

STUDIES ON THE DIGESTION OF WOOL BY INSECTS

VII. SOME FEATURES OF DIGESTION IN THREE SPECIES OF DERMESTID LARVAE AND A COMPARISON WITH *TINEOLA* LARVAE

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Summary

Three species of dermestid larvae (*Anthrenocerus australis*, *Anthrenus verbasci*, and *Attagenus piceus*) are shown to be capable of digesting wool.

The midgut of these species is simple, without differentiation into zones, and the epithelium consists of simple columnar cells, together with nidi of regenerative cells. A peritrophic membrane is present. The midgut of *australis* and *verbasci* is virtually devoid of tracheae, and although the midgut of *piceus* is better supplied, its tracheation is comparable with that of *Tineola*, which is poorly tracheated compared with many other insects.

Examination under polarized light of wool in the digestive tract shows that many fibres are disintegrated as they pass down the gut.

The pH of the midgut approximates 7.0 and the oxidation-reduction potential falls in the range — 190 to — 230 mV.

The highly reducing conditions in the dermestid midgut reduce the disulphide bonds of wool keratin, permitting attack by proteolytic enzymes. Most of the cysteine thus produced is not degraded further and is excreted. Dermestid larvae, therefore, only under exceptional circumstances produce metal sulphides after ingestion of appropriate salts. The faeces remain of normal colour except when a coloured cysteine-metal complex (Co) or reduction product (Te) is formed. By contrast, in *Tineola* larvae, portion of the cystine is degraded further by a process that appears to be partly chemical (high pH) and partly enzymic (a desulphhydrase, capable of splitting off H₂S).

Available evidence indicates that neither dermestid nor *Tineola* larvae are capable of digesting the water-insoluble fraction (fibroin and sericin C) that forms the bulk of the silk fibre.

I. INTRODUCTION

A considerable amount is now known of the processes whereby wool is digested by larvae of the clothes moth *Tineola bisselliella* (Day 1951a, 1951b; Linderstrøm-Lang and Duspiva 1936; Powning, unpublished data; Powning, Day, and Irzykiewicz 1951; Waterhouse 1952a, 1952b). However, except for some observations of Pradhan (1949), the record that at least some species are unable to digest spongin (Arndt 1931), and the record of the presence of free —SH groups in the midgut (Duspiva 1936), there is no information available on the physiology of digestion of dermestid larvae. These larvae, together with Mallophaga (chewing lice) and the larvae of a few species of moths, are the only animals thought or known to be capable of digesting keratin. The report (Stankovic, Arnovljevik, and Mataverlj 1929) that the crop juices of a hawk, but not of a vulture, were capable of digesting feather keratin requires confirmation.

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It is of great interest, therefore, to examine the mechanism of keratin digestion in dermestid larvae, and to determine whether it follows the pattern already established for *Tineola* larvae. A number of features of digestion in dermestid larvae are described herein and these enable a comparison with *Tineola* to be made, which indicates both similarities and important differences.

II. METHODS

Almost all of the experiments were carried out with three species, *Anthrenocerus australis* (Hope), *Anthrenus verbasci* (L.), and *Attagenus piceus* (Oliv.), although *Anthrenus vorax* (Waterh.) and *Dermestes maculatus* Deg. were also used in some experiments. All cultures were maintained in "Agee" jars containing a layer about 1 in. deep of a diet of casein 83 per cent., finely powdered yeast 15 per cent., cholesterol 1 per cent., and Hubble's salt mixture 1 per cent. Folded pieces of woollen fabric (air-dried after dipping in a suspension of 5 g. finely powdered yeast and 0.25 g. cholesterol per 100 ml. water) were placed on top of the powdered diet. The gauze tops of the jars and the upper surface of the fabric were sprinkled lightly with the active ingredient of "Dimite,"* which prevented mite infestation without apparently affecting the insects in any way. Adults were kept under similar conditions but transferred at weekly intervals to fresh jars, producing larval cultures of approximately known age. Adults were also supplied with drinking water and 15 per cent. honey in water in cotton-wool-plugged tubes after it had been found that they lived better in the presence of free moisture. All stock cultures were maintained at 30°C. and 75 per cent. relative humidity.

In the dye and metal feeding experiments single larvae were kept at 30°C. and 94 per cent. R.H. (over a saturated solution of potassium nitrate) in individual gauze-covered tubes with a piece of treated fabric. The woollen fabric (either unreduced, or with disulphide bonds partially reduced by brief treatment with 0.5M sodium thioglycollate at 40°C. and pH 10) was first dipped in 70 per cent. alcohol to ensure wetting, washed in water, and then steeped in a solution of the desired compound in a filtrate of the yeast-cholesterol suspension mentioned previously. The treated fabric was air-dried before use. This procedure of keeping single larvae in tubes was necessitated by their rather irregular feeding habits. Thus, for some days before and after moulting and, indeed, at other times also, larvae might cease to feed. By examining the tubes daily for faeces it was possible to select larvae whose alimentary tract was filled with the food in question.

When highly coloured food was present in the beginning of the midgut this could be seen through the cuticle of the metathorax and first abdominal segment if the larva was examined ventrally under CO₂ anaesthesia. This proved a useful check on the colours of pH and redox indicators in the midgut of living larvae.

* 1,1-Bis(*p*-chlorophenyl)ethanol, Sherwin Williams Co., U.S.A.

III. RESULTS

With relatively few exceptions, which are specifically mentioned, *australis*, *verbasci*, and *piceus* larvae gave similar results in the experiments listed below.

(a) Morphology of the Alimentary Canal

Several authors (Braun 1912; Lison 1937; Mobusz 1897; Pradhan 1949) have dealt with various aspects of the morphology and histology of the alimentary canal of dermestid larvae. The foregut and midgut (Fig. 1 (a) and (b)) are simple, uncoiled, and possess no crypts or diverticula. The hindgut first runs forwards and then turns back upon itself to lead to the anus. There are six malpighian tubules which, after running to various regions of the abdomen, become associated in two pairs of three. These two groups then fuse and disappear, where the hindgut bends back on itself, into a sheath investing one side of the hindgut, and thus produce the cryptonephridial arrangement characteristic of the larval malpighian tubules of many Coleoptera, Lepidoptera, and ant lions (Lison 1937; Wigglesworth 1951). This arrangement is thought to play an important part in the conservation of water and salts. The anterior end of the hindgut in dermestid larvae is attached to the cryptonephridial sheath where it becomes enlarged in the region of the rectum.

