Origin and Structural Function of the Basal Cells of the Larval Midgut in the Mosquito, *Aedes aegypti* Linnaeus[1]

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Abstract: This study of a series of midgut whole mounts of larval and pupal *Aedes aegypti* shows that basal or regenerative cells first appear as a distinct cell type in the mosquito midgut at about the ninth hour of larval life. These cells seldom take part in forming the epithelial lining of the larval midgut. After their appearance, frequent mitotic divisions occur in the basal cells throughout the larval instars resulting in the presence of a large number of these cells in the prepupal midgut. During metamorphosis in the pupal stage, the basal cells remain to form the epithelial layer of the imaginal midgut.

Relatively little is known about the cytological development of the midgut in the mosquito, *Aedes aegypti* Linnaeus. Christophers' (1960) description of the *Aedes* digestive tract indicates that the midgut has received little attention from cytologists. Among the three types of cells comprising the larval midgut of *Aedes*, the regenerative or basal cells remained somewhat of a mystery as to their origin. Christophers stated that the origin of the basal cells is unknown. Berger (1938) reported finding regenerative cells in the larval midgut of the mosquito *Culex pipiens* but offered no explanation as to their origin. The fairly constant size, active divisions, and increasingly larger numbers of these cells during the larval stages indicate that they must perform some function other than to replace epithelial cells in the larval midgut. The question regarding the origin of the basal cells as well as the fact that such a large number of these cells is present in the later larval instars indicated the need for further cytological study of the midgut of *A. aegypti*. While the investigation is principally concerned with the larval midgut, pupal and adult midguts were also studied to determine the origin and structural function of the basal or regenerative cells.

MATERIALS AND METHODS

The *Aedes* larvae used in this study were obtained from the colony maintained in this laboratory (O'Brien, 1965). Beginning about 6 hours after hatching and at intervals of from 1 to 4 hours throughout the larval and pupal stages, the midguts were dissected from the specimens. They were then prepared as whole mounts, stained with the Feulgen reaction and counterstained with Orange G. The dissections, the fixation, and the staining procedure were performed on depression slides to eliminate loss or damage to the tissue (O'Brien, 1965).

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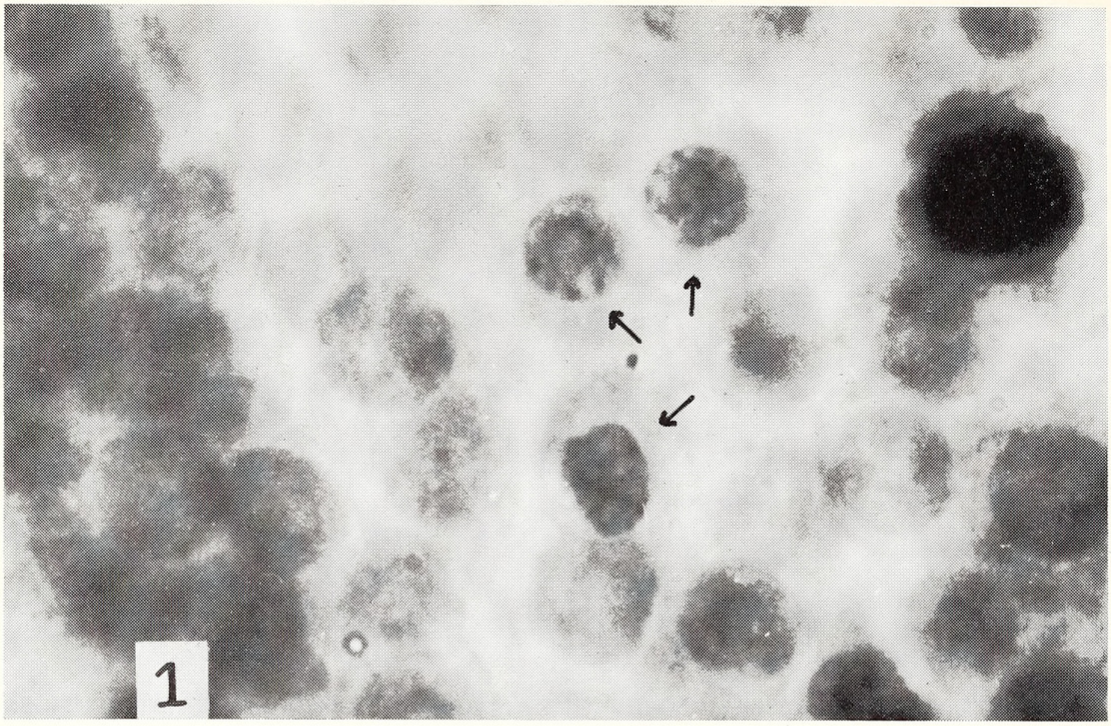

FIG. 1. Photomicrograph of portion of stomach area of 17-hour larva showing three potential regenerative cells (arrows). × 1,290.

