

METAMORPHOSIS OF THE MIDGUT OF A SIX-SPOTTED LADYBIRD BEETLE, *CHILOMENES SEXMACULATA* (COLEOPTERA: COCCINELLIDAE)

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Abstract—The larval regenerative cells have been found to reconstitute a transitory pupal epithelium at larval-pupal moult. A new epithelium is formed by the regenerative crypts or nidi in pupa at pupal-adult moult. The larval epithelium and transitory pupal epithelium are rejected *in toto*.

Index descriptors (in addition to those in title): Yellow body.

INTRODUCTION

ALTHOUGH the literature on the beetles is very extensive and much work has been done on the metamorphosis of the alimentary canal of various groups, controversies regarding the formation of adult midgut epithelium still persist. Deegener (1904) and Bushnell (1936) showed that the midgut epithelium regenerates from the regenerative cells of the larva. Poyarkoff (1910), Murray and Tiegs (1935) and Patay (1939, 1942), however, reported its formation from the posterior end of the foregut (anterior imaginal ring). Mansour (1927) similarly considered the metamorphosis of the midgut to be of two types. According to him, in *Ptinus* type the larval regenerative cells reconstitute the adult midgut epithelium but in *Calandra* type, the latter is derived from the posterior end of the foregut. In the present paper attempts have been made to re-examine the issue.

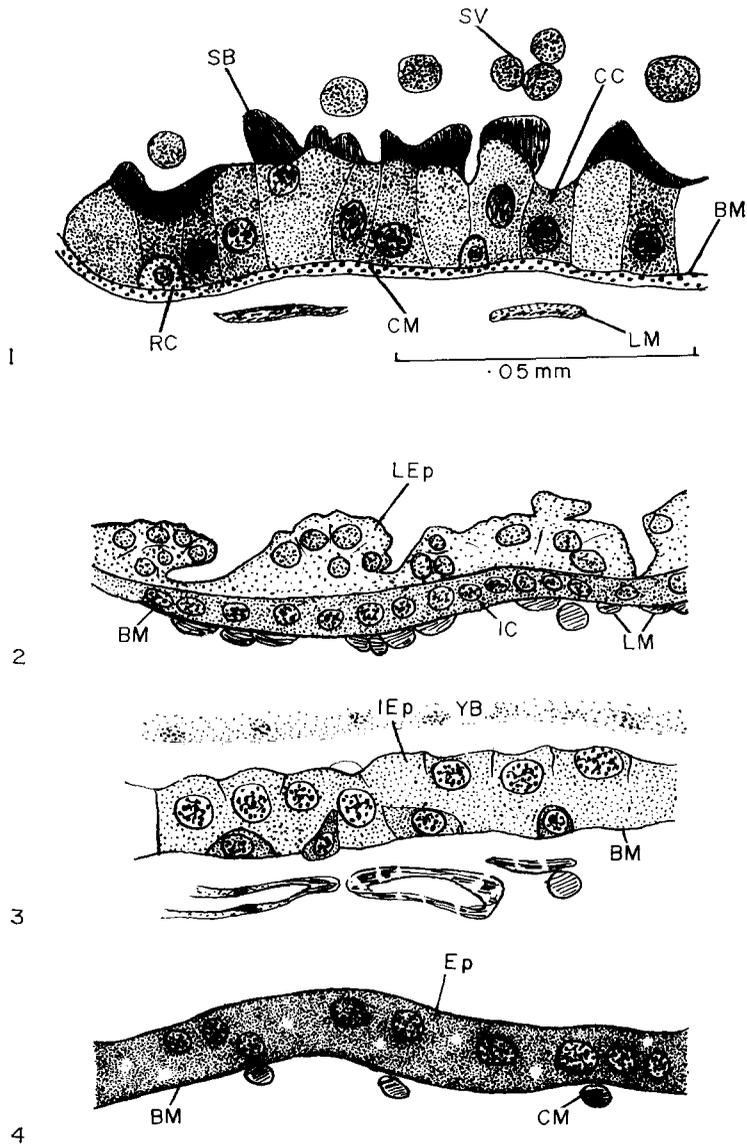
MATERIALS AND METHODS

The larvae of different ages were collected from *Madar* (*Calotropis* sp.) plants and reared in small rectangular jars. They were supplied daily with the yellow aphids, *Brevicoryne brassicae* L. upon which the larvae feed. The mature larvae were separated and kept in separate jars for pupation. Larvae and pupae were taken from the rearing jars at various times and dissected in normal saline. The gut was fixed in Bouin's solution for 6-8 hr and after usual dehydration and embedding, sections were cut at 6-8 μ . Heidenhain's iron haematoxylin, with eosin as counter stain, gave satisfactory results. Drawings have been made with the help of camera lucida.