Mobusz (1897) considered that a peritrophic membrane was absent in *Anthrenus verbasci*, although Aubertot (1934) recorded for *Attagenus pelli* the presence of a membrane that became more distinct and multiple-layered as it passed down the gut. It is true there is no well-defined membrane arising at the level of the oesophageal invagination. However, in the species examined in the present study (and particularly in *piceus*—Plate 1, Fig. 2) a membrane enclosing the food is clearly visible in the posterior half or two-thirds of the midgut. In *piceus* the membrane can sometimes be traced almost to the anterior end of the midgut, in *verbasci* it is often difficult to trace it anteriorly beyond the middle of the midgut, whereas *australis* occupies a position intermediate between these two species. Where it is first visible the membrane is rather diffuse in nature and its origin is uncertain. From the middle or posterior third of the midgut onwards, however, it becomes relatively tough and well defined and can be removed without damage with enclosed food. Its mechanical properties under these conditions indicate that it is an organized structure and not merely epithelial debris loosely enveloping the food.

(b) Histology and Tracheation of the Midgut

The histology of the midgut presents no unusual features. As observed by Mobusz (1897) there are small groups of regenerative cells, each separated by a variable number (5-20) of simple epithelial cells (Plate 1, Figs. 4-6). The latter have a conspicuous striated border, a small centrally placed nucleus, and a fine uniform cytoplasm with few or no granules visible with ordinary staining techniques. When granules were present they occurred in the cytoplasm between the nucleus and the gut lumen, but were not restricted to any particular region of the midgut. When tested by the Gallamine blue method

for calcium (Stock 1949) and by the rhodizonate method for barium and strontium (Waterhouse 1951) positive staining of the granules resulted. The granules also gave a weakly positive test for phosphate (cobalt sulphide method). Koehler (1920) reported the presence of numerous granules of calcium carbonate and oxalate in the midgut epithelium of *Dermestes fulvescens*, although it is not clear whether larvae or adults were examined. If

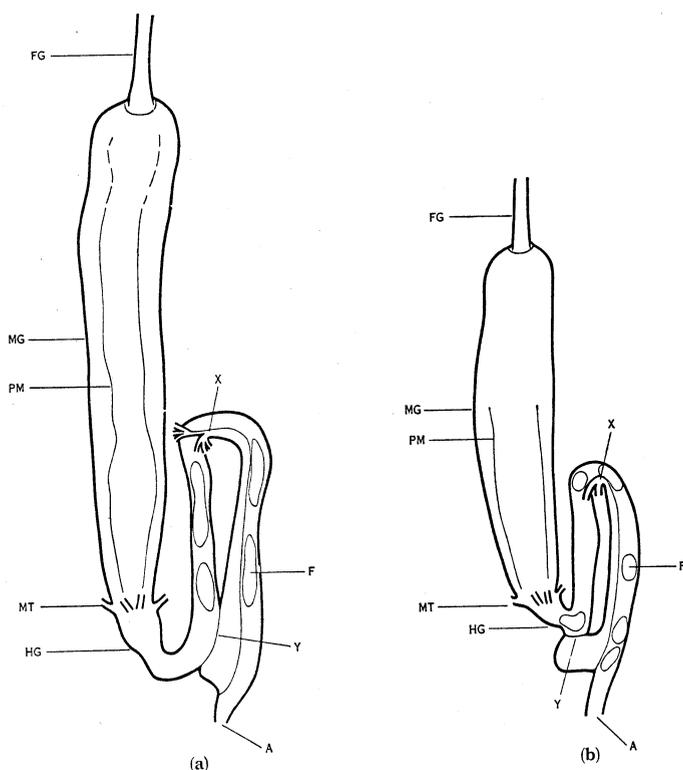


Fig. 1.—Ventral aspect of alimentary canal of dermestid larvae with hindgut rotated slightly to show entrance of malpighian tubules. (a) *Attagenus piceus*. (b) *Anthrenus verbasci*. A, anus; F, food mass in hindgut; FG, foregut; HG, hindgut; MG, midgut; MT, entrance of malpighian tubules into gut; PM, peritrophic membrane; X, entrance of malpighian tubules into cryptonephridial sheath; Y, region of attachment of anterior hindgut to cryptonephridial sheath.

larvae were used, either this species differs from those examined in the present study or the granules may be due to the diet (unspecified) upon which her insects were reared. It is of interest also to record that Braun (1912) mentioned a narrow zone of granules near the lumen border of the midgut epithelium of *Dermestes lardarius* larvae. The entire midgut epithelium appears to have a uniform structure and Bodian staining does not reveal any polymorphism of cell type as in *Tineola* larvae (Waterhouse 1952a). The entire epithelium is renewed at each moult (Braun 1912; Mobusz 1897).

It has already been noted that the midgut tracheation of *Anthrenus* and *Attagenus* larvae is less well developed than that of *Tineola* larvae which, in turn, has poor tracheation in this region compared with that of some other Lepidoptera (Day 1951*b*). Examination under dark field demonstrates that the midguts of last-instar *verbasci* and *australis* are supplied with very few tracheae and, occasionally, apparently with none at all. When tracheae are present they generally occur at the extreme anterior and posterior ends of the midgut, they branch very little, and they reach only a small area of midgut epithelium. The *vorax* midgut is better tracheated than either of these two species, although even it is supplied with only a few tracheae. Very occasionally in *verbasci* tracheoles are coiled upon themselves in an unusual fashion (Plate 1, Fig. 3) and the same tendency, although less marked, was also observed in *vorax*, *australis*, and *piceus*.

The tracheation of the *piceus* midgut is quite different, since there are many tracheae, which supply air to a good deal of the surface of the midgut (Plate 1, Figs. 1 and 2). Even this tracheal supply cannot, however, be regarded as particularly rich, since it is not unlike that of *Tineola* larvae. The *maculatus* midgut is more richly tracheated than any of the other species examined and it would appear to be more nearly comparable in this respect to the midgut of many other insects.

(c) Microscopical Examination of Wool undergoing Digestion

Pieces of wool in the digestive tract vary in length from about 40 to 120 μ , the average size increasing somewhat with advancing larval development.

When the digestive tract is examined under polarized light the wool in the anterior portion of the midgut is seen to be strongly birefringent. Further down the midgut many of the wool fragments have lost much of their birefringence, although others are little changed. The conspicuous and abrupt change in birefringence observed about one-third way down the midgut in *Tineola* larvae (Day 1951*a*) does not occur in dermestid larvae. This may be due, in part, to the fact that the wool is not "packeted" and firmly enclosed throughout the entire midgut in a well-defined peritrophic membrane as in *Tineola*. As a result, some mixing of wool fragments by peristaltic movements may be permitted.