RESULTS

In 6-hour larval midguts, only two cell types are present, the longitudinal and circular rows of muscle cells and larger cells forming the epithelial lining of the midgut. At about the twelfth hour of larval life, growth of the midgut has resulted in an increase in size of the epithelial cells, making the epithelial cells easily distinguishable from the smaller regenerative cells which have appeared by this time. Examination of whole mounts of midguts between 6 and 12 hours old shows that at about 9 hours, some of the original midgut epithelial cells are undergoing mitotic division. Such a division gives rise to two cells that are smaller than the neighboring cells. These smaller cells are regenerative cells. Up to this time, the midgut wall is only two cell layers thick, the outer cells being the rows of muscle cells and the inner layer the epithelial cells. The division of some of the initial epithelial cells results in the formation of the smaller cells that lie on the basement membrane, at the bases of the epithelial cells—hence the term "basal" cells.

Study of the cells of the midguts obtained from larvae between 6 and 9 hours old reveals the presence of large, uniformly sized epithelial cells resting on the basement membrane. A few of these cells exhibit nuclei that appear to be in early prophase of mitotic division, while the neighboring cells contain normal "resting" nuclei. Since all the cells are of about the same size, the cells appearing to be in early prophase must be the potential basal cells (Fig. 1). The origin of the basal cells from epithelial cells was confirmed when mitosis was observed

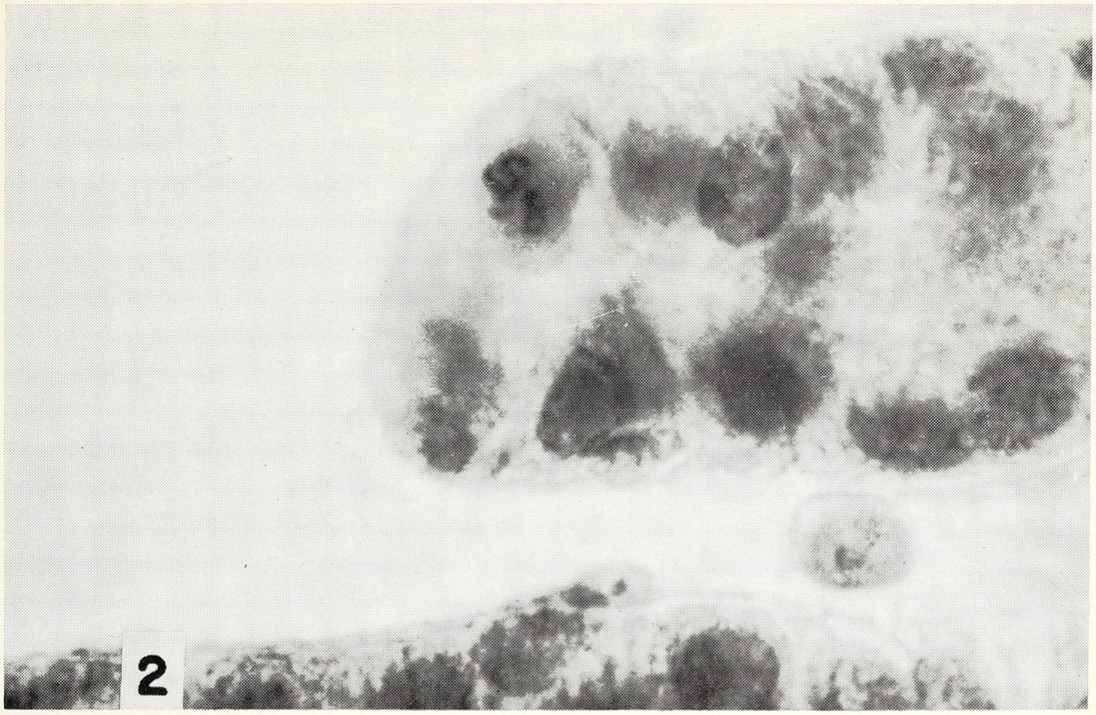

FIG. 2. Photomicrograph of portion of a pouch of gastric ceca of 24-hour larva showing one of the primordial epithelial cells in mitotic prophase. × 1,290.

in epithelial cells of the gastric ceca (Fig. 2) where regenerative cells appear at a later stage and in fewer numbers than in the stomach area of the midgut.

