ULTRASTRUCTURAL STUDIES ON THE SALIVARY GLANDS OF TICKS: *HAEMAPHYSALIS LEACHII LEACHII* (AUDOUIN) [IXODOIDEA: IXODIDAE] AND *ORNITHODOROS (ORNITHODOROS) MOUBATA* (MURRAY) [IXODOIDEA: ARGASIDAE].

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Title: Ultrastructural studies on the salivary glands of ticks: Haemaphysalis leachii leachii (Audouin) (Ixodoidea: Ixodidae) and Ornithodoros (Ornithodoros) moubata (Murray) (Ixodoidea: Argasidae).

Abstract

Salivary glands of the unfed tick Haemaphysalis leachii contain four types of alveoli. Type I alveoli have the morphological characteristics of transporting epithelia. Types II, III and IV are granule-secreting alveoli and are considered to secrete the enzymes and cement components required during feeding. After female attachment, types II, III and IV alveoli are initially concerned with the secretion of granular materials but become involved with fluid secretion as feeding progresses.

Salivary glands of the unfed ticks Ornithodoros moubata contain two types of alveoli. Type I is similar to that of H. leachii. Type II are granule-secreting alveoli but structurally differ from those of H. leachii. These alveoli do not secrete cement and are not significantly active during feeding.

Salivation processes in the tick H. leachii and O. moubata seem to be mainly under neural control.

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INTRODUCTION AND REVIEW OF THE LITERATURE

Ticks are obligate ectoparasites which infest man and animals, and feed on their blood. They are reservoirs and vectors of pathogenic protozoa (Neitz, 1956; Mehlihorn et al., 1978), rickettsiae and viruses (Hoogstraal, 1966, 1967; 1974). Ticks are also known to cause toxaemia, paralysis, anaemia and severe irritation to humans and domestic animals (Hoogstraal, 1974). Interest has focused on the tick salivary glands as playing an essential role in pathogen development (Schein and Friedhoff, 1978) and the glands constitute the major route through which pathogens are released and transmitted in the salivary secretion to susceptible hosts.

A thorough knowledge of tick salivary gland fine structure is important in the explanation and evaluation of disease transmission by ticks. However, the internal milieu of the salivary glands of each tick species has special structural and functional properties in the prefeeding, feeding and post-feeding states. Pathogen transmission and vector potential are influenced by this structure as well as other individual specific characteristics.

The purpose of the present study was to investigate the ultrastructure of the salivary glands of the ixodid yellow dog-tick, Haemaphysalis leachii leachii (Audouin), vector of human Rocky Mountain spotted fever Rickettsia rickettsii.

Coxiella burnetii, the causative organism of Q fever (Hoogstraal, 1956) and tick-borne typhus in Africa, Asia and the Americas (Hoogstraal, 1976; Okereke, 1976), and the argasid hut-tampan tick, Ornithodoros (Ornithodoros) moubata (Murray) vector of human African tick-borne relapsing fever Borrelia duttoni, Q fever (Coxiella burnetii), and Salmonella enteritidis, the etiologic agent of food poisoning (Hoogstraal, 1956; Okereke, 1976).

The fine structure of ixodid and argasid salivary glands has been studied by several authors in recent years (Dzhafarov, 1965a, b; Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973; Roshdy and Coons, 1975; Megaw, 1976). The salivary glands were considered to contain two functionally distinct types of alveoli: nongranular-secreting alveoli concerned with the elimination of excess fluid, the other with the secretion of granular materials. However, recent evidence suggests that the structure of ixodid salivary glands may be more complicated than those of argasids and more complicated than was originally appreciated.

It is well documented that argasid ticks eliminate excess ions and water through their coxal glands (Bone, 1943; Lees, 1946, 1947). The salivary glands of ixodids are responsible for osmoregulation during feeding (Tatchell, 1967a) and 200-500 ml. of fluid can be excreted by an ixodid female over a 12 to 48 hour

period (Kitaoka and Yajima, 1958; Tatchell, 1969c). Tatchell (1967a) demonstrated that in Boophilus microplus neither secretion through malpighian tubules nor cuticular transpiration were responsible for the rapid diuresis which occurs during feeding. Transpiration through the cuticle only accounted for less than 15 ml. of fluid during the final 12 hours of feeding, far less than that required to achieve the optimum concentration of the blood meals (Tatchell, 1967a). A dilute oral secretion formed by Dermacentor andersoni was suggested as a possible route for the excretion of excess fluid (Gregson, 1960). The fluid would be passed through the mouth parts of the tick during feeding and be reintroduced to the host's vascular system. Such a mechanism would account for the ease by which pathogens carried by ticks infect host tissue.

The nongranular-secreting alveoli possess morphological features indicative of epithelia involved with fluid transport. In fact a number of authors have described these alveoli as responsible for most of the fluid excretion, (Gregson, 1967; Tatchell, 1967a, 1969b; Balashov, 1968; Roshdy, 1972; Sauer and Hair, 1972; Coons and Roshdy, 1973; Roshdy and Coons, 1975). However, although the nongranular-secreting alveoli in salivary glands of H. leachii and O. moubata are similar to those of other ticks, alternative cells, found in the granule-

secreting alveoli of H. leachii and O. moubata may also be suited for this role.

Alveoli that appeared to be involved with both fluid and granule secretion were observed in the salivary glands of partially fed female Dermacentor andersoni (Meredith and Kaufman, 1973). Similar alveoli have been observed in the salivary glands of H. leachii in this study; the alveoli are initially concerned with the secretion of granular materials but become involved with fluid secretion as feeding progresses.

Oral secretions can be collected from ixodid ticks in capillary tubes placed over the mouth parts, although tubes which fail to compress the chelicerae do not elicit a response (Gregson, 1957). Injection of ticks with pilocarpine increases the yield of saliva (Howell, 1966; Tatchell, 1967b) and this technique was used to demonstrate the osmoregulatory role of the salivary glands during feeding (Tatchell, 1969c).

The secretion of a cement substance is characteristic of most genera of slow feeding ixodid ticks by which the tick ensures firm attachment of the mouth parts to the host. This phenomenon was observed by Cowdry and Danks (1933), Foggie (1959), Gregson (1960), Moorhouse and Tatchell (1966), Balashov (1968), Moorhouse (1969) and Chinery (1973). The origin of this material was for some time a matter of dispute for, although Cowdry and Danks (1933) described the cement as a tick secre-

tion, Foggie (1959) considered it to be a product of a host-parasite reaction. It was later shown that the mechanical laceration of host tissue during attachment of the tick was followed by the exudation of a white free-flowing fluid which bathed the mouthparts and later hardened to form a solid cone (Gregson, 1960). This observation was subsequently confirmed (Gregson, 1967; Moorhouse and Tatchell, 1966; Tatchell and Moorhouse, 1970) and the cement cone was considered to enhance the attachment of the tick to the host. Granule-secreting cells that appeared to be involved with cement secretion were described in the salivary glands of female Haemaphysalis spinigera (Chinery, 1965) and Boophilus microplus (Binnington, 1978).

Anticoagulating substances were reported in Argas persicus saliva (Nuttall and Stickland, 1908; Roshdy, 1972; Chinery, 1974). Hellman and Hawkins (1967) have described anticoagulins in the saline extracts of O. moubata salivary glands. Roshdy (1972) and Chinery (1974) described granule-secreting cells which are believed to be concerned with the secretion of anticoagulating substances. The saliva of Boophilus microplus, however, lacks anticoagulants (Tatchell, 1969a, Tatchell and Binnington, 1971).

Cytolysin and pharmacological properties of some ixodid and argasid ticks have been demonstrated by Lavoipierre and Rick (1955), Foggie (1959), Arthur (1962, 1965), Tatchell (1969a,b).

Geczy et al. (1971) and Moorhouse (1975). Saliva secreted by B. microplus contains an agent which causes an increased permeability of host capillaries and thus enhances the supply of blood to the mouthparts (Geczy et al., 1971; Tatchell and Binnington, 1971).

The salivary glands of ixodid ticks are thus concerned with three main activities during attachment to the host and the subsequent feeding period. The glands are responsible for the elimination of excess fluid ingested with the meal, the secretion of cement substances, and the secretion of agents which prevent blood coagulation and increase the supply of blood to the mouthparts.

The fine structure of the salivary glands of H. leachii and O. moubata is described here with special reference to the change in structure associated with attachment and secretion, followed by degeneration of the glands in H. leachii preceeding oviposition. Such changes have not been described before in the literature where authors have described mainly the structure found in unfed or partially fed ticks. An attempt is made in the discussion to relate structure to function in the complicated cells and alveoli of the salivary glands and to account for the changes found in the structure of the female salivary glands of H. leachii in comparison to the lack of fundamental structural change found in the male, and in the soft tick O. moubata.

Taxonomic Status:

Haemaphysalis leachii leachii, Audouin, 1827 (Fig. 1) is one of three subspecies of H. leachii (Hoogstraal, 1958) found in Africa. The other two subspecies are:

H.l. muhsami, Santos Dias, 1954 (found in Africa)

H.l. indica, Warburton, 1910 (found in the Oriental region)

Ornithodoros moubata, Murray, 1877, (Fig. 110), is one of seven widespread subgenera of the genus Ornithodoros, Koach, 1844, which are found in Africa. Clifford et al. (1964) reviewed the subgeneric and species groupings of the genus Ornithodoros and included the following species in the subgenus Ornithodoros.

O. moubata, Murray, 1877.

O. Savignyi, Audouin, 1826.

O. compactus, Walton, 1862.

O. opertus, Walton, 1862.

O. porcinus, Walton, 1862.

O. eremicus, Cooley and Kohls, 1941.

O. procaviae, Theodor and Costa, 1960.

MATERIALS AND METHODS

Haemaphysalis l. leachii and *Ornithodoros o. moubata* used in the present investigation were from colonies maintained at the London School of Hygiene and Tropical Medicine. The ticks were kept at 28°C and 80-90 per cent relative humidity.

H. leachii and *O. moubata* were fed on domesticated rabbits (Figs. 2, 3) and their feeding periods were 5 to 7 days and 15 to 20 minutes respectively.

A. Light Microscopy:

To study the general anatomy of the adult salivary glands of *H. leachii*, unfed, partially fed (1, 3, 5 and 7 days after attachment), and fully engorged (1, 3 and 5 days after dropping off from the host), as well as ovipositing females, were dissected. For the salivary glands of *O. moubata*, unfed, partially fed (5, 10, 15 and 20 minutes after attachment) and fully engorged adult ticks (5, 10 minutes and two hours after dropping off from the host) as well as ovipositing females, were dissected. Dissection was carried out in insect Ringer solution (Barka and Anderson, 1963) under a binocular microscope. An incision was made in the lateral and posterior margins of the tick's body by means of a sharp razor-blade. The dorsal cuticle was then gently raised from the posterior end and was carefully separated from the underlying tissues. Dissection of the internal organs was carried out with fine

needles, and van Gieson's stain was used (for unfed adults during feeding, fully-fed and postfed male and female ticks, as well as ovipositing females) to show up the salivary glands more distinctly. This stain was applied for two to three minutes and was then washed out with Ringer solution.

When required for histological or histochemical purposes, the entire mass of viscera was removed from the exoskeleton and transferred directly to the fixative without being stained.

For histological studies, formol-sublimate fixative (Culling, 1974) was used for 3 to 4 hours. The specimens were dehydrated in alcohol, cleared in xylol, and were embedded in paraffin wax. Serial sections were cut at a thickness of 5 µm in the transverse and longitudinal planes. The sections were stained with Mallory's triple stain and Giemsa (Culling, 1974).

Cytochemistry:

For histochemical studies, adult salivary glands of H. leachii unfed and five days after attachment, were used. In addition, adult salivary glands of unfed O. moubata and from adults two hours after dropping off from the host, were examined.

a) Carbohydrates and Mucopolysaccharides:

The following methods were used:

1. McManus PAS-technique and Best's carmine stain (Pearse, 1961) after formol-sublimate fixative were used to test the presence of glycogen. Sections treated with 1 per cent diastase enzyme or human saliva were used as control.
2. Colloidal iron (Barka and Anderson, 1963) and Alcian blue (Pearse, 1961) after Carnoy's fixative were applied for detecting acid mucopolysaccharides.
3. Toluidine blue and thionin techniques (Pearse, 1961) after formol-sublimate fixative were used to identify the various degrees of metachromasia.

b) Proteins:

The following methods were applied:

1. Mercuric bromophenol blue (Hg-BPB) (Bonhag, 1955) and naphthol yellow-s methods (Pearse, 1961) after Carnoy's fixative were used to identify basic proteins.
2. DMAB-nitrite method of Adams (Pearse, 1961), after one per cent trichloroacetic acid fixative, was used for detection of tryptophan.
3. Millon's reagent (Barka and Anderson, 1963), after 10 per cent formaline fixative, was applied for tyrosine.
4. Alpha-naphthol method (Pearse, 1961), after 10 per cent formaline, was carried out for arginine.
5. Performic acid-alcian blue (P.F.A.A.B.) method of

Adams and Slopper (Pearse, 1961) was used, after formol-calcium fixative, for the demonstration of -SS-groups.

6. Ninhydrine-Schiff method of Yasuma and Itchikawa (Barka and Anderson, 1963), after Zenker's fixative, was used for demonstrating protein-NH_2 groups.

c) Nucleic Acids:

The following methods were used:

1. Methyl green pyronin-Y method of Kurnick (Pearse, 1961), after Carnoy's fixative, was carried out for detection of RNA in the cytoplasma and nucleoli; and DNA in the nuclei.
2. Feulgen stain of Lillie (Barka and Anderson, 1963), after formol-sublimate fixative, for DNA nuclear chromatin.

The triple stain technique (Himes and Moriber, 1956), after formol-sublimate fixative, was used for selective demonstration of DNA, carbohydrates and proteins.

d) Lipids:

Lipids were detected in formol-sublimate, fixed material (Bharradwag and Love, 1959) and with sudan black-B (Pearse, 1961).

e) Enzymes:

The following methods were applied:

1. The lead nitrate method (Gomori, 1956), after cold acetone fixative, was used for detection of acid phos-

phatase.

2. The calcium cobalt method (Pearse, 1961), after cold acetone fixative, was applied for alkaline phosphatase.

B. Electron Microscopy:

For transmission electron microscopy, ticks were dissected in cold cacodylate-buffered glutaraldehyde fixative (3% glutaraldehyde buffered with 0.1 M Na. cacodylate pH 7.3) (Sabatini et al., 1963). Immediately after dissection, salivary glands were removed, cut into small pieces (to ensure rapid penetration of the fixative) and placed in fresh fixative for 2 to 3 hours at 4°C. The specimens were rinsed in 0.2 M Na. cacodylate buffer (1M sucrose added) for 15 minutes at 4°C. Tissues were postfixed in 1% OsO_4, buffered with 0.1 M. Na. cacodylate, for 1 hour at 4°C, dehydrated in a graded series of cold ethanol, transferred through three changes of propylene oxide, and embedded in Epon-812 (Luft, 1961).

Semithin sections (0.5 µm) were mounted on glass slides and stained with 1% Azure II as survey stain. Ultrathin sections cut with a glass knife on a LKB Ultramicrotome III, were mounted on 100 and 150 mesh formvar-coated copper grids, double stained in uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined under a Carl Zeiss EM9 at 60 Kv.

Cytochemistry

a) Demonstration of polysaccharides:

For the detection of glycogen, as well as of more complex polysaccharides, the PA-TCH-silver albumose technique by Thiery (1969) was applied to sections of salivary glands fixed in 3% buffered glutaraldehyde for 2 to 3 hours. Epon-embedded ultrathin sections were mounted on gold grids and floated on the following solutions:

Oxidation was performed by 1% periodic acid (PA) for 30 minutes, the grids carefully washed with redistilled water, and then treated with a 0.2% solution of thiocarbohydrazid (TCH) in 20% acetic acid for 2 hours, followed by careful washing in 10 and 5% acetic acid for 15 minutes each, and finally in three changes of redistilled water. The grids were then floated on a 1% aqueous silver albumose solution in the dark for 30 minutes, and then rinsed again in redistilled water.

Control sections were either oxidized with 1% H_2O_2 or incubation with TCH was omitted in the above procedure. The sections were examined without further staining.

b) Protein digestion:

Ultrathin sections from Epon-embedded salivary glands, fixed in cacodylate buffered 3% glutaraldehyde for 2 to 3 hours without postfixation in OsO_4, were mounted on 100-mesh

formvar coated copper grids, were floated for 6 hours on 0.1-0.3% Pronase (Merck 70.000 P.U.K./ g) in phosphate buffer (1/15 M), pH 7.4 at 4 °C (Kuhn et al., 1975). After pronase treatment the grids were floated three times on phosphate buffer and again washed on redistilled water. Contrast was enhanced by double staining with uranyl acetate and lead citrate.

c) Localization of acid phosphatase by tissue incubation:

Acid phosphatase was demonstrated by the lead-salt technique (Gomori, 1956) following the modification of Larson and Maunsbach (1975). After fixation in 3% glutaraldehyde buffered with 0.1 M Na.Cacodylate, for 60 minutes, small segments of salivary glands were incubated for 1-2 hours at 37°C in the following Gomori-medium:

0.25% Na- -glycerophosphate as substrate in 0.08% lead citrate in 0.02 M tris-malate buffer, pH 5.4. Washing was performed with two changes of the aldehyde-fixative and of 2% acetic acid alternately, for 15 minutes each. The material was further processed by double staining with uranyl acetate and lead citrate.

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R E S U L T S

I. HAEMAPHYSALIS L. LEACHII

(A) UNFED ADULTS

MORPHOLOGY:

The salivary glands of H. leachii are similar in male and female, and consist of a pair of loose, racemose, elongate, grape-like cluster of glandular organs which occupy a lateral position in the body cavity (Fig. 4). They extend anteroposteriorly along the body sides to the posterior margins of the spiracles (sp. Fig. 4), and are slightly larger in the female than in the male.

Each gland has a main salivary duct (m.s.d. Fig. 4), connected anteriorly to the buccal cavity or salivarium which opens into the food channel (Kemp and Tatchell, 1971). Posteriorly, and at the level of the fourth coxae, the salivary duct is divided into a pair of subsidiary branches, the inner (i.s.d. Fig. 4), of which is slightly longer than the outer (o.s.d. Fig. 4) one. The two branches run posteriorly and divide into smaller and smaller branches. Both the main duct and its two subsidiaries give rise to secondary ducts (s.s.d. Fig. 4) which give off smaller lobular ducts (l.s.d. Fig. 4). Alveoli arranged in grape-like clusters open either directly into the main salivary duct (or its two subsidiaries) or indirectly into its secondary ducts by means of their lobular ducts.

SALIVARY DUCTS:

In Epon-embedded sections, the main salivary duct (Fig. 5) of the unfed adult is a wide tube (Ca. 22µm diameter) and consists of a layer of epithelial cells which is lined by a thick layer of cuticle.

The epithelial layer is formed of flattened cells which contain thin oval nuclei and has no visible individual cell boundaries under the light microscope. It rests on an amorphous delicate basement membrane.

The cuticular layer is continuous with that of the buccal cavity. In sections stained with Mallory's triple stain after formol-sublimate fixation, three main cuticular regions are distinguished: a thin folded red-staining inner region (IR. Fig. 5) around the lumen of the duct; and next to this is a middle thick, light blue-staining region (MR. Fig. 5) which is surrounded by a thin brownish-orange staining outer region (OR. Fig. 5). The secondary ducts are smaller, but the histological structure of these as well as of the lobular ducts, is the same as that of the main duct. The cuticular lining extends up to the opening of the lobular duct into the lumen of the granular-secreting alveoli at which point it forms a valve-like structure (V. Figs. 8, 9) similar to that observed by Nordenskiold (1905), Till (1961), Chinery (1965) Coons and Roshdy (1973) and Binnington (1978). There is a large nerve associated with the main salivary duct (MSDN. Fig. 5).

Nerve within basement membrane is shown also in Fig. 5.

Ultrastructural studies of the main salivary duct (Fig. 12) show that the epithelial cells contain elongated nuclei, which measure 7 Um long and 1.8 Um wide, and contain less densely-staining compact nucleoli. The cytoplasm possesses distinctive small, electron-dense free ribosomes, abundant microtubules, small oval mitochondria with a moderately dense interior and possessing parallel cristae, small non-membrane bound vacuoles and few dense bodies (Fig. 12). Neither Golgi bodies nor rough endoplasmic reticulum have been demonstrated in the cytoplasm of the epithelial cells. Their apical plasma membranes form short microvilli (MV. Fig. 12) underlying the spiral thickening region, while the basal part is relatively straight. The lateral membranes are long, tortuous and frequently joined by septate desmosomes (SD. Fig. 12).

The three regions of the cuticular layer are differentiated in electron density and submicroscopic structure: (i) a thin, electron-dense inner region (IR. Fig. 12) (Ca. 0.08 Um wide) surrounds the lumen of the duct; (ii) a large (Ca. 5.5 Um wide), less dense middle region (MR. Fig. 12) of complex laminations; (iii) an electron-dense outer region (Ca. 0.6 Um wide) of spiral thickening (ST. Fig. 12). The fine structure of the lobular ducts is identical to those of the main and secondary salivary ducts but the alveolar ducts differ in

both nongranular and granule -secreting types, as described below.

A large nerve (Ca. 17 Um diameter) is associated with the main salivary duct (Fig. 13) and branches into smaller lobular nerves which follow the lobular ducts into the alveoli as alveolar nerves (AN. Figs. 20, 22, 39, 40, 41). The alveolar nerves are divided inside the alveoli into individual axons (AX. Figs. 16, 17, 20, 28, 39, 40, 41), which may form synaptic areas with the surrounding cells.

Electron microscopical histochemical techniques were used to detect polysaccharides, proteins and acid phosphatase in the main salivary duct.

The three cuticular regions and the cytoplasmic organelles of the epithelial cells are positive with the PA-TCH (Fig. 14) technique for polysaccharides, but the inner and the spiral thickening regions are denser than the middle region (MR. Fig. 14).

With protein digestion using the pronase enzyme technique, the inner and outer regions of the cuticular layer do not show any change, i.e. negative reaction (resistance is probably due to bound protein), while enzyme digestion of the large middle region reveals two different zones. There is a thick (Ca. 1.5 Um wide) outer zone full of parallel electron-dense collagen-like fibres and a thin (Ca. 0.3 Um wide) inner

zone which lacks these fibres (Fig. 15).

The main salivary duct is negative for acid phosphatase.

SALIVARY ALVEOLI

Four types of alveoli (Table 5) were recognised in the salivary glands of the unfed adult and are classified according to their position on the main salivary ducts and their branches from the anterior to the posterior direction. The first type of alveolus (Type I) is localised in the anterior region of the salivary glands whilst the other three smaller Types (II, III and IV) of granule-secreting alveoli form the major part of the gland (see Fig. 4, AB). The types II and III alveoli are mixed together and occupy anteriorly two thirds the length of the salivary gland while the type IV alveolus occupies the posterior one thirds region of the gland.

A. NON-GRANULAR SECRETING ALVEOLI:

TYPE I ALVEOLI:

These alveoli (Fig. 6) measure 26-28 Um in diameter, are confined to the anterior region of the gland and extend posteriorly to a short distance just before the duct branches into two. They are directly connected to the main salivary duct and open through their short lobular duct along its dorsal and mesial sides. In Epon-embedded sections (Fig. 6), no individual cell is recognised in these alveoli although there are

four to five rounded or oval nuclei present in the cytoplasm. One of these nuclei is larger (Ca. 9-10Um diameter) than the rest, and surrounded by a lighter and larger area of cytoplasm which nearly occupies the centre of the alveolus. The cytoplasm of the other cells is fibrillar (FC. Fig. 6) especially in the peripheral region. The fibrils run towards the centre of the alveolus and almost perpendicular to the basement membrane. Among these fibrils there are fine granules and many vacuoles (Va. Fig. 6). The cuticular lining of the lobular ducts extends through the alveolar base to end near to the alveolar lumen. In paraffin section, type I alveoli stained red with Mallory's triple stain and blue with Giemsa after formol-sublimate fixation.

Histochemical stains were employed to elicit the nature of alveoli components. The result of this histochemical study is given in table 1. The cytoplasm of these alveoli is PAS-negative, but the fine granules are intensely PAS-positive, (Fig. 11 I). The granules do not react with stain for acid mucopolysaccharides. These granules are PAS-negative after treatment with diastase enzyme and are positive with Best's carmine, indicating that the PAS-staining is due to glycogen. The alveoli stain blue with Hg-BPB for basic proteins, and with P.F.A.A.B. method for disulphide groups, but tryptophan and tyrosine containing proteins as well as arginine and NH_2 groups are not detectable. With methyl green pyronin-Y,

the cytoplasm is faintly positive for RNA while the nuclei stain positively for DNA and the nucleoli are RNA positive. The cytoplasm reacts positively for lipids and is negative for acid and alkaline phosphatases.

In ultrastructure, the alveolar duct (AD. Fig. 16) (Ca. 2 Um long) of type I nongranular alveoli is the extension of the short lobular duct in the alveolus (Fig. 20). It is a non-valvular cuticular structure (Figs. 16, 20), which enters the alveolar base as a tube, lacking spiral thickening to lead into the alveolar lumen. The middle region of the lining cuticle is interrupted at the junction between the lobular and the alveolar ducts (Fig. 16). This interruption of the alveolar duct of type I alveoli has not been reported in previous electron microscopic studies on tick salivary glands. The alveolar duct is surrounded by epithelial cells similar to those of the main salivary duct and its branches. These cells are joined to each other and to adjacent alveolar cells by means of septate desmosomes (SD. Fig. 16). No other cell junction specialization was observed in type I alveoli.

Type I alveolus possesses one large cell in the centre of the alveolus (CC. Figs, 16, 17) surrounded by three to four smaller cells. The apical plasma membrane of the central cell borders the small alvular lumen (Lu. Fig. 16), while those of the smaller cells border the basal and lateral plasma membranes of the central cell.

In the basal region of the alveolus, the basal cell membranes of the small alveolar cells are greatly infolded (BR. Fig. 18). These infoldings enclose numerous mitochondria, non-membrane-bound vacuoles, free ribosomes and small electron dense granules. The elongated mitochondria (Ca. 2.6 Um long and 0.2 Um wide) are moderately dense internally and possess parallel cisternae. The mitochondria lie parallel to the infoldings and nearly perpendicular to the basement membrane (Ca. 0.02 Um wide) which surrounds the whole alveolus. The non-membrane bound vacuoles (Va. Fig. 18) lie in between the folded membranes. Some of them possess moderately electron-dense secretion. They are few and small (Ca. 0.7 Um diameter) in the basal region but increase in number and size (Ca. 2.7 Um diameter) toward the intermediate region (IR. Fig. 18) of the alveolus.

The apical region possesses no infoldings, a few electron dense bodies (Db. Fig. 16) of variable size, and few small, short isolated cisternae of rough endoplasmic reticulum (opened arrows Fig. 17) as well as numerous microtubules.

The plasma membrane of the large central cell is not folded. The low dense cytoplasm of the central cell (CC. Figs. 16, 17) contains electron-dense free ribosomes, many microtubules and few small mitochondria. Small curved Golgi bodies (Gb. Fig. 16) are observed in the cytoplasm of the central cell giving rise to small spherical vesicles. The apical plasma

membranes of the large and small cells lack microvilli.

One or more axons (ax. Fig. 17) are observed embedded between the alveolar cells and contain neurosecretory vesicles. These axons may form a synaptic area with the surrounding cells (white arrow Fig. 17).

Polysaccharides were detected only in the small peripheral cells of the nongranular alveoli using electron microscopical histochemical techniques. The cytoplasmic organells are positive with PA-TCH (Fig. 19) especially the infolded membranes, ribosomes, mitochondria, glycogen particles and the content of the non-membrane-bound vacuoles.

For detection of acid phosphatase, the reaction is negative.

GRANULE-SECRETING ALVEOLI:

The salivary glands of unfed H. leachii adult contain three different types (II, III and IV) of granule-secreting alveoli. Each type of alveolus can be distinguished by its specific staining reaction and the size of the secretory granules of its alveolar cells as described below (see Table 1, Fig. 11).

Type II alveolus contains four types of cell (a, b, c and d) (Table 5), while types III and IV alveoli contain five types of cell in each alveolus (Table 5). The types of cell of alveolus III are e, f, g, h and i; and those of alveolus IV are j, k, l, m and n. The letterings of cell types represent the arrangement position of the cells in the alveolus (Fig. 11).

The types c, h and m cell as well as types d, i and n cell cannot be distinguished by light microscopy even at maximum magnification.

In ultrastructure, each alveolus of the granule secreting alveoli is connected to the main salivary duct or its branches via the lobular duct (LD. Figs, 22, 39, 40, 41). The latter is up to 14 Um in diameter and is structurally identical to that of the main salivary duct. An alveolar nerve (AN. Figs, 22, 39, 40, 41) is also present. Both the lobular duct and the alveolar nerve are occasionally surrounded by the same basement membrane which surrounds the whole alveolus. The basement membrane is up to 0.04 Um in width and invaded by small branches of tracheae (Tr. Fig. 32).

The alveolar duct (AD.Figs.22,39, 40, 41) of the granule-secreting alveolus is up to 8 Um in length and extends into the alveolar base to end in a valve-like structure (V. Figs. 39, 40, 41) guarding the alveolar lumen. The cuticle of the alveolar duct is the extension of both the inner and the middle cuticular regions of the lobular duct (i.e. without spiral thickening, see Fig. 22). The surface of the inner cuticle of the alveolar duct is indented. The middle region of the lining cuticle is interrupted (arrows Fig. 22, 39, 40, 41) at the junction of the lobular and the alveolar ducts (i.e. the interrupted region lacks the middle cuticular region and the spiral thickening).

The valve-like structure in each granule-secreting alveolus is formed of constructed, knob-like apices guarding a narrow opening and formed from the thin inner and middle cuticular regions extending from the alveolar duct; the inner cuticular surface is not indented. The opening leads into a narrow valvular canal (VC. Figs, 22, 39, 40, 41) which is formed by a thin cuticular lining extended from the thin inner cuticular region of the valve which narrows and leads into the non-cuticular alveolar lumen (AL. Figs. 23, 28, 29, 30, 32, 33, 34, 39, 40, 41). The lumen borders the apices of all the alveolar cells.

The valvular canal in each secretory alveolus is surrounded by two large curved epithelial cells. The latter are extensions from the epithelial cells which surround the alveolar ducts. The plasma membranes of these epithelial cells border the alveolar lumen and project short microvilli, measuring up to 0.3 Um long and 0.1 Um diameter (MV. Fig. 23). Their lateral plasma membranes are connected with the neighbouring cells by means of macula adherans and septate desmosomes (MA. SD. Fig. 23). Their cytoplasm contains a few, small, rounded mitochondria (up to 0.4 Um diameter) with a moderately dense interior. Numerous microtubules are observed in the cytoplasm especially near the cell membranes and the junctions with the neighbouring cells. The cytoplasm also possesses free ribosomes which increase in number near

the cell membranes. Nerve axons (up to 1.4 Um diameter) with numerous neurosecretory vesicles (Fig. 28,30) are enclosed often within the cell membranes.

The types a, e and j cells of types II, III and IV alveoli are granule-secreting cells, and are similar to each other (see Table 1) in position, cytological structure and cytochemical reactions. In addition, types c, h and m cells of types II, III and IV alveoli are non-granular-secreting cells, and resemble each other. They are known as "cap cells" (Meredith and Kaufman, 1973; Binnington, 1978). Their name is derived from their position, covering the apical surface of the other cells. Types d, i and n cells of types II, III and IV alveoli are nongranular-secreting cells, and are similar to each other. They represent the narrow interstitial epithelial cells which surround all the other cells in the three types of granule-secreting alveoli. They were called "water-cells" by Meredith and Kaufman (1973). Types b, f and k cells are granule-secreting cells, and differ in types II, III and IV alveoli. Moreover, types g and l cells are granule-secreting cells, and are similar in types III and IV alveoli. Types b, g and l cells almost occupy the fundus of the alveoli.

Some axons containing neurosecretory material were observed exhibiting synaptic junctions with the nongranular and granule-secreting cells of the three different types of granule-secreting alveoli.

TABLE 1: HISTOCHEMICAL RESULTS ON *H. LEACHII* SALIVARY GLANDS (UNFED ADULTS)

METHODS	TYPE I ALVEOLI	TYPE II ALVEOLI				TYPE III ALVEOLI					TYPE IV ALVEOLI				
		a	b	c	d	e	f	g	h	i	j	k	l	m	n
A. CARBOHYDRATES															
PAS	+	+	++			+	++	±			+	-	±		
GLYCOGEN: DIASTASE/PAS	-	-	-			-	-	-			-	-	-		
BEST'S CARMINE	+	-	-			-	-	-			-	-	-		
ACID MUCOPOLYSACCHARIDES															
ALCIAN BLUE	-	-	+			-	+	-			-	-	-		
COLLOIDAL IRON	-	-	+			-	+	-			-	-	-		
METACHROMASIA															
TOLUIDINE BLUE	-	-	+			-	±	-			-	-	-		
THIONIN	-	-	+			-	±	-			-	-	-		
B. PROTEINS															
BASIC PROTEINS:															
MERCURIC BROMOPHENOL BLUE	++	+++	±			+++	+	+			+++	++	+		
NAPHTHOL YELLOW-S	++	+++	±			+++	+	+			+++	++	±		
TRYPTOPHAN: D.M.A.B.-NITRATE	-	++	-			++	-	-			++	+	-		
TYROSINE : MILLON'S REAGENT	-	-	-			-	-	-			-	++	-		
ARGININE : α - NAPHTHOL	-	-	-			-	-	-			-	-	-		
-SS-GROUPS: P.F.A.A.B. METHOD	+	-	-			-	-	-			-	-	-		
AMINO- GROUPS: NINHYDRIN SCHIFF	-	-	-			-	-	-			-	-	-		
C. LIPIDS															
SUDAN BLACK-B	++	++	-			++	-	-			++	++	-		
D. NUCLEIC ACID: METHYL GREEN PYRONIN	±	-	±			-	±	-			-	++	-		
FEULGEN REACTION	+	+	+			+	+	++			+	+	++		
E. ENZYMES:															
ACID PHOSPHATASE: LEAD NITRATE METHOD	-	-	-			-	-	-			-	-	-		
ALKALINE " : Ca.COBALT METHOD	-	-	-			-	-	-			-	-	-		

a,b, ...n indicates types of cells ± sign indicates a faint positive reaction

The number of plus signs indicates the relative intensity of staining by comparison with strongest reaction in gland or other tick tissues.