In the posterior third of the midgut and in the hindgut partly detached scales can sometimes be seen and striations are conspicuous in many of the fibres, due no doubt to the removal of some intracellular cementing substance. Most, but not all of the faecal pellets contain wool fragments in various stages of digestion. Most of these fragments are either conspicuously striated or the original wool fibres have broken down into slender microfibrils.

The presence of incompletely digested wool in the faeces is, perhaps, not surprising in rapidly feeding larvae, which may produce as many as 300 faecal pellets per day, although 30-100 is a more usual number. From counts of faecal pellets produced after removing feeding larvae from fabric and from feeding dyed fabrics, it appears that food may frequently pass completely through the digestive tract in 8-12 hr. at 30°C. (cf. 8 hr. for *Tineola* (Day 1951*a*)).

(d) *The pH of the Digestive Tract*

Table 1 shows the results obtained when larvae were fed on fabrics impregnated with saturated solutions of pH indicators. The contents of the entire midgut generally had a pH of 6.8-7.0 (green with brom-thymol blue). There was a certain amount of variation, however, since in a small proportion of larvae phenol red, and less frequently cresol red, exhibited a pinkish orange colour in the midgut. If the gut wall of these larvae was punctured and the contents spread out it could be seen that neither indicator was displaying its full alkaline coloration, which was readily produced on addition of dilute alkali. It appears therefore that at times the midgut pH may be as high as 8.2. On no occasion did thymol blue change colour, indicating a pH of less than 8.4. Pradhan (1949) records the pH of the midgut contents of *Anthrenus vorax* (= *fasciatus*) as 6.8.

TABLE 1
pH OF ALIMENTARY CANAL CONTENTS OF THE MAJORITY
OF INDIVIDUALS OF THREE SPECIES OF DERMESTID
LARVAE

Indicator	Midgut	Hindgut
Brom-phenol blue	>4.0	>4.0
Brom-cresol green	>4.6	4.4-4.8
Chlor-phenol red	>5.8	<5.8
Brom-cresol purple	>6.2	<6.0
Brom-thymol blue	6.8-7.0	<6.7
Phenol red	<7.8	<7.6
Cresol red	<7.8	<7.8
Thymol blue	<8.4	<8.4
Range	6.8-7.0	4.4-4.8

The pH of the hindgut and faeces was 4.4-4.8. However, in occasional larvae of *australis* the value ranged to lower than pH 2.0, some faeces from these larvae being red and some yellow after feeding on thymol blue.

The zone of pH change between midgut and hindgut varied a little, according to how recently before examination food had passed on. Normally the change occurred about the level of entry of the malpighian tubules, but occasionally it was slightly further down the hindgut, particularly in *australis*.

When larvae were fed on fabric treated with dilute indicator solutions there was little colour visible in the midgut, although the contents of the hindgut were often definitely coloured. This suggests that indicators may be absorbed by the midgut and either discharged into the hindgut via the malpighian tubules or the hindgut epithelium. However, in none of the larvae was there any visible accumulation of indicators in the gut epithelium or in the malpighian tubules.

(e) *The Oxidation-reduction Potential of the Digestive Tract*

The results obtained by feeding oxidation-reduction indicators with the diet are shown in Table 2. If the midgut pH is taken to be 7.0 (Table 1), the fully reduced colour of potassium indigo disulphonate and the partly oxidized colour of brilliant alizarine blue indicates a midgut potential in the range —190 to —200 mV. (Hewitt 1950).

TABLE 2
OXIDATION-REDUCTION POTENTIAL OF THE ALIMENTARY CANAL CONTENTS OF
THREE SPECIES OF DERMESTID LARVAE

Indicator	E'_0 at pH 7 (mV.)	Condition of Indicator in	
		Midgut	Hindgut
1-Naphthol 2-sulphonate indophenol	+123	Fully reduced	Oxidized
Thionine	+ 63	Fully reduced	Oxidized
Methylene blue	+ 11	Mostly fully reduced	Oxidized
Indigo tetrasulphonate	— 46	Fully reduced	Oxidized
Tetrazolium blue		Fully reduced	Reduced
Indigo trisulphonate	— 81	Fully reduced	Oxidized
Indigo disulphonate	—125	Fully reduced	Oxidized
Brilliant alizarine blue	—173	Partly reduced	Oxidized
Phenosafranin	—252	Oxidized	Oxidized
Range		—190 to —252 mV.	+260 mV.

In individuals with a pH of about 8.0 (see earlier) a potential in the vicinity of —230 mV. is indicated.

Methylene blue gave somewhat anomalous results, some of the fabric in the midgut exhibiting a pale bluish grey coloration. This, however, was very much paler than the deep bluish grey coloration of the ingested food, indicating a considerable degree of reduction. When the larvae were dissected under 0.1 per cent. triphenyltetrazolium chloride no pink or red colour was produced, this differing markedly from the results obtained for *Tineola* larvae in which reduction occurred (Day 1951a). The negative results in dermestid larvae may possibly be due to the absence of sufficient endogenous substrate in the gut for reduction to occur under these conditions.

Little is known of the systems responsible for the maintenance of reducing conditions in the midgut. It is highly probable that cysteine is present, since the contents give an intensely positive nitroprusside reaction for —SH groups. Furthermore, both Pradhan (1949) and Powning (unpublished data)

record the presence of cystine in the faeces of dermestid larvae. Ingested tetrazolium blue is reduced in the midgut but this reduction probably cannot be effected by cysteine at the pH (7.0) of the digestive juices (Rutenburg, Gofstein, and Seligman 1950), although G. Rogers (personal communication) obtained reduction of the related triphenyltetrazolium chloride in the presence of 0.08M cysteine at pH 7.4 and 35°C. Hydrogen sulphide reduces tetrazolium blue quantitatively, but evidence from metal metabolism (see below) suggests that little or no H₂S is formed during digestion in dermestid larvae. The production of the blue formazan may probably therefore be taken as evidence of dehydrogenase activity in the midgut (Rutenburg, Gofstein, and Seligman 1950).

In the hindgut a potential more oxidizing than +260 mV. is indicated.