All divisions of the basal cells are normal mitotic divisions, exhibiting the somatic pairing of homologous chromosomes characteristic of dipteran cells (Figs. 2, 3, 4).

After the basal cells appear in the midgut, their number increases rapidly by repeated divisions. These cells lie at the bases of the large primordial epithelial cells which continue to grow larger during the larval instars and never divide after about 24 hours of larval life. By the fourth instar, the regenerative cells form almost a complete layer of cells, intermediate in size between the large primordial epithelial cells and the smaller muscle cells, against the basement membrane. The number of basal cells found in the gastric ceca is considerably smaller than in the stomach area of the midgut.

Examination of the pupal midgut shows that the regenerative cells form the new epithelial lining of the imaginal midgut. During the larval instars, few of the basal cells help to form the epithelial layer of the midgut. But, after the onset of pupation, the primordial epithelial cells quickly separate from the basement membrane, are sloughed off into the lumen of the midgut, and begin to disintegrate. The basal cells that have been increasing in number throughout larval life continue their divisions and soon form the epithelial lining of the imaginal midgut. Since the adult midgut contains no structure similar to the pouches of the larval gastric ceca, the basal cells that formed in the region of

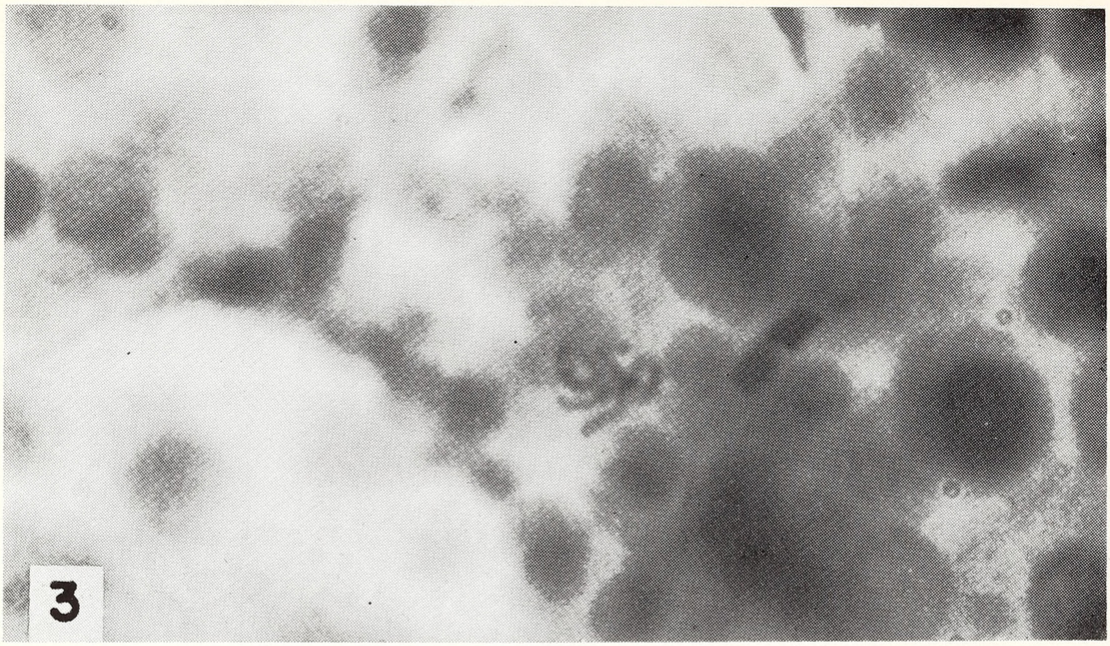

FIG. 3. Photomicrograph of portion of gastric ceca of 17-hour larva showing a large epithelial cell in mitotic prophase. × 1,290.

the gastric ceca during the larval instars combine with the cells of the cardiac region and those of the anterior portion of the stomach area to form the epithelium of the anterior region of the adult midgut.

