STUDIES ON THE DIGESTION OF WOOL BY INSECTS

V. THE GOBLET CELLS IN THE MIDGUT OF LARVAE OF THE CLOTHES MOTH (*TINEOLA BISSELLIELAL* (HUMM.)) AND OTHER LEPIDOPTERA

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Summary

Goblet cells and columnar cells occur, together with regenerative cells, in the midgut epithelium of lepidopterous larvae. The columnar cells have an appearance typical of the simple epithelial cells that occur in the midgut of many insects. The goblet cells are highly differentiated and, although there are marked variations between species, such as in frequency of occurrence, in shape, in staining reactions, and so on, their basic structure is very similar. Bodian's 'Protargol' staining technique provides excellent differentiation of goblet cells. Each goblet cell has a basally situated nucleus and contains an internal cavity, which is bordered by a faintly striated lining. No opening permitting direct movement of material from the cavity into the lumen has been observed. Available evidence suggests that materials moving out of the cavity pass through a bounding membrane. Unlike columnar cells, goblet cells do not possess a striated border on their lumen surface.

There is no satisfactory evidence for the occurrence of goblet cells in orders other than Lepidoptera.

I. INTRODUCTION

The regular occurrence of two principal cell types (columnar cells and goblet cells) in the midgut epithelium of lepidopterous larvae has been known for many years. Much of the early literature on the structure of the goblet or caliciform cell and its relationship to the columnar cell has been reviewed by Buchmann (1928), Henson (1929), Lotmar (1941), and Woke (1941). It was once thought that goblet cells were produced from columnar cells by discharge of material, followed by an invagination of the striated border, but there is no satisfactory evidence to support this view. On the other hand, it is now known that goblet cells are present before the larva has hatched from the egg (Woke 1941). Furthermore, when the epithelium is cast off and renewed at each moult, goblet cells are differentiated directly from the regenerative cells and are visible, with their developing internal cavity, before the new epithelium has taken on any digestive function (Lotmar 1941).

Several authors (Day 1951; Lotmar 1941; Woke 1941) have suggested that the goblet cells are secretory and function principally in the production of digestive enzymes, which are stored in the goblet cavity prior to discharge

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into the lumen of the gut. As yet, there is no evidence to support this hypothesis, although there are some data that render it improbable (Waterhouse 1952*b*). In larvae of the clothes moth, *Tineola bisselliella,* the goblet cells do not contain mucoid material, ascorbic acid, or alkaline phosphatase, although ascorbic acid granules and a weak alkaline phosphatase have been reported in the columnar cells (Day 1949*a*, 1949*b*, 1949*c*). However, in *Tineola* and in several other lepidopterous larvae, goblet cells play an important part in metal and dye metabolism, high concentrations of metallic salts and dyes being accumulated in the goblet cavity or in the cytoplasm of the cell (depending upon the species and the material accumulated). On the other hand, metal accumulations cannot, with few exceptions, be detected in the columnar cells (Waterhouse 1952*a*, 1952*b*). In conjunction with these experiments on metal detoxification and metal regulation it became desirable to re-investigate the occurrence and structure of goblet cells in lepidopterous larvae. Although goblet cells have a very similar basic structure, their appearance differs from species to species and in *Tineola* larvae, at least, there is a distinct dimorphism. In some species (*Deilephila, Galleria, Vanessa*) the goblet cells are reported to be fairly evenly distributed throughout the midgut. In others (*Pyrausta*) they are more numerous in the middle and posterior regions and in others again (*Dictyoploca*) they are more numerous in the anterior midgut (see Lotmar 1941). It is highly desirable, therefore, to establish whether the ability of *Tineola* larvae to digest wool and to maintain an unusually high pH and low oxidation-reduction potential in their midgut (Waterhouse 1952*b*) is associated with any unusual features of goblet cell structure or distribution.

II. Methods

Larvae of various instars of 18 species of Lepidoptera belonging to 17 Families were fixed in alcoholic Bouin, neutral formol alcohol, or Carnoy and generally stained in Bodian, Mallory, or haematoxylin. Bodian's 'Protargol' technique following alcoholic Bouin fixation was, in general, found to provide the best differentiation.

