Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 33 (1976)
Heft: 2

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DOI: https://doi.org/10.5169/seals-312223

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Ultrastructural Comparison of the Midgut Epithelia of Fleas with Different Feeding Behavior Patterns (*Xenopsylla cheopis, Echidnophaga gallinacea, Tunga penetrans,* Siphonaptera, Pulicidae)

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Introduction

Many insects are ectoparasites of mammals and birds, which suck and digest their blood. By different evolutionary pathways, convergent digestive processes now exist in insects of different orders. Comparative morphological and physiological studies are promising to identify fundamental processes of blood storage and digestion in the insect midgut (GOODING, 1972). Moreover, the fact that many of these insects are vectors of diseases together with the fact that pathogens always reach or pass through the midgut and its physiological environment, is stimulating intensified study of the midgut and its digestive processes.

Most bloodsucking insects have a midgut which can morphologically and physiologically be divided into two or more parts. In the following paragraph the most important groups of bloodsucking insects are compared with each other and their midgut structure and function are briefly reviewed. The midgut of *Cimex* and *Rhodnius* (Heteroptera) is divided into an anterior stretchable section in which the blood is stored, and a posterior thin section in which digestion takes place (WIGGLESWORTH, 1972; PACHECO & OGURA, 1966; PACHECO, 1970). In mosquitoes the situation is the reverse. The anterior part is thin and the posterior part stretchable (CHRISTOPHERS, 1960). In the anterior part, absorptive processes seem to dominate during blood digestion, whereas in the posterior part, synthesis and secretion of digestive enzymes into the midgut lumen seems to predominate (HECKER, et al. 1971a, b; 1974; GOODING, 1973). A morphologically similar midgut has been found in *Tabanus* (CRAGG, 1920) and in *Phlebotomus* (GEMETCHU, 1974). *Glossinae* possess three histologically recognizable midgut parts (WIGGLESWORTH, 1929). Digestive enzymes are normally synthesized in the 2 posterior parts (GOODING, 1974). The midgut of lice *(Siphunculata)* shows small diverticulae in the anterior part, which is thicker than the posterior part (MARTINI, 1952). The fleas *(Siphonaptera)* stand alone in having an apparently undivided and simple midgut (MINCHIN & THOMSON, 1914; FAASCH, 1935; WAGNER, 1935; WASSERBURGER, 1961; VASHCHENOK & SOLINA, 1969). The processes which are responsible for blood digestion seem to take place throughout the whole length and diameter of the midgut. Secretion of digestive enzymes and resorption of digested nutrients proceeds within the same epithelium. Secretion and synthesis of digestive enzymes begins immediately

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after blood intake. At the same time the epithelial cells start to absorb small free organic molecules of the blood as amino acids, carbohydrates and fatty acids (AKOV, 1972; WIGGLESWORTH, 1972; TURUNEN, 1975). The latter process is intensified once the activity of digesting enzymes increases in the lumen. Such small molecules are rarely absorbed through pinocytotic vesicles in insects, rather they are able to cross directly through the microvillar membrane (SMITH, 1968). In the epithelial cells they are generally metabolized and transported via basal labyrinth to the hemolymph although in some cases they are transported back into the midgut lumen (TREHERNE, 1957; BERRIDGE, 1970; GOODING, 1972; MURDOCK & KOIDL, 1972). At least some, if not all of these transport mechanisms depend on intra- and extracellular concentration gradients of water and small inorganic ions as sodium, potassium and chloride (EDMONDS, 1970; OSCHMAN & BERRIDGE, 1970, 1971; LEE & ARMSTRONG, 1972; OSCHMAN & WALL, 1972; FRIZZELL et al., 1973; ZEUTHEN & MONGE, 1975).

Most fleas are temporary parasites that exhibit a parasitic behavior which is similar to mosquitoes or tsetse flies (SMIT, 1973). They ingest a full bloodmeal which is digested in a few days, after which they are ready to take another bloodmeal. Under this rhythmic nutritive cycle the flea is free from the host most of the time. Such a cyclic behavior is called *'behavior pattern A'*. In some flea species (e.g. genus *Echidnophaga*) the females have to change their behavior before successful egg development can proceed. They become stationary or sedentary on the skin of the host by inserting their proboscis under the skin and this action results in the anchoring of the flea to its host (sticktight-type, SUTER, 1964). This non-cyclical pattern is called *'behavior pattern B'*. *Echidnophaga oschanini* (VASHCHENOK, 1966) and the rabbit flea *Spilopsyllus cuniculi* (MEAD-BRIGGS, 1964; ROTHSCHILD et al., 1970; ROTHSCHILD & FORD, 1973a, b) undergo characteristic changes when they switch from 'behavior pattern A' to 'B'. The cells of the midgut epithelium change their form and become elongated. The defecation rate increases and this indicates that the rate of blood feeding is increased (ROTHSCHILD & FORD, 1964). The rhythmic cycle of digestion is now replaced by a continuous form of digestion. The ten known flea species of the genus *Tunga* have developed a closer parasitic relationship to their host than the sticktight-type of *Echidnophaga* (SMIT, 1973). The chigoe or chigger flea, *Tunga penetrans,* by far the most common species of its genus, is abundant in tropical America and Africa. The female of *T. penetrans* takes its first bloodmeal according to the behavior pattern A. But within several hours it may change to behavior pattern B. It then sinks into the callous skin of its human host and the abdomen begins to enlarge enormously between the 2nd and 3rd abdominal segment. The hypodermis proliferates and enlarged cells can be found. Subsequently the internal organs of the flea hypertrophy and the whole flea reaches the size of a pea (GEIGY & HERBIG, 1949, 1955).

The process of hypertrophy in the midgut of termite queens has been studied at the ultrastructural level by NOIROT & NOIROT (1965). This seems to be the only EM study on hypertrophy in adult insects. Other examples of hypertrophy in adult insects are known from social parasites *(Termitoxeniide,* WEBER, 1966), and the dipteran *Ascodipteron* (*Streblidae,* THEODOR, 1957), which shows a parasitic behaviour very similar to *Tunga,* but this has not been studied histologically. WEBER (1966, p. 341) has mentioned that in several insect groups the change of temporary to stationary parasitism is accompanied by hypertrophy of internal organs such as the midgut and the ovary. This seems to point to some basic adaptation processes whereby parasites are able to react to changing environmental factors.

In this paper, we analyze the ultrastructure of the midgut epithelia of the three flea species *Xenopsylla cheopis, Echidnophaga gallinacea,* and *Tunga*

penetrans, during the process of blood digestion. The results permit a direct comparison with the process as it occurs in other blood sucking arthropods (PACHECO & OGURA, 1966; PACHECO, 1970; GRANDJEAN & AESCHLIMANN, 1973; HECKER, et al., 1971a, b, 1974; GEMETCHU, 1974, HECKER & BRUN, 1975). Hitherto few details on the midgut ultrastructure of unfed fleas have been published (RICHARDS & RICHARDS, 1968, 1969; REINHARDT et al., 1972). The three species studied here form a sequence of increasing adaptation to a parasitic life. The comparison of the midgut ultrastructure in this sequence shall lead us to a correlation between the degree of host-parasite association and the ultrastructure of the corresponding midgut epithelial cells.

Materials and Methods

1. *Flea Material*

Our stock of *Xenopsylla cheopsis* (ROTHSCHILD, 1903) originates from East Africa and has been kept on golden hamsters (SUTER, 1964). *Echidnophaga gallinacea* (WESTWOOD, 1875) was isolated in 1971 from cocks in Ifakara (Tanzania) and it has been possible to keep this species on hamsters for three years according to the method of SUTER (1964). Parasitizing *Tunga penetrans* females were collected from the skin of natives in Ifakara (Tanzania) in the summer 1972. Isolated hypertrophied chigger fleas often eject mature eggs, which allows the rearing of larvae and unfed adult fleas (HICKS, 1930; GEIGY & HERBIG, 1955). The fleas developed best in glass tubes containing sand, sawdust and some dried hen blood at room temperature (25–30 °C) and 100% humidity.

2. *Stages*

The three following stages represent the stages of 'behavior pattern A' (temporary parasite) and these were studied in both sexes of all three species. a) Unfed fleas, three days old; b) fleas immediately after the first bloodmeal. The length of a bloodmeal showed great individual fluctuations. In the case of *X. cheopis* that length could not be exactly determined (fed on hamsters for 6–12 h), whereas the other two species could be controlled exactly (fed on the author's arm for 5–120 min); c) three days after the first bloodmeal.

The next analysis is of stages throughout the period of 'behavior pattern B' (stationary parasites) which is a characteristic only of females of *E. gallinacea* and *T. penetrans.*

E. gallinacea ♀: a) One day sticktight (two days after first bloodmeal). The females of this stage start laying eggs (SUTER, 1964); b) eight days sticktight (nine days after first bloodmeal). The females of this stage have reached their maximal egg-laying activity which gradually decreases in several more weeks (SUTER, 1964); c) three days after having been artificially isolated from the host at stage (b). About 50% of these females died during that period and those which survived did not lay any more eggs.

T. penetrans ♀ (see GEIGY & HERBIG, 1949, for details): a) Stage I of hypertrophy (1–2 days after first bloodmeal). This stage exhibits the first signs of intersegmental growth around the fleas abdomen; b) stage II of hypertrophy (3–4 days after first bloodmeal). This stage shows increased growth of the intersegmental epidermis. Females of this stage may start laying eggs; c) stage III of hypertrophy (5–7 days after first bloodmeal). This stage has the form of a small sphere as a consequence of the great growing zone around the abdomen.

All females of this stage are oviparous; d) stage IV of hypertrophy (8–10 days after first bloodmeal). This is the final stage of the chigger flea by which time they attained the size of a pea. This fully oviparous flea may continue to lay eggs for the next several weeks.

3. *Preparation for Electron Microscopy*

The fleas were opened with iris scissors in insect saline by cutting off the thorax and the last two abdominal segments. In the hypertrophied stages the whole midgut was dissected out and cut into several pieces. *X. cheopis* and *E. gallinacea* specimens were fixed in 5% glutaraldehyde whereas *T. penetrans* gave best results with 2% glutaraldehyde. Fixation in OsO4, dehydration in acetone, and embedding in epon were carried out according to the method of HECKER et al., 1971a. 1 µm sections were stained with a 1:1 mixture of azure II and methylene blue. Ultrathin sections were made with a diamond knife on a LKB Ultrotome III or a Reichert OmU_2, stained with Reynold's lead citrate and photographed with a Philips EM 300.

4. *Morphometry (quantitative electron microscopy)*

The mean nuclear volume (Vnu) and the mean nucleus/cytoplasm ratio (nu/cy) of fully differentiated midgut cells were estimated by morphometric methods (WEIBEL, 1972; HECKER et al., 1972, 1974). Diagrams 1–4 present the means and standard errors (s.e.) for the two parameters Vnu and nu/cy in all stages of *E. gallinacea* and *T. penetrans* (n = 6 individual measurements for each value).

a) The mean absolute volume of the nucleus (Vnu) of an average midgut cell has been estimated from 1 µm cross sections of the midgut (HECKER et al., 1974; REINHARDT, 1975).