As a general rule the indicators were not accumulated in visible quantities by the epithelial cells. However, methylene blue is accumulated by some larvae in the epithelium of the anterior fifth of the midgut, and by others in the posterior half of the midgut, although in most larvae no accumulations were seen. Occasionally the cells of the malpighian tubules and fat body adjacent to the midgut contained oxidized methylene blue. Oxidized indigo disulphonate was accumulated by one *australis* larvae at the very posterior end of the midgut.

(f) Metal Feeding Experiments

Larvae were fed on woollen fabric impregnated with many of the salts used in the *Tineola* digestion experiments (Waterhouse 1951a). Faeces produced after ingesting the great majority of salts (e.g. those of Ni, Fe, Cd, Cu, Pb, Hg) were not, as a rule, any different in colour from those of control insects, even when the impregnation solution was concentrated (e.g. 15 per cent. NiSO₄). Where the fabric was coloured by the impregnation treatment (e.g. HAuCl₄), the faeces had the same colour as the fabric ingested. A small number (less than 0.5 per cent.) of larvae gave anomalous results in that dark faeces were produced when they were fed on lead or copper-impregnated fabrics and these larvae apparently produced sulphides. It is clear, however, that, with these possible exceptions, the larvae do not produce metal sulphides, which are so characteristic of the metal metabolism of *Tineola* larvae (Waterhouse 1951a). By transferring the larvae that had produced what are provisionally regarded as sulphides to thymol blue fabric it was shown that there was no correlation between capacity for sulphide formation and unusually low pH of the faeces (see earlier).

It appeared at first to be an anomalous result when larvae fed on fabric impregnated with 5 per cent. cobaltous chloride or saturated (3 or 4 per cent.) sodium tellurite or tellurate, had dark brown or black food respectively in the midgut and hindgut and produced brown or black faeces. However, on mixing equal volumes of M/10 cysteine and M/10 metal salts in either maleinate buffer at pH 6.9 (Smits 1947) or, if the buffer was incompatible, in water, cobalt produced a soluble dark brown reaction product and sodium tellurate slowly formed a finely divided black precipitate. Sodium tellurite produced initially a pale yellow precipitate that later blackened. Salts of other metals produced

white or nearly white precipitates (e.g. Zn, Cd, Sn, Pb, Hg, Ag) or lightly coloured or colourless solutions (e.g. Ni, Mn). Copper produced a light grey precipitate and iron a hydroxide-like precipitate, sometimes preceded by a transient blue colour. The implication is that metal-cysteine reactions may occur in the digestive tract of dermestid larvae, in contrast with sulphide formation, which is the predominant reaction in *Tineola*. Where the compounds are light in colour (all elements tested except cobalt and tellurium) the uric acid and other materials present in the faeces mask their colour and the faeces appear normal.

A search in the literature revealed that cobalt forms several complexes with cysteine, the cobaltic complex



being the most stable, being soluble and having a dark, yellow-brown colour. The pH and oxidation-reduction potential in the dermestid midgut are such as to permit this stable complex to be formed. Its formation depends upon the presence in the complexing molecule of a —SH group and a —NH₂ group, although there is some doubt whether a —COOH group is also involved (Albert 1952). Thus thioglycollic acid will produce a brown cobalt complex, but not cysteine ethylated at the —SH group. Cystine alone gives no colour reaction with cobalt (Michaelis 1929; Michaelis and Barron 1929; Michaelis and Yamaguchi 1929; Michaelis and Schubert 1930; Schubert 1931). It is highly probable that the dark brown colour produced in the dermestid gut following ingestion of cobaltous chloride is a complex formed with the cysteine or cysteine-peptides produced by digestion of keratin, particularly since the material responsible for the colour in the faeces was water-soluble as is the cobalt-cysteine complex produced *in vitro* (Kendall and Holst 1931; Shinohara and Kilpatrick 1934).

The formation of a white crystalline mercury-cysteine complex from mercuric and mercurous salts and from metallic mercury is recorded (Barron, Flexner, and Michaelis 1929), as is also a red, weakly coloured (in dilute solution) nickel-cysteine complex (Michaelis and Barron 1929). Iron, copper, and manganese catalyse the oxidation of cysteine to cystine by the formation of complexes (Michaelis 1929; Michaelis and Barron 1929), but there is no indication that highly coloured stable complexes are ever formed with these metals. The results of feeding metals to dermestid larvae, therefore, are consistent with the hypothesis that metal-cysteine reactions occur in the midgut.

Little information is available on the nature of the black material formed by feeding sodium tellurite, although it may be metallic tellurium, since this substance is readily reduced to the elementary condition. After larvae had fed on the tellurium fabric for some time a small zone of cells at the anterior end of the midgut sometimes became dark with accumulated material. However, the amount accumulated was too small to permit histological localization in the cell. Where food was present in the foregut, it was black, and mixing of the contents of the foregut and midgut by peristaltic and anti-peristaltic action could sometimes be observed. When larvae were fed on ferric chloride fabric and tested by the Prussian blue reaction, ferric iron could be demonstrated throughout the midgut, there being no regular zones in which particularly large concentrations appeared.

(g) *Comparison of Cysteine Breakdown in Dermestid and Tineola Larvae*

From evidence presented above and by Waterhouse (1952a) it appears that hydrogen sulphide is produced at least transiently by *Tineola* larvae, but not by dermestid larvae, its source being the cysteine formed by digestion of keratin. A greater degree of degradation of cysteine in *Tineola* than in dermestids is supported by the analyses of Powning (unpublished data), who found that *piceus* faeces contain about 12 per cent. cystine, compared with 6-7 per cent. for *Tineola* larvae.

This difference in capacity for cysteine breakdown may be due to the possibilities that H_2S is liberated at pH 10 (in *Tineola*) but not rapidly enough (or not at all) at pH 7 (dermestids) to produce visible sulphide formation, or that *Tineola* larvae possess a desulphydrase capable of splitting off H_2S from cysteine (Fromageot 1951; Symthe 1945), but that this is absent or very weak in dermestid larvae. Irrespective of the presence of a desulphydrase it is highly probable, from what is known of the ease of cleavage of carbon-sulphur bonds (Tarbell and Harnish 1951) that cysteine and cysteine peptides would liberate H_2S very much more readily at pH 10 than at pH 7. However, there is some evidence that splitting of H_2S in *Tineola* larvae is, in part, under enzyme control. It is known that cysteine desulphydrase is completely inhibited by 0.001M (or less) hydroxylamine or semicarbazide (Lawrence and Symthe 1943). When *Tineola* larvae were fed on woollen fabric that had been soaked in a 10 per cent. solution of either inhibitor plus 5 per cent. nickel sulphate, some nickel sulphide was produced in the midgut and appeared in the goblet cells and in the faeces.