The following species were examined:

Heterocera

Tineidae *Tineola bisselliella* (Humm.)
Plutellidae *Plutella maculipennis* (Curtis)
Gelechiidae *Sitotroga cerealella* (Ol.)
Tortricidae *Tortrix postvittana* (Walk.)
Galleriidae *Galleria mellonella* (L.)
Phycitidae *Plodia interpunctella* (Hubn.)
Phycitidae *Ephestia kuhniella* Zell.
Arctiidae *Spilosoma canescens* (Butl.)
Anthelidae An unidentified species
Noctuidae *Dasygaster hollandiae* Guen.
Agarastidae *Phalaenoides glycine* Lew.
Sphingidae An unidentified species
Boarmiidae *Mnesampela privata* Guen.
Bombycidae *Bombyx mori* (L.)

Rhopalocera

Papilionidae *Papilio aegeus* Don.
Satyridae *Heteronympha merope* (Fabr.)
Nymphalidae *Pyrameis itea* (Fabr.)
Pieridae *Pieris rapae* (L.)

III. Results

A careful study was made of the goblet cells in *Tineola* larvae which will, therefore, be described in some detail. Other species will then be dealt with more briefly.

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

The general features of the histology of the larval midgut have been described by Lotmar (1941) and only the distribution and structure of the cell types will, therefore, be considered here. In the anterior region of the midgut (approximately the first quarter) goblet cells are so numerous that they are separated by no more than one or two columnar cells, and occasionally they appear to touch one another (Waterhouse 1952*a*, Fig. 1*A*, *B*). Then follows a middle region (about half the length of the midgut) in which goblet cells occur only infrequently and columnar cells predominate. This leads into the posterior region (the last quarter) in which goblet cells occur almost as frequently as in the anterior region. Small groups of regenerative cells are scattered along the entire midgut and lie near the basement membrane. These regenerative cells are inconspicuous following a moult, but become more numerous and more apparent as the next moult approaches.

The columnar cells in the middle region of the midgut (Waterhouse 1952*a*, Fig. 1*C*) have an appearance typical of simple epithelial cells which occur in the midgut of many insects. The nuclei lie at about the centre of the cell and each cell possesses a conspicuous, but low, striated border. On the other hand, owing to the presence of numerous goblet cells, the cylinder cells in the anterior and posterior regions are often narrow at the level of the nucleus and wider proximally and distally (Waterhouse 1952*a*, Fig. 1*B*). In these regions the striated border, although variable, is comparatively high.

The goblet cells of *Tineola* larvae do not conform in structure to past descriptions of goblet cells in other lepidopterous larvae, principally in the fact that the cavities of the cells do not appear to open into the lumen, even when the cells are examined in thin (4 μ) sections. The cells narrow as they approach the lumen, but may expand again a little to form a "cap" before they terminate apically with a sharp demarcating line. This line has the appearance of a bounding cell membrane. There is no sign of a striated border on the lumen boundary of the goblet cells. The tips of the goblet cells generally terminate level with the general line of the base of the striated border of the columnar cells (Waterhouse 1952*a*, Fig. 1*B*, *C*). However, they sometimes lie below this level (illustrated for *Galleria* in Plate 2, Fig. 6) communicating with the lumen by narrow passages between the higher columnar cells. Whatever their level in relation to the striated border, the goblet cells expose relatively little

surface to the gut lumen. The goblet cells in the anterior and posterior regions of the midgut differ in shape from those in the middle region (Waterhouse 1952*a*, Fig. 1*B*, *C*). In the anterior and posterior regions the cells are cigar-shaped, being fairly uniform in width until they start to narrow towards the gut lumen. If there is a slight variation in width, the maximum is reached in the distal half of the cell (Plate 1, Figs. 1 and 3). In the middle region of the midgut, the goblet cells are more flask-shaped, the basal portion of the cell being enlarged and the distal being reduced in diameter (Plate 1, Figs. 2, 4, 5, 6).

