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The Ultrastructure of Midgut Epithelium in *Aedes aegypti* (L.) (Insecta, Diptera) Males

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Introduction

The ultrastructure of the midgut epithelium in *Aedes aegypti* females has been studied in the context of blood uptake and digestion as well as of transmission of pathogens by several authors (BERTRAM & BIRD 1961, STAEUBLI et al. 1966). In a previous paper (HECKER et al. 1971) the present authors have shown that, upon emergence of the female, the midgut was not yet functional, and they described the final differentiation of the epithelium which takes place during the first few days of adult life.

The question arose as to whether the delayed midgut formation was to be considered a sign of prolonged metamorphosis, independent on the mode of feeding, extending over emergence or, rather, a consequence of adaptation to haematophagy. The question is of interest for basic parasitology and we, therefore, examined the conditions in the non-haematophagous males of *Aedes aegypti.*

Material and Methods

The mosquito stock used in the present study, its breeding, maintenance, and processing for electron-microscopy was described elsewhere (HECKER et al. 1971). Males were collected at the following intervals after emergence: 30–60 min., 6, 12, 18, 24 hours, 2, 3, 4, 10, 25 and 40 days. Males older than two days were allowed to feed on sugar water ad libitum.

Results

Midgut epithelium

In all stages examined the midgut epithelium consists of a single layer of cells (figs. 1 and 2), nearly uniform, with the exception of some differences in contrast. In addition, in the basal portion sometimes undifferentiated small, dark regenerative cells are visible (fig. 2). Rarely cells are seen which contain dark granules (figs. 13 and 14). These are separated from the limiting membrane by a light space; they possibly are secretory vesicles. Structurally, the epithelial cells seem to be differentiated from the very beginning. First, their shape is rather flat or cubical, though; it gradually becomes cylindrical (figs. 1–4). At all times, among the cylindrical cells, groups of comparatively flat cells can be observed. From the beginning, too, the cells are fitted firmly together (fig. 1). The membranes of adjacent cells are separated by a more or less constant intercellular space, in places interrupted by vesicular-shaped stretches of loose contact (fig. 7). Septate desmosomes are always present in the apical and middle part of the cells and become more distinct with age (figs. 5–7). Desmosomes of the macula adhaerens type (FAWCETT 1966) are never encountered.

In newly emerged mosquitoes meconium is present in the gut lumen. It persists up to the second day.

Microvilli

The microvilli are always evenly distributed on the apical side of the cells (figs. 1–4). Their density is comparatively low at first; it increases strongly within two to three days, most likely due to the cells' altered shape. Their fibrillar contents extend to the apical zone of the cell body and give this region a filamentous aspect (figs. 8 and 9). The glycocalyx is not clearly limited everywhere; it seems to undergo close contact with the meconium, or possibly other gut contents, in some places.

The diameter of the microvilli measures approximately 0.1 to 0.18 μ. Their length increases from 1.1–3 μ in younger stages (30–60 min.), to 4 μ in older stages (10 days).

Exocytosis and pinocytosis are inconspicuous.

Basal labyrinth

In all stages the basal labyrinth is well developed (figs. 1–3 and 14). After emergence already, the infolds of the basal cell membrane reach approximately up to the middle part of the cells; the extra-cellular clefts are rather wide. Later on, the features of the basal labyrinth are varying, the extracellular clefts being sometimes irregular and wide, sometimes narrow with the limiting membranes running more or less parallel.

Basal lamina

In the stages examined the structure of the basal lamina shows hardly any variations (figs. 1, 3 and 12). It is amorphous, fine floccular

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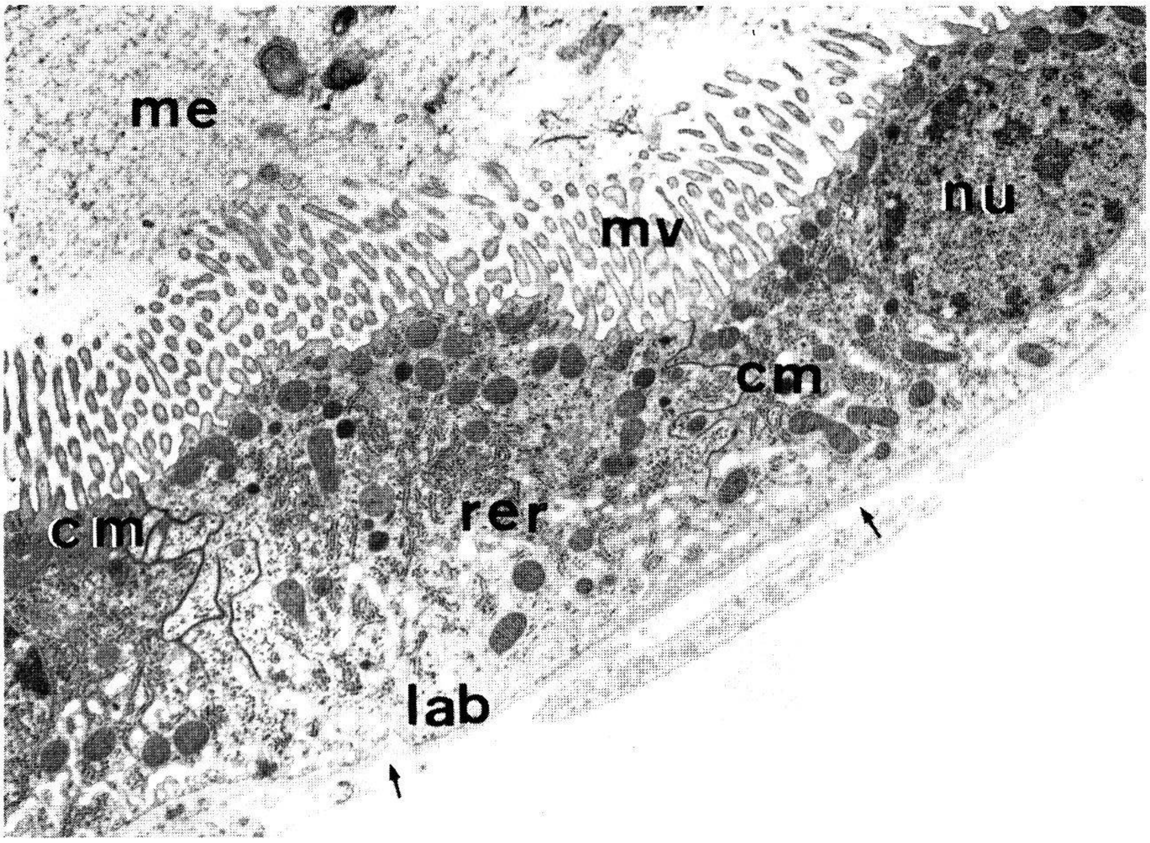
Fig. 1. Aedes aegypti male, newly emerged: flat epithelial cells firmly fitted together; meconium (me); low density of microvilli (mv), evenly distributed; basal labyrinth (lab), basal lamina (→), cell membranes (cm), rough surfaced endoplasmic reticulum (rer) and nucleus (nu) with heterochromatin. 7,000 ×.

Fig. 2. 18 hours: epithelial cells cubical to columnar; note different electrodensity of the cells. Undifferentiated regenerative cell at the base of the epithelium (re); higher density of microvilli (mv); mitochondria (mi) somewhat concentrated in the apical part of the cells; nucleus (nu) with relatively compact nucleolus (nl); muscle cell (mc) and tracheole (tr). 7,000 ×.



