# STUDIES ON THE DIGESTION OF WOOL BY INSECTS

## VIII. THE SIGNIFICANCE OF CERTAIN EXCRETORY PRODUCTS OF THE CLOTHES MOTH, *TINEOLA BISSELLIELLA*, AND THE CARPET BEETLE, *ATTAGENUS PICEUS*

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*Summary*

The excreta of clothes moth larvae (*Tineola*) bred on a standard undyed woollen fabric, the black carpet beetle (*Attagenus*) living on wool, and the potato moth (*Gnorimoschema*) feeding on potato tubers, have been examined. Water-soluble nitrogen constitutes most of the total nitrogen in both *Tineola* and *Attagenus* excreta. The fraction of this water-soluble nitrogen contributed by uric acid or its salts is high in *Tineola*, but considerably lower in *Attagenus*. Up to 3 per cent. urea and an appreciable quantity of ammonia are also present. Small quantities of urea (0.14 per cent.) were found in the dissected midguts of *Tineola* larvae feeding on wool, but none was found in several non-keratin-feeding insects. The concentration present in the *Tineola* midgut is too small to be of significance in the digestion of wool through any direct denaturing action on keratin.

In *Tineola* the sulphur of wool is not excreted to any large extent as inorganic sulphate or as sulphur dioxide but mainly as cystine. *Attagenus* excretes almost twice as much cystine as *Tineola* on a similar diet. Lanthionine was not found in *Tineola* excreta. *Tineola* larvae fed wool treated with nickel salts excrete black faecal pellets having a much reduced cystine content. It is suggested that the cystine is broken down (probably enzymically) to yield sulphur for the formation of nickel sulphide.

## I. Introduction

An examination of the end products of digestion in keratin-feeding insects should provide valuable data for the study of the digestive processes in these insects. Although some analyses do appear in the literature, a number of questions, some assuming greater importance in the light of recent work, remain unanswered. The fate of the keratin sulphur, after digestion by insects, is of considerable interest as the presence of sulphur is essential to the structure and stability of the keratin molecule (Geiger and Harris 1942). Babcock (1912) suggested that sulphur dioxide might be a metabolic product of clothes moth larvae feeding on wool, while Schultz (1925) concluded that the sulphur of keratin is excreted essentially as sulphate. The source of the sulphur required for the formation of metallic sulphides in the *Tineola* gut (Waterhouse 1952*a*) is also of interest. Wool is chemically changed with formation of lanthionine after treatment with alkalis (Cuthbertson and Phillips 1945) and recent work of Blackburn (1950) shows that wool thus treated becomes more easily digested by papain-bisulphite mixture than is normal wool. It was suggested that diges-

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tion of wool in the alkaline *Tineola* gut (about pH 10 (Linderstrøm-Lang and Duspiva 1936; Waterhouse 1952*b*)) might depend to a large extent on this reaction. Linderstrøm-Lang and Duspiva (1936) drew attention to the presence of urea in the gut of *Tineola* and inferred that urea might be involved in the digestion of wool in the larvae through some denaturation of the keratin. No quantitative data, however, were presented with which to determine the significance of the amounts of urea found in the larval gut.

## II. Methods

### (*a*) *Insect Material*

Larval excreta from cultures of *Tineola bisselliella* (Humm.) feeding on white woollen fabric, were obtained by sieving and winnowing with a small air jet until free from loose wool fragments. Samples of excreta from (i) *Tineola* which had infested a large number of dead beetles (*Aphodius howitti* Hope), (ii) from the black carpet beetle (*Attagenus piceus* (Oliv.)) on wool, and (iii) from potato moth (*Gnorimoschema operculella* (Zell.)) on potato tubers, were collected in a similar manner. Insect material required for the urea and ammonia estimations in the gut was drawn from laboratory cultures of *Tineola bisselliella, Locusta migratoria* L., *Agrotis infusa* (Boisd.), and *Tribolium confusum* Duv., and from field-collected *Aphodius* and melalonthid and dynastid larvae.

