate is not required in the diet and that the presence of starch is somewhat detrimental. Without cholesterol no growth takes place and the larvae die at an early stage. Yeast is equally an essential constituent of the diet; no growth takes place if yeast is omitted. There are no indications that addition of wool improves the efficiency of the diet (Table 1). Addition of a fat (wheat-germ oil) does not alter the growth rate (Table 1).

Requirements of vitamins of the B group

An investigation of the importance of the water-soluble vitamins, contained in yeast, was made by the method described in an earlier publication (Fraenkel & Blewett, 1943*c*). Instead of yeast a mixture of pure vitamins of the B group was given and in addition the diet contained 5 parts of 'insoluble' yeast (yeast exhaustively extracted with boiling water to remove water-soluble constituents). The diet then consisted of

			μg./g. of dry diet
Casein	50 parts	Thiamin (B_1)	50
Glucose	50 ,,	Riboflavin	50
'Insoluble yeast'	5 ,,	Nicotinic acid	50
Cholesterol	1 part	Pyridoxin (B_6)	50
Salt mixture	2 parts	Pantothenic acid	50
Water	10 ,,	Inositol	500
		Choline chloride	500
		p-Amino-benzoic acid	10

On this diet, called subsequently 'all vitamins' diet in Fig. 1 and in Table 1, growth was in every respect as good as on the original diet, which contained 5 parts of yeast. Leaving out single vitamins of the B complex, one at a time, affected the efficiency of the diet to a different degree with different vitamins (Fig. 1). Growth was equally good with or without

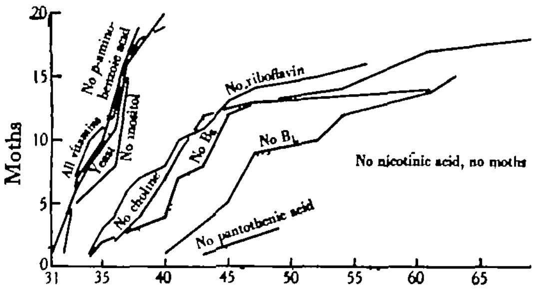

Fig. 1. Growth of *Tineola bisselliella* on an artificial diet, described above, which contains the known members of the vitamin B complex in pure substance ('all vitamins') compared with similar diets from which single vitamins have been left out, one at a time.

inositol or *p*-amino-benzoic acid. Growth became increasingly slower if either riboflavin, choline, pyridoxin or thiamin were omitted, but most larvae completed development. In the absence of pantothenic acid growth was slower still, and only three out of twenty reached the adult stage, while no growth took place in the absence of nicotinic acid.

In view of the fact that *Tineola* eventually pupates in the absence of any one of the B-group vitamins, except nicotinic acid, an attempt to determine the minimum optimal requirements was only made with nicotinic acid. A graded growth response was obtained with graded doses of nicotinic acid, and growth was optimal with 8–16 per g. of the diet (Fig. 2).

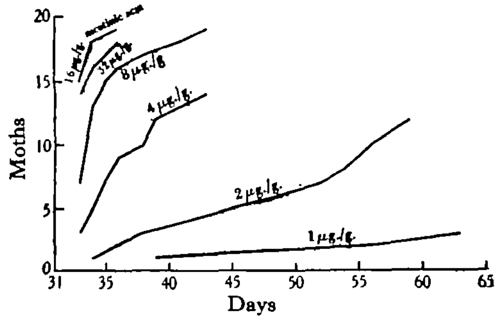

Fig. 2. Growth of *Tineola bisselliella* on a nicotinic acid-free diet with the addition of graded doses of nicotinic acid.