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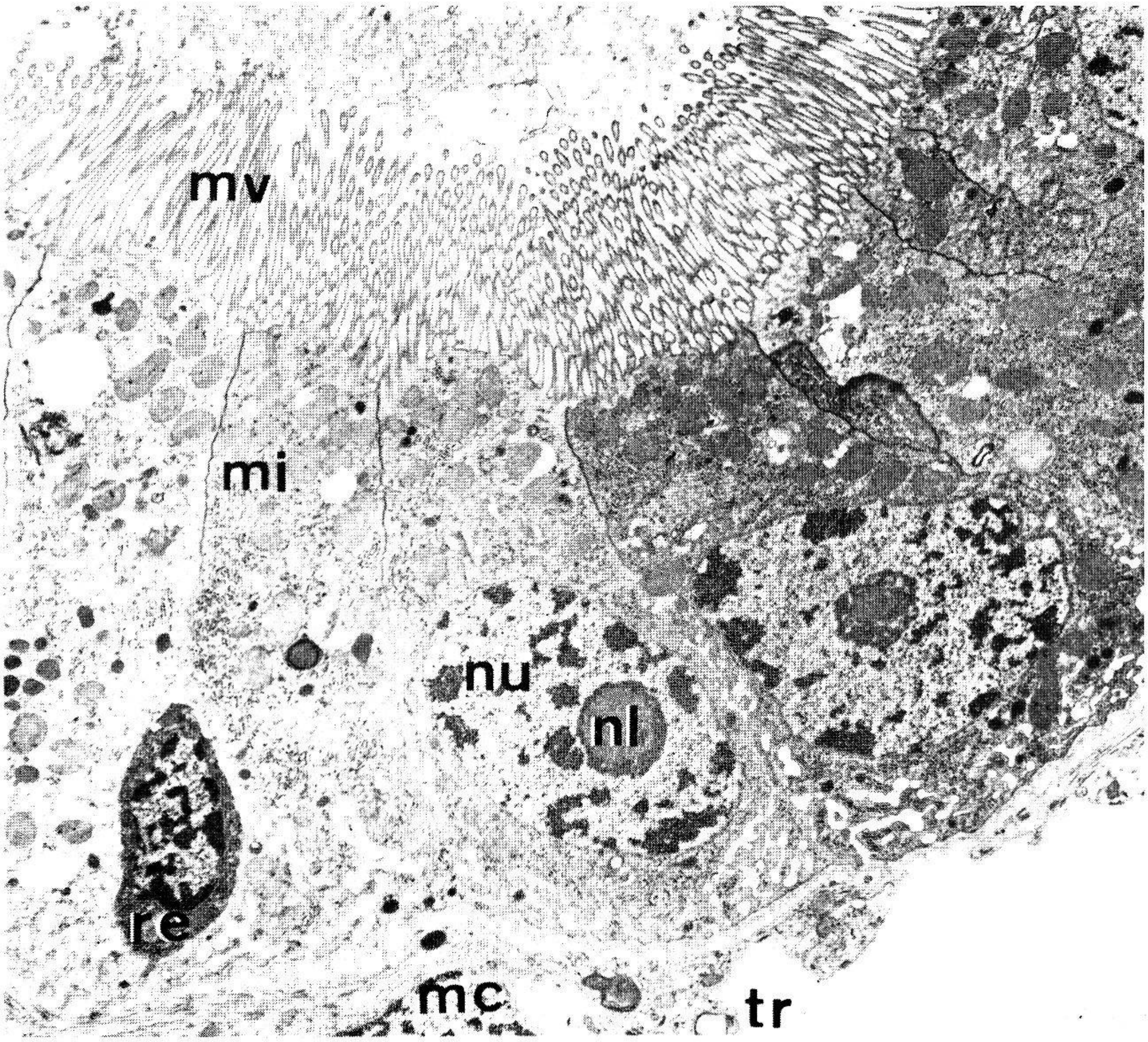

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and lacks the typical "striation" described in females (BERTRAM & BIRD 1961, TERRAKIS 1967, HECKER et al. 1971). In preparations of a single midgut few electron-dense rods (BERTRAM & BIRD 1961) arranged in a short chain-like fashion could be seen. These chains, however, showed no dinstinct orientation (fig. 12, onset).

Nucleus

The nucleus varies only little in size. Its mean diameter measures 4 to 6.5 μ at the beginning and 6 to 7 μ on the fourth day. Upon emergence the nucleus occupies practically the whole cell's height (figs. 1 and 3). In flat cells it often causes a protuberance on their apical side. When the cells become higher the nucleus lies in a central or in a somewhat basal position (figs. 2 and 4). In all stages electron-dense heterochromatin is present. The nucleolus is always quite compact, fibrillar to fine-granular in its center and more coarsely granular at the periphery (figs. 2 and 3).

Ribosomes and endoplasmic reticulum

The rather floccular appearance of the cytoplasm becomes more granular as the number of free ribosomes increases somewhat with age. The rough-surfaced endoplasmic reticulum (rer) is present from emergence (fig. 1). It undergoes only little variations in all stages observed. It consists of single or grouped cisternae (fig. 10), which may be arranged in parallel fashion. In the apical and basal part of the cells rer profiles are less abundant. "Whorls" or fingerprint-like structures, which are typical for the midgut cells of female *Aedes aegypti* (BERTRAM & BIRD 1961, STAEUBLI et al. 1966, HECKER et al. 1971) are absent.

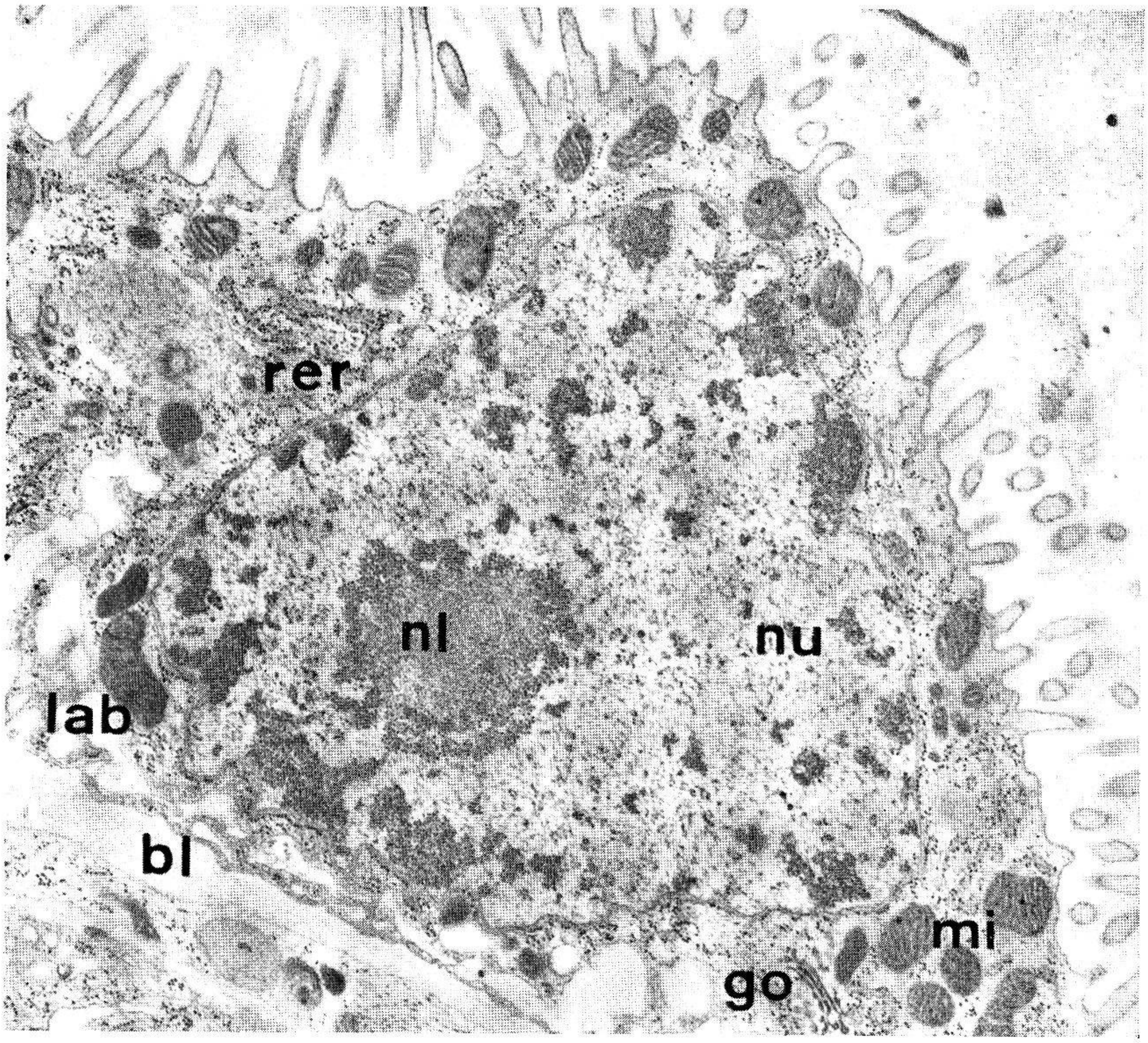
The smooth endoplasmic reticulum consists of but a few vacuoles and remains inconspicuous.