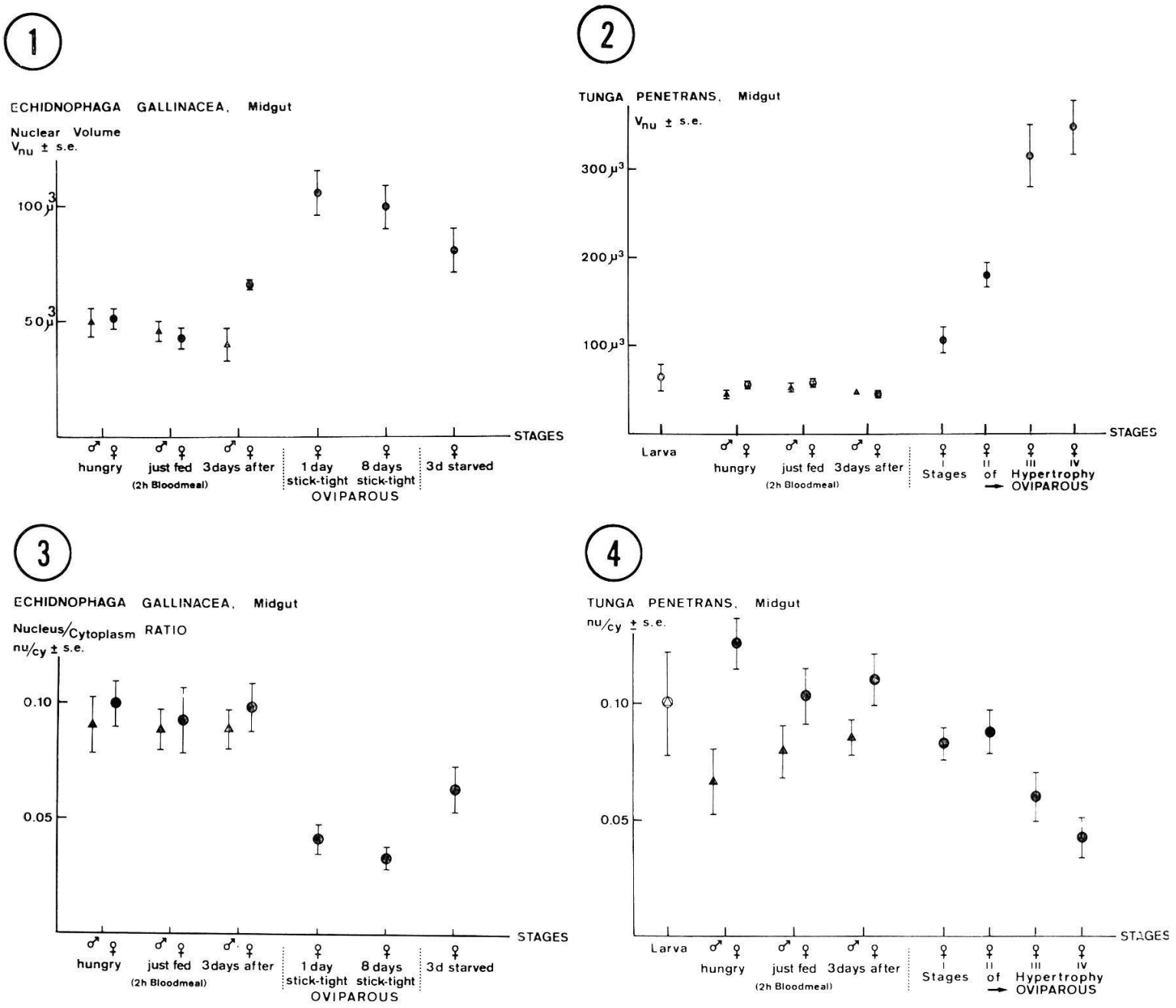
b) The mean value of the nucleus/cytoplasm ratio (nu/cy) has been estimated in the same animals as for (a) by means of low magnification EM micrographs (530 ×) according to the method of HECKER et al. (1974).

c) The mean value of the cytoplasmic volume (Vcy) can be calculated from a) and b) for each stage.

Results

1. *Ultrastructure of the Midgut Epithelium of Unfed Fleas*

The midgut epithelium of the three flea species *X. cheopis, E. gallinacea,* and *T. penetrans* consists of a simple tube of epithelial cells (Fig. 1). In all three species and in both sexes three cell types have been observed which appear in a constant and specific distribution along the whole length of the midgut: (a) differentiated or functional midgut cells, (b) regenerative cells, and (c) secretory cells. In both sexes of *E. gallinacea* a polynuclear cell type has also been observed, which occurs at the base of the epithelium. It is most abundant in starved henfleas and may be a pathologically altered epithelial cell (REINHARDT, 1975).



Diagrams 1–4. Quantitative parameters of digestive cells of the midgut epithelium in different stages of digestion and hypertrophy.

Basal to these epithelial cells, a hexagonally structured basal lamina adjoins as previously described for *X. cheopis* ♀ (REINHARDT et al., 1972) and for *Ctenophthalmus* spec. ('beaded layer', RICHARDS & RICHARDS, 1968). Between this beaded layer and the hemolymph collagen (spacing = 650 Å), muscle cells, tracheoles and nerve cells can be found (Figs. 3, 4). These elements are enclosed in an additional basal lamina which forms the border adjacent to the hemolymph (Fig. 3).

a) The *differentiated midgut cells* are cylindrical and form the main portion of the epithelium (Fig. 1). The nuclear volume and the nucleus/cytoplasm ratio have shown to be in the range of 50 μm^3 and 0.1, respectively, for both sexes of *E. gallinacea* and *T. penetrans* (diagrams 1–4). Each cell apex protrudes slightly into the lumen and is lined with short and regularly formed microvilli (Figs. 1, 5). An irregularly folded basal cell membrane forms the basal labyrinth which opens only at a few narrow channels towards the hemolymph (Fig. 3). The ultrastructure of these cells resembles that of absorptive cells in other insect midguts (SMITH, 1968; AKAI, 1970; BRODY et al., 1972; CHEUNG & MARSHALL, 1973; NOPANITAYA & MISCH, 1974). However, some aspects may be correlated with an accumulating or secreting function. Big unstained vesicles exist in the unfed stage, near the cell apex (Fig. 1). Within a few minutes of the bloodmeal these vesicles have disappeared from the epithelial cells (see paragraph 2., Fig. 2).

b) *Regenerative cells* lay at the base of the epithelium as single cells or in small nests (2–3 cells or nests per cross section of a midgut). They do not make contact with the midgut lumen and their cytoplasm is more electron dense than that of neighbouring cells (Fig. 1). The nucleus/cytoplasm ratio has not been measured, but is obviously much

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Fig. 1–7. Midgut epithelium of temporary parasitic fleas (behavior pattern A).

Fig. 1. E. gallinacea ♀, unfed. The midgut is surrounded by muscle cells (mc), tracheoles and nerve cells (nv). The digestive cells are cylindric and often contain unstained vesicles (ve) in their apex. A nest of regenerative cells (re) can be recognized by the electron dense cytoplasm. Midgut lumen (lu), 1800 ×.

Fig. 2. E. gallinacea ♀, fixed immediately after a 20 min. bloodmeal. Intact erythrocytes (ec) lay in the lumen (lu). The midgut epithelium is stretched, even the nuclei have changed their form (arrow). 1800 ×.

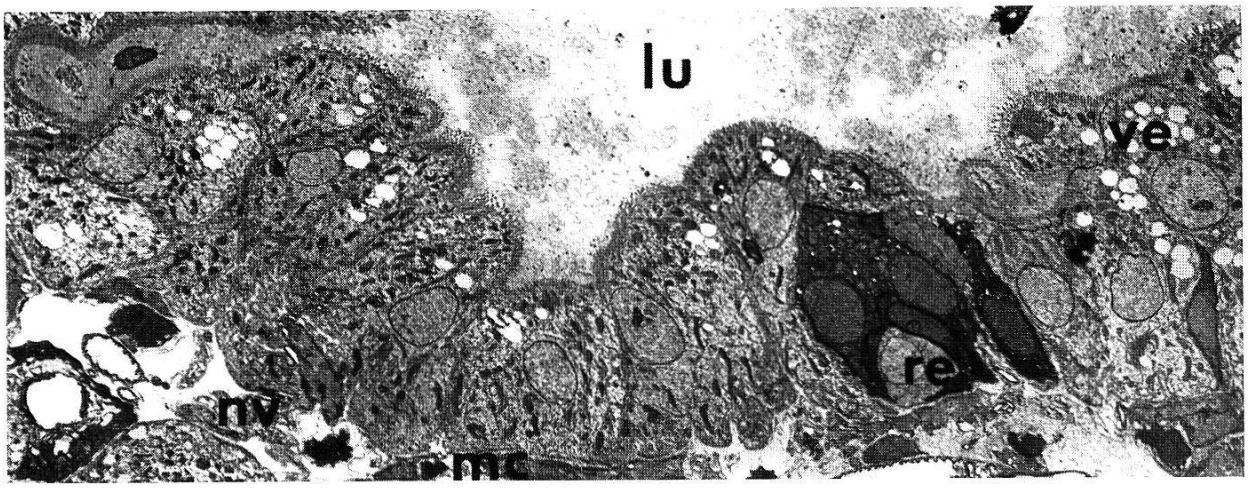
Fig. 3. X. cheopis ♀, unfed. A ring muscle cell (mc) lays basal the basal lamina (bl) of the midgut. An additional basal lamina (arrow) isolates muscle cells, tracheoles (tr) and nerve cells from the hemocoel (he). 14 000 ×.

Fig. 4. X. cheopis ♀, unfed. Collagen fibrils (co) with a 650 Å spacing pattern (arrows) are abundant near the basal lamina of the midgut (bl) and the muscle cells (mc). 86 000 ×.

Fig. 5. E. gallinacea ♂, unfed. A secretory cell contains many dense vesicles (arrow) and has only few microvilli (mv). 14 000 ×.

Fig. 6. E. gallinacea ♂, unfed. Dense vesicles (arrow) of a secretory cell in the midgut. The morphology of these vesicles resembles neurosecretory vesicles of nerve cells (fig. 7). 86 000 ×.

Fig. 7. E. gallinacea ♂, unfed. A synapse between two nerve cells found around the midgut. Neurosecretory vesicles (arrow) are seen in the presynaptic cell. 86 000 ×.