1. TYPE II ALVEOLI:

These granule-secreting alveoli (Fig. 7) measure 18-20 Um in diamater and occupy two-thirds the length of the glands mixing with type III alveoli (see Fig. 4). Only types a and b cells can be recognised at the light microscopic level (Fig. 7). They are 9-10 cells in number in each alveolus and possess secretory granules. The alveolar cells surround a small lumen which communicates with a smaller lobular duct.

At the electron microscopic level, two other types of cell, c and d, are recognised. They are 4-6 cells lacking secretory granules. Types a, b and c cells are separated from one another by type d cells which are the narrow interstitial epithelial cells (Figs. 21, 39).

i. Type a cells:

They are two in number (Fig. 7) occupying the bases of the alveolar duct. Type a cells were called "orifice cells" by Balashov (1965). They contain closely packed coarse granules which stain red with Mallory's triple stain and dark violet with Giemsa stain after formol-sublimate fixative. The nuclei measure about 7 Um in diameter and are located in the basal region or in the corners of the cells. In Epon-embedded sections stained with azure II, the granules are rounded with dark spots arranged on the edges of these granules while the middle is lighter in colour.

Histochemically (Table 1; Fig. 11 1I), the granules of type a cells are PAS-positive, while the cytoplasm is negative. The red colour of the granules after PAS does not change after treatment with diastase enzyme. These cells react negatively for acid mucopolysaccharides. For basic proteins, the granules are stained deep blue with Hg-BPB and intense yellow with naphthol yellow s, while the cytoplasm is moderately positive with both of them. These cells react positively for tryptophan but are negative for tyrosine, arginine, -SS-groups and $-NH_2$ groups. With sudan black-B, only the cytoplasm reacts moderately positively for lipids. The nuclei stain moderately green with Himes and Moriber triple stain for DNA while RNA is undetectable in both the cytoplasm and the nucleoli. No staining was found for acid and alkaline phosphatases.

In ultrastructure, the cytoplasm of type a cells is packed with oval or polygonal membrane-limited granules (Ca. 2.7 Um diameter) which are composed of membrane-bound subunits (Ca. 0.4 Um diameter) (sb, Fig . 24, 25). Some of these subunits are denser than the rest (Fig. 25) and are embedded in a less dense lattice matrix (Figs. 24, 25, 26). The latter consists of many small electron-lucent vacuoles (Ca. 0.05 Um wide) limited by pentagonal or hexagonal darker fine membranes (Ca. 0.01 Um thickness). Some of these small membrane-limited vacuoles are more dense than the rest (Fig.

24). Few subunits are observed isolated and embedded in the cytoplasm (Fig. 26). The various granular substructures probably represent different stages of granule formation.

The nuclei of type a cells contain dark clumped chromatin separated by less dense areas. The nucleolus is less dense and has a spongy appearance. Parallel rows of the rough endoplasmic reticulum surround the nucleus and may occupy the lateral areas of the cell from which the granules are absent (Fig. 25). Some small and isolated cisternae are observed between the membrane-limited granules. The cytoplasm contains many dark free ribosomes, some microtubules and small rounded or oval mitochondria with moderately dense interior parallel cisternae.

Ultrastructural histochemical techniques were carried out for detection of polysaccharides in the type a cells using PA-TCH. The membrane limited granules and their membrane-bound subunits as well as the lattice matrix and the other cell organells are faintly positive for polysaccharides (Fig. 26).

For proteins, the reaction is intensely positive with digestion of the membrane-bound subunit inclusions by pronase enzyme (Fig. 27). The lattice matrix is not affected by the enzyme.

Type a cells are negative for acid phosphatase.

ii. Type b cells:

They are 6-8 cells (Fig. 7) occupying the greatest volume of the alveolus. They contain loosely packed granules which stain faint blue with Mallory's triple stain and blue with Geimsa after formol-sublimate fixative. The large elongated nuclei (12 Um long and 4 Um wide) occupy the greatest part of the cell.

Histochemically (Table 1; Fig. 11 II), these cells are positive for polysaccharides, while they are faintly positive for basic proteins. There was no reduction in the intensity of PAS staining in type b cells following diastase digestion, indicating that PAS staining is not due to glycogen. Best's carmine test also gave negative results. In toluidine blue-stained preparations, the granules stained shades of purplish-violet, and reacted positively with colloidal iron and alcian blue, indicating a metachromasia of acid-mucopolysaccharides which stain with PAS. The nuclei are DNA positive with Feulgen raction while only the cytoplasm is faintly positive for RNA with methyl green pyronin. Lipids, acid and alkaline phosphatases are undetectable.

Ultrastructural studies of the type b cells (Figs. 21, 28) show that these cells are packed with a few sperical or oval electron-dense granules (Ca. 1 Um diameter). The cytoplasm possesses free ribosomes, some electron-lucent non-membrane-

bound vacuoles and parallel rows of rough endoplasmic reticulum which are distributed mainly in the apices and the sides of the cells. The lumen of the cisternae is pale. Mitochondria are distributed either in the cytoplasm between the nucleus and the apical plasma membrane or at the periphery of the cells. They are typically small, oval in shape with a moderately dense interior and possess parallel cisternae. No Golgi bodies are observed in the cytoplasm of these cells. The nucleus is large and occupies most of the cell. The nuclear membrane is indented in an irregular pattern giving an irregular outline of the nucleus. The latter possesses a compact nucleolus. The basal cell membrane is slightly infolded to form small narrow channels which are occupied by portions of the interstitial epithelial cells (Type d cells). The apical cell membrane lacks or contains few microvilli. Laterally, and occasionally apically, these cells are connected with the type c cells (the cap cells) and/or with the interstitial epithelial cells (Type d cells) by means of inter-cellular junctions (thick arrows, Fig. 28).

Ultrastructural histochemical staining showed that the granules of b cells are polysaccharides positive and negative for proteins and acid phosphatase.

iii. Type c cells:

In unfed H. leachii adults, these cells cannot be distinguished

by light miscroscopy even at maximum magnification.

At electron microscopy level, type c cells are 2 to 3 irregular shaped cells. They do not contain any secretory granules. Their lateral plasma membranes abut those of the interstitial epithelial cells (type d cells) and the granular secreting cells (types a and b) over short distances where there are intercellular junctions(Fig.28).The electron-lucent cytoplasm is mostly occupied by a large nucleus (Ca. 5 Um diameter) which possesses a thin peripheral electron-dense areas. The rest of the nucleus is electron-lucent containing small and dark patches of chromatin (Fig. 28). The cytoplasm possesses a few mitochondria with a moderately dense interior, free ribosomes, some non-membrane-bound vacuoles, few, short and narrow cisternae of rough endoplasmic reticulum and microtubules. Golgi bodies are not observed in the cytoplasm. The apical cell membrane borders the alveolar lumen and there are a few short microvilli (Ca. 0.06 Um diameter and 0.1 Um long). In some preparations, a part of the cell membrane appears swollen into the alveolar lumen (Fig. 29).

iv. Type d cells:

It is only possible to study type d cells by electron microscopy because they cannot be distinguished at the light microscopic level.

Ultrastructurally, they are 3 to 4 narrow interstitial epithelial cells (Figs.21, 28, 39) lying between the different alveolar cell types. They extend throughout the alveolus from the basement membrane to the alveolar lumen. Part of their plasma membrane has a few microvilli bordering the alveolar lumen. These cells contain small nuclei (Ca. 3.5 µm diameter), scattered small, oval mitochondria (Ca. 1.8 µm long and 0.5 µm wide) and microtubules which are particularly abundant apically and in areas bordering the other alveolar cells. Intercelluiar junctions (Fig. 28) are observed between these epithelial cells and the other cell types especially type c cells. One or more nerve axons which frequently contain neurosecretory vesicles are enclosed between the membranes of these cells (ax. Fig. 28).

2. TYPE III ALVEOLI:

These granule-secreting alveoli (Fig. 8) measure 20-23 µm in diameter and occupy two-thirds the length of the glands, mixing with type II alveoli (see Fig. 4).

Type III alveoli contain five types of cell e, f, g, h and i, but the first three types of cell can only be distinguished at light microscopic level. Types e, f and g cells are 10-13 cells in number in each alveolus. Type e cells are similar to that of type a cells in alveolus II. Type f cells contain secretory granules, while type g cells lack these granules

(Fig. 8). As in type II alveoli, all the alveolar cell types surround the alveolar lumen which communicates with the lobular duct.

At the electron microscopy level, another two types of cell h and i, can be revealed. They are 4-6 cells and similar to those of types c and d cells in type II alveolus. Types e, f, g and h cells are separated from one another by type i cells, which are the narrow interstitial epithelial cells.

i. Type e cells:

As mentioned before, type e cells of alveolus III are the same as type a cells in type II alveoli.

The fine structure of type e cells resembles that of type a cells.

ii. Type f cells:

There are 3 to 4 large cells (Fig. 8) occupying the greatest volume of the alveolus. They contain loosely packed granules which stain blue with Mallory's triple stain and faint pink with Giemsa after formol-sublimate fixative. The nuclei are about 12 µm in diameter and are present in the middle of the cells.

Histochemically (Table 1; Fig. 11 III), these cells react as type b cells in type II alveoli.

Ultrastructural studies of type f cells (Fig. 30) show that these cells are packed with rounded and irregular electron-

dense granules of variable size (Ca. 0.6-3.5 Um diameter). The cytoplasm is rich in free ribosomes which occupy most areas of the cytoplasm between the granules. Mitochondria (Ca. 0.4 Um diameter) are rounded or oval in shape with parallel cristae and moderate dense interior. The nucleus occupies the central region of the cell and possesses a compact nucleolus. The nuclear membrane is indented in an irregular pattern giving an irregular outline of the nucleus. Neither Golgi bodies nor rough endoplasmic reticulum are observed throughout the cytoplasm of these cells.

Many macrotubules (Ca. 0.8 Um long and 0.2 Um diameter) with electron-dense double membranes are observed throughout the cytoplasm (Fig. 31). Some of these macrotubules enclose an electron-lucent tubular structure with fine membrane (Fig. 31). These structures have not been reported in previous electron microscopic studies on tick salivary glands.

The cell plasma membranes are slightly infolded and their apical regions bordering the alveolar lumen lack microvilli. The lateral and the apical cell membranes are occasionally joined to the cap cells (type h cells) or to the interstitial epithelial cells (type i cells) by means of septate desmosomes.

Histochemical staining showed that type f cell granules are polysaccharides-positive using the PA-TCH technique, while they are slightly digested by the pronase enzyme. The reaction is negative for acid phosphatase.

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Histochemical staining showed that type f cell granules are polysaccharides-positive using the PA-TCH technique, while they are slightly digested by the pronase enzyme. The reaction is negative for acid phosphatase.

iii. Type g cells:

Type g cells (Fig. 8) are 6-7 small cells and form a group at the base of the alveolus. They lack granules and stain faint blue with both Mallory's triple stain and Giemsa. The nuclei are about 7 Um in diameter and occupy the greater part of the cell.

Histochemically (Table 1, Fig. 11 III), these cells react faintly with PAS and stain with mercuric bromophenol blue and naphthol yellow s for basic proteins.The nuclei are moderately positive for DNA.

In electron micrographs, type g cells (Fig. 32) lack secretory granules. The cell cytoplasm is rich in free ribosomes and contains small oval mitochondria. The nuclei are centrally placed and contain compact nucleoli. The nuclear membrane is indented giving an irregular outline to the nucleus. Neither Golgi bodies nor rough endoplasmic reticulum are observed throughout the cytoplasm. The apical regions of some cells contain non-membrane-limited electron lucent vacuoles. The basal and lateral cell membranes are slightly folded while the apical ones project microvilli into the lumen of the alveolus. Occasionally, some alveoli possess a narrow lumen in which the microvilli are crowded together (Fig. 33). The apical borders of the lateral cell membranes of type g cells are occasionally joined over a short distance with those of the interstitial epithelial cells (type i cells)

by means of macula adherans and septate desmosomes (Fig. 33).

iv. Type h cells:

As mentioned before, this type of cell cannot be distinguished at the light microscopic level.

The fine structure of these cells is the same as type c cells in type II alveoli described before.

v. Type i cells:

As previously mentioned, this type of cell is difficult to distinguish at the light microscopic level.

The fine structure of type i cells is similar to type d cells described before for type II alveoli.

3. TYPE IV ALVEOLI:

These granule-secreting alveoli (Fig. 9) measure 20-23 Um in diameter and occupy the posterior one-third of the length of the glands (see Fig. 4).

Type IV alveoli contains five types of cells j, k, l, m and n, but only the first three types of cell can be distinguished at the light microscopic level. Types j, k and l are 12 to 13 cells in number in each alveolus. Type j cells resemble types a and e cells of types II and III alveoli. Type k cells contain secretory granules, while type i cells lack these granules. As in type II and III alveoli, all alveolar cell types surround the alveolar lumen which communicates with

the lobular duct via the alveolar duct.

At the electron microscopic level, another two types of cell, m and n, can be distinguished. They are 4 to 6 cells similar respectively to those of types c and d cells in type II alveoli and types g and h cells in type III alveoli. Types j, k, l and m cells are separated from one another by type n cells, which are the narrow interstitial epithelial cells.

i. Type j cells:

As mentioned before, type j cells (Fig. 10) of type IV alveoli are the same as types a and e cells of types II and III alveoli (see Table 1).

The fine structure of type j cells (Fig. 34) is exactly similar to those of types a and e cells.

ii. Type k cells:

These are four large cells (Fig. 9) occupying the greatest volume of the alveolus, and adjacent to type j cells. They are readily distinguishable by their large granular inclusions (Fig. 9). They stain pink with both Mallory's triple stain and Giemsa after formol-sublimate fixative. The nuclei are 10-12 Um in diameter and occupy the central part of the cells.

Histochemically (Table 1; Fig. 11 IV), type k cells are negative with PAS for polysaccharides but are moderately positive

for proteins. They react positively with DMAB-nitrite and Millon's reagent for tryptophan and tyrosine containing proteins. The reaction is positive for lipids (Fig. 10). The cytoplasm as well as the nucleoli react moderately positive for RNA with Himes and Moriber triple stain and methyl green pyronin. The nuclei are DNA positive with the Feulgen reaction. Type k cells are negative for both acid and alkaline phosphatases.

The electron micrographs of type k cells (Fig. 34, 35) show that the cytoplasm is packed with rounded granules appearing as a reticulum of membrane-bound aggregates of electron-dense material (Fig. 35). These granules are variable in size (Ca. 0.3-2 µm diameter). The cytoplasm possesses a characteristic well developed dense rough endoplasmic reticulum which is often in whorls (Fig. 36) and occupies most of the cytoplasm between the granules. The reticulum has a beaded appearance due to alternate distended and non-distended regions. The lumen of the cisternae is pale. Some cisternae possess small intercisternal granules, while others are fully packed (Fig. 36). The cytoplasm contains free ribosomes and small, oval mitochondria with moderately electron-dense interior. The large nuclei occupy the upper basal corners of the cells and contain small compact nucleoli. The plasma membranes are slightly folded laterally and apically due to protruding of some granules into the alveolar lumen (Fig. 34). No microvilli are observed in the apical cell membranes.

Ultrastructural histochemical micrographs of type k cells show that these cells are negative for polysaccharides with PA-TCH, but are partially digested by pronase enzyme (Fig. 37). No acid phosphatase was detected in type k cells.

iii. Type l cells:

These cells were called "fundus cells" by Balashov (1968). Type l cells (Fig. 9) are 6 to 7 small cells and form a group in the alveolar fundus. They are similar in position and structure to type g cells described for type III alveoli.

The fine structure of type l cells (Fig. 38) is similar to that of type g cells described for type III alveoli.

iv. Type m cells:

As mentioned before, this type of cell cannot be distinguished at the light microscopic level.

The fine structure of type m cells is the same as type c cells of type II alveoli and type h cells of III alveoli described before.

v. Type n cells:

As previously mentioned, this type of cell is difficult to recognise at the light microscopy level.

The fine structure of type n cells (Fig. 34) is similar to type d cells of type II alveoli and type i cells of type III alveoli described before.

(B) FEEDING ADULTS

a) FEEDING AND OVIPOSITING FEMALES

MORPHOLOGY:

One to three days after attachment, the salivary glands increase slightly in size (Table 2) during the slow feeding process (Till, 1961; Balashov, 1968; Binnington, 1978). After three days, and until the ticks drop from the host, they increase enormously in size (Table 2, Fig. 54) during the rapid feeding process (Till, 1961; Balashov, 1968; Binnington, 1978). The salivary glands degenerate gradually after the dropping off from the host and reach their maximum degeneration directly after ovipostion.

SALIVARY DUCTS:

One to three days after attachment, no appreciable changes in the histological appearance of the salivary ducts were observed under the light microscope except that their epithelial cells are slightly increased in size, and their flattened nuclei become oval in shape. In Epon-embedded sections, the basement membrane which surrounds the salivary ducts become irregularly shaped giving an irregular outline to the salivary duct. The three cuticular regions are clearly observed and their inner region is still folded.

On the fifth day, the lumen of the salivary duct is greatly distended and the cuticular lining of the inner region lacks

the folds formerly seen (compare Fig. 42 with Fig. 5). Both the epithelial layer and the cuticular lining become thinner. This is in response to the increase in the width of the ducts. Large irregular vacuoles (Va. Fig. 42) appear in the epithelial layer. These vacuoles increase in size and irregularity during tick attachment and even up to five days after dropping off from the host.

During oviposition, the epithelial cells are completely degenerated leaving an amorphous cuticular layer.

Ultrastructurally, no appreciable changes were observed in the structure of the salivary ducts on the first day after attachment.

On the third day, the epithelial cells of the main salivary ducts as well as their nuclei are slightly enlarged in size (Ca. 10 µm diameter). The nucleoli are irregularly shaped with a spongy appearance and some scattered vacuoles.

The cytoplasm becomes less dense, and contains free and aggregated ribosomes, many microtubules, some membrane-like structures, mitochondria and scattered dense bodies. Some mitochondria, particularly those lying near the basal membrane, become more elongated (Ca. 0.4 µm diameter and Ca. 2 µm long) with dark granules in their low dense homogeneous matrix.

The basal membrane of some epithelial cells becomes infolded and these infoldings are directed towards the cuticular lin-

ing. The basement membrane surrounding the main salivary duct becomes thicker than in unfed adults and extends in between these infoldings.

On the fifth day after attachment and until dropping off from the host, the cytoplasm of the epithelial cells possesses large areas of an osmiophilic aggregate (Os. Fig. 56) (which are probably observed as vacuoles at light microscopic level), as well as membrane-like structures and electron-dense bodies especially in the apical region of the cells (Fig. 56). Most mitochondria become smaller, with a dark interior, and are mostly found in the areas lacking the osmiophilic aggregates. The nuclei are irregularly shaped with dark and compact nucleoli. Some nuclei are pycnotic and are apparently no longer functional, particularly on the seventh day after attachment. The infoldings of the basal cell membrane are more pronounced and come to surround most cytoplasmic areas which possess the osmiophilic aggregates (Fig. 56). The apical cell membranes underlying the cuticular lining form many short microvilli (MV. Fig. 56). The cuticular lining becomes thinner, fenestrated and irregular (compare Fig. 56 with Fig. 12).

At 5 days of attachment, the axons of the main salivary duct nerve have lost most of their neurosecretory vesicles (M.S.D.N. Fig. 56).

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At the beginning of oviposition, the epithelial layer is shrunken and the cytoplasm contains a few lysosomes (Ly. Fig. 57), and aggregates of ribosomes (r. Fig. 57).

The cuticular lining is completely collapsed, probably due to the shrinkage of the epithelial cells, and its lumen is very narrow.

TABLE 2: EFFECT OF THE BLOODMEAL ON THE DIAMETER OF THE ALVEOLI OF H. LEACHII

ADULT SALIVARY GLANDS DURING PREFEEDING, FEEDING AND POSTFEEDING PERIODS

	PRE-FEEDING PERIOD	FEEDING PERIOD				POSTFEEDING PERIOD		
		1 day	3 days	5 days	7 days	1 day	3 days	5 days
FEMALE: TYPE I ALVEOLI	27.5 26.0-28.0	28.0 27.0-30.0	25.0 23.5-27.5	21.0 20.0-22.0	20.0 19.0-21.0	18.5 17.5-20.0	18.0 16.5-19.0	17.0 16.0-18.5
" II "	19.0 18.0-20.0	22.5 21.0-24.5	42.5 40.0-45.0	67.5 66.0-69.0	81.0 79.0-82.5	71.0 70.0-73.0	55.0 52.5-57.5	43.5 40.0-46.0
" III "	22.5 20.0-23.0	26.5 25.0-30.0	50.0 48.0-50.5	66.5 66.0-69.0	74.5 73.0-77.5	66.5 65.5-69.0	53.5 52.5-57.5	39.0 37.0-40.0
" IV "	21.0 20.0-23.0	24.5 21.0-25.0	47.0 45.0-48.0	63.0 60.5-65.0	70.5 69.0-72.5	63.0 60.0-65.0	51.0 49.0-52.5	35.0 33.0-37.0
MALE: TYPE I ALVEOLI	26.5 26.0-28.0	26.5 25.5-28.0	19.0 18.0-20.0	14.0 12.5-15.0	10.5 10.0-12.0	8.0 6.5-9.5	5.5 4.5-6.5	4.0 2.5-5.0
" II "	18.5 16.5-20.0	21.5 19.0-22.5	23.5 22.0-25.0	25.5 22.5-27.5	26.5 25.5-29.5	25.0 23.0-27.0	21.0 19.0-24.0	16.0 15.0-19.0
" III "	23.5 21.5-25.0	25.0 23.0-25.5	26.0 25.5-27.0	25.5 23.5-28.5	24.5 22.0-25.5	21.5 19.0-23.0	17.5 15.5-20.0	12.5 10.0-14.0
" IV "	20.5 20.0-23.0	24.5 23.0-25.5	28.0 27.0-30.0	31.5 30.0-35.0	34.0 31.5-35.0	33.0 31.0-36.5	30.5 27.5-33.0	27.5 26.0-30.0

NOTE: ALL measurements in Um

Mean
(Range)

TABLE 3: HISTOCHEMICAL RESULTS ON H. LEACHII SALIVARY GLANDS (PARTIALLY FED ADULTS)

METHODS	TYPE I ALVEOLI	TYPE II ALVEOLI a	b_1	b_2	c	d	TYPE III ALVEOLI e	f	g	h	i	TYPE IV ALVEOLI j	k	l	m	n
A. CARBOHYDRATES																
PAS	++	-	++	±			-	+++	+++			-	+	+		
GLYCOGEN: DIASTASE/PAS	-	-	-	-			-	-	-			-	-	-		
: BEST'S CARMINE	-	-	-	-			-	-	-			-	-	-		
ACID MUCOPOLYSACCHARIDES																
: ALCIAN BLUE	-	-	+	+			-	+	-			-	-	-		
: COLLOIDAL IRON	-	-	+	+			-	+	-			-	-	-		
METACHROMASIA:																
: TOLUIDINE BLUE	-	-	++	++			-	++	++			-	-	+		
: THIONIN	-	-	++	++			-	++	++			-	-	+		
B. PROTEINS																
BASIC PROTEINS:																
· MERCURIC BROMOPHENOL	+++	+	++	++			+	+	++			+	+++	++		
BLUE (Hg-BPB)	+++	+	++	++			+	+	++			+	+++	++		
TRYPTOPHAN· DMAB-NITRITE METHOD	±	±	-	+			±	-	-			±	-	+		
TYROSINE: MILLON'S REAGENT	±	-	-	-			-	-	++			-	++	-		
ARGININE: ∝ - NAPHTHOL	-	-	-	-			-	-	-			-	-	-		
-SS-GROUPS: P.F.A.A.B.-METHOD	+	-	-	-			-	-	-			-	-	-		
$-NH_2$-GROUPS: NINHYDRINE SCHIFF	-	-	-	-			-	-	-			-	-	-		
C. LIPIDS: SUDAN BLACK-B	±	+	-	++			+	-	±			+	±	-		
D. NUCLEIC ACIDS:																
:METHYL GREEN PYRONIN Y	-	±	-	+			±	±	±			±	±	+		
:FEULGEN REACTION	-	±	++	++			±	++	++			±	+	+		
E. ENZYMES:																
ACID PHOSPHATASE: LEAD NITRATE METHOD	-	-	-	-			-	-	-			-	-	-		
ALKALINE " :Ca.COBALT METHOD	±	+	+	+			+	++	++			+	+	+		

a, b, ... n indicates types of cell ± sign indicates a faint positive reaction
The number of plus signs indicates the relative intensity of staining by comparison with strongest reaction in gland or other tick tissues.

SALIVARY ALVEOLI

NON-GRANULAR SECRETING ALVEOLI

TYPE I ALVEOLI:

No appreciable changes are observed on the first three days after attachment except that the basement membrane of some alveoli become infolded in an irregular shape.

On the fifth day after attachment until dropping off from the host, these alveoli become slightly smaller (Fig. 54) while the irregularity in shape is increased more than before. In semithin sections stained with azure II, the fibrillar cytoplasm lacks the parallel pattern found in unfed females and contains many dark granules (Fig. 43). The nuclei become slightly darker with compact nucleoli.

During oviposition type I alveoli are mostly degenerated.

Histochemically (Table 3, Fig. 55, I) on the fifth day after attachment, there is a marked increase in the intensity of PAS staining as well as an increase in protein material as revealed by the intensity of staining with mercuric bromophenol blue and naphthol yellow s. These alveoli are faintly positive for tryptophan and tyrosine while they react positively for -SS-groups. There is a marked decrease in the intensity of staining with sudan black-B. The reaction is faintly positive for alkaline phosphatase while it is negative for acid phosphatase.

Electron micrographs do not show any marked changes in type I alveoli on the first day after attachment.

On the third day, some lysosomes of variable size (Ca. 0.3 Um 0.8 Um diameter) appear in the cytoplasm of the basal region of the alveoli, (compare Fig. 58 with Fig. 18). Some of these lysosomes contain moderately dense vacuoles of 0.4 Um in diameter (Ly. Fig. 58). The non-membrane-bound vacuoles (Va. Fig. 58) lying between the infoldings of the basal cell membranes become slightly smaller and some of them have lost most of their secretory content. Small membrane-bound vacuoles with low dense interior and variable in size are present in the cytoplasm (small arrows Fig. 59). The mitochondria are slightly increased in size (2 Um long and 0.4 Um diameter) and are oval or spherical in shape. Low dense gaps are found between their cristae (M. Fig. 58) and most of the mitochondria contain small dense granules in their matrix (white arrows, Fig. 59). These dark granules are found in mitochondria in tissues transporting large amounts of ions or water (Fawcett, 1966). The cytoplasm contains numerous scattered free ribosomes. The nuclei are slightly enlarged in size and possess large, low dense areas. Some alveoli become very irregular in shape, and as a result of this irregularity, a type I alveolus is found in section enclosing portions of types f and i cells of type III granule secreting alveoli (Fig. 60).

On the fifth day after attachment, the cytoplasm possesses osmiophilic aggregates (Fig. 61), lying between the infoldings of the plasma membrane. In some alveoli, these infoldings also contain small membrane-bound vacuoles with a low dense interior (Figs. 61, 62). In addition, large membrane-bound vacuoles are found in the middle of the same alveoli. This is a result of the great distension of the infoldings in the intermediate and apical regions of the alveoli (Fig. 61). Most non-membrane-bound vacuoles are empty. The mitochondria are greatly distended and some of them show relatively few cristae in a homogeneous matrix that contains a few mitochondrial granules (Fig. 62).

On the seventh day after attachment and until detachment, type I alveoli become full of lysosomes occupying the denser areas of the cells which also contain numerous microtubles. The nuclei are spherical or oval in shape and lie in the lower dense areas of the cells. The cytoplasm here contains few dense bodies, scattered microtubles and free ribosomes.

The cell organelles of some type I alveoli are still in their normal condition although their cytoplasm may contain osmiophilic aggregates or lysosomes. Moreover, some type I alveoli start to breakdown earlier than others and this is probably as a result of the asynchronous growth of type I alveoli in the salivary gland as a whole.

After tick detachment and during oviposition, the whole alveolus is irregularly shaped and the cytoplasm consists of osmiophilic aggregates containing numerous lysosomes, dark pycnotic nuclei and many vacuoles of variable size. At this time other type I alveoli are completely degenerated.

Ultrastructural histochemical reactions were carried out on the fifth day after attachment. Type I alveoli are still positive for polysaccharides with PA-TCH and are still negative for acid phosphatase.

GRANULE-SECRETING ALVEOLI:

Tick attachment to the host serves to stimulate secretory activity. Alveolar size increases slowly one to three days after attachment and rapidly three to seven days after attachment (Fig. 54), but their growth is asynchronous in the gland as a whole. Moreover, even the cells of the same type in one secretory alveolus can be seen in different stages of secretory activity at the same time. Granule-secreting alveoli reach their maximum size on the seventh day after attachment (Table 2, Fig. 54). They start decreasing gradually in size after dropping off from the host, and are completely degenerated and autolized during, or after, oviposition leaving the cuticular lining of their alveolar and lobular ducts as dark marks.

Some haemocytes start to appear around the alveoli on the

fifth day after attachment and increase in number, particularly after the tick has dropped from the host.

Ultrastructurally, the effect of the bloodmeal on the cytological structure of the epithelial cells surrounding the lobular and alveolar ducts is similar to that described earlier in the epithelial cells of the main salivary duct.

On the first day after attachment, the proximal ends of the last two large curved epithelial cells (formerly described in unfed females) surrounding the valvular canal extend further and deeply between the alveolar cells to join the narrow interstitial epithelial cells (types d, i and n cells of types II, III and IV alveoli) in each granule-secreting alveolus. These two epithelial cells contain long bundles of microtubules (Ca. 4 µm long) on the third day after attachment. Their mitochondria measure Ca. 1.5 µm in length and Ca. 0.5 µm in diameter with rounded low dense gaps between their cristae.

The cap cells in each granule-secreting alveolus extend further to join the distal ends of the two curved cells surrounding the valvular canal and ultimately touch the valve-like structures. This extension can be seen at the light microscopic level (Fig. 49). The cytological structure of the cap cells and the two curved epithelial cells is the same during feeding and they are almost degenerated at the time of oviposition.

Ultrastructurally, an interrupted electron-dense line appears in the basement membrane of the granule-secreting alveoli on the fifth or seventh day after attachment (Figs. 92, 97, 98, 99). The density and thickness of this line are markedly increased after dropping off from the host and at the beginning of oviposition. The basement membrane of some alveoli become thicker and irregularly infolded (Fig. 99), particularly after dropping off from the host.

In the final stages of feeding, many haemocytes with different shape and structure are observed around or attached to the alveoli (Figs. 105a, b). The number of these haemocytes increases enormously after dropping off from the host and during oviposition.

Haemocytes are single cells with a very irregular surface which bear small pseudopodia (Fig. 105a, b). The nucleus is rounded (Fig. 105a) or elongated (Fig. 105b) and occupies the major part of the cell. The cytoplasm possesses some dense granules, which are probably lysosomes, many isolated cisternae of rough endoplasmic reticulum, large numbers of free ribosomes, some scattered mitochondria and few vesicles of variable size.

During oviposition, some of the granule-secreting alveoli are observed completely degenerated, while others are autolized. By the end of oviposition, all the alveoli were autolized (Fig. 104) and nothing can be distinguished other

than the cuticle of the alveolar duct with its epithelial cells and the irregularly shaped, electron-dense line of the basement membrane of the alveoli (Fig. 104). An unknown alveolus with autolized fundus cells was found on the first day after attachment (Fig. 63). This probably represents an earlier abnormal alveolar degredation.

1. TYPE II ALVEOLI

On the first day after attachment, these alveoli start to increase in size to reach their maximum (81 µm diameter) on the seventh day (Table 2, Fig. 54), becoming the largest alveoli in the salivary glands of the feeding female. During feeding, the alveolar duct, as well as the alveolar lumen, contain a coagulated secretory mass staining blue with Mallory's triple stain and pink with Giemsa after formol-sublimate fixation.

i. Type a cells:

On the first day after attachment, type a cells do not show any marked increase in size and neither do the secretory granules show any change in appearance. On the other hand, the ground cytoplasm shows some increase in activity through increase in basophilia.

On the third day, these cells are reduced in size (Fig. 44) and most of them lack many of their granules. This reduction

in size and absence of the secretory granules is most apparent by the fifth and seventh days after attachment. The nuclei degenerate, shrivel and stain diffusely with the Feulgen reaction.

After dropping off from the host, these cells are usually absent or appear as small clumps bordering the valve of the alveolar duct.

Histochemically (Table 3), the few granules found in type a cells on the fifth day after attachment react positively for proteins and faintly positive for tryptophan, while the cytoplasm and the granules show a positive reaction for alkaline phosphatase.

Electron micrographs at the first day after attachment show that the limiting membranes of the subunit granules fuse together (Fig. 64) forming larger subunits with a low dense interior in the same membrane-bound granules. A few isolated subunits are still embedded in the cytoplasm.

Small Golgi bodies with low dense parallel cisternae appear lying between the nucleus (in the basal region) and the granules (in the apical region). The Golgi bodies are very close to the isolated cisternae of the rough endoplasmic reticulum (Fig. 64) and give rise to small vesicles of variable size.

On the third and fifth days after attachment, the granules of type a cells are observed releasing their content into the alveolar lumen (Fig. 65). In the apical region of the cell, membrane-bound granules are found close to the apical cell membrane, which lacks microvilli. The limiting membranes of both the large granule and one of its subunits (lying nearer to this plasma membrane) fuse together and ultimately with the apical plasma membrane to discharge the granular content into the lumen (Fig. 65). In the case of the isolated subunit granules, they either discharge their content as the large granules or are pinched off into the lumen as an individual cytoplasmic unit (Fig. 66).