Golgi zones

Near rer often several Golgi zones are found (fig. 10). They are made of some parallel cisternae and numerous small, electron-dense vesicles (figs. 3, 10 and 11). They can be seen soon after emergence, but seem to become more numerous later on.

Fig. 3. Newly emerged: nucleus (nu) causing protuberance of the apical cell side; nucleolus (nl) fine fibrillar to fine granular in the centre, more coarsely granular at the periphery; rer profiles (rer), Golgi zone (go), mitochondria (mi), basal labyrinth (lab) and basal lamina (bl). 15,000 ×.

Fig. 4 25 days: large lysosome-like organelles, probably residual bodies (rb), located between nuclei (nu) and microvilli (mv). Note apical concentrations of mitochondria (mi). 7,000 ×.



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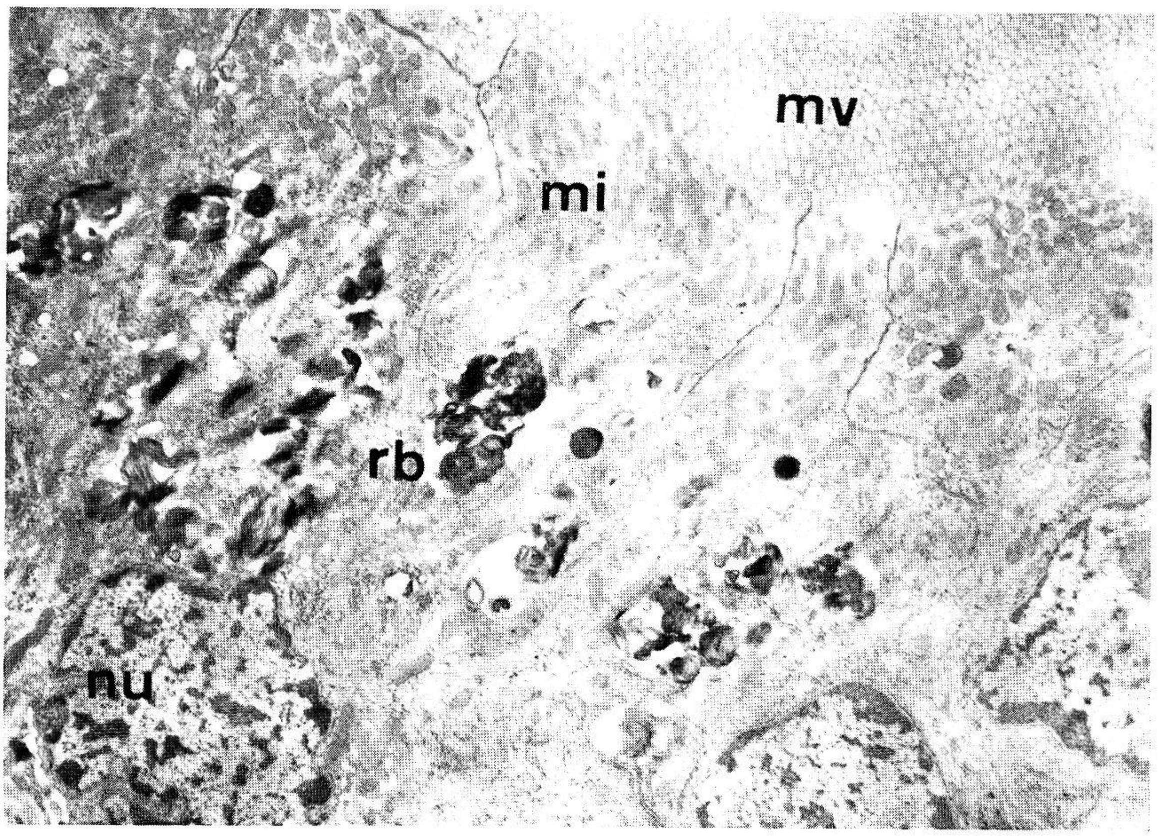

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Multivesiculate bodies and lysosomes

The multivesiculate bodies are present in all stages. In young males (30–60 min. after emergence) some relatively large dense bodies can be seen. Later (12–24 hours) dense bodies become more numerous and variable in size. Beside these, also lamellar bodies are now found (figs. 10 and 11). At the age of from 10–40 days complex large structures with heterogeneous contents arise (fig. 4); they possibly are residual bodies. Most of these lysosome-like structures are located in the vicinity of Golgi areas, between the nucleus and the cell apex. Their number increases with the mosquito's age.

Mitochondria

Mitochondria can be observed from the beginning (figs. 2–4). They are characterized by parallel cristae and a matrix of intermediate electron-density. They contain distinct mitochondrial granules. The mitochondria concentration seems to be higher in the apical part of the cell (figs. 2 and 4).

Microtubules

Microtubules are found at all stages. They are scattered throughout the cytoplasm, without evident orientation (figs. 5 and 11).

Regenerative cells

The basal cells (O'BRIEN 1966) in the midgut of *Aedes aegypti* males contain little, but electron-dense cytoplasm with poorly developed organelles and a heterochromatic nucleus (fig. 2).