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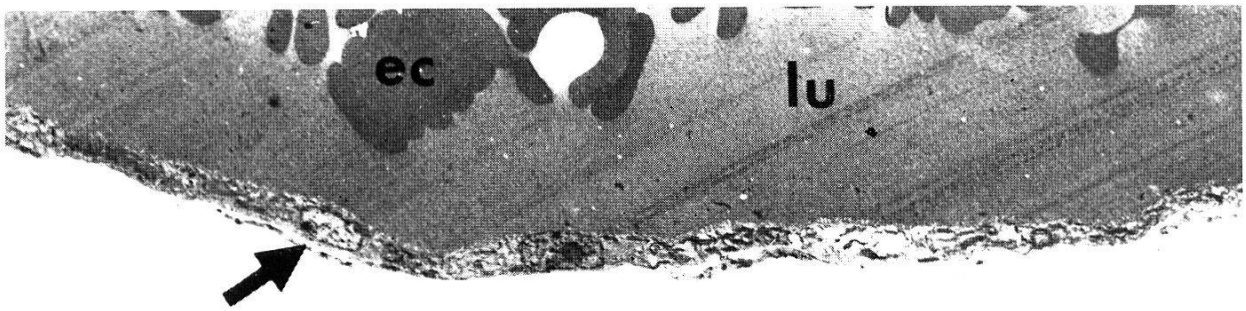

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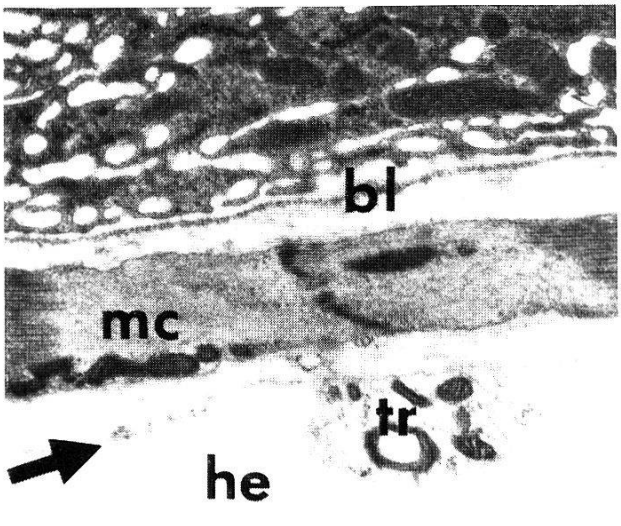

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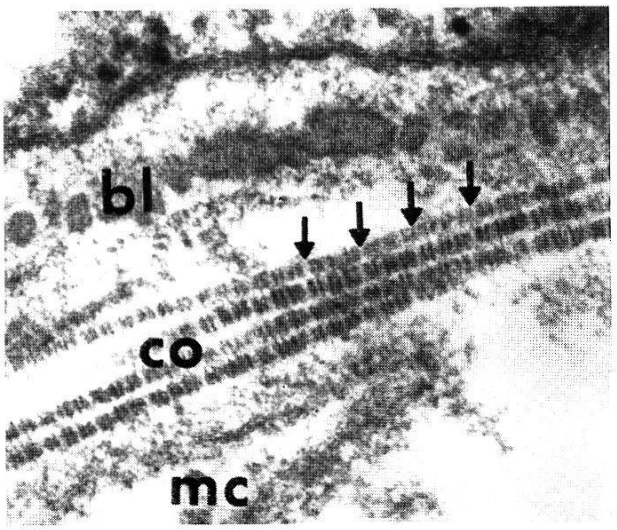

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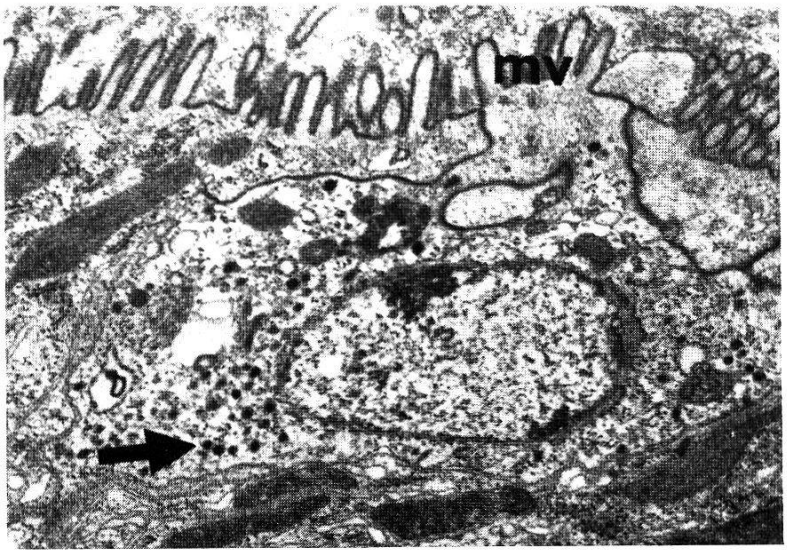

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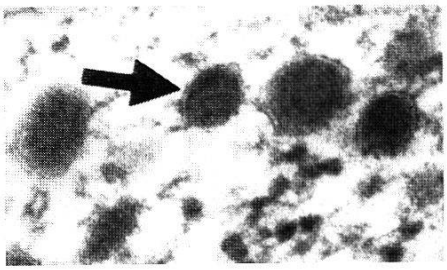

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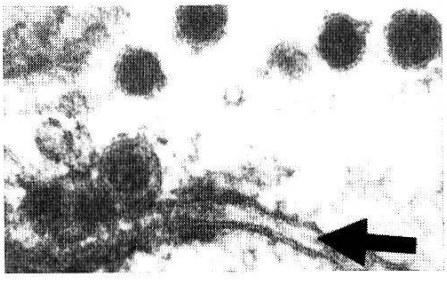

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higher than in the differentiated cells. The cytoplasm contains predominantly free ribosomes. Neither microvilli nor a basal labyrinth exists in the regenerative cells. In *X. cheopis* presumptive stages of outgrowth have been observed (REINHARDT, 1975). The intercellular space between an outer regenerative cell of a nest and the adjoining differentiated cell seems to widen near the apex and is filled with electron dense material. The first microvilli of the outgrowing regenerative cell are formed inside this widened intercellular space, which seems to open into the gut lumen as soon as the growth of the cell proceeds.

c) *Secretory cells* are always solitary and they are less numerous than regenerative cells (about 1 secretory cell per cross section of a midgut). Their cytoplasm is often more opaque than that of adjoining cells, and they always accumulate small dense vesicles (Figs. 5, 6). These vesicles resemble neurosecretory vesicles which can be found in the nerve cells that are responsible for muscle innervation of the midgut (Fig. 7). Microvilli and basal labyrinth are only rudimentary, whereas golgi cisternae are abundant and have a narrow relationship to the dense vesicles.

2. *Influence of the First Bloodmeal (behavior pattern A)*

Both sexes of all three flea species exhibit 'behavior pattern A' at the time of the first bloodmeal. A full bloodmeal induces a flattening and stretching of the midgut epithelial cells and it sometimes induces a high degree of cell deformation (Fig. 23). However, the quantitative parameters (Vnu, nu/cy, diagrams 1–4) are not significantly influenced. Immediately after a short bloodmeal (5–10 min) unstained vesicles can no longer be found in the stretched epithelial cells

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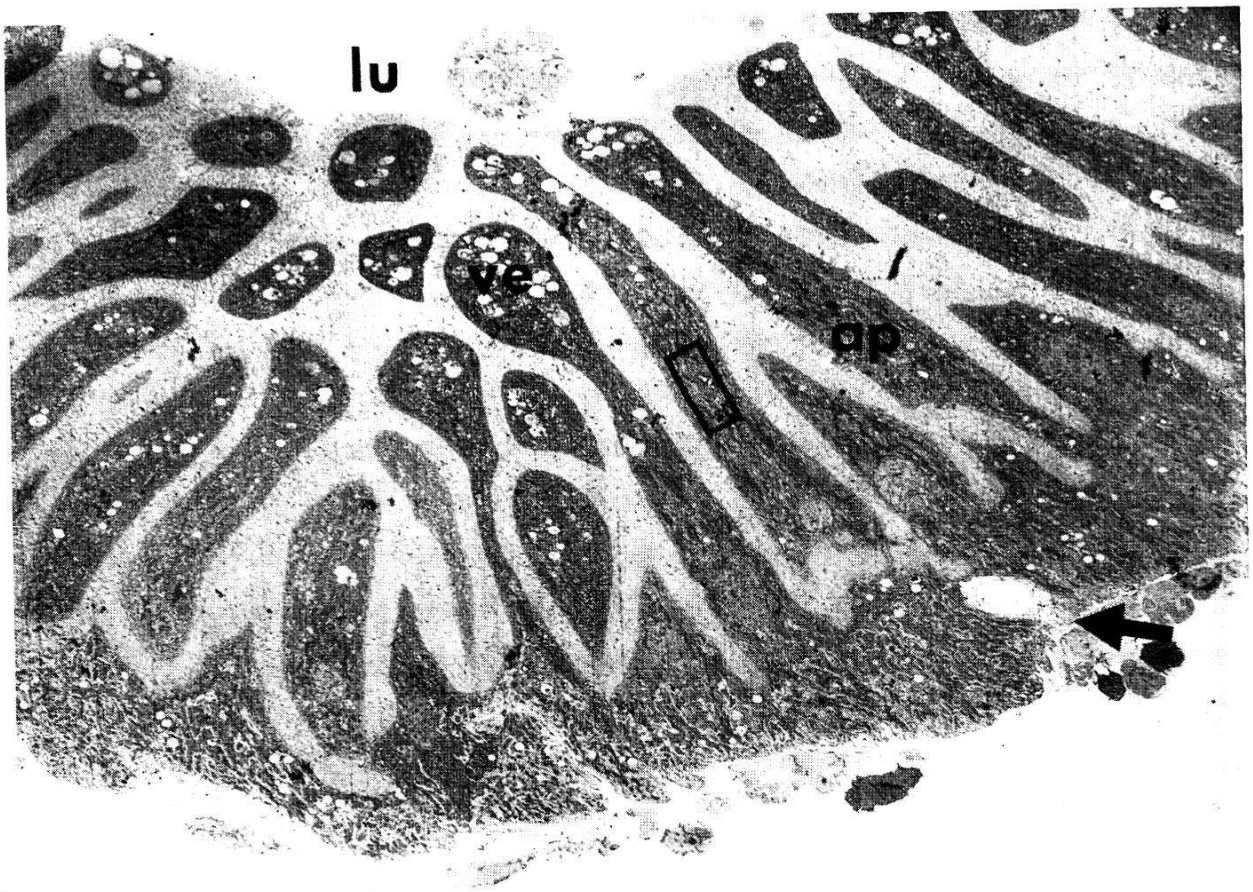
Fig. 8–10. Midgut epithelium of the stationary parasitic and oviparous females of *E. gallinacea.*

Fig. 8. A cross section through the midgut of a highly oviparous female exhibits digestive cells with long apical extensions (ap). The club-shaped top accumulates unstained vesicles (ve) and is sometimes nipped off into the lumen (lu). A secretory cell (arrow) can be recognized between the digestive cells due to its low contrast. 1400 ×.

Fig. 9. A higher magnification of the middle area of an apical cell extension (see fig. 8) reveals many aligned microtubules (mt). The lumina of the rough endoplasmic reticulum (rer) is widened and contains amorphous material (arrow). Mitochondria (mi). 33 000 ×.