The nuclei occupy the basal region of the cells. A spheroidal, moderately dense inclusion body is occasionally found in the middle of the nucleus (Fig. 67), and is readily distinguished from the nucleolus, chromatin and other components of the nucleoplasm. Some isolated distended portions of the rough endoplasmic reticulum may contain dark osmiophilic granules (Fig. 67).

On the fifth day after attachment, and up to the third day after detachment, most type a cells are shrivelled and their cytoplasm appears as osmiophilic aggregates.

On the seventh day after detachment, the majority of type a cells are completely degenerated, although a few of them can be differentiated even after the complete degeneration

of the other types of cells at oviposition.

ii. Type b cells:

These cells are in contrast to type a cells. On the first day after attachment, type b cells start increasing in volume without showing any change in size or number of their secretory granules.

On the third day, Epon-embedded sections show that these cells are distinguished into two different sub-types of cell according to their cytoplasmic activity and their different types of granules. Cells b_1 are two to three cells adjacent to type a cells. They possess the same secretory granules, formerly described in unfed females, which stain blue with Giemsa, but now these granules are increased in number and accumulate in the apical region of the cell (Fig. 44). Their nuclei increase slightly in diameter and occupy the centre of the cell. Cells b_2 are five to six large cells occupying the greatest volume of the alveolus. Their original secretory granules (as those of cell b_1) have already been discharged or altered and new spherical secretory granules occupy the apical region of the cells. The corners of the basal region of these cells are occupied by another type of secretion which appears as small vacuoles (Figs. 44, 45). Both the granules in the apical region and the small vacuoles in the basal region stain faint pink with Giemsa.

On the fifth and seventh days after attachment, sub-type b_1 cells show some increase in size as well as in the number of their secretory granules. Type b_2 cells reach their maximum size and activity on the seventh day after attachment (Fig. 46). They become full of new fine and spherical secretory granules which accumulate in the apical region of the cells (Fig. 46). The lateral and basal regions of sub-type b_2 cells contain some scattered granules, similar to those in the apical regions, as well as some vacuoles (which were also observed on the third day) of variable size (Fig. 46). The fine granules stain dark pink or red with Giemsa while the vacuoles still stain faint pink.

After dropping off from the host until the female starts oviposition, sub-types b_1 and b_2 cells do not show any more activity but begin to shrink. During oviposition, these cells are almost completely degenerate.

Histochemical tests of sections of salivary glands on the fifth day after attachment (Table 3, Fig. 55 II) show that the secretory granules of sub-type b_1 are moderately positive with PAS while those of sub-type b_2 are faintly positive. The cytoplasm of b_1 cells is faintly positive with PAS and with naphthol yellow s, for polysaccharides and basic proteins. The PAS intensity is unaffected by diastase digestion. The cytoplasm of some b_2 cells is positive for both

polysaccharides and proteins, while others are only positive for proteins and negative for polysaccharides. The vacuoles in the basal region of sub-type b_2 cells are strongly positive with PAS. Sub-types b_1 and b_2 cell granules as well as the b_2 cell vacuoles stain shades of purplish-violet with thionin and toluidine blue, indicating metachromasia of acid mucopolysaccharides, which also stain with PAS and reacted positively with colloidal iron and alcian blue. Some b_2 cells reacted positively with DMAB-nitrate for tryptophan. With sudan black B, sub-type b_1 cells are negative for lipids, while the vacuoles only of b_2 cells are positive. Nuclei of sub-type b_1 and b_2 cells are DNA positive with the Feulgan reaction while only the cytoplasm of b_2 is positive for RNA with methyl green pyronin. Both sub-types of cell are negative for acid phosphatase and positive for alkaline phosphatase.

Ultrastructurally, the electron micrographs of the salivary glands on the first day after attachment show that type b cells increase in size as well as in some cytoplasmic activity. Their cytoplasm contains small conspicuous parallel stack arrays of Golgi bodies cisternae. The Golgi regions contain small condensing vacuoles of variable size (Ca. 0.04 µm - 0.4 µm diameter) and density. Golgi bodies are surrounded by many free ribosomes and numerous cisternae of rough endoplasmic reticulum which occupy most of the cell. Mito-

chondria, about 0.4 µm in diameter and 0.8 µm in length, are distributed throughout the cytoplasm, especially beneath the apical cell membrane (Fig. 68). Their moderately dense matrix contains few small dense granules. Small, low dense gaps can be recognised between their parallel cristae (Fig. 69). The apical region of some cells contains a few completely dense granules as well as small vesicles (arrow heads Fig. 70), underlying the plasma membrane. Other cells, particularly those occupying the bottom of the alveolus, lack these granules. Some low dense membrane-bound vacuoles are found in the cytoplasmic matrix or attached to the apical plasma membrane, while others are observed in the alveolar lumen (Fig. 70).

On the third day after attachment, type b cells can be differentiated into the two different sub-types of cell. Sub-type b_1 cells are 2 to 3 cells and are similar to the previous type b cells (of one day after attachment). They are increased slightly in size and contain very dense granules occupying the apical region of the cells. The cytoplasm possesses numerous free ribosomes and mitochondria with few dense granules in their moderately dense matrix. Microvilli are present at the apical cell membrane (Fig. 71). The granules of sub-type b_1 cells discharge their content individually (i.e. they do not fuse together) but the mechanism of releasing their content has not been observed.

Sub-type b_2 cells are 5 to 6 large cells occupying the great volume of the alveolus. Their apical regions contain new, moderately dense membrane-bound granules measuring Ca.0.5-2 Um in diameter. These granules replace the previous ones which probably have been discharged or altered. There is an aggregate of a dense substance with filamentous material in the matrix of the granules (fm. Figs. 71, 72, 73). Two or three granules may fuse together via their limiting membrane before releasing their content (Figs. 71, 75A). After fusion, the limiting membrane of the large new granule may then fuse with the plasma cell membrane to release its granular content into the alveolar lumen (Lu. Figs. 73, 75A) leaving a large empty vacuole of low dense interior (Va. Fig. 73) underlying the plasma cell membrane. This large vacuole contains some of the filamentous material (fm. Fig. 73) of the discharged granules.

Microvilli are present at the apical surface of the cells. The apical lateral cell membranes are attached to those of type c cells (the cap cells) by means of macula adherans and septate desmosomes. Mitochondria are distributed throughout the cytoplasm and their low dense matrix contains small dense granules. Golgi bodies are numerous but small and are found often in the lateral and basal regions of the cells. The curved arrays of parallel Golgi cisternae are often associated with one end of rough endoplasmic reticulum stacks (Figs. 74,

75B) which are oriented parallel to the cell axis. Free ribosomes are present in the cytoplasmic matrix. Small vesicles (0.04 Um diameter) (Figs. 74, 75B) are found in the Golgi region and appear to carry a synthesised material. These vesicles arise by budding off from areas of the rough endoplasmic reticulum which have lost their ribosomes. Golgi bodies are associated with condensing vacuoles (cv.Figs. 74, 75B) which are larger in size (Ca. 0.6 Um diameter) than the small vesicles and contain moderately dense contents. They are converted into mature membrane-bound granules, probably by progressive filling and concentration of their content. The mature granules (G. Fig. 75B) are larger (Ca. 1.7 Um diameter) and denser than the condensing vacuoles.

The basal region of sub-type b_2 cells is occupied by a large oval nucleus measuring Ca. 14 Um in diameter and Ca. 22 Um in length. The basal cell membrane may invaginate and its invagination contains portions of the interstitial epithelial cell (type d cells).

On the fifth and seventh days after attachment, sub-type b_1 cells do not show any changes or increase in activity and still contain their dense secretory granules, which can be seen in the cells, even after detachment from the host. Their cytoplasm contains a few small Golgi bodies, and the isolated cisternae of the rough endoplasmic reticulum are slightly distended and

contain a low dense intercisternal matrix. The nuclei have not been changed.

However, on the fifth day, the cytoplasm of sub-type b_2 cells becomes more active, especially in the lateral and basal regions of the cells. In addition to the granules which contain filamentous material, new dark granules are seen in the Golgi regions (Fig. 76a). In the basal region, cisternae of the rough endoplasmic reticulum are distended, becoming rounded (Ca. 2.5 µm diameter) with a low dense intercisternal matrix (Fig. 76b), while others of these cisternae are smaller, irregular in shape and distributed in the middle and apical regions of the cell. The nucleus consists mainly of areas of low electron density with a few small, dark patches of chromatin (N. Fig. 77). The nucleolus is compact, granulated and possesses less dense vacuoles of variable size (n. Fig. 77).

On the seventh day after attachment, sub-type b_2 cells reach their maximum size and activity. The previous granules (which contained the filamentous material) have already been discharged, as described before, while the dark granules (which appeared in the Golgi regions on the fifth day after attachment) are greately increased in number. The cytoplasm of some b_2 cells becomes packed with these dark granules (Fig. 79), while the cytoplasm of the other cells contains few of them, especially in the apical region. Numerous, low dense membrane-bound

vacuoles of variable size occupy the middle and the apical regions of these cells (Fig. 79). The basal and, occasionally, the apical regions of the b_2 cells possess very large moderately dense vacuoles (up to 13 Um wide and 16 Um long) which are a result of the great distension of the cisternae of the rough endoplasmic reticulum (Fig. 80). Many lysosome-like structures are found between the small vacuoles and the distended cisternae of rough endoplasmic reticulum lying in the basal region (Ly. Fig. 80). The nucleus has completely lost its regularity and appears in section to contain low dense membrane-bound vacuoles (Fig. 78).

After detachment, and up to the beginning of oviposition, some sub-type b_2 cells continue to possess numerous dark secretory granules, low dense vacuoles as well as moderately dense granules, which probably represent the degenerated phase of the dark granules. The cytoplasm is not so dense and contains many osmiophilic aggregates.

During oviposition, sub-types b_1 and b_2 cells are completely degenerated and nothing can be distinguished.

Ultrastructural histochemical reactions were carried out on the fifth day after attachment to detect polysaccharides, proteins and acid phosphatase. All the dark granules of sub-type b_1 and b_2 cells are positive for polysaccharides with PA-TCH and partially digested with pronase enzyme. Acid phosphatase is not detectable in sub-types b_1 and b_2 cells except that some small condensing vacuoles, as well as lamellae-like

structures lying in the Golgi region of sub-type b_2 cells, are positive (Fig. 81).

iii. Type c cells: (The cap cells)

These cells still cannot be distinguished at the light microscopic level.

Ultrastructurally, no appreciable changes were observed in the position or cytological structure of type c cells during or after feeding except that they are slightly increased in size and now touch the distal extensions of the two curved epithelial cells surrounding the valvular canal from the first day after attachment.

Occasionally on the third day after attachment, the plasma membrane of some c cells is greatly distended and their cytoplasm, as well as the nuclei, contain membrane-bound vacuoles with low dense content (Fig. 82). These cells are completely degenerated at the time of oviposition.

iv. Type d cells: (The interstitial epithelial cells)

These cells still cannot be recognised at the light microscopic level.

Ultrastructurally, micrographs at the first day after attachment show connections between type d cells and the proximal extensions of the two curved epithelial cells surrounding the valvular canal.

On the third day after attachment, d cells start to form complicated canaliculi full of elongated mitochondria (Ca. 11.5 Um long and Ca. 0.2 Um diameter), free ribosomes, many microtubules and some electron-dense bodies. The complexity of these canaliculi increases gradually to reach their maximum on the seventh day after attachment (Fig. 83) and after detachment. These canaliculi surround all the alveolar cells and attach with their plasma membranes by means of intercellular junctions (Fig. 83). Type d cells are almost degenerated at oviposition.

Generally, the activity and complexity of the interstitial epithelial cells of type II alveoli is less than those of types III and IV alveoli.

2. TYPE III ALVEOLI

These alveoli (Fig. 47) also enlarge considerable in size and their diameter is 74.5 Um in females attached to the host for seven days, but are not as large as type II alveoli (Table 2, Fig. 54).

i. Type e cells

The effect of the bloodmeal on type e cells (e. Fig. 47) is the same as on type a cells of alveolus II and follow the same changes. Occasionally, some granules of type e cells are observed of a peculiar shape on the third day after attachment (Fig. 84). These granules contain a distended, spheri-

cal low dense centre which occupies the greater volume of the granule (i.e. lacking the subunit granules formerly seen in unfed females), surrounded by an irregular dark lattice which is occasionally thicker from one side than the other. There is usually a dense spherical spot lying in the lattice matrix (Fig. 84).

ii. Type f cells:

On the first and third day after attachment, the semithin sections show that the number and size of the secretory granules of type f cells are greatly reduced (compare Fig. 47 with Fig. 8). The cytoplasm is clearly visible between the granules, which mostly accumulate in the apical region of the cells. The nuclei are smaller and occupy the basal region of the cell. In paraffin sections stained with Giemsa after formol-sublimate fixation, the cytoplasm of these cells stain blue while the granules are faint red.

On the fifth day after attachment, a further reduction is observed in the number of secretory granules. In Epon-embedded sections stained with azure II, these secretory granules become variable in intensity of staining. The nuclei are reduced in size and lie in the lateral region of the cell.

On the seventh day after attachment, type f cells are completely reduced (Fig. 48), degenerated and nothing can be distinguished in them.

Histochemically (Table 3, Fig. 55 III), the few granules found in type f cells on the fifth day after attachment show a marked increase in the intensity of PAS staining for polysaccharides, while no change has been observed in the protein materials with mercuric bromophenol blue and naphthol yellow s staining. The intensity of PAS colour does not change after diastase digestion. Type f cells are negative for tryptophan, tyrosine, arginine, -SS-groups and $-NH_2$ groups. The cytoplasm is faintly positive for RNA with methyl green-pyronin Y and the nuclei are moderately positive for DNA with the Feulgen reaction. These cells are negative for lipids and acid phosphatase while they are moderately positive for alkaline phosphatase.

In ultrastructure, type f cells show secretory activity on the first to third days after attachment. Their cytoplasm becomes full of isolated, slightly distended, cisternae of rough endoplasmic reticulum with a moderately dense intercisternal matrix. The apical region is occupied by some granules, mitochondria, a few small Golgi bodies and membrane-bound vacuoles (up to C.8 µm diameter). Most of the secretory granules are irregularly shaped and their peripheral area is more dense than the middle. Mitochondria (M. Fig. 85) are up to 1.7 µm in diameter with obvious rounded or oval gaps between their parallel cristae. Golgi bodies (Gb. Fig. 85) are small (Ca. 1.3 µm long) with parallel and slightly curved cisternae giving rise to a few condensing vacuoles.

A few dark irregularly shaped lysosome -like bodies are observed near the Golgi region (Ly. Fig. 85). The lateral plasma membrane in the apical region is attached to those of type h cells by means of macula adherans and septate desmosomes. The basal region is occupied by a small nucleus (Ca. 3 µm diameter) surrounded by numerous isolated cisternae of rough endoplasmic reticulum.

Type f cells in some alveoli do not show much change except an elongation of their cells as well as their nuclei due to the great distension of the alveolar lumen. The macrotubules formerly seen in the unfed females are present in the cytoplasmic areas lacking secretory granules. In addition, another type of macrotubule with regular very low dense single membranes was also present beside the first one.

On the fifth day after attachment, type f cells are increased in size and the plasma membrane becomes irregularly indented. The isolated cisternae of rough endoplasmic reticulum are enormously distended, particularly in the basal and lateral regions of the cell. Parts of these cisternae have lost their ribosomes (Fig. 86). A few secretory granules still lie in the apical region while the most basal region is occupied mainly by an irregularly shaped nucleus. The apical cell membrane bordering the alveolar lumen possesses a few short microvilli. The mechanism of releasing the secretory

granules has not been observed. The basal region of the cell contains the new type of macrotubule, which are long (Ca. 2.8 µm long and Ca. 0.1 µm diameter)and surrounded with ribosomes which are arranged on their outer surface (Fig. 86). These new macrotubules probably are modified types of those described in the unfed female.

On the seventh day after attachment, until dropping off from the host, type f cells do not show any more activity but in contrast they start to degenerate, become very dense and nothing can be distinguished.

Ultrastructural histochemical reactions were carried out on the fifth day after attachment to detect polysaccharides, proteins and acid phosphatase. The granules of type f cells are intensely prositive for polysaccharides with PA-TCH, and are partially digested with pronase enzyme. Acid phosphatase is undetectable in type f cells.

iii. Type g cells:

On the first day after attachment, these cells increase greatly in size and reach their maximum size and activity by the fifth day. Semithin sections show that they contain secretory granules not seen in the unfed female. These accumulate in the apical region of the cell with a few granules scattered elsewhere in the cytoplasm (Fig. 47). The nucleus is greatly enlarged in size (20 µm long and 10 µm

wide) (compare Fig. 47 with Fig. 8) and occupies the basal region of the cell. In paraffin sections stained with Giemsa stain, the cytoplasm of these cells stained shades of blue while the secretory granules were pink.

On the seventh day after attachment, type g cells begin to decrease in size. They reach their maximum degeneration on the first day after oviposition. In Epon-embedded sections, portions from the basal region of these cells are found isolated in the interstitial epithelial cells (type i cells) (Fig. 48). This is probably due to the plane of the section and the irregular outline of type g cells at this time.

Histochemically (Table 3, Fig. 55 III), the granules of these cells are PAS-positive and the colour intensity does not change after diastase digestion indicating that the PAS staining is not due to glycogen. The granules stain orthomatically with toluidine blue and thionin and are positive for basic proteins while the cytoplasm and granules are moderately positive for tyrosine with Millon's reagent (unlike the cells in the unfed female). The cytoplasm and nucleoli are faintly positive for RNA while the nucleus is positive for DNA. These cells are negative for acid phosphatase while they are moderately positive for alkaline phosphatase.

In ultrastructure, type g cells possess electron-dense secretory granules (G. Fig. 85) and oval membrane-bound vacuoles (up to 2 Um diameter) (Va. Fig. 85) from the first day after attachment as well as isolated cisternae of rough endoplasmic reticulum. Some of the vacuoles were attached to the apical and lateral plasma membranes while others lie between the lateral plasma membrane of type h cells and type g cells (Fig. 85). Mitochondria, measuring Ca. 1.8 µm in length and Ca. 0.2 µm in width, contain low dense gaps between their parallel cisternae.

The basal region of type g cells is mainly occupied by the enlarged nucleus. Small Golgi bodies (Gb. Fig. 87) are observed in the cytoplasm on both sides of the nucleus and are associated with small condensing vacuoles. As in type f cells, lysosome-like bodies are found near to the Golgi region (Fig. 87).

On the third day after attachment, some granules in the apical region of type g cells show streaming of their contents, producing a tail (Ca. 0.2 µm long and Ca. 0.06 µm diameter) from the main body. These tails can often be traced into the microvilli projected from the apical cell membrane (Fig. 88). In the basal region, most of the isolated cisternae of the rough endoplasmic reticulum are distended, especially in the basal corners of the cell. Golgi bodies are active giving rise to more condensing vacuoles.

The basal cell membrane is irregularly folded (Fig. 89).

On the fifth day after attachment, all type g cells become irregularly shaped. Their apical region contains microtubules, membrane-like structures, and a few undischarged granules surrounded by many cisternae of rough endoplasmic reticulum which become rounded and variable in size with low dense matrix (Fig. 90). Most of these cisternae have lost their ribosomes.

On the seventh day after attachment, up to the seventh day after dropping off from the host, type g cells are greatly reduced in size and their surface becomes irregularly shaped, particularly in the basal region of the cell. As a result of this irregularity, cytoplasmic areas from the basal region appear as detached portions in section and contain a few isolated and distended cisternae of rough endoplasmic reticulum as well as a few undischarged granules and some mitochondria (Fig. 91). Some isolated cisternae reach maximum size of distension to be Ca. 30 Um in length and Ca. 20 Um in width with low dense intercisternal matrix (Fig. 92). The lateral cell membranes project microvilli which intersect with those of type h cells (the cap cells) (Fig. 93).

On the first day of oviposition, type g cells are completely degenerated and nothing can be distinguished in them.

Ultrastructural histochemical micrographs show that the dense secretory granules, as well as the mitochondria, of type g cells are polysaccharide positive with PA-TCH (Fig. 94). These cells are slightly digested by the pronase enzyme for protein while acid phosphatase is not detectable.

iv. Type h cells: (The cap cells)

Type h cells still cannot be distinguished at the light microscopic level.

Ultrastructurally, type h cells are more active than type c cells of type II alveoli.

On the first day after attachment, these cells cover the apical membranes of the other alveolar cells and extend between the different types of cells of type III alveolus to join the two curved epithelial cells which surround the valvular canal. This junction has not been observed in the unfed females. The apical lateral membranes of h cells are attached with the other alveolar apical cell membranes by means of macula adherens and septate desmosomes (Fig 85). As the tick continues to engorge, type h cells continue to extend between the alveolar cells.

On the seventh day after attachment, type h cells and their nuclei (Ca. 10 Um diameter) have enlarged greatly in size. These cells are irregular in outline. They extend to the distal parts of the alveolus to lie between either the

interstitial epithelial cells (Fig. 95), or type g cells. Their lateral membranes form septate desmosomes or tight junctions for long distances with the plasma membrane of type i cell canaliculi (Long arrows, Fig. 95). These extensions form trough-like extensions (Fig. 93) containing mitochondria, free ribosomes and numerous microtubules. The lateral membrane of these troughs projects microvilli which interdigitate with those projected from the lateral membrane of type g cells. The other sides of the troughs attach with type i cells (interstitial epithelial cells) by means of intercellular junctions (J, Fig. 93).

From the first to the seventh day after dropping off from the host, rounded, membrane-bound vacuole-like structures are found in the cytoplasm of type h cells and contain low dense contents. Their limiting membrane projects small microvilli into the lumen of these vacuoles (Fig. 96). These may be irregular extensions of the alveolar lumen.

At the beginning of oviposition, h cells are completely degenerated and nothing can be recognised.

v. Type i cells: (The interstitial epithelial cells)

These cells can only be seen under the light microscope on the fifth or seventh day after attachment as a light area full of fine fibrils and small dark granules and surrounding the other alveolar cells (Fig. 48).

Ultrastructurally, type i cells of alveolus III are more active than type d cells of alveolus II.

On the first day after attachment, these cells have narrow extensions filling the gaps between the alveolar cells. These extensions possess numerous free rinosomes, glycogen particles, and a few dense bodies.

On the third day after attachment, the extensions of these cells extend between the folded basal membranes of the other alveolar cells (Fig. 89).

On the fifth and seventh days after attachment, type i cells are branched extensively to form complicated canaliculi (Ca. Fig. 95).

In the basal region of type III alveoli, the distal extensions of these canaliculi form arm-like structures (Fig. 97) which are arranged regularly to be very close to the basement membrane of the alveolus (Fig. 97). The ends of these arms are swollen and contain rounded mitochondria (Ca. 0.4 Um diameter) with parallel cristae (Fig. 97). The basal region may contain canaliculi of type i cells enclosing various sizes and shapes of mitochondria (i.e. rounded, oval, elongated and dumb-bell shaped mitochondria) (Fig. 98). Some of these have the typical internal structure with transverse cristae while others have an unusual morphology with longitudinally oriented cristae at one side and transversely oriented cristae at the other, or longitudinally or trans-

versely oriented cristae at one side while both intersect together at the other (Fig. 98). Some partially discharged granules are observed in the canaliculi of type i cells, while the limiting membranes of some of these granules are fused with the plasma membranes of the canalculi (Fig. 98). Many lysosomes are found in these canaliculi engulfing mitochondrial remnants (Fig. 97) or small vesicles and membrane-like structures (Fig. 98).

After dropping off from the host and until oviposition, these canaliculi become packed with lysosome-like structures, degenerated and autolized granules, many low dense membrane-bound vacuoles, few membrane-like structures, numerous vesicles and various shapes of mitochondria (Fig. 99). Some type i cells have large membrane-bound vacuoles with many fine microvilli which project from the limiting membrane and are directed towards the alveolar lumen of these vacuoles (Fig. 96).

Three to five days after starting oviposition these cells are completely degenerated and nothing can be distinguished.

3. TYPE IV ALVEOLI:

These alveoli increase slightly in size between the first and third days after attachment. On the fifth day, their volume is appreciably greater and reaches a maximum on the seventh day after attachment (Table 2, Fig. 54).

At the beginning of engorgement, the lumen in these alveoli is greatly distended and, as a result of this distension, the fundus cells (type l cells) become flat (Fig. 49). As the tick continues to engorge this distended alveolar lumen becomes narrow. This is due to the gradual increase in size of the alveolar cells, especially the type n cells (the interstitial epithelial cells).

i. Type j cells:

The effects of the bloodmeal on type j cells are similar to that of type a cells of type II alveoli and type e cells of type III alveoli described before and follow the same developmental changes.

ii. Type k cells:

On the first and third days after attachment, the semithin sections show that the secretory granules of these cells are markedly reduced in size and number (compare Fig. 49 with Fig. 9). Their nuclei are about 6 Um in diameter and occupy the basal region of the cells. In paraffin sections, the granules are dark pink while the cytoplasm stains blue with Giemsa stain.

On the third and fifth days after attachment, these cells contain some scattered granules of variable size (Fig. 50). The nuclei become rounded (Ca. 10 Um in diameter) and occupy the apical region of the cells. The nucleolus is compact

At the beginning of engorgement, the lumen in these alveoli is greatly distended and, as a result of this distension, the fundus cells (type 1 cells) become flat (Fig. 49). As the tick continues to engorge this distended alveolar lumen becomes narrow. This is due to the gradual increase in size of the alveolar cells, especially the type n cells (the interstitial epithelial cells).

i. Type j cells:

The effects of the bloodmeal on type j cells are similar to that of type a cells of type II alveoli and type e cells of type III alveoli described before and follow the same developmental changes.

ii. Type k cells:

On the first and third days after attachment, the semithin sections show that the secretory granules of these cells are markedly reduced in size and number (compare Fig. 49 with Fig. 9). Their nuclei are about 6 Um in diameter and occupy the basal region of the cells. In paraffin sections, the granules are dark pink while the cytoplasm stains blue with Giemsa stain.

On the third and fifth days after attachment, these cells contain some scattered granules of variable size (Fig. 50). The nuclei become rounded (Ca. 10 Um in diameter) and occupy the apical region of the cells. The nucleolus is compact

and stains dark blue with Giemsa stain.

On the seventh day after attachment, the secretory granules of type k cells start to be broken down into a finely granular homogeneous material.

One to three days after detachment, these cells are completely degenerated.

Histochemically, (Table 3, Fig. 55 IV) the few granules found in these cells are positive for polysaccharides with PAS, while they show a marked increase in protein staining with mercuric bromophenol blue and naphthol yellow s. They react positively for tyrosine and negatively for tryptophan, arginine, -ss-groups and amino groups. There is a marked decrease in staining for lipids with sudan black-B. Their cytoplasm and nucleolus are faintly positive for RNA, and the nucleus is DNA-positive. These cells are negative for acid phosphatase while they are positive for alkaline phosphatase.

Ultrastructurally, type k cells show a great reduction in the number and size of their secretory granules on the first day after attachment. The whorls of dense rough endoplasmic reticulum of the unfed tick become isolated and show slightly distended cisternae with low dense intercisternal content (Fig. 100). The intervening cytoplasm is filled with closely packed ribosomes. The mitochondria (Ca. 0.6 µm dia-

meter) are distributed throughout the cytoplasm and contain few, small dense granules in their mitochondrial matrix. They are oval in shape, and their cristae are transversely oriented with small low dense gaps in between, while a few of them are irregular dumb-bell in shape (Fig. 100).

The nucleus occupies the basal region and does not show any appreciable change. The apical membranes of these cells lack microvilli. Golgi bodies have not been observed in their cytoplasm.

On the third day after attachment, no appreciable changes are observed except the further distensions of some isolated cisternae of rough endoplasmic reticulum and a few of them contain irregular membrane-bound structures (Fig. 101). The plasma cell membrane becomes folded in an irregular pattern. The mechanism of release of the granular content has not been observed.

On the fifth and seventh day after attachment, the irregularity of the cell surface is increased and accompanied by great reduction of the cell size. Many broken down granules are scattered in the cytoplasm (Fig. 102). The nuclear membrane is highly infolded giving an irregular outline to the nucleus. The nucleolus is compact and granulated with small vacuoles.

On the first day after dropping off from the host, these cells

are completely degenerated.

Ultrastructural histochemical reactions were carried out on the fifth day after attachment. The granules of type k cells are negative for polysaccharides with PA-TCH, while they are still positive for proteins with pronase enzyme. Some small granules are positive for acid phosphatase but the deposition of the lead particles is only on the peripheral zone of the granules (Fig. 103).

iii. Type l cells:

The activity of these cells after attachment is more or less as type g cells described before for type III alveoli.

On the first day after attachment, these cells do not show any marked change, while on the third day, they show an appreciable increase in size. In semithin sections, their cytoplasm contains few, fine and scattered granules. In paraffin sections, the cytoplasm stains light blue with both Mallory's triple stain and Giemsa, while the granules are red.

On the fifth and seventh days after attachment, these cells become irregularly shaped and constitute more than half the volume of the alveolus (Fig. 50). They still contain few scattered fine granules. Their nuclei are very much enlarged.

These cells degenerate gradually after dropping off of the tick from the host to reach the maximum degeneration at the

beginning of oviposition.

Histochemically, (Table 3, Fig. 55 IV), on the fifth day after attachment, the fine granules of these cells are positive with PAS and the colour is unchangeable after diastase enzyme. The cytoplasm and the granules stain orthochromatically with toluidine blue and thionin, and react positively for basic proteins with mercuric bromophenol blue and naphthol yellow s (Table 3). The cell granules are positive for tryptophan while they are negative for tyrosine, arginine, -SS- and NH_2-groups. Type 1 cells are negative for lipids and acid phosphatase while they are moderately positive for alkaline phosphatase.

In ultrastructure, these cells do not show any appreciable change on the first day after attachment except that the mitochondria possess small dense granules in their matrix.

On the third day after attachment, the apical region of type 1 cells is occupied by secretory granules similar to those of type g cells of alveolus III but are smaller in size (Ca. 0.5 µm in diameter) and less frequent. The apical membrane projects small microvilli into the lumen. Some small and compact Golgi bodies with parallel cisternae are distributed throughout the cytoplasm. The latter possesses low dense membrane-bound vacuoles, few scattered dark bodies and numerous slightly isolated distended cisternae of rough

endoplasmic reticulum with moderately dense lumen. The intervening cytoplasm is filled with closely packed ribosomes. The nuclei are rounded or oval in shape and occupy the most basal region of the cell.

On the fifth and seventh days after attachment, these cells begin to degenerate. The apical regions of type 1 cells are occupied by small dense secretory granules, scattered mitochondria and many slightly distended cisternae of rough endoplasmic reticulum (Fig. 102). Some of their isolated cisternae of rough endoplasmic reticulum [illegible], lying in the basal region of the cell, become highly distended to form large vacuoles (Ca. 12 µm long and Ca. 3.5 µm wide), with low dense lumen, surrounded by smaller vacuoles. The nuclei become irregularly shaped and occupy the major part of the cell with dark and compact nucleoli. Some small granules are scattered between the distended cisternae of rough endoplasmic reticulum. The apical part of the lateral cell membranes of type 1 cells project microvilli (Fig. 102). Often, the microvilli of the lateral membrane of two or three neighbouring 1 cells may intersect with each other when there are no type m cells (the cap cells) or type n cells (the interstitial epithelial cells) between them. The microvilli of the lateral membranes of both types 1 and m cells are often observed intersecting with each other (Mv. Fig. 102). In addition, parts of the lateral membrane of both types 1 and

and m cells are attached to each other by means of tight junction (thick arrows, Fig. 102).

The degeneration of type l cells increases after dropping off from the host to reach the maximum of degeneration at the beginning of oviposition.

iv. Type m cells:

Type m cells develop in the same way as type h cells of type III alveolus during and after the bloodmeal.

v. Type n cells:

In Epon-embedded sections, these cells can be seen as faint fibrillar structures lying between the alveolar cells only in the final stages of feeding (Fig. 50).

The fine structure and activity of type n cells after attachment, after dropping off and at the beginning of oviposition are similar to type i cells previously described for type III alveoli.

b) FEEDING MALES

In feeding males the salivary alveoli increase slightly in size but do not attain the same size as those of feeding females (Table 2, Fig. 54).

The histological and cytological structures of the main salivary ducts and the different types of alveolar cells (a-n) do not respond to the bloodmeal in the same way as described

for the feeding and ovipositing female.

On the fifth day after male attachment, type a, e and j cells do not show appreciable changes in either the cell size or the number of secretory granules (Figs. 51, 52, 53).

At the electron microscopic level, no Golgi bodies have been seen in type a cells similar to those of feeding females. In contrast to the feeding females, the mechanism of releasing the granular content of type a cells has not been observed in the feeding males. Types a, e and j cells of feeding males do not show any reduction in size, even on the seventh day after detachment, while these cells in feeding females become shrivelled on the fifth day after attachment.

Types c, h and m cells (the cap cells) and types d, i and n cells (the interstitial eputhelial cells) of types II, III and IV alveoli do not show any change in activity during and after feeding. The cap cells of the granule-secreting alveoli neither extend deeply between the different alveolar cells nor form troughs as in those of feeding females. Occasionally, as in the feeding female, membrane-bound vacuoles are found in the middle of the cap cell nuclei of type II alveolus (Fig. 106). In contrast to the feeding females, the interstitial epitholial cells of the granule - secreting alveoli of the feeding males neither form complicated branched canaliculi nor contain structurally complicated mitochondria.

The dark interrupted line, which was observed in the basement membrane of females during the final feeding stages, and the complexity of this basement membrane, were not found during or after feeding in male alveoli.

In type II alveoli, type b cells can be classified into the two sub-types b_1 and b_2 on the first day after attachment. The secretory granules of these two sub-types of cell are similar to those which were found in apical regions of b cells on the third day after female attachment. In contrast to feeding females, these granules do not change at all, even after the males drop from the host (Figs. 51, 106). Some nuclei of sub-type b_2 cells, however, are completely degenerated on the fifth day after male attachment and contain variable sizes of vacuoles (Fig. 107).