### (*b*) *Chemical Methods*

Dry matter content: dried to constant weight at 105°C. Water-insoluble matter: excreta heated with water on boiling water-bath for 1 hr., filtered, and dried to constant weight at 105°C. Ammonia and urea: urease method of Hawk, Oser, and Summerson (1947, p. 822). The xanthydrol method of Engel and Engel (1947) was used as a check on the urea figures. Nitrogen: micro-Kjeldahl. Amino nitrogen: micro-titrimetric ninhydrin method of Van Slyke, MacFadyen, and Hamilton (1941) modified by using Thunberg tubes instead of the recommended U-tubes and flasks, the barium hydroxide being held in the hollow stoppers. Creatine and creatinine: alkaline picrate method of Folin and Wu (1919) using tungstate filtrate. Creatine hydrolysed with HCl in an autoclave at 20 lb./sq. in. pressure for 20 min. Total and soluble sulphur: Micro-Carius digestion followed by precipitation of benzidine sulphate (Niederl *et al.* 1940). The precipitate was dissolved in hot N/50 NaOH and back-titrated with N/50 HCl, using neutral red as indicator. Sulphate sulphur: gravimetric as barium sulphate. Cystine: the colorimetric method of Shinohara (1935) using the reagent of Newton (1937). Total cystine was estimated on the hydrolysate obtained after refluxing for 4 hr. in 5N HCl. Sulphur dioxide: the method of Grant (1947) was applied. All figures for soluble constituents of the excreta were obtained on hot water extracts except amino nitrogen, sulphate, and cystine, in which dilute hydrochloric acid extracts were used.

## III. Experimental and Results

The results of the analyses of excreta from the present work are presented in Table 1, together with figures from other work on clothes moths. Some analyses (Brown 1937) of the excreta of *Melanoplus bivittatus* are included for

Table 1

ANALYSES OF EXCRETA OF *TINEOLA* AND OTHER INSECTS

| | *Tineola bisselliella* | | | *Tinea pellionella* | | | *Gnorimoschema operculella* | *Melanoplus bivittatus* | *Attagenus piceus* |
|---|---|---|---|---|---|---|---|---|---|
| | This Paper | | Hollande and Cordebard (1926) | Babcock (1912) * | Schultz (1925) | Mosher (1941) | This Paper * | Brown (1937) * | This Paper * |
| | * (Wool) | * (Beetle) | | | | | | | |
| Dry matter | 95·2 | 91·2 | 99 | | | 92·6 | 93·8 | 92·5 | 93·3 |
| Water-insoluble | 25 | 30 | 18 | | 28·4 | 25 | 59 | 56·5 | 31 |
| Ash | 1·06 | 9·92 | 5·5 | | | 9·67 | 11·8 | | |
| Total Nitrogen | 24·9 | 19·0 | 19·7 | 26·66 | | 19·74 | 1·80 | 4·24 | 22·5 |
| Soluble nitrogen | 21·1 | 16·2 | | | 6·39 | | 1·03 | | 17·8 |
| Purine bases nitrogen | | | | 0·13 | | | | | |
| Amino nitrogen | 1·2 | | | | | | | 0·32 | |
| Uric acid | 41 | 31 | 28 | 38·1 | | 28·46 | 2·0 | 4·7 | 2·8 |
| Ammonia | 4·1 | 2·8 | 3·0 | 3·31 | | 2·04 | 0·05 | 0·08 | 0·97 |
| Urea | 3·0 | 1·5 | 0·4 | 10·1 | | Trace | 0·06 | 0·34 | 3·0 |
| Creatinine | <0·1 | | | | | Nil | | | |
| Creatine | <0·1 | | | 7·2 | | Trace | | Nil | |
| Allantoin | | | | | | Nil | | | <0·1 |
| Total sulphur | 4·5 | 0·95 | | | † | | 0·28 | 1·0 | |
| Soluble sulphur | 3·1 | | | | | | | | |
| Soluble $SO_4$ sulphur† | 0·24 | Nil | Trace | | 0·76 | | Nil | | |
| Ethereal sulphate sulphur | Nil | | | | Nil | | | | |
| Insoluble sulphur | 1·4 (by diff.) | | | | 1·43 | | | | |
| Total cystine | 10·0 | 1·4 | | | Not found | | Nil | Doubtful | |
| Soluble cystine | 6·2 | | | | | | | | 11·8 |
| Soluble lanthionine | Nil | | | | | | | | |
| Insoluble lanthionine | Nil | | | | | | | | |

* These figures are quoted on a dry weight basis.

† Schultz (1925) states that total sulphur by the Liebig method corresponded to the sulphate sulphur.

comparison. All results from the present work are quoted to the nearest significant figure.

The water-insoluble fraction of a sample of *Tineola* larval excreta was examined by microscope and appeared to consist entirely of small fragments of undigested or partly digested wool fibres. This insoluble fraction, together with a sample of the original woollen fabric, was analysed for cystine content.