However, the amount of nickel sulphide produced was distinctly less, and the colour of the faeces far lighter, than in controls on nickel sulphate alone. Larvae fed on inhibitor plus lead acetate fabric showed a similar, but less marked inhibition of sulphide formation. When *Tineola* larvae were fed on silk that had been immersed in solutions containing 5 per cent. nickel sulphate and 1 per cent. cysteine hydrochloride, or 3 per cent. methionine or glutathione with and without 10 per cent. inhibitor, there was, once again, an indication of some inhibition of sulphide formation in the presence of inhibitors. In spite of the fact that larvae feeding on all inhibitor-treated foods produced fewer faeces than usual (and hence ingested less food and nickel sulphate) there were clear indications that sulphide formation was being interfered with. Furthermore, since these experiments were carried out, the presence of a desulphydrase has been demonstrated *in vitro* using extracts of *Tineola* larvae (Powning, unpublished data).

The results with silk and sulphur compounds suggest that there is more than one H_2S -splitting enzyme present, since cysteine desulphydrase is unable to split H_2S from methionine (Fromageot 1951). Alternatively the enzyme may be a non-specific desulphydrase unless the methionine and glutathione are first transformed to cysteine in the larval midgut before H_2S is split off, which is improbable.

Lower concentrations of inhibitor (5 or 1 per cent.) produced progressively less marked effects. This is possibly due to the fact that the inhibitors may

easily combine with other compounds in the larval gut and may not be available for desulphhydrase inhibition. The continued production of small amounts of sulphide in the presence of inhibitor either indicates that inhibition is incomplete or that a significant amount of H_2S is liberated under the alkaline conditions (pH 10) by a purely chemical reaction without the intervention of an enzyme.

(h) Digestion of Silk

Raw silk thread consists of two homogeneous strands of water-insoluble silk fibroin (forming some 75 per cent. of the fibre) cemented together and surrounded by sericin. Sericin may be separated into three components, two (sericins A and B) being water-soluble and the third (sericin C) water-insoluble. Sulphur-containing amino acids have not been detected in silk, although there is a small amount of inorganic sulphide associated with sericin C (Shaw and Smith 1951).

Ever since the work of Abderhalden (1925) it has been believed that larvae of *Anthrenus museorum* are capable of digesting silk. However, there are several features about his experiments that throw doubt on the validity of this claim, at least so far as silk fibroin is concerned. Firstly, Abderhalden's analyses, which showed the sulphur content of his *museorum* larvae to be 0.5 per cent., suggest very strongly that the silkworm cocoons on which they were bred were contaminated by other materials. It is possible that they may even have contained dead pupae. Secondly, he was unable to detect microscopically any digestion when silk was incubated with larval digestive juices, although digestion "appeared" to occur if the silk was first ground as finely as possible. Thirdly, it seems highly probable, in view of their well-known sterol and vitamin requirements, that the silk on which the *Anthrenus* larvae thrived for several generations was contaminated by other materials, which would not only supply growth factors, but may also have provided the proteins etc. required for development. More reasonable are Abderhalden's statements that larvae would not develop on pure silk fibroin or on Canton silk.

When *australis* larvae were transferred to degummed silk (i.e. sericins A and B had been removed) they ingested it readily. Microscopical examination under ordinary and polarized light failed to reveal any indication that the fibres had been attacked by digestive enzymes. The faeces consisted almost entirely of undigested silk, which showed many signs of mechanical damage caused during ingestion, but their size and form, including indentations caused by incomplete severing of the fibre by the mandibles, appeared to be identical in the midgut and in the faeces. Similar results were obtained with larvae feeding on clean, empty silkworm cocoons on which they lived many months without increasing in size and produced large numbers of faeces consisting mainly of undigested silk. In view of the high content of tyrosine in fibroin (some 10 per cent.) it seemed possible that, if digestion of silk occurred, any tyrosine in excess over metabolic needs would appear in the faeces, just as cystine is present after feeding on keratin (Powning, unpublished data). However, a Millon's test on a 0.1N HNO_3 extract of faeces from silk-fed larvae indicated that tyrosine was absent. There is thus no evidence yet available

to indicate that silk fibroin is digested, whereas there is good evidence that certainly no more than a minor amount of digestion takes place.

The position with regard to sericins *A* and *B* is rather different. Because of their water solubility they would be expected to be removed from the raw silk fibre during passage through the alimentary tract. It remains, however, to be shown whether the water-soluble sericins are actually degraded by the digestive enzymes although their amino acid composition (Shaw and Smith 1951) suggests that this is probable. On the other hand, Duspiva (1950) found that, although the sericinase-containing fluid regurgitated by the silkworm moth just before emergence dissolved the sericin and softened the silk, very little breakdown of sericin could be detected.

The position may be summarized, therefore, by saying that, whereas dermestid larvae may be able to obtain some nourishment from the water-soluble constituents of raw silk, they do not appear to be able to digest processed silk (sericin *C* + fibroin), which forms the great bulk of the raw silk fibre. Available evidence indicates that this statement applies equally well to larvae of the clothes moth, *T. bisselliella*.

IV. DISCUSSION

It is clear from these experiments that some species of dermestid larvae are capable of digesting wool and that, although the basic mechanism of digestion (reduction followed by enzyme attack) is similar to that in *Tineola* larvae, there are some notable differences in digestive physiology.

The histology of the midgut is simple, without differentiation into zones as in *Tineola*. Tracheation of the midgut is virtually absent in *australis* and *verbasci*, but in *piceus* is comparable with that of *Tineola*, which has relatively poor midgut tracheation (Day 1951*b*). This is not surprising since it would be difficult to conceive how intensely reducing conditions could be maintained in a midgut which is well oxygenated by means of a plentiful tracheal supply.

Some notable differences are evident when the present results for the pH of midgut contents are compared with those for *Tineola* larvae (Waterhouse 1952*b*). Firstly, no regions of varying pH were detected; secondly, there was no visible accumulation of indicator in the midgut epithelium of dermestid larvae; and thirdly, the midgut pH was very much less alkaline, falling within the range 6.8 to 8.2, compared with 9.8 to 10.0 for the most alkaline region of the midgut of *Tineola*. It appears that midgut pH may be more characteristic of the taxonomic group (although not necessarily of the order) to which a species belongs than of any unusual food habits possessed by individual species (Waterhouse 1949).