Type f cells of type III alveoli do not show any marked change during and after feeding (Fig. 52). At the electron microscopic level, the macrotubules of these cells found in the female have not been observed in the male.

Type k cells of type IV alveoli show some reduction in the cell size and in the number of secretory granules (Fig. 53) during and after feeding.

Types g and l cells lying in the fundus of types III and IV alveoli are similar to each other and do not show any appreciable changes at the light microscope level during and after feed-

ing (Figs. 52, 53).

Histochemical reactions were carried out on the fifth day after male attachment. It was found that all cells react as those of the unfed adult (Table 1, Fig. 11), except that subtypes b_1 and b_2 cells are differentiated and react as those of the feeding female on the fifth day after attachment.

At electron microscopy level, types g and l cells contain a few moderately and low dense scattered granules (Ca. 0.8 µm diameter) (Fig. 108). The cytoplasm is packed with distended and non-distended cisternae of rough endoplasmic reticulum (Fig. 108).

Ultrastructural histochemical reactions were applied on the fifth day after attachment. The secretory granules of types g and l cells are faintly positive for polysaccharides with the PA-TCH technique and partially digested after treatment with pronase enzyme. The two types of cell are negative for acid phosphatase.

Some nuclei of type l cells are degenerated on the fifth day after attachment and appear dark in the electron micrograph, with vacuoles in their peripheral region (Fig. 109).

2. ORNITHODOROS MOUBATA

(A) UNFED ADULTS

MORPHOLOGY:

The salivary glands of O. moubata are identical in both sexes and comprise a pair of compact, elongated organs resembling a bunch of grapes lying on either side of the body (Fig. 111). They are much smaller than those of unfed H. leachii adults, but they extend from the base of the first pair of legs to the base of the fourth pair of legs (Fig. 111).

In contrase to H. leachii, the two main salivary ducts (m.s.d. Fig. 111) of O. moubata do not divide into a pair of subsidiary branches but give rise to secondary ducts (s.s.d. Fig 111) which give off smaller lobular ducts (l.s.d. Fig. 111) which are similar to those of H. leachii.

SALIVARY DUCTS

Epon-embedded sections show that the structure of the main salivary ducts of the unfed O. moubata adult (Fig. 113) is similar to that of H. leachii except that the main duct lumen (Lu. Fig. 113) is more distended and the lining cuticular layer is thinner and less infolded. The middle cuticular region (MR. Fig. 113) of the main duct is thinner (Ca. 3.4 Um wide) than that of H. leachii (compare Fig. 113 with Fig. 5). For paraffin sections stained with Mallory's triple stain, the three cuticular regions of the main duct of O. moubata

stained in the same way as those of unfed H. leachii.

The cuticular lining of the secondary and lobular ducts is similar in structure and staining reaction to that already described in H. leachii. The lobular duct extends up to the alveolar lumen as the alveolar duct. In the nongranular-secreting alveoli , the alveolar duct is similar to that of unfed H. leachii while,in the granule-secreting alveoli of unfed O. moubata, it divides in the alveolar base into a valve-like structure and cuticular arms as described below.

The fine structure of the main, secondary and lobular ducts are similar to each other and to those of unfed H. leachii.

As in H. leachii, several nerves are observed closely associated with the salivary duct system and these divide into individual axons extending into the nongranular and granule-secreting alveoli. These axons contain neurosecretory material.

Ultrastructural histochemical techniques were carried out to detect polysaccharides, proteins and acid phosphatase in the main salivary duct. For polysaccharides, the reaction is similar to that of H. leachii except that the outer cuticular region (the spiral thickening) of the main duct of O. moubata stains less densely with PA-TCH.

For proteins, the inner and the middle cuticular regions of the main duct react similarly to those of H. leachii with

pronase enzyme. In contrast to H. leachii, the outer zone of the middle region does not show the dark parallel collagen-like fibres but shows low dense parallel cuticular lamellae. Moreover, the spiral thickening is partially digested by the enzyme and contains fine flocculent textured material. Acid phosphatase is undetectable in the main duct of the unfed adult.

SALIVARY ALVEOLI:

Only two types of alveoli were found in the salivary glands of the unfed O. moubata adult (Table 4, A and B Fig. 111) and are classified, as in H. leachii, according to their position on the main salivary ducts. Type I alveoli (Ca. 40-50 µm diameter) are disposed along the anterior one-third of the main duct but only on its dorsal, mesial and ventral sides. Type II alveoli (Ca. 45-60 µm diameter) are more numerous than type I and occupy the posterior two-thirds of the gland, and also occur anteriorly along the outer side of the main duct where the type I alveoli are absent. As in unfed H. leachii, type I alveoli are connected directly to the main duct while type II alveoli are connected either directly to the main duct or indirectly to the secondary salivary ducts via their short lobular ducts.

TABLE 4: HISTOCHEMICAL RESULTS ON *O. MOUBATA* SALIVARY GLANDS (UNFED ADULTS)

METHODS	TYPE I ALVEOLI	TYPE II ALVEOLI a	b	c	d	e
A. CARBOHYDRATES:						
PAS	+	var-to +++	var-to ++	±		
GLYCOGEN: DIASTASE/PAS	-	-	-	-		
: BEST'S CARMINE	+	-	+	-		
ACID MUCOPOLYSACCHARIDES:						
: ALCIAN BLUE	-	++	-	-		
: COLLOIDAL IRON	-	++	-	-		
METACHROMASIA						
: TOLUIDINE BLUE	-	+++	-	-		
: THIONIN	-	+++	-	-		
B. PROTEINS						
BASIC PROTEINS:						
: MERCURIC BROMOPHENOL BLUE	++	var-to ++++	var-to ++	++		
: NAPHTHOL YELLOW-S	++	var-to ++++	var-to ++	++		
TRYPTOPHAN: D.M.A.B.-NITRATE METHOD	-	-	-	++		
TYROSINE: MILLON'S REAGENT	-	-	++	-		
ARGININE: ∝ - NAPHTHOL	-	-	-	-		
-SS-GROUPS: P.F.A.A.B. METHOD	+	-	-	-		
AMINO GROUPS: NINYDRIN SCHIFF	-	-	-	-		
C. LIPIDS						
SUDAN BLACK-B	++	-	var-to +++	-		
D. NUCLEIC ACIDS: METHYL GREEN PYRONIN Y	±	-	+	±		
: FEULGEN REACTION	+	±	+	±		
E. ENZYMES:						
ACID PHOSPHATASE: LEAD NITRATE METHOD	-	-	-	-		
ALKALINE " : Ca.COBALT METHOD	-	+	-	-		

1. a - e indicates types of cells 2. ± sign indicates a faint positive reaction. 3. The number of plus signs indicates the relative intensity of staining by comparison with strongest reaction in gland or other tick tissues.

NONGRANULAR-SECRETING ALVEOLI:

TYPE I ALVEOLI:

This type of alveolus is much less numerous in the salivary glands of unfed O. moubata than in H. leachii. It is similar in structure and staining reaction to the type I alveolus previously described for unfed H. leachii.

Histochemically, these alveoli react exactly as those of the unfed adult of H. leachii.

GRANULE-SECRETING ALVEOLI:

TYPE II ALVEOLI:

Epon-embedded sections show that the salivary glands of adult O. moubata contain only one type of granule-secreting alveolus (Fig. 114).

The type II alveolus contains five types of cell: a, b, c, d and e. The letterings of the cell types represent the arrangement of the cells in the alveolus. Types d and e cells cannot be distinguished at the light microscopic level even at the maximum magnification. The alveolus is invested in a delicate basement membrane.

In ultrastructure, the alveolar duct is divided in the alveolar base into small inner and larger outer projections. The small projections appear in section as a short valvo-like structure (Ca. 0.13 µm long) with pointed tips in longitudi-

nal sections (white arrow, Fig. 116), and is directed toward the alveolar lumen. It guards a narrow opening leading into a narrow valvular canal (Vc., Fig. 116), similar to that previously described for the unfed H. leachii adult, which opens into the alveolar lumen (AL., Fig. 116). The valve-like structure is the extension of the middle and the inner cuticular regions of the alveolar duct. As in unfed H. leachii, the thin inner cuticular region extends further to form the valvular canal, which narrows and leads into the noncuticular alveolar lumen.

The large outer projections appear as two cuticular arms (Ca. 4.5 µm long) (CA., Fig. 116) which are the extensions of the middle cuticular region only of the alveolar duct, embedded between the lateral epithelial cells.

The valvular canal in each secretory alveolus is surrounded by extensions from type d cells (the cap cells), (two large arrows, Fig. 116).

Types a, b and c cells are packed with secretory granules. Type d cells (the cap cells) and e cells (the interstitial epithelial cells) are similar to those described for the unfed adult of H. leachii.

Haemocytes, structurally similar to those described for feeding H. leachii females, are found in the haemolymph of both sexes of the unfed adult of O. moubata. Some of them are found very close to types I and II alveoli whilst some others were

observed attached to the surrounding connective tissue.

i. Type a cells:

These are 3 to 4 cells in each alveolus (Fig. 112, 114). At least one or two of these cells are small in size, and surround the base of the alveolar duct while the rest are larger, and can be found between the other types of alveolar cells. The small a cells are packed with small secretory granules while the large cells are packed with large granules. The granules are rounded and differentiated into a middle and a peripheral zone. Semithin sections stained with Azure II show that the middle zone stains shades of pink-blue while the peripheral one stains violet. In paraffin sections, the middle zone stains red with Mallory's triple stain and blue with Giemsa, while the peripheral one stains shades of red-blue with Mallory and purple with Giemsa. The network of cytoplasm between the granules stains blue with both Mallory and Giemsa. Nuclei are normally marked by overlapping granules.

Histochemically, (Table 4, Fig. 112), the peripheral zone of large granules in the large a cells are characterised by an intensive positive reaction with PAS, while their middle zone varies in staining from moderately positive to negative with bromophenol blue and naphthol yellow s for basic proteins. The peripheral zone of the small granules of the small a cells

vary from negative to moderately positive with PAS, while their middle zone reacts more intensely and positively for basic proteins than that of the large granules. The intensity of PAS staining has not been reduced after treatment with diastase enzyme indicating the absence of glycogen. Type a cells react negatively for tryptophan , tyrosine, arginine, -SS and NH_2 -groups. In thionin and toluidine blue-stained preparations, the granules are differentiated into blue middle and purplish peripheral zones,indicating a metachromasia for acid mucopolysaccharides in the zone which stains with PAS peripherally. The presence of acid mucopolysaccharides was confirmed by positive staining with alcian blue and colloidal iron (Table 4). No staining of granules or cytoplasm was found with sudan black for lipids. DNA was detectable in the nucleus and RNA was detectable in the cytoplasm and nucleolus. Acid phosphatase was not detected, but alkaline phosphatase was found in the cytoplasm of these cells.

In ultrastructure, the cytoplasm of type a cells is packed with irregularly shaped or rounded membrane-bound granules (up to 4 µm diameter) (Fig. 117), which possess a small, very electron-dense cores (up to 0.9 µm diameter) and large, peripheral zones which are dark in some granules, while they are low in density in others (Fig. 117). In the narrow cytoplasmic areas between these mature granules, short parallel cisternae

of Golgi bodies are observed giving rise to small, dark vacuoles (Ca. 0.1-0.3 µm diameter) (black arrows, Fig. 118) of the same density as those of the middle cores of the mature granules. In Golgi regions, some of these small dark vacuoles are observed surrounded by a less dense peripheral zone (opened arrow, Fig. 118) which represent the condensing vacuoles. In another area of the same cell, dark vacuoles are found enclosed in one side of electron-lucent vacuole -like structures (Fig. 119). The exact origin of these vacuole-like structures is not known. Most of them possess material (Small arrows, Fig. 119) similar in density to those of the peripheral zone of the condensing vacuoles which are found in the Golgi region and appear as if coming out from the dense core (opened arrows, Fig. 119). This possibly represents an earlier stage of the condensing vacuoles formation. These condensing vacuoles are converted into mature granules, probably by the progressive filling and concentration of their content. Numerous free ribosmes and a few, small scattered mitochondria are observed throughout the cytoplasm. Nuclei are oval, small in size (up to 4 µm diameter), occupying the basal region of the cell and contain smaller nucleoli (up to 1 µm diameter) of spongy appearance. The apical cell membrane does not project microvilli.

Ultrastructural histochemical techniques were carried out for

detection of polysaccharides, proteins and acid phosphatase. The limiting membrane of the mature granules in type a cells is intensively positive for polysaccharides while the peripheral zones of these granules are faintly to moderately positive with PA-TCH. The middle cores are almost polysaccharide negative (Fig. 120).

For protein detection, the middle cores of all type a cell granules are completely digested with pronase enzyme (Fig. 121), while some large granules of the large a cells are partially digested and show flocculent textured material, which is probably due to the incomplete digestion with the enzyme.

Acid phosphatase was not detected in these cells.

ii. Type b cells:

These are 6-8 large cells occupying the major volume of the alveolus (b, Fig. 114). These cells are packed with granules of variable size, some of which are the largest granules in the whole alveolus.

Semithin sections stained with Azure II show that these granules stain shades of pink, violet and blue. In paraffin sections, these granules stain shades of red, orange, violet and blue with Mallory's triple stain and shades of blue with Giemsa. The cytoplasm mostly stains faint blue with both

Mallory and Giemsa. As in type a cells, the nuclei are usually masked by the overlapping granules.

Histochemically (Table 4, Fig. 112), the granules of type b cells vary from negative to moderately positive for polysaccharides and basic proteins with PAS and mercuric bromophenol blue, respectively. The intensity of PAS reaction is unaffected by diastase enzyme indicating the absence of glycogen. However, some bright red fine particles were observed in the cytoplasm after the PAS reaction and these disappeared after treatment with diastase enzyme. These fine particles stained red with Best's Carmine, indicating glycogen in the cytoplasm. Type b cell granules stain shades of yellowish-orange with Millon's reagent while the reaction is negative for tryptophan with DMAB-nitrite. They stain orthochromatically with toluidine blue and thionin, indicating the absence of acid mucopolysaccharides which is confirmed by the negative reaction with alcian blue and dialized iron. Only the largest granules of type b cells react strongly positive for lipids (Fig. 115) while the other granules vary from a negative to a positive reaction. Acid and alkaline phosphatases are undetectable.

Electron micrographs show that type b cells are packed with uniformly electron-dense mature granules which vary from 3

to 13 Um in size. Their limiting membrane is normally of the same density as the granule to which it is closely applied (see Fig. 124). A dense, fine textured material appears in some granules (Fig. 123). The cytoplasm contains many well developed Golgi bodies (Gb. Fig. 122) with curved parallel arrays. Material similar in density to the mature granules was found in the flat cisternae of the Golgi bodies as well as in the small condensing vacuoles (Cv. Fig. 122). Apparently, the mechanism of the granular formation is similar to subtype b_2 cells of feeding females of H. leachii on the third day after attachment. Small vesicles (arrows Fig. 122) are found in the Golgi regions. They arise from areas of rough endoplasmic reticulum which surround the Golgi bodies. The cytoplasm also contains rounded or oval mitochondria (up to 1.3 Um diameter) with parallel cristae and numerous free ribosomes. Nuclei are rounded (Ca. 6 Um diameter) and are found in the middle of the cells. The apical surface of type b cells lack microvilli

Ultrastructural histochemical micrographs show that the granules of type b cells and their limiting membrane, as well as the Golgi bodies and the condensing vacuoles, are polysaccharide positive with PA-TCH (Fig. 124, 125). These granules are unaffected by pronase enzyme (resistance probably due to bound proteins). Acid phosphatase is undetectable.

iii. Type c cells:

These are one or two small cells (C. Fig. 114) possessing the smallest granules in the whole alveolus. Type c cells are usually found in the fundus of the alveolus between the large a and b cells (Fig. 112). In Epon-embedded sections, the granules stain blue with Azure II, while they stain shades of blue with Mallory and pink with Giemsa in paraffin sections.

Histochemically (Table 4, Fig. 112), the granules of these cells react faintly positive for polysaccharides with PAS and moderately positive for basic proteins with bromophenol blue while the cytoplasm is faintly positive for basic proteins. There was no reduction in the intensity of PAS staining after diastase enzyme, indicating that the PAS staining is not due to glycogen. Best's carmine test also gave negative results. These cells do not show metachromasia with toluidine blue indicating the absence of acid mucopolysaccharides.

The reaction was moderately positive for tryptophan, while it was negative for tyrosine, -SS-groups, arginine and amino groups. There was no staining with sudan black.

Ultrastructurally, type c cells are packed with rounded, oval or irregularly shaped small granules (up to 1.5 µm size).

Each granule possesses two different dense zones, one of them is completely electron-dense while the other is less dense (G. Fig. 126). The size of each zone is different from one granule to another according to the angle of section.

Golgi bodies are found in a well developed compact ellipsoidal form with continuous, multi-layered cisternae (Gb. Fig. 126) and associated with small condensing vacuoles (Cv. Fig. 126). Golgi regions are surrounded by slightly distended cisternae of rough endoplasmic reticulum (RER. Fig. 126), with moderately dense intercisternal matrix. which appears as isolated cisternae in the plane of the section. The cytoplasm also contains numerous free ribosomes, few dense bodies and some scattered rounded or oval mitochondria (Ca. 0.4 µm diameter) with parallel cisternae. Nuclei are small (Ca. 1.8 µm size), oval or irregularly shaped, and lie in the middle of the cell.

Granule formation is apparently associated with Golgi regions and their adjacent distended cisternae of rough endoplasmic reticulum.

Ultrastructural histochemical reactions show that the dense zones of the granules are moderately positive for polysaccharides with PA-TCH while the low dense zones are faintly positive. For proteins, the dark zones are partially digested with the pronase enzyme while the low dense zones are unaffected. As in types a and b cells, acid phosphatase is undetectable.

iv. Type d cells: (The cap cells)

As in unfed H. leachii adults, these cells cannot be distinguished at light microscopy level.

Ultrastructurally (d. Fig. 116), they are identical in position and in cytological structure to types c, h and m cells of types II, III and IV alveoli of the unfed H. leachii adult described before. Type d cells extend to the base of the alveolar duct to touch the valve-like structures as well as to surround the valvular canal (arrows, Fig. 116).

v. Type e cells: (The interstitial epithelial cells)

As in unfed H. leachii, this type of cell is difficult to be recognised at light microscopic level.

The fine structure of type e cells (Fig. 116, 126) is similar to types d, i and n cells of types II, III and IV alveoli described before for unfed adults of H. leachii.

B. FEEDING ADULTS AND OVIPOSITING FEMALES

MORPHOLOGY:

The salivary glands of O. moubata adults do not show any increase in size during feeding or in the fully fed and post-fed male and female ticks, or in ovipositing females.

SALIVARY DUCTS

No appreciable changes were observed in the structure of the main, secondary and lobular ducts during and after male and female feeding periods, as well as during female oviposition. Occasionally, at 15 minutes after female attachment, the electron micrographs show that some irregularly shaped membrane-like structures are found in the lumen of the female secondary salivary ducts (arrows, Fig. 128).

SALIVARY ALVEOLI

There is no apparent change in the size of both types of salivary alveoli during and after male and female feeding periods, or in ovipositing females.

A. NONGRANULAR-SECRETING ALVEOLI

TYPE I ALVEOLI

Semithin and paraffin sections of type I alveoli do not show any appreciable changes in the structure of these alveoli except that there is an increase in the basophilia of their

cytoplasm during engorgement.

Histochemically, no changes in the intensity of the reactions were observed in this type of alveoli except that there is an appreciable increase in the intensity of RNA in the cytoplasm with methyl green/pyronin Y.

Ultrastructurally, no marked changes occured in the fine structure of type I alveoli at 5, 10 and 15 minutes after male and female attachment. At 20 minutes after attachment, the inclusions of the non membrane-bound vacuoles (as those described for type I alveoli of unfed H. leachii adult) found inbetween the basal membrane infoldings are absent, but are shown again at 2 hours after dropping off from the host.

After the adult attachment and up to the oviposition period of the female, the cytoplasm possesses a few scattered lysosome-like structures (Ca. 1 µm diameter) but without the vacuoles found in female H. leachii on the third day after attachment. Mitochondria are normal but a few of them contain some small dense granules similar to those found in the mitochondrial matrix of type I alveoli on the third day after H. leachii female attachment. Neither osmiophilic aggregates nor small or large membrane-bound vacuoles (which appeared on the fifth day after H. leachii female attachment) were found in the cytoplasm during or after male and female engorgement and in the ovipositing female. Numerous alveoli of type I

remained in the same condition as that found in unfed ticks.

Ultrastructural histochemical micrographs of type I alveoli show that the reaction results for polysaccharides, proteins and acid phosphatase at 2 hours after male and female detachment remain the same as those of unfed ticks.

GRANULE-SECRETING ALVEOLI

TYPE II ALVEOLI:

The effect of the bloodmeal on the granule-secreting alveoli of adult O. moubata is not so pronounced as that found in fed H. leachii females. There is no appreciable increase in the size of type II alveoli during feeding.

Epon-embedded sections do not show any marked change in the structure of these alveoli at 5, 10 and 15 minutes after male and female attachment. At 20 minutes after attachment and up to two hours after detachment, some of type II alveoli possess a highly distended alveolar lumen. The alveolar cells become smaller in size, particularly types a, b and c cells (Fig. 130). Other alveoli do not show such changes and remain in the condition found in unfed ticks.

Ultrastructurally, no changes were observed in the fine structure of the epithelial cells surrounding the alveolar duct during or after male and female engorgement. Occasionally, during feeding, an electron-dense discharged coagulated suc-

retory mass is observed streaming from the alveolar lumen into the alveolar duct and passing through the narrow valvular canal (arrow, Fig. 129).

The basement membrane of type II alveoli of O. moubata salivary glands do not show any structural change similar to that found in female H. leachii during and after feeding.

Numerous haemocytes, similar to those described for fed H. leachii female and unfed O. moubata, were observed during and after engorgement.

i. Type a cells:

Epon-embedded sections of these cells show no increase in the cell size or changes in the appearance of the secretory granules.

At 20 minutes after attachment and up to 2 hours after detachment, some type a cells have decreased slightly in size, as well as in the number of their secretory granules and there is an appreciable increase in the basophilia of their cytoplasm. Some vacuoles of variable size are found in their cytoplasm, especially in the large a cells. Semithin sections stained with Azure II and paraffin sections stained with Mallory and Giemsa show that the appearance and the intensity of the staining of the granules remain the same as those of unfed ticks, even up to female oviposition.

Histochemical tests carried out at 2 hours after male and female detachment show that the reaction results are the same as those of unfed ticks except that there is an appreciable increase in the intensity of RNA staining in the cytoplasm.

Electron micrographs of salivary glands at 5, 10 and 15 minutes after attachment show that the middle cores of most secretory granules of type a cells appear granulated.

At 20 minutes after attachment and up to 2 hours after detachment, some a cells show a great reduction in size and in the number of their secretory granules (Fig. 130). Others contain electron-lucent membrane-bound vacuoles of variable size, while a few of these vacuoles contain an osmiophilic aggregate material or membrane-like structures. The apical plasma cell membrane may project some short microvilli into the alveolar lumen.

The exact mechanism of discharging the granular content of type a cells has not been observed except that at 15 minutes after male and female attachment, a small number of ungranulated cores appear to be very close to the cell membrane.

No other changes in fine structure were observed in type a cells up to female oviposition.

Ultrastructural histochemical micrographs of 2 hours after tick detachment show the same reaction results for polysaccharides, protoins and acid phosphatase as those of unfed ticks.

ii. Type b cells:

These cells behave similarly to type a cells before and after attachment except that the cell size and the number of the granules are greatly reduced, particularly at two hours after dropping off from the host. There is a great increase in the basophilia of the cytoplasm.

As in type a cells, no changes were observed in the histochemical reactions of type b cells at 2 hours after detachment except that the cytoplasm stained intensively for RNA with methyl green/pyronin.

The fine micrographs of 5, 10 and 15 minutes after attachment show that the cytoplasm of some b cells appears granulated (white arrow, Fig. 131), while other cells contain membrane-bound vacuoles of low density and of different size (Va. Fig. 131). The nuclei are found in the basal regions of the cells (N. Fig. 131).

At 20 minutes after attachment and up to 2 hours after detachment, as well as in ovipositing females, type b cells, in some alveoli, appear flattened (Fig. 132). This is due to the great reduction in their secretory granules and the great distension of the alveolar lumen (Lu. Fig. 131). In some other alveoli, the b cells do not show great morphological change except that their cytoplasm contains a few rounded membrane-bound vacuoles with osmiophilic aggregates

which accumulate in the middle of these vacuoles (arrows, Fig. 133). The latter probably represent a degradation granular phase.

As in type a cells, the ultrastructural histochemical reactions of type b cells at two hours after detachment remain the same as those of the unfed adult.

iii. Type c cells:

As type a and b cells, the semithin and paraffin sections of these cells do not show any difference in their structure before or after attachment except that there is a slight reduction in the cell size and the number of their secretory granules.

No changes were observed in their histochemical reactions at 2 hours after detachment.

Ultrastructurally, these cells do not show great morphological change during and after feeding, even during female oviposition, except that a few of them are slightly reduced in size and have lost some of their secretory granules. Their cytoplasm contains low dense areas and a few secretory granules. Their nuclei (Ca. 2.4 µm diameter) occupy the most basal region of the cell.

As in types a and b cells, the ultrastructural histochemical reactions of type c cells at 2 hours after detachment are

the same as those of unfed ticks.

iv. Type d cells: (The cap cells)

These cells cannot be distinguished at light microscopy level.

In ultrastructure, these cells do not show any appreciable changes either in position or in structure during or after feeding except that some lysosome-like structures were present in the cytoplasm at 20 minutes after attachment.

v. Type e cells: (The interstitial epithelial cells)

These cells,as type d,still cannot be recognised at light microscopy level.

Electron micrographs of these cells do not show any appreciable change in their structure during or after tick engorgement except that in some areas of the cell, particularly those lying in the basal region of the alveoli, show a few branched canaliculi similar to those seen in fed H. leachii female (Fig. 132).

DISCUSSION

THE SALIVARY GLANDS IN ADULT H. LEACHII AND O. MOUBATA

MORPHOLOGY:

The salivary glands of unfed H. leachii and O. moubata adults are similar in both sexes. Those of H. leachii follow the general pattern found in other ixodid ticks in having a pair of loose glandular organs and their main salivary ducts divide into two subsidiary branches (Vitzthum, 1943; Till, 1961; Balashov, 1965, 1968; Chinery, 1965; Binnington, 1978). In contrast, those of O. moubata follow the general pattern found in other argasids in having a pair of compact glandular organs and their main ducts run undivided to the posterior end of the gland (Christophers, 1906; Robinson and Davidson, 1913; True, 1932; Vitzthum, 1943; Roshdy, 1961, 1962, 1963, 1966, 1972; Balashov, 1968; Sonenshine and Gregson, 1970; Guirgis, 1971; Chinery, 1974).

SALIVARY DUCTS

The main salivary duct is structurally similar in both H. leachii and O. moubata except that in H. leachii the duct lumen is narrower, the inner cuticular layer is more folded and the middle cuticular layer is thicker than those of O. moubata (compare Fig. 5 with Fig. 113).

These differences are probably due to the longer feeding periods, the larger quantity of secreted saliva and the larger amount of the bloodmeal taken in by H. leachii compared to O. moubata, a common and normal difference between feeding in ixodids and argasids (Balashov, 1968).

Sonenshine and Gregson (1970) observed that the salivary duct of O. Kelleyi and its secondary branches were formed of a thin squamous epithelium and a cuticular lining supported by regular spiral thickenings. This observation is most probably due to the epithelial layer of the main salivary duct containing nuclei at different levels. Squamous epithelium in the epithelial layer of the main salivary duct has never been described in any other tick so far studied.

The spiral thickenings (i.e., the outer cuticular region) in both H. leachii and O. moubata salivary ducts have also been observed in other tick species. Nordenskiold (1905) stated that these thickenings in Ixodes ricinus (=reduvius) may function as a regulator for the salivary duct. Robinson and Davidson (1913) mentioned that these thickenings in A. (P). persicus serve to maintain the potency of the ducts. In O. moubata they may aid in dilatation and collapse due to pharyngeal movements, and in turn may assist the flow of saliva (Bertram, 1939).

The fine structure of unfed H. leachii and O. moubata adult main salivary ducts is quite similar to that of Dermacentor variabilis male (Coons and Roshdy, 1973) and A. (P). arboreus (Roshdy and Coons, 1975), respectively.

On the third day after female attachment in H. leachii, the epithelial cells of the main salivary duct were slightly enlarged and their basal membrane became infolded, increasing the cell surface area. Their cytoplasm was less dense, and some mitochondria, particularly those lying near the basal membrane, became more elongated with dark granules in their matrix. The basement membrane surrounding the main salivary duct thickens and extends between the basal membrane infoldings of the epithelial cells (see large arrows Fig. 56). These features suggest that the epithelial duct layer may participate in some sort of an active transport across the basement membrane and supports Meredith and Kaufman's opinion (1973) that reabsorbtion of solute in D. andersoni might occur in the duct system.

The salivary glands of the female of H. leachii reach their maximum size on the final stages of feeding periods. The cuticular lining then appears thinner, fenestrated and collapsed (see Fig. 57) due to the extreme expansion of the main salivary duct. In addition, the cytoplasm of the epithelial duct cells contains pycnotic nuclei, many lysosome-

like structures and osmiophilic aggregates. These changes in the structure of the salivary duct are signs of a broken down tissue which was followed by complete degeneration during or after oviposition. In contrast, the male of H. leachii, as well as O. moubata adults, have not shown similar degeneration of tissue in the ducts during or after feeding. They neither secrete as much saliva nor imbibe such large quantities of blood as the female of H. leachii does during feeding. Furthermore, O. moubata adults, as in other argasid ticks, feed several times during their lifetime.

The ultrastructural histochemical techniques in the present work have provided new information about the composition of the salivary ducts.

Collagen-like fibres (Fig. 15) have been detected in the outer zone of the middle cuticular region of the main salivary duct of unfed H. leachii adult after treatment with pronase enzyme. The presence of these fibres suggests that this region gives the cuticular lining support and elasticity, particularly when the salivary ducts increase in diameter in the final stages of female feeding. In contrast, the salivary ducts of unfed O. moubata adults lack this structure.

SALIVARY ALVEOLI:

The presence of four similar types of alveoli in the sali-

TABLE 5: CELL TYPES AND NOMENCLATURE FROM RECENT STUDIES OF IXODID SALIVARY GLANDS

	SPECIES & AUTHORS	TYPE I ALVEOLI	TYPE II ALVEOLI F	TYPE II ALVEOLI M	TYPE III ALVEOLI F	TYPE III ALVEOLI M	TYPE IV ALVEOLI F	TYPE IV ALVEOLI M	NOTES
LM.									
1.	R. appendiculatus (Till, 1961)	I	a,b	a,b,f	c,d,e	c,d,e	-	g	
2.	H. spinigera (Chinery, 1965)	I	a,b	a,b,f	c,d,e	c,d,e	-	g,h	
3.	I. ricinus (Balashov, 1968)	Pyramidal alveoli	a,b	a,b					a=orifice cell
	H. asiaticum		a,b,c	a,b,c	d,e	d,e			b=fundus cell
4.	B. microplus (Binnington, 1978)	I	a,b,c_1-c_4 ep.cells	a,b,c_1-c_4 ep.cells	d,e,f	d,e,f			ep.cells= epithelial cells
EM									
1.	H. leporipalustris nymph (Kirkland, 1971)	I	A & B						A→A_1 after feeding B→B+C " "
2.	D. variabilis male (Coons & Roshdy, 1973)	I		a,b,c_1-c_4 ep.cells		d,e,f ep. cells			
3.	D. andersoni female (Meredith & Kaufman, 1973)	I	EG,NG,VC, CP,WC		EG,NG, VC,CP, WC				EG=encapsulated-granule cells, NG=naked-granule cells VC=vacuolar cells, CP=cap cells WC=water cells

4. *B. microplus* (Megaw, 1976)	I	a,b,c ep.cells	a,b,c ep.cells	d,e, f,g	d,e,f ep.cells			e→vacuolar cells after ♀ attachment in alveolus III ep.cells=f + g after attachment in alveolus III
5. *H. leachii* (This Study)	I	a,b,c,d	a,b,c,d	e,f, g,h, i	e,f,g,h, i	j,k, l,m, n	j,k, l,m, n	a=e=j c=h=m=cap cells d=i=n=interstitial epithelial cells b→b_1+b_2 after adult attachment

Note: Type IV alveoli of *H. leachii* = Type III alveoli of the other species

F = Female M = Male

vary glands of both sexes of unfed adult H. leachii has not been reported before in other ixodid ticks (Table 5). Under light microscopy, three types of alveoli have been described in the females and four in the males of Rhipicephalus appendiculatus (Till, 1959, 1961), Haemaphysalis spinigera (Chinery, 1965), Hyalomma asiaticum (Balashov, 1968) and Boophilus microplus (Binnington, 1978). Balashov (1968) described two alveoli only for Ixodes ricinus.

Under electron microscopy, two types of alveoli were distinguished in the nymphal stage of Haemaphysalis leporispalustris (Kirkland, 1971) while three types of alveoli were reported in Dermacentor variabilis male (Coons and Roshdy, 1973) and Dermacentor andersoni female (Meredith and Kaufman, 1973). Megaw (1976) was able to differentiate three types of alveoli in both sexes of Boophilus microplus adult.

The presence of two different alveoli in both sexes of O. moubata is common among the argasid ticks (Christophers, 1906; Robinson and Davidson, 1913; True, 1932; Vitzthum, 1943; Dzhafarov, 1965a, b; Balashov, 1968; Sonenshine and Gregson, 1970; Guirgis, 1971; Roshdy, 1972; Chinery, 1974; Roshdy and Coons, 1975). However, as in other argasids, there are fewer alveoli in the salivary glands of O. moubata than in H. leachii, or other ixodids studied so far.