TABLE 2

CYSTINE CONTENT OF WATER-INSOLUBLE MATERIAL FROM *TINEOLA* EXCRETA

Results expressed as percentage of dried material

| | Cystine | | |
|---|---|---|---|
| | 1 | 2 | Mean |
| Insoluble matter from *Tineola* excreta | 12·1 | 12·8 | 12·5 |
| Woollen fabric | 10·5 | 10·9 | 10·7 |

A paper chromatographic examination using phenol-water and acetone-water mixtures, and specific reagents for sulphur compounds, including iodoplatinate (Winegard, Toennies, and Block 1948) failed to show the presence of lanthionine in *Tineola* excreta, either before or after hydrolysis. The possible excretion of sulphur dioxide by clothes moth larvae was checked by analysis of the atmosphere passing over a group of 300 *Tineola* larvae feeding on wool. The air was drawn over the larvae at the rate of 5 l. in 2 days and passed through an absorbent of dilute alkali. No sulphur dioxide was detectable ($< 0.2$ $\mu$g.).

TABLE 3

ANALYSES OF EXCRETA FROM *TINEOLA* LARVAE ON TREATED WOOL

All results reported as percentage of dried excreta

| Treatment | Cystine (Soluble in dilute HCl) | Nitrogen (Soluble in dilute HCl) | Nitrogen (total) |
|---|---|---|---|
| Nickel-treated fabric | 1·4 | 4·6 | 19·1 |
| Untreated fabric | 6·7 | 7·8 | 26·0 |

An examination was made of a sample of excreta from *Tineola* larvae fed on woollen fabric treated with nickel salts. The standard white fabric was used as in the other experiments but it contained about 25 per cent. of its original weight of nickel sulphate, and a control test was carried out with untreated fabric. The analytical method for cystine (Shinohara 1935) was shown to be unaffected by the presence of nickel sulphate or nickel sulphide, in the amounts

expected. The excreta from the larvae on the treated fabric were almost black in comparison to the normally straw-coloured excreta. The results of the analyses are presented in Table 3, and are discussed later.

The amount of urea in tissues of *Tineola* larvae and of some other insects is reported in Table 4. Both the clothes moth and the grasshopper (*Locusta*) were divided up into appropriate tissues for the analyses. *Tineola* was divided into (*a*) the hind part of the body together with the hindgut, (*b*) the midgut, and (*c*) the remainder of the body including head, while the *Locusta* gut was separated into (*a*) crop, (*b*) caeca, and (*c*) hindgut.

TABLE 4

UREA AND AMMONIA CONTENT OF INSECT TISSUES

All results reported as percentage of fresh tissues

| Insect Material | Urea | $NH_3$ |
|---|---|---|
| *Tineola*, whole larvae .. .. .. | 0·14 | 0·04 |
| *Tineola*, hind (*a*) .. .. .. | 0·16 | 0·20 |
| *Tineola*, midgut (*b*) .. .. .. | 0·14 | 0·05 |
| *Tineola*, body (*c*) .. .. .. | 0·05 | 0·03 |
| *Agrotis*, larvae, entire gut .. .. | Nil | 0·01 |
| *Locusta*, crop (*a*) .. .. .. .. | Nil | 0·02 |
| *Locusta*, caeca (*b*) .. .. .. | Nil | 0·06 |
| *Locusta*, hindgut (*c*) .. .. .. | Nil | 0·05 |
| *Aphodius*, larvae, entire gut .. .. | Nil | 0·04 |
| Melalonthid larvae, entire gut .. .. | Nil | 0·07 |
| Dynastid larvae, entire gut .. .. | Nil | 0·02 |
| *Tribolium*, whole larvae .. .. .. | Nil | 0·01 |

## IV. DISCUSSION

The three common nitrogenous excretory products, uric acid, urea, and ammonia, are all present in *Tineola*, *Attagenus*, and *Gnorimoschema* excreta. The figures for the latter are quite low compared to those for *Tineola* and *Attagenus*, probably owing to the relatively low protein content of the food of the potato moth. After the ingestion by an insect of a high sulphur-containing diet such as keratin, it might be expected that ammonia would be required to assist in the excretion of sulphate (Wigglesworth 1931). A calculation from the figures in Table 1, however, shows that only about 0.1 per cent. ammonia would be required to combine with the small quantity of sulphate in *Tineola* excreta. Although the water-soluble nitrogen figures for both *Tineola* and *Attagenus* are of the same order (21.1 and 17.8 per cent. respectively), ammonia, urea, and uric acid can account for as much as 86 per cent. of soluble nitrogen from *Tineola* but only about 17 per cent. from *Attagenus*. The low uric acid figure is notable in *Attagenus* and it was therefore thought that nitrogen might be excreted in the form of allantoin as in certain other insects, e.g. *Lucilia* (Robinson 1935). However, no allantoin was found and no further attempts were made to establish the identity of the nitrogenous excretory products.