The basal quarter or fifth of each goblet cell contains the nucleus and dense cytoplasm similar to that of the columnar cells; the distal three-quarters of the cells is largely occupied by a centrally placed "cavity" with uniformly staining, structureless contents and, at times, some granules. The contents of the cavities (and the nuclei of the goblet, columnar, and regenerative cells) are heavily impregnated with silver following Bodian's technique. The cytoplasm of the columnar cells and the striated border is, by contrast, impregnated only very lightly (Plate 1, Figs. 1-6).

In the goblet cells of the anterior and posterior midgut the region of each cell bordering the basal half of the cavity has a different appearance from the remainder of the cytoplasm. This region sometimes appears to be faintly striated (Plate 1, Fig. 3) and evidently corresponds to the more definitely striated lining to the cavity of the goblet cells in some other lepidopterous larvae (see later). It has a different appearance and staining reactions from the striated border of the columnar cells.

In the goblet cells in the middle portion of the midgut, the region that stains heavily with Bodian often appears to extend around on either side of the nucleus towards the base of the cell (Waterhouse 1952*a*, Fig. 1*C*). This extension may either be uniform or take the form of a series of lobes invaginated into the cytoplasm. These lobes may be simple or branched (Plate 1, Figs. 4-6). Sections stained with aniline blue, safranin, or eosin show that the inner region of the Bodian-stained material constitutes the cavity of the cell. Occasionally there is some slight indication of striations in the outer region which, therefore, corresponds with the striated lining of other goblet cells.

(*b*) *Goblet Cells in Other Lepidoptera*

From Figure 1*A*, *B* and Plates 1-3 it can be seen that the goblet cells of a number of other species of lepidopterous larvae (both moths and butterflies) have a structure generally similar to the cigar-shaped goblet cells of *Tineola*. Principal variations occur in:

(i) The depth to which the goblet cavity extends into the cells. Although there is a certain amount of variability within a single species, the goblet cavity frequently occupies a characteristic position in the cell. It extends almost to the base of the cell in *Bombyx* (Plate 1, Fig. 11) and *Plutella* (Plate 1,

Fig. 12), but only to about the centre of the cell in *Plodia* (Plate 1, Fig. 10). Other species occupy intermediate positions.

(ii) The appearance of the lining of the goblet cavity. In most species the lining is only faintly striated, but in others, e.g. *Galleria* (Plate 2, Figs. 6 and 7), there are comparatively long and conspicuous filaments, somewhat reminiscent of the striated border of the columnar cells. In *Heteronympha* (Plate 1, Fig. 7) and *Plutella* (Plate 1, Fig. 12) striations can also be quite readily seen, although the "filaments" are more compact than in *Galleria*.

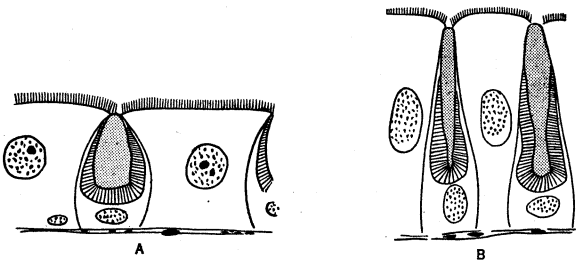

Fig. 1.—A, diagram showing goblet and columnar cells of *Plutella*. *B*, diagram showing goblet and columnar cells of *Heteronympha*.

(iii) The staining reactions of the goblet cavity. The goblet cavity of most species stains heavily with Bodian, but, in others, it stains comparatively lightly and the latter is usual in most species immediately after moulting. Furthermore, the tip of the goblet cell in some species (e.g. *Galleria, Plodia, Papilio aegeus*) is strongly fuchsinophilic, whereas in others there is very little red staining.

(iv) The accumulation of granular material at the tip of the cell. Heavily stained granular material at the lumen border of the cell was a conspicuous feature of most species (e.g. *Papilio aegeus* (Plate 2, Fig. 2) and *Pyrameis itea* (Plate 2, Fig. 5)), although it occurred less frequently in others.

