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Histological Investigations on the Intestine of *Palingenia longicauda* Oliv. (Ephemeroptera)

By

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With 12 Figures

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The structure and function of the digestive tract of Invertebrates and especially Insects have already been studied by several authors. These investigations have been carried out on intestine of larvae in different developmental states and adult animals respectively (HUBER and HAASSER 1950, ANDERSON and HARVEY 1966, SMITH 1968, BERRIDGE 1970, HECKER et al. 1971a, 1971b, DE PRIESTER 1971, 1972).

In spite of the fact that there are some references as to the structure of Ephemeroptera intestine (SMITH 1968), in case of *Palingenia longicauda* no such investigation exists. Only the younger larvae of this species have significant intake of food; in the last larval stages and in the course of metamorphosis this intake decreases and the imago itself does not take nourishment at all, its intestine gradually degenerates. For studying this degeneration process it is necessary to study the fine structure of the digestive tract in younger larval stages.

Our observations concerning the structure of the digestive tract in later larval stages, in larvae right before metamorphosis and in the imago will be published later.

Material and Methods

In the present study the intestine of 1 and 2 years old larvae were used. The intestines were fixed for light- and electron microscopic investigations. The sections of 5 to 7 μm thickness were stained with haematein-eosin, Best-carmin, van Gieson, iron-haematoxilin, gallocyanin, PAS, methylgreen-pyronin, wherease for the demonstration of the acid phosphatase the method of Gömöri was employed (KISZELY 1958).

For electron microscopic investigations the freshly dissected intestines were minced in 1 to 2% OsO_4 in veronal-acetate buffer (*p*H 7,2 to 7,4) and after the fixation period washed with the same buffer. Dehydration took place in cold graded ethanol series and embedding in Araldite via propylene oxide. The material was stained with 3% uranyl acetate in 70% ethanol in blocks and with Reynolds's (1963) lead citrate in section. The electron microscopic pictures were made in Tesla BS 242 D and JEM 100 B electron microscopes.

Results and Discussion

The digestive tract of *Palingenia longicauda* larvae similarly to that of other Insects, is a straight tube which is composed of three regions: foregut midgut and hindgut (ANDERSON and HARVEY 1966, SMITH 1968, BERRIDGE 1970).

The foregut, which broadens out backwards begins at the chewing mouth parts and terminates at the borderline between thorax and abdomen. The midgut passes over to hindgut in the second abdominal segment having thicker wall than the former two regions. According to the observations only the foregut and hindgut have villiform structures on the inner surface of the intestine covered by cuticle.

The three parts of the digestive tube have trilaminal histological structure: inner epithelium, intermediate connective tissue layer and the outer visceral musculature. Only in the thickness of the layer differences can be observed.

Foregut

The slightly protuberant villi of the foregut consist of a simple columnar epithelium and a very thin connective tissue layer (Fig. 1). The epithelial cells are high and have rather large, roundish nuclei. The basis of the cells is indented in consequence of the extensive infoldings of the basal plasma membrane and only in the apical region remains a thin unindented cytoplasm (Fig. 2). The extensions of cells show interwoven, tousled structure, being arranged only to a little extent perpendicularly to the surface of the epithelium. This indented basal part of the cells is rich in mitochondria and also the cisternae of the endoplasmic reticulum can sometimes be observed. The microtubuli which are characteristic of these cells can be followed from the basal extensions to the microvilli.

Fig. 1. *Palingenia longicauda*: foregut. On the inner surface of the intestine slightly protuberant villi (v) can be observed. At the basis of the epithelial cells connective tissue layer (ct) is seen (arrow), arranged in a sheet. m: muscle, tr: trachea

Fig. 2. Electron microscopic picture of the foregut epithelial cells. Microvilli (mv) protrude into lumen (Lu). At the infolded basal part of the cell there is a greater number of mitochondria (M). v: vacuole, Go: Golgi-apparatus, mt: microtubuli, GER: granulated endoplasmic reticulum

Fig. 3. More microvilli (mv) originate on common basis. Many vacuoles (v) are situated near to the apical surface of the cell

