

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.)

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Summary

The fate was investigated of 30 metallic and five non-metallic elements following ingestion by *Tineola* larvae. When incorporated in woollen fabric or in a yeast-casein medium, 19 elements that form insoluble sulphides produced characteristically coloured sulphides in the food undergoing digestion in the midgut. The production of the sulphides is brought about by the alkaline, highly reducing, midgut secretions, which cause the production of sulphhydryl groups by the reduction of the disulphide bonds of the cystine present in the wool. When metal is present in the diet less cystine is excreted than on a normal diet. Other sulphur-containing compounds (methionine, glutathione) also permit the formation of sulphides. Much of the sulphide formed passes down the digestive tract and is excreted. However, a certain amount forms highly dispersed colloidal solutions with the amino acids or polypeptides liberated by digestion of the food or present in the digestive secretions. These colloidal sulphides are taken up by the midgut epithelium, and granules of sulphides accumulate in the cavities of the goblet cells of the anterior and posterior regions of the midgut. Sulphides of fewer metals accumulate in the goblet cells of the middle region of the midgut. All goblet cell accumulations are eliminated during moulting, when the entire midgut epithelium is cast off and regenerated. The goblet cells of other lepidopterous larvae were also shown to accumulate some metals, although not as sulphides.

Elements incapable of forming insoluble sulphides do not lead to the formation of coloured compounds in the goblet cells of *Tineola* larvae. However, the alkaline earths are deposited as granules (mainly as phosphates), principally in the columnar cells of the anterior and posterior midgut. It is probable that small quantities of absorbed fluoride are deposited with calcium in these granules.

Tineola larvae are thus able to detoxify a wider range of metals and non-metals (many of which are ordinarily highly toxic) than most other animals.

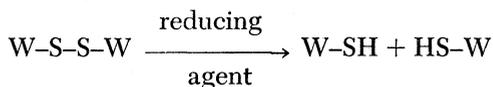
I. INTRODUCTION

The well-known resistance of wool to attack by proteolytic enzymes and its insolubility in many of the usual protein solvents is considered to be due to its high-molecular-weight polymeric structure. An important feature of this structure is the presence of disulphide cross-linkages between the polypeptide chains of the wool protein keratin. The proteolytic enzyme complex present in the digestive tract of the clothes moth larva, which is able to digest keratin,

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is very similar to that of other insects (Powning, Day, and Irzykiewicz 1951). Peculiarities in the nature of this enzyme complex, therefore, do not appear to be adequate to explain the ability of *Tineola* larvae to live on wool. It appears that this ability is largely due to their alkaline digestive juices (pH 10) and to the unusually low oxidation-reduction potential (in the vicinity of -250 to -280 mV.) in the midgut (Linderstrøm-Lang and Duspiva 1936; Waterhouse 1952*b*). These conditions result in the production of sulphhydryl groups from the disulphide linkages. Wool in which the disulphide linkages have been reduced *in vitro* is attacked by proteolytic enzymes, as is also the root portion of the wool fibre, where much of the sulphur is present as sulphhydryl groups as yet unoxidized to disulphide linkages (Geiger and Harris 1942; Geiger *et al* 1941).

When chemically reduced wool is reoxidized the disulphide linkages are reformed from the sulphhydryl groups and the wool regains its original stability. If the reduced wool is treated with an aliphatic dihalide, pairs of sulphur atoms are linked through short hydrocarbon chains thus



where W represents the portions of the wool unconnected by the disulphide groups and X a halogen atom. When trimethylene dibromide is used for the reaction, so that $\text{W-S-(CH}_2)_3\text{-S-W}$ linkages are formed, the modified wool is decidedly more stable than untreated wool to chemical agents, and furthermore it is attacked much less readily by clothes moth or carpet beetle larvae. As an example of the degree of protection conferred, wool in which the cystine content had been lowered to 6.5 per cent. suffered only 2 per cent. of the loss of weight suffered by control wool with a cystine content of 12.2 per cent. (Geiger, Kobayashi, and Harris 1942).

Linkage rebuilding in reduced wool can also be achieved by the use of metal salts, which are thought to produce W-S-metal-S-W linkages (Farnworth, Neish, and Speakman 1949; Stoves 1942). If a toxic material, such as a mercury salt, is used the resulting modified wool might be expected either to be indigestible or, if digested, with consequent rupture of metal sulphur linkages, to be highly toxic to clothes moth larvae. When wool containing mercury was fed to *Tineola* larvae the colourless fibres blackened in the middle region of the midgut and the faeces were black. This darkening was considered to be due to the liberation of mercury following rupture of the -S-Hg-S- linkages (Day 1951).

A surprising feature of these tests was that the larvae did not appear to be affected adversely by their diet. It therefore appeared to be of considerable interest to examine in *Tineola* larvae various aspects of the metabolism of those metals that might be incorporated in the chemical structure of wool as toxic agents.

The present account deals mainly with the fate of a number of metals and non-metals following ingestion by clothes moth larvae and provides data on the mechanisms whereby many toxic metal ions are detoxified by the larvae.

II. METHODS

Larvae of *Tineola bisselliella* were fed at 30°C. on a standard woollen fabric, on silk, or on a yeast-casein mixture, to which diets salts of various elements, on occasion, were added. The treated fabrics were prepared in two ways. In the first method the fabric was dipped in alcohol to ensure complete wetting, washed in distilled water, immersed in a solution of an appropriate salt (various concentrations up to 20 per cent. were used) and subsequently air-dried. Those metals the salts of which form insoluble compounds with water were added in acid solution and the acids removed *in vacuo* in the presence of alkali. In the second method a number of metals were incorporated chemically in the fibre, excess metal being removed by washing in running water. The form of linkage of these metals is not at present known although reaction with acid side-chains probably occurs (Lipson, personal communication). Mercury (up to 25 per cent.), and on one occasion lead (5.0 per cent.), was added by the method of Farnworth, Neish, and Speakman (1949) mentioned earlier. Salts were added to the artificial diet either in aqueous solution or as fine powders, which were ground into the food material.

After feeding for several days on the treated food the larvae were dissected under saline and examined fresh or after fixation in 10 per cent. neutral formalin in 70 per cent. alcohol or in alcoholic Bouin. Tests specific for the metals added to the food were also applied to the fixed larvae, details of the procedures being obtained from the B.D.H. Book of Organic Reagents (1948), from Feigl (1947), and from references contained therein.

Sections were prepared in the usual manner, counterstained with eosin or aniline blue, and mounted in "Lustrex"* in mesitylene. This polystyrene mounting medium has the advantage over balsam of inertness so that, for example, the Prussian blue colour characteristic of iron does not fade (Lillie, Windle, and Zirkle 1950).

III. RESULTS

(a) *Histology of the Midgut of Tineola Larvae*

The histology of the larval midgut has been investigated in some detail (Lotmar 1941; Waterhouse 1952a). There are three regions distinguished by the cell types (Fig. 1). In the anterior and posterior regions goblet cells are almost as numerous as columnar cells, the goblet cells have a fairly uniform diameter (Fig. 1B), and the striated border of the columnar cells is comparatively high. In the middle region each goblet cell is flask-shaped (Fig. 1C), it is separated from its neighbour by several columnar cells, and the striated border of the columnar cells is relatively low.

* Monsanto Chemicals Ltd. product.

The columnar cells have an appearance similar to those of the simple epithelial cells that occur in the midgut of many insects. The goblet cells have a basally placed nucleus surrounded by dense cytoplasm similar to that

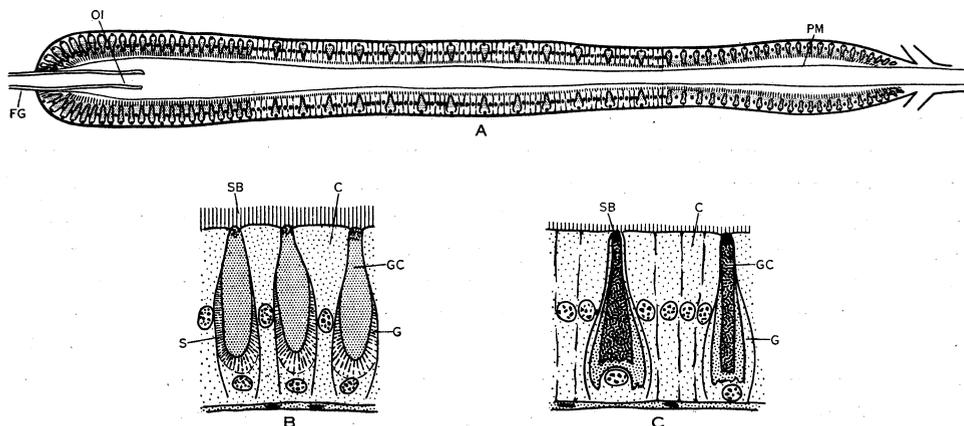


Fig. 1.—A, Midgut of a *Tineola* larva showing the distribution of columnar and goblet cells.

B, The columnar and goblet cells of the anterior and posterior regions.

C, The columnar and goblet cells of the middle region.

C, columnar cell; FG, foregut; G, goblet cell; GC, goblet cell cavity; OI, oesophageal invagination; PM, peritrophic membrane; S, striated lining to goblet cell cavity; SB, striated border.

of the columnar cells. The distal three-quarters of the goblet cell encloses a centrally placed cavity with a narrow, faintly striated lining. The tips of the goblet cells usually terminate about level with the striated border of the columnar cells and these cells, therefore, expose relatively little surface to the gut lumen in comparison with the columnar cells. The cavities of the goblet cells do not appear to open into the lumen, although the possibility of a narrow opening cannot be entirely discounted.

