# STUDIES ON THE DIGESTION OF WOOL BY INSECTS

## I. MICROSCOPY OF DIGESTION OF WOOL BY CLOTHES MOTH LARVAE (*TINEOLA BISSELLIELLA* HUMM.)

By M. F. Day

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*Summary*

The larvae of *Tineola bisselliella* digest wool fibres. The scales, resistant to most enzymes, are, except for the epicuticle, as readily digested as are the cortical cells.

Partly hydrolyzed wool, as it occurs in fabrics or after weathering of raw wool, is digested. Raw wool, in which no sulphydryl groups are present, is digested just as readily.

Visible evidence of digestion of keratin fibres may first be seen in a restricted section of the midgut and appears with dramatic suddenness when the passage of food is followed down the gut.

The epithelium of the region in which digestion is first visible differs histologically from the preceding region, and an unusually high reducing potential is maintained in the lumen in the same region.

## I. Introduction

Significant work has been done on the physiology of digestion of wool by the larvae of the clothes moth (Linderstrom-Lang and Duspiva 1936), but the actual course of digestion is still under discussion, and it has never been determined whether the larvae are capable of digesting native wool with all its labile sulphur in the form of disulphide linkages. The changes in wool fibres during digestion have been described by Reumuth (1946) who has shown that the first sign of proteolysis of the wool is seen as longitudinal striations in the cortex of the fibres and that wool scales are as readily digested as are cortical cells. But Mandels, Stahl, and Levinson (1948), who studied the effects of certain bacteria and fungi on wool fibres, found that the microorganisms were unable to dissolve the scale cells. They concluded that "the evidence which indicates that solution does occur (as a result of digestion by any organism) is inadequate and needs confirmation."

The digestion of fibres by larvae of *Tineola* has been examined and Reumuth's observations have been extended in several respects. It is the purpose of this paper to provide the confirmation of his results required by Mandels, Stahl, and Levinson, and to complement these observations with new information on the physiology of the *Tineola* alimentary canal.

* Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

## II. Microscopical Observations on Digestion of Fibres in the Tineola Gut

The fibres are bitten into pieces of roughly equal length, averaging 70 to 150 $\mu$, depending on the larval instar. When wool fragments are found in the foregut no signs of digestion can be seen in them. These fragments are "packeted," are passed through the unusually long oesophageal invagination, and thence to the midgut. Plate 1, Figures 1 and 2, shows photomicrographs with polarized light of wool fragments contained in the peritrophic membrane from the fore and hind parts respectively of the midgut of a *Tineola* larva. The fragments in the region just caudad to the oesophageal diverticulum are strongly birefringent (Plate 1, Fig. 1) and the scales are clearly visible (although only slightly so in the photograph). In the posterior part the fibres have lost much of their birefringence but are not swollen. Scales are not visible in the freshly dissected condition, after methylene blue staining, or after treatment with chlorine water (Allwörden reaction). Moreover, striations between the cortical cells are conspicuous, presumably due to the removal of some of the intercellular cementing substance (Plate 1, Figs. 2 and 3). Some isolated cortical cells may be observed in the lumen (Plate 2, Fig. 6). These changes in the ingested fragments are also made clear by appropriate staining. Methylene blue does not stain the fragments in the anterior part of the midgut. The further digestion has proceeded, the greater the distance the stain penetrates the cut ends of the fibres. Finally, the cortical cells all stain evenly. Results with Machida's stain (Glasgow 1934) are even more striking; the undigested fibres are yellow, whereas the separated cortical cells are red. Further details can be observed with the phase contrast microscope (Plate 2, Fig. 9), but clearer results are obtained with the peritrophic membrane and its contents than with the entire alimentary canal. In the hindgut, discrete fragments of wool fibres are usually not found, digestion having been complete. However, in larvae feeding rapidly, some fragments are not completely digested and may readily be seen in the centre of faecal pellets squashed in liquid under a coverglass (Plate 1, Fig. 4). This has been studied more fully by Mercer using electron microscopy (personal communication). Reumuth (1946) reported "droplike aggregations of digestive fluids" around the fibres in the gut. Although droplets may sometimes be seen in the gut lumen, no information has been obtained to confirm Reumuth's contention that they are concerned in digestive processes.