The interrupted area (Figs. 16, 22) found at the junction of the lobular and alveolar ducts in the nongranular and granule-secreting alveoli of both unfed H. leachii and O. moubata adult has been reported only for the granule-secreting alveoli of Dermacentor variabilis male (Coons and Roshdy, 1973). Apparently, this area provides flexibility. In addition, the valve-like structure at the end of the alveolar duct base in the granule-secreting alveoli of unfed H. leachii and O. moubata adults has been observed in other tick species by Nordenskiold (1905), Robinson and Davidson (1913), Till (1961), Chinery (1965), Guirgis (1971), and Coons and Roshdy (1973). Both Till and Chinery (loc. cit.) suggested that this valve prevents the back flow of saliva during pharyngeal dilatation. Furthermore, the arm-like structure extended from the end of the alveolar duct of the granule-secreting alveoli of O. moubata adult has been reported before only in Argas (Persicargas) arboreus (Roshdy and Coons, 1975). This cuticular arm may act as a skeletal support for the alveolus as a whole, particularly as the granule-secreting cells in each alveolus are always packed with secretory granules (i.e. in unfed and fed ticks).

I. NONGRANULAR-SECRETING ALVEOLI:

TYPE I ALVEOLI

The nongranular alveoli (Fig. 6) appear to be identical in both H. leachii and O. moubata and to those described in other argasid and ixodid ticks. These alveoli have been identified under the light microscope as "glandes venimeuses" by Bonnet (1906) on account of their structural resemblance to the venom secretory cells in the poison glands of certain arthropods and snakes. Samson (1909) recognised them as "Pyramidenzellen" in I. ricinus. Both authors described these alveoli as unicellular but the number of their nuclei indicates their multicellular nature. Dzhafarov (1965a) and Balashov (1968) called them "Pyramidal alveoli".

The fine structure of the nongranular alveoli (Figs. 16, 17, 18, 20) is remarkably similar in the unfed H. leachii and O. moubata adult and to those of the unfed D. variabilis male (Coons and Roshdy, 1973), A. (P.) arboreus adult (Roshdy and Coons, 1975) and the partially fed D. andersoni female (Meredith and Kaufman, 1973). The latter authors described these alveoli as composed of only two fibrillar cells surrounding a lightly stained internal cell whereas in H. leachii and O. moubata the alveoli are composed of three to four cells with infolded basal membranes surrounding a large central cell.

The basal membrane infoldings (Fig. 18) in the nongranular secreting alveoli are common ultrastructural features of the salivary glands of both argasid and ixodid ticks. They are similar to those of the epithelial plasma membranes of transporting cell tissues (Fawcett, 1962; Diamond and Tormey, 1966a, b); Diamond and Bozzert, 1967, 1968; Berridge and Oschman, 1972). They are also similar to the osmoregulatory salt cells of birds (Komnick, 1963; Balashov, 1968) and reptiles (van Lennep and Komnick, 1970); the malpighian tubules of insects (Berridge and Oschman, 1969) and mites (Coons and Axtell, 1971); coxal glands of arachnids (Rasmont, 1960; Hecker et al., 1969; Groepler, 1969); salivary glands of insects (Kessel and Beams, 1963; Kendall, 1969; Oschman and Berridge, 1970).

Gregson (1967), Roshdy (1972) and Coons and Roshdy (1973) mentioned that the salivary glands of ticks possibly have an osmoregulatory function and fluid transport activity. According to Lees (1946, 1947), female Ixodes ricinus, near the end of feeding, lost up to two thirds of the water and salts from the host blood. High succinic dehydrogenase activity was revealed in the pyramidal alveoli of O. moubata (Dzhafarov, 1965a). This enzyme participates in sodium transport in the avian salt cells (Natochin, 1962). Tatchell (1967a, 1969c) has proved experimentally that the feeding

female Boophilus microplus eliminates with the saliva much excess sodium chloride and up to 60-70% of the host blood water ingested during feeding. Tatchell's results have been supported by Kaufman and Philips (1973a, b, c) after working with Dermacentor andersoni female. Ixodids lack the specialized osmoregulatory coxal organs found in argasid ticks and the role of the malpighian tubules in eliminating excess water is negligible (Balashov, 1968).

Type I alveoli of both H. leachii and O. moubata adults are similar in position, structure and histochemical reactions to those of other ixodid and argasid ticks. In this respect, they probably play a role in the osmoregulatory processes as found in B. microplus and D. andersoni. In addition, the distended mitochondria with dark granules in their mitochondrial matrix that were observed on the third day after attachment of H. leachii females, have been described in tissues transporting large amounts of ions or water (Fawcett, 1966).

Transfer of water requires energy consumption (De Robertis et al., 1975). Numerous mitochondria have been detected within the nongranular alveoli cells and in close contact with the cytoplasmic membrane infolding. Apparently they provide this process with essential energy.

Lipid was detected in the paraffin sections of these alveoli. The fine micrographs also show many nonmembrane-bound vacuoles (Va. Fig. 18) lying between the infoldings, and contain moderately electron-dense secretion. These vacuoles and their content are structurally similar to lipid inclusions which have been described in various cells and tissues (Tokin, 1961; Polikar and Bo, 1962; Palade, 1962). During feeding of both H. leachii and O. moubata adult, the content of these vacuoles was absent and was shown again only in the nongranular alveoli of O. moubata adult at two hours after dropping off from the host, while the vacuoles remain empty in adult H. leachii, even during the female oviposition period. The vacuoles are renewed in adults of O. moubata which, as in other argasids, feed several times during their lifetime, but not in H. leachii adults, which as in other ixodids, feed only once in a lifetime. Palade (1962) suggested that the close contact between lipid inclusions and mitochondria represented oxidation of fat reserve in order to provide the cells with necessary energy. The lipid inclusions within the nongranular-secreting alveoli cells of H. leachii and O. moubata salivary glands may be used for the same purposes. This is very probable since direct association between mitochondria and lipid inclusions was detected in some regions of the cells. The origin of these lipid inclusions is not known.

During the final stages of feeding females of H. leachii, the parallel orientation of the fibrillar cytoplasm of the nongranular alveoli found in the unfed ticks has been lost. The ground cytoplasm possesses osmiophilic aggregates, small and large membrane-bound vacuoles, numerous lysosome-like structures and pycnotic nuclei. These changes in the internal substructure of type I alveoli are signs of breakdown which increase after the female detachment, and are then followed by complete degeneration during or after oviposition.

In the male of H. leachii and O. moubata adults, type I alveoli have not shown these changes in substructure during or after feeding. They do not imbibe such a large amount of blood as in the female of H. leachii during attachment. Hence, the active transport processes in their nongranular alveoli are not so great as those of feeding females. In addition, the coxal glands are the main osmoregulatory organs in the argasid ticks (Balashov, 1968), which secrete much less saliva than ixodids during feeding (Kirkland, 1971); thus the O. moubata salivary glands have less osmoregulatory significance.

The large central cell (CC. Figs. 16, 17) found in type I alveoli of H. leachii and O. moubata adults communicates with the nonvalvular alveolar duct and may collect the fluids from the adjacent cells and pass them into the duct lumen. However, other cells in the nongranular alveoli may also communicate

with the duct, thus obviating the need for indirect fluid passage through the large central cell.

II GRANULE-SECRETING ALVEOLI

The presence of these granule-secreting alveoli containing different secretory and nonsecreting cell types in the salivary glands of both sexes of H. leachii, has been reported before only in the male of some ixodid tick species (Table 5). In addition, the occurrence of four different types of cell in type II alveolus has been reported in the salivary glands of unfed Dermacentor variabilis male (Coons and Roshdy, 1973) and unfed Boophilus microplus adult (Megaw, 1976; Binnington, 1978); while five types of cell were reported in alveolus II of the unfed female of Dermacentor andersoni (Meredith and Kaufman, 1973). However, the presence of five different types of cell in types III and IV alveoli has been reported only for the D. andersoni female (Meredith and Kaufman, loc. cit) (Table 5).

In order to avoid confusion between the different cells of the granule-secreting alveoli of H. leachii and O. moubata during discussion, the granule-secreting cells of each species will be discussed separately (due to their difference in structure and function from one species to another). The similar nongranular secretory cells (e.g. the cap and interstitial epithelial cells) of both species will be discussed together.

Moreover, the similar granule-secreting cells of H. leachii (e.g. types a, e and j cells as well as types g and l cells) will be discussed together. Furthermore, the comparison of the structure and function of the granule-secreting cells between the two species will be given in a general discussion.

1. GRANULE-SECRETING CELLS:

A. H. Leachii (Types a, e and j cells)

Under light microscopy, types a, e and j cells of unfed H. leachii are similar in position and structure to each other and to types a and c cells of unfed Haemaphysalis spinigera (Chinery, 1965), types a and d cells of the unfed adults of both Hyalomma asiaticum (Balashov, 1968) and Boophilus microplus (Binnington, 1978). The secretory granules of types a, e and j cells of unfed H. leachii adults react positively for carbohydrates and contain protein rich in tryptophan, confirming the histochemical findings of the previous authors.

Untrastructurally, the a, e and j cell membrane-bound granules are composed of variable dense membrane-bound subunits. The latter are embedded in a lattice matrix (Fig. 24) consisting of dark fine membranes which form pentagonal or hexagonal electron-lucent vacuoles or are isolated in the cytoplasm. Some of these vacuoles are more dense than the rest (arrows, Fig. 24), and probably possess the primary material of the subunits and may represent an earlier stage of the subunit

growth. Balashov (1968) described large vacuoles in type a and d cells of H. asiaticum divided into two types, "simple vacuoles" filled with highly electron-dense homogeneous substance and "complex vacuoles" formed of a mass of much smaller vacuoles, which are surrounded by a limiting membrane. Balashov's "simple vacuoles" are similar to the subunits found isolated in the cytoplasm of types a, e and j cells of H. leachii. Kirkland (1971) reported composite granules composed of electron-lucent subunits in type A cells of Haemaphysalis leporispalustris nymph. Coons and Roshdy (1973) described granules containing electron-dense subunits in types a and d cells of unfed Dermacentor variabilis male. The last authors stated that these membrane-bound subunits probably originated from smooth membranous elements in the cytoplasm which aggregate to outline the subunits before electron-dense material is secreted. It is quite possible that the a, e and j cell subunit granules in H. leachii have been originated as suggested by Coons and Roshdy. The fine structural difference between granules noted by Balashov, Kirkland, Coons and Roshdy, and in H. leachii described here, may result from technical differences in electron microscopy or to different secretory properties of each species or of developmental stages of a single species.

Histochemical fine micrographs of types a, e and j cells of

the unfed H. leachii adult showed that the lattice matrix and the subunit granules are polysaccharide positive (which also reacted positively with PAS under light microscopy) (Fig. 26). After treatment with pronase enzyme, the subunit granules have been completely digested (see Fig. 27) indicating a protein material (which reacted positively with bromopehnol blue and was rich in tryptophan under light microscopy). The polysaccharide positive reaction of the subunits, and their complete digestion after treatment with the enzyme, suggest that they are protein in nature with traces of polysaccharides.

Types a, e and j cells were reduced in size and lost most of their secretory granules starting on the first day after female attachment. The same findings were also reported for other ixodid females during feeding (Till, 1961; Chinery, 1965; Balashov, 1968; Kirkland, 1971; Megaw, 1976; Binnington, 1978). On the other hand, type A granules in type II alveoli of Haemaphysalis leporispalustris (Kirkland, 1971) were found to have been replaced by second A_1 granules during female feeding showing structural change during the feeding period.

Three membranes were involved in the mechanism of releasing the granular content of types a, e and j cells (see Fig. 65). The two limiting membranes of the subunit and the main granules fuse first with each other, and ultimately with the

plasma membrane. This mechanism of exocytosis has not been reported before. Some isolated subunits released their inclusions after the fusion of their limiting membranes with the plasma membrane without loss of cytoplasmic content from cell (i.e. eccrine secretion) (Toner and Carr, 1971). Other subunits can be released by pinching off into the alveolar lumen as an individual cytoplasmic bounded subunit (see Fig. 66) (i.e. macroapocrine secretion) (Kurosumi, 1961). The surrounding cytoplasm probably breaks down in the alveolar lumen to release the subunit inclusion but, although several morphologically distinct membrane-encompassed bodies have been found in the lumen of the alveolus, particularly in the final stages of feeding, their origin and relationships to one another are unknown.

Spheroidal moderately dense inclusions were found in the middle of the nucleus (Fig. 67) on the third day after female attachment. Weber et al. (1964) recognised intranuclear inclusions in parynchymal cells of the bovine adrenal cortex with a central mass of dense particulate matter. In addition, Jones and Fawcett (1966) have observed similar bodies in the nuclei of pancreatic acinar cells, plasma cells and various other cell types. These authors concluded that these nuclear inclusions may prove to be a nuclear structure of very widespread occurrence, but their precise nature and significance are quite obscure. Bird (Pers. Com.) reported that these inclusions

can be expected in cells which secrete extensive amounts of lipids and proteins, and as revealed under light microscopy, the cytoplasm of these cells is lipid positive, while the granular subunits contain pure protein as revealed under the electron microscopy.

Contrary to the observations in the female of H. leachii, types a, e and j cells of the male have not shown any appreciable changes during or after feeding, confirming the observations of Chinery (1965) and Binnington (1978). On the other hand, Till (1961) and Balashov (1968) suggested that these cells behave during feeding as those of the females. Binnington (loc. cit) mentioned that the difference in the structure between the salivary glands of the male and the female of Boophilus microplus during feeding is due to the behavioural differences between the sexes i.e. the male remains on the host for up to 70 days (Hitchcock, 1955) during which time it copulates up to six times (B.F. Stone, from Binnington, 1978).

Type b cells:

In unfed H. leachii adult, type b cell inclusion are composed of mainly carbohydrate material, containing few proteins, and show a positive reaction for acid mucopolysaccharides. They react somewhat similarly to type b cells of Haemaphysalis spinigera female (Chinery, 1965), Hyalomma asiaticum adult (Balashov, 1968) and unfed Dermacentor variabilis male

(Coons and Roshdy, 1973).

After attachment, type b cells in the female increase greatly in size. As a result, type II alveoli became the largest in the salivary glands and this is found also in other ixodid female ticks.

The characteristic fine structure of the b cells (Fig. 28) of unfed H. leachii is similar to that of Dermacentor variabilis male (Coons and Roshdy, 1973), except that the secretory granules of these cells in H. leachii do not have the amorphous structure and the electron-lucent areas, as well as the alternating light and dark concentric layers, found in D. variabilis granules.

On the first day after female attachment, some small vesicles were observed in the apical region underlying the plasma membrane of type b cells (arrow heads, Fig. 70). De Robertis et al. (1975) suggested that patches of membranes can be invaginated from the surface as small vesicles that move back into the Golgi region, to be re-utilized in the packing of more secretion. He has supported his hypothesis by the finding of an increase in the number and size of Golgi region components after intense in vitro stimulation of the vertebrate pancrease by carbamylcholine. Large numbers of different types of membrane-bound granules were synthesised by type b cells especially in its subtype b_2 cells (i.e. on the

third, fifth and seventh day after attachment) (Figs. 71, 76a, 79) and Golgi bodies were involved in their packing as described during the attachment of H. leachii female.

Successive types of granules have been secreted by sub-type b_2 cells during the female feeding period. The moderately dense granules with filamentous material (fm. Fig. 72) were observed on the third day, the dark granules on the fifth day, and the low dense vacuoles on the seventh day after attachment. They were different in density from each other, although they have been synthesised by the association of Golgi bodies and the rough endoplasmic reticulum.

The mechanism of secretion in vertebrate pancreas has been demonstrated by Palade (1975). The newly synthesised proteins penetrate into the cisternal cavity of the endoplasmic reticulum, from the polyribosomes present on its surface, to reach the Golgi zone, probably by way of continuities with the endoplasmic reticulum, which may be permanent or, more probably, transient. The protein is first diluted and fills large vacuoles of the Golgi complex. Then it is progressively concentrated, forming zymogen granules surrounded by a Golgi membrane. By progressive condensation, the granules migrate into the apical region of the cell where they will be delivered by exocytosis (i.e. eccrine secretion) accompanied by fusion of the limiting membrane of the granule with the apical cell

membrane. The secretory granules with the filamentous material which were observed on the third day after female attachment, were possibly synthesised (Figs. 74, 75B) and released their content in the same way as in the vertebrate pancreatic cell, leaving large empty vacuoles underlying the apical plasma membrane (Figs. 73, 75A). These empty vacuoles were due to the fusion of the limiting membrane of a granule with a small portion of the plasma membrane through which the granular content were discharged. The membrane of a second granule may fuse with one whose membrane was already continuous with the plasmalemma and a third may join this. Thus, in rapid release of secretion, chains of three or more granules may communicate with one another and ultimately with the alveolar lumen, forming a flask-shaped concavity at the cell apex (Figs. 73, 75A). The fate of the filamentous material of these granules has not been followed in the lumen of the alveolar and salivary ducts.

Information is available about energy requirements of the various steps in the secretion and discharge of zymogen granules in vertebrate cells producing protein. Redman (1967) has shown that the transport of the newly synthesised polypeptide chain from the ribosomes into the cisternae of rough endoplasmic reticulum does not require additional energy, and seems to be controlled mainly by the structural relationship of the

large ribosomal subunit with the membrane of the reticulum. Jamieson and Palade (1968a) showed that the intracellular transport of synthesised protein was not dependent on further synthesis. Jamieson and Palade (1968b) also showed that transport from the endoplasmic reticulum to the condensing vacuoles was dependent on cell respiration (but not glycolysis). Schramm (1967) showed that zymogen release in the pancreatic cell (which occurs by eccrine secretion) is also dependent on cellular respiration, but not glycolysis. In vertebrate pancreas, the cytological appearance in the opaque cells of an energy source immediately before the production of a new batch of zymogen granules is evidence for the possible presence in the opaque zone cells of the energy dependent lock, between the rough endoplasmic reticulum and the Golgi, as shown in the exocrine cells of the vertebrate pancrease by Jamieson and Palade (1968b). However, glycogen has not been detected in the sub-type b_2 cells of feeding female of H. leachii. Thus, it is possible that the eccrine release of the synthesised material is dependent on cellular respiration in H. leachii, but not glycolysis, as is found in the vertebrate pancreas (Schramm, 1967). De Robertis et al. (1975) stated that part of the energy required for discharging the zymogen granules is related to the process of membrane fusion-fission taking place during exocytosis, but it is possible that energy could be consumed in the propulsion

of the granule to the cell apex.

On the fifth day after attachment, acid phosphatase was detected in the condensing vacuoles as well as in lamellae-like structures in the Golgi region of sub-type b_2 cells (Fig. 81). Novikoff et al. (1976) found reaction product for acid phosphatase in cisternae, probably "rigid-lamellae" (of Claude, 1970), and in immature secretory granules (i.e. condensing vacuoles) in a cell of transplantable insulinoma of Syrian golden hamster, while the reaction was negative for Golgi apparatus. Novikoff (1976, 1977), Novikoff and Novikoff (1976) and Novikoff et al. (1976) showed that these rigid lamellae and the condensing vacuoles are two smooth endoplasmic reticulum components of three which form the acid phosphatase-rich region. Novikoff (1976) used thionine pyrphosphatase and acid phosphatase cytochemistry to distinguish between this region and Golgi apparatus. He found that this region reacts positively for acid phosphatase while Golgi apparatus reacts negatively and vice versa with thiamine pyrophosphatase. The acronym GERL has been given to this region (Novikoff, 1964) because it is located at the inner or "trans" aspect (Ehrenreich et al., 1973) of the Golgi apparatus and because it appears to produce various types of lysosomes (Novikoff, 1973).

On the seventh day after attachment, numerous lysosome-like

structures of variable shape and size were found in some b_2 cells between the small vacuoles and the distended cisternae of the endoplasmic reticulum (Ly. Fig. 80) (or near to the Golgi regions of some other types of cell e.g. types f and g cells on the first day after attachment) (Ly. Fig. 87). These lysosomes are probably originated by the lamellae-like structures and the condensing vacuoles in which acid phosphatase has been detected on the fifth day after attachment (as described by Novikoff, 1976).

The endoplasmic reticulum cisternae occupying the basal region of b_2 cells become distended on the fifth day after female attachment, to reach the maximum size of distension on the seventh day after attachment (Fig. 80). It was obvious that intercisternal material had been diluted, probably due to filling with fluid produced from direct active transport across their membrane. De Robertis et al. (1975) stated that active transport may take place across the membranes of the endoplasmic reticulum. However, the main function of the salivary glands of H. leachii female is almost over on the seventh day after attachment (i.e. approximately one day before detachment from the host). It is possible therefore that these distended cisternae maintain this amount of fluid to be used after detachment or, before the breakdown of the cells.

On the first day after attachment, type b cells of the male

H. leachii begin to differentiate into the sub-types b_1 and b_2 cells (Fig. 106) (which were distinguished on the third day after female attachment) and contain the same granules. The sub-type b_2 cell granules lack the filamentous material seen in the female. No more secretory activity at all has been distinguished in these cells in the male.

Type f cells:

These cells are different in structure and histochemical reactions from the other granule secreting cells described in this work, as well as to those studied previously in other ixodid female salivary glands. As mentioned before, only the salivary glands of H. leachii adult contain an extra type of alveolus, and this type seems to be alveolus III in this work.

In the unfed adult, these cells reacted histochemically somewhat similarly to type b cells of alveolus II, while they were completely different after attachment. On the fifth day after attachment, a considerable amount of carbohydrate containing little protein has been detected histochemically in type f cells. The granules stain shades of purplish-violet with toluidine blue and thionin, indicating metachromasia of acid mucopolysaccharides, which stain with PAS, and reacted positively with colloidal iron and alcian blue.

The secretory granules of these cells begin to be reduced in number and size directly after attachment, to a minimum by

the fifth day after attachment.

Ultrastructurally, many electron-dense double membrane "macrotubules" were found throughout the cytoplasm of these cells in unfed and in the feeding adult. Some of these macrotubules enclosed electron-lucent structures with fine membranes (Fig. 31). The term "macrotubules" has been given to these cell organelles due to their tubular shape and their difference in size and structure from the known microtubules. This type of cell organelle has not been reported before in previous electron microscopic studies on tick salivary glands.

During feeding, another type of macrotubule appeared with regular very low dense single membranes beside the first type on the third day after attachment, particularly in the basal region of the cell (arrow, Fig. 86). The origin and function of these macrotubules are not known. The cisternae of rough endoplasmic reticulum were enormously distended particularly in the basal region, and parts of them had lost their ribosomes on the fifth day after attachment. The secretory process has not been observed. These cells started to degenerate on the seventh day after attachment.

Types g and 1 cells

In the unfed adult of H. leachii, these two types of fundus cells (Figs. 32, 38) are similar in position and structure to each other and to type c cells of alveolus II of the sali-

vary glands of unfed Hyalomma asiaticum (Balashov, 1968). They are unlike the other ixodid fundus cells in lacking secretory granules at the unfed stage.

Under light microscopy, on the first day after attachment, these cells increase greatly in size and reach their maximum size and activity on the fifth day after attachment. They contain secretory granules which accumulate in the apical region of the cell. Types g and l cells degenerate gradually at the final stages of feeding and after detachment to reach the maximum of degeneration at the beginning of oviposition.

Histochemically, small amounts of carbohydrates and proteins were detected in the cytoplasm of these cells of unfed adults. On the fifth day after female attachment, the granules of these cells were positive for carbohydrate and protein which is probably a carbohydrate-protein complex and which also stainsorthochromatically with toluidine blue and thionin. Type g cell granules contain proteins rich in tyrosine while those of type l cells contain proteins rich in tryptophan. Alkaline phosphatase was detected in these cells.

Ultrastructurally, on the third day after attachment, some granules lying in the apical region of type g cells showed streaming of their content producing a tail from their main body (arrows, Fig. 88); these tails can often be traced

into the microvilli projected from the apical cell membrane. Similar tails have been described by Lehane (1976) in the membrane secretory vacuoles of the midgut cells of the haematophagus fly Stomoxys calcitrans. Schramm et al. (1972) showed that tailing in the membrane bound vacuoles of the rat paratoid glands can be induced by cyclic adenosine monophosphate (cyclic AMP) which may direct the tail towards the acinar lumen. The exact mechanism of discharging the granular content has not been recognised, but the tails of the secretory granules may fuse with the microvilli to release the content into the alveolar lumen.

In the basal regions of types g and l cells, Golgi regions were more active giving rise to more condensing vacuoles, and were surrounded with slightly distended cisternae of rough endoplasmic reticulum. This probably means that both Golgi bodies and rough endoplasmic reticulum were involved in the granular formation.

On the seventh day after female attachment, the cisternae of rough endoplasmic reticulum facing the basement membrane became enormously distended (particularly in type g cells) with low dense intercisternal matrix (as in Fig. 92). These cisternae may act as those of sub-type b_2 cells in maintaining a large amount of fluid to be used somewhere else before or during oviposition, or possibly as a sign of the initial

degeneration of the cells.

During attachment of the male, these cells did not show any change in structure from the unfed adult ticks.

Type k cells

In unfed H. leachii, type k cells were readily recognisable by their large granular inclusions. This type of cell is common in alveolus III of the salivary glands of the other ixodids. They are structurally similar to type d cells of R. appendiculatus (Till, 1961), to type d cells of H. spinigera (Chinery, 1965), to type e cells of D. variabilis (Coons & Roshdy, 1973), to the naked-granule cells of D. andersoni (Meredith and Kaufman, 1973), and to type e cells of B. microplus (Binnington, 1978), see Table 5.

The histochemical reactions of unfed adult, and five days after female attachment, resemble those described before (see above) of d cells of H. spinigera, and e cells of B. microplus. Their granules were characterised in possessing lipid and proteins rich in tryptophan and tyrosine.

As in other ixodids, these cells begin to reduce in size, and in the number of their secretory granules, directly after female attachment to reach the maximum reduction on the fifth day after attachment.

In ultrastructure, type k cells of unfed ticks are identical

to type e cells of unfed D. variabilis (Coons and Roshdy, loc. cit). Their cytoplasm contains highly developed cisternae of rough endoplasmic reticulum which was often in whorls (Fig. 36) and occupied most of the cytoplasmic areas between the granules. Rough endoplasmic reticulum is especially well developed in cells actively engaged in protein synthesis, such as those of the pancreatic acinus of vertebrates (De Robertis et al., 1975).

Ultrastructural histochemical results of these cells on the fifth day after attachment confirmed those which have been given under light microscopy. Moreover, acid phosphatase was detected in the peripheral zones of some small granules (arrows, Fig. 103) but not in the larger granules. Hand and Oliver (1975, 1976a, b, 1977) showed that immature secretory granules (i.e. the condensing vacuoles) may display acid phosphatase activity. They also considered that the immature granules to be part of an acid phosphatase-rich region (GERL). The small granules of type k cells with acid phosphatase in their peripheral region may be immature granules which are part of GERL region as showed by Hand and Oliver, or, more probably on the fifth day after attachment at which the most k cells are breaking down, represent a degredation phase of a secretory activity.

TABLE 6: CELL TYPES AND NOMENCLATURE FROM RECENT STUDIES OF ARGASID ADULT SALIVARY GLANDS

SPECIES & AUTHORS	NONGRANULAR SECRETING ALVEOLI	GRANULE-SECRETING ALVEOLI	
LM			
1. O. papillipes (Balashov, 1968)	Pyramidal alveoli	-	Different types of secretory granules
2. O. kelleyi (Sonenshine & Gregson, 1970)	A	B	Different types of secretory granules
3. A. arboreus (Guirgis, 1971)	A	B	a, b, c
4. A. persicus (Roshdy, 1972)	A	B	a, b, c
5. A. persicus (Chinery, 1974)	I	II	a, i
EM			
1. O. moubata (Dzhafarov, 1965a,b)	Pyramidal alveoli	-	Different types of secretory granules
2. A. arboreus (Roshdy & Coons, 1975)	I	II	a, b, c
3. O. moubata (This study)	I	II	a, b, c, d, e

B. O. Moubata

Type II alveoli of the salivary glands of adult O. moubata contain three different types of secretory cells a, b and c (Table 6, Fig. 114). The same number of secretory cells was found in the granule-secreting alveoli of A. (P.) arboreus (Guirgis, 1971; Roshdy and Coons, 1975) and A. (P.) persicus (Roshdy, 1972) while Chinery (1974) described only two secretory cells for the last species. Dzhafarov (1965 b), Balashov (1968) and Sonenshine and Gregson (1970), working with O. moubata, O. papillipes and O. kelleyi respectively, have described only granular alveoli with different secretory granules and mads no mention of different types of granule-secreting cells.

Type a cells:

These cells with readily distinguishable granules are identical in structure and histochemical reactions to type c cells of the salivary glands of A. persicus (Roshdy, 1972) and A. arboreus (Roshdy and Coons, 1975). They are recognised by PAS-and protein-positive granules which are differentiated into central and peripheral zones. Histochemical tests suggest that these cells contain a carbohydrate - protein complex exhibiting a variable degree of metachromasia. In ultrastructure, the secretory granules of these cells are

identical to those of type c cells of unfed adult of A. arboreus (Roshdy and Coons, 1975). In O. moubata, apparently, the electron dense cores of the granules are first manufactured in the Golgi cisternae and are first observed in vacuole-like structures. Thereafter, these dense cores were found in condensing vacuoles in the Golgi region (see Fig. 118). These observations support the findings of Roshdy and Coons (loc. cit.) on type c cells of unfed A. arboreus. These authors added that numerous, less dense, vesicular bodies bud off and accumulate around the centre. They have not described how these less dense vesicular bodies bud off, and from what, or how they accumulate around the centre as they have mentioned.

The exact origin of the vacuole-like structures observed in the a cell is not known. They possess a material lower in electron density (see Fig. 119) than that in the peripheral zone of the condensing vacuoles associated with the Golgi regions. Possibly they are the intermediate stage between the dense cores and the condensing vacuoles. According to the histochemical reactions, using light and electron microscopy, the peripheral region of the mature granules is carbohydrate positive while the dense core is protein (see Fig. 121). Neutra and Leblond (1969) have proved experimentally, using glucose labelled with tritium, that the Golgi cisternae of the goblet cells of the rat large intestine are the membrane

site where carbohydrate is added to protein to form glycoprotein. They also referred to the Golgi bodies as responsible for the synthesis of the carbohydrate part of the mucus secreted by the salivary glands of the rat.

Berg and Young (1971), and Young (1973) have also proved experimentally, using inorganic sulphate labelled ^{35}S ($H_2{}^{35}SO_4$), that the sulphate was detected in the smooth membranes and vesicles of the Golgi complex of cells which were engaged in the synthesis of several different sulphated compounds, including mucopolysaccharides, glycoproteins, lipids and esteroids. Acid mucopolysaccharides were detected in the peripheral regions of type a cell granules. Hence, and according to the previous authors, the material of the peripheral region of these granules was first synthesised in the Golgi cisternae and is not added until after the protein molecule has been built on the ribosomes which are the site of protein synthesis. This means that the small, dark vacuoles (the dense core) associated with the Golgi bodies of type a cell of O. moubata contain a carbohydrate-protein complex. The latter are bounded by a limiting membrane which has been donated by the Golgi bodies (Neutra and Leblond, 1969; De Robertis et al., 1975). Thereafter, the limiting membrane of these dark vacuoles probably fuses with the membrane of the vacuole-like structure, and by the end only the dense core material (carbohydrate-protein) is

located inside the vacuole.

Dzhafarov (1965b) stated that no Golgi bodies have been observed in the secretory alveoli cells of the salivary glands of unfed adult of O. moubata, although he has described different types of membrane-bound granules. This is incorrect because the three different types of granule-secreting cells contain well developed Golgi bodies.

During feeding of O. moubata, type a cells do not show any activity comparable to the change in structure found in the females of H. leachii. However, Coons and Roshdy (unpublished data) have observed a rapid discharge of granules from type c cells in A. arboreus (which are similar to type a cells of O. moubata) as early as three minutes after tick attachment to the host. Coons and Roshdy's observations have been confirmed in type c cells of feeding A. persicus (Samir, unpublished data) in which the peripheral regions only of all the granules have been discharged while some of their central core remains within the cell. The mechanism of discharging the secretory granules has not been observed in O. moubata except that a small number of ungranulated cores appear to be very close to the cell membrane. They probably discharge their granular content by "eccrine" secretion (Toner and Carr, 1971) or "macroapocrine" secretion (Kurosumi, 1961).

Type b cells:

The secretory granules of these cells stained different colours with the Mallory staining technique. This finding indicates changes in the chemical composition of the granular content. This was also found in the staining of type b cell of A. arboreus (Guirgis, 1971), in type b cells of A. persicus (Roshdy, 1972), and a cell of A. persicus (Chinery, 1974). These authors have attributed the different colours of the secretory product, found in the same cell after differential histological staining, to the different chemical nature of the granules.

Histochemically, type b cell granules contain polysaccharides, proteins and lipids. Their proteins are rich in tyrosine. These findings are similar to those of type d cells of H. spinigera (Chinery, 1965), e cells of B. microplus (Binnington, 1978) and k cells of H. leachii (this study).

Ultrastructurally, the uniformly electron-dense mature granules of type b cells of O. moubata are similar to those of type b cells of A. arboreus (Roshdy and Coons, 1975). Some b cells contain granules with dense fine textured filamentous material (Fig. 123). It is uncertain whether these granules represent a degredation phase, abnormal formation, or a stage in normal granule formation. Apparently, the rough endoplasmic reticulum and the Golgi bodies are involved

in the granular formation of type b cell granules (see Fig. 122). The mechanism is similar to that described for type b_2 cells of the feeding female of H. leachii, and type b cells of A. arboreus (Roshdy and Coons, loc. cit.).

During feeding, type b cells are greatly reduced in size and in the number of their secretory granules in the alveoli. Other cells contain membrane-bound vacuoles with osmiophilic aggregates. The latter probably represent a degredation phase of the secretory granules. The mechanism of discharging the granular content unfortunately has not been observed.

Type c cells:

Proteins rich in tryptophan have been detected in the secretory granules of these cells. This was also found in type a cells of A. persicus (Roshdy, 1972).

Ultrastructurally, these granules possess two different dense zones, one of them is completely electron-dense while the other is less dense (G. Fig. 126). The fine structure of these granules has not been described before in the previous electron microscopy studies on the same species by Dzhafarov (1965b), or in the other argasid ticks. These cells are characterised by the presence of well developed ellipsoidal Golgi bodies surrounded by slightly distended cisternae of rough endoplasmic reti-

culum (Fig. 126). Apparently, as in type a and b cells, the rough endoplasmic reticulum and the Golgi bodies are involved in the granular formation, but the exact mechanism has not been observed.