OBSERVATIONS

Larval midgut

The larval midgut is covered externally by the usual circular and longitudinal muscles. A distinct basement membrane is present. The epithelium is composed of simple columnar cells, resting on the basement membrane (Fig. 1). The cytoplasm is granular and vacuolated.



FIGS. 1-4. 1. L.S. of larva. 2. C.S. of prepupa at early stage. 3. L.S. of prepupa at late stage. 4. L.S. of 3-5-hr old pupa. The scale for all figures is same as shown in Fig. 1.

Abbreviations used in figures

- | | |
|--------------------------------------|----------------------------|
| BM = basement membrane | LEp = larval epithelium |
| CC = columnar cell | LM = longitudinal muscle |
| CEp = columnar epithelium | Mu = muscle |
| CM = circular muscle | Ni = nidi |
| DPEp = degenerating pupal epithelium | PREC = pro-epithelial cell |
| Ep = epithelium | RC = regenerative cell |
| IC = imaginal cell | SB = striated border |
| IEp = imaginal epithelium | SV = secretion vesicle |
| YB = yellow body | |

The large nuclei possess one or two nucleoli. Each cell measures $27 \times 36 \mu$. Small, single interstitial or regenerative cells are present between the columnar cells. Each interstitial cell contains a small nucleus and granular cytoplasm. The epithelium is lined by a striated border. During the active feeding period, the cells exhibit secretory activities and give out secretory vesicles.

Metamorphic changes

The metamorphosis of the midgut begins towards the end of the last larval instar, about one day before the pupal moult. During the prepupal phase undigested food material is expelled from the midgut into the hindgut and the lumen gradually diminishes in size. The interstitial cells increase in size as well as in number and arrange themselves under the larval epithelium (Fig. 2). A few hours before the prepupa moults, the larval epithelium is sloughed off from the basement membrane and its cells show vacuolization and disintegration, so that very soon they get dissolved to form the 'yellow body'. This material fills up the empty midgut. With the disintegration of the larval midgut epithelium, the interstitial cells, which now behave as imaginal cells, increase in number and in due course of time spread over the entire basement membrane to form a new epithelium (Fig. 3). The imaginal cells are $9 \times 12 \mu$ in size, and show prominent nuclei. The 'yellow body' is still present in the lumen and contains some round, basophilic particles.

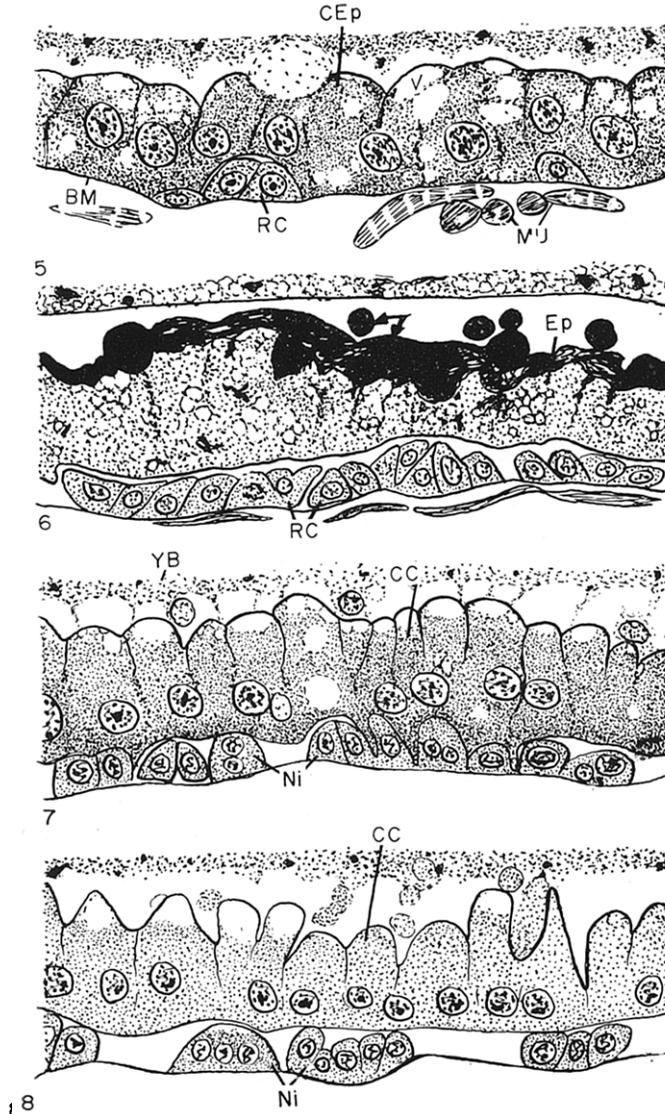
The prepupa moults into a crescent-shaped pupa in which the cells of the ventral side of the midgut appear to be larger, flat and stretched and more crowded than those on the dorsal side. The cell boundaries, although evident in the ventrally placed cells, are indistinct in the dorsal cells. Near the cardiac valve, the cells are extraordinarily large, columnar in shape and exhibit secretory activities. The epithelium near the valve appears to be folded into short villiform ridges.