With all the B vitamins present in the diet, but without the insoluble part of yeast, growth was very slightly retarded. Addition of biotin (0·1 μg./g.) to this diet possibly somewhat improved it (Fig. 3). The

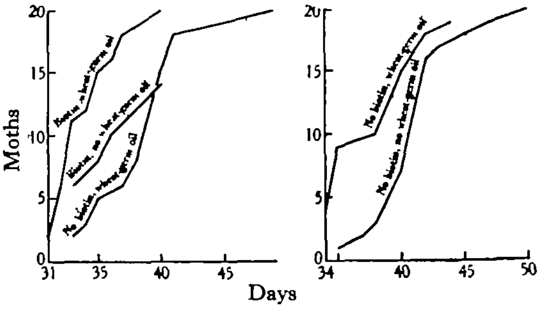

Fig. 3. Growth of *Tineola bisselliella* on artificial diets in the presence or absence of biotin or wheat-germ oil.

following vitamins are therefore of some importance in the nutrition of the *Tineola* larva: thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, choline and possibly biotin.

Fat requirements

The artificial diet described on p. 156 contains no fat, apart from such small amounts which the yeast contained. Extracting the yeast with ether has not the slightest effect on the efficiency of this diet (Table 1). This is not a conclusive result, as a Soxhlet extraction might not remove all traces of fat. There was, furthermore, no evidence that addition of fat, neither wheat-germ oil nor the fat extracted from yeast with petrol ether, improved the diet (Table 1). It may be argued that in all these diets very small amounts of fat were supplied with yeast, and therefore a further comparison was made using diets which did not contain yeast or 'insoluble yeast' and where the B vitamins were supplied in pure substance (Fig. 3). On this diet, both in the presence and absence of biotin, growth was slightly speeded up by the addition of wheat-germ oil. This indicates that some fat-soluble substance may be of slight advantage for the development of *Tineola bisselliella*.

Growth on flour and other natural food substances

The ease with which the clothes moth can be grown on mixtures of pure food substances made it probable that some dried foods might also constitute an adequate food. In fact, an unwanted infestation of certain dried foods, stored in the laboratory, e.g. yeast and fishmeal, has frequently occurred with us. Results of the breeding of *Tineola bisselliella* on several dried foods are shown in Figs. 4 and 6. On flours of different extraction the speed of development is dependent on the rate of extraction and falls

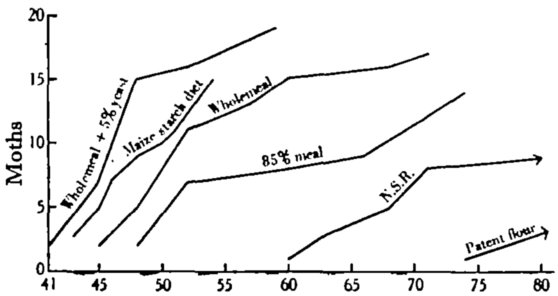

Fig. 4. Growth of *Tineola bisselliella* on flour of different rates of extraction (wholemeal = 100 %, National Flour = 85 %, National Straight Run = 72 %, Patent Flour = 40 %) and on an artificial diet which contains starch.

as the extraction is lowered (Fig. 4). This phenomenon, which has often been demonstrated with other insects (e.g. Fraenkel & Blewett, 1943*b*), is almost certainly mainly due to the smaller amounts of vitamins of the B group in low extraction flour. Even wholemeal flour is not an optimal food and can be greatly improved by adding 5 % yeast. In attempting to find out whether a single factor is responsible for this deficiency in wholemeal flour, tests were carried out with wholemeal flour to which riboflavin or nicotinic acid was added. Riboflavin was chosen, because wheat is relatively short of this factor, and nicotinic acid, because *Tineola* appears to require this factor most. There was certain an improvement with nicotinic acid, but not with riboflavin (Fig. 5).