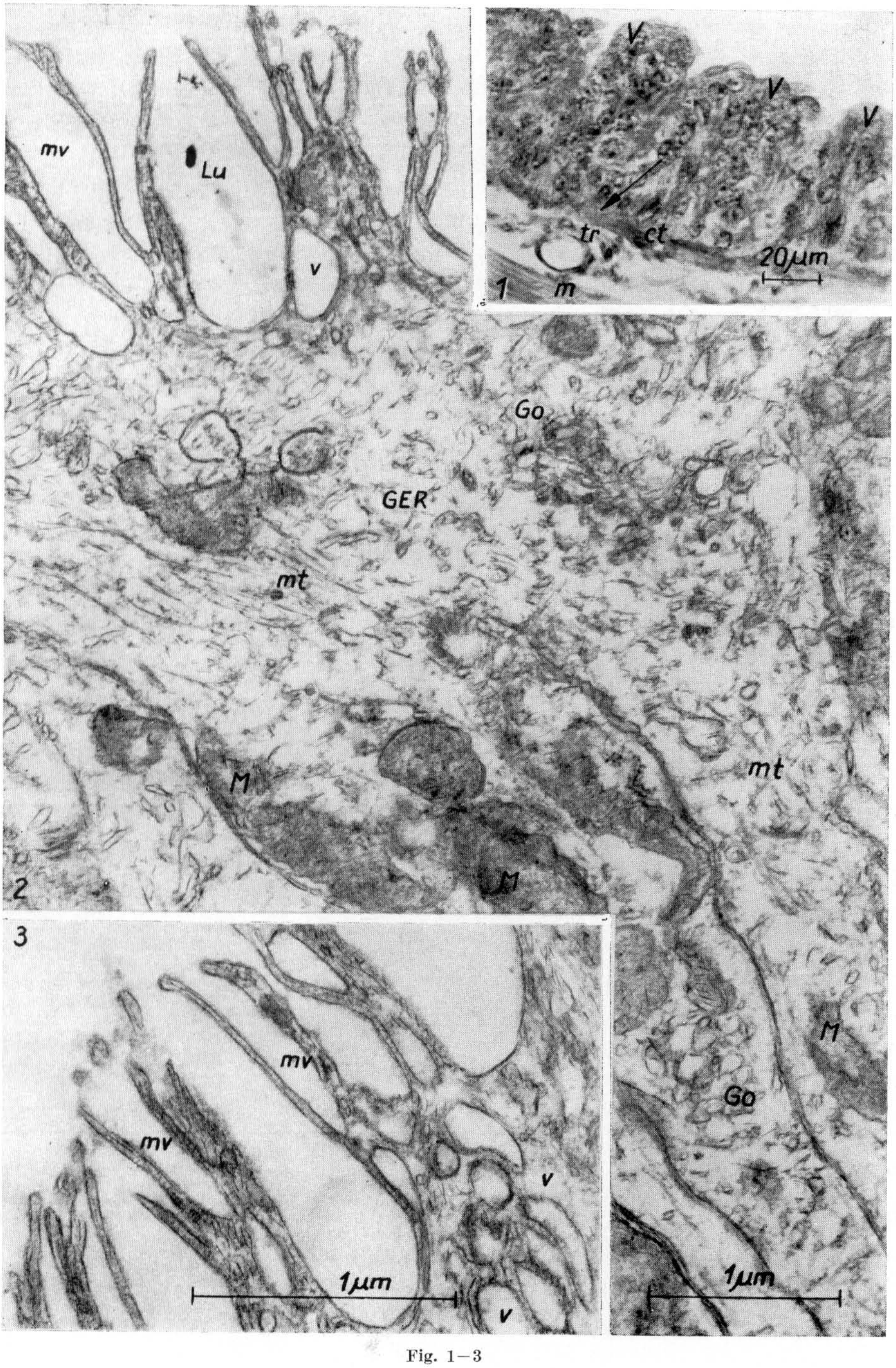

Fig. 1−3

On the apical side of the cells microvillial structures can be observed, differing slightly from the similar structures found in the midgut (Figs. 2 and 3). In the foregut they are relatively short (1,2 to 1,5 μm) and often dividing. Variegated form and arrangement is characteristic of their appearance but they are similar in being originated in groups on common, broad basis, which often contains mitochondria and microtubules. 60 to 80% of microvilli have nearly the same diameter. At their basis they often surround mostly empty vacuoles limited by independent membranes (Figs. 2 and 3), the size of which increases towards the surface (0,2 to 0,5 μm).

The surface of the cells is covered by cuticle slightly differing structurally from the cuticle covering the whole body (Smith 1968).