Only once, two days after emergence, regenerative cells growing upwards between epithelial cells could be observed (figs. 14 and 15). Their cytoplasm, in addition to the nucleus, shows free ribosomes, pieces of rer, distinct Golgi zones, some dense-bodies and mitochondria. Microtubules are clearly visible, especially in narrow parts of the cells (fig. 14, inset). One cell exhibits dark granules as mentioned above, separated by a light space from the limiting membrane (figs. 13 and

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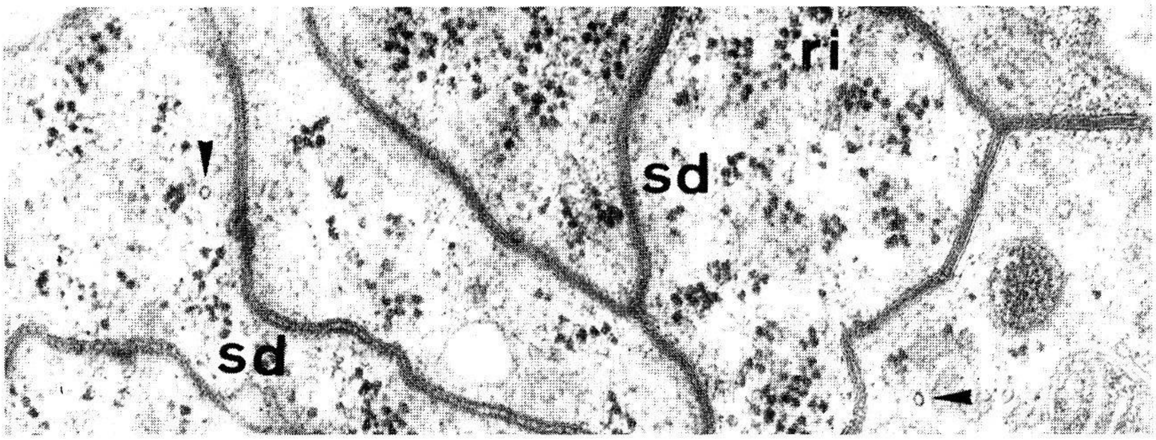
Fig. 5. Newly emerged: first septate desmosomes (sd); free ribosomes (ri) and cross-sections of microtubules (←). 4,400 ×.
Fig. 6. 4 days: distinct septate desmosome (sd); nuclear membranes (nm). 110,000 ×.
Fig. 7. 40 days: vesicular-shaped stretches of loose contact (lc) and septate desmosome (sd). 44,000 ×.
Fig. 8. 4 days: fibrillar contents of microvilli (mv) extending into apical cytoplasm (—▶). 44,000 ×.
Fig. 9. Newly emerged: obliquely sectioned microvilli (mv); glycocalyx in close contact with gut contents (▶). 44,000 ×.



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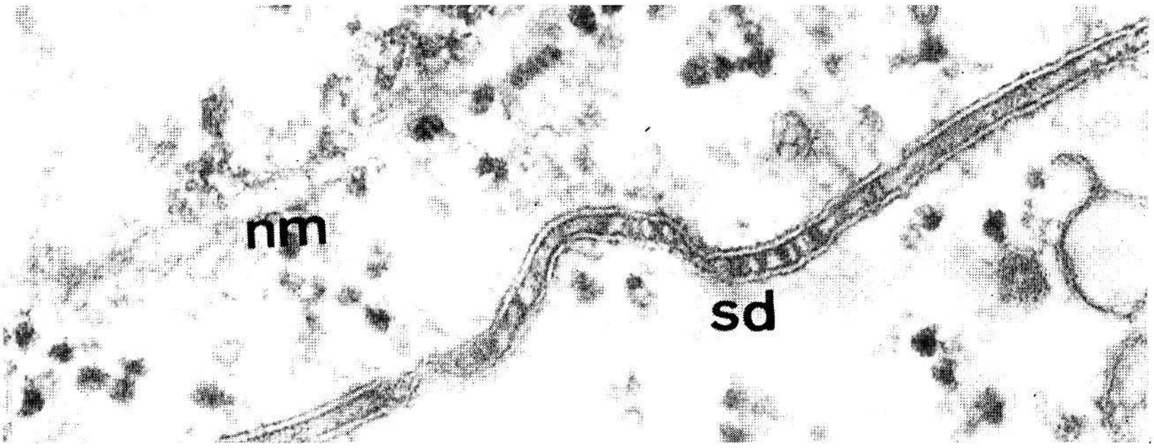

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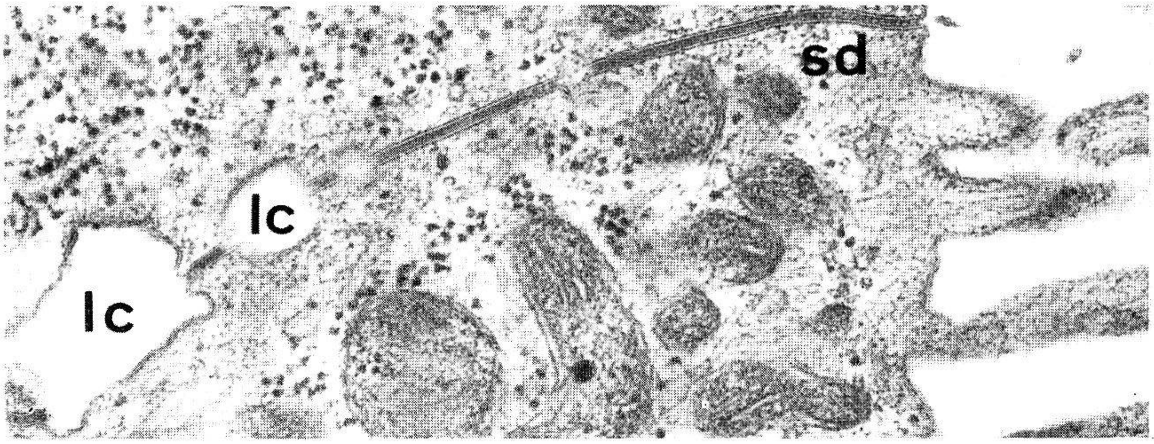

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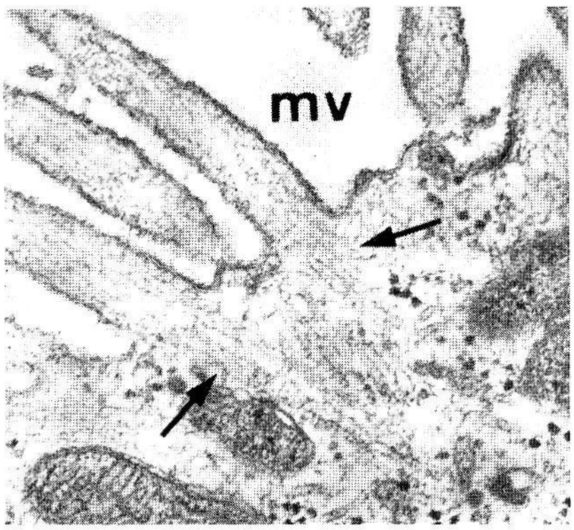

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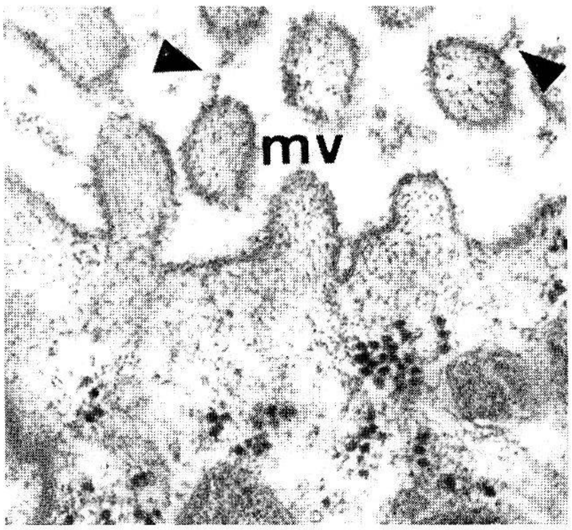

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14). Another cell shows the formation of the basal labyrinth and of microvilli. The formation of the latter sets in before the cell reaches the gut's luminal surface (figs. 14 and 15). The microvilli are then closely packed like the petals of a bud. They already possess their fibrillar content and the glycocalyx.

Further observations

Muscle, tracheal and nervous cells in conjunction with the male midgut (figs. 2, 12 and 14) are similar to those observed in female *Aedes aegypti* (HECKER et al. 1971).

