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Aspects histologiques, cytologiques et physiologiques de la digestion du sang chez la Tique *Ornithodorus moubata* Murray (*Ixodoidea, Argasidae*)

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Thèse de doctorat de Olivier Grandjean

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Forme réduite de la thèse pour l'obtention du grade de docteur ès sciences de l'Université de Neuchâtel

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IMPRIMATUR POUR LA THÈSE

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UNIVERSITÉ DE NEUCHATEL

FACULTÉ DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel, sur le rapport des membres du jury,

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Neuchâtel, le *1er septembre 1986*

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BLOOD DIGESTION IN *ORNITHODORUS MOUBATA* MURRAY *SENSU STRICTO* WALTON (IXODOIDEA : ARGASIDAE) FEMALES. I. BIOCHEMICAL CHANGES IN THE MIDGUT LUMEN AND ULTRASTRUCTURE OF THE MIDGUT CELL, RELATED TO INTRACELLULAR DIGESTION

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BLOOD DIGESTION IN *ORNITHODORUS MOUBATA* MURRAY *SENSU STRICTO* WALTON (IXODOIDEA : ARGASIDAE) FEMALES I. BIOCHEMICAL CHANGES IN THE MIDGUT LUMEN AND ULTRASTRUCTURE OF THE MIDGUT CELL, RELATED TO INTRACELLULAR DIGESTION

BY Olivier GRANDJEAN *

DIGESTION
CHEZ LES
ARGASIDAE
STOCKAGE
DE
L'HÉMOGLOBINE
DIGESTION
INTRACELLULAIRE
HÉTÉROPHAGOSOMES

RÉSUMÉ : La digestion du sang chez les femelles d'*Ornithodorus moubata* Murray *sensu stricto* Walton (Ixodoidea : Argasidae). I. Changements biochimiques dans la lumière du mésentéron et ultrastructure des cellules mésentérales, en rapport avec la digestion intracellulaire.

La digestion du sang par la Tique *O. moubata* a été étudiée, tant par des méthodes physico-chimiques, pour le contenu de la lumière intestinale, que par des méthodes cytologiques, en particulier par la microscopie électronique, pour les cellules intestinales. Il en résulte que la lumière intestinale ne participe pas à la digestion, malgré une chute du pH observable chez les Tiques, plusieurs jours après leur nutrition. En effet, l'hémoglobine non digérée est stockée (sous forme de cristaux orthorhombiques dans le cas du Cobaye) et peut être retrouvée intacte dans l'intestin de Tiques jeûnant depuis des mois. La digestion est donc intracellulaire et correspond à un concept lysosomal : Les vacuoles de phagocytes et les vésicules de pinocytose deviennent des hétérophagosomes par la fusion avec des lysosomes primaires, produits au niveau des dictyosomes et qui contiennent des enzymes digestifs intracellulaires. Les modifications ultrastructurales dans le cadre du cycle digestif sont rapportées dans un autre travail (GRANDJEAN, 1983).

DIGESTION
IN
ARGASIDAE
STORED BLOOD
INTRACELLULAR
DIGESTION
HETEROPHAGOSOMES

ABSTRACT : Digestion of blood by the soft tick *O. moubata* is investigated by physico-chemical methods in the extracellular compartment of the midgut lumen and by electron microscopy in the intracellular compartment of the midgut cells. Undigested blood may be stored (in form of orthorhombic crystals, in the case of guinea-pig blood) over months in the midgut lumen, although a drop of the pH is observed there some days after bloodmeal. Digestion is intracellular indeed and corresponds to a classical lysosome concept, leading to heterophagosomes by the fusion of phagocytosed material or pinocytotic vesicles with intracellular enzymes produced by the GERL complex. Ultrastructural changes related to the digestive cycle are related in a following paper (GRANDJEAN, 1983).

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1. INTRODUCTION

The digestion of a bloodmeal plays an essential role in the biology of the tick. Important processes, such as molting or vitellogenesis, follow bloodmeals and require considerable energy and mass changes, the source of which is provided from the nutrient, by means of the digestive processes.

As digestion is intracellular in ticks, the midgut lumen contents undergo only few important modifications, namely in relation to blood concentration, erythrocyte haemolysis and lysis of midgut cells which completed intracellular digestion. Due to the buffering capacity of fresh blood, the midgut lumen is probably not too unfavorable for survival of transmissible pathogens, such as spirochetes of African Relapsing Fever (BURGDORFER, 1951), although bactericidal substances have been described in tick midguts (DUNCAN, 1926, PODBORONOV & *al.*, 1975). Our experiences, carried out with Ciba-Geigy, Basle (Dr. J. NÜESCH), also proved midguts of *O. moubata* to be sterile.

The present study should contribute to better understanding of the actual mechanisms involved in digestive activity, both in the intracellular and extracellular compartments. But the basic cellular processes must also be placed in a dynamical context of cell changes, during the digestive cycle (GRANDJEAN & AESCHLIMANN, 1973, GRANDJEAN, 1983).

2. ON SOME BIOCHEMICAL CHANGES (pH, HAEMOGLOBIN) OCCURRING IN THE MIDGUT LUMEN, AS RELATED TO THE DIGESTIVE CYCLE

2.1. Material and Methods

Ticks : An *O. moubata sensu stricto* strain, originally from Tanzania (Ulanga District), was bred for several years according to GEIGY & HERBIG (1955). Both male and female ticks were fed on guinea-pigs and their crude midgut contents were withdrawn at various intervals after bloodmeal.

pH-measures : The collected crude sample (20-100 μl) were measured with a Metrohm E 300 B pH-meter with a fine electrode, immediately after sacrifice of the tick.

Spectroscopical methods : The crude samples were placed under a carbone monoxyde atmosphere to freeze the momentary equilibrium between bivalent (oxyhaemoglobin) and trivalent iron (methaemoglobin). Non-crystallised material was diluted with acetate buffer (pH = 5) and measured with a Beckmann DB and Acta V Double Beam Spectrophotometer of the Friedrich Miescher Institute, Basle, Switzerland (Prof. K. H. WINTERHALTER).

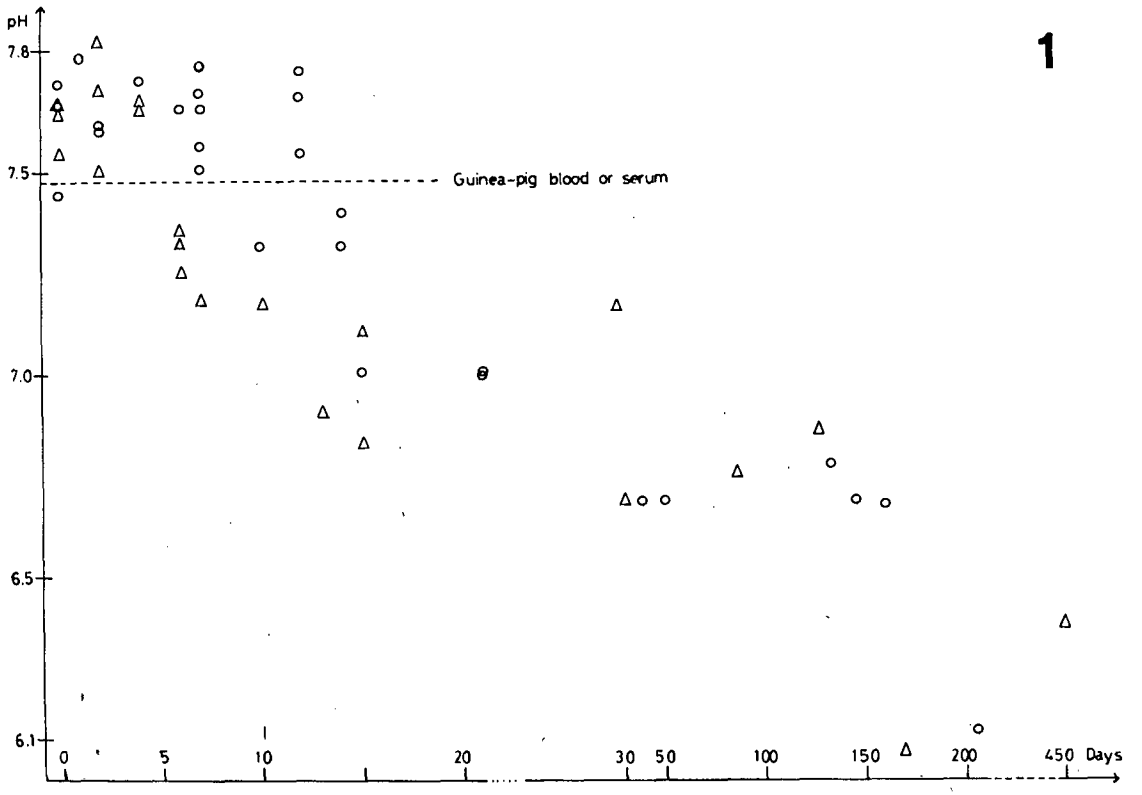
Crystallised haemoglobin placed in ammonium sulfate was measured with a Zeiss UMSP I (Universal Microspectrophotometer) at Wissenschaftlicher Dienst, Kriminalpolizei, Zürich, Switzerland (Dr. R. HALONBRENNER).

Part of the results have already been published (SMIT & *al.*, 1977) and will not be further discussed.

2.2. Changes of pH in the midgut lumen, related to digestive cycle and compared to pH of haemolymph

a) *Results*

The pH of the midgut content has revealed not to be constant, but first to rise as compared to that of guinea-pig blood before ingestion, and later to decline rapidly (Fig. 1). These results were coherent in females and males, but the falling off started earlier in males (after about 5 days) than in females (10 to 15 days).



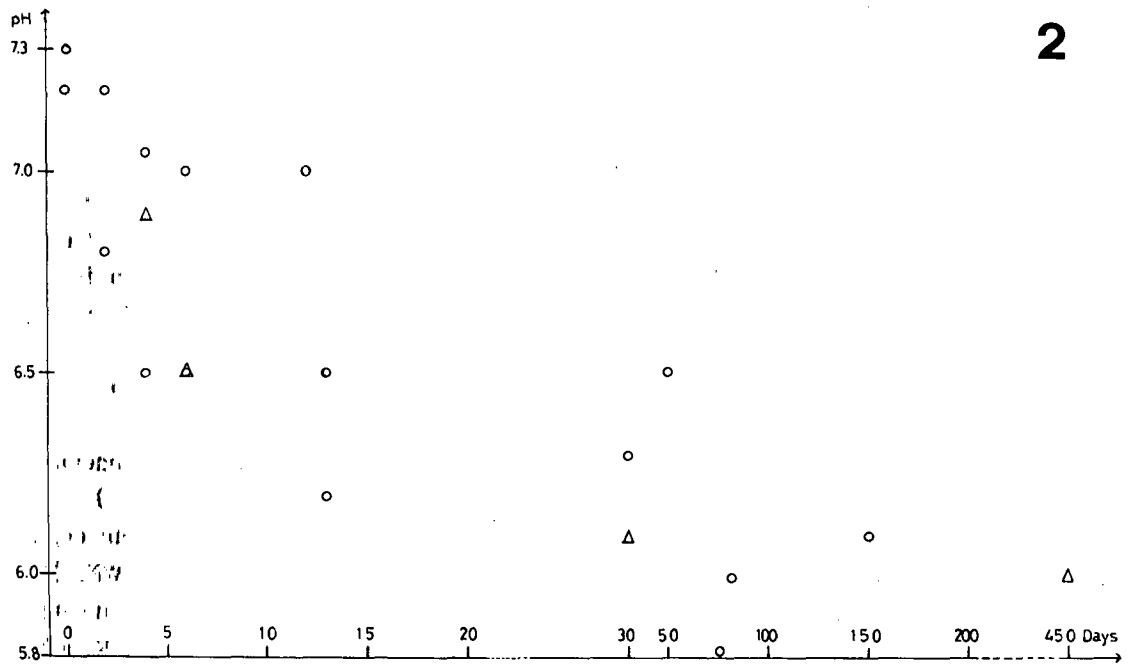


FIG. 1 : Changes of pH in the midgut lumen of *O. moubata* as related to digestive cycle (O = ♀, Δ = ♂).

FIG. 2 : Changes of pH in the haemolymph of *O. moubata*, as related to the digestive cycle (O = ♀, Δ = ♂).

A similar initial rise in pH linked with feeding has also been found in the haemolymph of the ticks (Fig. 2). pH of haemolymph appears to be always lower (about half a pH unit) than that of the midgut content, and it starts decreasing earlier than in the latter.

b) *Discussion*

It may be assumed that the blood taken up initially conserves its own pH within the midgut. The following fall of the pH would be connected with digestive activity (release of acid lysosomal contents of lysed midgut cells).

The pH-values obtained for tick midgut contents do not coincide with the much lower pH-optima (2,5-4) for their proteases (TATCHELL, 1964, BOGIN & HADANI, 1973, AKOV & *al.*, 1976). This may be linked to the fact that intracellular digestion does not involve enzymatic activity within the midgut lumen (AKOV & *al.*, 1976). Thus storage, rather than digestive processes, is the prevalent function in the midgut lumen.

2.3. Changes of midgut content, as revealed by microscopy

a) *Results*

Thrombocytes of the guinea-pig blood taken up disappear almost within the first day after bloodmeal, being phagocytosed, and leucocytes have virtually vanished after two days. Erythrocytes are often rounded. If they are not phagocytosed by midgut cells, they remain intact up to five or six days after bloodmeal. Then haemolysis starts and the haemoglobin released from lysed erythrocytes starts crystallising within the midgut lumen. Numerous crystals (Fig. 3) grow into the lumen and become mixed with haematin granules released as digestive residues by the midgut cells. The midgut content changes in colour from light red (before haemolysis) to brownish red (during crystallisation) and finally brownish black (with dominating haematin granules).

FIG. 3 : Scanning electron micrograph of a haemoglobin crystal from the midgut of a female *O. moubata*, 8 days after bloodmeal. Preparation : fixation 2,5 % glutardialdehyde, 2 % osmium tetroxyde ; dehydration to absolute ethanol and to 100 % amyl acetate, air dried, covered with carbon / gold (acceleration : 10 kV ; SEM-Laboratory, University of Basle, Switzerland, Dr. R. Guggenheim). Axes of the orthorhombic crystal are shown (arrows) ; scale in micrometers (μm).

b) *Discussion : Intraluminal haemolysis and crystallisation*

Haemolysis has been shown to occur earlier in males than in females (OSTERHOFF & GOTHE, 1966), which possibly corresponds to our findings of the earlier decrease of pH (Fig. 1) and earlier change from red to brown observed in midgut contents of males, as compared to those of females of *O. moubata*.

The almost tetraedrical structure of haemoglobin crystals (Fig. 3) (SMIT & *al.*, 1977) corresponds to that of guinea-pig haemoglobin crystallised either *in vitro* (REICHERT & BROWN, 1909, BOOR, 1930) or within midguts of other haematophagous arthropods (BIOCCA, 1950, PICK, 1965).

2.4. Spectroscopical results

a) *Uncrystallised fraction of the midgut content*

During digestion, the haemoglobin content of the midgut lumen diminishes and has almost disappeared in fasting ticks (Fig. 4). The midgut lumen of female ticks, up to 6 days after blood-meal, contains oxyhaemoglobin as does fresh guinea-pig blood (absorption peaks of carboxy-haemoglobin, A = 540, A′ = 570 nm in Fig. 5). It later contains mainly haemichrome (C = 530, C′ = 565 nm in Fig. 5). Tests with additional cyanide (see SMIT & *al.*, 1977) show that methaemoglobin is absent from non-crystalline material.

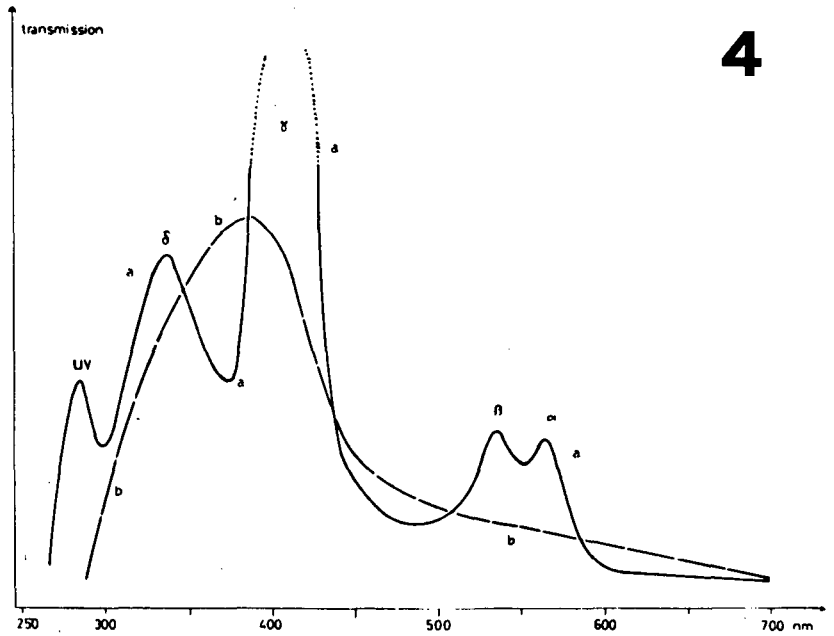


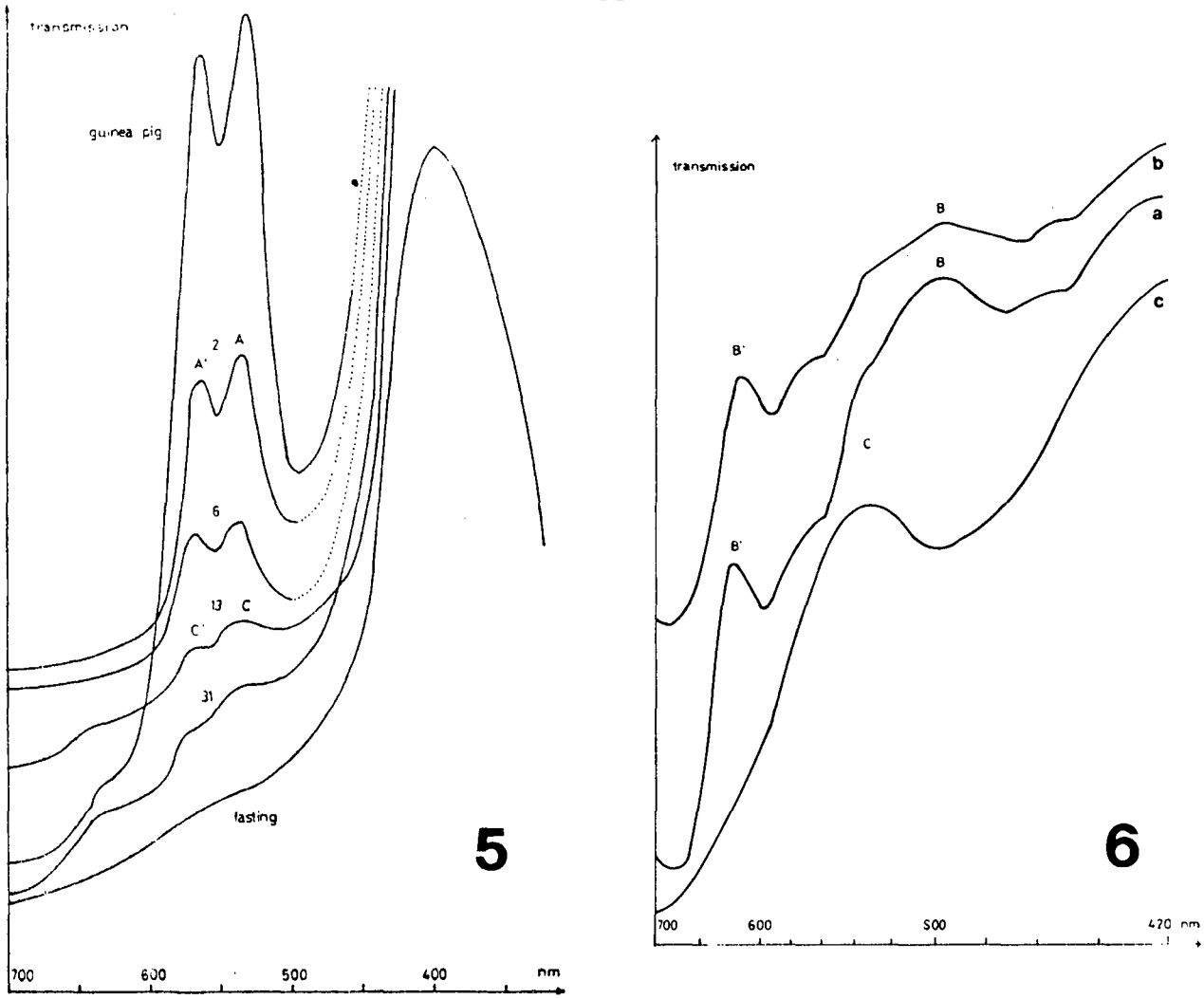
FIG. 4 : Spectroscopical analyses of the non-crystallised fraction of midgut content in *O. moubata*. a = female, 4 days after blood-meal, b = fasting female ; absorption maxima of carboxyhaemoglobin : α = 570, β = 540, γ = 420, δ = 345, UV = 275 nm (Acta V DB Spectrophotometer).

b) *Crystallised fraction of the midgut content*

Analysis of the oxydation state of haemoglobin iron with visible absorption spectroscopy shows that haemoglobin crystallises in its oxy-form (A & A′ in Figs 7a, 8b, c) and then gradually changes to methaemoglobin (B = 500, B′ = 630 nm in Figs 6a, b, 7b, c, 8a). Conversion is not synchronous is all crystals as an important part of oxyhaemoglobin may be found still in older crystals (e.g. Figs 7c, 8d).