In the final stages of feeding, type c cells of O. moubata adult have decreased greatly in size and lost most of their secretory granules. The mechanism of discharging the granular content is not known.

(2) NONGRANULAR-SECRETING CELLS

a) The Cap Cells (In H. leachii and O. moubata)

The cap cells of alveolus II (type c cells of H. leachii female) were observed touching the distal ends of the two curved epithelial cells on the first day after attachment. In addition, the cap cells of alveolus IV (type m cells) were observed touching the valve-like structures on the first day after attachment.

In unfed O. moubata adult, the cap cells (type d cells) were extended to touch the valve-like structures as well as to surround the valvular canal. Thus the cap cells of the unfed H. leachii appear to be similar to those of the unfed O. moubata adults, extending to touch the valve-like structures and to surround the valvular canal.

The cap cells are structurally similar in unfed H. leachii and O. moubata adults. Their lateral cell membranes abut those of the granule-secreting cells and the interstitial epithelial cells by means of septate desmosomes.

On the third day after female H. leachii attachment, the plasma membranes of type c cells in alveolus II are greatly distended and their cytoplasm contains membrane-bound vacuoles as well as the nuclei which probably represents an abnormal core (see Fig. 82). Types h and m cells of alveoli III and IV have formed trough-like shapes and extend between the alveolar cells (as in Fig. 93). The lateral

cell membranes of these troughs project microvilli which interdigitate with those projected from the lateral membranes of type g and l cells, while their other sides attach with types i and n cells (the interstitial epithelial cells) by means of intercellular junction. These observations may interpret the function of the cap cells in the granule secreting alveoli. They may help in transferring the discharged secretory content, from the distal parts of the secreting cells, the lateral and basal regions of the cells (from which the secretory content has been released), to the proximal parts of the alveolus, the alveolar lumen, via their microvilli bordering this lumen, or further to the valvular canal by means of their extensions surrounding this canal. Some transfer of secretory product seems to be necessary especially with the great enlargement of the granule secreting alveoli at the final stages of feeding in the female. Meredith and Kaufman (1973) suggested that the cap cells may help to hold the alveolus together during secretion when the luminal hydrostatic pressure may be considerably higher, but this does not explain the presence of microvilli in these cells

The interstitial epithelial cells: (In H. leachii and O. moubata) Types d, i and n cells of unfed H. leachii adult and type e cells of unfed O. moubata adult are similar in position and structure. They are similar to the interstitial epithelial cells which have been described for the unfed male of D. varia-

bilis (Coons and Roshdy, 1973). These authors suggested that these cells provide support to the granule-secreting alveoli. They have based their suggestion on the extensive junction specializations between the epithelial cells and the granule secreting cells. Coons and Roshdy have concluded that these cells may possibly differentiate into new secretory cells during feeding, but this has not been observed during feeding in the female of H. leachii. Roshdy and Coons (1975) described epithelial cells in unfed adult of A. arboreus, which are somewhat different from those of unfed H. leachii and O. moubata adults. The epithelial cells in A. arboreus form basal caps over the granule secreting cells and possess extensive membranous infoldings, with a complex system of tortuous canaliculi.

During attachment in female H. leachii, types i and n cells of types III and IV alveoli were more active than type d cell of type II alveolus. Activity was evident on the first and third days after attachment. These cells fill the gaps between the alveolar cells, as well as the interdigitations created by the folded basal membranes of the granule-secreting cells.

On the fifth and seventh days after attachment of the female of H. leachii, these cells are extensively branched and form complicated tortuous canaliculi (Ca. Figs. 95, 97) structurally similar to the fluid transporting epithelia described by Ber-

ridge and Oschman (1972), to the "water cells" of Dermacentor andersoni nymph (Meredith and Kaufman, 1973) and to the epithelial cells reported for A. arboreus (Roshdy and Coons, 1975).

The question then arises, how the interstitial epithelial cells increase in length and complexity during this feeding period. Some partially discharged unknown granules of variable size were found in the canaliculi of the epithelial cells and their limiting membranes are fused with the plasma membrane of the canaliculi (small arrow, Fig. 98). These unknown granules may be derived from subunits secreted by other cells in the alveolus.

In the basal regions of alveoli III and IV of the female of H. leachii, the distal extensions of the canaliculi have formed arm-like structures with swollen ends possessing rounded mitochondria and arranged regularly to be very close to the basement membrane surrounding the alveolus (Fig. 97). The presence of the mitochondria near the basement membrane may provide the essential energy for the active-transport process which may take place across the basement membrane and the plasma membrane of the epithelial cell canaliculi. In addition some mitochondria were observed with unusual morphology in the basal regions of type III and IV alveoli while some others appeared with perforated cristae (M. Fig. 98). Berridge and Oschman (1972) reported unusual mitochondria where high energy demands were placed upon them. Mitochondria with perforated cristae have been found in the salivary glands of

the blowfly Calliphora erythrocephala (Berridge and Oschman, 1972), in the flight muscle of the same insect (Smith, 1963) and in the cardiac muscle of canaries (Slautterback, 1965), all of which are tissues with a high metabolic demand.

An interrupted electron-dense line appears in the basement membrane (thick arrow, Fig. 98) surrounding the granule-secreting alveoli, especially in alveoli III and IV, during the final stages of the female H. leachii feeding period. This line is markedly increased in density and thickness and is accompanied by extensive infoldings, particularly after the tick has dropped off from the host. Oschman and Berridge (1971) reported that the basement membrane may act as a filter. Thus the dark line is probably due to a deposition of an organic or inorganic material in the basement membrane during the osmotic filteration processes, and then stains strongly with osmium during grid processing. Increasing density and thickness during the final stages of feeding and after detachment, possibly indicates that the activity of transport, as well as the osmotic filteration processes, increases during the final stages of feeding and have not been stopped even after detachment.

During the final stages of feeding and after the female detachment, large lysosomes were found in the canaliculi of the interstitial epithelial cells, in some of which were mito-

chondrial remnants, small vesicles or membrane-like structures (Ly. Figs. 97, 98). De Robertis et al. (1975) and Novikoff (1976) have described autophagic vacuoles (cytolysosome or autophagosome) as special cases of secondary lysosomes in which the lysosome contains part of the cell in a process of digestion (e.g. a mitochondrion or portions of the endoplasmic reticulum). By this mechanism, the cell can achieve the degredation of its own constituents without irreparable damage (Allison, 1967; De Duve, 1967).

Meredith and Kaufman (1973) considered the "water cells" (which are identical to the interstitial epithelial cells of H. leachii female) in type III alveoli to be the possible site of fluid secretion rather than the fibrillar cells in the nongranular alveoli. They also reported that, unlike ixodids, salivary glands of argasids secrete little fluid during feeding and seem to have no fluid-secreting cells.

The structure and function of the interstitial epithelial cells of the granule-secreting alveoli of H. leachii female during feeding support Meredith and Kaufman's suggestion that the "water cells" in Dermacentor andersoni female excrete the bulk of the water during engorgement. Megaw (1976) postulated that alveolus I of Boophilus microplus may secrete salts, while Binnington (1978) suggested that alveolus I present in both argasids and ixodids instars, which do not have a rapid engorge-

ment phase, may add water and salts to granular secretion throughout feeding. In female H. leachii, type I alveoli, according to their fine structure, may help the interstitial epithelial cells in eliminating the excess fluid during feeding.

The development of the interstitial epithelial cells is not found in feeding H. leachii male and O. moubata adults, because they possibly do not have the same requirements for water and salt excretion as the female.

GENERAL DISCUSSION

1. ACID AND ALKALINE PHOSPHATASE:

Acid phosphatase has not been detected in the salivary glands of either H. leachii or O. moubata before or after attachment. It is possible that acid phosphatase may be present but was not demonstrable by the techniques used. It is also possible that acid phosphatase may be present but in such a low concentration that it remains undetectable. In contrast, alkaline phosphatase has been detected in the cytoplasm and the granules of all cell types of the four types of alveoli on the fifth day after the attachment of female H. leachii. In addition, at two hours after the attachment of adult O. moubata, the cytoplasm and a few granules of some a cells of type II alveoli have shown a positive reaction for alkaline phosphatase. The significance of this is not clear because, in spite of the ubiquitous distribution of phosphatases, relatively little is known of their biological function (Burstone, 1966). However, the presence of alkaline phosphatase in all the cell types of H. leachii and type a cell of O. moubata adult after attachment would tend to indicate that this reflects a cycle of metabolic activity in both cytoplasm and in secretory granule formation. It is also interesting to note that the observations of Khalil (1972) indicated physiologically and biochemically that b cells in the salivary glands of A. arboreus were metabolically very active.

2. HAEMOCYTES:

Haemocytes (Fig. 105a, b) were found in the haemocoel and close to the salivary glands of the female of H. leachii in the final stages of feeding and increased in number after the tick detached from the host. These haemocytes were not observed so commonly in the feeding male.

In O. moubata adults, haemocytes were found in both unfed and fed ticks, very close to the salivary alveoli or to the overlying connective tissue. The surface of haemocytes is produced into pseudopodia, while their cytoplasm contains dense structures that probably represent lysosomes containing hydrolytic enzymes. The structure of the tick haemocytes is similar to that found in haemocytes of the blowfly Calliphora shortly before pupation (Crossely, 1964, 1965). In insects, the haemocytes are associated with immune responses (Smith, 1968), forming cysts or capsules (Salt, 1963, 1967, 1970), or form a compact isolating tissue around an invading body (Grimstone, et al., 1967). Wigglesworth (1965) stated that insect haemocytes have been implicated in a variety of functions including blood clotting, the movement of nutrients about the body and the manufacture of haemolymph proteins. According to Wigglesworth (1965), the haemocytes in fed female ticks may help in movement of the undischarged and undegenerated secretory granules from the alveolar secreting

cells of the salivary glands to the ovary, probably to be used as nutrients for synthesising ova, particularly after dropping off from the host.

The increase in the haemolymph cells after feeding of the female of H. leachii is probably a recognition of cellular breakdown in the alveoli and a reaction to maintain the other organs until oviposition. The reaction is not found in the male where cellular breakdown does not take place in the salivary glands and where the male does not live for long after detachment.

The haemolymph cells were found in unfed and fed O. moubata adult, possibly because argasids have developed several adaptations ensuring long survival without food, as well as a requirement for a blood meal every time before oviposition.

3. FLUID ELIMINATION:

Numerous studies have shown that ticks concentrate the ingested meal by excreting large quantities of excess fluid (Lees, 1946; Sutton and Arthur, 1962; Arthur, 1965, 1970; Seifert et al., 1968; Snow, 1970; Sauer and Hair, 1972; Koch et al., 1974). As the blood meal is ingested, excess ion and water are moved across the gut epithelium into the haemocoel and in ixodid ticks secreted back into the host via the salivary glands, (Tatchell, 1967a, 1969c; Kirkland, 1971; Kaufman and Philips, 1973a, b, c; Meredith and Kaufman, 1973).

Sauer (1977) suggested that the main organs of fluid elimination are the salivary glands in ixodids and the coxal glands in most argasids. The interstitial epithelial cells of the granule-secreting alveoli, particularly alveoli III and IV of the salivary glands of H. leachii female, are well developed during the final stages of the feeding period (rapid phase), and became very complicated in structure with increase in the surface area. This structure suggests that these cells are responsible for fluid elimination and serve as active transport epithelia. Certainly the ultrastructural features of type I alveolar cells suggest that they are also very active in this way. However, group I alveoli are not numerous in the glands and are found only in the proximal part of the gland. Each group I alveolus possesses only a small lumen. Therefore, the interstitial epithelial cells of types III and IV alveoli with characteristic developed structure from day 5 after attachment are the better candidates for secreting the bulk of fluid than type I alveoli. The latter may be involved in the secretion of another material. Megaw (1976) suggested that type I alveoli in both argasid and ixodid ticks may secrete salts, or may add water and salts to the granular secretions throughout feeding (Binnington, 1978).

Meredith and Kaufman (1973) have described "water cells" (similar to the interstitial epithelial cells of H. leachii

female) in alveolus III of D. andersoni female. These cells were present in both III and IV alveoli of H. leachii. This difference between species may suggest that females of H. leachii eliminate greater amounts of fluid than D. andersoni when concentrating the ingested blood meal. Male argasids and ixodids are known to secrete saliva during copulation (Feldman-Muhsam et al., 1970) as well as during feeding. These authors postulated that the salivary secretion ensures the smooth transfer of the spermatophore to the mouthparts of the male where the spermatophore is held during transfer to the female. The salivary glands of O. moubata adult and H. leachii male showed little development of the interstitial epithelial cells during feeding, which also corresponds with the low salivary fluid secretion observed in argasids and male ixodid ticks. However, coxal glands are lacking in larvae of the argasid tick A. persicus and Moorhouse (1975) suggested that the concentration of the blood meal may also be brought about by salivary secretion at this stage in argasid ticks.

4. SECRETION OF CEMENT

The salivary glands of most slow feeding ixodid ticks secrete cement to ensure firm attachment of the mouthparts to the host. This phenomenon is characteristic of most genera of ixodids (Cowdry and Danks, 1933; Foggie, 1959; Gregson, 1960; Moorhouse and Tatchell, 1966; Balashov,

1968; Moorhouse, 1969 and Chinery, 1973). Exceptions are some Ixodes species, such as I. ricinus and I. holocyclus, which do not produce cement but insert the mouth parts deeply into the host skin tissues (Moorhouse, 1969, 1973; Arthur, 1970). Moorhouse (1969) reported that the tick genera with short mouthparts (e.g. Haemaphysalis and Boophilus) are capable of penetrating deeply into the host dermis and, in addition, a secondary secretion of cement is used to provide an additional support for the tick (Moorhouse and Tatchell, 1966). These authors postulated that the cement in these species is inert and does not provoke the development of host-parasite factors inimical to the tick. The cement of feeding females of H. spinigera consists of an external cement, forming a cone and an internal cement tube extending deeper into the host skin (Chinery, 1973). The latter author concluded that the deposition of all the cement occurs early in the feeding process of H. spinigera and not during the final phase of rapid engorgement, as reported by Moorhouse and Tatchell (1966).

Chinery (1973) considered that the secretory granules of types a, d and e cells of alveoli II and III of H. spinigera are responsible for the deposition of the cement material due to their similarity in histochemical reactions i.e. protein in nature and rich in tyrosine and tryptophan. Chinery added

TABLE 7: THE POSSIBLE FUNCTION OF THE GRANULE-SECRETING CELLS OF THE TICKS, H. LEACHII AND O. MOUBATA

MAIN CHEMICAL COMPONENTS OF THE CELL GRANULES	H. LEACHII			O. MOUBATA	POSSIBLE FUNCTION
	II	III	IV	II	
Types a, e and j cell granules contain protein rich in tryptophan and traces of carbohydrate while those of k cells contain lipid and protein rich in tyrosine.	a	e	j + k	-	Cement Secretion
They contain a carbohydrate-protein complex exhibiting a variable degree of metachromasia that increases during the feeding period particularly in b and f cells.	b, b_1+b_2	f	-	a	Anticoagulant Secretion
They contain carbohydrate and protein rich in tyrosine.	-	g	k	b	Cytolysin Secretion
They contain small amounts of carbohydrate and protein rich in tryptophan.	$a+b_2$	e	j + l	c	Pharmacological agent

Note: In H. leachii:
1. The a, e, j, k and f cell granules are found in unfed ticks and are discharged after attachment.
2. The b cell granules are found in unfed ticks and are distinguished into sub-types b_1 and b_2 after attachment.
3. The b_2, g and l cell granules appear only after tick attachment.

In O. moubata: The a, b and c cell granules are found before and after attachment.

that types a and e cells are the cement precursors of the external cement layer owing to their anterior position in the alveoli, while d cells are responsible for the deposition of the internal cement layer. Moorhouse and Tatchell (1966) recorded that the cement substance of the cattle tick, B. microplus is histochemically positive for lipoprotein, tryptophan and tyrosine. The same reaction was found for types a, d and e cells granules of alveoli II and III of the same species (Binnington, 1978).

Types a, e, j and k cells of types II, III and IV alveoli appear to be likely candidates for the secretion of cement precursors in unfed H. leachii adult (Table 7) and analogous to those described for B. microplus and H. spinigera. They occupy similar positions in their respective alveoli, have similar histochemical properties (types a, e and j cells contain lipid, protein-rich in tryptophan, and traces of carbohydrate, while k cells are positive for lipid and protein-rich in tyrosine and tryptophan), are filled with secretory granules before attachment, and, in the female, are apparently non-functional from approximately three days after attachment. The early discharge from these alveoli is significant since it is known that the first secretion of cement occurs within five minutes of tick attachment (Binnington, 1978). This author suggested that the presence of

phenols and phenol oxidase shown in cells of a, d and e of type II and III alveoli of B. microplus indicated a parallel with insect cuticular tanning in the mechanism for cement hardening.

In the feeding male of H. spinigera, the cement is similar to that in the female, but the cone and the tube in the host skin are much reduced (Chinery, 1973). This is probably the reason why types a, e, j and k cells of the feeding male of H. leachii do not show so much activity as those described in the feeding female.

Most authors believe that rapid-feeding argasid ticks, in contrast to ixodid ticks, do not secrete a cement substance (Balashov, 1968; Chinery, 1974). Balashov (1968), Kirkland (1971), Chinery (1973), Coons and Roshdy (1973), Meredith and Kaufman (1973), and Roshdy and Coons (1975) relate the subgranular formation in ixodid alveoli to the cement substance. The granule secreting cells in O. moubata do not show the subgranular structure found in the hard ticks and also no cement substance is known to be secreted in this species of soft ticks studied so far.

5. ANTICOAGULANT SECRETION:

Histochemical tests suggest that types b and f cells of types II and III alveoli in the salivary glands of unfed H.

leachi adult contain a carbohydrate-protein complex exibiting a variable degree of metachromasia that increases during the female feeding period (i.e. in sub-types b_1 and b_2 cells as well as type f cells). Strong metachromatic substances, known to be PAS-positive (Pearse, 1961), include highly sulphated acid mucopolysaccharides such as heparin with high anticoagulant activity (Foster, 1955; Hale, 1957). According to Foster, this anticoagulating activity is associated with the degree of sulphation. Less sulphated forms of mucopolysaccharides, such as heparin monosulphate, are PAS-positive and orthochromatic (Jorpes et al., 1948; Hale, 1957). Chinery (1965) believed that the anticoagulant factor in H. spinigera resided in the granules of type b and c cells, is an orthochromatic, low sulphated, and consequently less active form showing little variation with PAS staining. He suggested a close relationship between anticoagulants in this form and the long feeding periods of hard ticks. Balashov (1968) suggested that two different types of salivary secretion can be distinguished in ixodid ticks. The first secretion occurs during the initial attachment phase and forms the cement sheath. The second (and later) secretion, which is elaborated after attachment, is more transparent and is postulated to contain a component that prevents blood coagulation. Balashov further speculated

that the active salivary coagulant may be a carbohydrate-protein complex inhibiting the enzymatic activity of thrombokinase and having high thermostability. However, anticoagulants have not been shown unequivocally in ixodid ticks; they are present in Hyalomma asiaticum but definitely absent in Dermacentor andersoni and Boophilus microplus (Tatchell, 1969a; Tatchell and Binnington, 1971). In argasids, on the other hand, Hellman and Hawkins (1967) reported a marked anticoagulant action in the salivary glands extract of O. moubata. Howell (1966) showed that O. savignyi saliva, collected after pilocarpine injection, contained an extremely potent anticoagulant. Type a cells of O. moubata shown in this study contain readily recognisable granules which are differentiated into central and peripheral zones with differential staining reaction. These granules are identical to the c cell granules in A. persicus (Roshdy, 1972) and A. arboreus (Roshdy and Coons, 1975). Roshdy suggested that the moderately metachromatic, PAS and protein-positive granules of c cells of A. persicus may be responsible for producing a moderately sulphated anticoagulant which probably becomes active as the tick feeds. The a granules in O. moubata may also possess a similar property (see Table 7). The salivary glands of A. arboreus (Coons and Roshdy, unpublished data from Roshdy and Coons, 1975) and A. persicus (Samir, unpublished data) have shown submicroscopic changes in the granule-secreting cells and a

rapid discharge of cell granules as early as a few minutes after attachment. However, type a cells of the salivary glands of O. moubata do not show this activity reported for both A. arboreus and A. persicus.

6. CYTOLYSIN SECRETION AND PHARMACOLOGICAL AGENTS:

Although cytolysins or pharmacological agents have not been demonstrated directly in the saliva of H. leachii and O. moubata, the cytolysin and the pharmacological properties of some other ixodid and argasid ticks have been demonstrated by Lavoipierre and Riek (1955), Foggie (1959), Arthur (1962, 1965), Tatchell (1969a, b), Geczy et al. (1971), and Moorhouse (1975). The reported data in this aspect are clearly inadequate and depend only on the analysis of the tick oral secretion, using Gregson's capillary tube technique (1957) or pilocarpine stimulation (Tatchell, 1967b).

Balashov (1968) speculated that ixodid saliva hinders host blood coagulation, and also causes lysis of surrounding cells and tissues. Tatchell and Moorhouse (1970) and Tatchell and Binnington (1971) hypothesized that the feeding lesions result from extra-oral digestion through the action of cytolysins in the salivary secretions. Tatchell and Binnington also reported that the saliva of B. microplus contains a pharmacologically active material which caused a contraction of a rat fundus preparations, and an increase in capillary

permeability after intradermal injection into a bovine host. Prostaglandin has been reported in the saliva of B. microplus (Higgs et al., 1976) after stimulation with subcuticular injections of pilocarpine hypochloride. These authors suggested that prostaglandins could be an important factor in the initiation and maintainance of the host lesion. Chinery and Ayitey-Smith (1977) have found the salivary gland homogenates of Rhipicephalus s. sanguineaus antagonize and potentiate, respectively, the action of histamine and acetylcholine on guinea pig ileum. Howell et al. (1975) indicated the presence of proteolytic enzymes and cholinesterase activities in the oral secretion of O. savignyi.

Type g cell granules of type III alveoli in the salivary glands of the feeding female of H. leachii, and some type b cell granules of type II alveoli of O. moubata adult react moderately for carbohydrate and proteins. These cells possibly contain neutral mucopolysaccharides firmly bound with proteins (Chinery, 1965). It is interesting that these cell types contain tyrosine and, in this respect, are similar to the d cells in H. spinigera (Chinery, loc. cit.), k cells of H. leachii (which are probably involved in cement secretion), b cells of A. persicus (Roshdy, 1972), and b cells of A. arboreus (Roshdy and Coons, 1975). Roshdy (loc. cit.) reported that the presence of tyrosine-rich protein granules in the b cells of unfed A. persicus may lead one

to expect an active role of this cell type during feeding. Tatchell (from Roshdy, 1972) postulated that as type b cells of A. persicus have been shown to contain an active esterolytic enzyme; this may be subsidiary to a major proteolotic action, e.g. Chymotrypsin. Therefore, type g cells of the feeding H. leachii female, and type b cells of O. moubata adult may be the production site for an argasid and ixodid lytic salivary component, respectively (see Table 7).

Although type g cell granules of H. leachii female have been observed only after tick attachment, and probably play a role in lytic action during feeding, type b cell granules of O. moubata were found before tick attachment, but have not shown the morphological changes seen in the g cells of H. leachii females, or even as in the b cells of A. persicus and A. arboreus during attachment, as suggested by Roshdy (loc. cit.) and Roshdy and Coons (1975), respectively. Furthermore, sub-type b_2 and type 1 cell granules of the feeding H. leachii female, as well as type c cell granules of unfed and fed O. moubata adult contain small amount of carbohydrate and protein rich in tryptophan groupings. Compounds containing tryptophan are known to occur in cells with zymogenic activity and may include pharmacologically active indole derivatives such as serotonin-like substances (Pearse,

1961). Tatchell (1969a) and Tatchell and Moorhouse (1970) attributed host tissue destruction, during B. microplus attachment, to host neutrophils rather than to specific lytic action of the saliva. Therefore, it is suggested that the secretion of sub-type b_2 cells and type 1 cells of H. leachii and c cells of O. moubata may contain substances related to the pharmacological properties of tick saliva (Table 7). In addition, types a, e, and j and k cell granules also contain protein rich in tryptophan before attachment, and in this respect, one may suggest that their secretions probably play a role as pharmacological agents directly after attachment and during cement formation.

7. TOXIC COMPONENTS

In certain ixodid ticks, the saliva is thought to contain a strong toxin which causes paralysis of domestic animals and man (Emmons and Mclennan, 1959, 1960; Esplin et al. 1960; Cherington and Snyder, 1968; Balashov, 1968; Gregson, 1973). Ross (1926) injected salivary gland extracts from Ixodes holocyclus into mice and produced signs resembling tick paralysis. Gregson (1957) stated that paralysis does not appear until ixodid ticks had been feeding for approximately four days, the time when oral secretions become abundant. Murnagham (1960) reported that paralysis produced in the dog by Dermacentor andersoni is due to nerve blockage produced by the tick toxin on the somatic motor fibres. Gregson

(1973) concluded that tick saliva is a prerequisite for the toxin. He recognised three possible origins of the toxin: as a product of the tick tissues, as a product of the metabolic breakdown of host tissues, or as a product of an organism from the tick.

In argasid ticks, Howell (1966) suggested that a toxin might be present in the saliva of O. savignyi, and later Neitz et al. (1969) purified a toxic component from the same species. Howell et al. (1975) have collected oral secretions after stimulating salivary secretion in O. savignyi with pilocarpine hydrochloride. The secretion showed the presence of at least ten different protein fractions after electrophoresis and chromatographic analysis, of which only one was toxic. The authors hypothesized that the remaining fractions, although not toxic as such, may play an important role in the syndrome produced by the secretion.

Toxic substances have not been tested in H. leachii and O. moubata, and analysis of the salivary secretion awaits further physiological, pharmacological and biochemical studies.

8. CONTROL OF SALIVATION:

A large nerve is associated with the main salivary duct of both sexes of H. leachii and O. moubata, and this branches into smaller lobular nerves which follow the lobular ducts

into the alveoli, as the alveolar nerves. The latter are divided inside the alveoli into individual axons containing neurosecretory vesicles. Synaptic areas between axons and surrounding cells were observed in the nongranular and granule-secreting alveoli. These findings suggest that the secretory activity of the salivary glands of H. leachii and O. moubata is under neural control and conform to recent observations of Coons and Roshdy (1973); Kaufman (1973), Roshdy and Coons (1975), Megaw (1977) and Binnington (1978). Coons and Roshdy (loc. cit.) have described synaptic areas in the granule-secreting alveoli of the unfed D. variabilis male. These authors suggested that each granule-secreting cell is innervated. Meredith and Kaufman (loc. cit.) found a nerve close to the basal surface of the group III acinus of D. andersoni, but they did not know whether such nerves influence secretion, or some other function, although they believed that acinus III contributed most of the fluid portion of the saliva. Roshdy and Coons (1975) reported axons containing neurosecretory material in both nongranular and granule-secreting alveoli of A. arboreus and Amblyomma americanum, and in close association with the basal region of the interstitial epithelial cells. They suggested that the secretory activity and the epithelial cells are under neural control. Megaw (1977) stated that the salivary glands of the ixodid tick Boophilus microplus are at least partially innervated by a branch of

the pedipalpal nerve. It is also suggested that the transmitters involved with the control of salivary activity are catecholomines (e.g. adrenaline, noradrenaline and dopamine), and these induce secretion from ixodid salivary glands both *in vivo* and *in vitro* (Kaufman and Philips, 1973; Megaw, 1976, 1977); Megaw and Robertson, 1974; Sauer *et al.*, 1974; Kaufman, 1976) thus suggesting that salivation normally is under nervous rather than hormonal control. Sauer *et al.* (1974) found that the stimulatory factors for salivary secretions are not found in the tick haemolymph, and suggested that catecholaminergic molecules may stimulate alveolar axons to release local neurosecretory material. The presence of the neurosecretory granules in axons showing synaptic junctions with a granule-secreting cells in both *H. leachii* and *O. moubata* supports this suggestion. Whitehead (1970, 1971) found two types of secretory control mechanism after morphological and pharmacological studies on salivary glands of the cockroach *Periplaneta americana*. The first is a chemical mediator found in synaptic vesicles associated with a thickened postsynaptic membrane, and the second is in the neurosecretory granules. Oschman and Berridge (1970) suggested that the salivary glands of adult blowfly, *Calliphora erythrocephala* have a third type of secretory control involving hormones. As stated by Whitehead, all three types may

serve to mediate the control of salivary secretion in different animals.

9. DISEASE TRANSMISSION:

The role of the salivary glands in the development and release of pathogens, unfortunately, has not been researched at the ultrastructural level in H. leachii and O. moubata.

It is widely believed that disease-causing agents are transmitted to the host in secretions of the salivary glands (Burgdorfer, 1951; Varma, 1962; Rehacek, 1965), but detailed information is limited about the relationship between salivary gland structure and parasite development and release into the host. Martin et al. (1964) showed that type II and III alveoli of the salivary glands of Rhipicephalus appendiculatus are important in the cyclic development and transmission of Theileria parva, the agent of East Coast fever of cattle in East Africa. Purnell et al. (1969) found the greatest number of mature infective particles of T. parva in the salivary glands of ticks which had fed either four or five days on rabbits. These authors found a progressive decrease from five days in the number of infected acini. Purnell et al. (1973) further demonstrated that cattle became infected when injected with supernatent fluids from triturated ticks that had attached for four to nine days to the infected host. The most highly infective supernatent fluids were prepared from five day fed

ticks and these results were correlated with the histological detection of mature parasites in the tick's salivary glands. As described in the feeding female of H. leachii, in this study, some types of granule-secreting cells (e.g. a, e, j, k and f) start to release their secretion directly after attachment to be insignificantly active on the fifth day after attachment while others (e.g. b, g and l cells) start to be active directly after attachment to reach their maximum activity on the fifth day after attachment. Although these active cells are probably involved in the secretion of coagulating, cytolysin and pharmacological substances, they are possibly the more suitable environment for cyclic development of the parasite to be released on the fifth day after the tick attachment, and before the tick detachment, as well as before the degeneration of these cells. However, further work on infected ticks will show the specificity of the parasite for cell types within the complex salivary glands described here. Any specificity would determine the timing of transmission after attachment of the tick to the primary host.

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1 HAEMAPHYSALIS LEACHII LEACHII

Fig. 2. Feeding of H. leachii adults in a sleeve attached on the back of the rabbit.

Fig. 3. Feeding of O. moubata adults on the ear of the rabbit.


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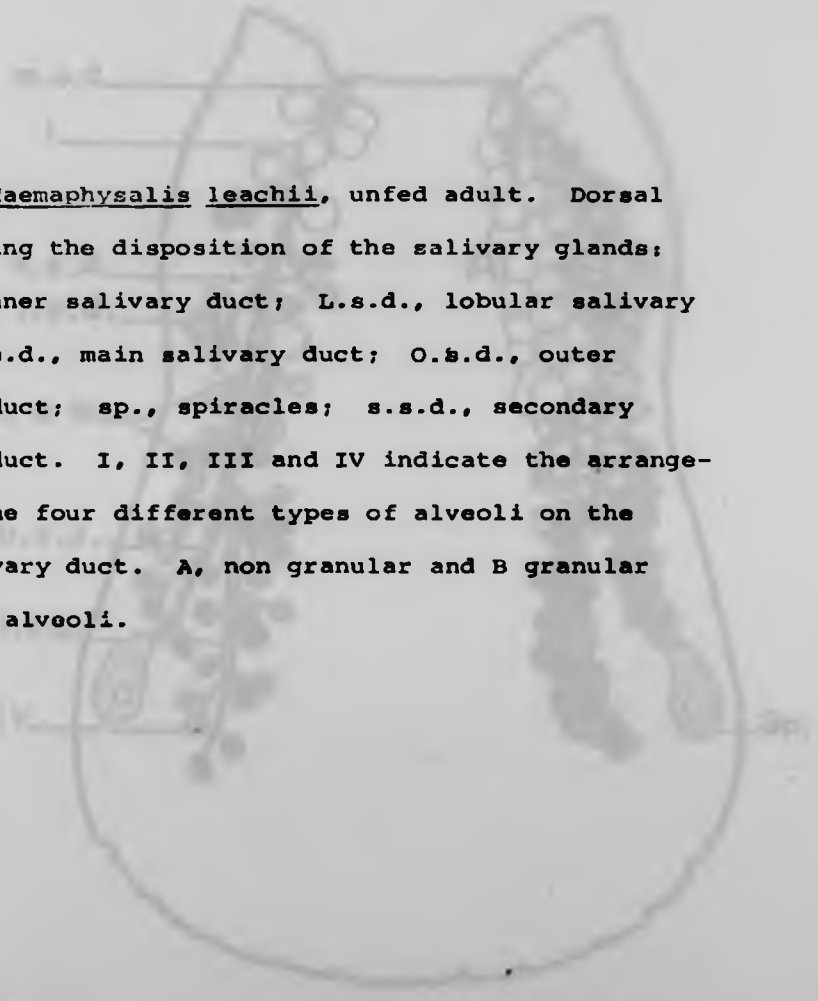
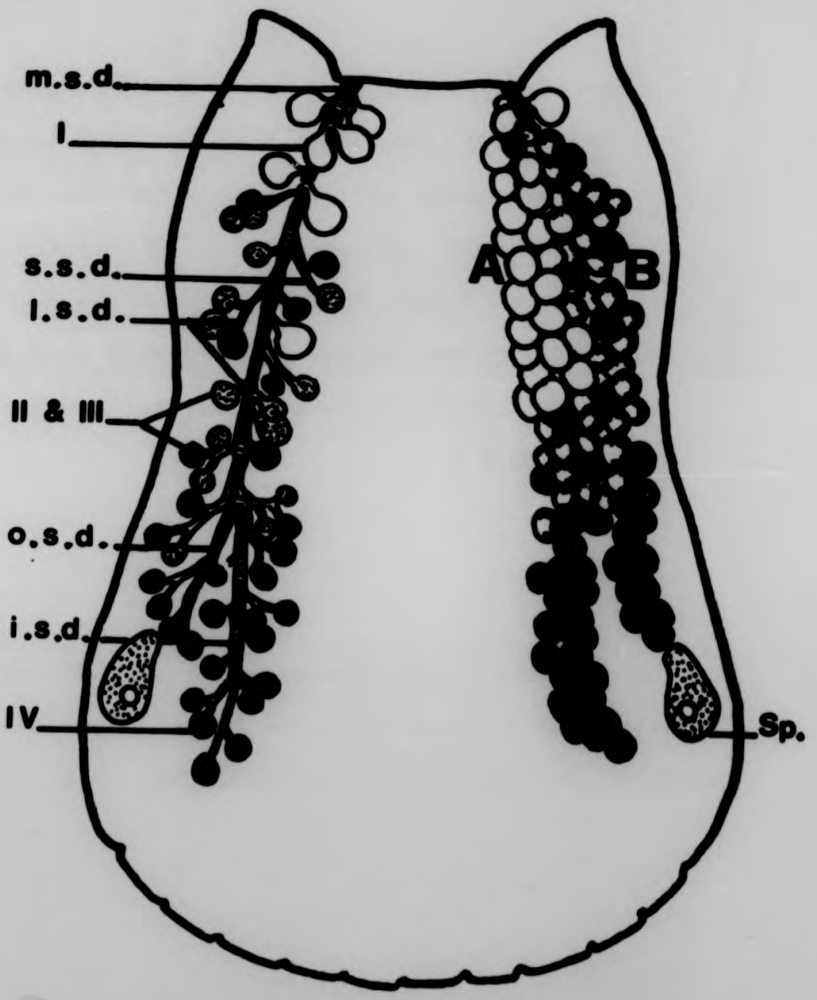


Fig. 4. Haemaphysalis leachii, unfed adult. Dorsal view showing the disposition of the salivary glands: i.s.d., inner salivary duct; L.s.d., lobular salivary duct; m.s.d., main salivary duct; O.s.d., outer salivary duct; sp., spiracles; s.s.d., secondary salivary duct. I, II, III and IV indicate the arrangement of the four different types of alveoli on the main salivary duct. A, non granular and B granular secreting alveoli.