In their appearance these cells remind of the epithelial cells described by de Priester (1971) as absorptive cells in the midgut of *Calliphora erythrocephala*. At the same time some relationship exists with the so-called "goblet cells" of Lepidoptera midgut attributing role in the osmoregulation (Smith et al. 1969). Beyond that, Anderson et al. (1966), emphasize the absorptive and secretory function of these cells, too.

In our opinion these cells may play a role both in osmoregulation and in secretion. Structural features referring to absorption, however, can only be very seldom observed.

Midgut

The midgut epithelium consists of a single layer of closely aggregated (firmly fitted together) cells (Fig. 4). According to light microscopic investigations they stain evenly, but following electron microscopic preparation processes "lighter" and "darker" cells can be distinguished (Figs. 5 to 8). The latter are isolated or arranged in smaller groups under or sometimes among large numbers of "light" cells.

The connection of the thin, high "light" cells with the lumen of intestine can always be well observed. Their large nuclei have basal location and sometimes two of them can be demonstrated in one cell (Fig. 6). On the luminal surface of the cells densely packed microvilli can be seen, which exhibit strong PAS reaction in good agreement with the literature data

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Fig. 4. Midgut (PAS-reaction). The surface of the epithelial cells arranged in a row exhibits strong PAS reaction (arrows). N: nucleus, m: visceral musculature

Fig. 5. Epithelial cells in the midgut (semi-thin section). The light and dark cells (asterisk) can be well distinguished. N: nucleus, mv: microvilli, llg: lipid-like granules

Fig. 6. Electron microscopic picture of the midgut epithelial cells. The cells are in many instances multinucleate (N). The arrows show cell-connecting structures. M: mitochondrium, Go: Golgi-apparatus, GER: granulated endoplasmic reticulum, mv: microvilli, llg: lipid-like granules, x: vacuole with dense wall