Discussion

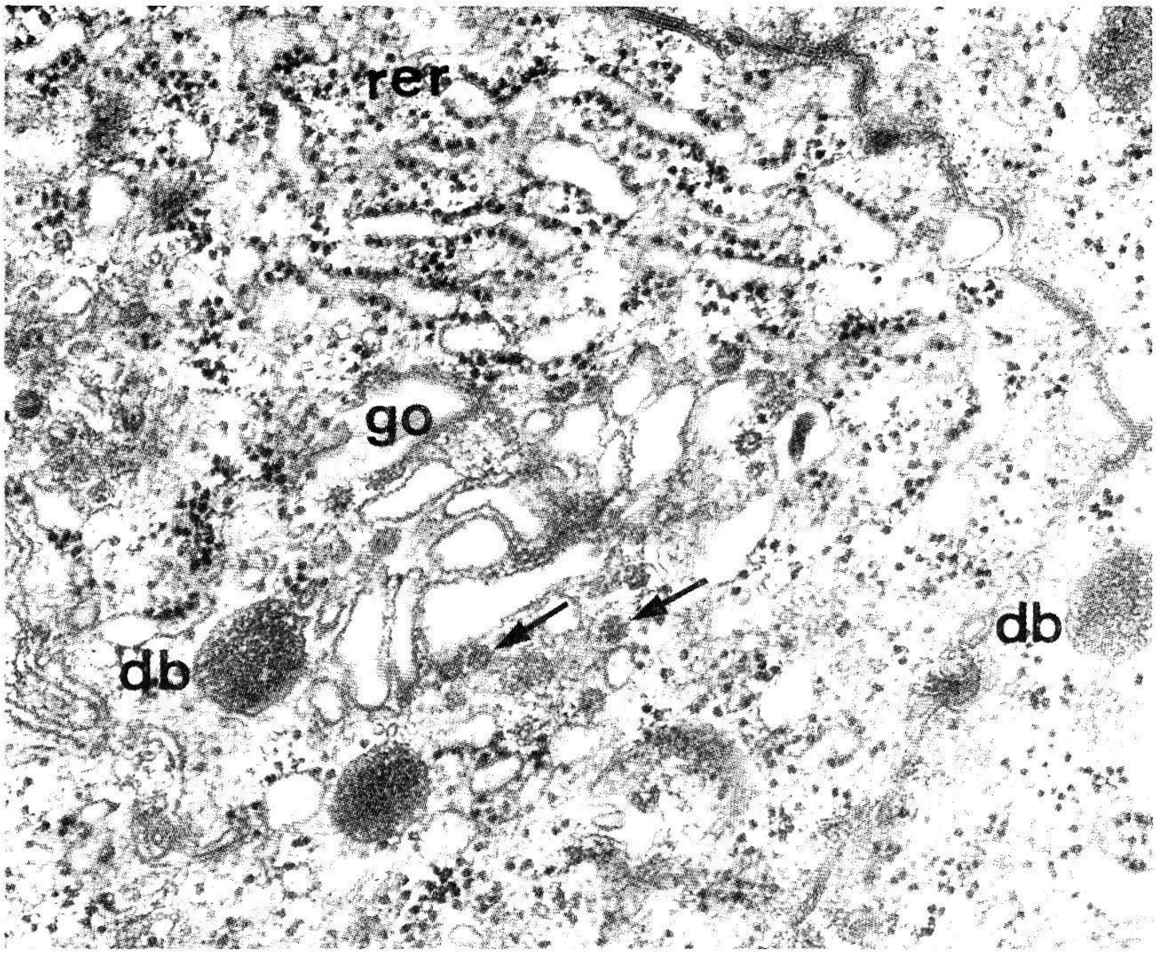
The epithelial ultrastructure in both sexes

The results presented show that, with *Aedes aegypti*, there are distinct differences in the fine structural organization of the midgut epithelium of the male as compared to the one of the female (HECKER et al. 1971). Some of the more notable differences are:

1. In the male, upon emergence, the epithelial cells seem to be differentiated with respect to the cell organelles. Especially the mitochondria are fully differentiated. Furthermore, the nucleus always shows heterochromatin and its nucleolus is quite compact.
2. At the same time the cells are already firmly fitted together.
3. Desmosomes of the macula adhaerens type (FAWCETT 1966) are completely absent.
4. The basal lamina lacks the typical "striation" described in females (BERTRAM & BIRD 1961; TERRAKIS 1967; HECKER et al. 1971). The rare presence of short electron-dense rods (BERTRAM & BIRD 1961), arranged in an unorientated chain-like fashion, leads to the suggestion that a predisposition for striated structures might be present.
5. The rough endoplasmic reticulum (rer) is quite abundant, but fingerprint-like structures or "whorls" (BERTRAM & BIRD 1961, STAEUBLI et al. 1966, HECKER et al. 1971) are never found.
6. The epithelial cells of the male, after emergence, do not show "apical extensions" as observed in females; the microvilli are evenly distributed on the luminal surface.

Fig. 10. 3 days: close connection between *rer*, Golgi zones (go) and lysosome-like structures (dense bodies = db); numerous small electrondense Golgi vesicles (→) perhaps contributing to db formation. 44,000 ×.

Fig. 11. 3 days: Golgi zone (go), Golgi vesicles (→), multivesiculate body (mvb), dense bodies (db) and lamellar bodies (lb) close together; microtubules (➛) 44,000 ×.



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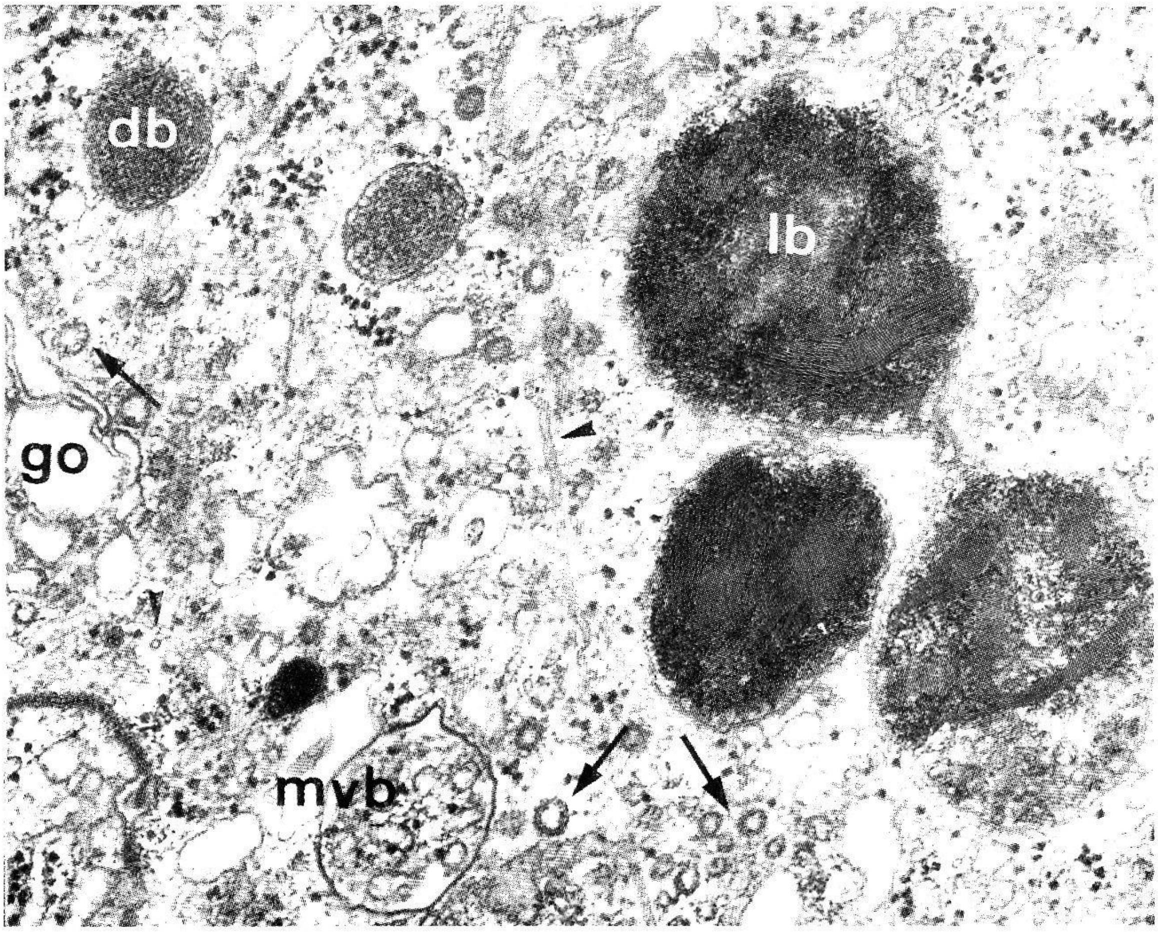