(b) *The Fate of Ingested Elements Capable of Forming Insoluble Sulphides*

Observations on larvae fed on diets enriched with salts of 19 elements are summarized in Table 1. It can be seen that both the food in the midgut and the faeces have a colour typical of the sulphides of the elements concerned and that the epithelium of both anterior and posterior regions of the midgut generally contains similarly coloured accumulations. Five of the elements resulted in sparsely scattered accumulations in the middle region of the midgut and one (tellurium) in heavy accumulations in this region. At times several additional metals (e.g. nickel) also resulted in barely detectable accumulations in this region. The colour of the faeces was sometimes influenced by the large amount (some 30-40 per cent.) of uric acid present in the pellets, which tended to give pellets a lighter shade than typical for the respective sulphide. Fuller data follow on representative metals to indicate the type of detailed results obtained. Except for the higher concentrations of arsenic, and to a

TABLE I
 COLOURS PRODUCED BY FEEDING *TINEOLA* LARVAE ON FABRICS ENRICHED WITH VARIOUS ELEMENTS
 Blanks indicate colour similar to control. Asterisks indicate metals strongly accumulated by the goblet cells

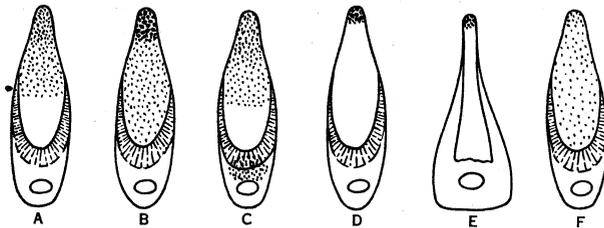
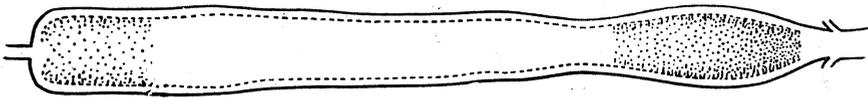
Element	Formula and Colour of Sulphide	Colour of Accumulations in Midgut				Colour of Food Undergoing Digestion	Salt Used
		Faeces	Anterior	Mid	Posterior		
Zinc *	ZnS white	Normal, light brown, or grey	White Very light brown or few black masses (often no colour)	White Light brown, sometimes black (often no colour)	Whiter than usual	ZnSO ₄ FeCl ₃	
Iron	FeS black						
Cadmium	CdS yellow-orange	Light yellow	Very light yellow		Light yellow	CdCl ₂	
Thallium	Tl ₂ S, Tl ₂ S ₃ black	Grey	Black		Brown to black	Tl (CH ₃ COO)	
Cobalt	Co ₂ S ₃ , CoS ₂ black; CoS brown	Brown to black	Light brown	Very light brown	Dark brown	CoCl ₂	
Nickel *	NiS black	Dark brown or black	Black	Black	Dark brown	NiS NiSO ₄ SnCl ₄	
Tin	SnS ₂ straw yellow	Greenish yellow	Very pale yellow	White specks	Dark brown	Pb (CH ₃ COO) ₂	
Lead *	PbS black	Dark brown to black	Black		to black		
Antimony *	Sb ₂ S ₃ orange	Greenish yellow	Orange	Orange specks	Light orange yellow	SbCl ₃ Sb ₂ S ₃ Bi(NO ₃) ₃	
Bismuth	Bi ₂ S ₃ brown	Brown or black	Brown to black	Brown to black	Dark brown		
Arsenic	As ₂ S ₃ , As ₂ S ₅ red or yellow	Light yellow to brownish yellow	Very light yellowish brown (sometimes)	Very light yellowish brown (sometimes)	Light brown	Na ₃ AsO ₃	
Copper	CuS, Cu ₂ S black	Brown	Light brown	Light brown to black	(sometimes)	CuSO ₄	
Tellurium *	TeS ₂ black	Black	Black	Numerous black masses	Dark brown	Na ₂ TeO ₃ Na ₂ TeO ₄ OsO ₄	
Osmium *	OsS ₂ , OsS ₄ black	Grey to black	Grey to black	Grey to black	Dark brown to black		
Mercury *	HgS, Hg ₂ S black	Grey to black	Black	Black specks	Black	Hg (CH ₃ COO) ₂ Ag albuminate, AgNO ₃ Pd Cl ₂	
Silver	Ag ₂ S black	Black	Black	Few scattered	Brown to black		
Palladium	PdS, PdS ₂ brown to black	Dark brown	Brown		Dark brown		
Platinum *	PtS ₂ black-brown	Light brown	Reddish brown	Very light brown	Dark brown	PtCl ₄	
Gold *	Au ₂ S ₃ , Au ₂ S brown to black	Golden brown to brown	Golden brown to brown	Golden brown specks to brown	Golden brown	HAuCl ₄	

lesser extent thallium and tin, none of the elements appeared to have any adverse effects on the larvae.

(i) *Iron*.—The light brown, metal-linked fabric containing iron was seen to change in colour as it passed along the alimentary canal. In the foregut and the anterior fifth of the midgut the food was brown; it then changed to a dark green or black, which later gave way to a dark grey in the posterior end of the midgut. It appears that digestion of the fabric first results in visible liberation of iron about one-fifth of the distance down the midgut.

When fed on fabric dipped in 4 per cent. ferric chloride the epithelium of the anterior and posterior midgut regions had a light rusty-brown colour; with iron saccharate there were sometimes occasional black masses in the extreme anterior end and in the posterior fifth of the midgut.

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Fig. 2.—Distribution of ferric iron in the midgut of *Tineola* larvae fed on iron-enriched wool. Iron accumulations indicated by stippling.

Fig. 3.—The site of metal accumulations in the goblet cells of *Tineola*. A, iron; B, nickel; C, copper; D, E, mercury; F, barium.

After staining for ferric iron by means of the Prussian blue reaction (potassium ferrocyanide and dilute hydrochloric acid) the anterior and posterior regions of the midgut stained heavily (Fig. 2). There was no staining of the remaining tissues, except very occasionally of the fat body or of the intima lining the hindgut. Sections of the midgut revealed that staining occurred only of the contents of the cavities of the goblet cells and then principally in the distal portion of the goblet (Fig. 3A). The cytoplasm of these cells and of the adjacent columnar cells remained unstained. Staining was generally heaviest in the goblet cells of the posterior midgut zone, less heavy in those of the anterior zone, whereas the goblet cells of the middle region failed to stain, indicating that goblet cells do not all function similarly. Except for the cut ends of the fibres, which reacted rapidly, the metal-linked fabric stained slowly after immersion in the ferrocyanide solution. However, the incompletely digested pieces of wool and other material in the faeces

stained very rapidly. Control larvae occasionally stained lightly for ferric iron in the anterior and posterior regions of the midgut.

Two hours after larvae had commenced feeding on a piece of iron-enriched fabric, which had also been dyed with eosin, dyed fibres could be seen to have reached the posterior end of the midgut. When these larvae were tested for iron the anterior midgut always became stained, whereas the posterior midgut reacted less frequently and then very weakly. The more usual condition with the posterior midgut staining more heavily for ferric iron than the anterior midgut became established only after some hours feeding. When larvae were transferred from iron-enriched fabric to control fabric the intensity of staining of the goblet cells gradually diminished over several days. However, it seldom disappeared entirely until moulting occurred. During moulting the old columnar and goblet cells are cast off into the midgut (Lotmar 1941) and the latter carry with them their accumulated iron.

When iron-fed larvae were tested for ferrous iron (potassium ferricyanide and dilute hydrochloric acid) the goblet cells of the posterior midgut stained intensely. In some larvae, but not all, there was some staining of the goblet cells in the anterior midgut, whereas the rest of the midgut remained unstained.

The occurrence of ferrous iron principally in the posterior midgut suggests that the oxidation-reduction potential of the goblet cell contents of this region is lower than that of the anterior midgut and it is noteworthy that black deposits of ferrous sulphide were observed more frequently in the posterior than in the anterior midgut.

The metal-linked fabric stained only very lightly and very slowly for ferrous iron and a similar behaviour was observed for the undigested wool in the faeces. However, the exterior of the faeces stained deeply and rapidly.

(ii) *Nickel*.—In living larvae fed nickel-enriched diets the anterior and posterior zones of the midgut could be seen through the transparent larval cuticle to contain elongated black masses. The actual distribution of dark masses within these zones depended upon the larval food. For example, on the nickel-linked wool, the masses occurred throughout the entire anterior midgut and were almost never seen in the posterior midgut (Fig. 4A). The light-coloured fabric in the anterior portion of the midgut became slightly darkened when it reached the middle of the midgut and the faeces were normal in colour. When larvae were fed on the fabric that had been dipped in 2 per cent. nickel sulphate the masses were present throughout the anterior midgut and in the central region of the posterior midgut (Fig. 4B). Larvae feeding on fabric dipped in 20 per cent. nickel sulphate (resulting in a 25 per cent. increase in fabric weight) accumulated dense black deposits throughout the entire anterior and posterior midgut (Fig. 4C), but no deposits could be seen in the middle region. The food throughout the midgut (and any fluid present in the foregut) was brown and the faeces were black. However, when the faeces were kept in a humid environment they became a greenish grey, presumably owing to the oxidation of nickel sulphide to nickel sulphate. Larvae fed on the yeast-casein diet containing 4 per cent. nickel sulphate accu-

mulated their densest and darkest masses in the middle of the posterior midgut (Fig. 4D). The food in the midgut was brown and the faeces black when excreted. It is clear, therefore, that the artificial diet contains enough sulphur to enable sulphide formation to occur readily, and in fact 0.5-1.0 per cent. cystine is present.