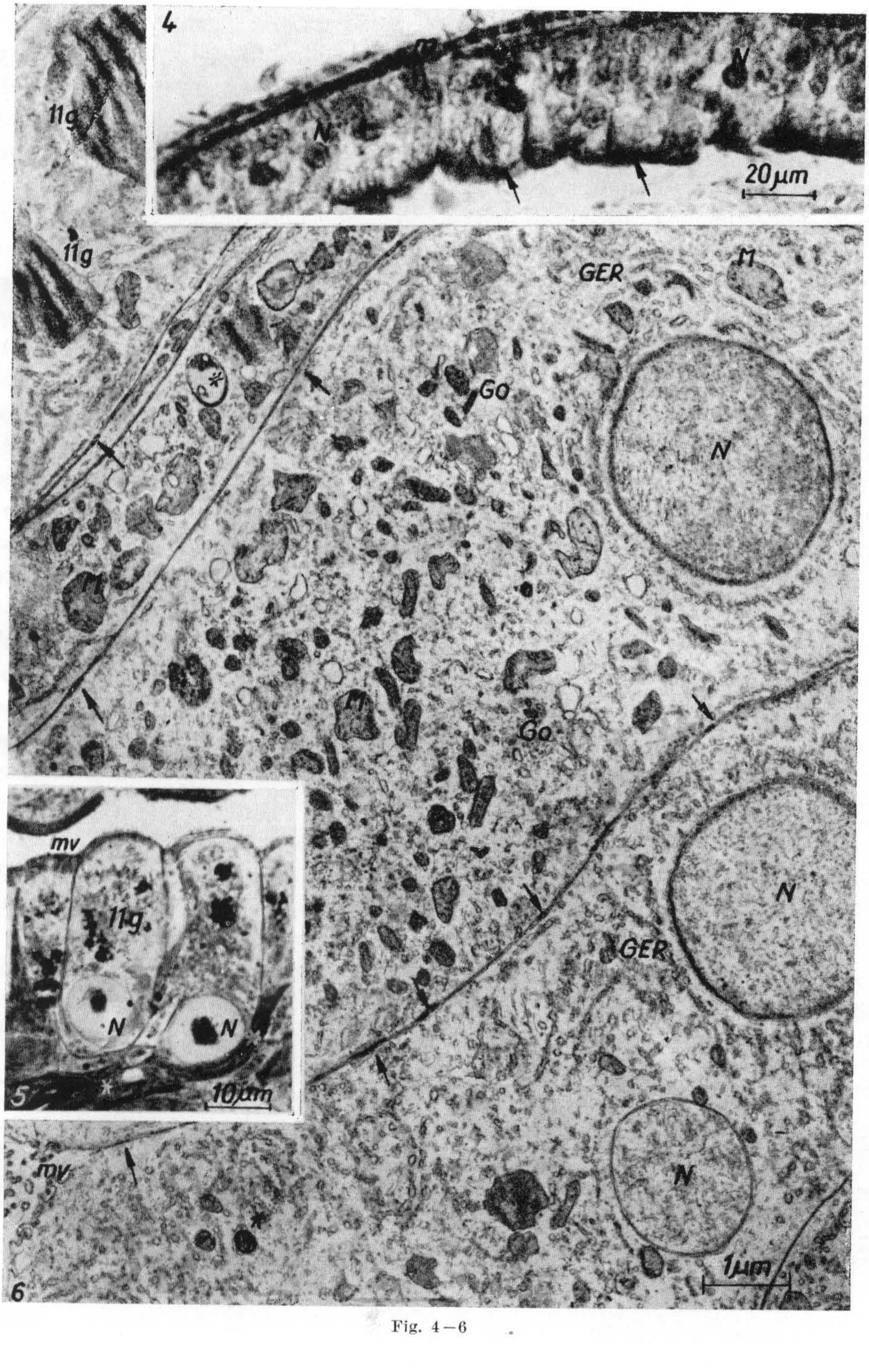

Fig. 4—6

(Beams and Anderson 1957) (Fig. 4). Between the microvilli small pits are sometimes formed, pinching off into the cell, indicating the possibility of pinocytotic intake of materials (Hecker et al. 1971b). The terminal web is characterized by filaments, vesicles and few mitochondria. Similar structure can be observed in the midgut epithelial cells of *Periplaneta americana* L. and *Kalotermes flavicollis* Fab., too. Deeper in the cytoplasm more mitochondria, lipid-like granules (Figs. 5 and 6) and vacuoles with characteristically thickened wall can be seen among the dilated granular endoplasmic reticulum, free ribosomes and well-developed Golgi-apparatus.

The other cell-type of the midgut may be called "dark" cell. The fine structural observations reveal the explanation of the difference in electron density, these "dark" cells being extremely rich in cytoplasmic organelles compared with the surronding "light" cells. The quantity of cell organelles falling on unit cytoplasm volume is multiple and also the ratio of the different organelles differs. It possesses granular endoplasmic reticulum of large extent densely packed with ribosomes. Free ribosomes are also abundant in the cytoplasm. The density of these cells is increased by the extended Golgi-apparatus and the mitochondria of varied size, too. In addition to these organelles dark secretory droplets are often observed, too. From the structure described above the conclusion can be drawn that in the "dark" cells intensive enzyme synthesis takes place, but the possibility can not be excluded that the cells are involved besides the intracellular digestion (de Priester 1972) in the absorption process, too. The absorption process, however, may occur mainly in the "light" cells. According to de Priester (1971), three types of epithelial cells can be distinguished in the midgut of Calliphora, namely: absorptive, regenerative and granulated cells. On the basis of their location and the relevant literature data (Smith 1968, Hecker et al. 1971a, 1971b, de Priester 1972) the "dark" cells observed by us in the midgut of *Palingenia* larvae ought to be regarded as regenerative cells having role mainly in replacing the destroyed absorptive cells. This process, however, is far from being elucidated. We also failed in our material to find cytological evidences in favour of this presumed regeneration.

Fig. 7. Light and dark epithelial cells. The dark cells are extremely rich in cell-organelles. GER: granulated endoplasmic reticulum, M: mitochondrium, r: ribosomes, Go: Golgi-apparatus, N: nucleus. The arrows point to close cell-to-cell attachments

Fig. 8. Attachment points (arrows) between light and dark or light and light cells respectively. The intercellular space between the attachment points is often dilated. GER: granulated endoplasmic reticulum, M: mitochondrium, N: nucleus

Fig. 9. Attachment structures in higher magnification between dark (asterisk) and light cell. M: mitochondrium, GER: granulated endoplasmic reticulum