Fig. 10. The base of midgut cells of an oviparous female often contains many lipid vacuoles (li) which lay close to the membranes of the basal labyrinth (la). Nucleus (nu), lumen (lu). 6900 ×.



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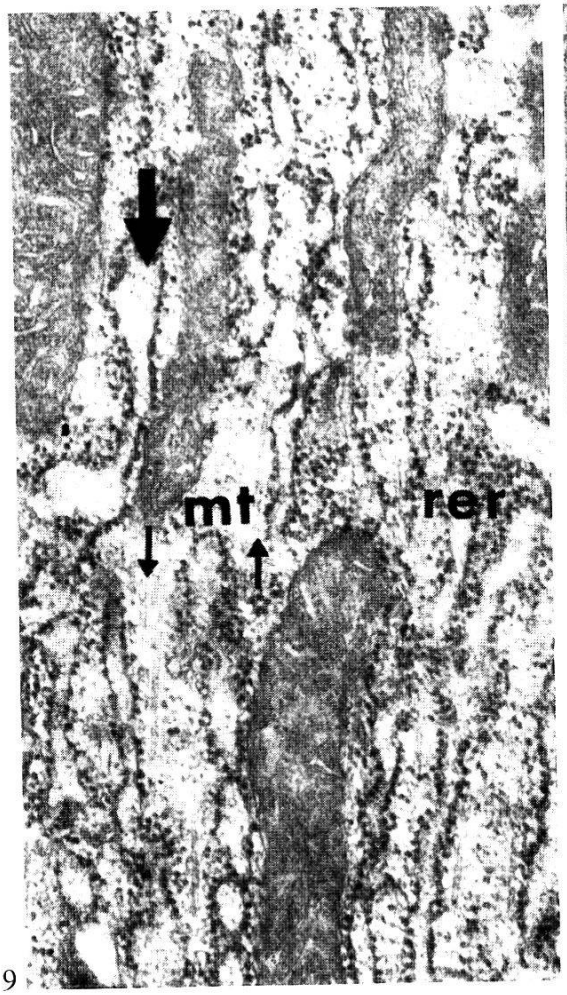

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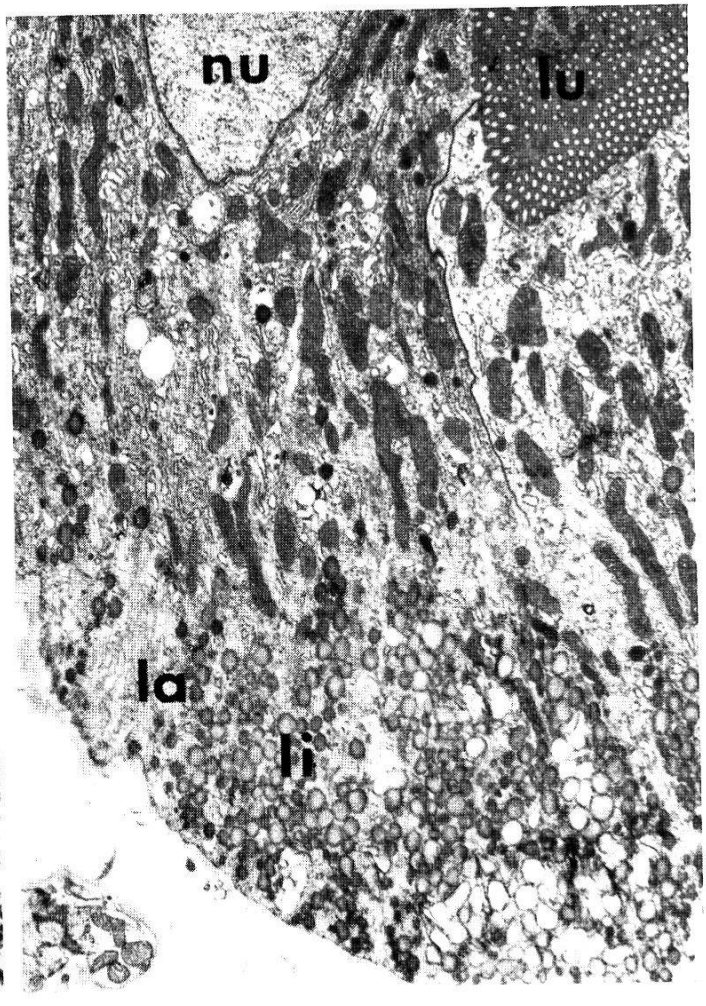

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(Fig. 23). 30–60 min after blood intake, lipids are often accumulated in large vacuoles. Females seem to have a faster absorption rate for these lipids than males. Vesicles of the rough endoplasmic reticulum (rer), golgi vesicles, and microbodies are more abundant, which suggests a slightly higher metabolic activity (NOVIKOFF & HOLTZMAN, 1970) compared to the unfed stage.

Towards the end of the digesting cycle (three days after bloodmeal) roughly the same cell form and organelle set can be found as in the unfed stage. Lysosomes are slightly more abundant. Significant nuclear volume changes were observed in female *E. gallinacea* and *T. penetrans*, which are increasing and decreasing, respectively (diagrams 1, 2).

3. *Influence of the Bloodmeal During 'behavior pattern B' (stationary parasite)*

'Behavior pattern B' is a prerequisite for successful vitellogenesis and oviparity in *E. gallinacea* and *T. penetrans* females. This is not the case in the oviparous *X. cheopis*, since it always shows unaltered 'behavior pattern A' (SUTER, 1964).

Fig. 11–16. Midgut of *T. penetrans* ♀ in the process of compensatory hypertrophy (stages I–IV).

Fig. 11. This cross section through the midgut of an unfed female illustrates the original cell size and shape of the epithelium. The unstained vesicles can already be recognized in this light micrograph (arrows). 500 ×.

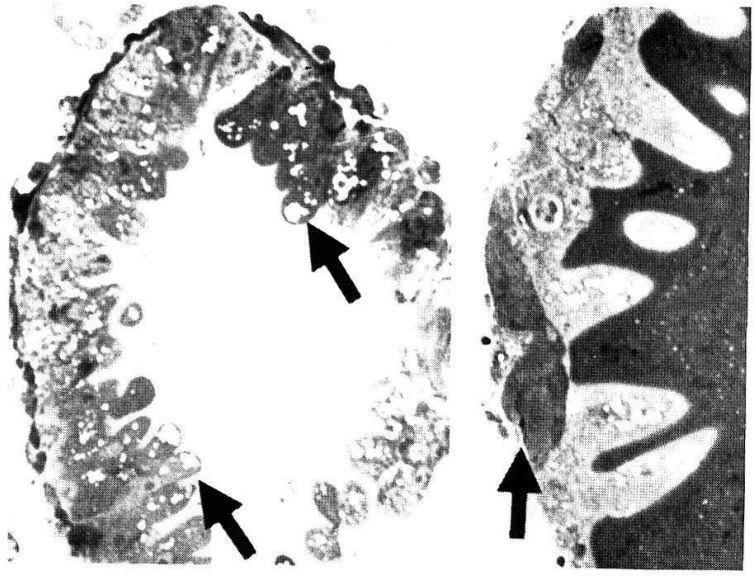
Fig. 12. Stage II of hypertrophy. The proliferation of regenerative cells is reflected in the formation of large nests (arrow). The digesting cells start to undergo cellular hypertrophy. 500 ×.

Fig. 13. Stage IV of hypertrophy. The diameter and length of the midgut has increased many times. The digestive cells are hypertrophied and their apices lap deep into the lumen (lu). 500 ×.

Fig. 14. Stage I of hypertrophy. The base of the epithelium is lined with proliferating regenerative cells (re). 2100 ×.

Fig. 15. Stage I of hypertrophy. These digestive cells start to undergo cellular hypertrophy. The nuclei (nu) are euchromatic and the nucleolus (nc) is split up into several parts showing an extensive nucleonema (arrow). A rer cisterna (arrowhead) is often seen near the nuclear periphery which indicates the first signs of the nuclear halo formation. Unstained vesicles (ve), 6900 ×.

Fig. 16. Stage IV of hypertrophy. The digestive cells are hypertrophied and their nuclei (nu) possess a broad nuclear halo (arrow). The cytoplasm is filled with rer, golgi, and mitochondria. Mitochondria are accumulated at the cell base and along the apical cell membrane. 1800 ×.



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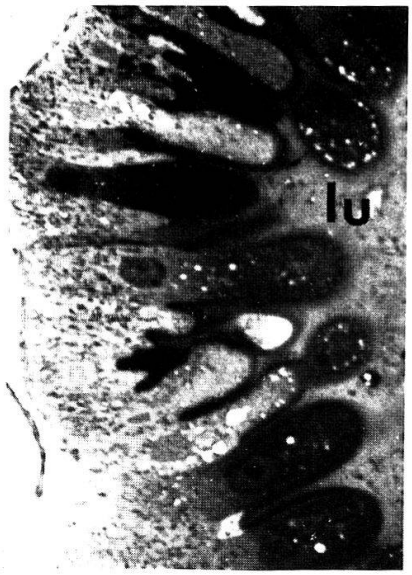

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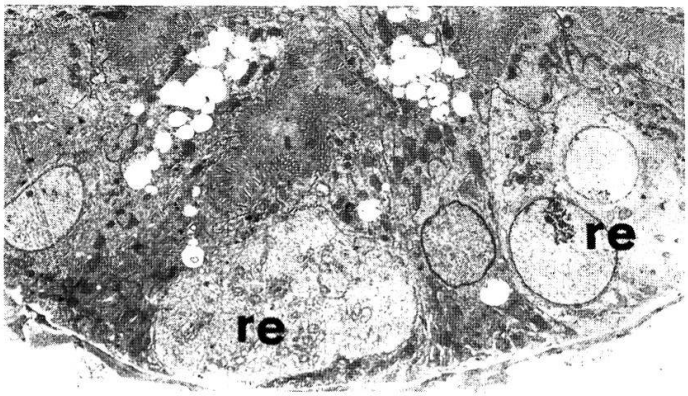

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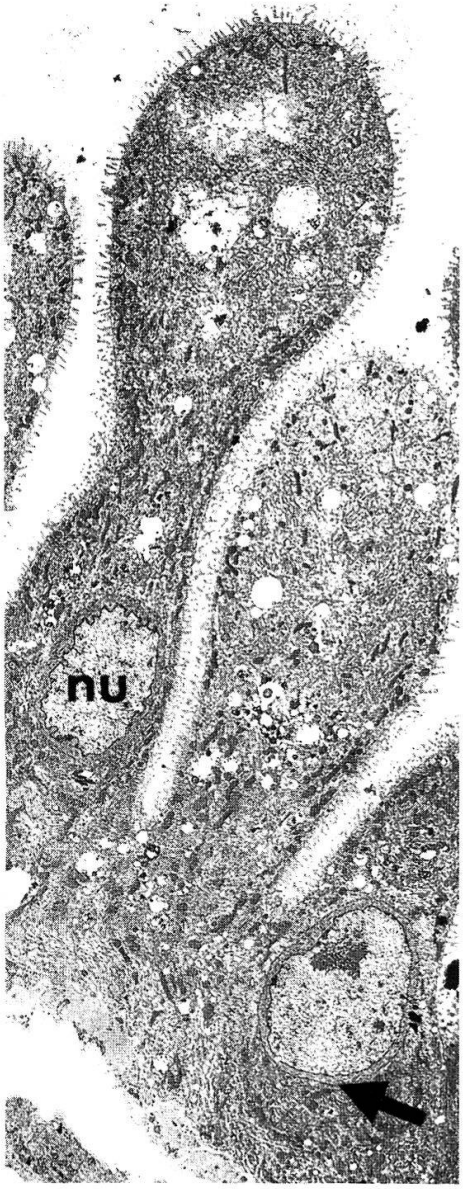