Linderstrøm-Lang and Duspiva (1936) suggested that reduction of wool in the insect gut might be due to the presence of a hydrogen-transferring enzyme such as is involved in the Schardinger reaction. It seemed possible, in view of the high uric acid excretion in *Tineola* (Table 1), that xanthine oxidase, an enzyme responsible for the production of uric acid, might be involved. While this may be true in *Tineola* it seems much less likely in *Attagenus,* which has a low uric acid excretion, although it digests wool readily. The 41 per cent. uric acid excreted by *Tineola* appears to be high, but it is not exceptionally so, as the dried urine of the non-keratin-feeding *Rhodnius* contains 64-84 per cent. (Wigglesworth 1932). The possibility still remains that dehydrogenase activity takes part in the digestive processes of some keratin-feeding insects.

Three per cent. of urea was found in both *Tineola* and *Attagenus* excreta when feeding on wool, and *Tineola* feeding on *Aphodius* remains excreted 1.5 per cent. On general grounds one would not expect urea to be a "favourable vehicle" for the excretion of nitrogen in these keratin-feeding insects because of the relatively dry diet (Wigglesworth 1931). However, it is clear that an appreciable amount of urea is a normal constituent of the excreta of these insects and that it is of metabolic origin, in contrast to that of *Rhodnius,* which is largely derived from the blood upon which the insects feed (Wigglesworth 1931).

Small quantities of urea were found in whole *Tineola* larvae (Table 4) and it was first thought that this could be accounted for by the urea content of the faecal pellets in the hindgut. However, on dissection, it was shown that the *Tineola* midgut contains 0.14 per cent. urea, while other species examined contained none, although the ammonia concentrations were comparable. The presence of this urea in *Tineola* gut might appear to be of significance in the digestion of wool by insects in view of the known effects of urea on proteins, e.g. the denaturation of proteins (Hopkins 1930) and the depolymerization of actin (Szent-Gyorgi and Joseph 1951). However, these authors show that high concentrations of urea (about 3-5M) are required for the effects to appear. Assuming that the gut contents occupy one-half the volume of the total gut, the urea concentration may become about 0.28 per cent. or about 0.05M in the contents. It is known that certain changes in the contents of the gut, such as the loss of birefringence of wool and other indications of breakdown, occur quite rapidly and in a small clearly defined part of the *Tineola* midgut (Day 1951*a*). If all the urea found in the *Tineola* gut were concentrated in the region of this noticeable activity, approximately one-tenth of the length of the gut, the maximum local concentration of urea would still only be about 0.5M, which is very much lower than that required for any denaturing action.

It is also interesting to note in view of the restricted air supply to the *Tineola* gut (Day 1951*b*) that the speed of induction of gels in concentrated albumin solutions by urea is increased under anaerobic conditions (Huggins, Tapley, and Jensen 1951). However, since these authors have shown that this gelation follows the formation of a lattice structure by a rearrangement of sulphydryl and disulphide groups, it would seem that the action of concentrated urea under these conditions would tend to preserve the three-dimensional

molecular structure of keratin rather than disrupt it. Although the effect of dilute urea solutions on proteins under anaerobic conditions is not known, it is unlikely that the concentrations of urea in *Tineola* gut could contribute to the digestion of wool by any direct and independent action on the fibres. This is supported by Mercer's (1949) observation that urea solutions dissolve the "unstabilized lower levels" of the wool root but have no effect on hardened keratin. No analysis of *Attagenus* gut has been made for urea concentration but it can be expected to be similar to *Tineola* in view of the similar concentrations in the excreta.

When considering the combined effects of urea and enzyme systems it is notable that Steinhardt (1938), De Jaffe (1947), and others have found that pepsin, trypsin, and some other enzymes are not inhibited by up to 10 per cent. urea. Furthermore, Lennox (1952) has found that urea increases the digestion of wool by some enzymes in the presence of reducing agents. The gut of both *Tineola* and *Attagenus* are very reducing, but nothing is known of the effect of urea on the activity of the proteinases of these insects. However, the maximum local concentration of about 0.5M would seem to be of little significance when the rate of digestion of wool by the urea-activated papain only increases about one-third at this urea concentration (Lennox 1952).