The methaemoglobin within the crystals from tick midguts (Fig. 6a) is similar to that formed by oxydation of guinea-pig blood in the air (Fig. 6b). Presence of methaemoglobin is proved by conversion into cyanmethaemoglobin (Fig. 6c, 7d : disappearance of peak B′). Evidence show that methaemoglobin may be converted to haemichrome, without cracking of the crystal (Fig. 6c and SMIT & *al.*, 1977 : Fig. 4). The latter transformation would indicate a partial degradation of the globin chain of methaemoglobin.

No fundamental differences are found between crystals taken from midguts of males (Fig. 7) and those from females (Fig. 8).



FIGS 5-6 : Blood digestion in *Ornithodorus moubata*.

A, A′ = peaks of carboxyhaemoglobin
B, B′ = peaks of methaemoglobin
C, C′ = peaks of haemichrome

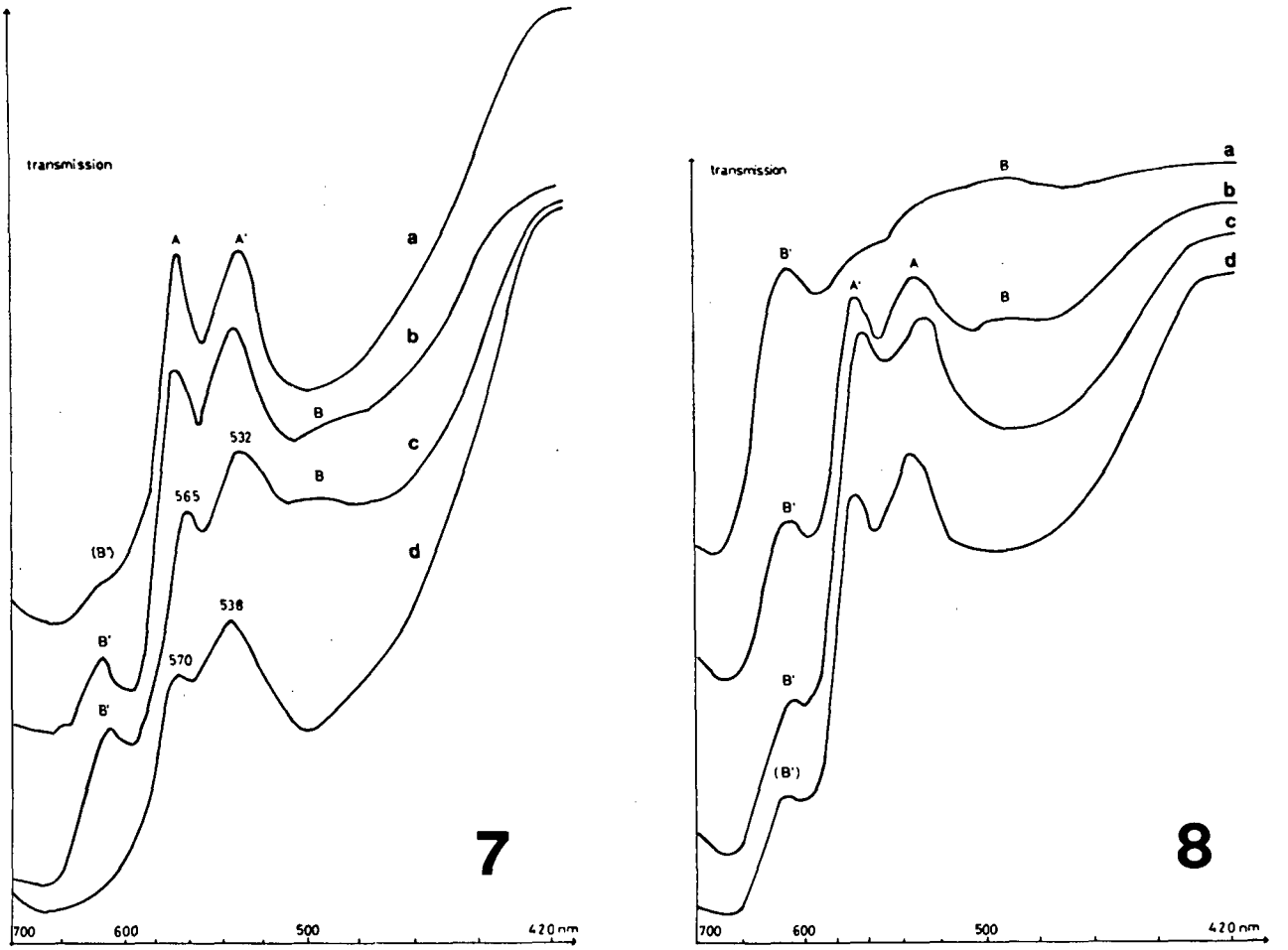
5 : Spectroscopical analysis of change occurring in the non-crystallised fraction of midgut content in *O. moubata* females, as related to digestive cycle. (Beckmann DB Spectrophotometer). Numbers indicate days after bloodmeal.

6 : Spectroscopical analysis of : a = guinea-pig haemoglobin oxydised in air, not under CO-atmosphere ; b = haemoglobin crystal from the midgut of a female, 30 days after bloodmeal ; c = as b, treated with cyanide (0,01 M KCN, see SMIT & *al.*, 1977).

c) *Discussion on the fate of crystallised oxyhaemoglobin*

Conversion to methaemoglobin is intracrystalline, as no methaemoglobin was found in the non-crystallised fraction of the midgut content. Conversion from oxy- to methaemoglobin appears to be faster in ixodids (O'HAGAN, 1974) than in argasids (TATCHELL, 1964) : our results also show that crystals may still contain considerable amounts of oxyhaemoglobin over prolonged periods in *O. moubata*.

It is not known how larger crystals are made available for midgut cells (SMIT & *al.*, 1977) ;



FIGS 7-8 : Blood digestion in *Ornithodorus moubata*.

Spectroscopical analysis of changes occurring in the crystallised fraction of midgut content of *O. moubata*, as related to digestive cycle : males (a = 6, b = 30, c = 450 days after bloodmeal, d = as c, treated with cyanide). Numbers indicate wave-lengths (nm).

8 : As fig. 7 :
females (b = 6, c = 12, a = 30, d = 85 days after bloodmeal).

smaller ones may be eventually phagocytosed. Our findings of a possible conversion to haemichrome crystals could indicate haemoglobin crystals to get enzymatically attacked and to loose their globin moieties. This could also agree with the high amino-acid content found of the midgut lumen in *Argas* (BOCTOR & ARAMAN, 1971), in some contradiction to the intracellular way of digestion.

The final fate of haemoglobin in the tick is to become digested within midgut cells up to the haematin residues (WIGGLESWORTH, 1943) which are partly reused for synthesis of vitellogenins in the female ticks (KITAOKA, 1961, DIEHL, 1970, O'HAGAN, 1974) or for body coloration in some ixodids (FRICK, 1936).

2.5. Description of a whitish acellular mass found in the midgut of several ticks

Since 1972, few ticks of our laboratory strain revealed to contain some acellular whitish mate-

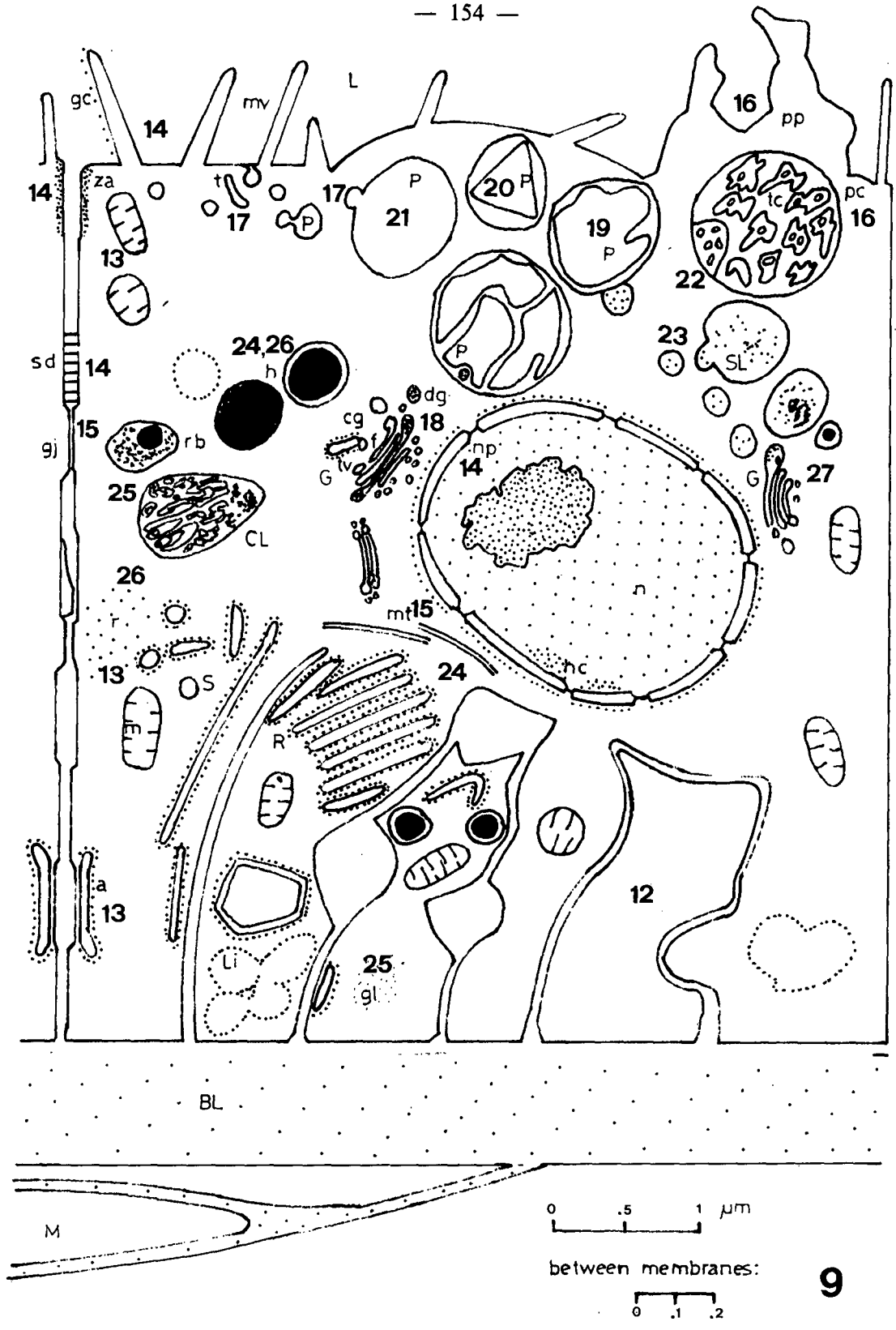


FIG. 9 : Schematic drawing of the midgut cell of *O. moubata*, summarising ultrastructural observations (see also text ; numbers indicate other figures).

rial. The number of suck ticks increased. Both males and females contained such material, at quite different stages of their digestive cycle. The whitish material therefore seems not to be related to digestion. It has a fibrillar structure (Fig. 10 : f) with a central stalk bearing small corpuscules (arrows) in grape-like formations. Fibrils covered with agglutinated corpuscules were often seen in the vicinity of debris of lysed cells or of midgut cells which contained vesicles with fine granular material (Fig. 11). We suppose that the fibrillar material, which contains (muco)polysaccharides and possibly chitin, is produced by midgut cells. Only in ticks containing such material, we found particular dictyosomes which produce moderately opaque, finely granular and homogeneous material (Fig. 11) as described also for periproventricular cells (GRANDJEAN, in prep., Fig. 30).

The moderately opaque, apical vesicles are comparable to those containing precursor material for the peritrophic membrane in the mosquito *Anopheles stephensi* (STAEUBLI & *al.*, 1966).Involvement of both typical and periproventricular midgut cells could also be suggestive of a peritrophic membrane (ZIMMERMANN & PETERS, 1971). Yet, if there are striking similarities in the histological structure between the fibrillar material in *O. moubata* and the peritrophic membrane of *Leiobonum* sp. (Opiliones : Phalangidae ; PETERS, 1967), ultrastructure of the peritrophic membrane of the Acarine *Dermatophagoides farinae* (WHARTON & BRODY, 1972) differs from our results. It is not known which conditions were favorable for such a phenomenon in our laboratory strain, as compared to native animals.

3. CYTOLOGY OF MIDGUT CELLS AND INTRACELLULAR DIGESTION

3.1. **Methods in Transmission Electron Microscopy** (TEM)

Midgut pieces are prefixed in glutardialdehyde (rather 2 — 2,5 % than 5 % ; in 0,1 M Na-cacodylate buffer, 3 h, room temperature), washed in Na-cacodylate buffer (0,2 M + 4 % saccharose, over night) and postfixed with cold osmium tetroxyde (1 % in 0,2 M Na-cacodylate buffer, 2 h), all at pH 7,2 — 7,3. For several series, some of the above values were moderately changed (see also GRANDJEAN & AESCHLIMANN, 1973). Block staining was generally performed with uranyl-acetate (1 — 2 %, either in acetone 50 % or in distilled water), before dehydration in acetone and embedding in Taab's Spurr (or in Epon 812, after treatment with propylene oxyde).

Ultrafine sections were stained with uranyl and lead salts and observed with an electron microscope (Philips EM 201 at the Zoological Institute, Neuchâtel, and, to a lesser extent, Philips EM 300 at the Botanical Institute, Fribourg, and Zeiss EM 9a at the Swiss Tropical Institute, Basle Switzerland).

Key to abbreviations used in the figures 9-27.

a = special cell membrane – associated, paired RER saccules, cg = clear Golgi vesicle, dg = dark or dense Golgi vesicle, e = erythrocyte, f = forming face of the dictyosome, fs = fibrillar structure of whitish acellular mass, gc = glycocalyx, gj = gap junction, gl = glycogen, gv = great clear vacuole, h = haematin granule, hc = heterochromatine, m = mitochondria, mt = microtubules, mv = microvilli, n = nucleus, nl = nucleolus, np = nuclear pores, pp = pseudopod-like expansion, r = ribosomes, rb = residual body, sd = septate desmosome, t = tubular apical channel, tc = thrombocytes, tv = transitional vesicle, za = *zonula adhaerens*.

BL = basal lamina, CL = cytolysosome, G = dictyosomes (Golgi system), L = midgut lumen, Li = lipid inclusion, M = muscle cell, P = phagosome, R = RER, SL = secondary lysosome, W = *Wolbachia sp.* (rickettsia-like microorganism).

For each figure, the scale is expressed in micrometers (μm).

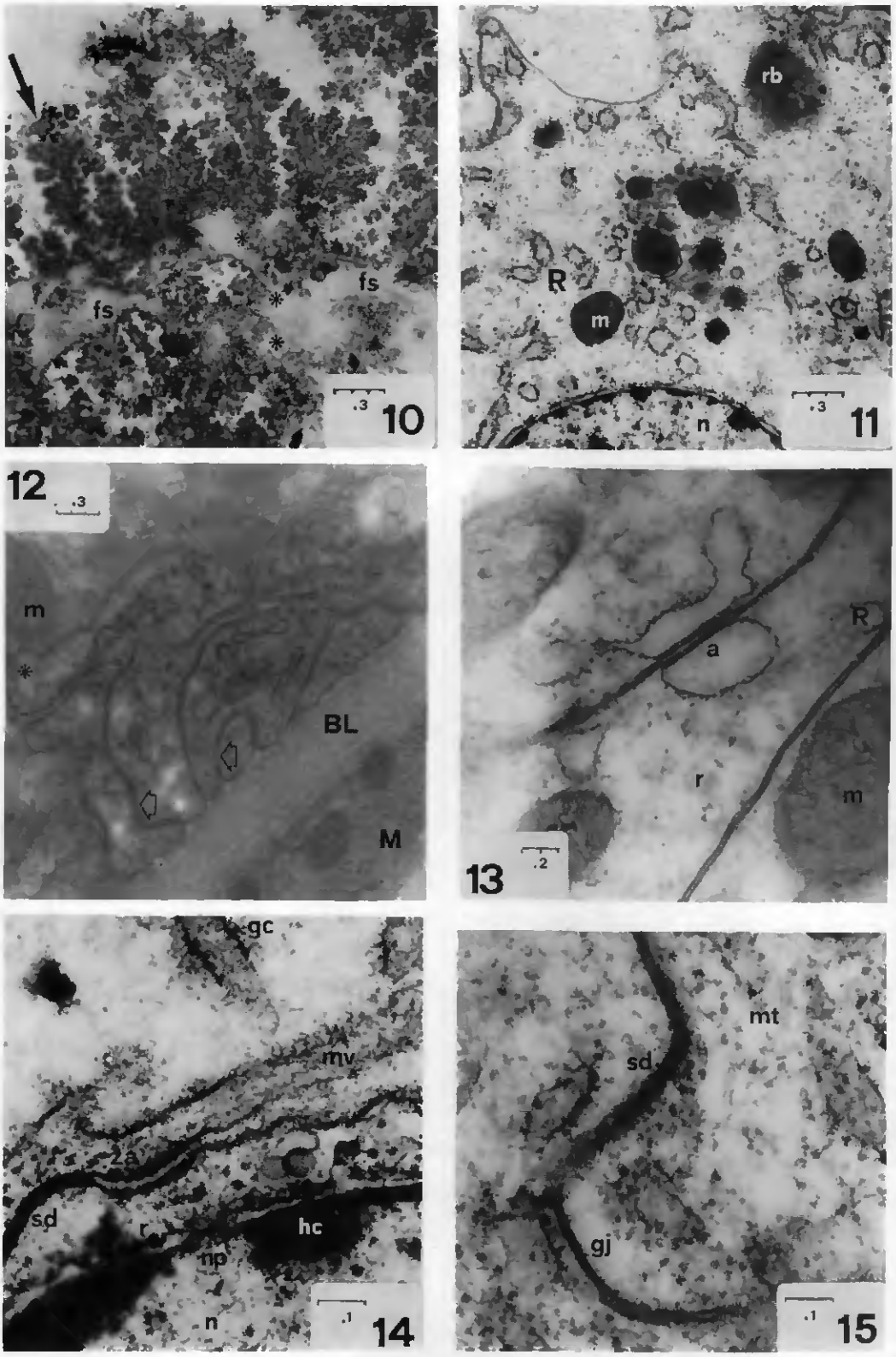


FIG. 10-15 : Blood digestion in *Ornithodorus moubata*.

10 : Detail of fibrillar structure in unfed tick (arrows = small corpuscular elements, * = dividing central fibril).

11 : Detail of cytoplasm of a midgut cell, 26 days after bloodmeal (* = moderately opaque content of Golgi vesicles, arrows = dense content within RER).

12 : Basal part of a midgut cell, 5 days after bloodmeal, showing invaginations of the basal cell membrane (arrows ; * = lacunar extracellular space).

13 : Basal part of a cylindrical midgut cell of a hungry tick (see GRANOJEAN, 1983).

14 : Nucleus and apical part of a distended midgut cell, of a gorged female.

15 : Lateral cell membranes of adjacent midgut cells, 6 days after bloodmeal, with septate desmosomes and gap junctions.