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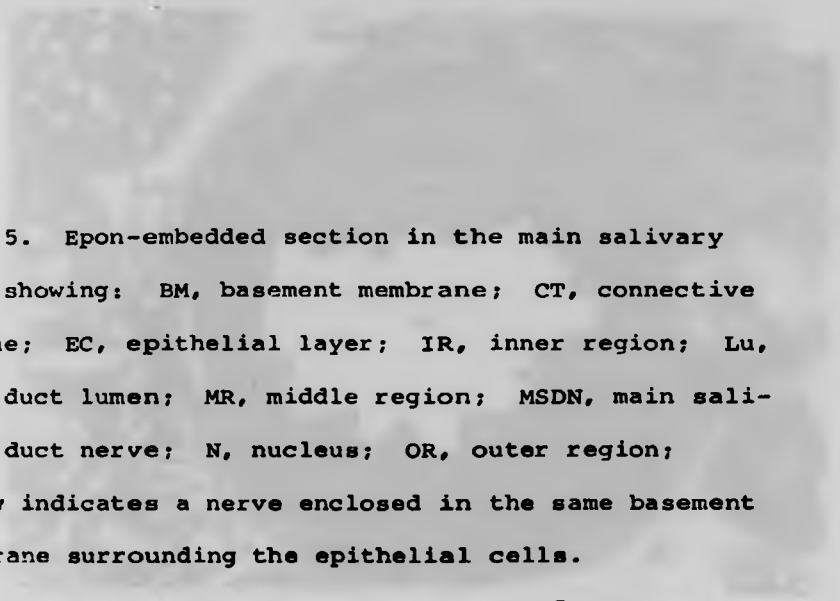

Fig. 5. Epon-embedded section in the main salivary duct showing: BM, basement membrane; CT, connective tissue; EC, epithelial layer; IR, inner region; Lu, main duct lumen; MR, middle region; MSDN, main salivary duct nerve; N, nucleus; OR, outer region; arrow indicates a nerve enclosed in the same basement membrane surrounding the epithelial cells.

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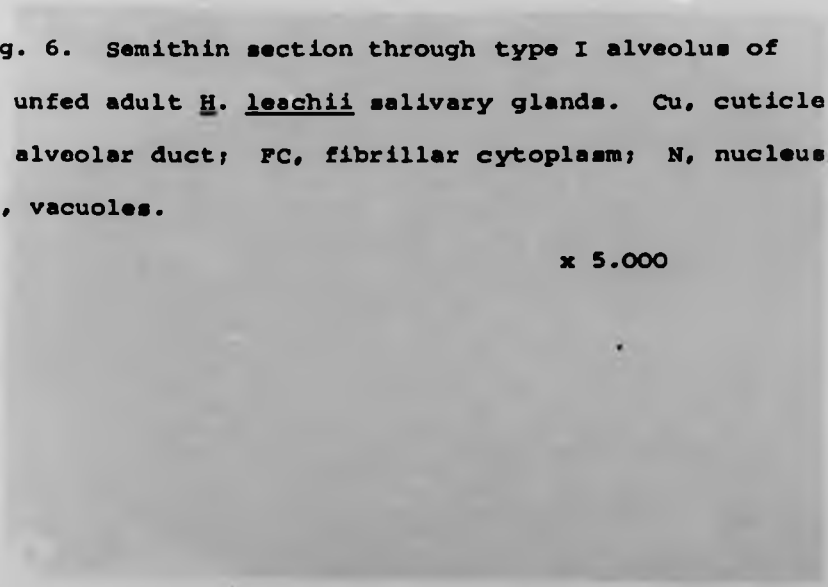

Fig. 6. Semithin section through type I alveolus of an unfed adult H. leachii salivary glands. Cu, cuticle of alveolar duct; FC, fibrillar cytoplasm; N, nucleus; Va, vacuoles.

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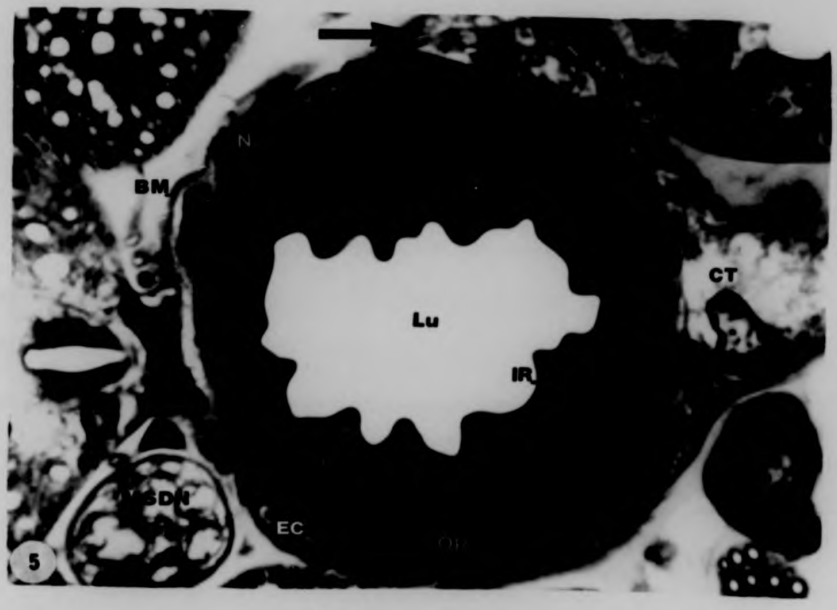
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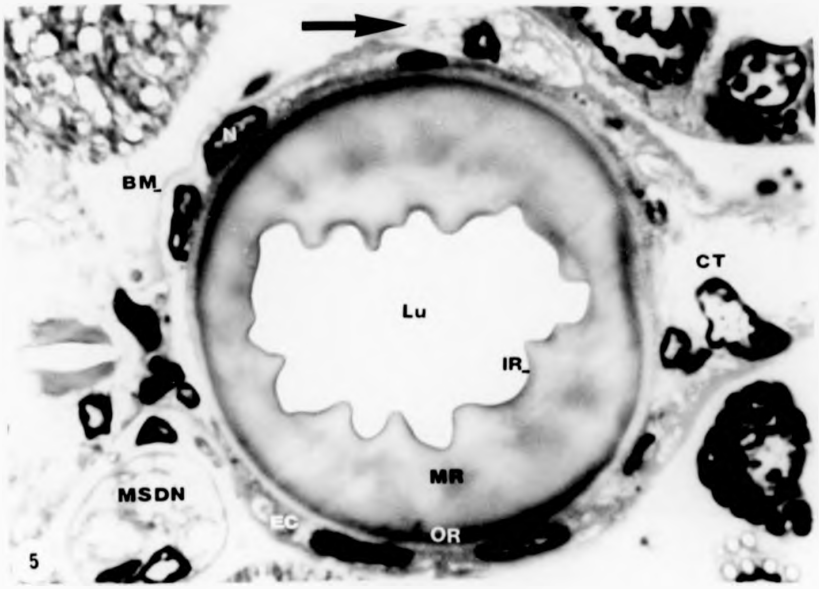

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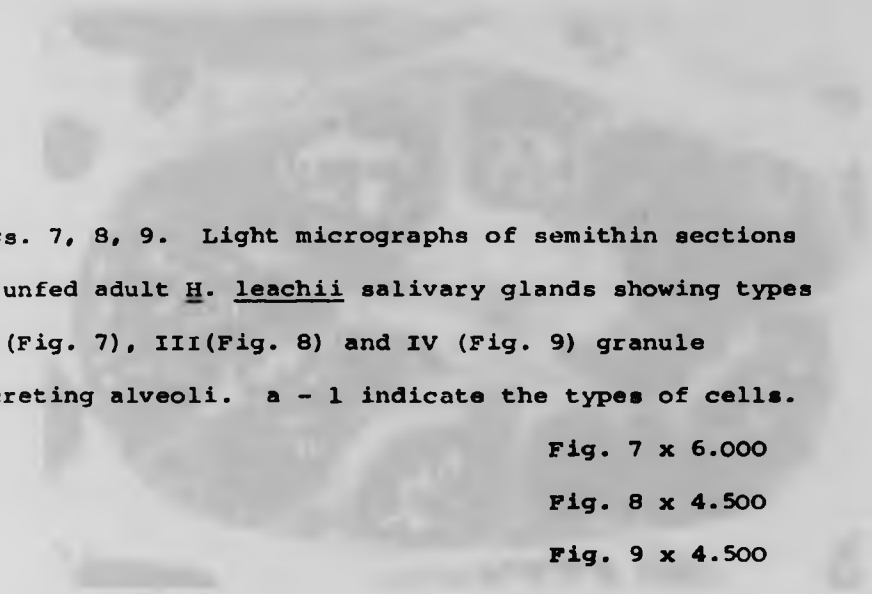


Figs. 7, 8, 9. Light micrographs of semithin sections of unfed adult H. leachii salivary glands showing types II (Fig. 7), III(Fig. 8) and IV (Fig. 9) granule secreting alveoli. a - l indicate the types of cells.

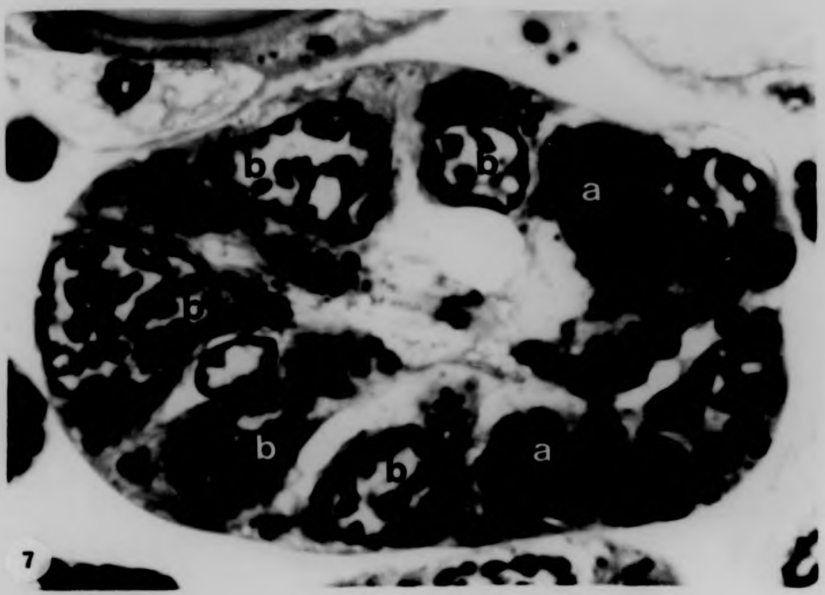
Fig. 7 x 6.000

Fig. 8 x 4.500

Fig. 9 x 4.500

Fig. lO. Paraffin sections of type IV alveoli after treatment with sudan black-B. As seen here, type j cells are moderately positive while type k cells are intensely positive.

x 3.000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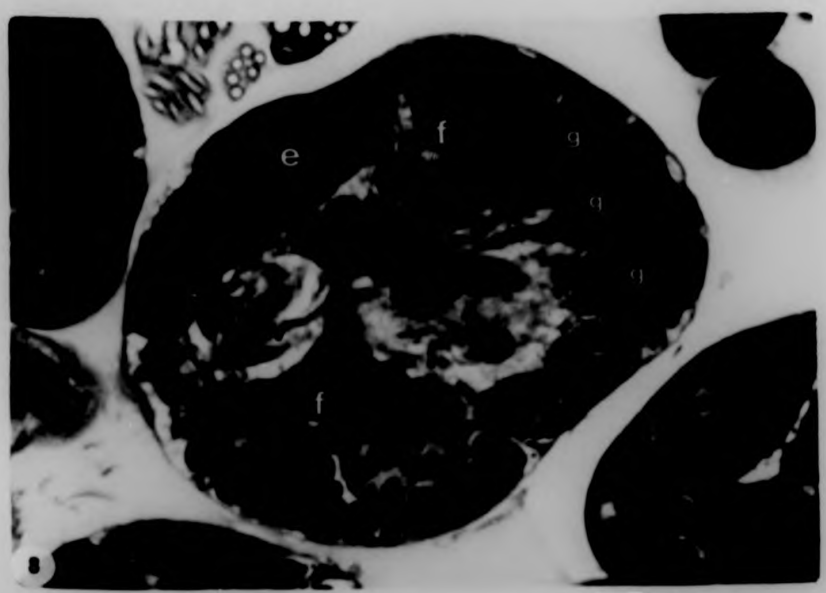
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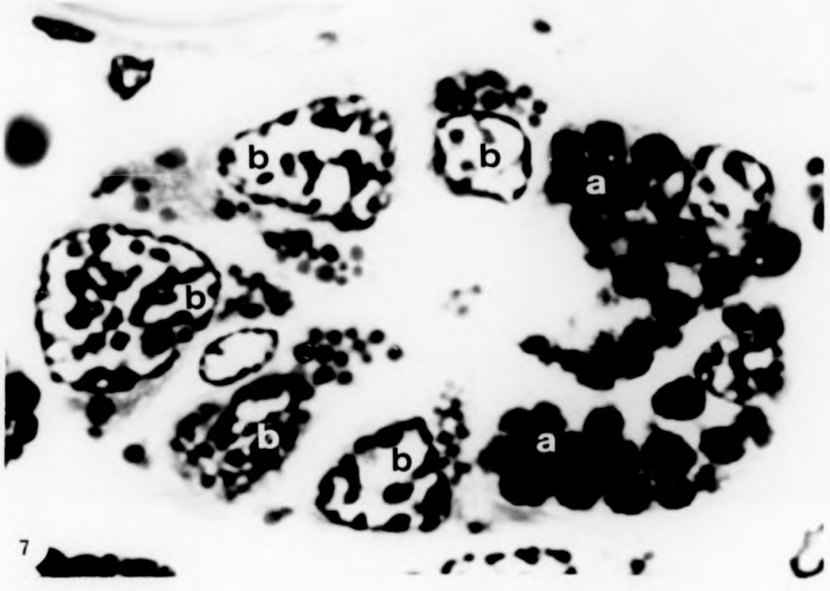

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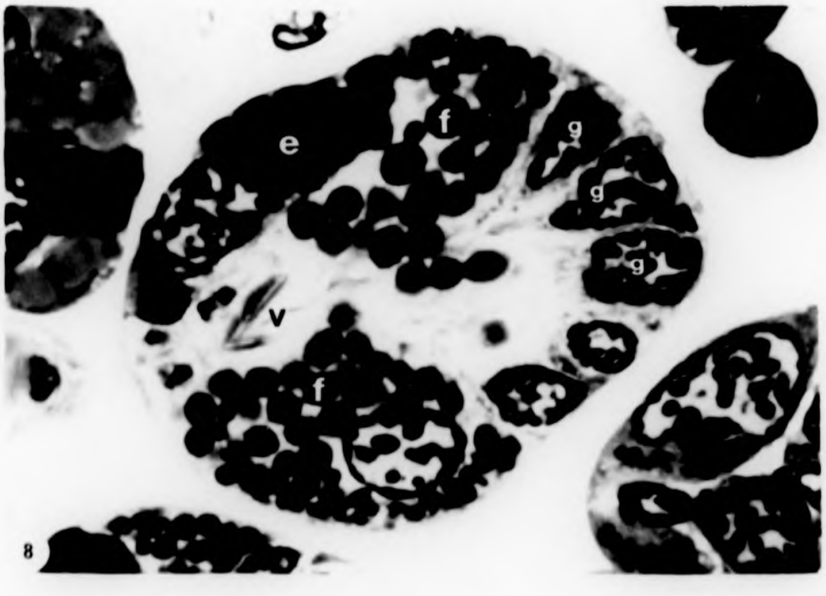
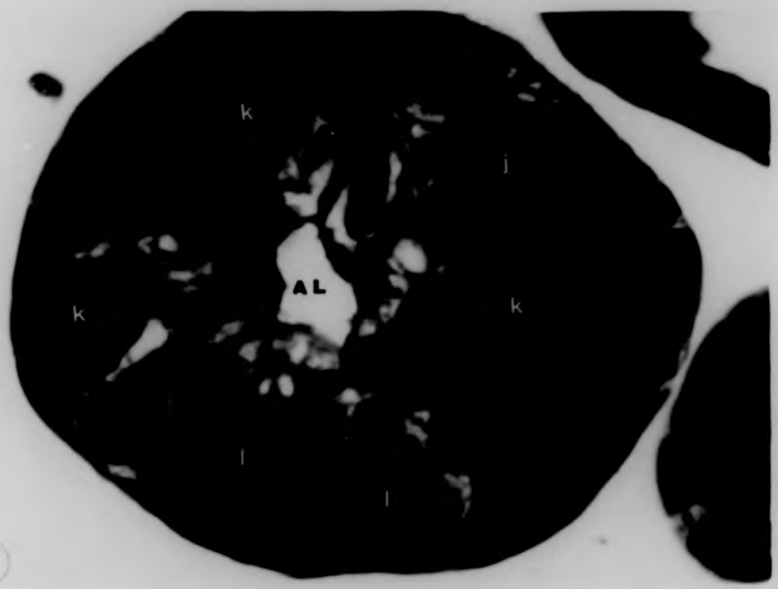


8



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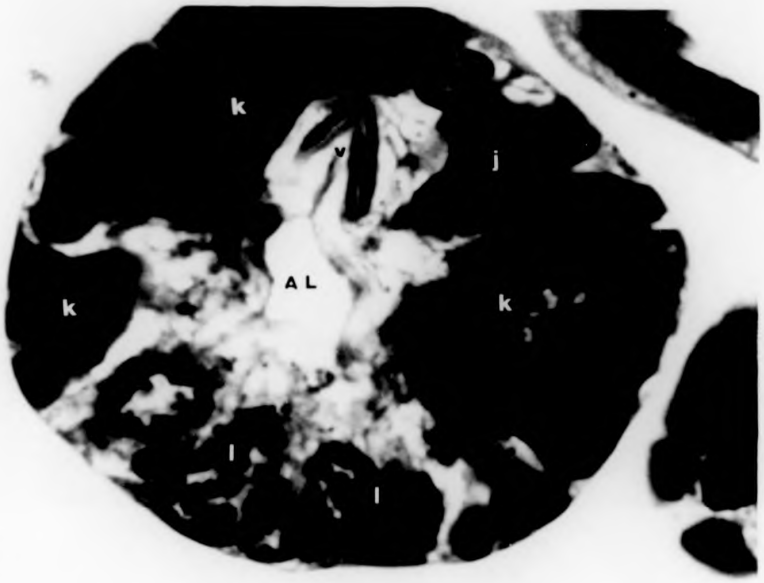

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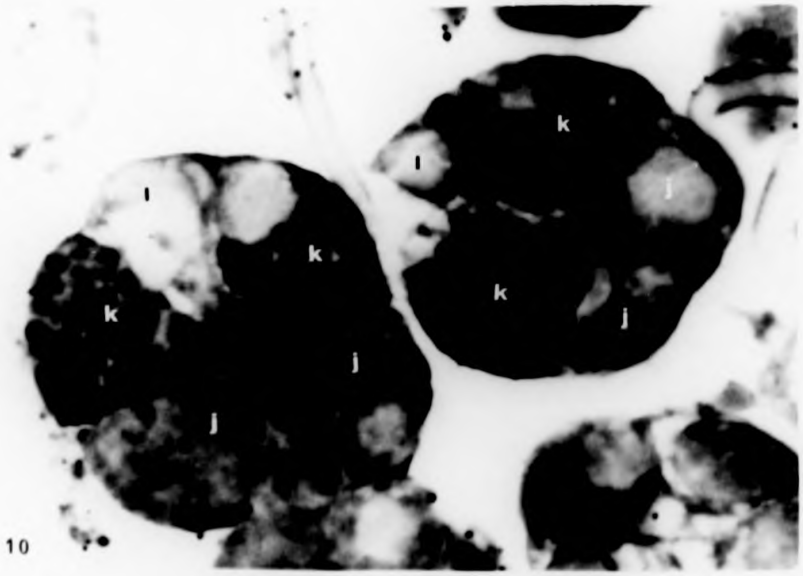

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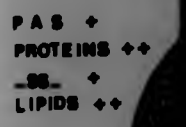
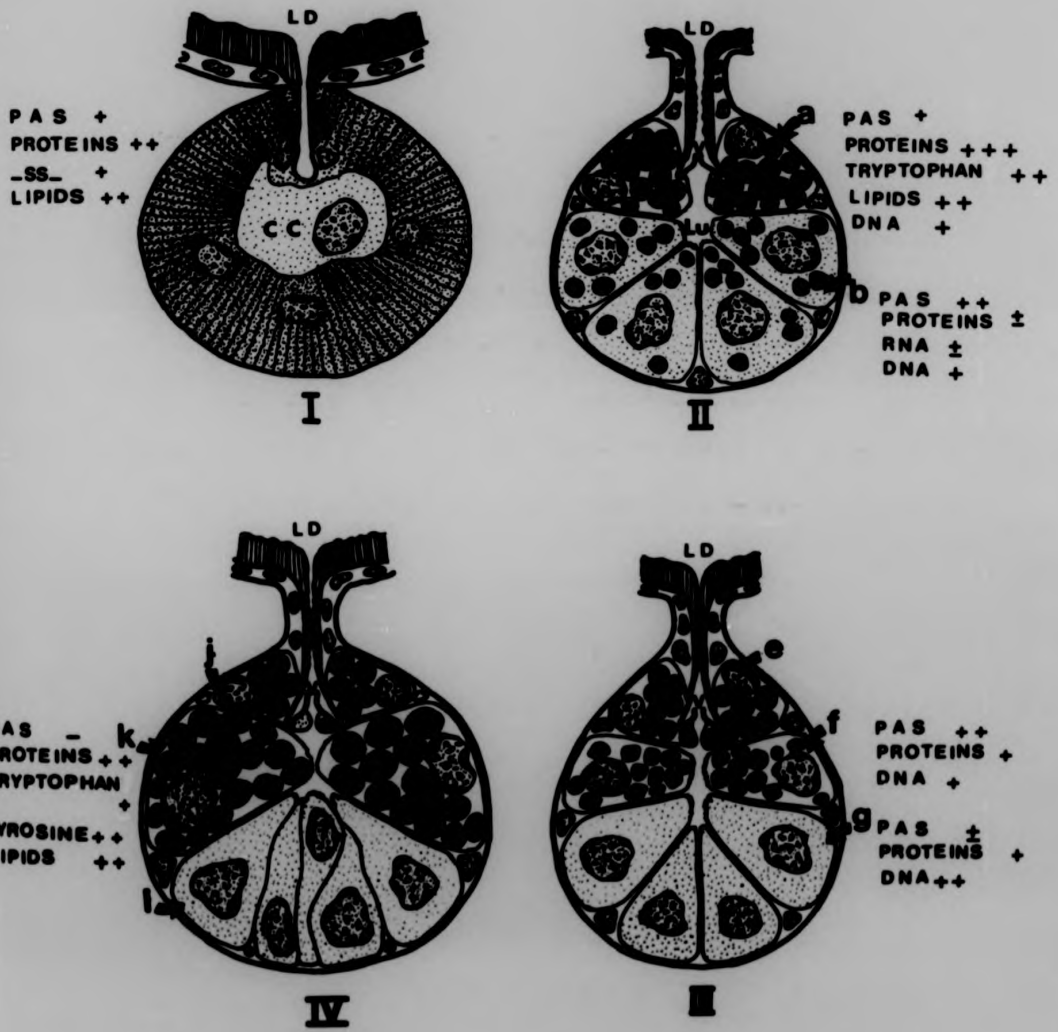


Fig. 11. Drawing of types I, II, III and IV alveoli in unfed adult H. leachii salivary glands to show the histochemical nature of the cells of these alveoli. Note that a, e and j cells are histochemically similar to each other. In addition, types g and l cells give the same histochemical reactions. Lu, alveolar lumen; cc, central cell; LD, lobular duct; V, valve-like structure.


LD
PAS +
PROTEINS ++
-SS- +
LIPIDS ++
CC
I
LD
a
PAS +
PROTEINS +++
TRYPTOPHAN ++
LIPIDS ++
DNA +
b
PAS ++
PROTEINS ±
RNA ±
DNA +
II
LD
PAS - k
PROTEINS ++
TRYPTOPHAN +
TYROSINE ++
LIPIDS ++
l
IV
LD
f
PAS ++
PROTEINS +
DNA +
g
PAS ±
PROTEINS +
DNA ++
III

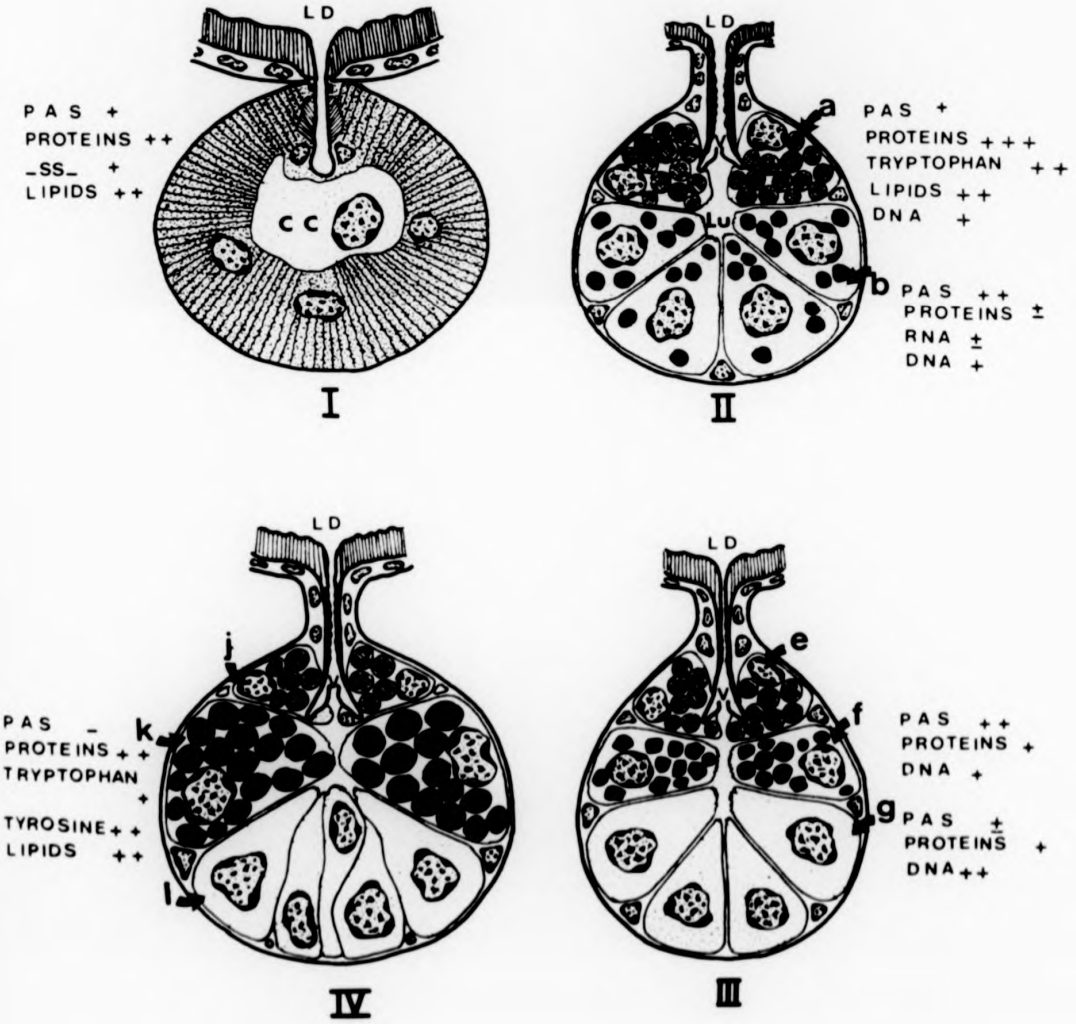
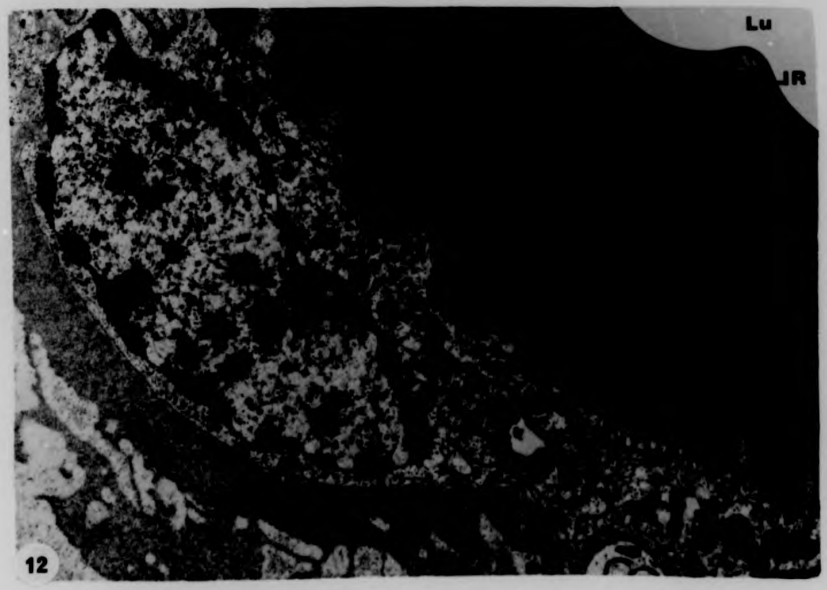

LD
PAS +
PROTEINS ++
-SS- +
LIPIDS ++
CC
I
LD
a
PAS +
PROTEINS +++
TRYPTOPHAN ++
LIPIDS ++
DNA +
Lu
b
PAS ++
PROTEINS ±
RNA ±
DNA +
II
LD
j
PAS -
k
PROTEINS ++
TRYPTOPHAN +
TYROSINE ++
LIPIDS ++
l
IV
LD
e
f
PAS ++
PROTEINS +
DNA +
g
PAS ±
PROTEINS +
DNA ++
III

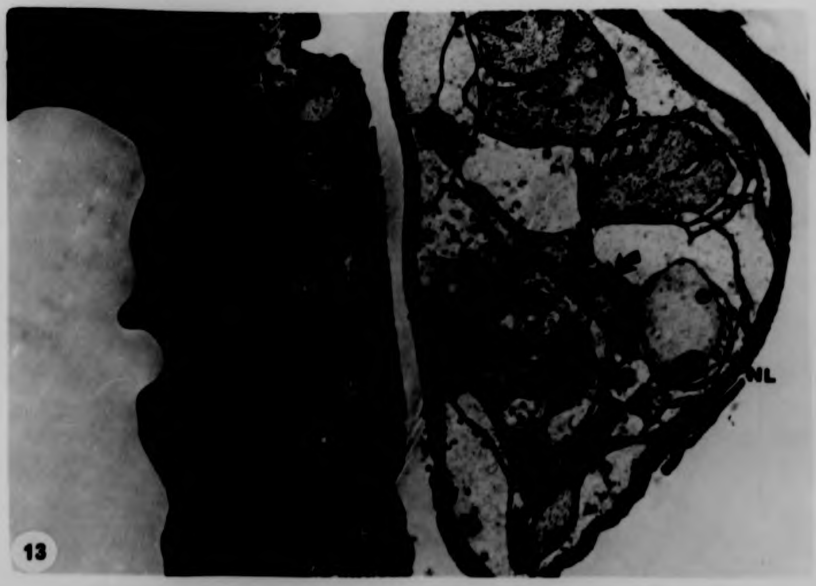
Fig. 12. Electron micrograph of a portion of unfed adult H. leachii main salivary duct. Arrow points to microtubules. BM, basement membrane; Db, dense bodies; IR, inner region; Lu, main duct lumen; M, mitochondria; MR, middle region; MV, microvilli; N, nucleus of epithelial cell; SD, septate desmosomes; ST, spiral thickening; Va, vacuoles.