The fate of the scale cells is of particular importance. They are more resistant than cortical cells to *in vitro* enzymolysis (Hock, Ramsay, and Harris 1941). Chemically they probably contain a higher proportion of proline than does the remainder of the fibre (Lindley 1947), and this amino acid is thought to confer increased resistance to enzyme attack because of the folding it produces in the polypeptide chain. And indeed, scale cells are more resistant than the remainder of the fibre to attack by microorganisms (Mandels, Stahl, and Levinson 1948). In *Tineola* larvae, however, scales may be seen separating from the fibres about the middle of the midgut (Plate 2, Fig. 7), and in more

posterior regions no residue of scales can be discerned by light microscopy. Reumuth's contention that they are digested is thus unquestionably confirmed. Whether the epicuticle (described by Lindberg, Philip, and Graten 1948) is resistant has yet to be determined.

The digestion of wool fibres treated in various ways was examined. Thus, fibres in which 10 per cent. polymethacrylic acid had been deposited as an internal polymer, or 5 per cent. of the same reagent as an external deposit, or which had been treated with a melamine-formaldehyde resin, were digested just as the fibres from untreated fabric. Fabric treated by the method of Geiger, Kobayashi, and Harris (1942), to produce bisthioether linkages in place of some of the disulphide groups, is rendered partially resistant to digestion by *Tineola* larvae. The gut of a larva fed such a fabric is full of wool fragments, and the only visible signs of digestion are in the striations in the cortex. The scales are intact even at the posterior end of the midgut. Wool treated by this method does not give the Allwörden reaction, and the scales of the wool fragments in the gut likewise fail to show changes microscopically in chlorine water.

The most striking feature of the change observed in the fibres in the *Tineola* larval midgut is its abruptness. This can be readily observed in the isolated midgut from any replete larva. It is illustrated in Plate 1, Figure 5, which shows marked changes in the properties of the fibres when viewed between crossed polars. Since the treatment necessary to produce, *in vitro,* fibres with the appearance of those shown below the arrow in Plate 1, Figure 5, consists of treatment with pepsin for some days (Hock, Ramsay, and Harris 1941), the effectiveness of the system *in vivo* is readily appreciated, especially when it is recognized that material passes completely through the alimentary tract within eight hours at 27°C. The structure and physiology of this region of the gut is, therefore, of considerable interest, and will be considered below (Sections IV and V).

## III. The Ability of Tineola Larvae to Digest Wool with all Disulphide Linkages Intact

Previous authors who have studied the digestion of wool have generally used fabric or weathered wool. In both of these, a proportion of the disulphide linkages, which are the basis of the resistance of keratin (Geiger, Kobayashi, and Harris 1942), are converted to sulphydryl groups.

In order to determine the effects of feeding wool keratin in which all disulphide linkages are intact, freshly shorn wool from a merino carrying a 3-in. fleece was thoroughly extracted in ether and in water. The absence of sulphydryl groups was determined in the half-inch nearest the skin by (1) the nitroprusside reaction, (2) absence of colour with triphenyltetrazolium chloride, (3) the phosphotungstic acid test in the hydrolysate produced by HCl in sealed tubes. This wool was resistant to digestion by trypsin or the proteases extracted from *Tineola* larvae. Microscopic examination of the contents of midguts of larvae of *Tineola* fed on this wool proved without question that they digested the fibres just as those fed on wool from fabrics (Plate 1, Figs. 1 and 2) in

which some of the disulphide linkages are reduced. Some of this sulphydryl-free wool was then partially reduced by calcium thioglycollate until it gave positive reactions with each of the above three tests; it was then found to be digested slowly *in vitro* by trypsin and by protease extracted from *Tineola* larvae.