3.2. Cytological results

Investigations have been carried out mainly on female ticks. Some male ticks were also sacrified and did not show fundamental differences in their midgut cytology, as compared to females. Cytological results are schematically summarised in Fig. 9.

a) *Cell membranes and basal lamina*

The *basal lamina* of the midgut is thicker than that of other tissues (foregut, muscle or nerve cells, tracheoles, fat body) : 0,5 — 0,6 μm. It consists of acellular, homogeneous and granular material without any periodical structure.

The *basal cell membrane* shows numerous infoldings building up a basal labyrinth (Fig. 12 : arrows), as typical for transporting epithelia (SJOESTRAND, 1956, ANDERSON & HARVEY, 1966). Adjacent folded membranes either form a *zona continua* or septate desmosomes (both with a width of 25 nm including the membranes), or may be separated by a wider, basal and extracellular lacunar system (Fig. 12 : *). The basal membrane infoldings may be associated with RER saccules of a special type, fitting with one face to the cell membrane and baring ribosomes on the other (Fig. 13 : a). Such elements are generally present on both sides of the slightly enlarged (33 nm) intercellular space, delimited by the basal cell membranes.

The *lateral cell membranes* have a similar structure as the basal infoldings, in their basal part. Centrally or apically, they are characterised by their junctional structures such as septate desmosomes (Figs 14, 15 : sd ; width, including membranes : 25nm, interseptal distance 16 nm) *zonulae continuae* and gap junctions (Fig. 15 : gj). The apical part of the lateral membrane consists of a *zonula adhaerens*, the slightly enlarged intercellular space being separated by the membrane from an internal covering with dense material (Fig. 14 : za).

The *apical cell membrane* is generally differenciated into microvilli (Figs 14, 17, 19 : mv) which are covered with the external dense material of the glycocalyx (Fig. 14 : gc).

b) *Absorption of nutrient by micropinocytosis or phagocytosis : formation of phagosomes*

In the apical part of the midgut cell, turned towards the lumen, material is taken up. This may be performed by formation of micropinocytotic vesicles from the apical cell membrane (Fig. 17), generally at the basis of the microvilli. Vesicles are surrounded by a coat of dense material ("coated vesicles", arrow) or, in several cases, rather tubular structures contain the absorbed material without being surrounded by a coat (Fig. 17 : t). Micropinocytotic vesicles may fuse together (*) and form a larger food vacuole or phagosome (P).

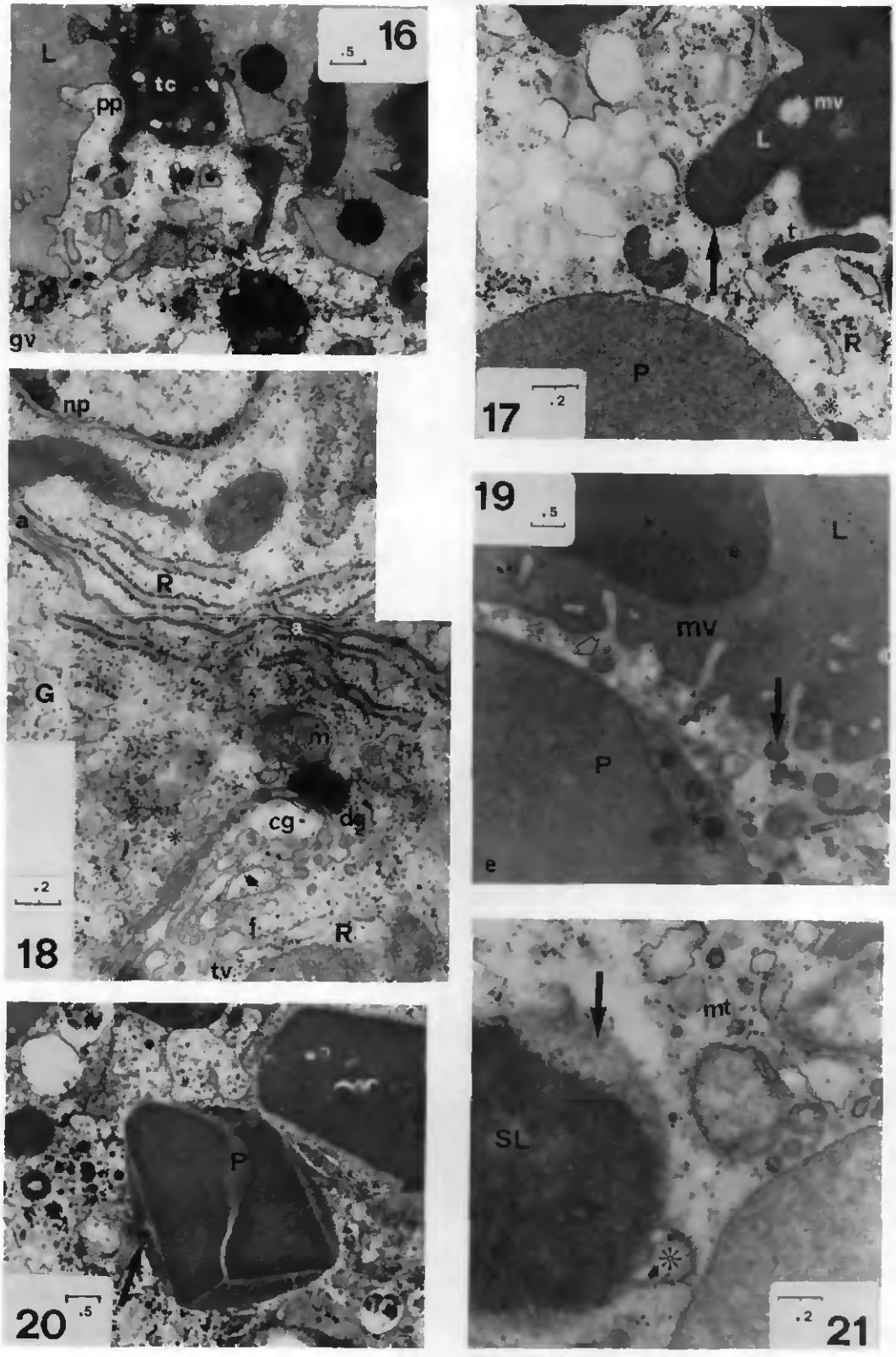
Alternatively the uptake, especially of cellular components of the blood to be digested, occurs by phagocytosis. The apical part of the cell is able to project poorly differenciated pseudopodia-like expansions (Fig. 16 : pp) which surround and engulf the nutrient particles. The latter may be thrombocytes (tc), leucocytes, erythrocytes (Fig. 19 : e) or crystallised haemoglobin (Fig. 20). They are all enclosed within a membrane-bound vacuole, the phagosome (P).

c) *Nucleus*

Nuclei of midgut cells are larger than those of other cells of the tick body (midgut cells : ∅ 4 — 6 μm, foregut or muscle cells : ∅ 2 — 3 μm). The perinuclear cisterna is covered on its cytoplasmic face with ribosomes (Fig. 14 : r) and is often associated with dense heterochromatin material (Fig. 14 : hc) on its inner, nuclear face. It is interrupted by nuclear pores (Figs 14, 18 : np, ∅ 65 nm). The nucleus contains one nucleolus (sometimes two). We could not observe any mitotic figure of the nucleus at an ultrastructural level.

d) *Rough endoplasmic reticulum (RER) and ribosomes*

Free ribosomes may be present over large cell areas (Figs 13, 26 : r), but are often bound to



FIGS 16-21 : Blood digestion in *Ornithodorus moubata*.

16 : Phagocytosis of thrombocytes in a protruding cell, shortly after bloodmeal.

17 : Apical part of a midgut cell, 1 day after bloodmeal, showing micropinocytotic activity (arrows = coated vesicles, * = fusion of vesicles.

18 : Central part of a midgut cell, 5 days after bloodmeal, showing nucleus, RER and dictyosome (* maturing face of dictyosome, arrow = small vesicle enclosed in a larger clear Golgi vesicle).

19 : Phagosome with an erythrocyte and undissolved Golgi vesicles (*) ; micropinocytotic coated vesicles (arrows), 2 days after bloodmeal.

20 : Phagocytosis of haemoglobin crystals : phagosome with undissolved Golgi vesicles (arrow), 7 days after bloodmeal.

21 : Fusion (*) of a small phagosome with vesicular material (arrows), first day after bloodmeal.

vesicular (Figs 13, 17 : R) or saccular structures (Fig. 18) of the RER, the latter being sometimes arranged in a stack of numerous adjacent saccules (Fig. 24). Specialised saccules, either adjacent to the cell membrane (Figs 13, 18 : a, see above) or surrounding glycogen inclusions are devoid of ribosomes on their face turned towards the cell membrane or the glycogen, respectively (in a similar way as the perinuclear cisterna). There are also some cytoplasmic vesicles completely devoid of ribosomes.

e) *Golgi apparatus and primary lysosomes*

The dictyosomes may be numerous in the midgut cells, either in perinuclear or apical position (Fig. 24) or in-between numerous food vacuoles (phagosomes ; Fig. 27). They show a distinct polarity between the convex forming face (Fig. 18 : f), in the vicinity of RER saccules (R) and transitional vesicles (tv), and the rather concave maturing face, where small vesicles containing granular material are budding off (*). Laterally, larger Golgi vesicles separate from the stack, either with a clear (cg) or with an opaque granular content (dg). The small vesicles and the large dark vesicles correspond in size and structure to the type 2 and type 1 Golgi vesicles described and succesfully tested for acid phosphatases by RAIKHEL (1975) in midgut cells of *Hyalomma asiaticum* (Ixodidae). They may be considered as primary lysosomes.

f) *Digestive vacuoles : Secondary lysosomes*

Actual intracellular digestive processes start with the fusion of primary lysosomes with phagosomes. We observed the fusion of phagosomes with vesicles (Fig. 21 : *) which contained smaller vesicles (arrows) comparable to those empacked in clear Golgi vesicles (Fig. 18 : arrows). In other cases we found dark Golgi vesicles within phagosomes, the content of the former seeming not yet to have escaped the vesicular membrane nor mixed with the phagosomal content (Figs 20, 27 : arrows, Fig. 17 : *). Thus there is the possibility of a delayed instead of an immediate fusion. Enzymes contained in the vesicles might fuse with the substrate of the phagosome not immediately, but after some delay.

Structural changes within the phagolysosomes (secondary lysosomes) appear more dramatic in the case of digestion of thrombocytes (Fig. 22 : SL 1 & 2) than in that of erythrocytes. In the latter case, the most apparent changes are the loss in opacity of the vacuole which also becomes smaller (Fig. 27). Membranous and vesicular elements are temporarily present in some phagolysosomes, but generally soon disappear.

Late secondary lysosomes have the aspect of small clear vacuoles (∅ ca. 2 μm) with granular and some membranous content (fig. 23). These vacuoles may fuse together (arrows and *) and build up huge vacuoles surrounded merely by a thin peripheral cytoplasmic layer (GRANDJEAN, 1983).

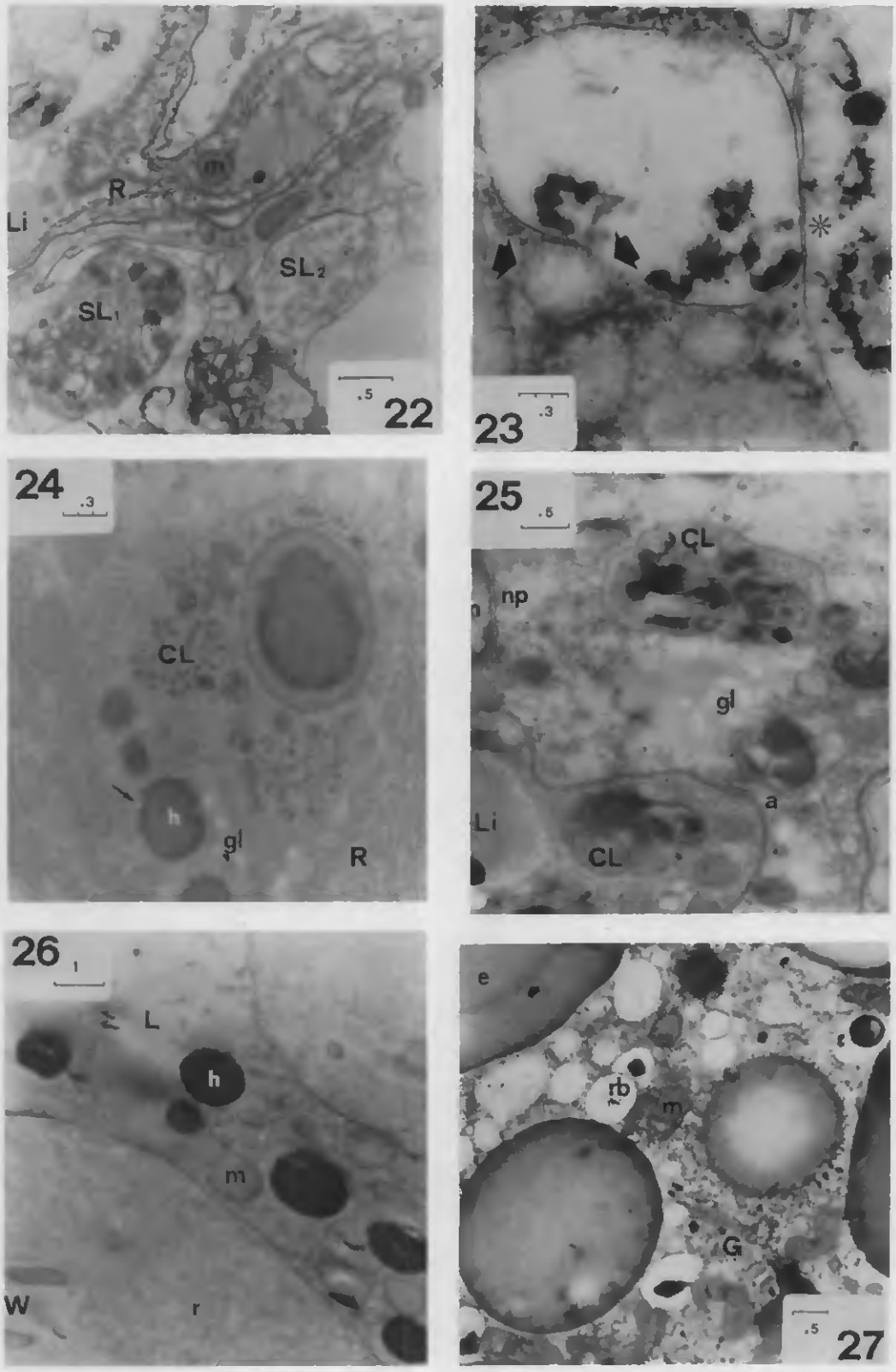
g) *Residual bodies*

Haematin granules : The protein part of haemoglobin is digested inside the secondary lysosome, the resulting aminoacids or peptides being presumably taken up across the lysosomial membrane into the cytoplasm. Residues of the haeme group are stored as haematin granules within the cell (∅ 0,5 — 2 μm or more). These granules with a high opacity are only rarely found within late secondary lysosomes. They are generally built up in vicinity of the RER, either basically or in the centre of the apical part of protruding midgut cells (Fig. 24). Some haematin granules are surrounded by a membrane (arrows), whilst others are not. After lysis of the midgut cell, the haematin granules released remain in the midgut lumen up to the death of the tick.

Cytolysosomes : Residual bodies of midgut cells having accomplished digestion (GRANDJEAN, 1983) contain granular (Fig. 24) or membranous material (Fig. 25), formed of disorganised cell structures or undigested residues. Residual bodies are stored within the cell and released into the midgut lumen when the cell disrupts (Fig. 26).

h) *Storage inclusions*

An important part of nutrient is made available for the ticks midgut cell for immediate metabolic



FIGS 22-27 : Blood digestion in *Ornithodorus moubata*.

22 : Detail of a midgut cell having digested thrombocytes, 2 days after bloodmeal.

23 : Apical part of a protruding midgut cell formerly loaded with phagosomes, showing the resulting late secondary lysosomes (clear vacuoles), 17 days after bloodmeal ; fusion (*, arrows) of late secondary lysosomes.

24 : Detail of a midgut cell, 5 days after bloodmeal, showing the well developed RER and a cytolysosome which contains granular material (arrow = membrane surrounding a haematin granule).

25 : Perinuclear part of a midgut cell of a fasting tick, showing several cytolysosomes.

26 : Apical part of columnar midgut cells of a fasting tick with microorganisms and free ribosomes, the content of one disrupted cell being released in the midgut lumen.

27 : Central part of a protruding midgut cell loaded with phagosomes, 5 days after bloodmeal, showing a dictyosome, (G), RER and residual bodies (arrow = undissolved vesicle).

activities (such as protein synthesis). Other food material may be stored, either as glycogen grains (Figs 24, 25 : gl) or as lipid vacuoles (Figs 22, 25).

i) *Mitochondria, microtubules, Rickettsia-like microorganisms*

Mitochondria have a typical elongate and cristate structure (NOVIKOFF & HOLZMAN, 1970). They are numerous in the basal part of the midgut cell (Fig. 12) and also present in the central (Fig. 18) and apical (Fig. 13) part of the cell.

Microtubules are not uncommon in midgut cells of *O. moubata*, especially in the subapical part of the cell (Fig. 15).

Rickettsia-like microorganism (*Wolbachia sp.*) are present in numerous midgut cells at different stages of the digestive cycle (Fig. 26), but do not seem to be linked with digestive processes of the tick [1].

3.3. **Discussion :**

a) *Lysosome concept of intracellular digestion*

Results on the ultrastructure of the midgut cells of *O. moubata* are in accordance with the general lysosome concept of intracellular digestion (de DUVE & WATTIAUX, 1966). The absorption of particulate food and formation of heterophagosomes by phagocytosis corresponds to a primitive cell function connected with the phylogenetically primitive character of intracellular digestion (SCHLOTTKE, 1934). Phagocytosis is the main way of entry of food into the midgut cell, but some material is also taken up by micropinocytosis. It seems, as compared with *Hyalomma asiaticum* (RAIKHEL, 1974b), that coated vesicles are more numerous in *O. moubata* than apical tubular channels. Larger phagosomes are formed by fusion of micropinocytotic vesicles.

The formation of heterophagosomes presumably triggers the synthesis of proteases, as already admitted for mammalian cells (HOLTZMAN, 1976). Proteases (amongst which cathepsin D appears to play a major role, AKOV & *al.*, 1976) are synthetised within the RER of the tick midgut cell and pass through the dictosomes. They form two types of primary lysosomes : smaller and larger vesicles, as also described in ixodids (RAIKHEL, 1975).

There is evidence of a possibly delayed fusion of the primary lysosome with the heterophagosome, as decribed for mammalian cells (de DUVE & WATTIAUX, 1966). Such delayed fusions could also play a role in the ability of *H. asiaticum* nymphs to store haemoglobin intracellularly for several days, up to the actual digestion (BALASHOV & RAIKHEL, 1976). They could further be a regulating mechanism in the context of digestion being faster in mated than in virgin *O. moubata* females (GRANDJEAN, 1983). Results of TATCHELL & *al.* (1972), showing the persistance of a certain protease level in virgin females of *Argas persicus*, and the statement of AKOV & *al.* (1976) that proteases are intracellular in the argasid midgut, would support such a hypothesis.

In the secondary lysosome (heterophagolysosome) of *O. moubata* midgut cells, erythrocytic membranes appear to become more readily digested than in other haematophagous cells, which often contain undigested residues after digestion of an erythrocyte : membranous and crystallised materials in *Entamoeba histolytica* (GRIFFIN & JUNIPER, 1971) or mammalian macrophages (COLLET & PETRIK, 1970). We therefore admit that phospholipases must be present within the primary lysosomes, which would be supported by the evidence of unspecific esterases being present within argasid midgut cells (TATCHELL, 1964, *A. persicus*). The transport mechanism of digested food from the heterophagolysosome through the lysosomial membrane is not exactly known (HOLTZMANN, 1976). We admit that aminoacids are reused by the midgut cell for protein synthesis and that haematin granules are built up in the vicinity of the RER (connections between RER

1. We found them also in greater numbers within midgut cells of male ticks and hereby complete the list of localisation of *Wolbachia sp.* (REINHARDT & *al.*, 1972).

and residual bodies are known, HOLTZMANN, 1976). Other substances, such as lipids and carbohydrates (glycogen) may be stored within the cell. The amounts stored appear to be less important in argasids than in ixodids (*H. asiaticum*, BALASHOV, 1961, RAIKHEL, 1974a), as already admitted by COOK (1973).

b) *Cytological comparison with midgut cells of other arthropods*

The basal lamina which surrounds the single-layered midgut epithelium is very thick in *O. moubata* (ca. 500 nm) as compared to that of ixodids (BALASHOV & RAIKHEL, 1974 : *H. asiaticum*, 80 — 100 nm ; BELOZEROV & TYMOPHEEV, 1971 : *Dermacentor marginatus*, 200 nm) or of insects (RICHARDS, 1975 : 50 — 100 nm). The basal lamina becomes strongly stretched during bloodmeal and its thickness diminishes : 300 nm in gorged *O. moubata* and 50 nm in gorged *D. marginatus* females (ixodids take up more blood than argasids). The apical microvilli of tick midgut cells are rather short (ixodids : 0,7 μm, BALASHOV & RAIKHEL, 1974 ; argasids (*O. moubata*) 1,5 μm), sparse and not as strictly arranged as in insect midgut cells (SMITH, 1970). This could be linked to the ability of tick midgut cells to perform phagocytosis. Intercellular junctions appear to be simple, without elaborate structures, in both argasids and ixodids. The apical *zonula adhaerens* is devoid of a *zonula occludens*.