In a 3-5-hr pupa, the midgut again becomes straight. A constriction develops as a result of folding, and divides the midgut into two chambers. The cell boundaries of the midgut epithelium are hardly visible and the epithelium appears syncytial (Fig. 4). The cytoplasm is granular and vacuolated. The nuclei are of variable sizes.

In a 9-hr pupa, the gut is still constricted and divided into two chambers. The cells have grown considerably and the cell boundaries make their appearance from now onwards. The cells are short, columnar to cuboidal in shape (Fig. 5). The cytoplasm is granular and vacuolated. The vacuoles are larger near tips of cells. The nuclei are prominent, basal or central in position. Owing to the secretory activities, the nuclei sometimes migrate towards the apical ends of the cells. A definite basement membrane is seen. In between the larger cells a few smaller cells are also visible at irregular intervals. These smaller cells are the regenerative cells.

The regenerative cells of the 9-hr stage again increase considerably in number so that in a 12-hr pupa, they are small, rectangular to cuboidal in shape (Fig. 6). The cytoplasm is thick and stains deeply. The nuclei are large and stain brightly. The large epithelial cells are columnar in shape and exhibit great secretory activities. A copious and darkly staining secretory material is seen at the tips of the cells and outside. The cytoplasm is thin, scanty and highly vacuolated. Nuclei are hardly visible.

In the 18-hr pupa, the columnar cells appear narrow and cylindrical in shape (Fig. 7). The basal cytoplasm is thick and granular, while in the rest of the cells it is highly vacuolated. The secretory activities continue. The regenerative cells, by now, arrange themselves into groups called nidi or crypts, which are present at regular intervals on the



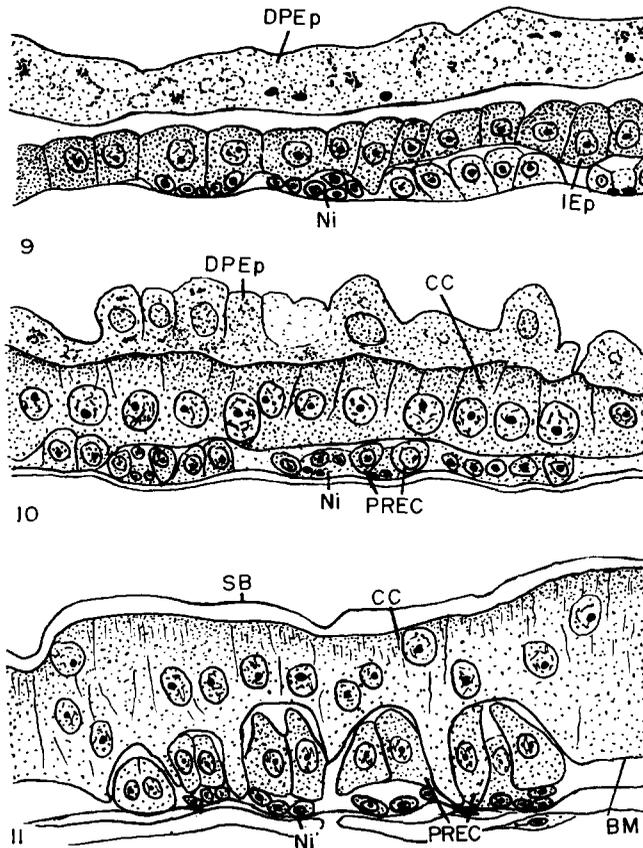
Figs. 5-8. 5. C.S. of 9-hr old pupa. 6. L.S. of 12-hr old pupa. 7. L.S. of 18-hr old pupa. 8. L.S. of 30-hr old pupa.