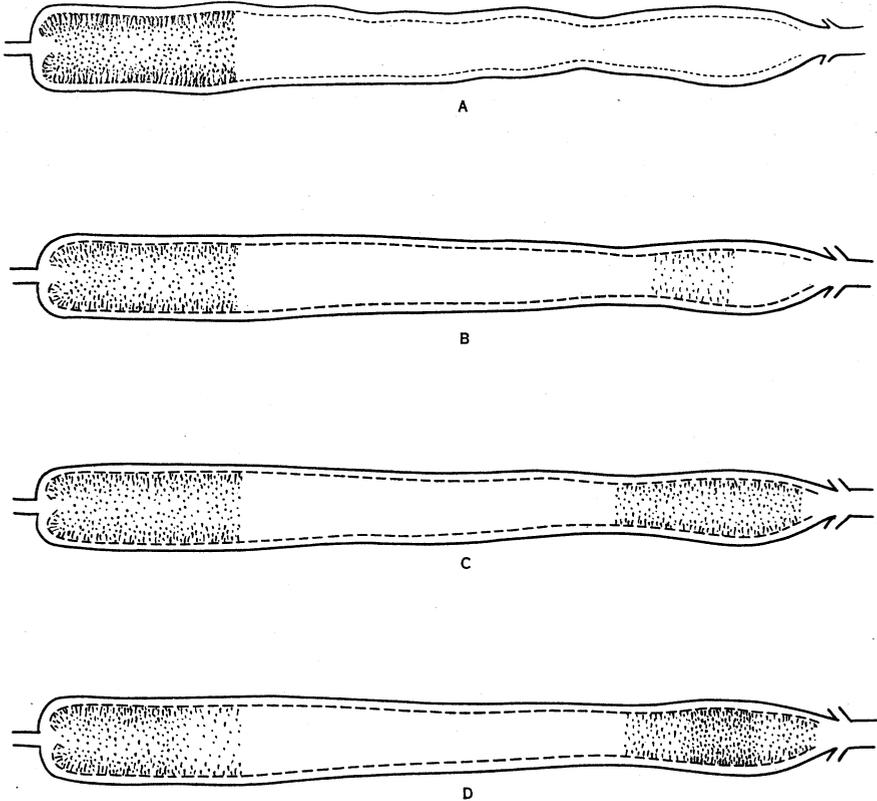


Fig. 4.—The distribution of nickel in the midgut of larval *Tineola*. See text for information on differences in diet resulting in distributions shown in A to D.

Histological preparations showed no black deposits if the sections were allowed to dry for several hours after flattening on the slides. However, the deposits were clearly visible when drying was speeded up by drying the slides in an evacuated, carbon dioxide-filled desiccator over phosphorus pentoxide. By this means oxidation of the moist deposits of nickel sulphide was prevented. Such histological preparations demonstrated that the black masses were the contents of the cavities of the goblet cells (Fig. 3B). The contents became progressively more heavily laden with black material towards the lumen of the gut and discrete black granules could be seen in the tips of many of the cells. The entire cavity appears to be filled after some days on a nickel diet whereas with some other metals (e.g. lead, Plate 1, Figs. 1 to 3) the deposits are restricted to the periphery of the cavity.

Following unsuccessful attempts with dimethyl glyoxime, salicylaldoxime, and α -furildioxime the presence of nickel was confirmed with a 0.1 per cent. solution of dithio-oxamide (rubeanic acid) in 70 per cent. alcohol. The deep purple nickel rubeanate formed proved fairly stable to normal treatment during sectioning and provided clear evidence that a nickel compound was distributed, as described above, in the cavities of the particular goblet cells concerned and nowhere else. Both uneaten fabric and faeces stained purple. No reaction was given by control larvae.

When larvae possessing conspicuous black goblet cell contents were transferred to control fabric the inclusions disappeared completely only during moulting. Prior to this there was some slight diminution of the amount of black material present. This appeared to be due, in part at least, to a sloughing off of entire goblet cells or to the discharge of the entire contents of their cavities since, in cleared whole preparations of the midgut, black cigar-shaped masses could occasionally be seen between the epithelial cells and the peritrophic membrane. It is also worth noting that a few goblet cells may be seen in sections to contain little or no black deposit, although the remainder are heavily laden.

(iii) *Copper*.—After feeding for several days on fabric dipped in 4 per cent. copper sulphate the anterior midgut was heavily laden with black masses (Plate 1, Fig. 4), whereas the posterior midgut contained fewer accumulations. The contents of the foregut and anterior midgut were light brown and those of the remainder of the midgut dark brown. When fed on lower concentrations of copper little or no accumulation could be seen, although the faeces were brown. However, when sections were prepared and mounted in sodium diethyldithiocarbamate in 20 per cent. alcohol, intense yellow staining of the anterior and posterior regions and faint staining of the middle region of the midgut were observed. This reaction was absent in control larvae. The most intense carbamate staining occurred either in the cytoplasm of the goblet cells immediately above the nucleus or in the distal two-thirds of the cavities of the goblet cells (Fig. 3C). The striated border throughout the midgut generally stained a light yellow and, in the anterior and posterior regions particularly, the lumen border of both goblet and columnar cells also stained. These observations indicate that a considerable amount of copper is present in these larvae in some form other than the free sulphide.

(iv) *Tellurium*.—Larvae that had fed on fabric treated with saturated solutions of sodium tellurate or tellurite accumulated black deposits in the goblet cell cavities in all regions of the midgut (Fig. 5). The faeces were black, although the fabric was white. These observations are of interest firstly because tellurium (a non-metal) is the only element observed to accumulate in quantity in the goblet cells in the middle region of the midgut, and secondly because they indicate that the phenomenon of the goblet cell accumulation (and colloidal dispersion with amino acids (see later)) is related to the sulphide nature of the compound rather than to its metallic nature.

(v) *Mercury*.—In larvae fed for long periods on fabric or on the artificial diet containing mercury (and they survived well on metal-linked fabric containing 25 per cent. by weight of mercuric acetate or cyanide) small black masses could be seen scattered throughout the epithelium of the anterior midgut (particularly at its anterior end) and of the anterior quarter of the posterior midgut (Fig. 6). There were also, quite often, small numbers of black masses present in the epithelium of the middle region and, on occasion, they were present only in this region. The black masses are jet black granules massed in the tips of some of the goblet cells (Plate 2, Figs. 1 and 2; Figs. 3D and E). In very occasional larvae, scattered black granules also occurred beneath the striated border of the columnar cells.

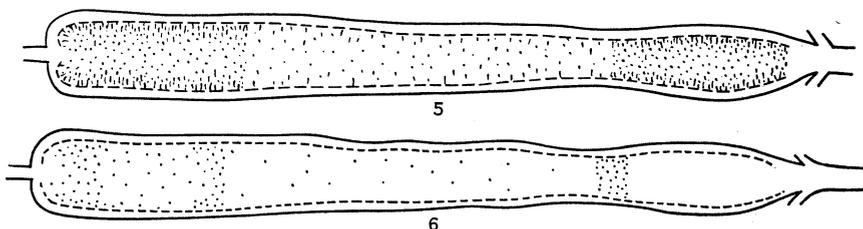


Fig. 5.—The distribution of tellurium in the midgut of larval *Tineola*.

Fig. 6.—The distribution of mercury in the midgut of larval *Tineola*.

Following long immersion in a freshly prepared saturated solution of diphenyl carbazone in 70 per cent. alcohol, the characteristic purple colour produced with mercury could be seen around the edges of the black granules. The cavities of a few of the goblet cells also stained a purplish pink, and uneaten fabric and undigested wool in the faeces became purple. No reaction was given by control larvae or control fabric.

(vi) *Gold*.—Larvae fed on gold-enriched fabric accumulated conspicuous golden-brown masses in the goblet cell cavities in the anterior midgut and golden-brown to brown masses in the posterior midgut. The tips of the goblet cells in the middle region also frequently contained small golden-brown masses. Gold produced accumulations in these cells far more readily than any of the other metals tested.

(vii) *Elements Producing No Midgut Accumulations*.—Fabrics containing a number of other elements were also fed to *Tineola* larvae (Table 2), but no coloured compounds were accumulated in the midgut epithelium. However, in most instances this can be explained on the basis of the properties of the respective sulphides. Thus aluminium, cerium, chromium, and zirconium do not form sulphides in the presence of water and there is some doubt whether molybdenum trisulphide and uranyl sulphide would be precipitated under the alkaline conditions in the gut. Tungsten tends to form soluble thiotungstates under alkaline conditions, the trisulphide only being thrown down if the solution is subsequently acidified.

Selenates are reduced to selenites by H_2S and these produce a precipitate

consisting of selenium and sulphur, selenious sulphide being too unstable to exist under these conditions. It is possible, therefore, that the yellow coloration of food in the gut and of faeces is due to the presence of free sulphur.