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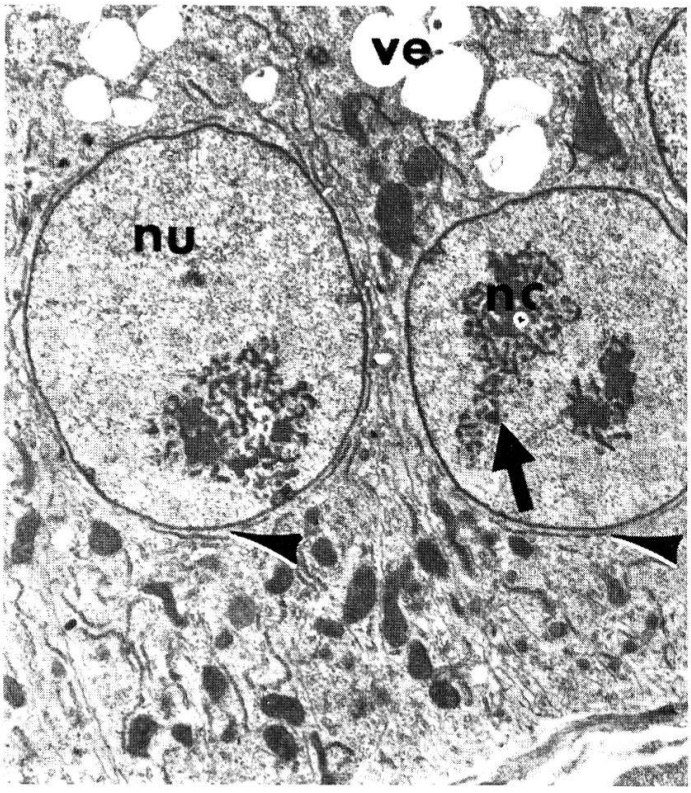

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In female *E. gallinacea* basic changes develop 24 h after attachment to the host. These changes in the midgut epithelial cells have never been observed during the blood digestion cycle throughout 'behavior pattern A'. Long apical cell extensions project into the lumen (Fig. 24). The most apical part contains many unstained vesicles (Fig. 8). The nuclear volume of these cells increases to 100 μm^3 and the nucleus/cytoplasm ratio decreases to 0.04 (diagrams 1, 3). Cisternae of the rer are very numerous. They exhibit a wider lumen which contains amorphous material (Fig. 9). Microtubules are very abundant and are mostly aligned in the longer direction of the cell extension (Fig. 9). Many lipid vacuoles are accumulated in the basal cell region between the membranes of the basal labyrinth (Fig. 10). The latter is much more extensive than in earlier stages.

Seven days later, the *E. gallinacea* females reach their highest egg production period (SUTER, 1964), and the midgut epithelium exhibits the above described features to an even greater extent. The now more elongated cell processes are club-shaped at their end (Fig. 8). Such

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Fig. 17–22. Midgut of *T. penetrans* ♀ in the process of compensatory hypertrophy (stages I–IV).

Fig. 17. Stage III of hypertrophy. Some lipid vacuoles (li) are found at the cell base. The rough endoplasmic reticulum (rer) contains amorphous or dense material (arrow). Mitochondria (mi). 15 000 ×.

Fig. 18–20. Formation of the nuclear halo during the process of cellular hypertrophy.

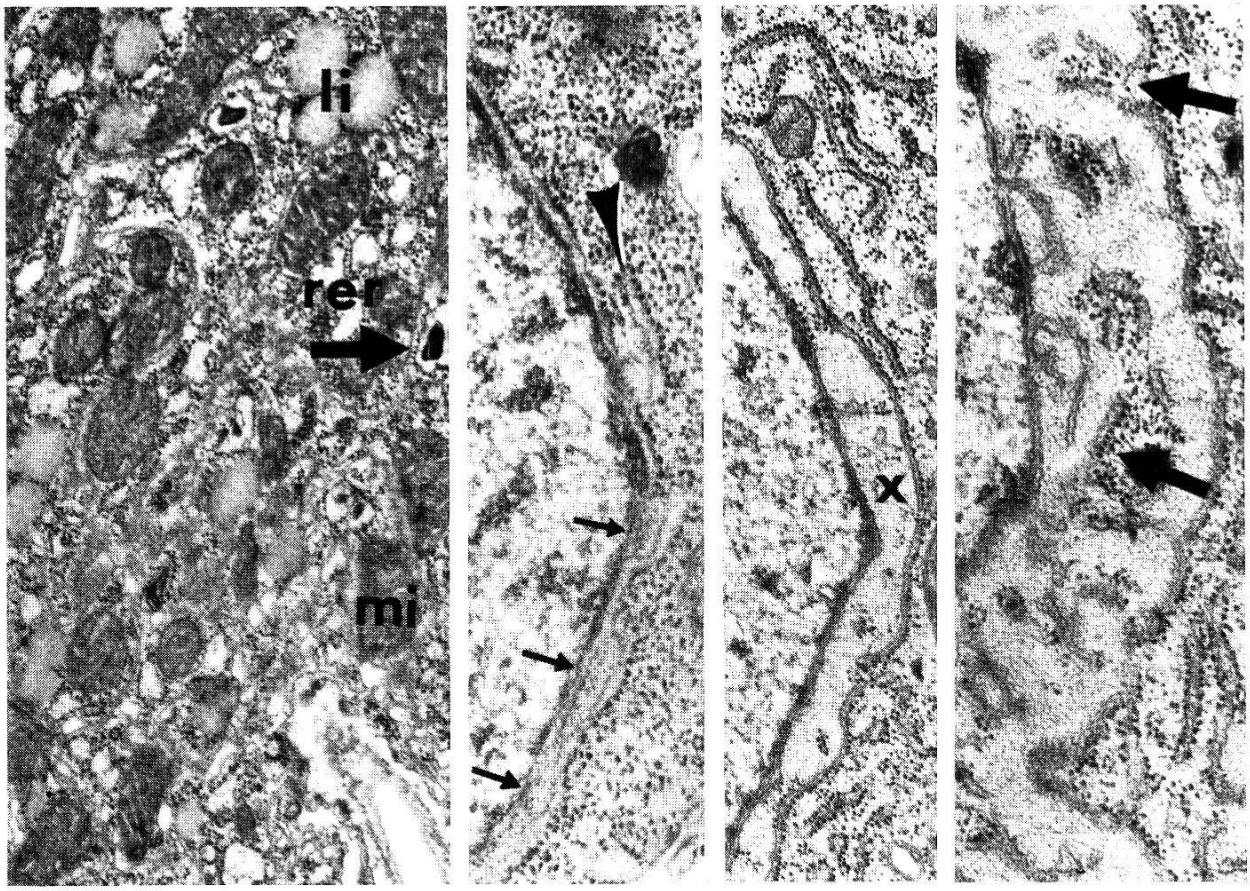
Fig. 18. The first filaments of the nuclear halo are always found near the nuclear pores (arrow). A rer cisterna (arrowhead) screens the filament-containing area from the cytoplasm. 34 000 ×.

Fig. 19. A more hypertrophied cell exhibits a typical nuclear halo with filaments (×) around the whole nuclear periphery. Ribosomes seem to be pushed out of the compartment of the nuclear halo. 22 000 ×.

Fig. 20. A fully hypertrophied cell possesses a thick nuclear halo. The screening rer cisterna is not quite continuous and laps at some places (arrow) into the halo. 30 000 ×.

Fig. 21. This micrograph presents a regenerative nest with cells in different stages of cellular hypertrophy (1–4). The nucleus of cell 2 did not yet form a nuclear halo whereas cells 3 and 4 show a progressing halo formation (arrows). 6900 ×.

Fig. 22. A higher magnification of the nuclear halo of a fully hypertrophied cell presents the structure of the filaments (arrows). These filaments are sometimes oriented towards the nuclear pores (arrowhead). The screening cisterna of the rough endoplasmic reticulum (rer) is lined with ribosomes (ri) only on the cytoplasmic side. 86 000 ×.



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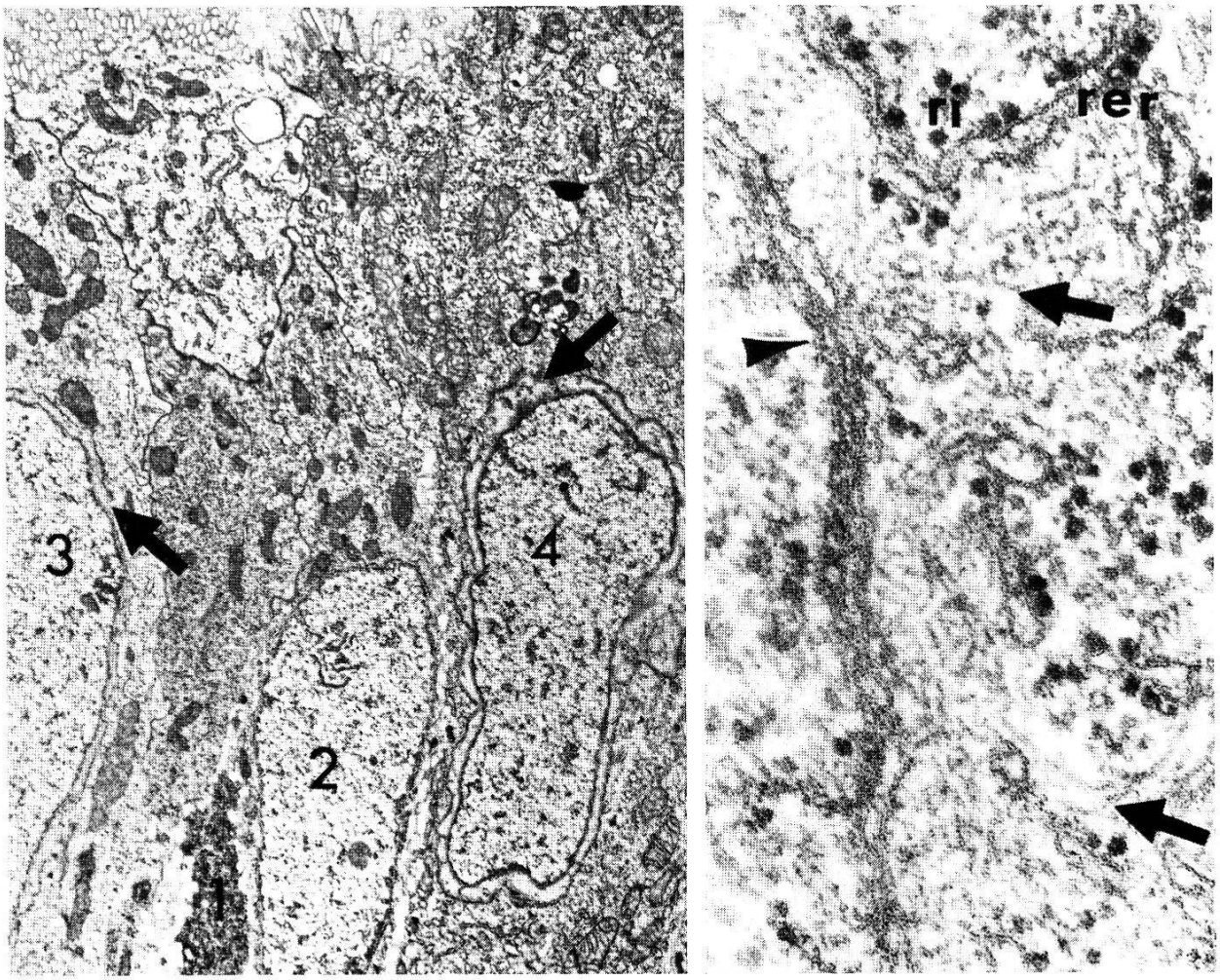