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7. The number of free ribosomes does not seem to be as high as in females, a fact which might be correlated with a smaller rate of structural protein synthesis.

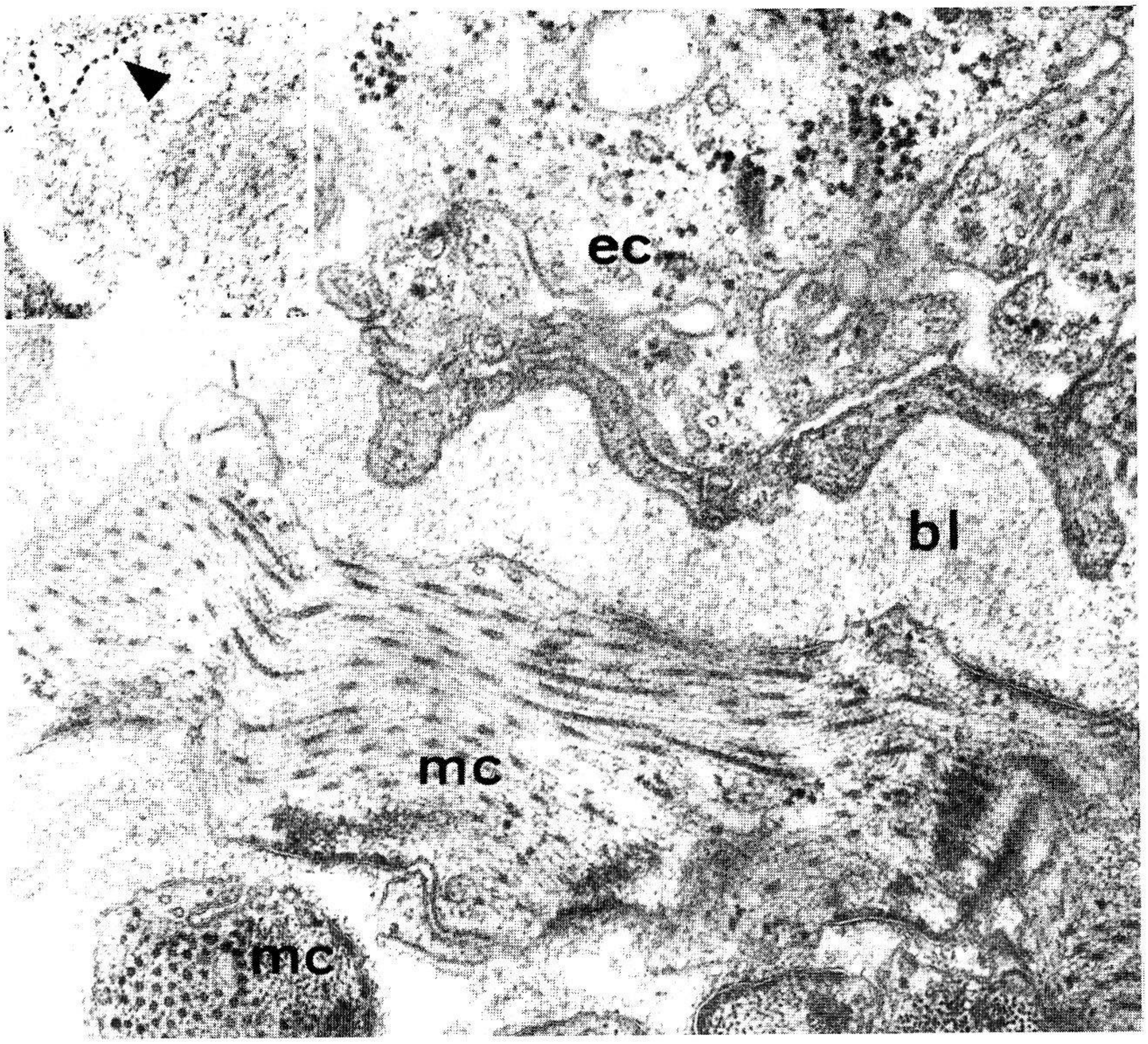
As to the association of nucleus, rer, Golgi zones and lysosomes, previously described for females, a similar situation is found in males. The presence of comparatively well developed rer, numerous small electron-dense Golgi vesicles and the increasing number of lysosome-like structures could support the view that the rer and the Golgi zones do contribute to the formation of lysosomes (DE DUVE 1963, NOVIKOFF & SHIN 1964, NOVIKOFF 1967). Residual body complexes arise similarly as in females. Their extrusion, as postulated for other cell types in mammals (HELMINEN & ERICSSON 1970), into the gut lumen could not affirmatively be observed. Their number increases with age; this supports the idea that deterioration processes do take place in males, too. With respect to cell organelles, it must be admitted, though, that 24 days old females exhibit a structurally more deteriorated state of the midgut epithelium than do males of 40 days. This result, however, may be purely accidental.

The otherwise uniform cells of the epithelium often differ in contrast. A similar observation was made by DOHRMANN (1970) with epithelial cells of the choroid plexus in mammals. The author explains this fact as being caused by differences in the status of hydration and did not consider it as being a preparation artefact. Two other phenomena, however, the vesicular-shaped stretches of loose contact between adjacent cell membranes and the variations in the extracellular clefts of the basal labyrinth, might be partly dependent on preparation influences such as inadequate dissection, fixation or embedding procedures.

The occurrence of regenerative cells has been discussed in female *Aedes aegypti* (HECKER et al. 1971). The situation seems to be similar in males. Once only, in one out of fifty midguts examined, a group of outgrowing "basal cells" (O'BRIEN 1966) was seen. The scarcity of such observations leads to the suggestion that regenerative cells do not play an important role in the adult midgut. The stimuli for the outgrowth of these cells are unknown.

The functions of the "dark granules", too, are unknown. Due to the strong resemblance to granules in alpha-cells of the bat pancreas (FAWCETT 1966), we tend to correlate them with secretory processes.

Fig. 12. 40 days: basal lamina (bl) amorphous and fine floccular, without visible "striation". Inset (1 day): a few "electron-dense rods" arranged in a short chain-like fashion (▶). Epithelial cell (ec) and muscle cells (mc). 44,000 ×.
Fig. 13. 2 days: cell containing dark granules (gr), separated by a light space from the limiting membrane, resembling alpha-granules; nucleus (nu). 27,000 ×.