Cerium and uranium caused the production of some faeces having a colour approaching that of the respective sulphides, but the remainder of the impreg-

TABLE 2
COLOURS PRODUCED BY FEEDING *TINEOLA* LARVAE ON FABRIC ENRICHED WITH
VARIOUS ELEMENTS

Blanks indicate colours similar to control larvae

Element	Sulphide	Faeces	Food in Gut	Salt Used
Aluminium	Is rapidly hydrolysed			$\text{Al}_2(\text{SO}_4)_3$
Cerium	Is rapidly hydrolysed	Pink to red		CeCl_3
Chromium	Is rapidly hydrolysed			KCrO_4
				$\text{K}_2\text{Cr}_2\text{O}_7$
Manganese	MnS green or pale pink			MnSO_4
				KMnO_4
Molybdenum	MoS_3 dark brown			$(\text{NH}_4)_2\text{MoO}_4$
Selenium	SeS_2 red-yellow	Yellow	Often light yellow	Na_2SeO_3
				Na_2SeO_4
Tungsten	WS_3 chocolate-brown			Na_2WO_4
Uranium	UO_2S brown-black	Grey		$\text{UO}_2(\text{CH}_3\text{COO})_2$
Zirconium	Is rapidly hydrolysed			$\text{Zr}(\text{NO}_3)_4$

nated fabrics caused the production of normal coloured faeces. Although some of the treated fabrics were evidently toxic, some larvae survived on every fabric for a week or more, indicating that none of the compounds used was highly poisonous.

An unexpected inclusion in this group of elements is manganese, which does not result in sulphide formation, although it is closely related chemically to zinc, which does. A possible explanation is that the hydroxide is formed and this is not converted to the sulphide at pH 10. It may be significant also that manganese sulphide is far more soluble than zinc sulphide.

(c) *The Identity of the Coloured Compounds Accumulated in the Goblet Cells*

It is far too striking to be purely coincidental that not only the metal accumulations in the goblet cells but also the food in the midgut and the faeces invariably have a colour typical of the respective metal sulphides where these are insoluble. Furthermore, a number of elements that do not form insoluble sulphides under the conditions existing in *Tineola* larvae fail to produce visible accumulations when fed in the diet. Solubility experiments, such as those shown in Table 3, also indicate that the visible metal accumulations react as would be expected of finely divided sulphides.

It is of course well known (see Albert 1950) that metals form chelation compounds not only with amino acids but also with many other compounds occurring in tissues. There would be every opportunity for complex formation

to occur when amino acids are liberated from wool in the course of digestion in the alimentary canal or at a later stage when the metals are taken up by the midgut epithelium, where amino acids occur. However, these complexes, those of metals with polypeptides, pteridines, purines, porphyrins, and riboflavine are seldom black so that, even if they are formed, they cannot be the compounds (so frequently black) with which we are concerned.

TABLE 3
EFFECT OF IMMERSING METAL COMPOUNDS ACCUMULATED BY *TINEOLA* LARVAE
IN VARIOUS SOLVENTS

U = unchanged, F = faded, — = decolorized

Solvent	Ni			Pb		
	15 min.	7 hr.	48 hr.	15 min.	7 hr.	48 hr.
Water	U	U	U	U	U	U
Ethanol	U	U	U	U	U	U
Xylene	U	U	U	U	U	U
1N HCl	U	F	—	U	—	—
0.1N HCl	U	U	—	U	F	—
1N HCl + HClO ₄	F	—	—	U	—	—
1N HNO ₃	U	F	—	F	—	—

(d) *The Source of the Sulphide Sulphur*

As outlined earlier, the highly reducing alkaline conditions encountered by wool in the larval midgut reduce the disulphide bond of cystine (which is present to the extent of about 13 per cent. by weight in wool) with the production of sulphhydryl groups. The presence of these groups can, in fact, be readily demonstrated in the midgut lumen by the nitroprusside reaction. The reduced cystine is probably hydrolysed to give H₂S. When digestion occurs in the presence of metals it would be expected, therefore, that metal sulphides would be formed and there is no reason to doubt that this is the reaction leading to the production of characteristic colours in the alimentary tract following ingestion of metal-impregnated fabric.

Confirmation of this mechanism comes from an examination of the excreta of metal-fed larvae and from feeding larvae on metal-impregnated silk, which contains no sulphur amino acids.

Analyses (Powning, unpublished data) of faeces of larvae fed on control fabric demonstrate that they contain a good deal of cystine (6.65 per cent. of dry weight), presumably because this amino acid is present in such great quantities in wool that the larvae can utilize only a fraction of it for protein metabolism in conjunction with the amounts of other amino acids also available. When nickel was present in the diet (a fabric dipped in 20 per cent. NiSO₄, resulting in a 25 per cent. increase in weight) the cystine content of the faeces was very much lower (1.44 per cent. of dry weight). This indicates that nickel has combined with, and removed, some of the hitherto unutilized

sulphur. It becomes easier now to see why the presence of many metals in the larval diet has no detrimental effect.

Tineola larvae are unable to develop on silk, although they ingest it and remain alive, or even eventually pupate and produce adults, if transferred to it from a more adequate diet. When larvae were transferred from the standard woollen fabric to silk that had been dipped in 5 per cent. nickel sulphate there was some darkening of the food in the midgut, but no visible accumulation of nickel sulphide in the goblet cells. Since silk contains almost no sulphur the darkening is presumably due to the presence of sulphhydryl groups carried over by the larvae from their earlier diet, and the presence of sulphhydryl groups was, in fact, demonstrable by the nitroprusside reaction. The absence of goblet cell accumulations may be due either to the small amount of metal sulphide in the midgut or to the absence or inadequate concentration of suitable solubilizing agents (see later). The former was shown to be the correct explanation by the following experiments:

(i) When nickel sulphide is added to silk or "Terylene"* the sulphide accumulates in the goblet cell cavities. The faeces of silk-fed larvae are largely composed of undigested silk, indicating that little, if any, digestion has occurred. "Terylene" is a synthetic fabric and is completely resistant to digestion. It follows that the midgut is able to provide suitable conditions for the absorption of sulphides even if protein digestion is not taking place.

(ii) When 1 per cent. cysteine hydrochloride or 3 per cent. methionine or glutathione was added to a nickel sulphate-silk diet the food in the gut was considerably darker than without these sources of sulphur, the faeces were dark, and accumulations of nickel sulphide were almost always visible in the goblet cells of the anterior midgut, but not elsewhere. The formation of sulphide from methionine indicates that *Tineola* larvae are capable of demethylating this compound, thereby exposing the sulphur for sulphide formation. Demethylation is not perhaps surprising in view of the importance and frequent occurrence of enzymic transmethylation, at least in higher animals. Since the methionine content of wool is low (about 0.7 per cent.) this mechanism is probably not of great importance in sulphide formation from metals added to woollen fabrics.

From these various lines of evidence it is concluded that:

(i) The coloured compounds formed during digestion of metal-impregnated wool are sulphides;

(ii) The sulphur comes predominantly from sulphhydryl groups produced by the reduction of cystine in the larval midgut;

(iii) There is a very considerable amount of unutilized cystine available for sulphide formation, and

(iv) Ingested sulphides can be absorbed by the midgut epithelium and accumulated in the goblet cell cavities.

* An ethylene terphthalate, a Dupont product.

(e) *Mode of Absorption of "Insoluble" Sulphides*

The accumulation in the goblet cell cavities of material having the same colour as that of the partly digested, element-enriched food would at first sight suggest that the sulphides are either produced *in situ* in the cavities, or that the coloured compounds are absorbed in solution from the gut lumen, and hence this could scarcely be as the insoluble sulphides. If the goblet cell cavity opened directly into the lumen it is, of course, possible that insoluble compounds could be taken up, but no opening can be seen in sections and there is also indirect evidence against such an opening. We have seen earlier that the compounds (e.g. those of iron and nickel) persist in the cavities for several days at least after nickel-fed larvae have been transferred to control fabric. This is evidence either that there is no discharge from the cavities into the gut lumen (and discharge is not yet proven, although it is generally accepted) or that discharge occurs either through a cell membrane or through a very narrow opening, which holds back much of the accumulated metal. If there is an opening through which the sulphide has been taken up it should be large enough to permit its discharge later. If, alternatively, there is a bounding cell membrane difficulties arise, as mentioned above, in providing an explanation for the uptake of insoluble compounds through it.

The capacity of the midgut epithelium to absorb and accumulate sulphides was clearly demonstrated by feeding larvae on woollen fabrics or silk smeared with the freshly precipitated, thoroughly washed, sulphides of several of the metals. A curious observation was that when ferrous sulphide was ingested a bluish green fluid could sometimes be seen between the peritrophic membrane and the epithelial cells, although black accumulations in the goblet cell cavities were not observed.

It appears that the mechanism by which the sulphides are rendered capable of being absorbed is that described by Neuberg and Mandl (1948). These authors showed that metal sulphides are "solubilized" by the presence of amino acids and polypeptides, and that, although the complexes produced are often colourless, some of those formed with iron sulphide are bluish green. They concluded that the sulphides formed true solutions with amino acids principally because of (i) their stability, in some cases for several days, (ii) their clarity, (iii) their lack of a Tyndall effect at ordinary observation, and (iv) the absence of flocculation with $\text{NH}_4\text{OH-NH}_4\text{Cl}$ or $\text{NH}_4\text{OH}-(\text{NH}_4)_2\text{SO}_4$. However, a re-examination of the problem (Harris, unpublished data) has shown that the "solutions" are colloidal since the metal sulphides can be readily flocculated by the addition of large amounts of NaNO_3 solution and much smaller amounts of CaCl_2 or alum. Furthermore, in the copper "solution" negatively charged colloidal particles were observed to migrate under the influence of a current.