(v) The diversity of goblet cell structure. No distinct dimorphism in goblet cell type (such as occurs in *Tineola*) has been observed in other species, although the latter have been studied far less carefully. The most striking changes are those that occur in different instars. In young larvae the epithelium is generally simple and the goblet cells and columnar cells are clearly separated. In later instars, in which the epithelium commonly becomes highly convoluted, the cells are relatively longer and narrower and the goblet cells frequently become crowded together. In the young larvae the goblet cavity is often wider than in the folded epithelium of more mature larvae. The change in appearance with advancing instar is exemplified in the photographs of young *Papilio aegeus* (Plate 2, Figs. 1 and 2) and a mature larva (Plate 2, Figs. 3 and 4).

(vi) The ratio of goblet to columnar cells. In different regions of the midgut it is common to find the ratio of goblet cells to columnar cells varying

from 1 : 1 to 1 : 5. Even when the goblet cells are fairly widely spaced two contiguous cells may occur (*Plodia,* Plate 1, Fig. 10). Where the epithelium is highly convoluted in later instars the crowded goblet cells appear to touch each other (Plate 2, Fig. 4). However, they must, in reality, generally be separated by columnar cells, whose nuclei (lying about the centre of the epithelium) and striated border are clearly visible.

(vii) The position of the goblet cell in relation to the folds of the epithelium. In some species (*Tortrix,* Plate 3, Figs. 7 and 8) the goblet cells occur most commonly at the bases of the epithelial folds. However, they may occur in all positions (*Pyrameis itea,* Plate 2, Fig. 5) and are shown on the side (*Diacrysia,* Plate 3, Fig. 4 also at base, Plate 3, Fig. 3) and at the top (anthelid, Plate 3, Fig. 6) of epithelial folds.

IV. Discussion

One of the striking features of this brief survey of goblet cell structure is the general similarity of these cells in most species. The cells that deviate most from the basic pattern are those occurring in the middle region of the *Tineola* midgut. It might be expected, therefore, that goblet cells will have a similar function in all lepidopterous larvae.

It appears that, very largely by analogy with the mucus-secreting goblet cells in the digestive tract of vertebrates, the cavities of the goblet cells have been considered to discharge their contents through an opening that communicates directly with the gut lumen. Except where fixation has been faulty, no such openings have been seen in the sections on which the present study was based and it is noteworthy that many authors have not figured any direct opening, although even they have not suggested that the cavity was closed. Very thin (3-4 μ) sections of several species (e.g. *Tineola, Plutella*) failed to demonstrate any direct passage, so that, if such does occur, it must either have a diameter less than 3 μ or follow an oblique path into the lumen. Under such circumstances, no direct passage would be seen. However, no passages could be identified in horizontal sections where channels as small as 0.5 μ in diameter should be visible. There is some functional evidence that, if any discharge of material from goblet cells occurs, this must take place through a membrane or, at most, through a very narrow passage, which appears to be readily blocked. This evidence is derived from the accumulation of the sulphides of many metals in the goblet cavities of *Tineola* larvae. These sulphides, together with ionized iron and copper compounds, are accumulated from metal-enriched diets and persist, after transfer of larvae to a normal diet, until the next moult, when the entire epithelium is cast off and regenerated (Waterhouse 1952*a*). Dyes and pH and oxidation-reduction indicators are also accumulated in the goblet cavities, although some of the more soluble pH indicators (e.g. phenol red) disappear before moulting if the larvae are transferred to a control diet (Waterhouse 1952*b*). Several authors have recorded what appears to be the production of secretions by the goblet cells (see Lotmar 1941), but it remains