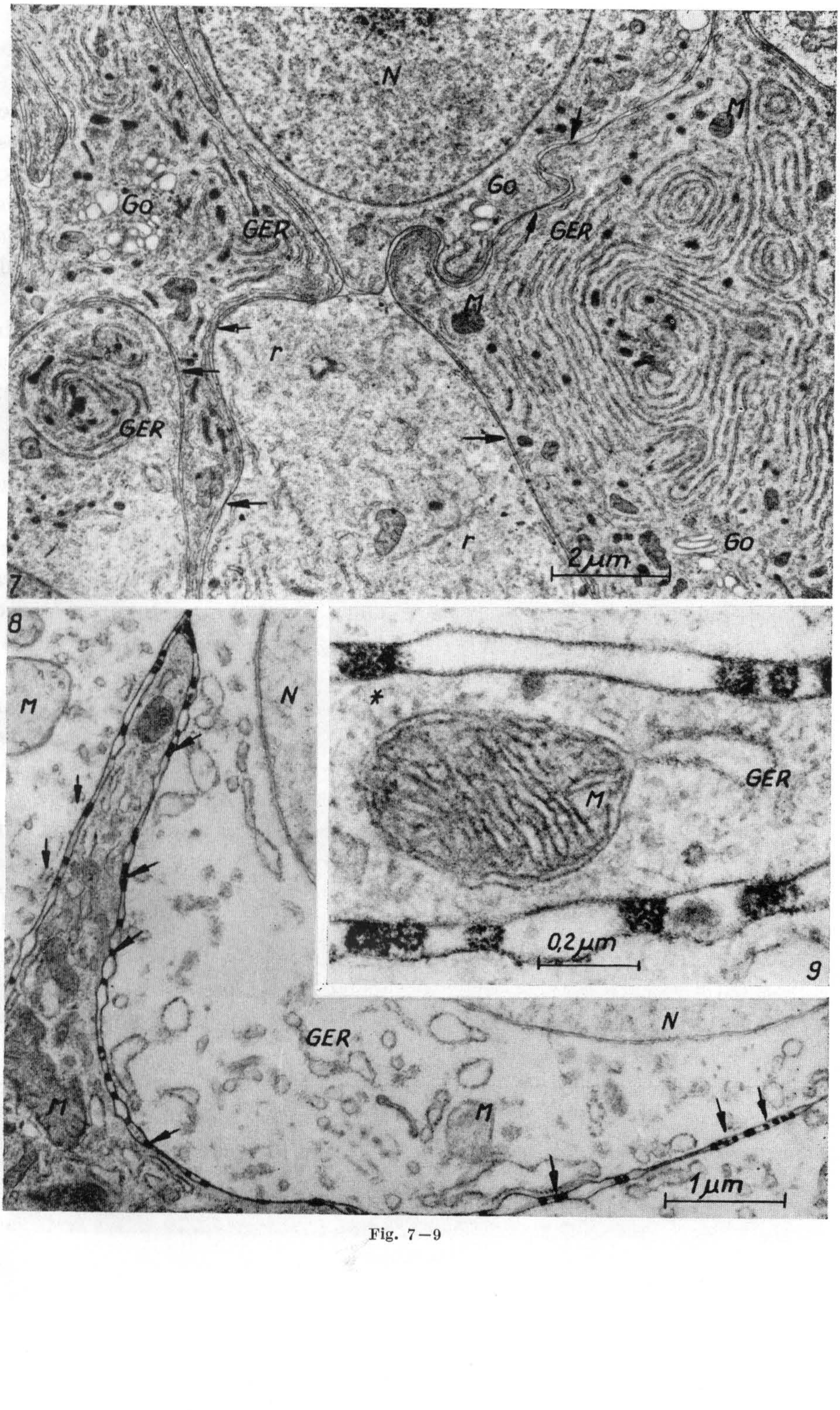

Fig. 7–9

Having analysed the observed fine structural characteristics of both "light" and "dark" cells, we consider none of them to be regenerative cells. We failed to observe granulated cells in the midgut, too.

From the linking structures of the invertebrate epithelial cells, besides the zonula adherens mainly the septate desmosome is though to be the most characteristic (Satir and Gilula 1970). According to other authors (Locke 1965, Smith et al. 1969), however, tight junction (zonula occludens) occurs preferably, especially near to the apical surface of the epithelial cells. In the midgut epithelium of *Orchesella cincta* (Collembola) zonula continua was reported by Dallai (1970).