On the other hand, the midgut potential of -190 to -230 mV. is not very different from -250 to -280 mV. recorded for *Tineola* (Waterhouse 1952*b*). There is no reason to doubt that wool is effectively reduced at pH 7 and -200 mV. in the dermestid midgut and it is possible that the materials responsible for the reducing conditions may also activate the proteolytic enzymes present. Lennox (1952), for example, has found that hydrosulphite

is less effective than bisulphite in promoting the digestion of wool by papain-urea in neutral solution although it is the stronger reducing agent.

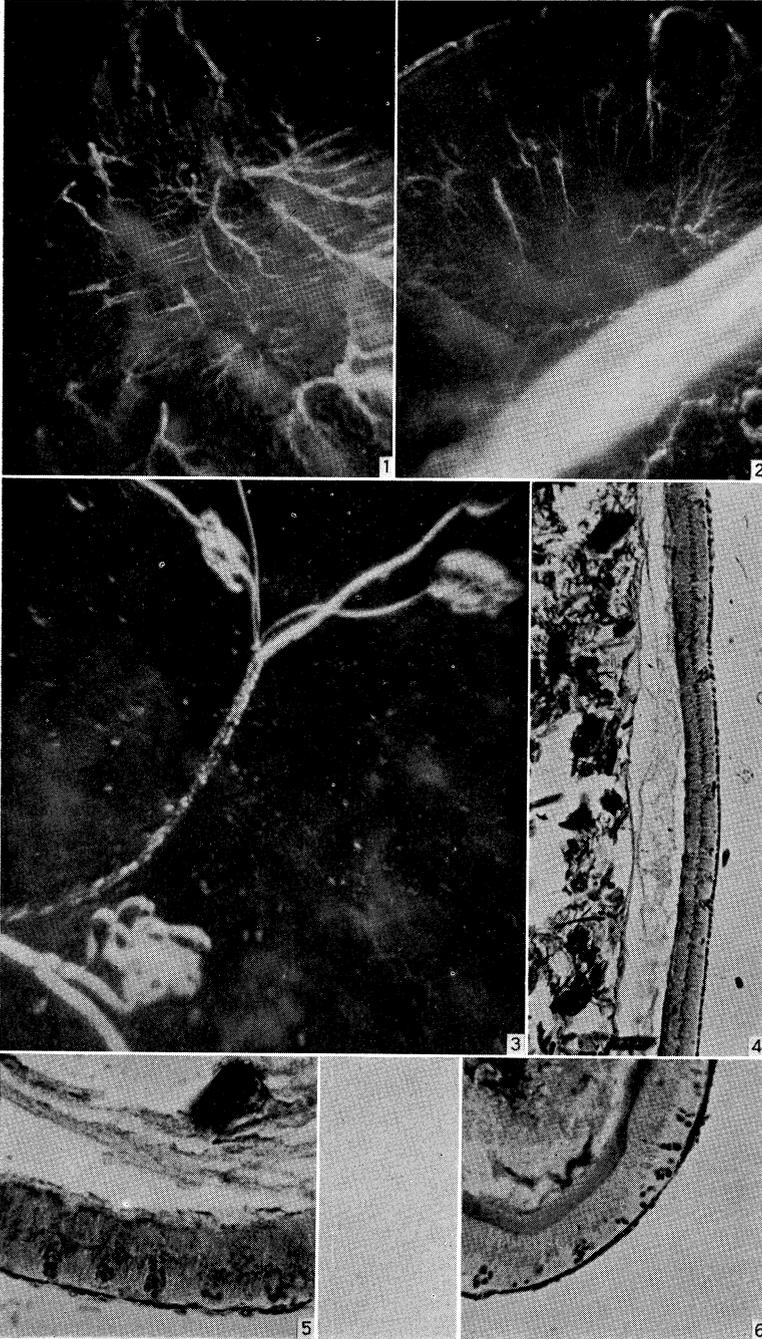
It appears therefore that, in dermestid larvae, the disulphide bonds of wool cystine are rapidly reduced under the neutral reducing conditions encountered and that cysteine or cysteine peptides are formed as a result of the simultaneous action of proteolytic enzymes. The occurrence of this process is supported by the demonstration of an intensely positive nitroprusside reaction in the wool undergoing digestion.