to be shown that this is not the result of faulty histological technique. If there were a continuous discharge of material from the goblet cavity through a passage into the lumen it is probable that any accumulated metallic salts would be discharged also. On the other hand, sparingly soluble materials would be retained if secretion occurred through a cell membrane, although the soluble dyes would be discharged without difficulty. The massed, densely staining, granular material, which has been stated to occur in the tips of many goblet cells of larvae feeding on normal diets would similarly occlude, under natural conditions, any passage leading from the cavity into the gut lumen. It is relevant to recall that, in larvae of *H. meropc* feeding on grass, the goblet cell cavities often contain dark brown material, particularly in the middle region of the midgut (Waterhouse 1952*a*). This brown material is presumably accumulated from the food just as are dyes in *Tineola* larvae. Finally it is clear from the careful studies of Lotmar (1941) on the formation of the new midgut epithelium in *Tineola* at moulting that the goblet cavity is formed within the developing cell, so that it has clearly not arisen by invagination of the lumen border. This would explain also the different appearance and staining properties of the striated lining to the cavity and the striated border of the columnar cells. These have frequently been considered of similar origin, although the differences in properties have been clearly recognized.

No evidence was obtained of replacement of goblet or columnar cells between moults. However, cigar-shaped, black masses were occasionally seen between the epithelium and the peritrophic membrane of *Tineola* larvae feeding on a diet rich in nickel (Waterhouse 1952*a*). This may have been the discharge of the massed accumulations of nickel sulphide from a few of the goblet cavities or, alternatively, a casting off of a few entire goblet cells following disruption of function due to blockage with nickel sulphide deposits.

The only demonstrated function of goblet cells is in metal and dye storage and it appears that they play an important role in storage excretion (Waterhouse 1952*a*, 1952*b*). Because of the small surface they expose to the gut lumen it would not be anticipated that they would play an important part in absorption. However, they must be capable of absorption, unless, as appears more likely, the metals are first absorbed by the adjoining columnar cells and subsequently transferred laterally to the goblet cells for storage.

Goblet-like cells have been reported to occur in the Thysanura and Ephemeroptera and true goblet cells in Trichoptera (Shinoda 1927). This report remains unconfirmed and appears to have very doubtful validity. A careful examination of an extensive series of Bodian-stained sections of *Ctenolepisma longicaudata* and *Heterojapyx evansi* (Thysanura) and of more than one species of larval Ephemeroptera showed no sign of goblet-like cells in the midgut and this is in agreement with published observations on other species of these orders. Certainly, therefore, Shinoda's statement cannot be regarded as a true generalization for the first two orders. Furthermore, goblet cells could not be detected in the course of a careful examination of representatives of three families of Trichoptera (Glasgow 1936). Sections of the midgut of larvae

of an unidentified species of Trichoptera were examined after staining with Bodian, but no goblet cells could be found. Since the species examined by Shinoda (*Philopotamus* sp., Fam. Philopotamidae) belongs to the same superfamily as two of the families examined by Glasgow, Shinoda's report of the presence of goblet cells must be treated with great reserve until it is confirmed.

V. References

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Explanation of Plates 1-3

Plate 1

All sections stained with Bodian

Fig. 1.—Anterior midgut of *Tineola* larva, showing cigar-shaped goblet cells alternating with columnar cells.

Fig. 2.—Middle region of midgut of *Tineola* larva, showing flask-shaped goblet cells.

Fig. 3.—Cigar-shaped goblet cell of *Tineola* larva, showing striated lining to base of cavity.