According to our observations, in the *Palingenia* midgut epithelium the zonula occludens is common (Fig. 6) especially on the apical surface or near to it, whereas in the deeper regions characteristic cell-to-cell attachment points occur, showing some similarity to the septate desmosome (Figs. 6 to 9). The intercellular gap between the adjoining cells is bridged by dense bodies, 600 to 800 Å in diameter, placed sometimes regularly in 0,05 to 1,5 μm interval (Fig. 8).

In higher magnification these bodies seem to be composed of globular elements (Fig. 9). The exact fine structural description of this structural element is not an object of this study. The dark bodies are in close contact with both of the cell membranes, never coming apart from them. This contact can be especially well observed in the poorly fixed areas where, as a sign of the swelling, the adjacent cell membranes have drifted apart with the exception of the above mentioned attachment points. These peculiar connecting structures were observed between both "light" cells and "dark" cells, as well as linking together "light" and "dark" cells, too.

In the midgut there is no direct connection between the food and the surface of epithelial cells, because they are separated by an extracellular sheath, the so-called peritrophic membrane. This membrane, however, does not hinder nutriment in getting to the surface of the gut cells (Huber and Haasser 1950, Waterhouse 1957, Smith 1968). This very thin sheath which easily becomes detached from the surface of cells is a secretory product of the epithelial cell in case of Orthoptera, Ephemeroptera, Odonata, Lepidoptera, Coleoptera and Hymenoptera larvae.

Fig. 10. Light-microscopic picture of the hindgut (haematein-eosin). Intestinal villi (V) infold into the lumen. m: visceral musculature, ct: connective tissue

Fig. 11. Electron microscopic picture of hindgut epithelial cells. A layer of dense bodies (Db) can be observed near to the apical surface of the cell. Under this layer pigment containing vacuoles (pr) are situated. M: mitochondrium, mv: microvilli