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club-shaped apical parts often seem to separate from the rest of the cell and float through the gut lumen. Some of the changes are irreversible. If one isolates females from the host in that egg production period, they stop egg production immediately and are barely able to survive for the next three days (REINHARDT, 1975). The value for the nuclear volume does not significantly decrease after such a three day starving period (diagram 1).

Parallel to the development of reproductive activity, the midgut of *T. penetrans* undergoes similar changes as described for *E. gallinacea* (Fig. 24). However, *T. penetrans* has to be attached to its host for about five days before the first eggs are produced (between stages II and III of hypertrophy) while *E. gallinacea* needs only one day. Moreover, the changes in *T. penetrans* are more extreme. Nuclear and cytoplasmic volumes of the differentiated cells multiply (diagrams 2, 4; Figs. 11–14, 16). Cisternae and vesicles of the rer are most numerous and always show a widened lumen containing amorphous or dense material (Fig. 17). In the stages I–III the midgut grows fast to about 20 × of its original length.

Fig. 23–24. Schematic architecture of the differentiated midgut cells of fleas during the digestion cycle ('behavior pattern A', fig. 23) and during the continuous phase of digestive activity of the stationary fleas ('behavior pattern B', fig. 24).

Fig. 23. The digestive midgut cells of temporary parasitic fleas undergo cyclic changes between A and B. *A.* The digestive cells of an unfed flea possess distinct microvilli (mv), a tubular basal labyrinth (la) and three kinds of cell junctions towards adjoining cells (septate junctions, sj; gap junctions, gj; maculae adhaerentes, ma). Besides the regular cell components (nucleus, nu; mitochondria, mi; rough endoplasmic reticulum, rer; lysosomes, ly; golgi apparatus, go) unstained vesicles (ve) are accumulated in the apex. *B.* Upon a blood meal the cells get stretched and their nuclei (nu) change to a disc shape. The unstained vesicles (ve) apparently are secreted into the lumen (arrow) and may contain digestive enzymes.

Fig. 24. Only the midgut cells of the stationary parasitic females *E. gallinacea* and *T. penetrans*) undergo the changes A → D, which is an irreversible process. *A.* The digestive cell of an unfed flea shows the same ultrastructure as in fig. 23A. *B.* The first bloodmeal triggers a similar deformation of the digestive cell as during the digestion cycle of 'behavior pattern A' (fig. 23B). *C.* As soon as the flea has switched over to a stationary parasitic life ('behavior pattern B') the digestive cells grow. Their nuclei (nu) probably undergo endomitosis. Besides many free ribosomes (ri) vesicles of the rer are abundant which contain amorphous material (arrow). *D.* The fully oviparous female of *E. gallinacea* and *T. penetrans* possesses enlarged digestive cells which have undergone cellular hypertrophy. Golgi (go) and rer are abundant and lipid vacuoles (li) are often accumulated near the membranes of the basal labyrinth. Unstained vesicles (ve) are concentrated in the very apex which may be nipped off (arrows) into the lumen together with lysosomal vesicles (ly).

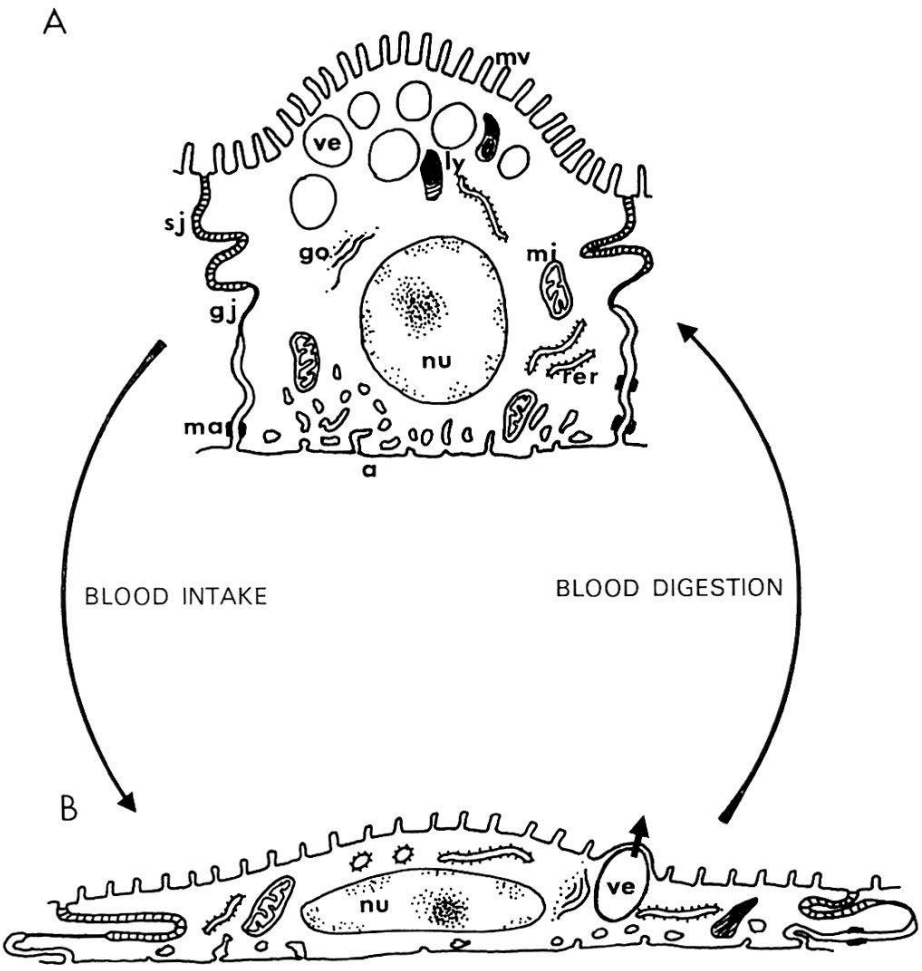
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Fig. 23

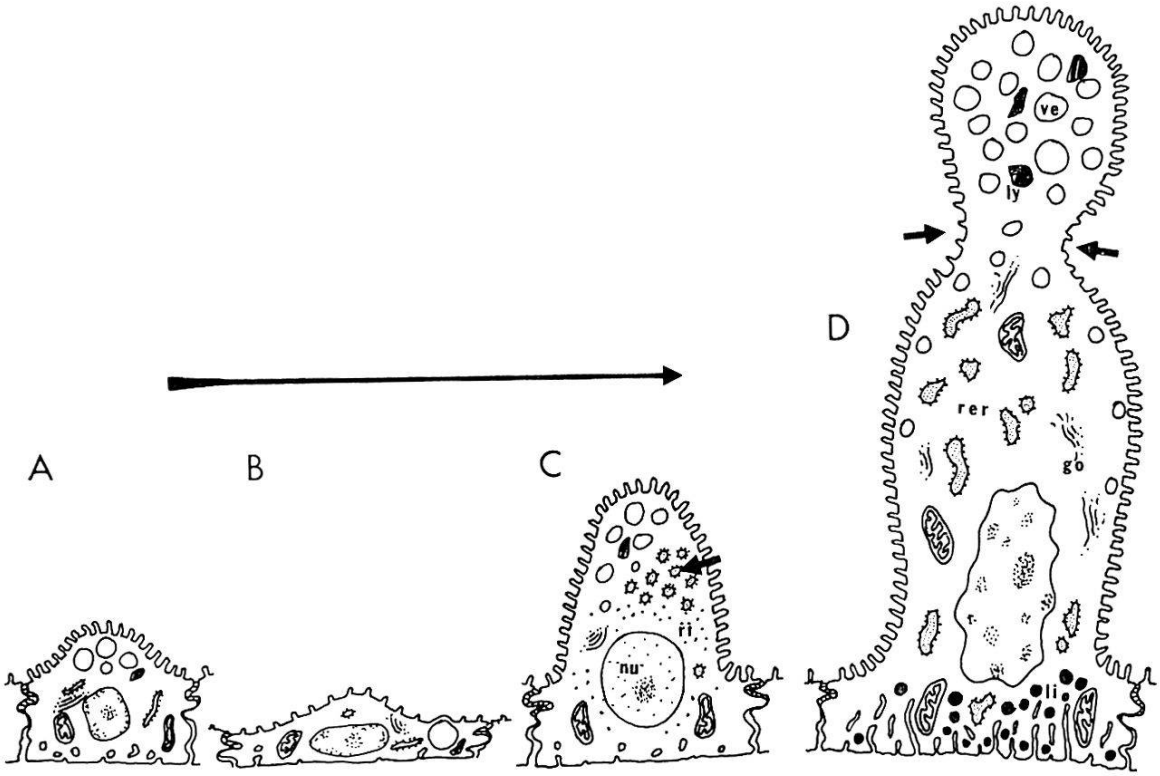
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Fig. 24

4. *The Phenomena of Hypertrophy in the Midgut of T. penetrans* ♀

The hypertrophy of the chigger fleas' midgut is due (1) to the extensive increase of each cell volume and (2) to a very active proliferation of the regenerative cells.