Septate desmosomes are probably responsible for no haemoglobin passing from the midgut lumen to the tick haemolymph, without entering the midgut cells. Gap junctions allow electrotonical coupling between cells (HUDSPETH & REVEL, 1971) and could play a role for the synchronisation of digestive activity in the whole epithelium.

The typical structure of the cell membrane — associated, paired RER saccules described in the present paper could be tentatively interpreted in the context of an adaptation for transport of material from the midgut cell into the extracellular space and the haemocoele.

Association of vesicles (of the smooth ER) with basal cell membrane infoldings have been described in *Triatoma infestans* midgut cells (BURGOS & GUTIERREZ, 1976), but in *O. moubata*, ribosomes are present on the vesicles. The similar structures which are externally coated with ribosomes and surround glycogen inclusions, could play a role in the transport of glycogen.

The RER is mainly vesicular in fasting ticks. Vesicles were found in greater numbers in *D. marginatus* (BELOZEROV & TIMOPHEEV, 1971) than in *O. moubata*.

The organisation of the RER starts only after completion of the bloodmeal in *O. moubata* (GRANDJEAN, 1983). It is worth mentioning that in ixodids, as feeding is more prolonged than in argasids, RER and dictyosomes are already developing during the several days of slow feeding (BELOZEROV & TIMOPHEEV, 1971 : *D. marginatus*). After bloodmeal, digestive activity is initiated by stimulation of protease synthesis, both in ixodids (BOGIN & HADANI, 1973) and in argasids. In the latter, protease activity has been shown to be related to the amount of blood ingested (AKOV & *al.*, 1976 : *Ornithodorus tholozani*). AKOV & *al.* suggest that the observed rise in energy and oxygen consumption in ticks after a bloodmeal (GALUN & WARBURG, 1968 : same species) could be linked to the increase of metabolic activity in midgut cells, which have to synthetise intracellular proteases.

Blood digestion is much slower in ticks than in insects (GOODING, 1972), possibly because of the intracellular mechanism of digestion of the former, the hydrolases, produced in the GERL-system being utilised according to actual needs within the midgut cell itself, instead of being released at once in considerable amounts into the lumen as in insects. Thus, in rapidly digesting mosquito females (BERTRAM & BIRD, 1961, STAEUBLI & *al.*, 1966, HECKER & *al.*, 1971), in *Stomoxys* (LEHANE, 1976) and eventually in *Glossina morsitans* (BOEHRINGER-SCHWEIZER, 1977), the midgut cells are highly organised with "whorls" of RER, already before feeding, and enzyme production may start at once with arrival of fresh blood, after unwrapping of the "whorls". Yet the RER is also poorly developed before feeding in fleas (REINHARDT, 1976).

In conclusion, the comparison of results of the present study with those of BALASHOV & RAIKHEL (1974, 1976) and RAIKHEL (1974b, 1975) shows that intracellular digestion in argasids and ixodids is largely conformable.

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BLOOD DIGESTION IN *ORNITHODORUS MOUBATA* MURRAY *SENSU STRICTO* WALTON FEMALES (Ixodoidea : Argasidae)

II. Modifications of midgut cells related to the digestive cycle and to the triggering action of mating

O. GRANDJEAN*

SUMMARY. The cellular organisation of midgut cells (see Grandjean, in press) depends on the digestive cycle which follows a bloodmeal. The phases of this cycle are characterised in *O. moubata*, and various "cell-types" are described, correlated and discussed.
Mating triggers digestion : a) by an increased intracellular digestion (including the formation of heterophagolysosomes) during the rapid phase of digestion ; b) by reactivating midgut cells in starving females, which then may perform vitellogenesis and lay few normal eggs.

La digestion du sang chez les femelles d'*Ornithodorus moubata* Murray *sensu stricto* Walton (Ixodoidea : Argasidae) :
II. Modifications des cellules du mésentéron, en relation avec le cycle digestif et l'action stimulante de copulations.

RÉSUMÉ. L'organisation cellulaire des cellules intestinales (voir Grandjean, sous presse) varie au cours du cycle digestif qui est déclenché par un repas sanguin. Les principales phases de ce cycle sont caractérisées chez *O. moubata*, et différents « types d'organisation » de cellules intestinales sont décrits, comparés et discutés.
La copulation active la digestion : a) chez les femelles nourries, elle intensifie la digestion intracellulaire, par une formation accrue d'hétérophagolysosomes ; b) chez des femelles à jeun, elle réactive les cellules digestives et permet ainsi à la Tique de mobiliser ses réserves nutritives pour la vitellogenèse et la ponte de quelques œufs normalement constitués.

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1 - Introduction

1.1. Digestive cycle after ingestion of a bloodmeal

The blood taken up by *O. moubata* is made available for the tick body mass and energy requirements by intracellular digestion (Grandjean, in press). This type of digestion is phylogenetically primitive, but well adapted to the ability of the ticks to survive over prolonged periods : the food reserves of the midgut lumen may be mobilised at a very low rate in a fasting tick. Due to the intracellular type of digestion, the midgut cells have to perform diversified functions which have to be coordinated between the single cells.

Sequential modifications of midgut cells are studied in relation with the digestive cycle. The latter is analysed mainly in mated females ; but virgin females were also considered in order to investigate the action of mating on digestion.

Midgut cells of males were also investigated to a lesser extent. No fundamental differences were found, as compared to those of females.

1.2. Action of mating

In adult females, energy and nutrient reserves provided by digestion of bloodmeals allow vitellogenesis and egglaying. Except in rare cases of parthenogenesis, mating is an absolute necessity for normal egglayings (Aeschlimann and Grandjean, 1973a).

Yet it is possible to induce vitellogenesis and egglaying in unfed females by delayed mating (without previous bloodmeal) or by injections of male gonad homogenates (Aeschlimann and Grandjean, 1973a ; Germond and Aeschlimann, 1977 ; Ducommun, in prep.). The process of vitellogenesis following mating is dependent on an increased mobilisation of food reserves, which means an activation of digestive functions of the midgut epithelium. The triggering action on midgut cells may be demonstrated both for mating occuring at feeding time and for delayed mating in starving ticks.

2 - Materials and Methods

2.1. Tick material

O. moubata sensu stricto females, originally from Tanzania (Ulanga District) and bred according to Geigy and Herbig (1955), were fed on guinea-pigs, either in presence or in absence of males, and taken at various intervals after bloodmeal for gravimetric, histological or cytological investigations.

Other virgin females were fed and fasted during 110 days before males were added to half of them for delayed mating.

2.2. Methods

Histology (H) : Tissue pieces fixed and embedded as for transmission electron microscopy were cut into sections of 1 μm thickness and stained with toluidine blue (1 %, 6 parts + sodium carbonate 2,5 %, 3 parts + ethanol 70 %, 1 part.).

Histochemistry (H) : Pieces of midguts were fixed in either Carnoy's fluid, formaline or cold acetone before embedding into paraffine. Reactions such as PAS (including a diastase-fast control), pyronine-methyl green stain, cyanol (Fautrez and Lambert, in Gabe, 1968), benzidine (Pickworth, in Pearse, 1972) and Perl's blue (Burck, 1969) were performed. Attempts to stain for phosphatases or lipids did not succeed in a satisfactory manner.

Transmission Electron Microscopy (TEM) : See Grandjean (in press).

Scanning Electron Microscopy (SEM) : Midgut pieces were either fixed with formaline or ethanol (70 %) and dried in liquid nitrogen, or fixed as for Transmission Electron Microscopy and dried in air or liquid carbon dioxyde (Boyde and Wood, 1969). They were covered with a gold-carbon layer before observation with a Cambridge Mark II Stereoscan (Zurich : Cytological Laboratory of the Institute of General Botany, University of Zurich, Professor Hohl ; Basle : SEM-Laboratory, Institute of Geology and Paleontology, University of Basle, Dr. R. Guggenheim, *fig. 26)*.

3 - Modifications of Midgut Cells Related to Digestive Cycle

3.1. Definition of midgut cell "types" related to digestive cycle

Histology (H, including histochemistry) and ultrastructure (SEM = scanning electron microscopy, TEM = transmission electron microscopy) of cell types are described according to the digestive phases (*fig. 1,23* ; table I ; see also Grandjean and Aeschlimann, 1973). After the unfed females (3.1.1, IV *in fig. 1,23*) have taken up blood, a "preparation phase" (3.1.2., I) is followed by a rapid phase of digestion (3.1.3., II and IIIa, up to IIIb and oviposition in mated females) which leads to the slow phase of digestion (3.1,6, IV) typical for fasting ticks.

3.1.1. *Unfed females*

H : Cubical or columnar midgut cells contain few stained inclusions, except haematin granules and some rare lipid or glycogen inclusions *(fig. 1 : IV)*.

SEM : The much infolded basal lamina is continuous with an organ investment layer, which covers also muscle, nerve and tracheolar cells *(fig. 24)*. Seen from the interior of the midgut lumen, the apical surface of midgut cells is homogenously warty *(fig. 25)*. Individual cells may not be distinguished.

TEM : "A-type" midgut cells : cubical (Grandjean and Aeschlimann, 1973 : *fig. 4*) or cylindrical midgut cells *(fig. 2)* contain numerous free ribosomes, some of which are associated with small vesicles *(fig. 3 : R)*.

Inclusions or organites other than mitochondria and haematin granules are rare. The basal labyrinth is generally well developed.

3.1.2. *Gorged females*

H : Cells of the midgut epithelium are submitted to an important stretching with feeding. Their inclusions are the same as in unfed ticks, at least immediately

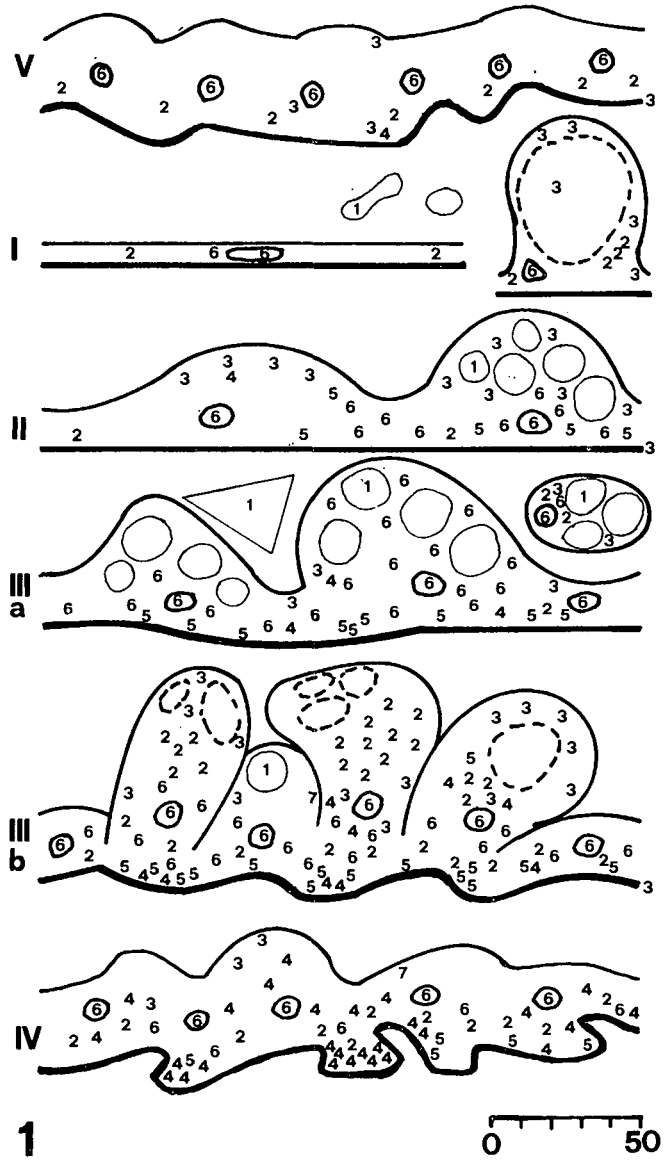


FIG. 1. — Schematic drawing summarising histochemical results on the midgut cells of *O. moubata*. I = epithelium of newly gorged female, II = of female starting digestion, about 2 days after bloodmeal, III = of female, 6 (a) to 15 (b) days after bloodmeal, IV = of fasting female ; 1 = haemoglobin, green with Peil + H_2O_2, cyanol, 2 = haematin, blue with Peil + H_2O_2, 3 = (muco)polysaccharides, diastasefast PAS positive, 4 = glycogen, PAS positive, not resistant to diastase, 5 = lipids, metachromatic toluidin blue or osmium tetroxyde, 6 = ribonucleic acids (RNA), stained with pyronine, 7 = free iron (Perl).

TABLE I. — Synoptic summary of results on the midgut of *O. moubata* according to the phases of digestion (BL = basal lamina ; Hb = haemoglobin ; Hn = haematin granules ; PAS, RNA : see text (methods) ; RBC = erythrocytes ; RER = rough endoplasmic reticulum).

Phase of digestion (Grandjean and Aeschlimann, 1973)	Unfed 3	Preparation 1	Rapid phase 2a	Rapid phase 2b		Slow phase 3
Cellular phase (fig. 1, 23)	IV	I	II	IIIa	IIIb	IV
Days after bloodmeal	fasting	0-2	2-5	5-10	11-20 (oviposition)	more than 20
Weight losses (Grandjean and Aeschlimann, 1973)	very slow	varying (delayed or rapid)	very rapid	rapid, enhanced in mated females	less rapid	slow
Histology of epithelium	cubical cells few inclusions	flattened cells start activity	growing, phagocytosis, micropinocytosis	protruding cells, much phagocytosis	protruding cells with Hn	cubical cells, few inclusions
Histochemistry	basal Hn	few / no inclusions	PAS +, some RNA, Hb-phagosomes some lipids	much RNA, Hb-phagosomes, some glycogen / lipids	much apical Hn, RNA, glycogen, lipids	basal Hn, glycogen, lipids
Scanning electron microscopy	BL : narrow folds, warty cell apices	stretched BL and smooth apices	BL : few sac-like expansions, apices irregular	BL : large sac-like expansions, apical microvilli + protruding phagosomes	BL : some sac-like expansions, apices irregular	BL : narrow folds, warty cells apices
Transmission electron microscopy	basal labyrinth free ribosomes	poor organisation, except « C-type » cells phagocyting thrombocytes	appearing RER + Golgi	highly organised RER + Golgi	highly organised RER, residual bodies	disappearance of RER, few more inclusions
Midgut lumen (Grandjean, in press)						
pH	low	comparable to that of fresh blood			sinks	low
colour	black	bright red	bright red	brownish red	brownish	brown-black
content	haematin granules	fresh blood, thrombocytes phagocytosed	RBC persist, disappearing leucocytes	starting crystallisation of oxy-Hb	Hb crystals grow	destruction of crystals (met-Hb, haemichrome) Hn granules

after feeding. Some protruding cells with a large vacuole reacted positively for (muco)-polysaccharides (diastase-fast PAS-reaction ; *fig. 1 : I)*.

SEM : The important stretching action of blood entering the midgut during the bloodmeal is revealed as well on the external side of the midgut epithelium *(fig. 26)* as on the internal side *(fig. 27)*, if compared with unfed females *(fig. 24, 25)*.

TEM : "B-type" : nuclei are stretched within the strongly flattened midgut cells *(fig. 4)* and the few organites or inclusions, such as mitochondria or haematin granules, are squeezed between the basal lamina and the apical cell membrane.

"C-type" : protruding cells with huge vacuoles and a thin peripheral cytoplasm layer (gv and pc *in fig. 3*, and Grandjean, in press : *fig. 16*) are either teared off or remain attached with the entry of fresh blood. In both cases they are able to perform early phagocytic activity in absorbing mainly thrombocytes.

3.1.3. *Start of digestion* (from the second day after bloodmeal).

H : The growing cells start reacting positively for RNA and often for (muco)polysaccharides. Glycogen and lipids may be found. Slightly protruding cells contain vacuoles with haemoglobin : pseudopodia-like expansions of the cell allow phagocytosis of erythrocytes.

TEM : "D-type" : midgut cells which start absorbing material from the midgut lumen by micropinocytosis have a well developed basal labyrinth. Their elongated RER saccules are found mainly along the lateral part and the basal invagination of

General key to abbreviations (excepting fig. 1 and 23).

a = special cell membrane-associated, paired RER saccules (See Grandjean, in press), e = erythrocyte, gl = glycogen, gv = great clear vacuole, h = haematin granule, ic = intercellular space, m = mitochondria, mp = micropinocytotic vesicles, mt = microtubules, mv = microvilli, n = nucleus, nl = nucleolus, pc = peripheral cytoplasm layer, r = ribosomes, rb = residual body, za = *zonula adhearens*.
BL = basal lamina, G = dictyosome (Golgi system),
L = midgut lumen, Li = lipids, M = muscle cell,
P = phagosome, R = rough endoplasmic reticulum,
SL = secondary lysosomes, T = tracheole, W = *Wolbachia sp.* (rickettsia-like microorganism).

For each figure, the scale is expressed in micrometers (μm).

Fig. 2. — Apical part of cylindrical midgut cells of an unfed female, with a cell undergoing lysis (*).

Fig. 3. — Detail of the apical part of midgut cells of unfed female, one cell possessing a large clear vacuole (gv).

Fig. 4. — Flattened, distended cell of a gorged female, immediately after completion of feeding, with a monocyte in the midgut lumen (arrow = "organ investment layer" surrounding midgut muscle and tracheal cells).

Fig. 5. — Flattened midgut cell, starting uptake of material from lumen, 1 day after bloodmeal (arrow as in *fig. 4*).

Fig. 6. — Detail of cell as in *fig. 5* (* = lacunar extracellular space ; note appearance of long RER saccules, R).

Fig. 7. — Growing and developing cubical midgut cell, 2 days after bloodmeal, with developing dictyosomes and stacks of RER, and with reserve inclusions.