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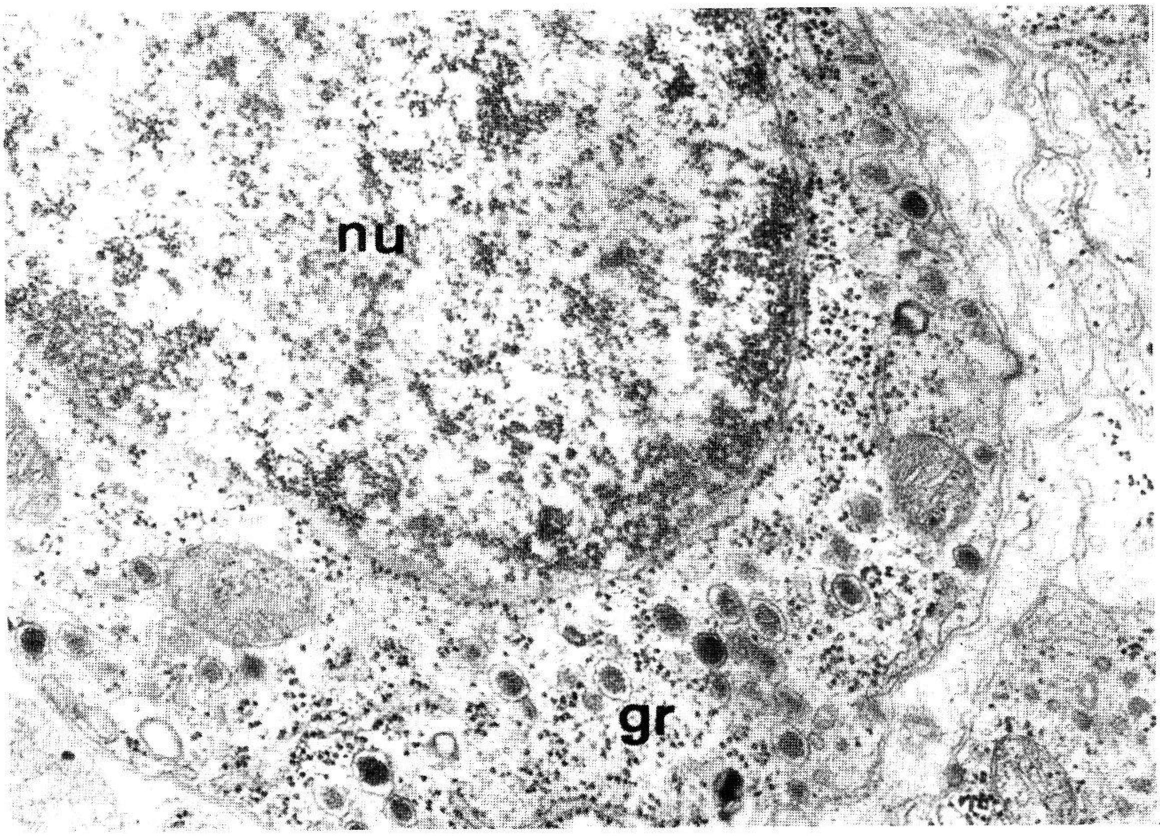

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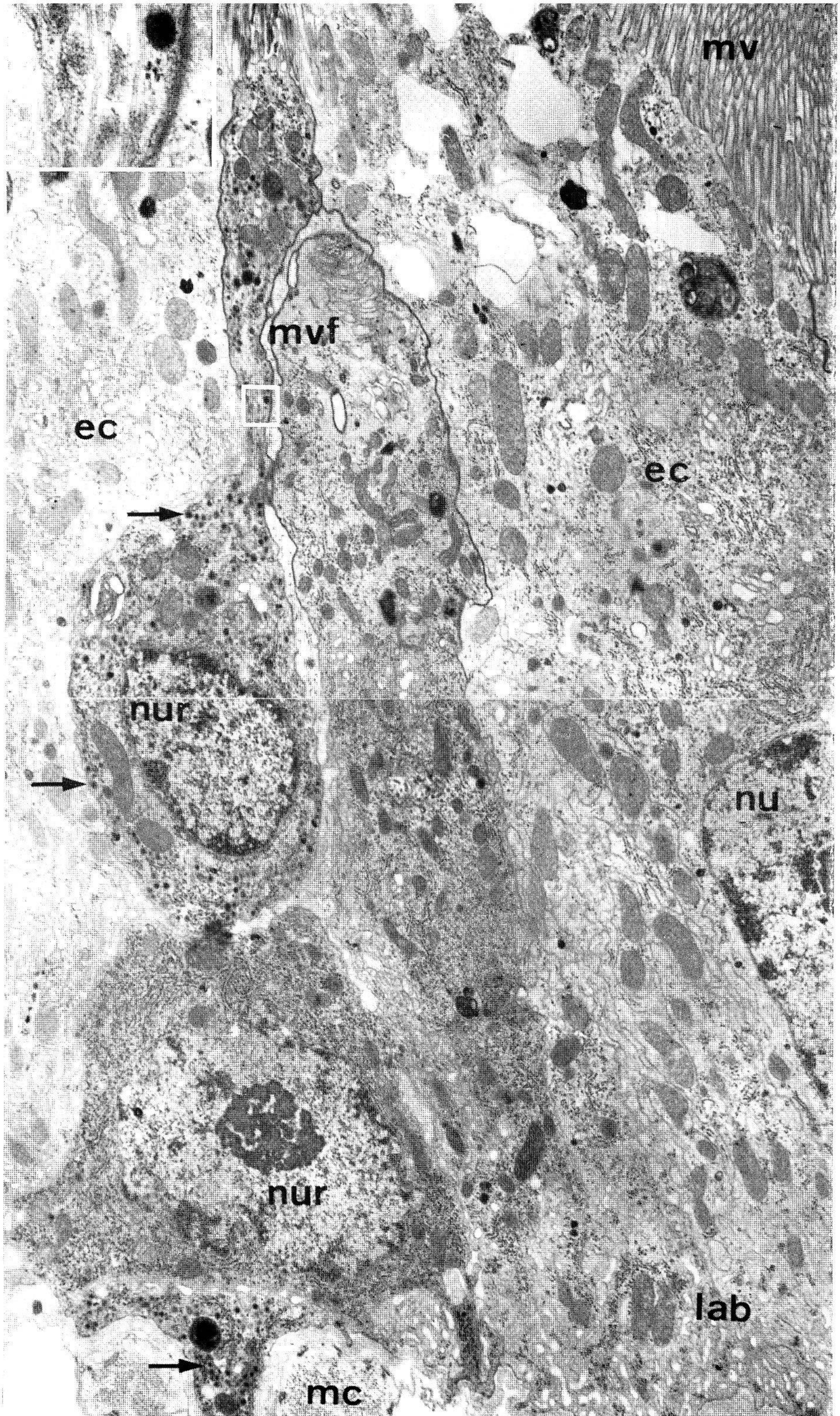