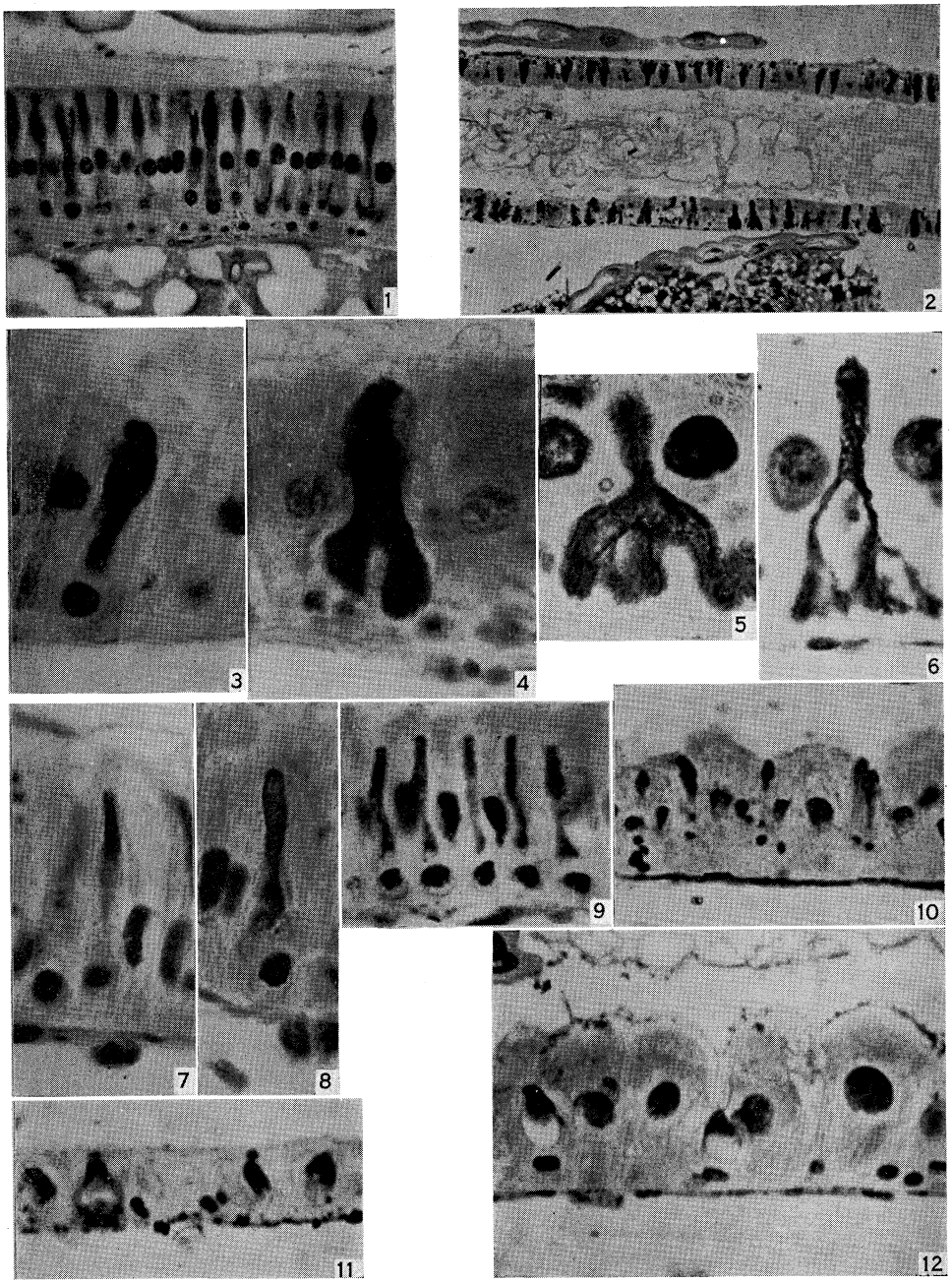
Fig. 4.—Flask-shaped goblet cell of *Tineola*, showing cavity extending around the basally situated nucleus.

Figs. 5 and 6.—Flask-shaped goblet cell of *Tineola* larva, showing lobes of cavity invaginated into cytoplasm.

Fig. 7.—Goblet cell of *Heteronympha* larva, showing striated lining to cavity.

Figs. 8 and 9.—Typical goblet cells of *Heteronympha* larva.