Fig. 12. Regularly arranged microvilli in higher magnification. mt: microtubuli

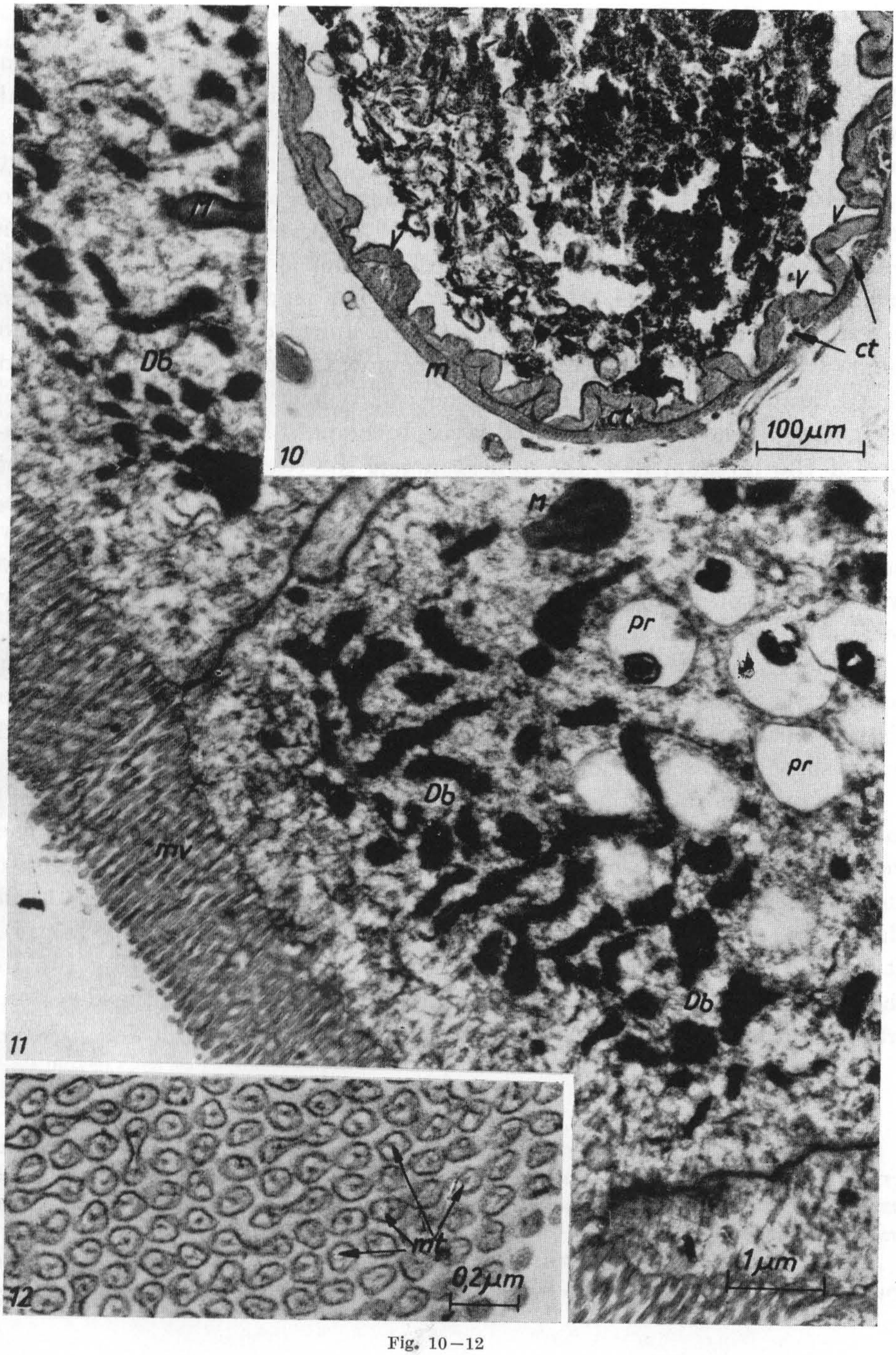

Fig. 10–12

Hindgut

The apical surface of the hindgut is covered by simple epithelium (Fig. 10). The structure of these epithelial cells is similar to the epithelial cells both in midgut and epidermis as well. The expressed microvillial structure of the hindgut epithelial cells refers to the relationship with the midgut epithelium (Fig. 11). The microtubuli running from the terminal web to the microvilli can be well demonstrated mainly in cross sections (Fig. 12). The nuclei are large and basally located. Well developed endoplasmic reticulum can be seen in the cells. At the same time the pigment and dense bodies (about 1 μm in size), observed beneath the apical cytoplasm refer to the similarity to the epithelial cells of the integument. These bodies of variegated shape can be seen scattered in the whole cell, but beneath the apical surface they form a nearly uninterrupted layer. Incompatible with the characteristics of the integument is the appearance of the cuticule on the surface of the epithelial cell, which does not form a continuous layer.

From a functional point of view the hindgut epithelial cells are probably not uniform. Concluding from their structure they may play a role in the water reabsorption, like the epithelial cells in vertebrate hindgut, but they secrete the cuticle as well.

Under the epithelial cells, especially in the fore- and hindgut, a very thin connective tissue layer is situated, which consists of mainly connective tissue (collagen) fibres and a few cells. These fibres form a well-pronounced wavy sheet at the basis of the epithelial cells (Fig. 1).

The outermost histological layer of the digestive tract is the visceral musculature. In its structure, this type of muscle shows similarity to the vertebrate striated muscle whereas its function is similar to the vertebrate smooth musculature. In the foregut the musculature is very thin (Fig. 1), in the midgut somewhat thicker, observable even by light microscope (Fig. 4). It is the thickest in the hindgut, forming a continuous layer (Fig. 10). In the terminal portion of the hindgut the muscle bundles of the digestive tract are in connection with the musculature of the body.

Summary

Histological and cytological investigations at both light- and electron microscopic level were performed on the digestive tract of young *Palingenia longicauda* larvae. The structure of the epithelium and the different cell types of the three regions of the digestive tract were studied in detail.

The most characteristic cell types of the foregut epithelium are the secretory and osmoregulatory cells, that of the midgut are the "light" — absorptive — and "dark" — mainly

secretory — cells, whereas in the hindgut the resorptive and secretory cells have to be emphasized.

Zusammenfassung

Die Verfasser geben eine auf licht- und elektronenmikroskopische Aufnahmen gestützte histologische Beschreibung des Darmepithels der Larven von *Palingenia longicauda*, mit besonderer Berücksichtigung der einzelnen Darmabschnitte.

Für das Epithel des Vorderdarms sind eigentümliche Sekretions- und Osmoregulationszellen, für den Mitteldarm „helle" absorbierende sowie „dunkle", hauptsächlich sekretorische Zellen und für den Enddarm Resorptions- und Sekretionszellen charakteristisch.

Acknowledgement

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