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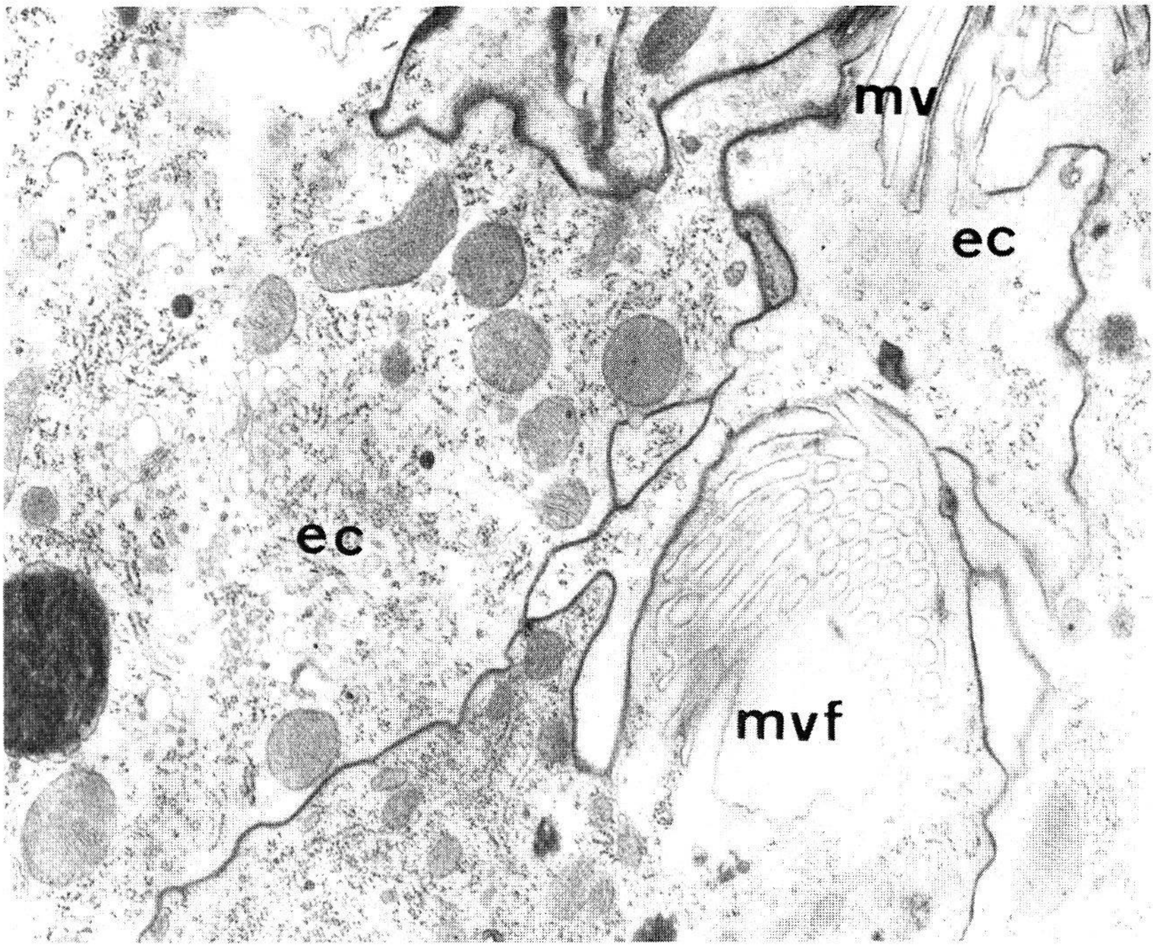

Fig. 15. 2 days: detail of microvilli formation (mvf) showing the bud-like arrangement of regenerative cell microvilli. Epithelial cells (ec) and microvilli (mv). 15,000 x.

Sexual dimorphism

From what has been said so far there follows that the main differences with respect to ultrastructure and formation of the midgut in males and females are:

1. The male epithelium seems to be less consolidated than the female epithelium.
2. Male epithelial cells lack the fingerprint-like "whorls" of rer which are typical of the female cells prior to blood meals.
3. At emergence time, differentiation of the male stomach is much farther ahead than differentiation of the female stomach.

In our previous paper on the differentiation of the female midgut epithelium (HECKER et al. 1971) we stated that it seemed obvious that

Fig. 14. 2 days (oblique section): regenerative cells growing upwards between epithelial cells (ec). Nuclei of regenerative cells (nur); dark granules (→); formation of microvilli (mvf). Microvilli (mv) of epithelial cells (ec), nucleus (nu) and basal labyrinth (lab). Muscle cells (mc). 8,500 x. Inset: microtubules in narrow part of outgrowing cell (□). 44,000 x.

at times of blood intake, the female stomach had to resist a substantial pressure from inside. We thought that the consolidation of the epithelium needed was brought about, in part, by the formation of numerous septate desmosomes and of maculae adhaerentes. In males, there are septate desmosomes, but no maculae adhaerentes. As to the basal lamina in females, we thought the "striations" to be an indication of solidification. No such "striations" were observed in males. The conclusion, therefore, seems to be reasonable that maculae adhaerentes as well as the "striations" of the basal lamina, both contribute to the consolidation of the midgut and that they represent additional structures, characteristic of the female sex in conjunction with its specific mode of feeding. A similar conclusion can be reached with respect to the higher degree of complexity of the rer in female epithelial cells: more elaborate features are necessary in the context of blood digestion.

Already upon emergence, in the males, the epithelial cells are firmly fitted together; they show microvilli and a basal labyrinth; and they contain most organelles, practically in their final differentiation. The only conspicuous change which the majority of cells is still to undergo is their shape. It is conceivable that their initial flat shape is due to extension by air, which is needed in the midgut for the act of emergence (CLEMENTS 1963). The subsequent change from flat or cubical to cylindrical shape, therefore, could be considered rather an adaptation to the new pressure situation, once emergence is completed, than a further differentiation process. If this assumption is correct, the formation of the midgut is completed with metamorphosis in the male sex, while it is not in the female sex.

The delayed midgut formation, the presence of maculae adhaerentes and "striations" of the basal lamina, as well as the higher complexity of the rer in females may be considered to represent a consequence of their special mode of feeding. Haematophagous Nematocera, with their different feeding behaviour in both sexes, represent an excellent model for the study of adaptation to a particular type of ectoparasitic life.

Acknowledgements

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Zusammenfassung

Im Vergleich zu den Verhältnissen bei *Aedes aegypti*-Weibchen wird die Ultrastruktur dem Mitteldarm-Epithels beim Männchen untersucht. Ein deutlicher Sexual-Dimorphismus wird aufgezeigt. Die wichtigsten Unterschiede bestehen darin:

1. daß beim Männchen die Bildung des Mitteldarmes mit der Metamorphose weitgehend abgeschlossen ist, während die endgültige Differenzierung beim Weibchen noch zwei bis vier Tage nach dem Schlüpfen beansprucht,

2. daß beim Männchen zweierlei submikroskopische Strukturen – die maculae adhaerentes und die Querstreifung der Basal-Lamina – fehlen, welche beim Weibchen wohl zur Verfestigung des Darmes beitragen, und

3. daß das rauhe endoplasmatische Retikulum in den Darmepithel-Zellen des Männchens keineswegs den komplexen Ausbildungszustand erreicht, wie er für die Weibchen vor Blutmahlzeiten typisch ist.

In der Diskussion wird die Hypothese aufgestellt, die ultrastrukturellen Unterschiede und die imaginale Differenzierung des Darmes beim Weibchen seien als Erscheinungen bzw. als Folgen der Anpassung an die haematophage ektoparasitische Lebensweise zu bewerten.

Résumé

L'épithélium de l'intestin moyen d'*Aedes aegypti* mâle a été étudié à l'aide du microscope électronique et comparé à l'épithélium de la femelle. On a observé un net dimorphisme sexuel. Les différences principales sont les suivantes:

1) Chez le mâle, la formation de l'intestin moyen est achevée à la métamorphose. Chez la femelle, la différentiation définitive ne se termine que deux à quatre jours après l'éclosion.

2) La femelle montre deux particularités dans la structure fine de son épithélium : les maculae adhaerentes et la striation de la Lamina basiliamis. Ces structures servent à renforcer l'intestin. Elles sont absentes chez le mâle.

3) L'ergastoplasme de l'épithélium intestinal du mâle n'atteind jamais un dévelopment aussi complexe que celui de la femelle avant la prise du repas sanguin.

Au cours de la discussion, les auteurs proposent, comme hypothèse que les différences enregistrées, de même que la différentiation explicative, retardée de l'intestin de la femelle, sont l'expression et les suites d'une adaptation à la vie ectoparasitaire et haematophage.