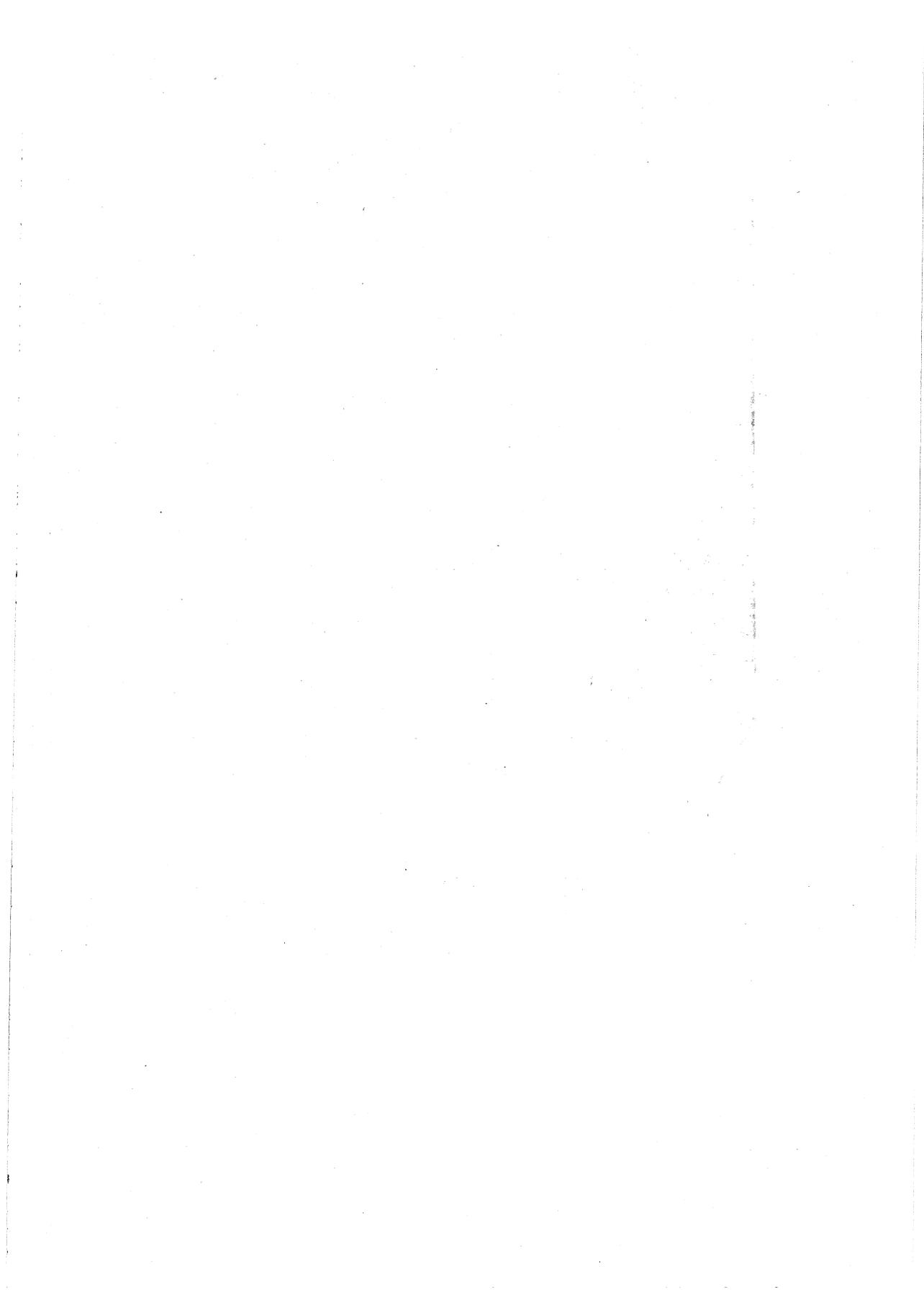
There is good evidence that the breakdown of cysteine does not proceed as far in dermestid larvae as in *Tineola* larvae. In *Tineola* larvae, H_2S is produced in the midgut (partly chemically and partly enzymically) as can be shown by the formation of characteristically coloured sulphides from metals ingested with their food (Waterhouse 1952a). In view of the greater insolubility of most metal sulphides than their cysteine complexes, it is unlikely that appreciable quantities of metal-cysteine compounds are formed in the *Tineola* midgut in spite of the fact that cysteine is freely available, as evidenced by the presence of some 6-7 per cent. cystine in *Tineola* faeces (Powning, unpublished data). In the majority of dermestid larvae no sulphides are produced. Coloured faeces are only produced by elements (Co or Te) that were found to form deeply coloured reaction products with dilute solutions of cysteine. The salts of most metals do not produce strongly coloured metal-cysteine compounds and these resulted in the production of normally coloured faeces. Furthermore, although the figures cannot be compared directly, the fact that there is twice as much cystine in *piceus* faeces as in *Tineola* faeces (Powning, unpublished data) lends support to the belief that, in dermestid larvae, cysteine is not utilized to the same extent as in *Tineola* larvae.

The small proportion of dermestid larvae (both *australis* and *piceus*) that produce faeces apparently containing metal sulphides poses some interesting problems. Insufficient larvae have been detected so far to determine whether their midgut pH is higher than usual (i.e. whether this group is also responsible for the few pH records of about 8.0) and hence approaches more closely that of *Tineola* larvae. The differences in cysteine breakdown by *Tineola* and dermestid larvae and possible sulphide formation in occasional dermestid larvae are under investigation.

It is apparent from the absence of mortality after feeding on fabrics containing lead, mercury, etc. that dermestid larvae are capable of detoxifying many metals, although this problem has not been investigated specifically. Where detoxification occurs the process is somewhat analogous to that of *Tineola* larvae. Instead of insoluble sulphides being produced the metals form undissociated complexes with cysteine or cysteine peptides, which are either insoluble or, if soluble, exert less toxicity than the metal in ionized form. It is interesting that this detoxification appears to be no less effective than that in *Tineola* larvae, although fewer compounds have been investigated. However, it is possible that the long period of starvation dermestid larvae are able to survive may account for the low mortality produced by some of the treated fabrics.

DIGESTION OF WOOL BY INSECTS. VII





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VI. REFERENCES

- ABDERHALDEN, E. (1925).—Beitrag zur Kenntnis der synthetischen Leistungen des tierischen Organismus. *Z. physiol. Chem.* **142**: 189-90.
- ALBERT, A. (1952).—Quantitative studies of the avidity of naturally occurring substances for trace metals. 2. Amino acids having three ionizing groups *Biochem. J.* **50**: 690-8.
- ARNDT, W. (1931).—Zur Frage der Verdaubarheit der Badeschwammgerustsubstanz. *Zool. Anz.* **93**: 199-207.
- AUBERTOT, M. (1934).—Récherches sur les peritrophiques des insectes et en particulier des Diptères. *Thèses Fac. Sci. Univ. Strasbourg Nancy E. No.* **44**: 1-357.
- BARRON, E. S. G., FLEXNER, L. B., and MICHAELIS, L. (1929).—Oxidation-reduction systems of biological significance. III. The mechanism of the cysteine potential at the mercury electrode. *J. Biol. Chem.* **81**: 742-54.
- BRAUN, M. (1912).—Das Mitteldarmepithel der Insektenlarven während der Häutung. *Z. wiss. Zool.* **103**: 115-69.
- DAY, M. F. (1951a).—Studies on the digestion of wool by insects. I. Microscopy of digestion of wool by clothes moth larvae (*Tineola bisselliella* Humm.). *Aust. J. Sci. Res. B* **4**: 42-8.
- DAY, M. F. (1951b).—Studies on the digestion of wool by insects. III. A comparison between the tracheation of the midgut of *Tineola* larvae and that of other insect tissues. *Aust. J. Sci. Res. B* **4**: 64-74.
- DUSPIVA, F. (1936).—Beiträge zur enzymatischen Histochemie. 21. Die proteolytischen Enzyme der Kleider und Wachsmottenraupen. *Z. phys. Chem.* **241**: 177-200.
- DUSPIVA, F. (1950).—Die enzymatischen Vorgänge beim Durchbruch des ausschlüpfenden Seidenspinners (*Bombyx mori* L.) durch die Kokonwand. *Z. Naturf.* **5b**: 273-81.
- FROMAGEOT, C. (1951).—Desulhydrases. Chap. 41 (pp. 1237-43) of 'The Enzymes' Vol. 1, Part 2, Ed. J. B. Sumner and K. Myrbäck. (Academic Press Inc.: New York.)
- HEWITT, L. F. (1950).—"Oxidation-reduction Potentials in Bacteriology and Biochemistry" 6th Ed. (E. & S. Livingstone Ltd.: Edinburgh.)
- KENDALL, E. C., and HOLST, J. E. (1931).—The oxidation of cobaltous cysteine. *J. Biol. Chem.* **91**: 435-74.
- KOEHLER, A. (1920).—Über die Einschlüsse der Epithelzellen des Bienendarms und die damit in Beziehungstehender Probleme der Verdauung. *Z. angew. Ent.* **7**: 68-91.
- LAWRENCE, J. M., and SYMTHE, C. V. (1943).—The partial purification and some of the properties of the enzyme in rat liver that forms hydrogen sulphide from cysteine. *Arch. Biochem.* **2**: 225-34.
- LENNOX, F. G. (1952).—Digestion of wool keratin by papain-bisulphite-urea and related systems. *Aust. J. Sci. Res. B* **5**: 189-209.
- LISON, L. (1937).—Sur la structure de la région cryptosoleniée chez les coléoptères *Tenebrio molitor* L. et *Dermestes lardarius* L. *Bull. Acad. Belg. Cl. Sci.* (5) **23**: 317-27.
- LINDERSTRØM-LANG, K., and DUSPIVA, F. (1936).—Studies in enzymatic histochemistry. XVI. The digestion of keratin by the larvae of the clothes moth (*Tineola bisselliella* Humm.). *C.R. Lab. Carlsberg* **21**: 53-83.