## IV. The Localization of the Reducing Region in the Tineola Midgut

Because of the extraordinary sharpness of the line in the midgut between wool showing no effects of digestion and the striated appearance described above, an attempt was made to find any characteristics of the gut that could be correlated with the ability to cause proteolysis in this region.

A useful indicator of reducing power is known in triphenyltetrazolium chloride (TTC) (Mattson, Jensen, and Dutcher 1947). When a midgut of a feeding larva is dropped into a 1 per cent. aqueous solution of TTC, a pink colour develops in the tissue within three minutes and is first usually apparent just at the zone where visible digestion of wool is seen. Later the colour spreads over the entire midgut and is found in some other tissues. For example, the body wall and the muscles give a positive reaction but the nerve cord, fat body, and the silk glands never do so. The midgut of larvae that have just emerged from the egg and have not yet fed gives no colour with TTC, nor does the midgut from a recently moulted larva. However, mature larvae continue to give an intense colour even after starvation for two days when no wool fragments remain in the gut, or after feeding from eclosion on an artificial diet containing no wool. The wool itself is negative but a faint colour can sometimes be seen within the peritrophic membrane. The main reaction is in the cytoplasm of the epithelial cells. It does not appear to be given by the contents of the goblet cells, but is given by their cytoplasm as well as by the cytoplasm of the columnar cells.

The nature of the reducing property of the larval midgut was studied by making up the TTC in 0.001M inhibitors and activators of enzyme systems, dropping the gut into the solution and determining the time taken for the appearance of the colour. In view of the similarity between a series of midguts of insects of the same age, this technique approximates the tissue slice method. The results are clear. Urea and cyanide are without effect, malonate slows the development of the colour, azide strongly inhibits it, whereas pyrophosphate strongly activates the reaction. These results suggest that a dehydrogenase is concerned in the maintenance of the reducing condition in the gut, and that it is most concentrated in the region of the gut most active in the digestion of wool fibres.

The localization of the reducing region in the midgut was confirmed in another way. Woollen fabric was reduced and cross-linked with mercuric cyanide by the method of Farnworth, Neish, and Speakman (1949) who believe that this treatment results in the incorporation of mercury between the sulphur atoms of the disulphide linkages. When such fabric is fed to *Tineola* larvae the wool is digested. The fabric is white in colour but the faecal pellets are black, probably due to the liberation of mercury following the rupture of the

-S-Hg-S- linkages. Further, the wool fibres in the gut are white in the anterior part of the midgut, but, at a point corresponding to the position where the TTC reaction is first observed, the fibres in the gut are blackened also, indicating precisely where reduction is taking place.

## V. The Histology of the Gut in Relation to Digestion

In view of the localization of strongly reducing conditions in the *Tineola* larval gut, it seemed desirable to determine whether these conditions were correlated with differences in the structure of the gut. Lotmar (1941) had already demonstrated that there are differences in histology in different parts of the midgut. However, it was not possible to correlate her observations with the TTC reaction until the two techniques were used on guts similar in size and history. It was found that good differentiation between the goblet cells and the columnar cells of the epithelium was obtained with Bodian's protargol technique following fixation in alcoholic Bouin's solution. It was immediately apparent that the reducing region of the midgut is poorly supplied with goblet cells whereas these are abundant anteriorly. The line between the two regions is very sharp (Plate 2, Fig. 10), and agrees exactly with the point where digestion of fibres becomes visible. Plate 2, Fig. 11, also shows the differences in form between the goblet cells of the two regions. Such differences have not been reported in the epithelium of other larval Lepidoptera. The posterior third of the midgut also contains abundant goblet cells and this region almost always gives a weak TTC reaction. It would thus appear that the columnar cells are responsible for the maintenance of the low potential.