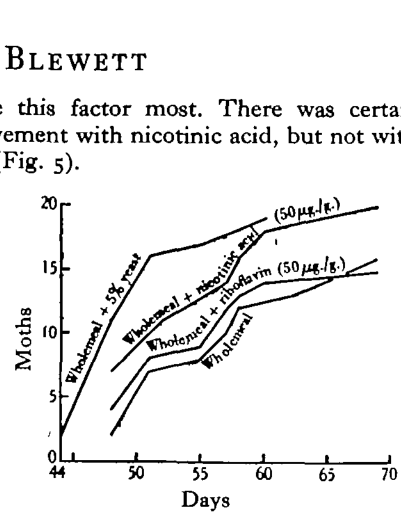

Fig. 5. Growth of *Tineola bisselliella* on wholemeal flour with the addition of riboflavin or nicotinic acid.

Tineola also grows successfully on fishmeal, meatmeal, wheat germ and yeast, but in no case was development as fast as on our artificial diets. Fishmeal again was greatly improved by the addition of 5 % yeast (Fig. 6).

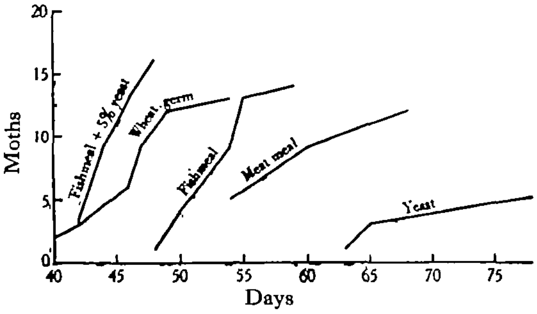

Fig. 6. Growth of *Tineola bisselliella* on different natural foods.

Growth at different relative humidities (Fig. 7)

The rate of growth of the clothes moth larva is greatly dependent on the relative humidity to which the diets are exposed. As in the case of many other insects (e.g. Fraenkel & Blewett, 1943*b*, 1944*b*) growth is faster at high humidities. But even at 30 % R.H. the majority of the larvae ultimately complete de-

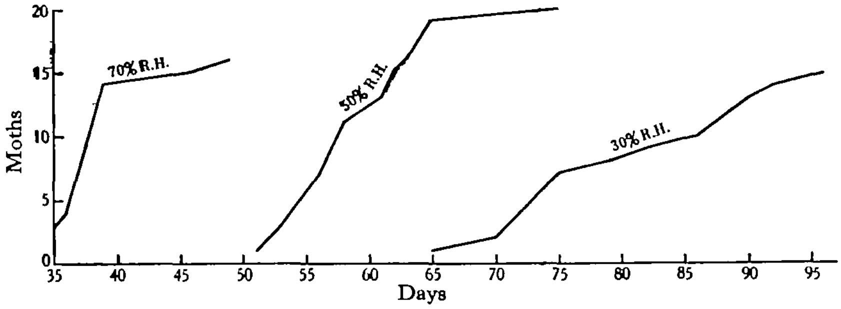

Fig. 7. Growth of *Tineola bisselliella* on an artificial diet described on p. 156, at different relative humidities.

velopment, though after a long delay. This agrees well with results by Griswald & Crowell (1936), who found that even at 20% R.H. twenty-four moths emerged out of a total of thirty larvae.

DISCUSSION

An analysis of the nutritional requirements of the clothes moth larva has been attempted once before (Crowell & McCay, 1937). These authors found that a supply of B vitamins, given in the form of a 'Harris B concentrate', was essential for growth. Purified casein appeared to be a not entirely adequate protein, but was satisfactorily supplemented by purified lactalbumin. The value of ash (from fishmeal) was doubtful. On the most successful diet consisting of 89·5% casein, 10% lactalbumin and 0·5% B concentrate the life cycle averaged 48·2 days, as compared with 55 days on fishmeal. This is considerably slower than growth on our best diets, although the temperature of the experiments was the same—25°. In the light of our present knowledge it seems most probable that Crowell & McCay's diet could have been improved by the addition of a sterol. As the casein and lactalbumin in that diet were 'vitamin-A free', one would expect them to be relatively free of sterol also. The diet must, however, have contained some sterol, possibly in the yeast concentrate, otherwise growth would have been impossible. In some of our diets casein was the sole source of protein and proved entirely satisfactory. This makes it improbable that the beneficial effect obtained by the addition of lactalbumin was due to a protein deficiency and suggests that the lactalbumin might have contained some impurity of importance.