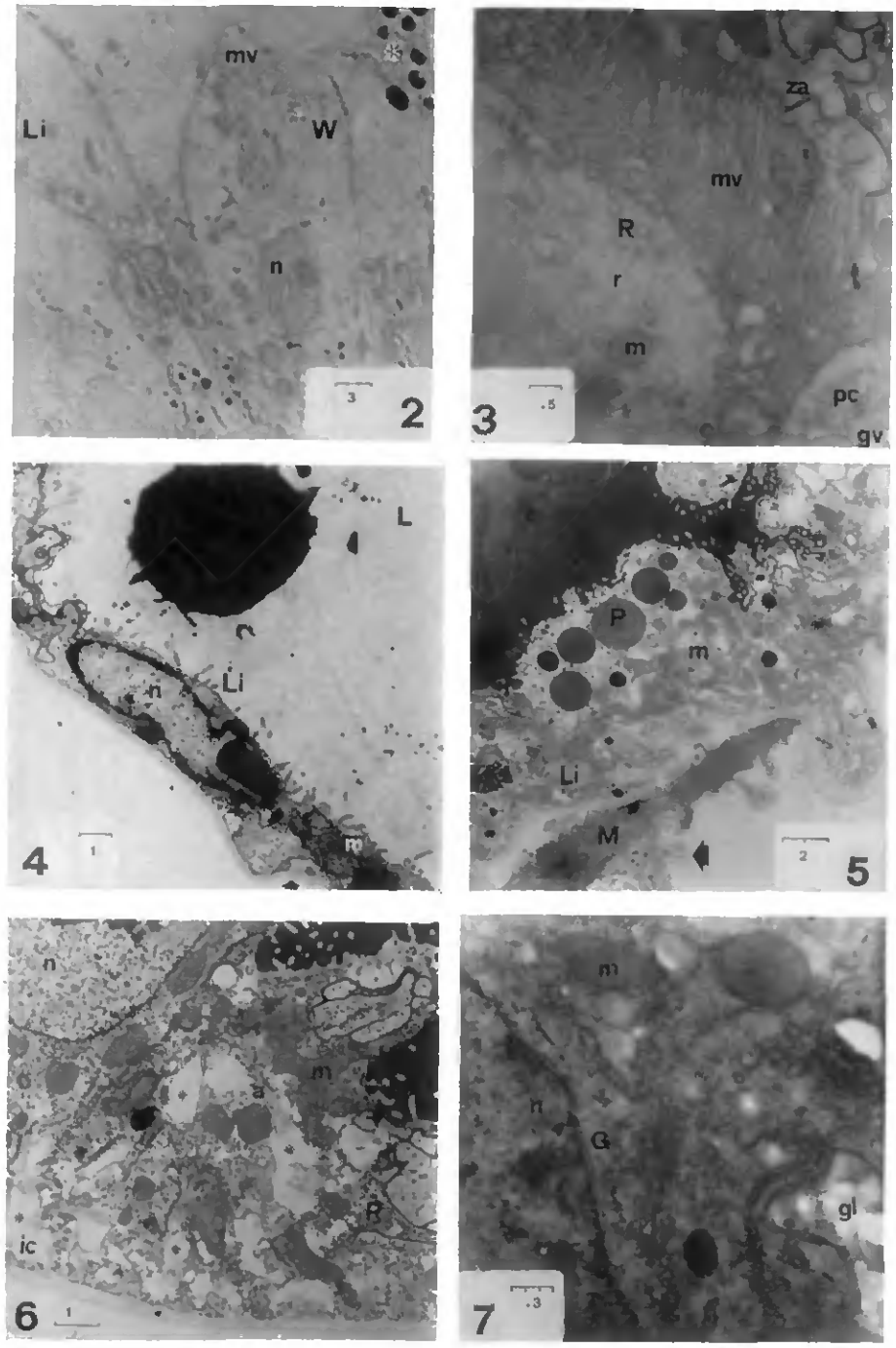


FIG. 2-7.

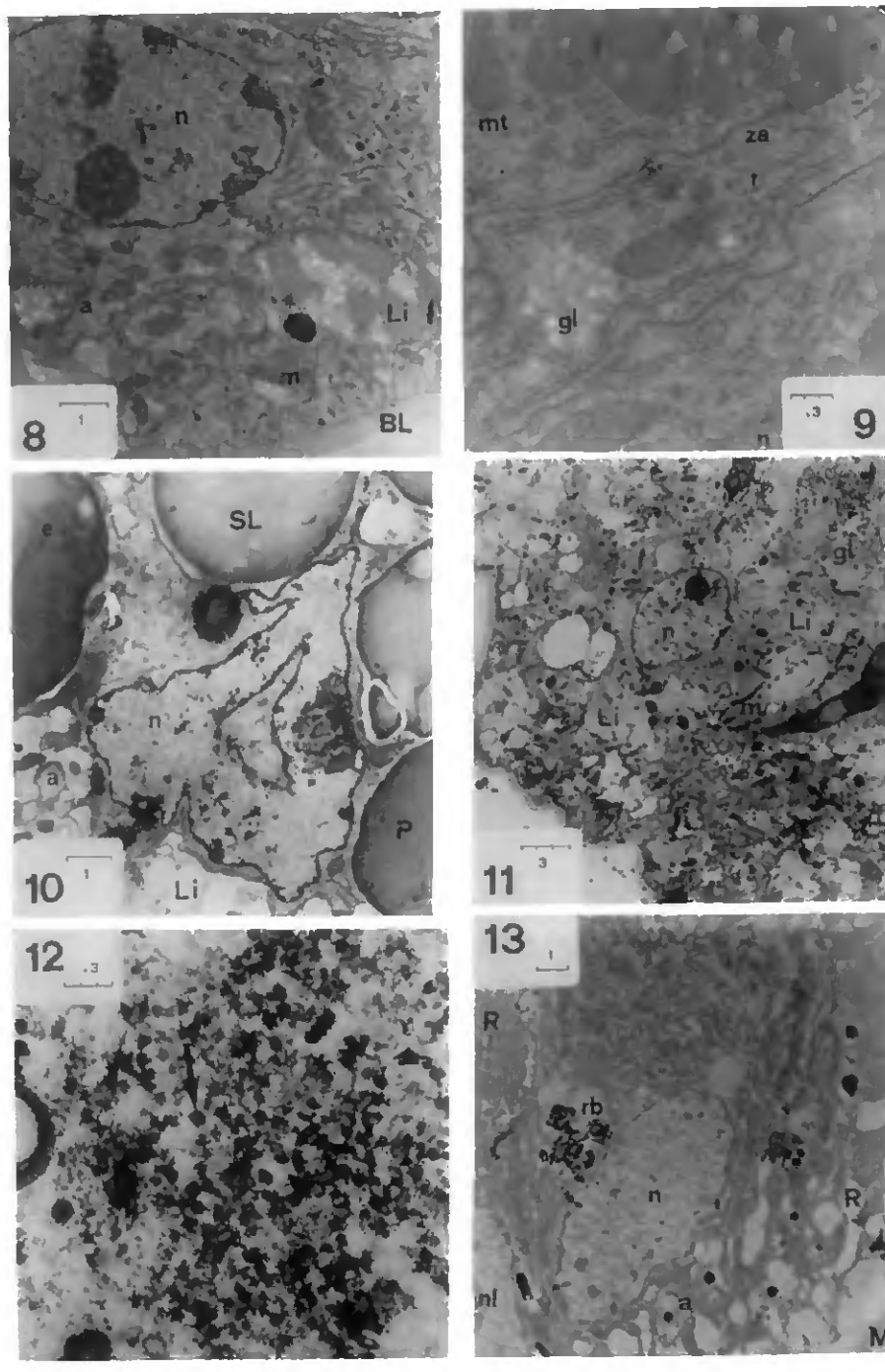


FIG. 8-13.

the cell membrane *(fig. 6)*. These cells contain some lipid inclusions in their basal part *(fig. 5 : Li)*.

"E-type" : growing midgut cells change from a flat to a cubical shape and undergo important differenciation with the appearance and the organisation of rough endoplasmic reticulum (RER) and of dictyosomes *(fig. 7)*. Lipid and glycogen reserves may be found in these cells.

"F-type" : protruding cells which absorb erythrocytes by phagocytosis *(fig. 14)* have a very similar cellular organisation as compared with cubical cells ("E-type").

"G-type" : some large protruding cells digest intracellularly phagocytosed thrombocytes *(fig. 23 : G)*.

3.1.4. *Full digestive activity* (about 5 to 8 days after bloodmeal) *H* : The numerous protruding cells contain haemoglobin in their vacuoles. Their cytoplasm is scattered with RNA material ; lipid inclusions and some glycogen may be found in the basal part of the cells.

SEM : Externally *(fig. 28)* the fully active midgut epithelium forms sac-like expansions between the muscle cords allow an ampliation of the epithelial basement surface. Individual protruding cells loaded with food material may be recognised if seen from inside the midgut lumen ; they are covered by a coat of well distinguishable microvilli *(fig. 29)*.

TEM : "H-type" : These cubical cells show a very high level of cellular organisation. RER saccules are often arranged in stacks *(fig. 15 : R)* and dictyosomes are well differenciated (Grandjean, in press : fig. 18). Lipid *(fig. 8)* and glycogen *(fig.9)* inclusions are present and micropinocytotic processes still may be observed (Grandjean and Aeschlimann, 1973 : *fig. 6a*).

"I-type" : protruding cells, loaded with food material from the lumen (mainly phagocytosed erythrocytes) do not greatly differ in their organisation from the cubical cells ("H-type", *fig. 15*). Basal lipid inclusions are often found *(fig. 10)*. Ultrastructural changes occuring within food vacuoles (phagosomes, phagolysosomes) have been described (Grandjean, in press).

"J or K-type" : few cubical or cylindrical cells contain food inclusions, numerous residual bodies *(fig. 13 : rb)* and a poorly developed, rather vesicular RER.

"L-type" : several cells were nearly filled with lipid or glycogen inclusions *(fig. 11, 12)*.

Fig. 8. — Cubical midgut cells, 5 days after bloodmeal, showing well differenciated cell structures (* = smooth vesicle with a haematin granule being formed).

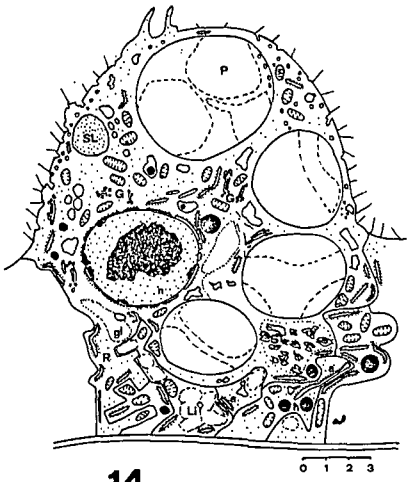
Fig. 9. — Apical detail of a midgut cell, 5 days after bloodmeal.

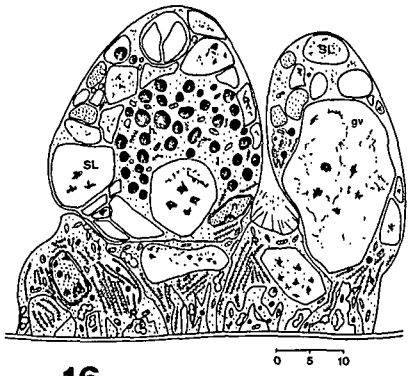
Fig. 10. — Detail of central part of protruding cell loaded with phagosomes (P) and early secondary lysosomes (SL), 6 days after bloodmeal (see also *fig. 15*).

Fig. 11. — Protruding midgut filled with reserve inclusions (glycogen and lipids), 8 days after bloodmeal.

Fig. 12. — Detail of *fig. 11* with alpha (thin arrow) and beta (small thick arrow) glycogen granules.

Fig. 13. — Basal part of midgut cell as drawn in *fig. 16*, with very much developed RER.





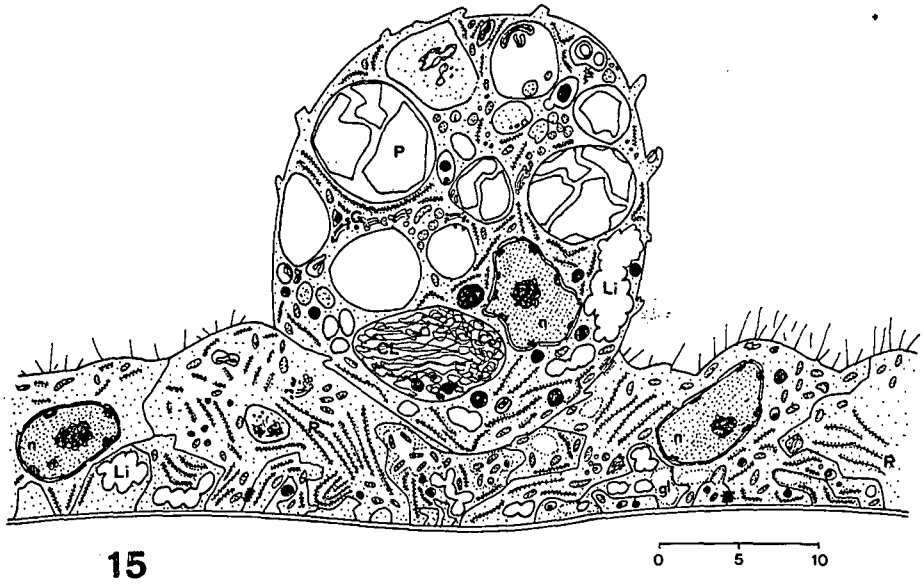


FIG. 14-16.

Fig. 14. — Schematic drawing of slightly protruding midgut cell filled with phagosomes (phagocytosed erythrocytes), 2 days after bloodmeal.

Fig. 15. — Schematic drawing of several cubical and one protruding midgut cells, the latter being filled with digestive vacuoles (secondary) lysosomes), 5 days after bloodmeal.

Fig. 16. — Schematic drawing of protruding midgut cells having completed digestion, 17 days after bloodmeal, with late secondary lysosomes (clear vacuoles with some granulations, see Grandjean, 1982) and accumulation of haematin granules.

3.1.5. *Advanced stages of rapid phase of digestion* (about 15 to 20 days after bloodmeal).

H : Numerous protruding cells are filled with haematin granules in their central part. Clear apical vacuoles, (muco)polysaccharides are present there. Lipid and glycogen reserves are mainly bound to the basal parts of the cells which contain also RNA material. Some free iron could be detected *(fig. 1)*, but this seems to be an exception, as iron normally remains bound in the haematin.

TEM : "M-type" : in the large protruding cells, the centrally accumulated haematin granules *(fig. 16)* are surrounded by numerous vacuoles (late secondary lysosomes, Grandjean, in press ; *fig. 23*). The basal part of these cells is filled with highly organised stacks of RER and with lipid inclusions *(fig. 13)*.

"N-type" : cubical cells of this stage contain a well developed RER and important lipid and glycogen reserves.

3.1.6. *Slow phase of digestion* (fasting females, more than 20 days after bloodmeal, after oviposition).

H : The mainly cubical midgut cells contain haematin granules, some lipid and more numerous glycogen inclusions. RNA material has almost disappeared.

SEM : External and internal surfaces of the midgut epithelium may hardly be distinguished from those of unfed females *(fig. 24, 25)*.

TEM : "O-type" : cell organites and inclusions at this phase may be compared to those of unfed females. RER is no longer arranged in stacks, but rather composed of few vesicular elements *(fig. 17)*. Lipids and glycogen are stored. Several protruding cells contain huge vacuoles *(fig. 17 :* gv ; *fig. 23* : "Oc-type").

3.2. Postulated relationships between cell "types"

3.2.1. *Cell transformations due to feeding activity of the tick.*

The arrival of fresh blood is responsable for a dramatical mechanical stretching of the midgut epithelium and the tearing off of numerous midgut cells, which will be lysed in the lumen. It also triggers phagocytic activity in some midgut cells which attack mainly thrombocytes (*fig. 23* : "C-type" cells which are present also in the epithelium of unfed or starving ticks as the "Ac-type").

3.2.2. *Growth and activation of cells after the bloodmeal.*

A deep cellular reorganisation of the stretched migdut cells is triggered by the arrival of blood. Differenciation includes the growth of the cell and leads to a well developed and functional system of organites, such as RER and Golgi apparatus, which will allow intracellular digestion of blood within a lysosomial system. The cellular reorganisation appears to be synchronous in most of the midgut cells and lasts for 1 or days, which correspond to the "preparation phase" before start of rapid digestion.

3.2.3. *Fate of protruding cells loaded with food vacuoles (phagosomes and secondary lysosomes).*

Midgut cells start protruding into the lumen by taking up food material (*fig. 23* :

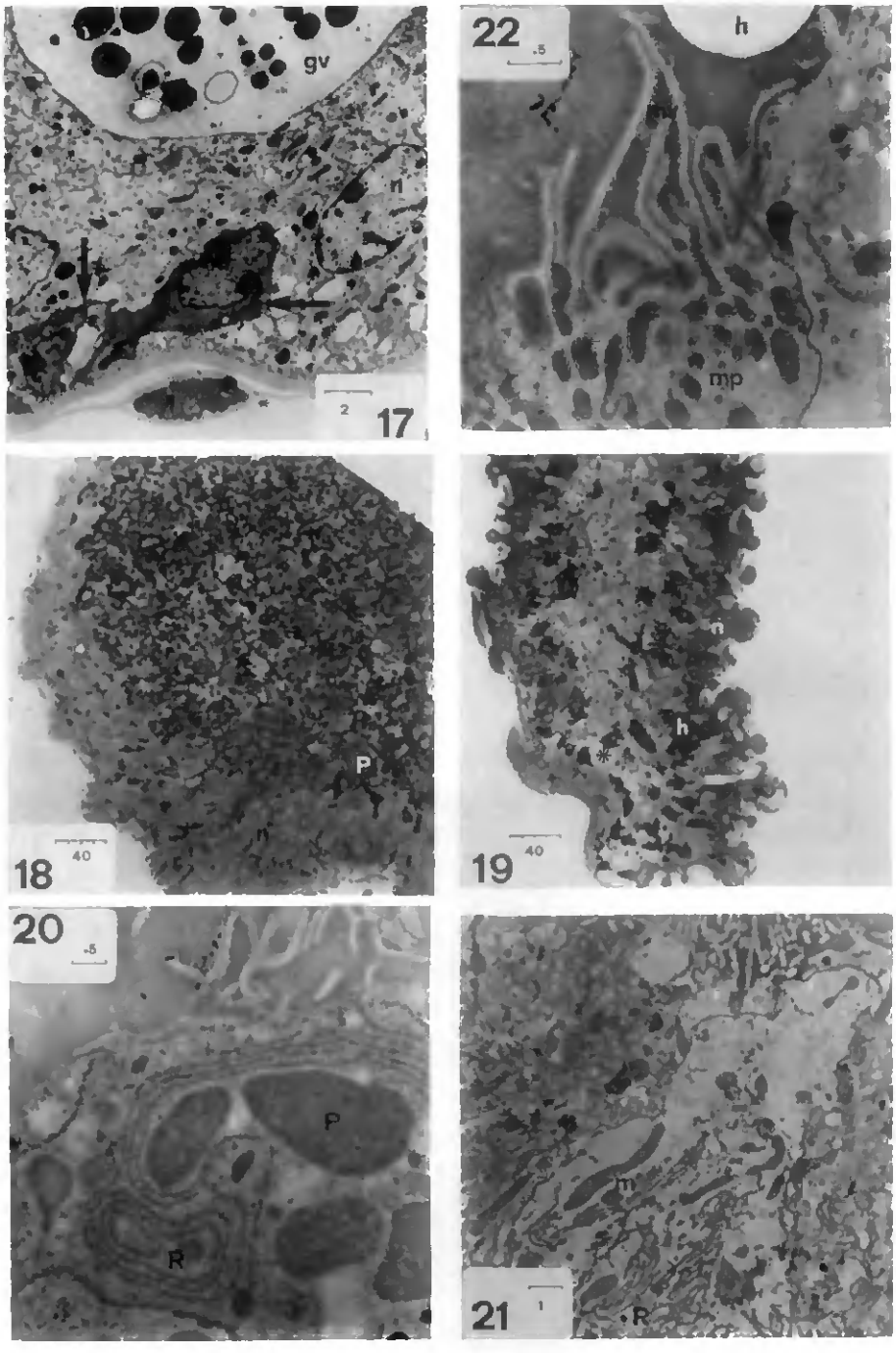


FIG. 17-22.

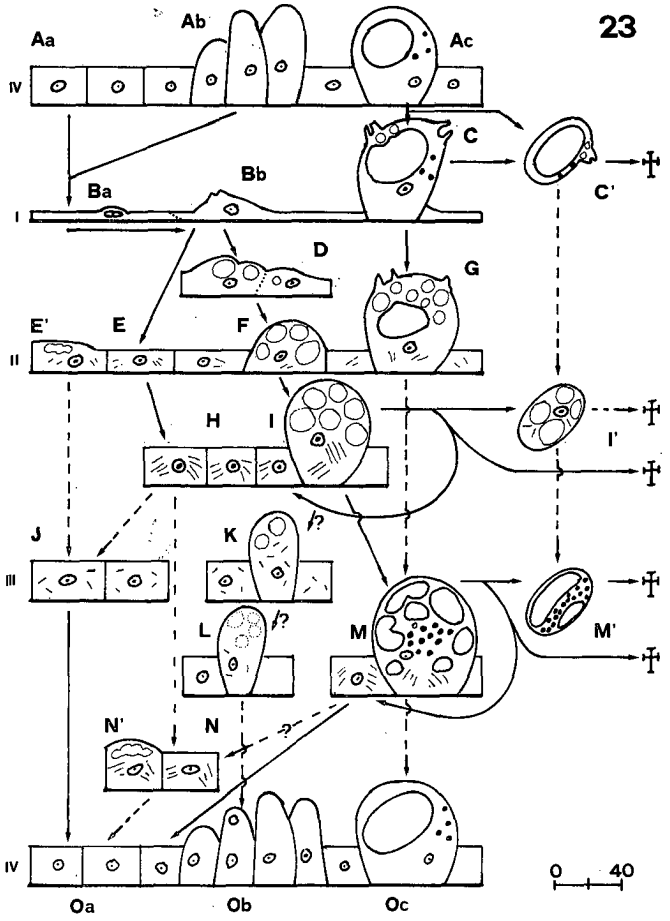


FIG. 23. — Schematic drawing summarising the cell types described and showing their postulated inter-relationships (A to O, see text, 3.1 ; I to IV, as in *fig. 1* and in Grandjean & Aeschlimann, 1973).