(1) The nuclei of the differentiated cells enlarge their volume about eight times. In the phase of greatest nuclear growth (stages I–III; diagram 2) the nucleus is almost spherical surrounded by a stretched nuclear membrane. It contains very little heterochromatin (Fig. 15). The nucleolus also undergoes distinct changes. Formerly compact in shape, it seems now to divide or segregate into small bodies which become distributed throughout the whole nucleoplasm. This growing phase is also characterized by a peculiar alteration of the periphery of the nucleus which we call formation of the nuclear halo. The first signs of that halo may be seen in some larval or hungry adult fleas. These consist of 70–100 Å thin filaments which are preferentially found around the nuclear pores (Fig. 18). A rer cisterna isolates these filaments from the surrounding cytoplasm. Ribosomes seem to be pushed away from this newly formed cell compartment. This suggestion is supported by the fact that in the progressive stages of hypertrophy the nuclear membrane lacks ribosomes (Figs. 19, 20). The filament containing compartment enlarges over the whole nuclear periphery like a halo, and may reach a thickness of over 0.5 μm in the last stage of hypertrophy. This nuclear halo has only been found in differentiated midgut cells. It has never been observed in undifferentiated regenerative cells, secretory cells of the midgut, or in cells of other organs of the chigger flea (malpighian tubules, foregut, hindgut, muscles, tracheae, nerves, ovary).

(2) In the stages I–III of hypertrophy the regenerative cells proliferate extensively. The regenerative nests enlarge and cover most of the basal surface of the epithelium (Fig. 14). In a single regenerative nest cells of different stages of differentiation can always be observed. Undifferentiated and dividing cells lie in the center of the next, whereas more differentiated cells are found towards the periphery (Fig. 21). The progressive differentiation is reflected in (1) the cell shape and position in the epithelium (progressive lumen contact and microvilli formation), (2) the increasing nuclear and cytoplasmic volume, (3) the decreasing nucleus/cytoplasm ratio, and (4) in the formation of the nuclear halo. All four of these phenomena appear in outgrowing regenerative cells as they have been found to appear in already differentiated cells throughout the process of hypertrophy of the whole midgut.

Discussion

1. *Ultrastructure of the Midgut Epithelium of Unfed Fleas*

a) The *differentiated midgut cells* are well prepared for their absorptive function during digestion (Fig. 23a). Their microvilli and basal labyrinth resemble those of other insects, where they are known to play a key role in absorption (ANDERSON & HARVEY, 1966; BERRIDGE, 1970; MILLS et al., 1970). The basal labyrinth encloses an almost isolated extracellular compartment which has only a few open channels with the hemolymph. It has been proposed that this form of basal labyrinth is effective in establishing concentration gradients for transport processes (BERRIDGE, 1970). In absence of any structural differences in different regions of the midgut of the fleas, I assume that the epithelium secretes digestive enzymes while maintaining an absorptive function throughout its whole length. The abundance and the position of the unstained vesicles suggests that they are secreted after a blood meal. In fact, they do disappear within a few minutes of the blood meal. Two more observations give additional evidence for the hypothesis that these unstained vesicles contain digestive enzymes. They contain acid phosphatase and are often next to lysosomes and different sizes of rer vesicles (REINHARDT, 1975).

b) The *regenerative activity* is not a conspicuous feature of the adult insect midgut (WIGGLESWORTH, 1972). However, several growing and differentiating cells have been observed in *X. cheopis* (REINHARDT, 1975). In such regenerative cells that are growing toward the gut lumen, the microvilli seem to be formed before the cell makes contact with the lumen. Very similar outgrowing processes have also been reported from other insect midguts (HECKER et al., 1971a; JEANTET, 1971; ANDRIES, 1972), whereas microvilli formation in the embryonic midgut seems to be different in that the microvilli are all formed at their prospective place at the cell apex (BONNEVILLE & WEINSTOCK, 1970; VAN DER STARRE & DE PRIESTER, 1972).

c) I have called the *'secretory cell'* in the flea midgut by that name because most features of its structures indicate a secretory function. Cells of a similar morphology have been described in a few insect midgut epithelia under different names, in *Aedes* 'cells containing dark granules' (HECKER et al., 1971a, b), in *Calliphora* 'granular cells' (DE PRIESTER, 1971), and in *Petrobius* 'cellules claires' (FAIN-MAUREL et al., 1973). Similar cells have also been observed in the louse *Pediculus vestimenti* (HECKER et al., unpublished) and in the tsetse fly *Glossina morsitans* (only in the posterior third of the midgut; BOEHRINGER, personal communication). It would be worthwhile to look for such cells in other insects because they may occur regularly in the adult

midgut but, due to their relatively low frequency, may have been overlooked in some earlier studies.

In the midgut of fleas the secretory cells are mostly solitary and not in close association with regenerative nests, as in *Calliphora* (DE PRIESTER, 1971) nor in the regenerative nests, as is the case in *Petrobius* (FAIN-MAUREL et al., 1973). The similarity of the secretory vesicles to neurosecretory vesicles and their association with the golgi apparatus has suggested an endocrine role for these cells (CASSIER et al., 1972; FAIN-MAUREL et al., 1973). It is still not clear whether this role consists of a control of digestive processes, of differentiation of regenerative cells, or of some other process.

2. *Influence of the First Bloodmeal (behavior pattern A)*

A full bloodmeal of *E. gallinacea* and of *T. penetrans* results in a remarkable stretching of the midgut epithelium (Fig. 23b). The cells are deformed but do not change their volumes. The formerly folded apical and basal membranes are flattened, and even the microvilli seem to shorten or disappear (REINHARDT, 1975). Within the first few minutes after starting a bloodmeal the unstained vesicles in the cells disappear and have probably been secreted into the lumen. This temporal coincidence suggests that the mechanical stretching triggers the release of the content of these vesicles into the lumen (Fig. 23b). As in mosquitoes the mechanical stress on the gut cells may stimulate the production of some digestive enzymes as esterases and lipases (GEERING & FREYVOGEL, 1975). However, protease synthesis depends there on the presence of proteins in the lumen (GOODING, 1973; BRIEGEL, 1975).

As digestion proceeds in the flea midgut, the cells regain their original form and the unstained vesicles reappear in their apex, which indicates the cyclic nature of the digestive processes (Fig. 23).

Thirty to sixty minutes after the first bloodmeal some lipid droplets appear in the cells for the first time. They may be stored until needed as an energy source for digestive and metabolic processes. Intracellular esterases in the midgut are able to mobilize such energy sources (GEERING & FREYVOGEL, 1974). The lipids disappear after digestion. Similar observations of a temporary storage of lipids in the midgut have been made in *Aedes aegypti* (GANDER, 1968; HECKER et al., 1974) and in *Phlebotomus longipes* (GEMETCHU, 1974).

Other ultrastructural changes besides those mentioned above are rather inconspicious after a first bloodmeal. However, the rer membranes seem to be more abundant during the digestion process. Quantitative measurements could reveal more information about the rer

membrane turnover as has already been demonstrated for *Aedes aegypti* (HECKER et al., 1974).

3. *Influence of the Bloodmeal During Stationary Parasitism (behavior pattern B)*

The females of *E. gallinacea* and *T. penetrans* ingest much more blood as soon as they are attached to the host and have changed to 'behavior pattern B'. This can be seen directly in the increased defecation rate of blood droplets (SUTER, 1964; ROTHSCHILD, 1966). The midgut always contains fresh blood mixed with partly digested blood.

The differentiated midgut cells undergo distinct changes in their form and volume as soon as behavior pattern B is established (Fig. 24). They develop long apical cell extensions which may partly be nipped off into the lumen (Fig. 24d). The same observations have been made in other stationary fleas. A histological study of VASHCHENOK (1966) on *Echidnophaga oschanini* reveals changes in the shape of the midgut cells as soon as the flea is attached to its host *(Ochotona pricei)*. The midgut cells of the oviparous female develop long cell extensions which contain similar unstained vesicles as in *E. gallinacea*. The irreversibility of this process has been mentioned. VASHCHENOK (1966) observed the expelling of whole cells into the lumen and suggested this was due to age and exhaustion of cells. Accordingly, cell replacement occurs starting with mitosis in the regenerative nests. This cell replacement has not been observed in *E. gallinacea*. However, the irreversibility of the growing process in the epithelial cells seems to be very similar in both species. In the phase of practically unlimited food, the digesting cells are unable to digest the gut content totally. This can be demonstrated by isolating the attached flea from the host and thus interrupting feeding (diagrams 1, 3). Oviposition stops immediately and within three days some of the fleas die with some blood still in their gut.

The European rabbit flea, *Spilopsyllus cuniculi,* presents another aspect of stationary parasitism which originates in the narrow relationship to its host. Standing between fleas with temporary and stationary parasitic behavior, *S. cuniculi* is called semi-sedentary (ROTHSCHILD, 1957). Both sexes may parasitize the rabbit for months without reaching maturation. In this stage the midgut epithelium resembles that of temporary parasitic fleas although *S. cuniculi* may be attached to the rabbit over long periods (ROTHSCHILD et al., 1970). The maturation of the rabbit flea is only induced by certain hormones of its host (adenocorticotrophic hormone, oestrogen, 'nestling factor'; ROTHSCHILD & FORD, 1964, 1966, 1973). Parallel to the maturation of the

flea the midgut epithelium undergoes similar changes to that seen in *E. gallinacea* and in *T. penetrans* females. Mainly the female's midgut cells form long apical extensions lapping into the lumen (ROTHSCHILD et al., 1970). However, the changes of these cells seem to be reversible in a manner parallel to the ovarian regression (MEAD-BRIGGS, 1964). Hormones of the host seem to influence other flea species too, and this is thought to be rather common in this parasitic insect order (ROTHSCHILD & FORD, 1973a, b; ROTHSCHILD, 1975).

The following discussion concerns three conspicious ultrastructural features of the enlarged and elongated midgut cells of both *E. gallinacea* and *T. penetrans*.

(1) It has already been discussed (see above) that the unstained vesicles may contain digestive enzymes which are released upon the first bloodmeal due to the mechanical stress in the epithelium. The stretching does not occur in the stages associated with 'behavior pattern B', discussed here. Another pathway of release of these vesicles seems to be activated. The vesicles are mostly accumulated in the very apex which is often nipped off into the lumen and then degenerates there (Fig. 24d). This would liberate the enclosed enzymes for digestive function. This 'nipping-off' of vacuolated cell parts has often been observed in insects and has also been interpreted as playing a role in enzyme secretion (WIGGLESWORTH, 1972). A similar secretion process has been found in ticks too, but its function seems to be quite different (GRANDJEAN & AESCHLIMANN, 1973). In that case the eliminated cells or cell apices are filled with hematin, the residue of intracellular haemoglobin digestion.

(2) The cytoplasmic volume of each differentiated midgut cell reaches the maximum in the highly oviparous females of *E. gallinacea* and *T. penetrans*. In this stage the rer is predominant (Fig. 24d). The rer cisternae and vesicles enclose amorphous material which can also be found in the golgi apparatus. The morphology of rer and golgi points to a very active metabolism of both organelles (NOVIKOFF & HOLTZMAN, 1970). Due to the enlarged cytoplasmic volume these active membrane systems suggest a much higher metabolic capacity for protein synthesis (probably digestive enzymes) of each cell. In the midgut of *A. aegypti* morphometrical and histochemical investigations have proved a temporal coincidence between increase of the cytoplasmic volume, and the amount of rer membranes as well as the synthesis of digestive enzymes (GOODING, 1973; HECKER et al. 1974). In fleas, however, it is not known whether the efficiency and the metabolic turnover rate of protein synthesis has increased. This has been demonstrated not to be the case in the hypertrophied fat body of a termite queen (WYSS-HUBER & LUESCHER, 1975).