*Tineola* larvae have been fed fabrics carrying a variety of dyestuffs. Certain dyes are released when the fibre is digested and may be absorbed. One such mixture of dyes* then sometimes appeared in the midgut epithelium as conspicuous needle-shaped crystals, which were metallic blue by reflected light and bright golden between crossed polars. These crystals occurred only in the columnar cells, and the line separating the region in which the crystals occurred coincided exactly with the point where digestion of the fibres became visible (Plate 2, Fig. 8). Woke (1941) has suggested that the goblet cells are especially concerned with the production of digestive enzymes. Since the columnar cells are concerned with absorption and with the maintenance of the oxidation-reduction potential, our results are not incompatible with Woke's hypothesis. There are differences in the frequency of occurrence of goblet cells along the length of a single region of a midgut. These differences are not constant from larva to larva from a single culture and indicate a cycle of secretory change presumably associated with phases in the processes of digestion.

* Said to be a commercial mixture of Alezarine brilliant blue PFN, fast light yellow 5GL, and acetyl rose 2GL. The individual dyes, which were kindly supplied by Hardie Trading Ltd., when applied to fabric did not, however, produce detectable crystals in the midgut epithelium.

## VI. Acknowledgments

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## Explanation of Plates 1 and 2

### Plate 1

Photomicrographs of fragments of wool fibres from larvae of *Tineola* photographed between crossed polars.

Fig. 1.—Wool contained in peritrophic membrane immediately caudad to oesophageal invagination. Note strong birefringence of fibres. This and the following figure are from an insect fed native wool.

Fig. 2.—Fibre fragments from the same peritrophic membrane from the region about two-thirds of the length of the midgut from the oesophageal invagination. Note loss of birefringence and striated appearance of fibres.

Fig. 3.—Striated appearance of digested fibres from insect fed a fabric containing wool and some indigestible fibres. One of these indigestible fragments is seen at *A*.

Fig. 4.—Macerated faecal pellet showing birefringent crystals (much of it is uric acid), and some partly digested wool fragments.

Fig. 5.—Contents of anterior third of peritrophic membrane of *Tineola* larva showing at arrow the extraordinarily sharp line of demarcation between the fragments showing no signs of digestion and those in which digestion is obvious.

PLATE 2

Fig. 6.—Partially digested fibre fragments from the midgut of a *Tineola larva.* Note the free cortical cells.

Fig. 7.—The same as Figure 6. Note the scale cells (marked with arrow) separating from the fibres.

Fig. 8.—Portion of midgut of *Tineola* larva fed a fabric containing certain dyestuffs. The dyes are released from the fabric on digestion, are absorbed by the midgut epithelium and produce crystals in the epithelial cells. Note the sharp line separating region where crystals are formed from the anterior portion of the midgut.

Fig. 9.—Partially digested fibre fragments within *Tineola* peritrophic membrane under phase contrast.