Amino acids and polypeptides are liberated from the food during larval digestion or are secreted with the digestive juices and it is suggested that these then produce an extremely finely divided colloidal dispersion of portion

of the metal sulphide also produced. Any undispersed sulphide is excreted. These processes can apparently be observed sometimes for ferrous sulphide, for bluish green fluid occurs between the peritrophic membrane and the epithelial cells. However, until more is known of the environment in which the sulphides are deposited in the cells and of the polypeptides and amino acids liberated from wool during its digestion by *Tineola* larvae, it will not be possible to explain the few minor irregularities observed in sulphide accumulation in the goblet cells.

Since the columnar cells expose a very much greater surface area to the gut lumen than the goblet cells it would be expected that most of the products of digestion would be absorbed by them. It is suggested, therefore, that the colloidal sulphides may first be taken up by the columnar cells and passed without accumulation into the goblet cells, which in turn discharge them into their cavities. A difficulty arises, however, in explaining on this basis the path of uptake in the central region of the midgut. Here, unlike the anterior and posterior regions, most of the columnar cells are not in contact with goblet cells (Fig. 1) and would, therefore, have greater difficulty in disposing of absorbed sulphides. It should be pointed out, however, that the goblet cells of this region accumulate sulphides and dyes (see later) less readily than the goblet cells of other regions and this may be correlated with a different absorptive capacity of the columnar cells.

The generally accepted function of goblet cells is that they accumulate secretions (notably digestive enzymes) in their cavities prior to discharge into the gut lumen. If this is so, the enzymes, salts, or other materials encountered in the cavities may well act as flocculating agents. Alternatively, if the dispersion-producing amino acids are reabsorbed the colloidal dispersion will become unstable and sulphide will be deposited in the cavity. If the secretions of the cavities are discharged continuously through a bounding membrane into the gut lumen the liberated particles of sulphide would be carried by the flow of secretion into the necks of the cavities and would first accumulate here and only later in the remainder of the cavity. This is what appears to happen.

(f) *The Fate of Ingested Metals Capable of Forming Insoluble Phosphates*

A mechanism has been demonstrated whereby *Tineola* larvae can detoxify elements that form insoluble sulphides. There are, however, other metals, such as barium and beryllium, that are toxic to many animals but form readily hydrolysable sulphides. It was therefore of interest to determine the effect of feeding these metals and also of feeding calcium and magnesium which have certain properties in common with barium and beryllium, notably that they all form relatively insoluble phosphates.

(i) *Barium*.—The presence of barium in granules that occur near the lumen border of the columnar cells in the anterior and posterior midgut of larvae feeding on a normal diet has already been demonstrated by the rhodizonate technique (see Waterhouse 1951, Fig. 2). After feeding on barium-

enriched wool these two regions of the midgut stain more heavily than usual, but the middle region, as before, remains unstained. In both anterior and posterior regions, the distribution of staining in the cells was similar (Plate 2, Fig. 3). Stained granules occurred in the inner ends of the columnar cells and, at the same level but far less frequently, in the goblet cells (Fig. 3E). In the columnar cells these granules are often separated from the striated border by a narrow zone of granule-free cytoplasm although, at times, the granules do occur directly beneath the striated border. The structureless contents of the cavities of the goblet cells may stain a uniform pink, particularly at the extreme anterior and posterior ends of the midgut. The nuclei of the goblet, columnar, and regenerative cells also stain a diffuse light pink with rhodizonate but the nucleoli are the only really conspicuously staining elements. At times the regenerative cells throughout the midgut were found to contain large numbers of granules which stained intensely for barium (see Waterhouse 1951, Plate 1, Fig. 2).

(ii) *Beryllium*.—Larvae were fed with little mortality for a week or more on fabrics dipped in various concentrations (up to 10 per cent.) of beryllium sulphate or nitrate. The histochemical distribution of beryllium in the midgut epithelium was examined by means of the naphthochrome green *B* technique (Denz 1949). On low beryllium concentrations the apple-green staining characteristic for beryllium was localized in the lumen border of the columnar and goblet cells in the region of the accumulation of the barium-rich granules. Light staining also occurred in the middle region of the midgut. With higher concentrations of beryllium the entire midgut epithelium stained a light green. It does not appear, therefore, that the resistance of larvae to beryllium poisoning is to any appreciable extent dependent upon its detoxification as insoluble phosphate, although some is undoubtedly deposited in this form. The low toxicity of beryllium to *Tineola* larvae is interesting in view of its high toxicity to mammals, where it has been shown to inhibit alkaline phosphatase and other magnesium-activated enzymes (Aldridge 1950). The present experiments suggest that alkaline phosphatase is not an important enzyme in the *Tineola* midgut, which is in agreement with the report that it could be detected only faintly in some of the columnar cells (Day 1949*b*).

(iii) *Calcium*.—Sections of *Tineola* midgut were stained by the Gallamine blue technique for calcium (Stock 1949). This indicated the presence of calcium in the granules, which occur in the anterior and posterior midgut in the columnar cells and less frequently in the tips of the goblet cells. Occasionally granules in the regenerative cells also stain for calcium. This finding confirms the report of Lotmar (1941) who used Kossa's method. The calcium is present in the granules which have been shown above to contain barium. They do not occur in all larvae feeding on fabric, although they are numerous in larvae on a calcium-enriched diet.

(iv) *Magnesium*.—Treatment of fixed control larvae with 0.2 per cent. Titan yellow, followed by the addition of a drop of 10 per cent. sodium

hydroxide produced a red stain in the midgut, the cut edges of the cuticle, and occasionally in the salivary glands. This is not surprising in view of the fact that magnesium is a normal tissue constituent. More intense staining occurred in larvae fed on a magnesium-enriched fabric. Staining was generally most intense in the anterior quarter of the posterior midgut, less intense in the remainder of the posterior midgut and in the anterior midgut, and comparatively light in the mid midgut. Similar results were obtained with quinalizarin and less satisfactory ones with *p*-nitrobenzene azo- α -naphthol (Magneson).

Permanent sections could not be made of material stained with Titan yellow since the red stain faded very rapidly in xylene to a pale yellow. However, if fixed tissues were sectioned and mounted in alkaline Titan yellow, the inorganic granules shown earlier to be the site of barium and calcium accumulations could be seen to have taken on a pink or red coloration. Quinalizarin-stained material enabled permanent stained preparations to be obtained.

The stained granules occurred most frequently near the lumen border of the columnar cells in the regions described above and were occasionally seen in the tips of the goblet cells. At times too, the cavities of the goblet cells were lightly stained. When the regenerative cells along the entire midgut commenced to enlarge in preparation for the next moult they frequently contained numerous granules that stained for magnesium.

(v) *The Presence of Insoluble Phosphates.*—The cobalt sulphide method for demonstrating phosphates (Danielli 1946) indicated that the granules, which have been shown under appropriate conditions to be sites of barium, beryllium, calcium, and magnesium deposition, also contain phosphate. Granules extremely rich in phosphate were particularly numerous in the inner ends of columnar cells of larvae that had fed on the yeast-casein diet enriched with calcium glycerophosphate (Fig. 7). A few granules occurred in the tips of some of the goblet cells and phosphate was also, at times, detected in diffuse form in the cavities of these cells. It is highly probable, therefore, that any slight excess in the diet of the above metals (and of any others capable of forming insoluble phosphates) will be immobilized as phosphates in the midgut epithelium. The addition of dilute acid to the midgut did not produce any visible evolution of gas, indicating that little if any carbonate can be present in the granules. It appears that the granules are not dissimilar in composition to those accumulated in the larval malpighian tubules of the blowfly *Lucilia cuprina* (Waterhouse 1950).

(g) *The Fate of Fluoride*

When larvae were placed on fabric that had been dipped in 1 or 4 per cent. sodium fluoride fairly high mortality occurred, although a number were still alive after one week. The presence of odd fibres in the midgut was evidence that these living larvae had ingested some of the fabric, although the

relatively few faeces produced indicated that it was distasteful to them. It appears, therefore, that the larvae are able to dispose of a certain limited amount of ingested fluoride. Since calcium is often present in sufficient amount to permit the formation of calcium-rich granules in the midgut it is suggested that absorbed fluoride may be prevented from exerting toxic effects by rapid deposition as calcium fluoride in the granules. The situation of the granules near the lumen border of the cells would enable this deposition to occur without the necessity of transporting the fluoride far into the cell interior. However, since the amount of calcium present in the fabric must be extremely small, many larvae evidently soon reach a stage at which all ingested fluoride cannot be detoxified.

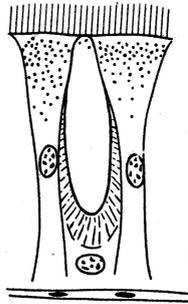


Fig. 7.—The distribution of phosphate granules (indicated by stippling) in the columnar cells of the anterior and posterior midgut.

(h) Effect of Feeding Boric Acid and Borax

Larvae developed satisfactorily and appeared quite normal on fabrics that had been dipped in warmed 10 per cent. solutions of boric acid or borax. This is interesting in view of the toxicity of these compounds to larvae of *L. cuprina*, which also develop on a diet rich in animal protein (Lennox 1941). However, the most important proteolytic enzyme in blowfly larvae appears to be adenosine deaminase which is inhibited by boric acid, and which must play little, if any, part in the digestion of wool by *Tineola* larvae.