In its food requirements, the clothes moth larva in general resembles other insects recently investigated by us (Fraenkel & Blewett, 1943*a*, *c*). Like other insects, it requires a sterol, and the amount of sterol present in 5% yeast proved insufficient. It also resembles other insects in its requirements for vitamins of the B group. The five factors of the B complex, thiamin, riboflavin, nicotinic acid, pyridoxin and pantothenic acid, which most insects seem to require, are also of importance in the nutrition of the clothes moth. But in contrast to the needs of certain other insects, only nicotinic acid and pantothenic acid proved indispensable. In the absence of any of the other B vitamins growth was delayed, but did not stop. In this the clothes moth resembles *Lasioderma* and *Sitodrepa*, which by virtue of the presence of intracellular symbiotic yeasts have become somewhat independent of the extraneous supply of B vitamins (Fraenkel & Blewett, 1944*a*). No intracellular symbiosis has, however, ever been reported from a lepidopter, and a cursory examination of the gut system of the clothes moth larva has not so far revealed the presence of either intracellular symbionts or of any conspicuous micro-organism in the lumen of the gut to which the production of B vitamins might be attributed. None the less, for optimal growth, most factors of the B complex are required. Even such a good source of B vitamins as wholemeal flour is not an optimal diet and is considerably improved by the addition of 5% yeast. It is interesting to note that the presence of riboflavin has been demonstrated in the Malpighian tubes of clothes moth larvae which had been grown on a food free of riboflavin (Busnel & Drilhorn, 1943).

The clothes moth, like all the other insects so far investigated, does not require inositol or *p*-aminobenzoic acid. In the presence of all the eight chemically known factors of the B complex growth is as good as on a diet which contains yeast. This shows that no further soluble or insoluble factors in yeast are required.

Particular effort has been made to find out whether any fat-soluble factor is required in addition to sterol. The search for such fat-soluble factors has so far proved unsuccessful in other insects, except in the case of caterpillars of the genus *Ephestia*, which in the absence of linoleic acid and vitamin E or some other unsaponifiable substance fail to emerge from the pupal case (Fraenkel & Blewett, 1945). Most of our tests suggested that no fat is required. In only two cases addition of fat proved very slightly beneficial (Fig. 3), and this occurred in diets which contained no yeast and where the presence of traces of fat was excluded. This makes it possible that in all the other diets yeast might have supplied the necessary fat. On the other hand, fat improved growth only very slightly, and there were no indications that the absence of fat had an unfavourable effect on the emergence of the adult as in the case of *Ephestia*.

The principal food of the clothes moth is generally considered to be woollen cloth. The main clue to this particular food habit is the ability of this insect to digest keratin. That pure keratin cannot serve as a satisfactory food has been shown again and again, and it would be very surprising indeed if it were otherwise. It is now well recognized that growth is only possible on cloth soiled by dirt, sweat or urine. Even so, it is astonishing enough that any higher organism can develop on a food of such poor quality. We recognize now at least five factors which go far to make the peculiar food habits of the clothes moth understandable: (1) its extraordinary ability to digest keratin (Duspiva, 1936; Linderstrøm-Lang & Duspiva, 1935), (2) its relative indifference to the absence of certain vitamins of the B complex, (3) its ability to grow under very dry conditions, e.g. at 20% R.H., (4) its indifference to the presence of a carbohydrate, and (5) of a fat in the diet. Even so the clothes moth grows only relatively slowly on cloth, very much slower than on our artificial diet and several other foods. This may be due to a number of factors. Although keratin is digested it may possibly not constitute an optimal source of amino acids. In experiments with rats it has been recently shown that keratin, under certain conditions, can serve as a source of protein, if supplemented by tryptophane, methionine, histidine and lysine, in which wool is low (Anon. 1944). With regard to B vitamins the supply may not necessarily be too unfavourable. As already mentioned clothes moths mostly feed on places soiled by sweat or urine. It has been known for long that water-soluble vitamins are excreted with the urine. Recently (Anon. 1943) it has also been shown that vitamins of the B complex, namely thiamin, riboflavin, nicotinic acid and pantothenic acid, are lost in sweat. The amounts concerned are small in relation to daily requirements, but it is conceivable that significant amounts would be concentrated over long periods on certain parts of a garment.