Fig. 17. — Cubical midgut cells, 26 days after bloodmeal, with one cell apically filled with a huge vacuole (gv) containing haematin granules and two cells with a gross granular content (arrows) ; * = organ investment layer.

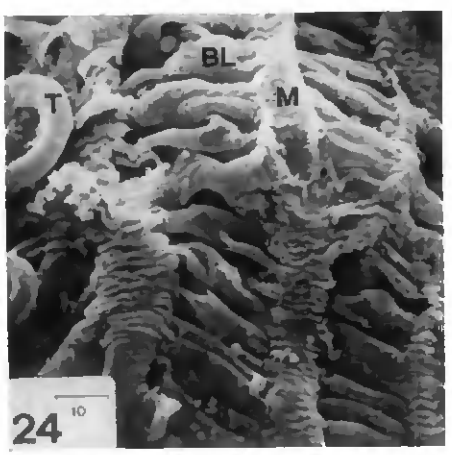
Fig. 18. — Midgut histology of virgin females, 17 days after bloodmeal, with protruding cells filled with undigested haemoglobin vacuoles (P).

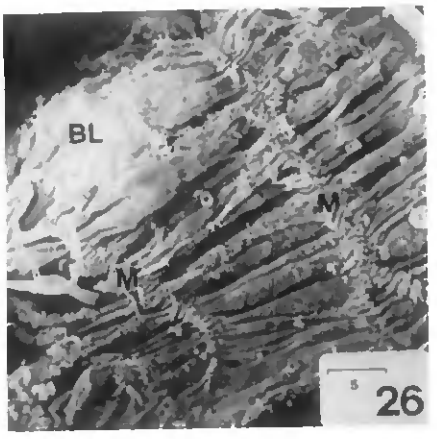
Fig. 19. — Midgut histology of mated female, 17 days after bloodmeal, with numerous cells filled with haematin granules (h) and clear vacuoles (*).

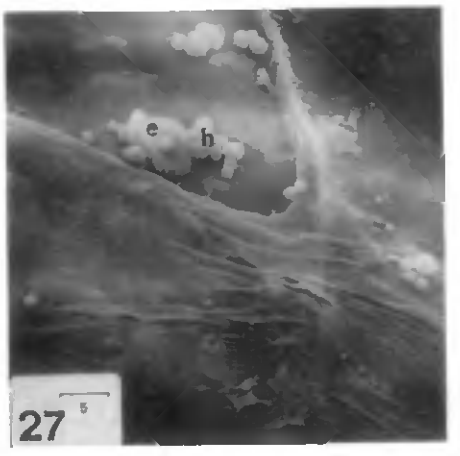
Fig. 20. — TEM : apical part of midgut cell of virgin female, 17 days after bloodmeal (note "whorl"-like arrangement of RER).

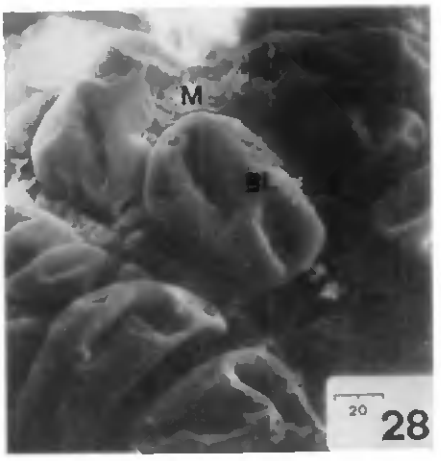
Fig. 21. — TEM : apical part of midgut cell of mated female, 17 days after bloodmeal.

Fig. 22. — TEM : apical detail of cell like *fig. 20* (virgin female).









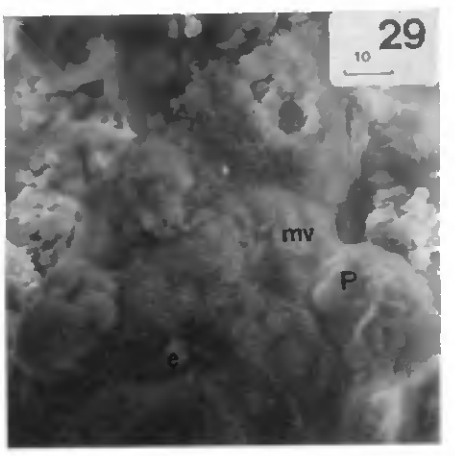


FIG. 24-29.

"D or F-type") up to important quantities (see "I-type"). With progress of intracellular digestion, clear vacuoles are formed (see "M-type") which may fuse together. Thus certain cells ("Oc-type") may contain huge vacuoles for very long periods ("Ac-type").

Some cells may be observed to disrupt after completion of digestion. Others detach from their bases, either with or without nucleus, and float within the lumen before lysis. Cubical cells in fasting ticks might originate from protruding cells having completed digestion and lost their apical parts *(fig. 23)*.

3.2.4. *Synthetic activities of the midgut cells*

Early differenciation of midgut cells is linked to the needs for synthesis of digestive enzymes (proteases ; Akov *et al.*, 1976, see also Grandjean, in press). Midgut cells having completed digestion (*fig. 23* : "M and N-type") still possess a highly organised RER, which suggests that proteins might be synthetised from material mobilised by digestion. As such cells are numerous during the preoviposition period of females, there is much evidence for them to play an important role for the synthesis of vitellogenins, as postulated earlier (Diehl, 1970). Cells of the fat body probably also are involved (Obenchain, personal communication). The mechanisms of exocytosis and release of vitellogenins from the midgut cells into the lumen still remain unknown ; special basal cell membrane-associated RER saccules (Grandjean, in press) could possibly be involved.

3.2.5. *Cubical cells and cell renewal*

During all digestive phases, some cells do not participate in the actual digestion of blood. They are generally cubical, may be highly organised and start to take up food and digest it at once. Cubical cells also result from mitotic divisions, even if the latter are hardly ever observed (Grandjean and Aeschlimann, 1973). Small cells with gross granular elements (*fig. 17* : arrows) are like the replacement cells in *Aedes aegypti* midguts (Hecker *et al.*, 1971a,b).

3.2.6. *Other cell types*

The other cell types described (*fig. 23* : "J, K, L-type") were not often encountered and apparently do play a less important role in digestion.

Fig. 24. — SEM : external face of midgut epithelium of unfed female, showing folded basal lamina (BL) which is continuous (arrows, as in *fig. 4*) with organ investment layer covering muscle (M) and tracheolar (T) cells (acceleration : 18 kV).

Fig. 25. — SEM : internal face of midgut epithelium of unfed female, showing homogenous warty appearance of the surface of cell apices (h = haematin granules ; acceleration 18 kV).

Fig. 26. — SEM : external face of midgut epithelium of freshly gorged female with stretched basal lamina (compare distance between muscle cords, M, with *fig. 24* ; acceleration 10 kV).

Fig. 27. — SEM : internal face of stretched midgut epithelium as in *fig. 26*, the surface of cell apices being smooth and poorly differentiated (e = erythrocyte ; acceleration : 20 kV).

Fig. 28. — SEM : external face of midgut epithelium of female, in the phase of rapid digestion (5 days after bloodmeal), showing sac-like expansions of basal lamina between muscular strings (acceleration : 20 kV).

Fig. 29. — SEM : internal face of midgut epithelium as in *fig. 28*, showing apices of protruding cells loaded with phagolysosomes (P) and covered with microvilli (mv ; e = erythrocyte : spherical, not yet lysed ; acceleration : 20 kV).

3.3. Homogeneity of digestive activity in the various midgut diverticula

In cell counts, the percentage of midgut cells which contained haemoglobin vacuoles was chosen as being representative of the cell population actually involved in processes of intracellular digestion *(table II)*. Shortly after bloodmeal, midgut cells absorbing haemoglobin are more numerous in the anterior and medial diverticula than in the other parts of the midgut. These diverticula have been found, mainly in their distal parts, to become less stretched with arrival of fresh blood than e.g. the central part of the midgut.

After some 5 to 8 days, all the parts of the midgut appear to be equally involved in absorptive and digestive activities, as the half to three quarters of the cells contain haemoglobin. About 2 weeks after bloodmeal, the number of cells with digestive activity diminishes more rapidly in the anterior and medial diverticula than in the central and posterior parts of the midgut. In fasting ticks or unfed females, few midgut cells are responsable for slow digestion ; they are more numerous in the central and posterior parts of the midgut which might therefore be considered to perform mainly storage functions.

TABLE II. — Percentage of midgut cells containing haemoglobin inclusions, according to their localisation in the midgut and at various intervals after bloodmeal

Days after bloodmeal	Number of cells counted	a, l (%)	p, c (%)	total (%)	$\frac{a, l}{p, c}$ — ratio*
fasting	357	1,6	5,6	4,2	0,28
0	384	26,5	11,6	16,6	2,28
1	322	25,7	26,1	31,7	0,99
2	283	67,9	44,0	50,0	1,54
5	370	66,3	49,1	58,6	1,35
6-7	148	49,0	52,0	53,4	0,94
8	290	74,8	72,4	71,8	1,03
12	111	30,0	34,0	32,4	0,88
15-18					
(m)	109	7,0	14,0	11,9	0,50
(v)	111	45,7	66,7	51,3	0,68
20-22	373	6,7	8,6	7,8	0,78
30-35	117	5,0	5,9	6,0	0,85

(a = anterior, l = lateral, p = posterior diverticula, c = central part of midgut, m = mated, v = virgin females).

* Ratio 1 means that digestive activity is homogenously present in all parts of the midgut,
ratio > 1 indicates predominance of digestive activity in the anterior and lateral diverticula,
ratio < 1 indicates predominance of digestive activity in the central part and the posterior diverticula of the midgut.

3.4. Discussion

3.4.1. *Phases of the digestive cycle*

A distinction between a rapid and a slow phase of digestion has been established for argasids, according to the protein content of the midgut (Akov *et al.*, 1976) and to general metabolic activity of the tick (Hajjar, 1971). It corresponds to definitions of digestive phases by Balashov (1961, from state of midgut content) and Tatchell (1964, from haemoglobin concentration of midgut content). According to our findings for *O. moubata* (Grandjean and Aeschlimann, 1973, Grandjean, in press and present paper ; resumed in *table I*), a short period of preparation, immediately after bloodmeal, is followed by a rapid and a slow phase of digestion. Midgut cytology and biochemistry, as well as weight losses of the tick are related to the digestive phases.

3.4.2. *Cell-"types" in the midgut epithelium of ticks*

a) *Comparison with other argasids*

The several "types" of midgut cells of *O. moubata* may be explained by following the fate of one single, changing cell (*fig. 23* ; e.g. stretched - growing - dividing - absorbing - digesting - lysis or persisting cell with a large vacuole - phygocytic activity after bloodmeal - digestion of thrombocytes - lysis). In this we would agree with Tatchell (1964 : *Argas persicus*) or Guirgis (1971 : *A. arboreus*), who assert that the cells may have various functions, rather than with Balashov (1972) who describes three distinct types of cells (reserve, digesting and secreting cells). The interpretation of cubical cells as remnants of partly detached protruding cells has been supported by autoradiographic evidence (Khalil, 1971 : *A. arboreus*).

The activity of early phagocytosis by "C-type" cells is not identical with that of cells digesting haemoglobin after differenciation and has not yet been reported so far. These cells could possibly be interpreted as identical to the secreting cells found shortly after bloodmeal by Balashov (1972).

b) *Comparison with ixodids*

During the slow feeding phase of *H. asiaticum* nymphs, Balashov and Raikhel (1976) have found secreting cells with cytological features different to any of the midgut cell "types" in *O. moubata*. They describe other cells which perform phagocytosis and pinocytosis, mainly in the central parts of the midgut. Such cells could be compared to "C or G-type" of early phagocyting cells in *O. moubata*. Few midgut cells filled with haemoglobin vacuoles (comparable to "I-type") were found by the same authors.

Cubical cells with developing RER, dictyosomes and appearing lipid inclusions in the midgut of *D. andersoni* females (Belozerov and Timopheev, 1971 : during slow phase of preliminary feeding) could be compared to the "E-type" found in *O. moubata* during the preliminary preparing phase of digestion after bloodmeal.

Large cells with numerous haemoglobin vacuoles increase in number during the rapid phase of feeding and final engorgement of ixodids (Belozerov and Timopheev,

1971 : Balashov and Raikhel, 1976). After ixodids have dropped off their host, their midgut cells must undergo a second differenciation before rapid digestion may be fully accomplished.

Histological modifications of midgut cells are well synchronised in ixodids (Balashov, 1972), whereas in argasids, they are less strictly dependent on the digestive cycle. This is coherent with the "all-or-none" sequence of biological events (such as vitellogenesis, egg-laying and death) observed to a larger extent in ixodids than in argasids (Aeschlimann and Grandjean, 1973a).

3.4.3. *Homogeneity of midgut diverticula*

Our results differ from those of Guirgis (1971) or Balashov (1972) in showing that posterior diverticula are able to perform important digestive functions and not only to serve as a storage organ. We agree with the conclusions of Khalil (1971), that the rapid phase of digestion involves rather anterior, and the slow phase rather posterior midgut cells. Yet midgut cells of posterior diverticula are also active in the rapid phase of digestion in *O. moubata*.

4 - Triggering Action of Mating

4.1. Effects of mating approximately synchronous with bloodmeal

a) *Effects on nutrition*

Nutrition is completely independent on mating, as well for the amount of blood taken in as for duration of bloodmeal (*table III*, see also Aeschlimann and Grandjean, 1973a).

TABLE III. — Nutritional parameters in mated and unmated *O. moubata* females.

	mated females (n = 14)	unmated females (n = 14)
$\frac{\text{fed weight}}{\text{unfed weight}}$ (%)	424 ± 68	420 ± 59
duration of bloodmeal (minutes)	47 ± 17	42 ± 20

b) *Effects on digestion : gravimetric analysis*

Virgin females loose their weight less rapidly than mated ones (Grandjean and Aeschlimann, 1973). The difference arises at the time of vitellogenetic activity of the mated tick (*table IVa* : about 10 days).

TABLE IV. — Weight losses of female ticks following mating, compared to unmated controls, both :

Days after mating	a) mating synchronous with bloodmeal			b) delayed mating	
	mated (n = 14)	unmated (n = 14)	t-test	mated (n = 10)	unmated (n = 6)
0	100,0	100,0	—	100,0	100,0
4				98,1	98,5
5	88,8 ± 2,2	89,0 ± 3,4	—		
7	86,5 ± 1,5	87,3 ± 3,6	—	95,5	96,6
8	85,0 ± 1,9	86,8 ± 3,8	(—)		
10	82,6 ± 1,6	85,0 ± 4,5	+	93,7	95,0
14				91,1*	93,0*
15	79,7 ± 1,7	82,4 ± 4,5	+		
18	79,0 ± 1,9	81,6 ± 4,8	+		
20	78,1 ± 0,5	80,7 ± 1,1	+		
21				85,8	89,4

a) after mating at the moment of bloodmeal ;
b) after delayed mating of females, fed 110 days before.
Weights are expressed in % of weight, at the moment of mating.
Student's t-test : + = significant for P = 0,95
— = non significant.
* value of t-test : t = 2,05, which is significant for P = 0,95.

c) *Effects on digestion : cytological analysis*

Intracellular digestion is very active in mated females, since 4 or 5 days after bloodmeal. Later (about 2 weeks affter bloodmeal, midgut cells containing haemoglobin vacuoles are more numerous in virgin than in mated females (*table II* : 15-18 days, *fig. 18, 19*). They contain a well arranged rough endoplasmic reticulum (RER) with "whorl"- like formations *(fig. 20 : R)*, whereas RER is formed by shorter, but numerous elements in the midgut cells of mated females *(fig. 21)*. Undigested food *(P in fig. 20)* will hardly be found in the latter. Micropinocytotic activity is less important in midgut cells of mated than of virgin females, in which the midgut cells still absorb material from the lumen *(fig. 22)*.

4.2. Effects of delayed mating on digestion in fasting ticks

a) *Gravimetric analysis*

Delayed mating induces a significant weight loss in fasting females *(table IVb)*. Normal eggs laid by females after delayed mating *(table V)* are less numerous than after mating synchronous with bloodmeal. After delayed mating, only 14 % of the weight of tick (if 100 % = at moment when the female starts laying eggs) are converted into eggs, as compared with 30 % if, mating coincides with bloodmeal *(table V)*.

TABLE V —. Parameters of egg-laying, both in natural case (normal oviposition following simultaneous bloodmeal and mating) and after delayed mating (occurring 110 days after bloodmeal).

	After mating synchroneous with bloodmeal (n = 3)	After delayed mating (n = 3)
Number of eggs laid	130	26
Mean weight of a single egg (mg)	0,504	0,497
Weight of tick having laid eggs, 25 days after mating (% of body weight at moment of mating)	50,3	74,2
Total weight of eggs (% of body weight of the tick starting to lay eggs)	30,5	13,5

b) *Histological analysis*

After delayed mating, histological evidence reveals an important triggering of digestive activity of midgut cells, linked with the mobilisation of food reserves stored in the midgut lumen (Aeschlimann and Grandjean, 1973a).

4.3. Discussion

4.3.1. *Effects of mating on nutrition*

Compared to ixodids, for which mating is an absolute necessity for completion of gorging (Aeschlimann and Grandjean, 1973a, including *Ixodes ricinus*, Graf, 1974), mating is not necessary for a normal nutrition in argasids.

4.3.2. *Effects of mating on digestion*

Our gravimetric results suggesting lower metabolic activity in virgin than in mated ticks agree with statements of Tatchell (1964) who found that haemoglobin concentration no longer diminishes after several days in unmated females, or with those of Galun and Warburg (1968) who could mimetic the slower protein digestion in virgin females by irradiating mated ones. Lowered digestive activity in virgin compared to mated females has also been reported from haematophagous insects (Edman, 1970, Pratt and Davey, 1972).

Our cytological results show that midgut cells of virgin females are able to store haemoglobin in phagosomes whithout actually digesting it immediately. Initiation of intracellular digestion by the fusion of primary lysosomes with such phagosomes could be delayed in the midgut of virgin ticks (Grandjean, in press). Protease level has been shown to remain constant over long periods in midguts of virgin females (Tatchell *et al.*, 1972), which would agree with the hypothesis of a delayed intracellular digestion. Proteases would be synthetised, but not readily used up by the midgut cells.

In midgut cells of virgin females, the "whorl"-like arrangement of RER could suggest poor cellular activity of protein synthesis, as in midgut cells of fasting haema-

tophagous insects (see Grandjean, in press). In relation to the slower digestion, nutrient reserves are stored in the midgut lumen of virgin females, even several months after bloodmeal (Aeschlimann and Grandjean, 1973a, Germond and Aeschlimann, 1977).

4.3.3. *Hypothetical endocrine relay of the triggering effect of mating on digestion*

Digestive activity of midgut cells appears to be regulated differently in mated or virgin females. It seems thus to be influenced by mating stimuli. Neurosecretory activity has been demonstrated to be linked to digestion (Gabbay and Warburg, 1976 : *O. tholozani*). Following delayed mating, neurosecretory cells become activated (Eisen *et al.*, 1973 : *Argas persicus*). An endocrinally mediated, triggering action of mating on digestion is not to be excluded. In autogenic argasids, which lay eggs without previous bloodmeal as adults (Feldman-Muhsam, 1973, Aeschlimann and Grandjean, 1973b), digestion could be activated in a similar way to that observed after experimental delayed mating.