(i) Selective Accumulation of Dyes by the *Tineola* Midgut

The selective accumulation of needle-shaped crystals in the columnar cells of the middle region only of the *Tineola* midgut after feeding on a dyed fabric has been recorded (Day 1951). It was therefore of interest to determine whether other dyes were similarly treated, for this would indicate a highly specific function for these particular cells.

A careful re-examination of the distribution of dye accumulated by larvae feeding on the same dyed fabric as used by Day demonstrated that the dye was invariably restricted to the goblet cell cavities and did not occur in the columnar cells. It was accumulated in a narrow band of goblet cells at the

end of the anterior midgut and also in the tips of the goblet cells of the middle region in the same location as the sulphides mentioned earlier.

Fabrics dipped in 1 per cent. ninhydrin, Trypan blue, or 12 vital dyes were also fed to *Tineola* larvae (Table 4). Ninhydrin and eight of the dyes were accumulated more or less strongly in the anterior midgut, and accumulations of many of these dyes could be seen in the goblet cells. The precise location of most dyes was not determined. However, methylene blue was

TABLE 4
THE DISTRIBUTION OF DYES IN THE MIDGUT OF *TINEOLA* LARVAE FED ON DYED FABRIC
+++ Very heavy; ++ distinct; + less heavy; ± just distinguishable accumulations;
blank, no accumulations

Dye	Midgut			Remarks
	Anterior	Middle	Posterior	
Benzopurpurin 4B				
Brilliant cresyl blue				
Brilliant vital red				
Chicago blue	+ goblet cells		±	
Dianyl blue	+ goblet cells			
Janus green	+ goblet cells			
Methyl violet	++ in few goblet cells	+++		
Methylene blue	++	+		Fabric blue in ant. end ant. midgut, elsewhere colourless
Neutral red	+++ all cells?			
Ninhydrin	++ pink mainly in goblet cells		±	Fabric blue in ant. and post. midgut, white in mid midgut. Faeces pink
Pontamine sky blue	+		±	
Trypan blue	+			
Vital new red				
Vital red				

rendered insoluble by the ammonium molybdate procedure and its distribution examined in sections. Fully coloured methylene blue occurred mainly in the cytoplasm of the anterior goblet cells and the lining of the cavities of the goblet cells of the middle region. When the epithelium was heavily laden with dye, small, scattered granules of methylene blue also occurred near the basement membrane and throughout the columnar cells. It is clear that these regions cannot be as intensely reducing as the midgut digestive juices. Methyl violet appeared in living larvae to have a similar distribution. Only three of the dyes were visibly accumulated by the posterior midgut epithelium. It is clear, therefore, that the three regions of the midgut accumulate dyes very differently. These experiments with dyes provide further evidence that the cells of the various regions of the midgut are capable of acting in an independent and highly specific manner.

(j) *Fate of Ingested Metals in Other Lepidopterous Larvae*

The structure of the goblet cells and midgut epithelium of the species mentioned below will be described elsewhere (Waterhouse 1952a).

Larvae of *Plutella maculipennis* (Curtis) (the diamond-back cabbage moth) were reared on seedlings of Chihili (the dwarf Chinese cabbage) whose roots were immersed in 0.5 per cent. solutions of ferric chloride or copper sulphate. When larvae that had fed on the iron-enriched diet were tested, an intense Prussian blue reaction was given by most of the goblet cells (Fig. 8A), but not by any of the columnar cells. Staining was confined to the cytoplasm. It was strongest between the nucleus and the striated lining of the cavity and often extended up around this lining towards the gut lumen. Iron occurred less frequently between the nucleus and the base of the cell. The nucleus, the striated lining, and the contents of the cavity were not observed to stain. This distribution of iron in the cytoplasm of the goblet cells is not inconsistent with the possibility that it has been transferred laterally after absorption by the columnar cells.

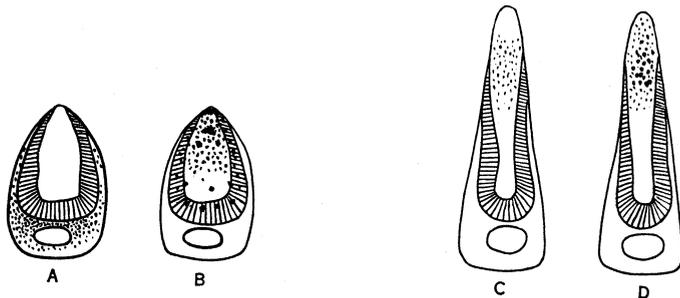


Fig. 8.—A, The distribution of iron in a goblet cell of *Plutella*.
 B, Copper in a goblet cell of *Plutella*.
 C, Iron in a goblet cell of *Heteronympha*.
 D, Copper in a goblet cell of *Heteronympha*.

After copper feeding, the carbamate test demonstrated that this metal was present mainly in the goblet cells, although it could be detected in small amounts elsewhere in the midgut epithelium. In the goblet cells copper was restricted to the cavity and its lining, appearing as numerous scattered golden-brown masses in these situations (Fig. 8B). The tip of the cell often contained a large mass of copper-rich material.

Larvae of *Heteronympha merope* (Fabr.) (the "Common Brown" butterfly) were reared on a variety of native grasses, the roots of which had been immersed in metal solutions. Small, dark brown masses are often present throughout the midgut epithelium of control larvae. These masses, which are most abundant in the middle region, were shown by sectioning to be the contents of the goblet cell cavities. After iron feeding, small accumulations of ferric iron could sometimes be detected in the distal half of the cavity of some of the goblet cells (Fig. 8C). No iron could be seen in the columnar

cells. After copper feeding the distal half of some of the goblet cell cavities and the adjoining striated lining stained yellow with carbamate (Fig. 8D). Some copper-rich masses were present, but the distribution of staining was generally far more uniform than in *Plutella*. At times the striated borders of the columnar cells stained a light yellow, but the remainder of these cells did not react.

Iron accumulations were also detected in the goblet cells of three other species. As in *Tineola* and *Heteronympha*, it was present in the goblet cell cavities of *Pieris rapae* and *Galleria mellonella* but, like *Plutella*, it occurred in the cytoplasm of the goblet cells in *Ephestia kuhniella*. From these scattered observations it is clear that the goblet cells of many lepidopterous larvae accumulate iron and copper and presumably other metals also.

Barium-rich granules were detected in the midgut epithelium of several species fed on a barium-enriched diet. These granules occurred principally near the lumen border of the columnar cells and less frequently in the goblet cells.

IV. DISCUSSION

The foregoing experiments demonstrate that the goblet cells in the midgut of *Tineola* and other lepidopterous larvae play a specialized and important part in the disposal of a large number of absorbed metals and one non-metal, tellurium. It is clear, however, that all goblet cells do not behave in an identical fashion. There are firstly species differences in the manner in which ferric iron is accumulated. In *Tineola*, *Heteronympha*, *Pieris*, and *Galleria* it is present in the cavity, whereas in *Plutella* and *Ephestia* it is in the cytoplasm. Secondly, in *Tineola larvae*, the goblet cells in the middle region of the midgut (in which relatively few metals were accumulated) function differently from those in the anterior and posterior midgut regions. This is perhaps not unexpected in view of the different morphological appearance of the goblet cells in these regions. Thirdly, and more remarkable, is the differential behaviour in *Tineola* of the goblet cells in the anterior and posterior zones to different metals. This cannot simply be a question of the availability of the sulphide. For example (Figs. 2, 4, 6) the goblet cells in the posterior midgut all accumulate iron at low concentrations in the diet, but only those at the anterior end of this region accumulate nickel unless the nickel concentration is high. Furthermore, only a few of those that accumulate nickel ordinarily accumulate mercury. This indicates that cells that have a similar histological appearance and behave similarly to one metal behave differentially to others. This differential behaviour is not confined, however, to the goblet cells, for the columnar cells in the anterior and posterior regions accumulate granules, which are not present in the columnar cells in the middle region. These granules enable the inactivation of a certain limited amount of absorbed alkaline earth metals and possibly also of fluoride. Except for small amounts of ascorbic acid (Day, unpublished data) no capacity for accumulation has yet been detected in the columnar cells of the middle region of the midgut, which may be concerned mainly with secretion. Differential accumulation is not restricted to cells of

the lepidopterous midgut, however, for iron and copper are accumulated only by certain midgut cells of *Lucilia* larvae (Waterhouse 1940, 1945) and *Drosophila* larvae (Poulson 1950a, 1950b).

Other interesting information on the distribution of materials in the midgut of *Tineola* larvae is also available. Thus the columnar cells throughout the midgut are reported to possess a weak alkaline phosphatase and to contain granules that give the silver reduction test for ascorbic acid (Day 1949a, 1949b). These latter granules occupy a position identical with those now shown to contain calcium, magnesium, and phosphate, and this raises some doubt of the validity of the positive test for ascorbic acid. The goblet cells, on the other hand, do not contain alkaline phosphatase, ascorbic acid, or mucoid material (Day 1949a, 1949b, 1949c).