(3) Many differentiated midgut cells accumulate lipid vacuoles in

the basal part of their cytoplasm. The possibility of their role as an energy source has already been discussed. Their position between the membranes of the basal labyrinth suggests another role. They seem to be transferred via the extracellular space of the basal labyrinth into the haemocoel. In *Pieris brassicae* it has been demonstrated that lipids may pass as diglycerides from the midgut epithelium to the hemolymph (TURUNEN, 1975). In contrast, the transport of lipids from the vertebrate absorptive cells to the extracellular space has been shown to be an exocytosis process (formation of chylomicra; FRIEDMAN & CARDELL, 1972).

4. *The Phenomena of Hypertrophy in the Midgut of T. penetrans Females*

The midgut of the oviparous female of *T. penetrans* has hypertrophied due to a cellular hypertrophy (volume increase of each differentiated cell) and to an extensive hyperplasia (proliferation of regenerative cells). Goss (1966) calls this kind of growth 'compensatory hypertrophy'. In contrast, the midgut of termite queens hypertrophies due to cell proliferation alone (NOIROT & NOIROT, 1965), and should instead be called hyperplasia. A 'compensatory hypertrophy' has also been reported for hypodermis, Malpighian tubules, and muscle cells of the chigger flea, whereas other organs such as the ovary undergo a hyperplasia (GEIGY & HERBIG, 1949). Since midgut and hypodermis start to hypertrophy before all other organs the inducing factor might be found there. In some other flea species, hormones of the host influence the growth of the midgut cells and the maturation of the ovary (ROTHSCHILD & FORD, 1973). Accordingly, the compensatory hypertrophy in the midgut of *T. penetrans* could be initiated by a certain level of hormones of the host which is only reached during 'behavior pattern B'.

The function of two distinct structural changes during the process of cellular hypertrophy are discussed next, (1) the growth of the nucleus, (2) the nuclear halo.

(1) The nucleus undergoes an almost eight-fold volume increase during the process of cellular hypertrophy. Similar observations of nuclear growth have been made during the postembryonic development of insects (DORN, 1973). There, the growth of the nucleus correlates with the polyploidy of the gene set, which is achieved by endomitosis without apparent nuclear division (GEITLER, 1938; NUR, 1968). Endomitosis is thought to occur predominantly in cells with a continuously high metabolic activity (KRAMER, 1958; ROMER, 1966). This seems to be the case in the midgut of the stationary chigger flea since

the digestive activity is very intensive without any sign of regression. The nuclei are euchromatic in all hypertrophied stages which points to their high metabolic activity. Moreover, the nucleoli enlarge and break down into small parts which could be a structural reflection of the metabolic and endomitotic activity of the midgut nuclei.

(2) The function of the filaments of the nuclear halo seems to be correlated with some additional functions of the enlarged midgut nuclei. The filaments at the nuclear periphery get more abundant as cellular hypertrophy progresses. The arrangement of these filaments strongly suggests a nuclear origin, and that they are transported through the nuclear pores to the compartment of the nuclear halo. The main flux of molecules from the nucleus to the cytoplasm consists in mRNA and rRNA which are transported probably through the nuclear pores (NOVIKOFF & HOLTZMAN, 1970). The filaments in the nuclear halo of the chigger flea could represent a filamentous form of the RNA which gets produced in large quantities inside the hypertrophied nucleus. This hypothesis is supported by the fact that the first filaments are always found near the nuclear pores and by some histochemical evidence which suggests that they contain RNA (REINHARDT, 1975). The rer cisterna which encloses the nuclear halo seems to screen the cytoplasm from the filamentous compartment. The function of the halo could then consist in a regulation of the transport mechanisms for RNA from the nucleus to the cytoplasm.

Summary

This morphological study describes the ultrastructure of the midgut of three flea species, including temporary parasitic fleas (both sexes of *Xenopsylla cheopis,* males and immature females of *Echidnophaga gallinacea* and *Tunga penetrans*) and stationary parasitic fleas (mature females of *E. gallinacea* and *T. penetrans*).

(1) Three cell types (a, b, c) constitute the midgut epithelium, each appearing in a characteristic and constant frequency along the whole midgut. a) The functional digestive cells form the main part of the epithelium as one layer of cylindric cells. Nuclear volume and nucleus/cytoplasm ratio have been estimated (with morphometric methods) to be the same in both sexes of *E. gallinacea* and *T. penetrans.* b) Some single regenerative cells or nests, containing 5–10 cells per section plane, lay at the base of the epithelium (2–3 cells or nests per cross section of a midgut). c) Secretory cells are characterized by their opaque cytoplasm which contains electron-dense vesicles. They have few microvilli and no basal labyrinth and are placed between digestive cells only as single cells (1–2 per cross section of a midgut).

(2) After the first bloodmeal some ultrastructural changes occur in the midgut of all fleas. These changes are interpreted as a structural reflection of metabolic processes such as secretion of digestive enzymes, resorption, storage and transport of digested nutrients, and synthesis of digestive enzymes.

(3) More conspicious changes occur in the midgut of the stationary parasitic

and maturing females of *E. gallinacea* and *T. penetrans*. The nuclear volumes of the digestive cells reach the two-fold and eight-fold value, respectively. The nucleus/cytoplasm ratio decreases by half. The corresponding cytoplasmic growth of each cell is reflected in an enlargement of the whole midgut. The digestive cells form long apical cell extensions. The nucleus, basal labyrinth, rer, and golgi complex all change their morphology, and this can be interpreted as the result of a higher level of metabolic activity than during the first bloodmeal.

(4) The midgut of the oviparous female of *T. penetrans* undergoes a process of compensatory hypertrophy which consists in the cellular hypertrophy of each digestive cell and in an extensive proliferation of the regenerative cells. A unique structure, called 'nuclear halo' appears within the process of cellular hypertrophy. This structure consists of a layer of 70–100 Å thick filaments along the periphery of the nucleus. The nuclear halo contains few ribosomes and is screened from the cytoplasm by an rer cisterna. The filaments may contain RNA molecules which are on their way to the cytoplasm.

It has been demonstrated that the structure of the midgut epithelium is influenced by the nutritive and parasitic behavior of the flea. The stationary and oviparous *E. gallinacea* and *T. penetrans* reveal an extensive and irreversible change of their midgut epithelium.

Acknowledgements

This paper is a short form of the Ph. D. Thesis of Reinhardt (1975), in which more ultrastructural details and micrographs are presented. I wish to thank Professor Dr. T. A. Freyvogel and PD Dr. H. Hecker for their helpful advice and supervision during my Thesis studies in the Swiss Tropical Institute, Professor Dr. H. A. Schneiderman (U. C. Irvine) for fruitful discussions and comments, Dr. D. Falk (U. C. Irvine) and Mr. C. Roseland (U. C. Irvine) for reading and correcting the manuscript and Mrs. S. Torri for typing the final text.

Zusammenfassung

Die Ultrastruktur des Mitteldarmes von adulten Männchen und Weibchen der drei Floharten *Xenopsylla cheopis* (tropischer Rattenfloh), *Echidnophaga gallinacea* (Hühnerkammfloh) und *Tunga penetrans* (Sandfloh) wird beschrieben.

1. Das Mitteldarmepithel baut sich aus 3 Zelltypen auf: a) hauptsächlich aus funktionellen Verdauungszellen, b) aus gelegentlichen Regenerationszellen und c) aus vereinzelten, sogenannten Sekretzellen.

a) Die funktionellen Verdauungszellen bilden ein einschichtiges Zylinderepithel. Kernvolumen und Kern/Cytoplasma-Relation sind an beiden Geschlechtern aller Stadien von *E. gallinacea* and *T. penetrans* bestimmt worden.

b) An der Basis des Epithels liegen gelegentlich Regenerationszellen von hoher Elektronendichte. Sie bilden z. T. Nester mit 5–10 Zellen in einer Schnittebene (2–3 Zellen oder Nester pro Mitteldarm-Querschnitt).

c) Die Sekretzellen sind weniger kontrastiert als die übrigen Epithelzellen. Sie sind relativ selten und liegen zwischen den funktionellen Verdauungszellen (1–2 pro Mitteldarm-Querschnitt). Sie enthalten viele kleine Vesikel, welche in ihrer Struktur neurosekretorischen Vesikeln gleichen.

2. Die Strukturveränderungen im Mitteldarm sind nach der 1. Blutmahlzeit in beiden Geschlechtern aller drei Arten verfolgt worden. Sie werden als Ausdruck verschiedener metabolischer Vorgänge interpretiert, wie Sekretion von Verdau-

ungsenzymen, Resorption, Speicherung und Transport von verdauten Nährstoffen, Synthese von Verdauungsenzymen.

3. Die Strukturveränderungen im Mitteldarm der stationär parasitischen Weibchen von *E. gallinacea* and *T. penetrans* sind sehr auffällig. Die morphometrisch bestimmten Kernvolumina erreichen bei *E. gallinacea* den doppelten, bei *T. penetrans* etwa den achtfachen Wert. Der Wert für die Kern/Cytoplasma-Relation sinkt auf die Hälfte. Die dementsprechend hohe Cytoplasma-Volumenzunahme äussert sich in einer Vergrösserung des ganzen Mitteldarmes. Die funktionellen Verdauungszellen bilden lange apicale Zellfortsätze aus. Ultrastrukturell kann eine deutliche Veränderung gewisser Zellorganellen festgestellt werden. Vor allem Kern, basales Labyrinth, RER und Golgi-Komplex lassen die Interpretation zu, metabolisch aktiver zu sein als bei der Verdauung der ersten Blutmahlzeit.

4. Der Mitteldarm des reifenden Sandflohes *T. penetrans* macht einen Prozess der kompensatorischen Hypertrophie durch. Dieser Prozess besteht aus der zellulären Hypertrophie jeder funktionellen Verdauungszelle sowie aus einer umfassenden Proliferation der Regenerationszellen. Im Zusammenhang mit der zellulären Hypertrophie zeigt sich bei den meisten Kernen ein sogenannter Kernhof (nuclear halo). Der Kernhof wird von einer RER-Cisterne vom übrigen Cytoplasma abgeschirmt und ist weitgehend frei von Ribosomen. Er enthält eine Schicht von 70–100 Å dicken Filamenten und umhüllt den ganzen Kern. Die Filamente im Kernhof werden mit aus dem Kern ausgeschleuster RNS in Verbindung gebracht.

Es hat sich gezeigt, dass sich der Bau des Mitteldarmepithels mit dem parasitischen Verhalten des Flohes ändert. Bei den stationär parasitischen Weibchen von *E. gallinacea* und *T. penetrans* sind diese Veränderungen tiefgreifend und irreversibel.

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