- MICHAELIS, L. (1929).—Oxidation-reduction systems of biological significance. VI. The mechanism of the catalytic effect of iron on the oxidation of cysteine. *J. Biol. Chem.* **84**: 777-87.
- MICHAELIS, L., and BARRON, E. S. G. (1929).—Oxidation-reduction systems of biological significance. IV. Comparative study of the complexes of cysteine with the metals of the iron group. *J. Biol. Chem.* **83**: 191-210.
- MICHAELIS, L., and SCHUBERT, M. P. (1930).—Cobalt complexes of thioglycolic acid. *J. Amer. Chem. Soc.* **52**: 4418-26.
- MICHAELIS, L., and YAMAGUCHI, S. (1929).—Oxidation-reduction systems of biological significance. V. The composition of the oxidised cobalt complex of cysteine. A colorimetric method for the microanalyses of cobalt. *J. Biol. Chem.* **83**: 367-73.
- MOBUSZ, A. (1897).—Ueber den Darmkanal der *Anthrenus* Larve nebst Bemerkungen zur Epithelregeneration. *Arch. Naturgesch.* **63**: 89-128.
- POWNING, R. F., DAY, M. F., and IRZYKIEWICZ, H. (1951).—Studies on the digestion of wool by insects. II. The properties of some insect proteinases. *Aust. J. Sci. Res.* **B 4**: 49-63.
- PRADHAN, K. S. (1949).—On the physiology of digestion in the larva of the woolly bear *Anthrenus fasciatus* Herbst (Insecta, Coleoptera, Dermestidae). *J. Zool. Soc. India* **1**: 107-19.
- RUTENBURG, A. M., GOFSTEIN, R., and SELIGMAN, A. M. (1950).—Preparation of a new tetrazolium salt which yields a blue pigment on reduction and its use in the demonstration of enzymes in normal and neoplastic tissues. *Cancer. Res.* **10**: 113-26.
- SCHUBERT, M. P. (1931).—Cobalt complexes of cysteine. *J. Amer. Chem. Soc.* **53**: 3851-61.
- SHAW, J. T. B., and SMITH, S. G. (1951).—Amino-acids of silk sericin. *Nature* **168**: 745.
- SHINOHARA, K., and KILPATRICK, M. (1934).—The stability of cystine in acid solution. *J. Biol. Chem.* **105**: 241-51.
- SMITS, G. (1947).—A new buffer for the pH range 5 to 7. *Biochim. Biophys. Acta* **1**: 280.
- STANKOVIC, R., ARNOVLJEVIC, V., and MATAVERLJ, P. (1929).—Enzymatische Hydrolyse des Keratins mit dem Kropfsafte des *Astur palumbarius* (Habicht) und *Vultur monachus* (Kuttengeier). *Hoppe-Seyl. Z.* **181**: 291-9.
- STOCK, A. (1949).—The determination of calcium in histological sections. *J. R. Micr. Soc.* **69**: 20-4.
- SYMTHE, C. V. (1945).—Some enzyme reactions of sulfur compounds. *Adv. Enzymol.* **5**: 237-47.
- TARBELL, D. S., and HARNISH, D. R. (1951).—Cleavage of the carbon-sulfur bond in divalent sulfur compounds. *Chem. Rev.* **49**: 1-90.
- WATERHOUSE, D. F. (1949).—The hydrogen ion concentration in the alimentary canal of larval and adult Lepidoptera. *Aust. J. Sci. Res.* **B 2**: 428-37.
- WATERHOUSE, D. F. (1951).—The occurrence of barium and strontium in insects. *Aust. J. Sci. Res.* **B 4**: 144-62.
- WATERHOUSE, D. F. (1952a)—Studies on the digestion of wool by insects. IV. Absorption and elimination of metals by lepidopterous larvae, with special reference to the clothes moth, *Tineola bisselliella* (Humm.). *Aust. J. Sci. Res.* **B 5**: 143-68.
- WATERHOUSE, D. F. (1952b)—Studies on the digestion of wool by insects. VI. The pH and oxidation-reduction potential of the alimentary canal of the clothes moth larva (*Tineola bisselliella* (Humm.)). *Aust. J. Sci. Res.* **B 5**: 178-88.
- WIGGLESWORTH, V. B. (1951).—“The Principles of Insect Physiology.” 4th Ed. (Methuen & Co. Ltd.: London.)

EXPLANATION OF PLATE I

- Fig. 1.—An area typical of the most richly tracheated portion of the midgut of *A. piceus*. Dark field.
- Fig. 2.—Typical tracheation of *A. piceus* midgut of starved larva, showing portion of contracted peritrophic membrane. Dark field.
- Fig. 3.—One of the occasional tracheae supplying the midgut of *An. verbasci* showing coiling of tracheoles. Dark field.
- Fig. 4.—L.S. midgut of *An. australis* larva showing simple epithelium, regenerative nidi, peritrophic membrane, and food in lumen. Mallory.
- Fig. 5.—T.S. midgut of *An. australis* larva showing epithelium and peritrophic membrane in more detail. Mallory.
- Fig. 6.—T.S. midgut of *An. verbasci* larva showing nuclei of epithelium, striated border, and fragment of peritrophic membrane. Bodian.