There are considerable differences between metals in the amounts of sulphide accumulated in the goblet cell cavities. Elements marked with an asterisk in Table 1 accumulate readily, whereas others accumulate slowly or only in occasional larvae. This is doubtless related to the properties of the various sulphide-amino acid colloidal dispersions and to the conditions they encounter in the midgut epithelium. When more is known it may be possible to explain, for example, why neither copper nor iron regularly appear in the cavities as sulphides, although their presence there in some other form can be readily demonstrated by appropriate histochemical tests. It is probable that these elements are being absorbed, in part, by a more usual mechanism than solubilization of their sulphides. It is not known, for example, whether the colour of the sulphide is masked, owing to the fact that these metals are still dispersed by amino acids. This raises the possibility that there may be definite, but invisible, accumulations of metals elsewhere in the epithelium. If this were so, the metals must be present in some form that does not react with available histochemical tests, for such tests have demonstrated that heavy accumulations of iron, nickel, copper, lead, and mercury are largely restricted to the goblet cell cavities.

It has been stated that digestion of wool first commences at the beginning of the middle region of the midgut, since visible changes in the fibres are first detected at this level (Day 1951). However, reducing conditions, which are capable of promoting the rupture of the disulphide bonds, are established in the anterior region also, as can be demonstrated by feeding larvae on oxidation-reduction indicators (Waterhouse 1952b). The appearance of iron in the anterior goblet cells (but seldom in those of the posterior midgut) by the time iron-enriched fabric has travelled to the end of the midgut suggests that some digestion and absorption take place in the anterior midgut also.

The function of the metal accumulations in the goblet cells warrants consideration. If the metals have been taken up, as is possible, via the columnar cells, then their location in the portion of the goblet cells adjacent to the lumen would suggest that this may be principally a form of storage excretion of metal absorbed in excess over metabolic needs, or that the metals are being prepared for active elimination into the gut. For the latter to be efficient,

however, the metals would have to be eliminated from the anterior midgut in a form that would not permit re-absorption further down the gut. The elimination of metal accumulations when the entire old epithelium is discarded at moulting strongly supports the view that they constitute a form of storage excretion.

Some information on the mode of secretion of the goblet cells is available from the persistence of metals in their cavities for several days after metal-fed larvae have been transferred to control fabric. This is evidence that, if any regular passage of material from their cavities into the lumen occurs, it is either through a very narrow opening which holds back much of the accumulated metal, or through a cell membrane. If the entire contents of the cavity are discharged into the gut either regularly or at a particular stage in cell maturation the accumulated metals would inevitably be discharged at the same time. If periodic discharge does occur the present observations indicate that the frequency of such discharge cannot be greater than every two or three days. It has been mentioned earlier that, in sections, there appears to be a bounding membrane between the goblet cell cavity and the gut lumen. The functional evidence just presented supports this interpretation and is regarded as being more satisfactory than the histological evidence. Firstly, the latter is contrary to the observations of previous authors who have considered that the cavity opens directly into the lumen, as in the differently functioning goblet cells of mammals. Secondly, it is almost impossible when examining even thin sections to decide whether one is focusing on a bounding cell membrane or on the obliquely cut end of a channel leading from the goblet cell cavity into the gut lumen. However, in the many sections of several species of larvae examined no goblet cell cavity could be seen communicating directly with the lumen unless fixation of the tissues was clearly faulty. Thus, although there is not yet conclusive evidence that the goblet cell cavity is closed available evidence favours this view. Alternatively, if the sulphides are absorbed directly from the lumen by the goblet cells it will be necessary to recast current ideas of the function of the goblet cells, namely that they are principally secretory in nature.

The foregoing experiments demonstrate that *Tineola* larvae, partly by virtue of their unusual diet and partly because of their digestive mechanisms, are singularly well equipped to detoxify often quite large amounts of inorganic materials that are ordinarily regarded as highly toxic to animal life. Probably the most toxic of the relatively few elements employed in the present tests that had adverse effects on the larvae were fluorine, arsenic, and thallium, but the larvae tolerated relatively high concentrations even of these. However, accurate data on toxicity were not obtained. Unless the larvae possess a more effective mechanism than was discovered for the detoxification of fluorides, fluorine would appear to offer better possibilities for mothproofing than the other elements tested. It is perhaps significant that fluorides (mainly silico-fluorides) are the only inorganic materials that have found any widespread use for mothproofing fabrics (Hartley, Elsworth, and Barrett 1943).

It is interesting to speculate on the possible importance in metal metabolism of the colloidal dispersion of insoluble sulphides. Sulphides are frequently formed in the digestive tract of animals, largely by bacterial action on organic sulphur compounds (Fromageot 1947). Under these conditions insoluble sulphides of many essential minerals are probably formed and these would be lost to the animal by excretion unless they are capable of being absorbed after colloidal dispersions have been formed. Since animals possess the capacity to oxidize sulphide to sulphate (Fromageot 1947) it is possible that the uptake of essential minerals as sulphides and their transport in the body in this form before utilization may be of fairly general occurrence. Metals absorbed as ions may also form sulphides or mercaptides after reaction with sulphhydryl groups in the tissues, and transport of sulphides and the liberation of ions by subsequent oxidation to sulphate may play an important part in mineral metabolism.

It is also worth considering the general evolutionary significance of the sulphide-detoxifying mechanism. This is reminiscent of the protection given by 2-3-dimercaptopropanol (British Anti-Lewisite) against poisoning by arsenic and a number of other metals. Clothes moth larvae possess an extremely effective biochemical mechanism for detoxifying many metals they would never normally encounter in their diet. Not only is the mechanism able to deal effectively with very large quantities of metals, but the range of insoluble sulphides formed is greatly extended by the fact that the conditions are quite alkaline. As Professor Dobzhansky has pointed out (in personal discussion) this is indeed a remarkable instance of biochemical "pre-adaptation" to a change which has not yet occurred, and may never occur, in their environment. It is possible that many other processes, which are now essential to the life of organisms, may have arisen initially as fortuitous by-products of some other metabolic process.

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DIGESTION OF WOOL BY INSECTS. IV