DIGESTION OF WOOL BY INSECTS. V



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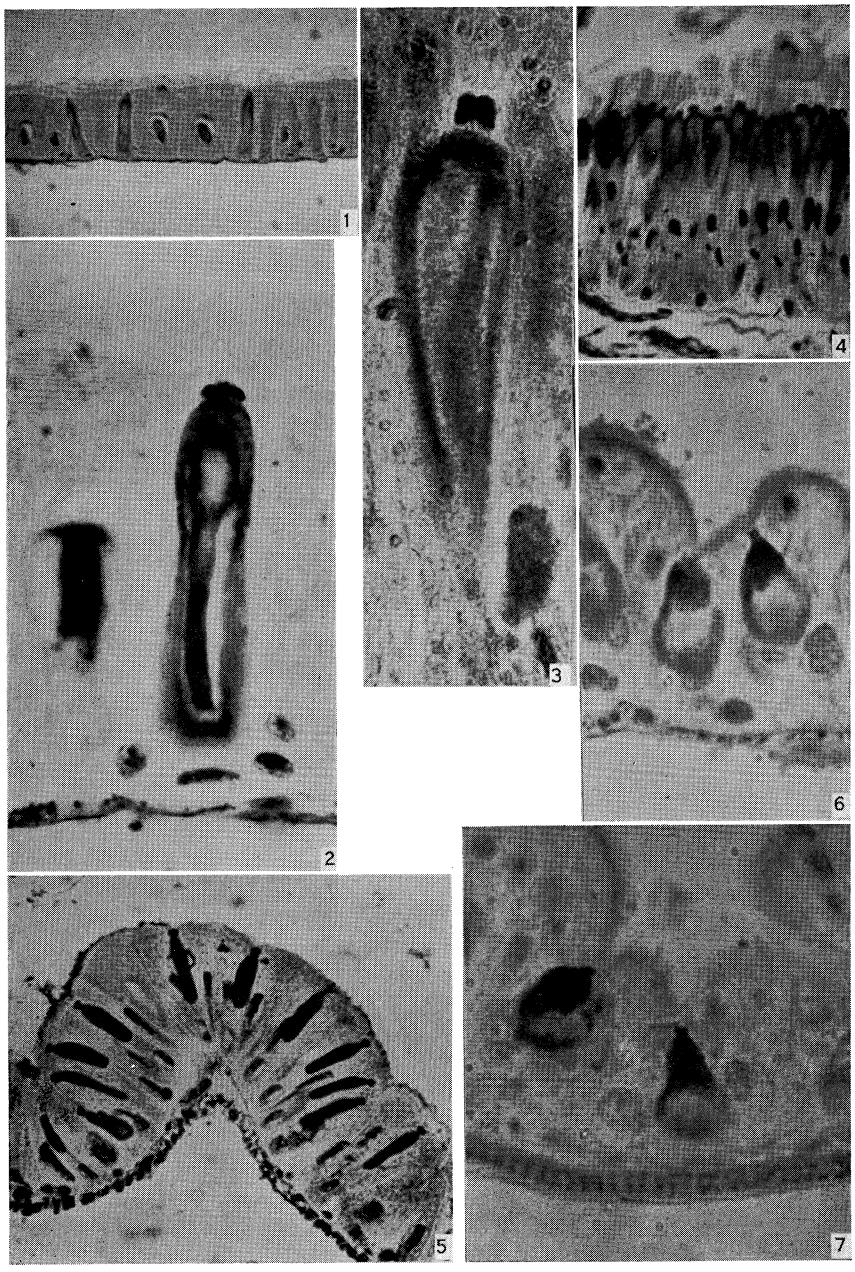