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HISTOLOGIE ET ULTRASTRUCTURE DE L'INTESTIN ANTÉRIEUR ET DES CELLULES PÉRIPROVENTRICULAIRES DE L'INTESTIN MOYEN DE FEMELLES *ORNITHODORUS MOUBATA* MURRAY *SENSU STRICTO* WALTON, EN RELATION AVEC LE CYCLE DIGESTIF

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AVEC 34 FIGURES

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HISTOLOGIE ET ULTRASTRUCTURE DE L'INTESTIN ANTÉRIEUR ET DES CELLULES PÉRIPROVENTRICULAIRES DE L'INTESTIN MOYEN DE FEMELLES *ORNITHODORUS MOUBATA* MURRAY *SENSU STRICTO* WALTON, EN RELATION AVEC LE CYCLE DIGESTIF

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AVEC 34 FIGURES

1. INTRODUCTION

Dans le cadre d'une étude sur la digestion du sang chez *Ornithodorus moubata* (GRANDJEAN 1983, 1984), l'anatomie de l'intestin a été décrite (GRANDJEAN et AESCHLIMANN 1973). L'intestin antérieur comprend l'œsophage et la valve proventriculaire. Des cellules particulières, nommées ici périproventriculaires, entourent immédiatement la valve; elles appartiennent déjà à l'intestin moyen.

A notre connaissance, l'ultrastructure de l'intestin antérieur n'a pas encore été étudiée chez les Tiques, voire chez les Chélicérates. On n'a pas non plus fait de recherches, au niveau de l'intestin antérieur, sur les modifications cellulaires liées au cycle digestif.

2. MATÉRIEL ET MÉTHODES

Les Tiques: Les intestins antérieurs ont été prélevés sur des femelles adultes d'une souche d'*O. moubata* Murray *sensu stricto* Walton 1962 (GRANDJEAN 1983, 1984), à différents intervalles après le repas sanguin (2 Tiques par stade mentionné dans les figures 32, 33 et 34).

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La microscopie électronique à transmission (MET): Pour les détails de préparation (fixation avec du dialdéhyde glutarique et du tétroxyde d'osmium, inclusion dans une résine époxy: voir GRANDJEAN 1984). Nous remercions Olivier Peter de son matériel, préparé de même et aimablement mis à notre disposition.

L'histologie: Les intestins sont fixés et inclus comme pour la MET; les coupes, épaisses d'un micromètre, sont colorées avec du bleu de toluidine (6 parts à 1% + 3 parts de carbonate de sodium à 2,5% + 1 part d'éthanol à 70%).

3. RÉSULTATS

3.1. *Le pharynx*

Nos observations histologiques du pharynx d'*O. moubata* correspondent aux résultats antérieurs publiés sur *Ornithodorus* (TRUE 1932, SEN 1935, BERTRAM 1939, SONENSHINE et GREGSON 1970; voir aussi SNODGRASS 1948). L'ultrastructure du pharynx n'a pas été étudiée ici.

3.2. *L'œsophage*

Reliant le pharynx à l'intestin moyen, l'œsophage passe à travers la masse du synganglion (EICHENBERGER 1970 et fig. 1, 5, 9: C). A l'extrémité de l'œsophage – qui se termine par la valve proventriculaire – se trouvent les cellules périproventriculaires de l'intestin moyen (voir 3.3.).

a) *Histologie:* L'épithélium de l'œsophage est formé d'une couche simple de cellules épithéliales. Du côté de la lumière de l'œsophage, les cellules sont recouvertes d'une cuticule de chitine (fig. 1: flèche). A la base de l'épithélium se trouvent des cellules musculaires et nerveuses (fig. 5: M et N), le tout étant entouré d'une couche triple (fig. 5: flèche) qui correspond à l'«organ investment layer» décrit dans l'intestin moyen d'insectes hématophages (RICHARDS 1975).

b) *Ultrastructure:* Au niveau du synganglion, l'épithélium forme des replis qui donnent à l'œsophage une forme «en étoile» sur les coupes longitudinales (fig. 10, 31). Les cellules épithéliales, peu épaisses, sont entourées de cellules musculaires et nerveuses (fig. 9). La membrane cellulaire forme de nombreux replis dans la partie basale des cellules (fig. 9, 10: f). Différents types de jonctions cellulaires existent: des *zonulae continuae*, des desmosomes septés (fig. 11: flèches) et, dans la partie apicale, des *zonulae adhaerentes* (fig. 11: za, comme dans les cellules de l'intestin moyen, GRANDJEAN 1984); la présence de «gap junctions» reste à démontrer.

Dans la partie apicale, la membrane cellulaire plissée (fig. 6, 7, 11: af) entoure le cytoplasme qui forme des expansions (fig. 10, 11, 12: e) dans la cuticule (cu). Cette dernière est formée d'une épicuticule externe, fortement opaque aux électrons (fig. 11: ec), et d'une épicuticule interne qui contient des éléments fibrillaires (fig. 2, 4, 6, 11: *; WEIS-FOGH 1970 a trouvé des structures comparables chez des insectes); l'épicuticule interne

n'est pas nettement séparée de la procuticule (cu); on retrouve les éléments fibrillaires dans cette dernière, ainsi que des plages homogènes et faiblement opaques aux électrons (fig. 11: d).

Dans les cellules épithéliales de l'œsophage, on peut observer des inclusions de réserves avec un contenu lipidique (fig. 10: flèche) ou de glycogène (fig. 11: gl). Les organites tels que le réticulum endoplasmique granulé (REG) et les dictyosomes n'apparaissent qu'à certaines phases de l'activité cellulaire (voir plus bas). Les microtubules sont fréquents (fig. 4, 7, 8). A proximité de la valve proventriculaire, les cellules sont plus allongées et plus nombreuses, donnant l'image d'un épithélium pseudo-stratifié (fig. 12).

c) *Modifications liées au cycle digestif* (fig. 32): 3 à 8 jours après un repas sanguin, la différenciation des cellules de l'œsophage est remarquable; elle touche notamment le développement du réticulum endoplasmique granulé (fig. 7, 8) et des dictyosomes (fig. 4, 7, 8). Ces derniers forment des vésicules lisses (fig. 8: * en bas) qui paraissent identiques aux vésicules apicales; celles-ci sont continues avec la procuticule (* en haut). Les cellules hautement organisées contiennent de nombreux microtubules.

Quelques jours plus tard, on trouve de grandes inclusions de glycogène (fig. 12). La masse du cytoplasme des cellules de l'œsophage augmente, ce qui est particulièrement visible par rapport à la position des replis de la membrane apicale (fig. 4, 6: af). La masse cuticulaire, fortement distendue lors de l'absorption du repas sanguin, s'épaissit fortement dans les jours qui suivent (comparer fig. 3 et 1; fig. 32); plus tard, son épaisseur diminue, alors que la taille des cellules augmente (fig. 5).

Alors que nous n'avons pas d'explication du premier mécanisme, nous pensons pour le second que les vésicules lisses produites par les dictyosomes pourraient participer à la digestion de la cuticule (sans toucher l'épicuticule; ce phénomène est connu chez les insectes, WIGGLESWORTH 1971). 5 à 12 jours après le repas sanguin, la masse cuticulaire est fortement réduite (fig. 5, 11); plus tard, elle augmente à nouveau (fig. 2: Tique à jeun).

3.3. *La valve proventriculaire*

a) *Histologie:* La valve proventriculaire fait saillie dans la lumière intestinale (fig. 13); elle est composée de cellules cylindriques allongées (fig. 14, 31). On trouve des éléments musculaires à leur base, qui semblent servir de sphincter (fig. 14: M). Une triple paroi (fig. 3, 14: SW, 3 flèches) forme la limite avec le sinus sanguin (ROSHDY et *al.* 1973), entre le synganglion et l'œsophage. Les cellules nerveuses trouvées ici (fig. 31: N) correspondent, par leur localisation, à l'organe neurohémal (ROSHDY et *al.* 1973, PETER, communication personnelle).

b) *Ultrastructure:* Les cellules cylindriques de la valve proventriculaire ne paraissent pas fondamentalement différentes des cellules épithéliales de l'œsophage décrites ci-dessus. La membrane cellulaire est fortement plissée dans la partie basale (fig. 21), où les mitochondries sont nombreuses

(fig. 20). Les jonctions intercellulaires correspondent à celles des cellules épithéliales de l'œsophage (fig. 23: sd) et les microtubules se voient fréquemment (mt). Le niveau de développement du réticulum endoplasmique réticulé et la présence de vacuoles claires (fig. 15: v) dépendent du degré d'organisation de la cellule (voir plus bas). On peut voir quelques inclusions lipidiques (fig. 20: Li) et du glycogène (flèches). La plupart des cellules contient encore un autre type d'inclusion, entouré d'une membrane: des grains fortement opaques aux électrons baignent dans une matrice finement granulée et moyennement opaque aux électrons (fig. 19, 20: db). La partie apicale de la membrane cellulaire latérale est généralement fortement plissée (fig. 15-18, 22, 23: cm); la zone des plis apicaux (af) correspond à celle qui a été décrite pour les cellules de l'œsophage.

Des expansions protubérantes de cytoplasme (e) font saillie dans la masse cuticulaire, ce qui se voit même au niveau de l'histologie (fig. 14). La cuticule est plus épaisse qu'au niveau de l'œsophage, mais possède la même structure, y compris les inclusions modérément opaques aux électrons (fig. 24: d, voir plus bas).

c) *Modifications liées au cycle digestif* (fig. 33): Dans les cellules du proventricule, les modifications cellulaires ne sont pas aussi importantes que dans les cellules du reste de l'œsophage. D'autres observations sont pourtant encore nécessaires.

Chez les Tiques à jeun, la masse cuticulaire est épaisse et, seules, quelques rares expansions cytoplasmiques, peu épaisses, dépassent le niveau des replis apicaux de la membrane cellulaire (fig. 15). Des vacuoles subapicales, au contenu clair (v), parfois continues avec la procuticule (cu), sont nombreuses. Après un repas sanguin, d'autres vacuoles claires apparaissent et restent cinq jours environ. Leur nombre diminue ensuite, tandis que les expansions cytoplasmiques pénètrent dans la cuticule, dépassant le niveau des replis apicaux de la membrane cellulaire (fig. 16, 17, 22). Trois semaines après le repas sanguin, les expansions cytoplasmiques diminuent, alors que des vacuoles apicales claires apparaissent (fig. 18). Les plages homogènes de la cuticule (fig. 24: d) sont présentes en grand nombre, entre 5 et 22 jours après le repas sanguin, soit pendant la période qui correspond à la phase rapide de la digestion (GRANDJEAN 1983).

3.4. *Les cellules périproventriculaires*

a) *Histologie:* Autour de la valve proventriculaire, la dizaine de cellules les plus proches diffèrent des autres cellules de l'intestin moyen; comme celles-ci, elles ont un noyau plus grand que celui des cellules de l'intestin antérieur (fig. 25); par contre, elles ne participent pas à la digestion. En outre, elles sont remplies de grains opaques aux électrons, qui ne sont pas de l'hématine (voir GRANDJEAN 1984), et leur lame basale est fine, contrairement à celle des cellules de l'intestin moyen (GRANDJEAN 1984, voir plus bas).

b) *Ultrastructure:* A la limite entre la valve proventriculaire et les cellules périproventriculaires, la couche apicale de chitine disparaît

(fig. 28: flèche). A son extrémité, la cuticule forme de nombreux replis; à ce niveau, elle est essentiellement composée d'épicuticule.

La membrane basale des cellules périproventriculaires est fine (fig. 29: bl), comparable à celle de l'intestin antérieur (fig. 12: bl) et non à celle, plus épaisse, de l'intestin moyen (fig. 29: BL). Cette dernière est reliée directement à la paroi du sinus sanguin (fig. 25, 29: *; fig. 31). Une analyse plus poussée des cellules périproventriculaires est encore nécessaire. Notre étude préliminaire révèle un réticulum endoplasmique granulé fortement développé (fig. 27: R), des dictyosomes avec des vésicules faiblement opaques aux électrons (fig. 30: *) et des corps denses très opaques (fig. 28: db).

c) *Modifications liées au cycle digestif* (fig. 34): Les cellules périproventriculaires sont approximativement cubiques chez les Tiques à jeun et ne changent guère de forme au moment de l'absorption du repas sanguin. Quelques jours après celui-ci, elles commencent à pousser et deviennent cylindriques, en forme de colonnes (fig. 25). Plus tard, leur partie médiane s'étrangle jusqu'à ce que la partie apicale se détache dans la lumière intestinale (fig. 26: flèche).

4. DISCUSSION

4.1. *Intestin antérieur (œsophage et valve proventriculaire)*

Les plis apicaux de la membrane cellulaire en contact avec la procuticule, décrits ici, sont comparables à ceux que l'on trouve dans l'intestin postérieur d'*Aedes aegypti* (TONGU et *al.* 1969). Chez cette dernière espèce, les diverticules de l'intestin antérieur présentent les mêmes types de jonctions intercellulaires que ceux que nous avons observés (HECKER et BLEIKER 1972). Contrairement à ce qui est décrit pour les insectes (SMITH 1970), nous avons trouvé chez *O. moubata* de nombreux replis de la membrane cellulaire basale. Nous ne pouvons toutefois pas déterminer si ces structures remplissent des fonctions de transport ou si elles permettent de répondre à de fortes extensions mécaniques. L'absorption de matériel contenu dans la lumière de l'intestin antérieur (d'ailleurs généralement vide) n'a pas été observée: les cellules ne participent pas à la digestion (contrairement aux insectes: TREHERNE 1967). Néanmoins, les activités des cellules de l'intestin antérieur subissent des modifications bien définies, au cours du cycle digestif. Les causes et les mécanismes de ces changements méritent d'être étudiés plus en détail.

Du point de vue histologique, la valve proventriculaire est semblable à celle des insectes (HOOGSTRAAL 1956, MOLOO et KUTUZA 1970). Son rôle semble être limité à celui d'une barrière qui empêche le contenu de l'intestin moyen de s'échapper à travers l'œsophage. On sait par contre que, chez des insectes, elle sert plutôt à régler l'admission de nourriture dans l'intestin moyen (BERRIDGE 1970). Cette fonction n'existe pas chez les Tiques, car leur intestin antérieur n'a pas de rôle de stockage de la nourriture.

La présente étude a démontré l'existence de muscles de type sphincter, à la base de la valve proventriculaire; ceux-ci n'avaient pas été trouvé par GUIRGUIS (1971) chez *Argas*. La limite de la couche de cuticule apicale de l'intestin antérieur, que SONENSHINE et GREGSON (1970) n'avaient pas pu démontrer, a été mise en évidence.

4.2. *Cellules périproventriculaires*

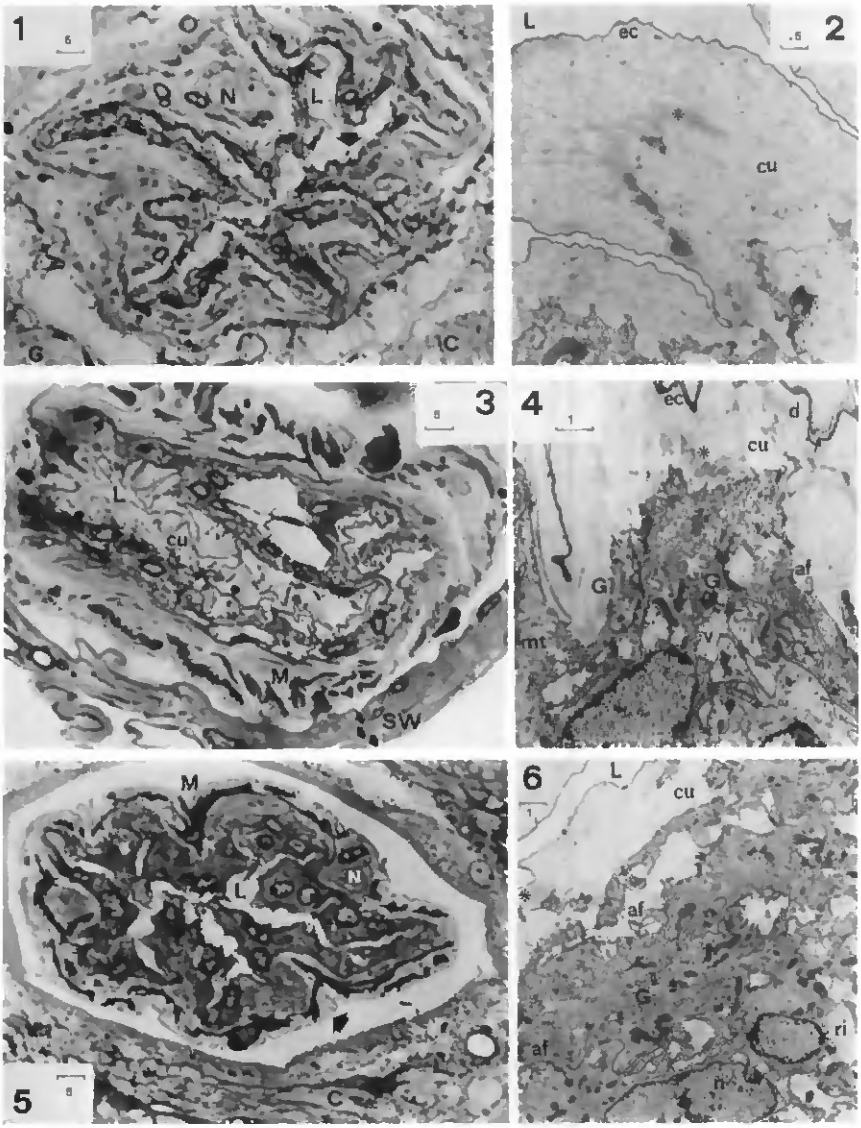
Par leur localisation, les cellules périproventriculaires d'*O. moubata* correspondent aux «cellules sécrétrices» de la «cardia» des Ixodides (ROESLER 1942, SCHULZE 1942) ou des Argasides (BALASHOV 1961). A l'intérieur de telles cellules, on a aussi trouvé des granulations pigmentées chez *Rhipicephalus sanguineus* (STELLA 1942). La fonction exacte de ces cellules reste à déterminer. Quoique non systématique, la présence de vésicules golgiennes faiblement opaques aux électrons (fig. 30) suggérerait une activité cellulaire comparable à celle de cellules intestinales de certaines Tiques, liée à la production de matériel acellulaire (GRANDJEAN 1984).

ABRÉVIATIONS UTILISÉES DANS LES FIGURES

af («apical folds») = replis apicaux de la membrane cellulaire, bl («basal lamina») = lame basale (intestin antérieur et cellules périproventriculaires), cm («cell membrane») = replis de la membrane cellulaire latérale, cu = procuticule, d («dots») = plages homogènes dans la cuticule, db («dense bodies») = corps denses, e = expansions du cytoplasme dans la cuticule, f («folds») = replis de la membrane cellulaire basale, gl = glycogène, m = mitochondries, mt = microtubules, mv = microvillosités, n = noyau, ri («reserve inclusions») = inclusions de réserves, sd («septate desmosomes») = desmosomes septés, v = vacuoles claires, za = *zonula adhaerens*.

BL («basal lamina») = lame basale (intestin moyen), C = masse cérébrale du synganglion, G = dictyosome (appareil de Golgi), L = lumière (intestin antérieur), Li = inclusions lipidiques, M = cellules musculaires, MC («midgut cells») = cellules de l'intestin moyen, ML («midgut lumen») = lumière de l'intestin moyen, N = cellules nerveuses, OE = œsophage, PC («proventricular cells») = cellules proventriculaires, R = réticulum endoplasmique granulé, SW («sinus wall») = parois du sinus sanguin, T = cellules trachéolaires.

Les chiffres sur les échelles correspondent à des micromètres (1 μm = 10^{-6} m).



Œsophage

Fig. 1, 3, 5. Coupes histologiques: fig. 1 femelle à jeun (flèche = couche cuticulaire), fig. 3 un jour après le repas sanguin (flèches: triple paroi du sinus sanguin), fig. 5 cinq jours après le repas sanguin (flèche = couche de l'«organ investment layer»).

Fig. 2, 4, 6. Micrographies électroniques de la partie apicale des cellules: fig. 2 à jeun, fig. 4 trois jours, fig. 6 cinq jours après le repas sanguin (* = matériel fibrillaire).