The figure for total sulphur in clothes moth excreta (Table 1) confirms that of Titschack (1922) (4.03-4.62 per cent.). Schultz (1925) observed some crystalline material in the faecal pellets of *Tinea* which resembled cystine crystals, but concluded that the sulphur of keratin is excreted mainly as inorganic sulphate and that cystine was absent. However, Pradhan (1949) examined the excreta of *Anthrenus fasciatus* Herbst. and, on the grounds of crystal structure and solubility, expressed the opinion that cystine was present. It may be calculated from Table 1 that 55 per cent. of the total water-soluble sulphur excreted by *Tineola* is cystine sulphur, but only 8 per cent. is sulphate sulphur. Cystine is also excreted in high concentration (11.8 per cent.) by *Attagenus*. It is clear that cystine rather than sulphate is the important sulphur-containing excretory product in *Tineola* and *Attagenus* and probably also in the closely related genera *Tinea* and *Anthrenus*.

The presence of cystine in keratin-feeding insects is interesting in view of the paucity of information on the excretion of amino acids by insects in general (Wigglesworth 1950). Since the keratin ingested by clothes moth larvae and carpet beetles is digested under highly reducing conditions, one would expect the production in the gut of cysteine rather than cystine. As we have seen, the sulphur of this cysteine is excreted principally as cystine and very little appears as sulphate. This provides an interesting parallel to the work on vertebrate cystinuria (Brand, Cahill, and Harris 1935) which shows that ingested cysteine is excreted as cystine, whereas the sulphur of ingested cystine appears as sulphate through a different catabolic process.

The black carpet beetle (*Attagenus*) excretes almost twice as much cystine as *Tineola* when feeding on a similar diet (Table 1). This is of interest as Waterhouse (1952*c*) has found that *Attagenus*, in contrast to *Tineola*, does not

form metallic sulphides in the gut when fed wool treated with metallic salts. It is notable that when *Tineola* excretes black faecal pellets after feeding on nickel-treated fabric, the cystine content is much lower than after feeding on normal fabric (Table 3). Any dilution of the cystine in this experiment by nickel compounds should be reflected in a drop in nitrogen content; however, this occurs to a much smaller extent than the drop in cystine content. These differences between the insects may be explained by the presence, in *Tineola*, of an enzyme that decomposes cysteine with the production of hydrogen sulphide, and its absence in *Attagenus*. Such an enzyme is cysteine desulphydrase as found in the liver of the rat and dog (Fromageot, Wookey, and Chaix 1941). One of the products of the action of this enzyme is pyruvic acid and this has been demonstrated, using 2-4-dinitrophenylhydrazine (Lu 1939), in both *Tineola* and *Attagenus* excreta. Preliminary tests *in vivo* (Waterhouse 1952*c*) have indicated that the clothes moth larva does contain an enzyme of this type and *in vitro* confirmation will be reported more fully in a later paper.

Table 2 shows that the water-insoluble fraction of *Tineola* excreta (undigested wool fragments) contains slightly more cystine than the original woollen fabric. This may be due to the differential digestion of wool fractions of different chemical composition, on passage through the insect gut. In support of this it has been shown that the scales of wool contain a higher proportion of proline (Lindley 1947) and cystine (Geiger 1944), and that they are less easily attacked by enzymes than the cortex (Hock, Ramsay, and Harris 1941; Reumuth 1946).

The proteinase of *Tineola* has no special characteristics that could explain the digestion of wool by the larvae (Powning, Day, and Irzykiewicz 1951) but it was thought that the highly alkaline nature of the larval gut could contribute in some way. The larva of *Tineola* has a midgut pH of about 10 (Linderstrøm-Lang and Duspiva 1936; Waterhouse 1952*b*), which is unusually high, although midgut alkalinity has been shown to be characteristic of the Lepidoptera whether carnivorous or nectar- or wax-feeding (Waterhouse 1949). A possible product of the action of alkali on wool is lanthionine (Cuthbertson and Phillips 1945) but none was found in a chromatographic examination of the water-soluble fraction of the excreta. Also, the excreted undigested wool fragments constituting the water-insoluble fraction (Table 2), contained more rather than less cystine than the woollen fabric upon which the insects had been feeding. It may be concluded that neither *Attagenus*, which has a midgut pH of about 7 (Waterhouse 1952*c*), nor *Tineola* depend solely upon gut alkalinity for the digestion of wool.

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