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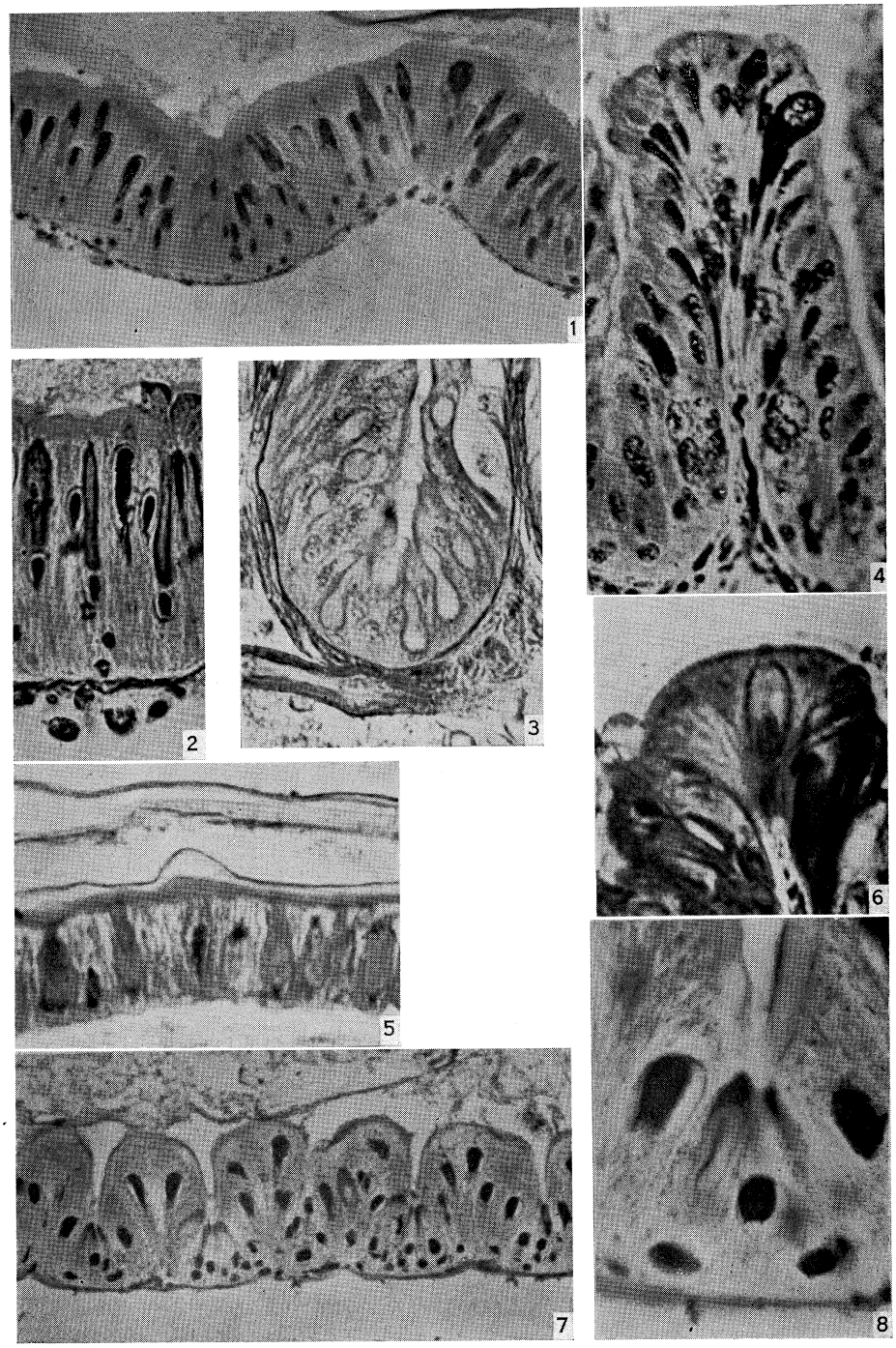

Fig. 10.—Goblet cells of *Plodia* larva.
Fig. 11.—Goblet cells of young *Bombyx* larva.
Fig. 12.—Goblet cells of *Plutella* larva, showing striated lining to cavity.

PLATE 2

All sections stained with Bodian

Fig. 1.—Goblet cells of young *Papilio aegeus* larva.
Fig. 2.—Goblet cell of young *P. aegeus* larva at higher magnification, showing densely staining cap.
Fig. 3.—Goblet cell of mature *P. aegeus* larva at high magnification, showing densely staining cap.
Fig. 4.—Goblet cells of mature *P. aegeus* larva.
Fig. 5.—Goblet cells of *Pyrameis itea* larva.
Figs. 6 and 7.—Goblet cells of young *Galleria* larva.

PLATE 3

Figs. 1 and 2.—Goblet cells of *Pieris* larva, Bodian.
Fig. 3.—Goblet cells of *Diacrysia* larva, Mallory.
Fig. 4.—Same, Bodian.
Fig. 5.—Goblet cells of *Phalaenoides* larva, Mallory.
Fig. 6.—Goblet cell of anthelid larva, Bodian.
Figs. 7 and 8.—Goblet cells of *Tortrix* larva, Bodian.