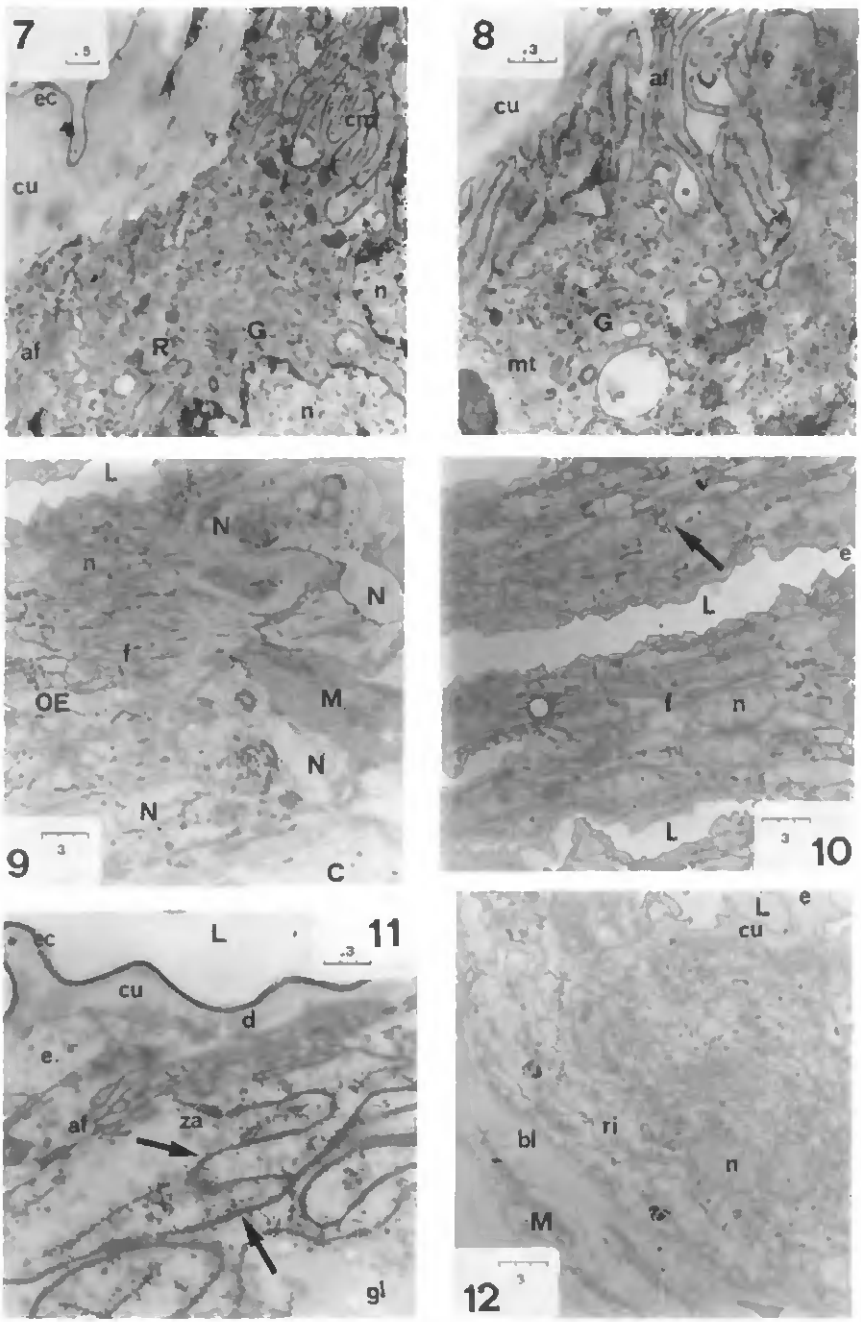
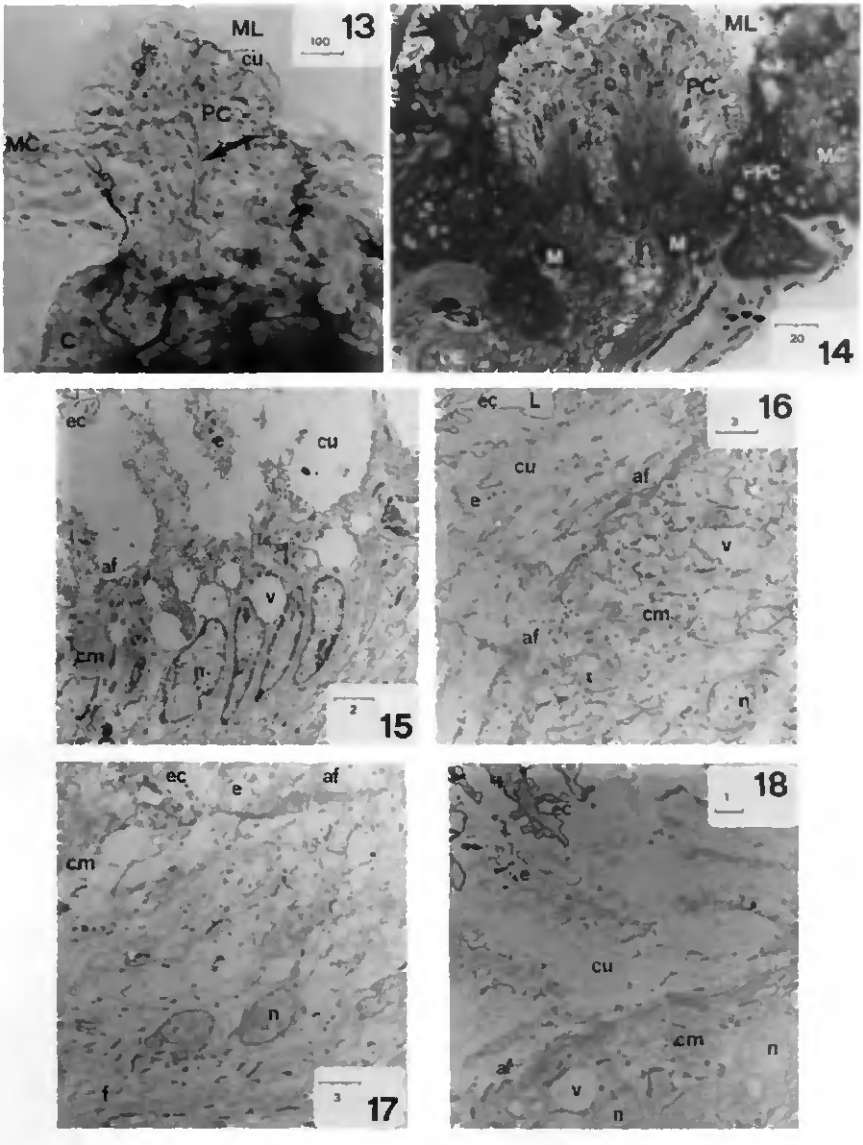


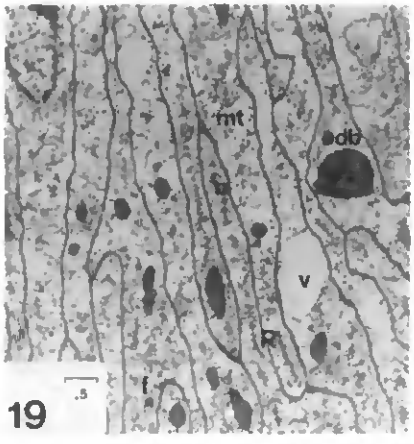
Fig. 7-12. Micrographies électroniques; fig. 7 partie apicale (cinq jours après le repas sanguin), fig. 8 détail (comme en fig. 7; * en haut = vésicules claires en contact avec la procuticule, * en bas = vésicules de Golgi), fig. 9-11 replis «en étoile» de l'œsophage au niveau du synganglion, voir fig. 31, permettant une succion? (flèche de la fig. 10 = vacuole lipidique, flèches de la fig. 11 = desmosomes septés, * (fig. 11) = matériel fibrillaire de l'épicuticule interne), fig. 12 cellules plus allongées, proches de la valve proventriculaire.

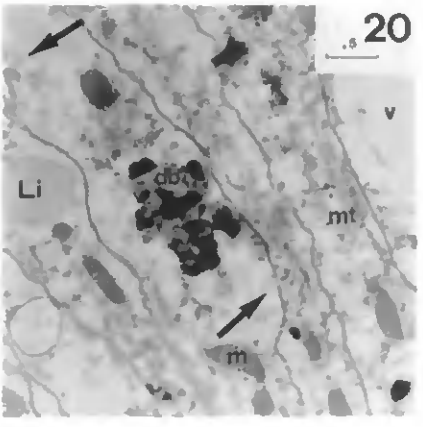


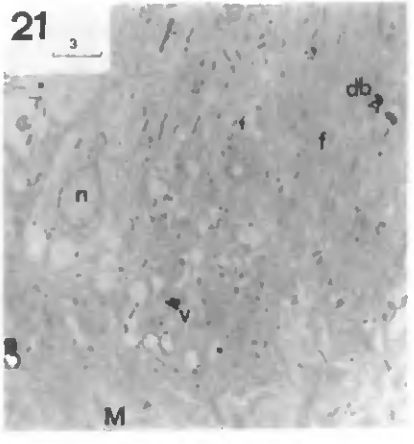
Valve proventriculaire

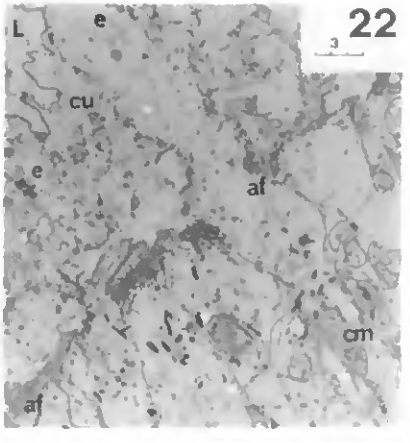
Fig. 13. Préparation «in toto» (flèche = lumière de l'intestin moyen), fig. 14 coupe histologique (flèches = triple couche du sinus sanguin).

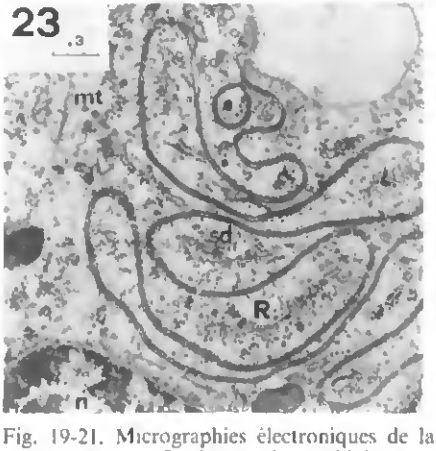
Fig. 15-18. Micrographies électroniques de la cuticule et de la partie apicale des cellules proventriculaires: fig. 15 à jeun, fig. 16 douze jours (face à la lumière de l'intestin antérieur), fig. 17 douze jours (face à la lumière de l'intestin moyen), fig. 18 vingt-deux jours après le repas sanguin.











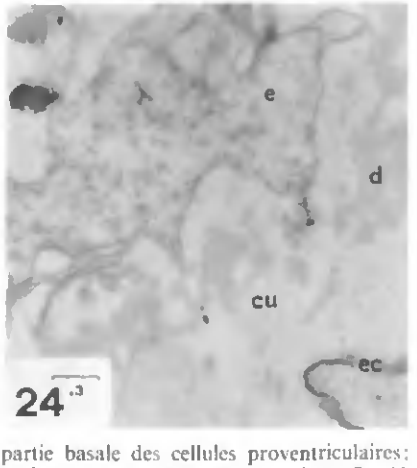
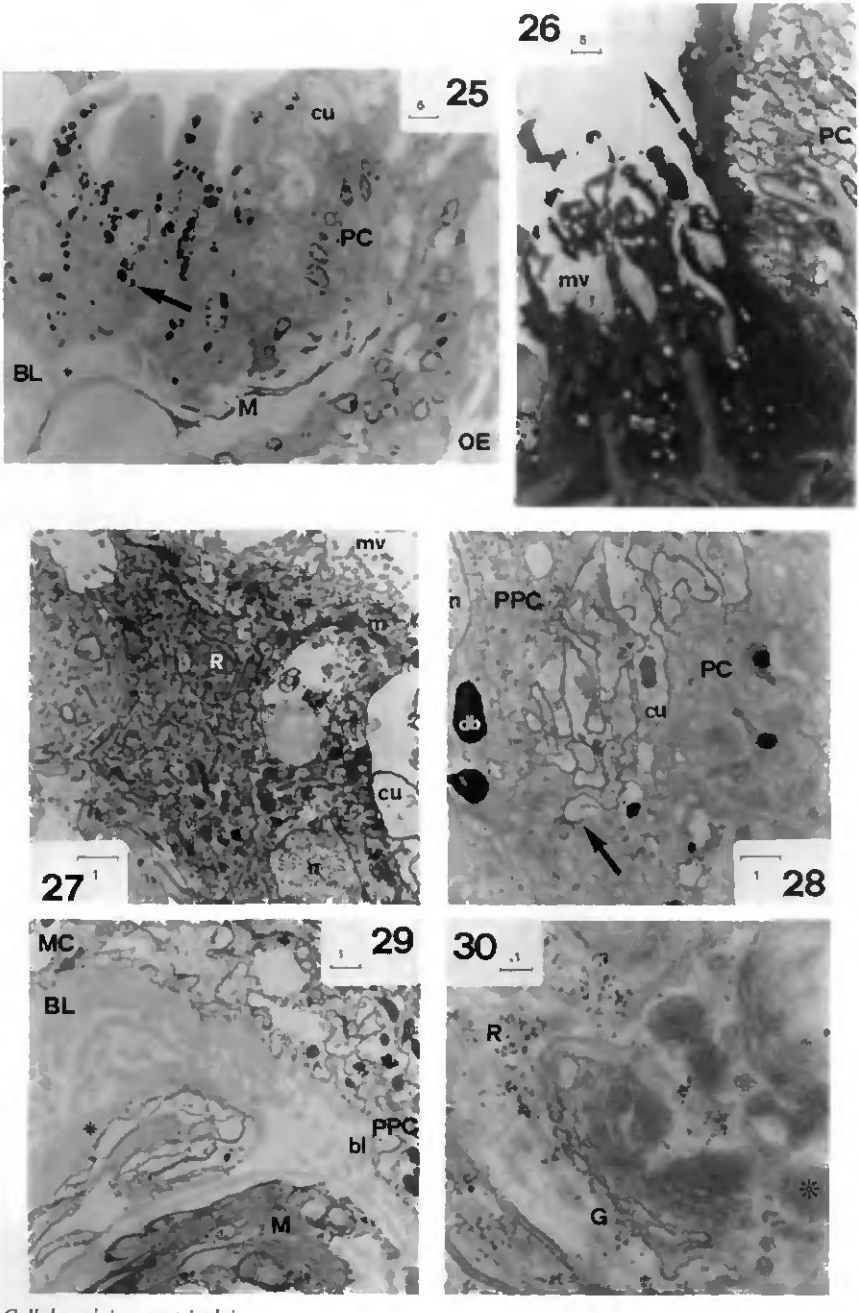


Fig. 19-21. Micrographies électroniques de la partie basale des cellules proventriculaires: fig. 19 à jeun, fig. 20 cinq jours (flèche = probablement des grains de glycogène), fig. 21 douze jours après le repas sanguin.

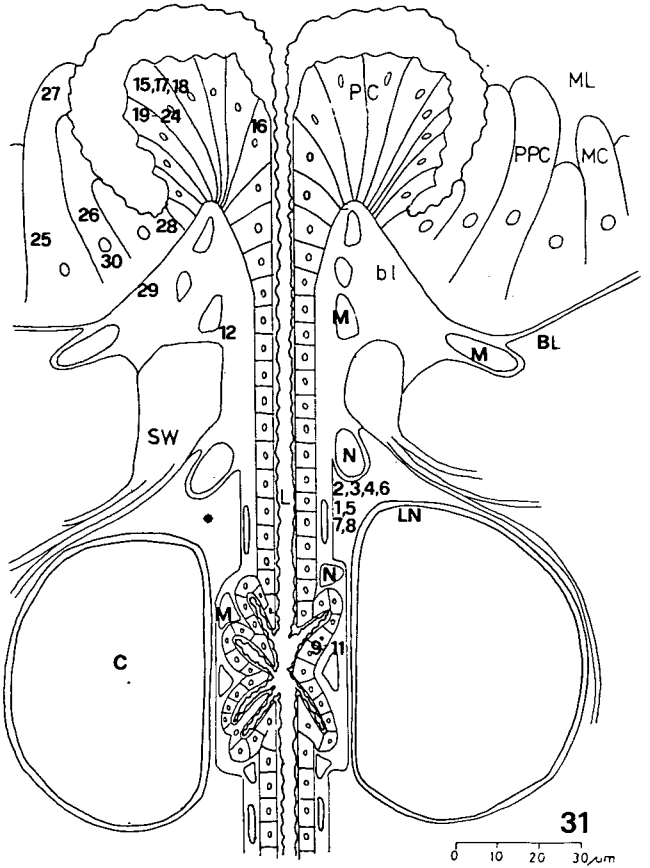
Fig. 22-24. Partie apicale: fig. 22 douze jours, fig. 23 à jeun, fig. 24 vingt-deux jours après le repas sanguin.



Cellules périproventriculaires

Fig. 25, 26. Coupes histologiques: fig. 25 cinq jours (flèches = corps denses), fig. 26 soixante-neuf jours après le repas sanguin (flèche = reste de la «tige», après détachement de la partie apicale).

Fig. 27-30. Micrographies électroniques: fig. 27 partie apicale (douze jours), fig. 28 partie centrale (un jour, flèche = terminaison de la cuticule de l'intestin antérieur), fig. 29 partie basale (à jeun), fig. 30 détail avec un dictyosome (un jour après le repas sanguin, * = vésicule golgienne avec un contenu modérément opaque aux électrons).



Schémas synthétiques
Fig. 31. Intestin antérieur (sans le pharynx): les chiffres indiquent le numéro des figures, * = sinus sanguin périganglionaire.

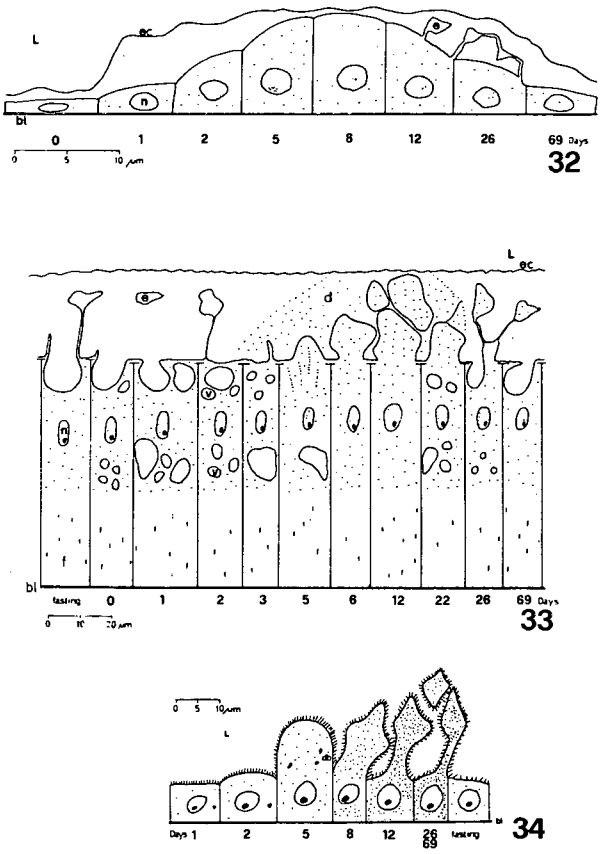


Fig. 32-34. Modifications liées au cycle digestif: fig. 32 cellules épithéliales de l'œsophage, fig. 33 cellules proventriculaires, fig. 34 cellules périproventriculaires.

Résumé

Les structures histologiques et cytologiques, ainsi que leurs modifications cycliques, sont décrites pour l'œsophage, la valve du proventricule et les cellules périproventriculaires de l'intestin moyen de femelles de l'espèce *O. moubata*. Les cellules de l'intestin antérieur ne participent pas à la digestion du sang. Elles sont pourtant soumises à un développement cyclique qui pourrait être lié à un renouvellement cuticulaire. Quant aux cellules périproventriculaires, d'autres études sont nécessaires pour déterminer leurs fonctions exactes.

Zusammenfassung

Oesophagus, Proventrikelöffnung und spezielle Mitteldarmzellen in unmittelbarer Nähe des Proventrikels wurden bei *O. moubata* Weibchen histologisch und zytologisch auf ihre Strukturen und Veränderungen im Verdauungskreislauf untersucht. Die Vorderdarmzellen beteiligen sich nicht an der Blutverdauung, zeigen jedoch eine zyklische Entwicklung, wahrscheinlich in Zusammenhang mit einer Kutikulaerneuerung. Die Funktionen der periproventrikulären Mitteldarmzellen bleiben vorderhand ungeklärt.

Summary

Histology, cytology and modifications linked to life and digestive cycle are described for oesophagus, proventricular valve and periproventricular cells of the midgut of *O. moubata* females. Foregut cells are not involved in actual digestion, but undergo a cyclical development which could be linked with cuticle turn-over. Further investigation is still necessary to elucidate the exact function of periproventricular cells.

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