basement membrane. Each nidus contains two to three cells. The yellow body material of the lumen diminishes in volume, probably because of absorption by the new epithelial cells.

In the 30-hr pupa, the nidi (Fig. 8) are substantially bigger in size and push the columnar cells towards the lumen. The epithelium formed by these cells will be termed transitory pupal epithelium from now onwards. Some degeneration signs are visible in the columnar cells.

In the 50-hr old pupa, the nidi further increase in size and each possesses 4-5 cells. A new epithelium under the transitory pupal epithelium is formed by the regenerative cells of the

nidi. This new epithelium is carried to the adult and becomes functional. The transitory pupal epithelium is completely detached from the basement membrane (Fig. 9) and seems to be pushed towards the lumen. The cells of the transitory pupal epithelium show such signs of degeneration as extreme vacuolization and disappearance of nuclei and cell boundaries. Finally, in the 66-hr pupa, disintegration is completed and the transitory pupal epithelium practically disappears. However, a few cells are still retained and remain attached with the new columnar cells developing from below (Fig. 10). Side by side, some of the cells, designated as pro-epithelial cells, grow rapidly from each nidus, and assume columnar appearance in the newly hatched adult.



FIGS. 9-11. Sections of midgut. 9. L.S. of 50-hr old pupa. 10. L.S. of 66-hr old pupa. 11. L.S. of newly emerged adult.

In a newly emerged adult, the columnar cells are located on top of the pro-epithelial cells (Fig. 11). The cytoplasm in each pro-epithelial cell is granular and consists of a prominent nucleus. A striated border is also seen. The midgut lumen, by now, becomes practically empty, probably because of the absorption of the 'yellow body' by developing cells. However, in due course of time more globular material again makes its appearance as a result of the secretory activity.

DISCUSSION

In *C. sexmaculata* the larval regenerative cells replace the midgut epithelium at larval-pupal moult and a transitory pupal epithelium is formed. The larval cells are rejected *in toto*, and degenerate during the early pupal period to form 'yellow body'. Deegener (1904) also observed replacement of larval midgut epithelium by transitory pupal epithelium in the beetle, *Cybister*. The pupal epithelium in *C. sexmulata* is again replaced by the adult epithelium, which in turn is formed by the regenerative cells or nidi at pupal-adult moult. A similar process was observed by Bushnell (1935), Patay (1948) and Dobrovasky (1951). But in *Galerucella* (Poyarkoff, 1910), *Calandra orizae* L. (Mansour, 1927 and Murray and Tiegs, 1935), *Leptinotarsa* (Patay, 1939) and *Tenebrio molitor* L. (Patay, 1942) both the pupal and adult epithelia are formed by the proliferation of cells from the posterior end of the foregut. In the hymenopteran, *Nasonia*, Tiegs (1922) however, observed that larval regenerative cells divide horizontally into two halves; the anterior half degenerates at pupal-adult moult and the posterior or basal half builds the adult epithelium. A similar process of midgut metamorphosis has also been recorded in ants (Glöckner, 1958; Nitschmann, 1959; and Schmidt, 1964).

The 'yellow body' was originally observed by Dufour (1846) and later confirmed by Weismann (1864). All subsequent workers (Ganin, 1876; Kowalevsky, 1887; Van Rees, 1888; Perez, 1910; Bushnell, 1936) considered the formation of 'yellow body' from the debris of larval epithelium and secretion of the imaginal cells. The same has been found in *C. sexmaculata*. Mansour (1927), however, considered that the 'yellow body' in *Calandra* is formed exclusively by the secretion of the imaginal cells. The 'yellow body' has been considered to provide nourishment to the growing imaginal cells and this appears to be quite consistent in view of gradual decrease in its amount in *C. sexmaculata*. Gray (1931) termed 'yellow body' as nourishing tissue in *Homaledra*.

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