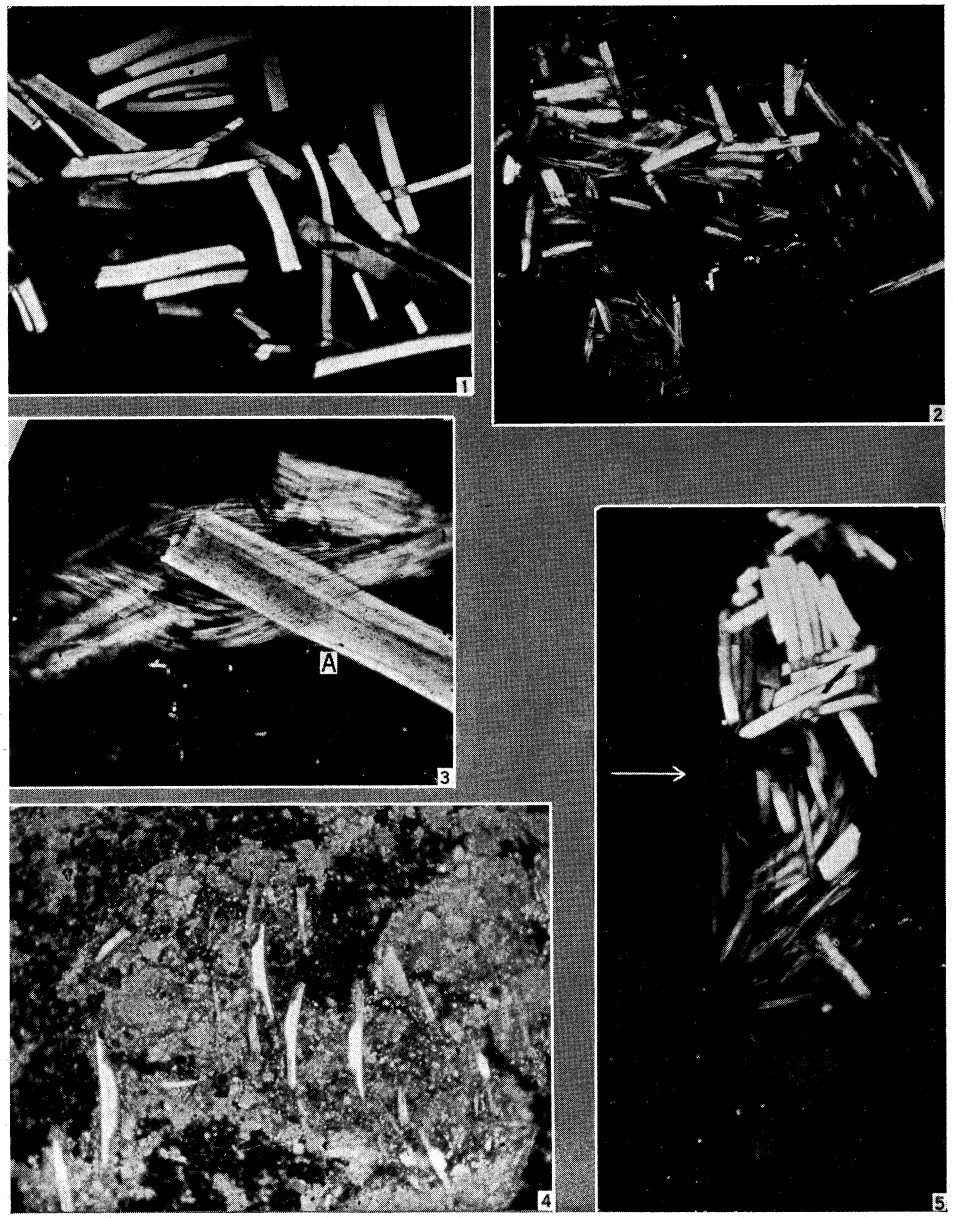
Fig. 10.—Longitudinal section of *Tineola* larval midgut showing sharp line between anterior portion in which goblet cells are abundant and region in which there are only occasional goblet cells among the columnar cells.

Fig. 11.—Longitudinal section of *Tineola* larval midgut at later stage in digestion. Note wider striated border, the shape of the goblet cells, and the increase in numbers of regenerative cells at the base of the epithelium.

## ADDENDUM

Experiments performed since the above was written have shown that the localization of the reducing conditions demonstrated by the use of an aqueous solution of triphenyltetrazolium chloride is not apparent if the dissection is performed beneath the fluid. Under these conditions, the most strongly reducing region is generally found to be the anterior part of the midgut. This observation suggests that the reducing conditions are rapidly destroyed upon exposure to the atmosphere. Although it is probable that the columnar cells are concerned with the maintenance of the reducing conditions, conclusions based on the localization of these conditions in the midgut must be revised. This will be discussed in greater detail in a subsequent paper in this series.

DIGESTION OF WOOL BY INSECTS. I



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