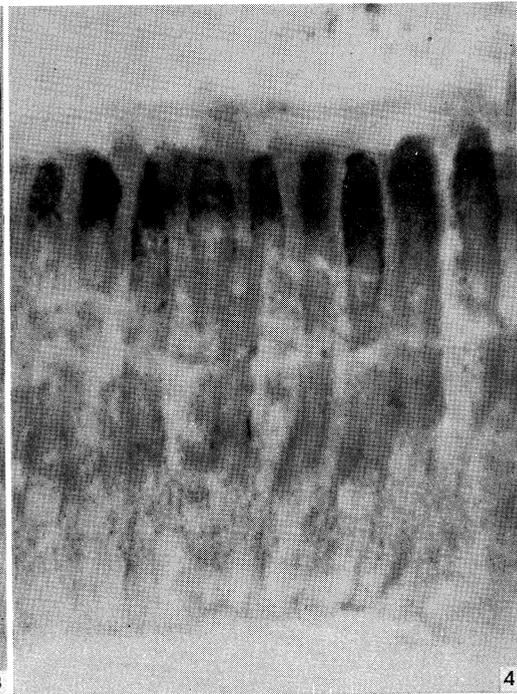
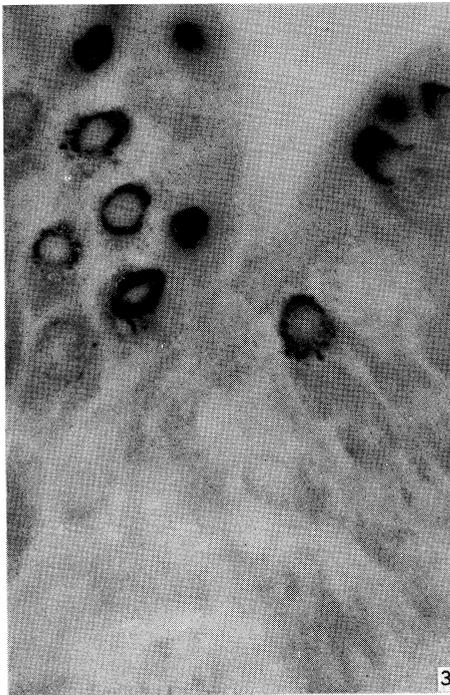
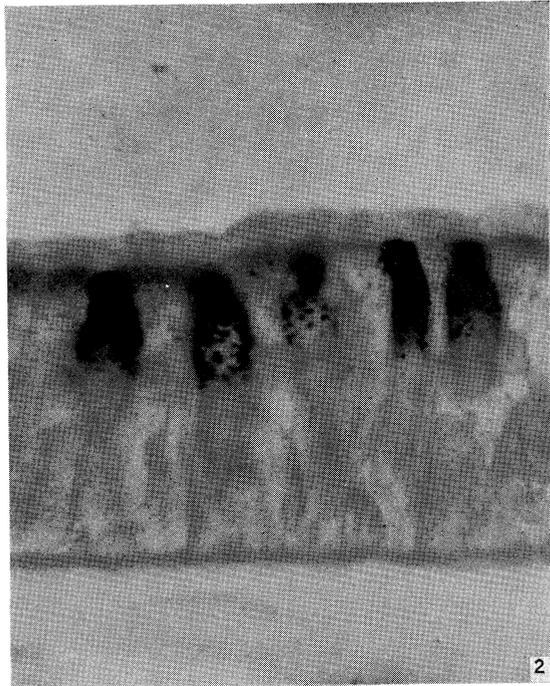
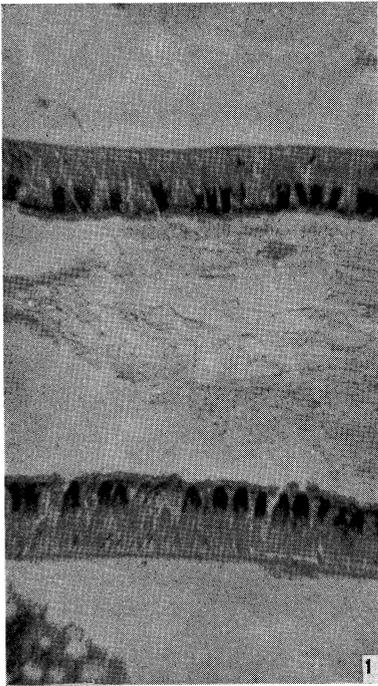
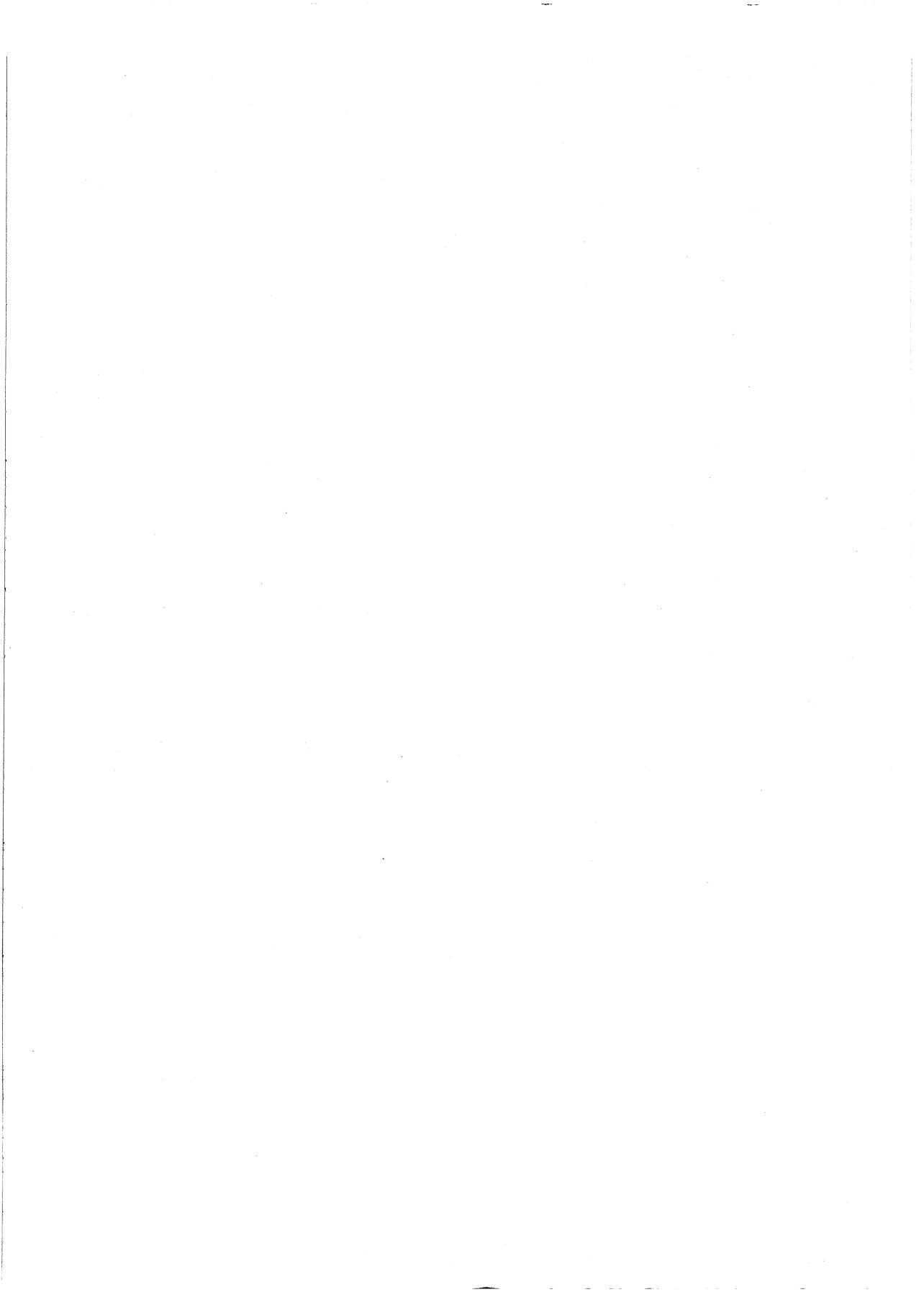


Fig. 1.—L.S. anterior midgut of a *Tineola* larva, showing accumulations of lead sulphide in goblet cells.

Fig. 2.—As Figure 1, but at higher magnification.

Fig. 3.—Tangential section showing lead sulphide at periphery of goblet cell cavities.

Fig. 4.—L.S. anterior midgut of a *Tineola* larva, showing copper sulphide accumulations.



DIGESTION OF WOOL BY INSECTS. IV

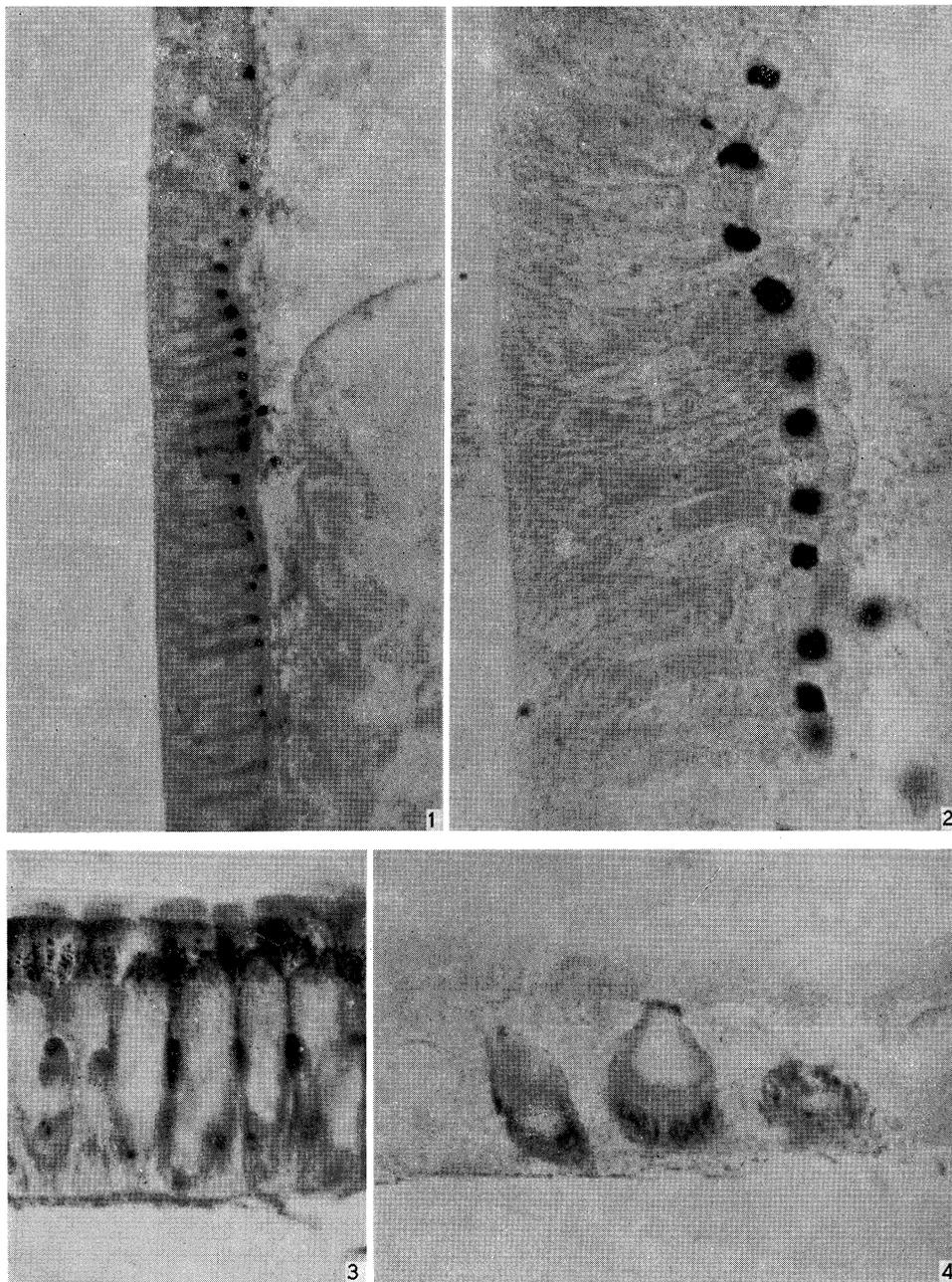


Fig. 1.—L.S. anterior midgut of a *Tineola* larva, showing accumulations of mercuric sulphide in tips of goblet cells.

Fig. 2.—As Figure 1, but at higher magnification.

Fig. 3.—L.S. beginning of anterior midgut of a *Tineola* larva, showing granules (stained for barium) near lumen border and principally in the columnar cells.

Fig. 4.—L.S. *Plutella* midgut, stained with the Prussian blue reaction, showing the accumulation of ferric iron in the cytoplasm at the base of the goblet cells.