DISCUSSION

The results of this study indicate the need for revising some statements based upon earlier findings. Berger (1938) reported that the cells comprising the epithelial lining of the larval mosquito midgut (the "primordial" epithelial cells) never undergo mitotic division but rather only increase in size during larval life. The basal cells were thought to function primarily as replacement cells for the worn-out epithelial cells in the larval midgut. But the findings here presented show that the early first-instar midgut contains only muscle cells and primordial epithelial cells and that very few of the basal cells function as replacement cells in the larval instars. Therefore, it seems that some of the primordial epithelial cells in the young larva become potential basal or regenerative cells soon after hatching. Once larval feeding and growth begin, these potential basal cells cease functioning as epithelial cells, undergo mitotic division, and become basal cells. In the process of this transformation, their places in the epithelial layer are taken by the nearby primordial epithelial cells which do not divide, but rather enlarge to fill the space left in the epithelial lining. Thus, some of the primordial epithelial cells do undergo division, but only during the early hours of larval life. The factor determining the time of transformation from primordial epithelial cells to potential basal cells is not known.

Since so few of the basal cells in the larval midgut function as replacement

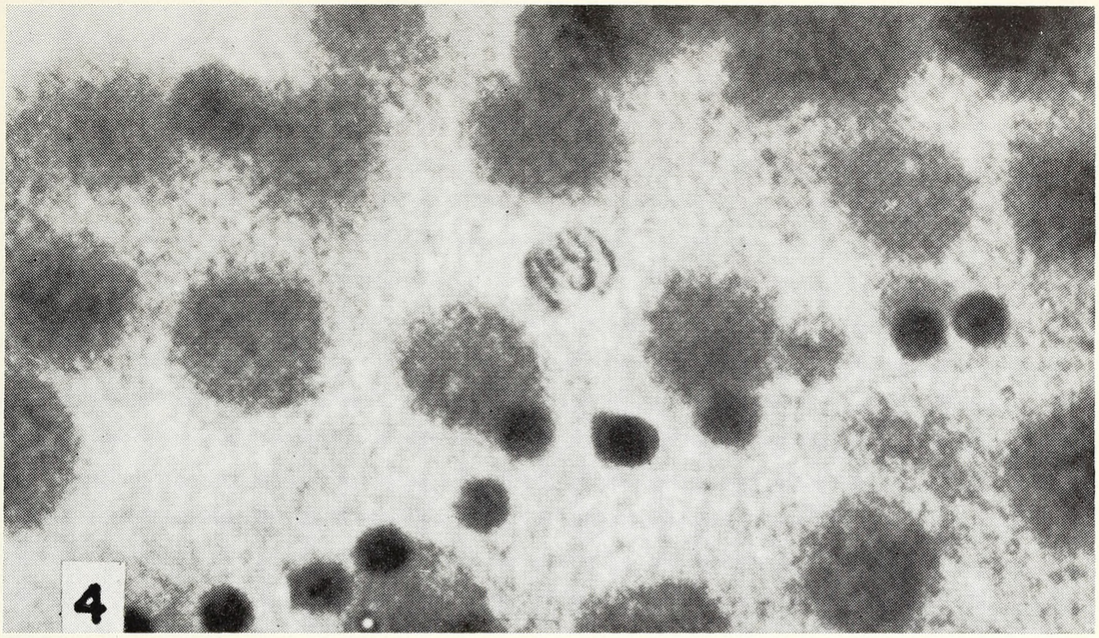

Fig. 4. Photomicrograph of portion of stomach area of 24-hour larva showing a basal cell in mitotic prophase. × 1,290.

cells in the epithelial coat, the main role of these cells must be to form the epithelial lining of the imaginal midgut. Therefore, the formation of the adult midgut does not take place principally in the pupa. Both the muscular coat for the midgut, basically that present in the prepupa (O'Brien, 1965), and the epithelial lining of the imaginal midgut, derived from the basal cells of the larval midgut, have been steadily developing throughout the larval stages.

SUMMARY

Regenerative or basal cells in the larval midgut of *A. aegypti* first appear about 9 hours after hatching.

The basal cells generally take no part in forming the epithelial lining of the larval midgut.

After their appearance in the early larval midgut, the basal cells undergo frequent mitotic divisions, resulting in the presence of a large number of basal cells in the prepupa.

Early in the pupal stage, the primordial epithelial cells of the larval midgut are sloughed off into the midgut lumen and the basal cells remain to form the epithelial lining of the imaginal midgut.

Literature Cited

Berger, C. A. 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. Pub. 496. Carnegie Institution of Washington.

Christophers, S. R. 1960. *Aedes aegypti* (L.). Cambridge Univ. Press.

O'Brien, J. F. 1965. Development of the muscular network of the midgut in the larval stages of the mosquito *Aedes aegypti* Linnaeus. Jour. N. Y. Ent. Soc. **73**(4): 226–231.