Of foods which we have tested our artificial diet allows the fastest development to take place, but a number of other foods have proved favourable breeding grounds for *Tineola*. Of these, wholemeal flour and fishmeal are obviously deficient in certain B vitamins, as is indicated by the improved growth rate after 5 % yeast has been added to these foods. On flours of different extraction, duration of development and mortality increases as the extraction is lowered. This has been shown already for a number of other insects (Fraenkel & Blewett, 1943*b*), and is explained by the smaller amounts of B vitamins in white flour. But even on wholemeal flour, supplemented with 5 % yeast where the concentration of B vitamins could be expected to be optimal, growth is much slower than on our best artificial diets. This is most probably due to the presence of large quantities of starch in flour. In an artificial diet, growth is much slower if starch is used instead of glucose, and about equal to that of flour plus yeast (Fig. 4).

Although flour and other dried foods tested by us are not optimal diets, it still remains unexplained why the clothes moth does not commonly become a pest on them, as development is even slower on cloth. According to Titschak (1925), total development on dirty woollen cloth, at 25°, takes on the average 71·9 days for males and 89·1 days for females. But damage to clothing may be caused by only a few growing larvae, whereas on food a much heavier infestation must develop before noticeable damage occurs. It may be suggested that in temperate climates the temperature would be too low to allow development of large numbers of clothes moths in stored foods. This is certainly not the case. At 15° the life cycle takes about 190 days on cloth (Titschak, 1925), and we can infer from this that on flour at the same temperature the life cycle would take about 120 days. Larvae survive very cold winter temperatures (Mansbridge, 1936), and though development stops below 5° (Yamada, 1940), it is resumed when the temperature rises in the spring (Zacher, 1933). One would therefore expect that, given sufficient time, probably over a year in southern England, large numbers of clothes moths could develop and ultimately become a pest on certain dried foods. In fact, on some occasions, clothes moths have been reported as pests on stored grain (Austen & McKenny Hughes, 1942; Cotton & Good, 1937), and during the present war outbreaks have occurred on stored flour.

Our investigations suggest that relative to the populations of such moths as the *Ephestia* species, for example, those of *Tineola* and its fellow clothes moths are small and that the economic importance of the clothes moth is very high relative to the size of populations and to the amount of food they consume.

SUMMARY

1. The larva of the clothes moth *Tineola bisselliella* Hum. grows well on an artificial diet consisting of casein, glucose, yeast, cholesterol, salt mixture and water. The presence of glucose is unnecessary, and that of cholesterol and yeast necessary for growth.

2. Yeast has been successfully replaced by a mixture of eight pure vitamins of the vitamin B complex. In the absence of either riboflavin, choline chloride, pyridoxin or thiamin growth is retarded, while little or no growth takes place in the absence of pantothenic acid or nicotinic acid. Inositol and *p*-amino-benzoic acid do not seem to be necessary, and the value of biotin for growth is doubtful.

3. The presence of a fat in the diet is of no or very slight value.

4. Like many other insects, the clothes moth grows faster on flour of high than of low extraction, but even wholemeal flour is far from being an optimal diet.

5. Growth is faster at 70 % relative humidity than at 50 and 30 %, but even at 30 % most larvae complete development.

6. These findings are discussed in connexion with the natural occurrence of clothes moth larvae on woollen cloth, flour and other dried foods.

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