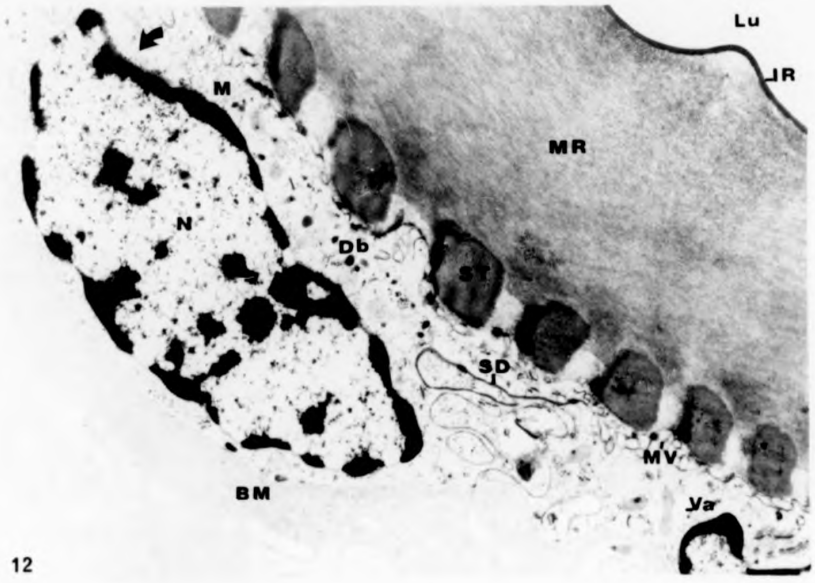
x 10.800

Fig. 13. Large nerve adjacent to the main salivary duct (MSD). ax, axons; NL, neural lamella. Arrows indicate neurosecretory vesicles.

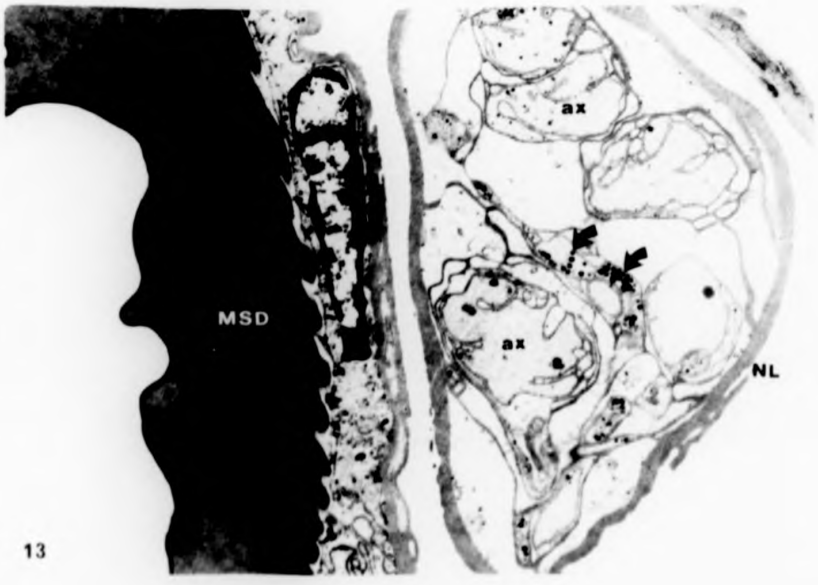
x 6.000


Lu
IR
12


NL
13



12



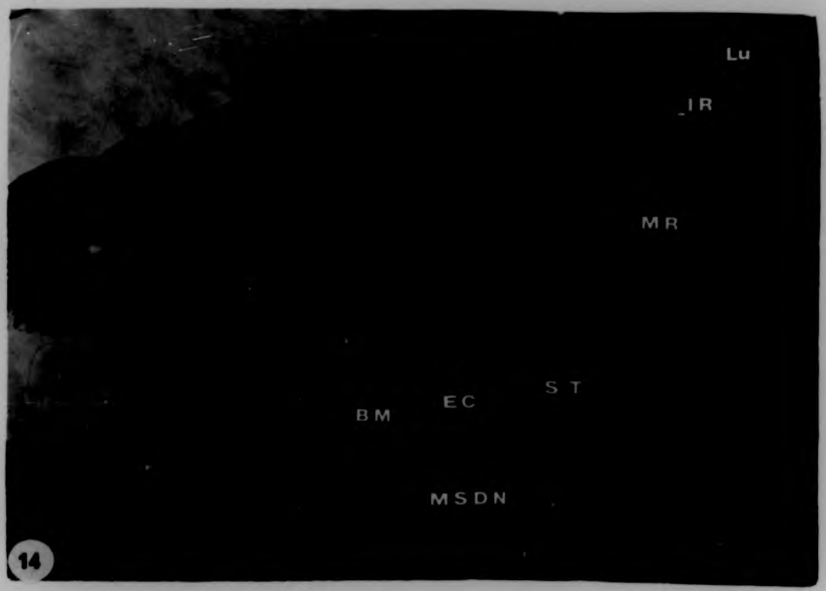
13

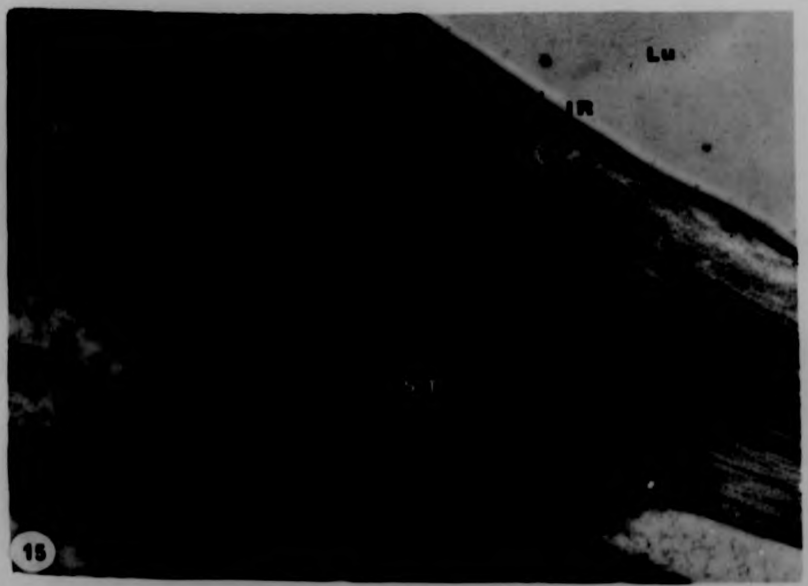
Fig. 14. Ultrastructural histochemical micrograph of unfed adult H. leachii main salivary duct after treatment with PA-TCH for polysaccharides. Note that the three regions of the cuticle and the epithelial cells (EC) as well as the neurosecretory vesicles (arrows) of the main salivary duct nerve (MSDN) are polysaccharide positive. The other letterings as in Fig. 12.

x 4.500

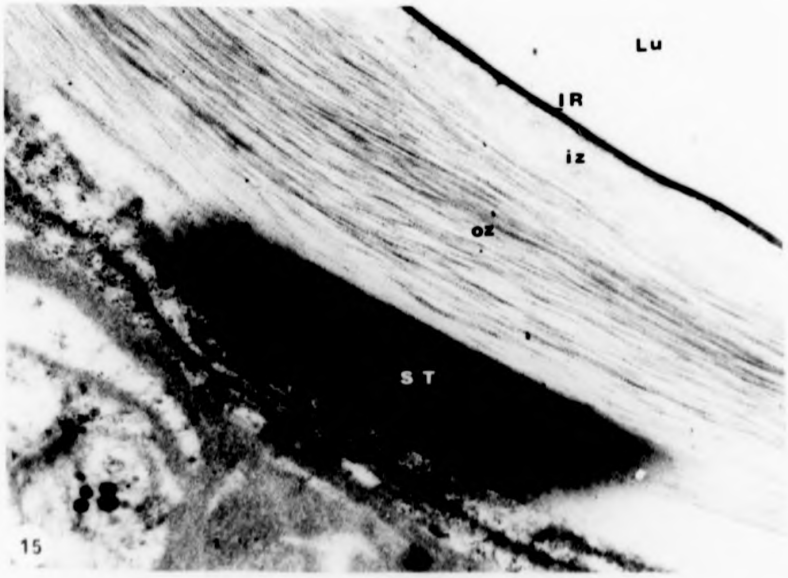
Fig. 15. The same as in Fig. 14 but after treatment with pronase enzyme. Note that the inner region (IR) and the spiral thickening (ST) are not digested, while the middle region is protein positive and two new zones are shown, an outer wide zone (oz) with parallel collagen-like fibres and an inner narrow zone (iz) lacking these fibres.

x 28.100


Lu
IR
MR
ST
EC
BM
MSDN
14


Lu
IR
15

14



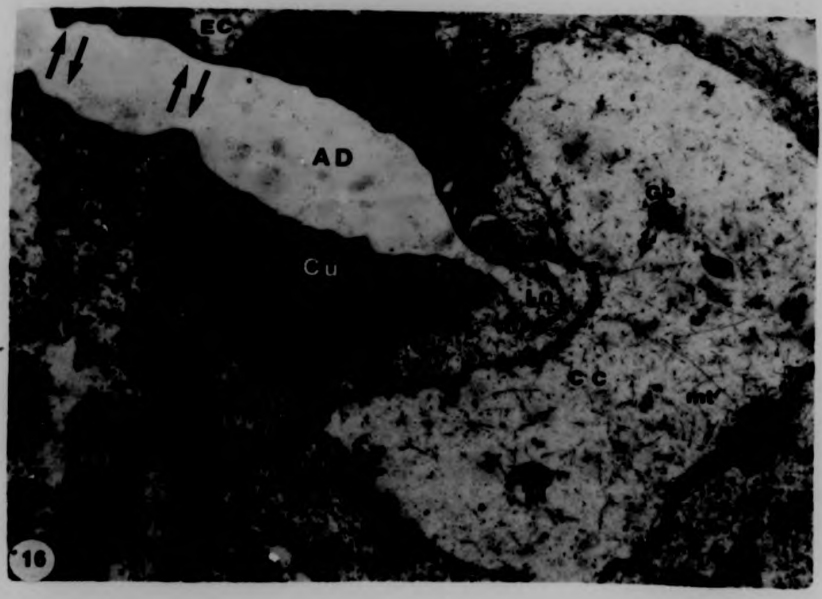
15

Fig. 16. Electron micrograph showing the apical region (AR) of type I non-granular alveolus. AD, alveolar duct; ax, axon; cc, central cell; Cu, cuticle lining of alveolar duct; Db, dense bodies; EC, epithelial cells; Gb, Golgi bodies; Lu, lumen of alveolar duct; M, mitochondria; mt, microtubules; SD, septate desmosomes. Large black arrows indicate area of flexibility. Small black arrows indicate small vesicles associated with Golgi bodies (Gb). Note that some microtubules are projected into the lumen of the alveolar duct.

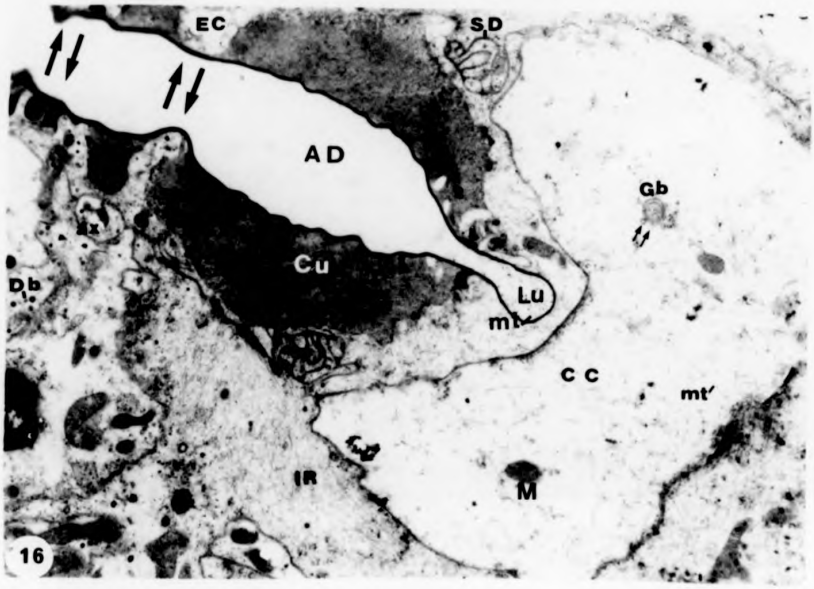
x 18.500

Fig. 17. The same as Fig. 16 but here showing the intermediate region (IR). Va, vacuoles. Black arrow indicates a junction between the basal membrane of the central cell (cc) and the apical membrane of a neighbouring small cell. White arrow indicates a synaptic area in non-granular alveolus. Empty arrows indicate small, short, isolated cisternae of rough endoplasmic reticulum. Note the difference in density of the cytoplasm of the central cell and its neighbouring cells.

x 18.000


EC
AD
Cu
Gb
CC
16


N
17


EC
SD
AD
Gb
Cu
Db
Lu
mt
CC
mt'
IR
M
16

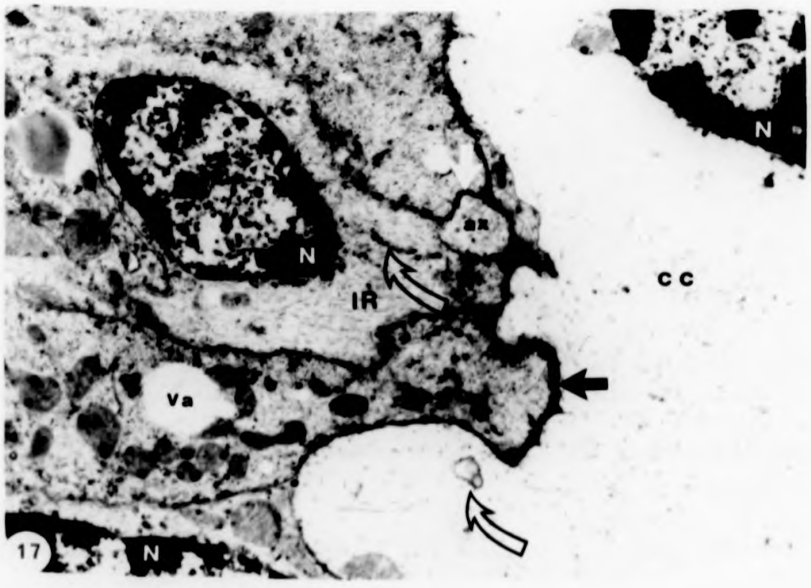

N
N
IR
CC
Va
N
17

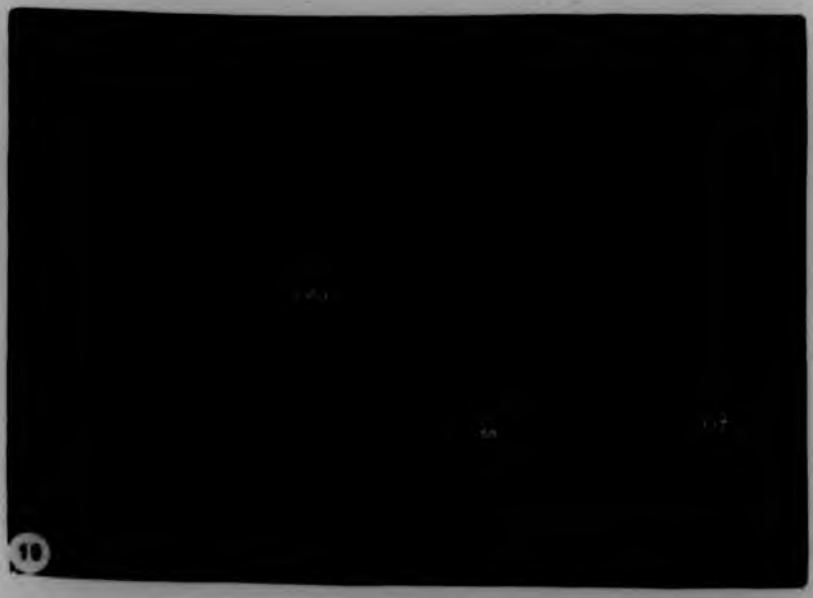
Fig. 18. Electron micrograph showing the basal region (BR) and the intermediate region (IR) of type I alveolus. Arrows indicate the numerous membrane infoldings; other letterings as in Fig. 17.

x 12.000

Fig. 19. Ultrastructural histochemical micrograph of type I non-granular alveolus in the area between the basal and intermediate regions, after treatment with PA-TCH. Note that the glycogen particles (Gl), vacuoles (Va), mitochondria (M), ribosomes (arrows) and the infoldings of the plasma membrane are polysaccharides positive.

x 38.300


IR
BR
ax
18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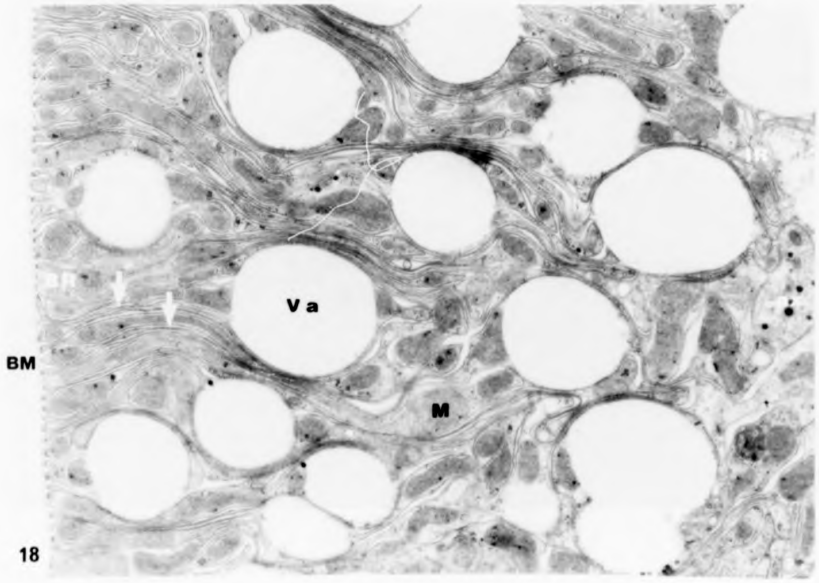
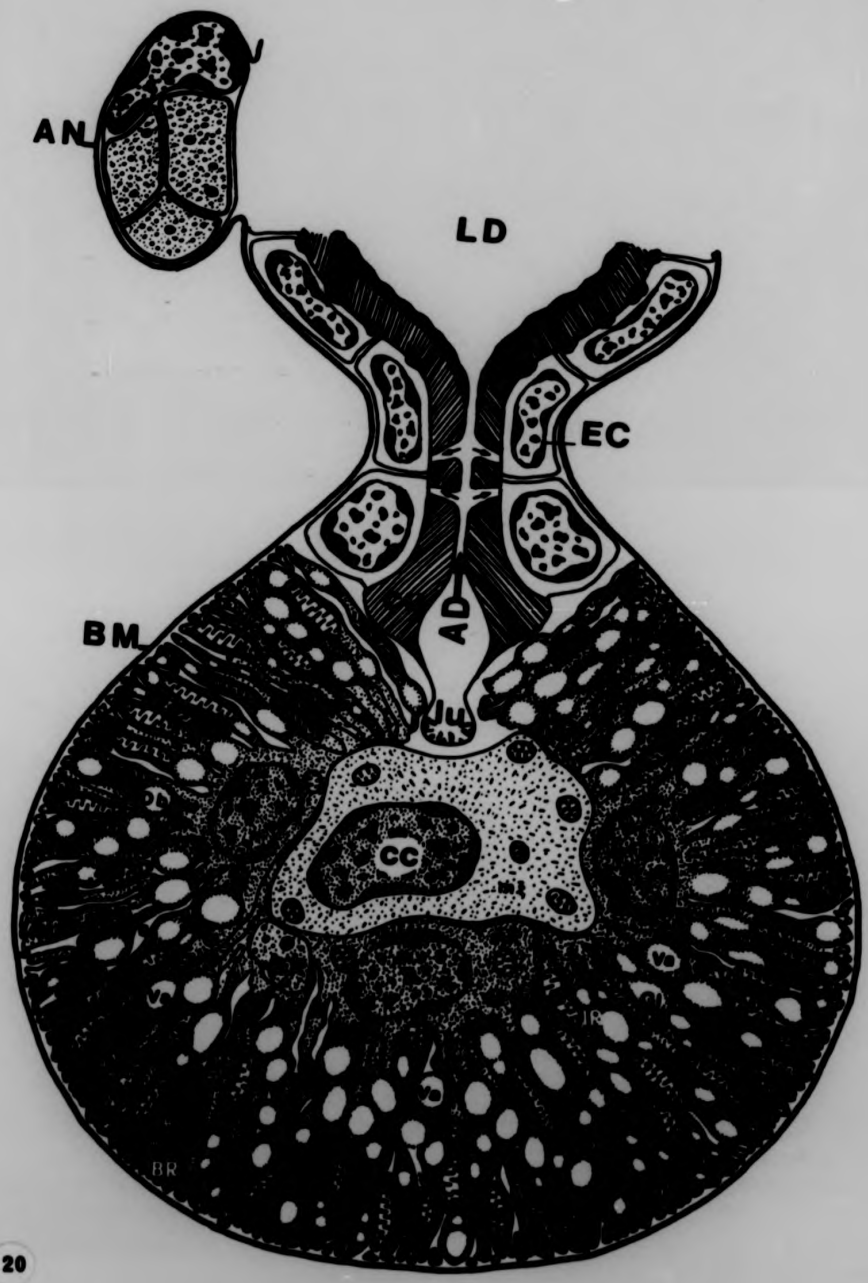


18

19

Fig. 20. Reconstructed line drawing of type I alveolus in unfed adult H. leachii salivary glands at the electron microscopic level. AD, alveolar duct; AN, alveolar nerve; AR, apical region; ax, axon; BM, basement membrane; BR, basal region; cc, central cell; Cu, cuticle; Db, dense bodies; Gl, glycogan particles; IR, intermediate region; Lu, lumen; M, mitochondria; mt, microtubules; Va. vacuoles. Arrows indicate area of flexibility.


AN
LD
EC
BM
AD
CC
BR

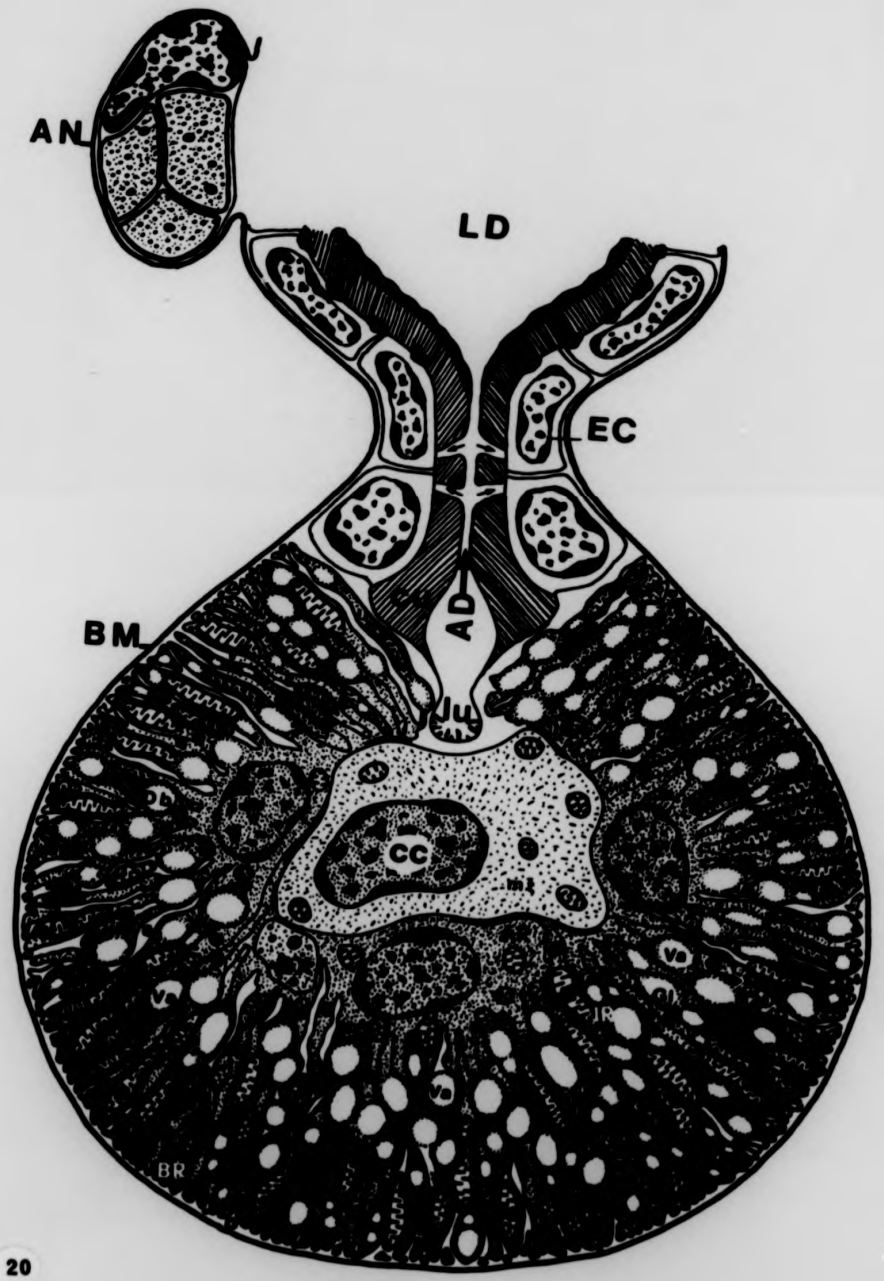
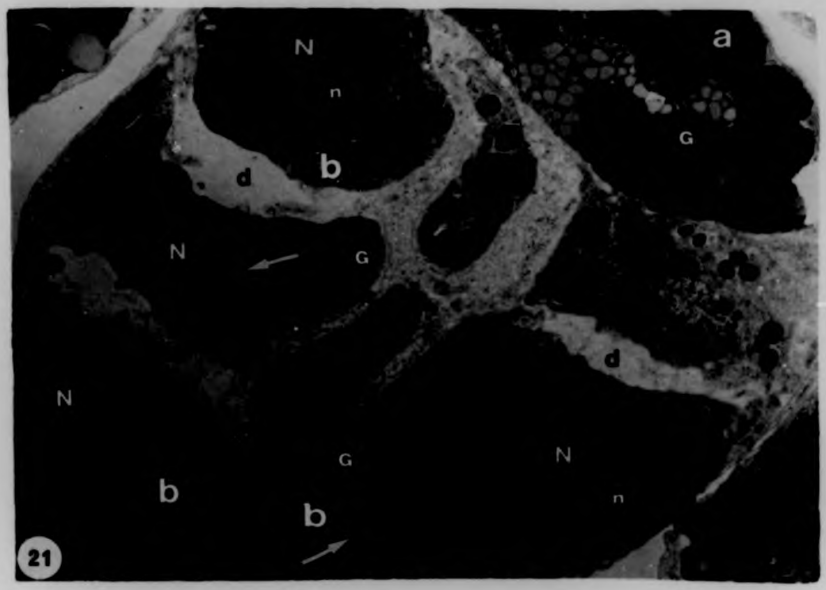

AN
LD
EC
BM
AD
CC
VD
BR

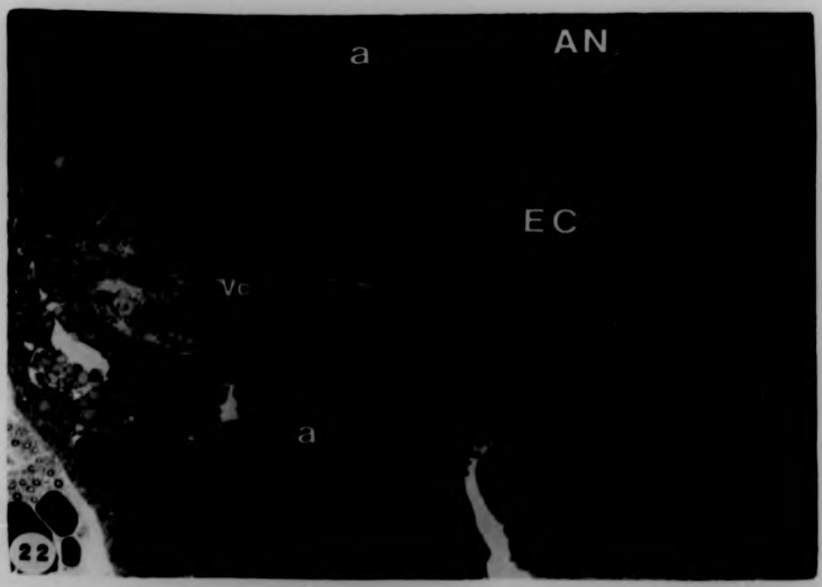
Fig. 21. Electron micrograph of type II granule-secreting alveolus of unfed adult H. leachii salivary glands. a - d, types a - d cells; G, secretory granules; N, nucleus; n, nucleolus; arrows indicate the indented surface of the nuclear membrane of type b cells.

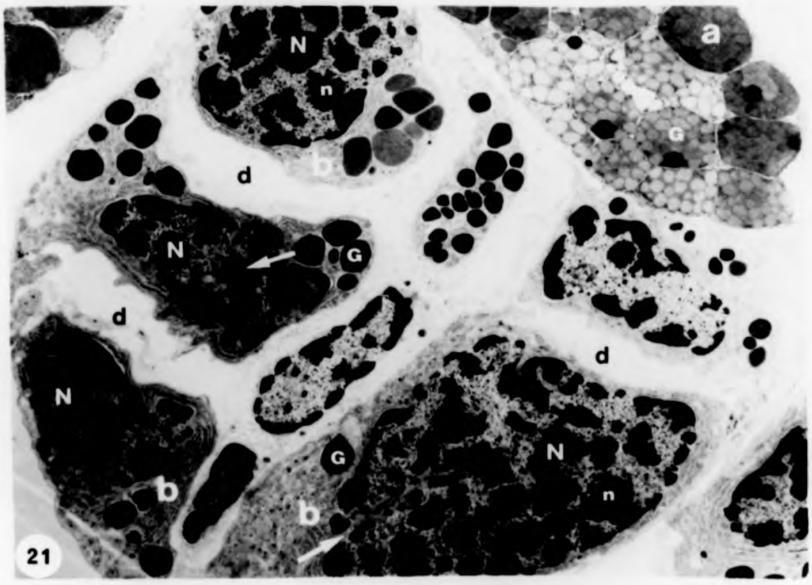
x 4.500

Fig. 22. Electron micrograph of a portion of type II alveolus showing the alveolar duct (AD) and the lobular duct (LD). a, type a cells, AN, alveolar nerve; EC, epithelial cells; VC, valvular canal. Arrows indicate area of flexibility.

x 4.300


N
n
a
G
b
d
N
G
d
N
G
N
b
n
b
21


a
AN
EC
Vc
a
22


N
n
a
G
d
N
G
d
d
N
G
N
b
b
n
21

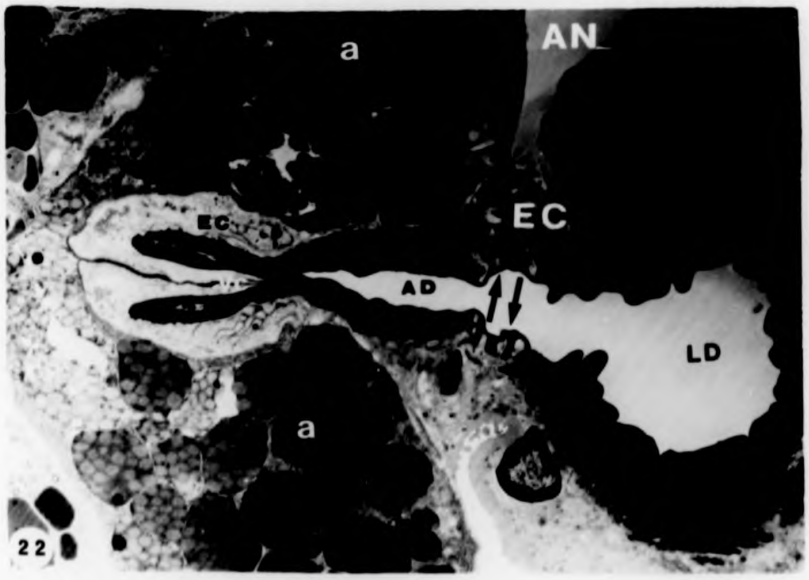
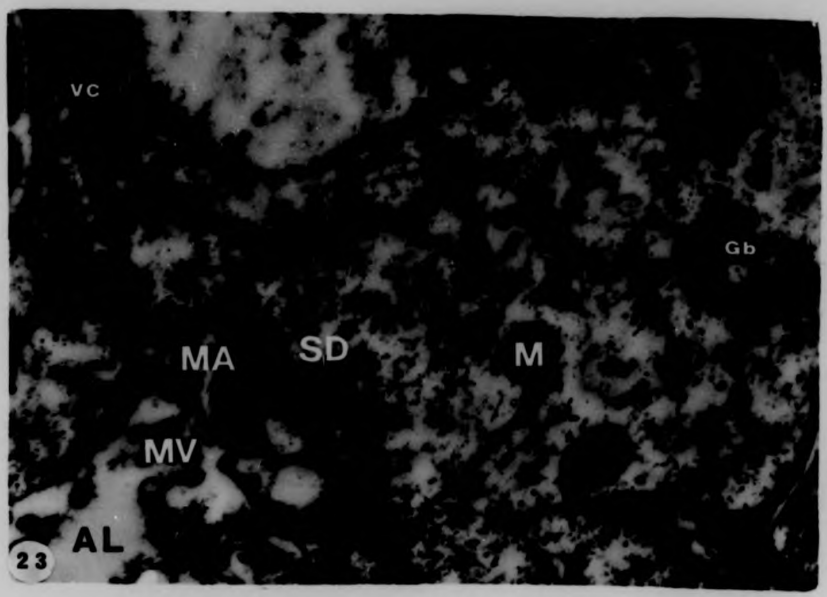

a
AN
EC
EC
AD
LD
a
22

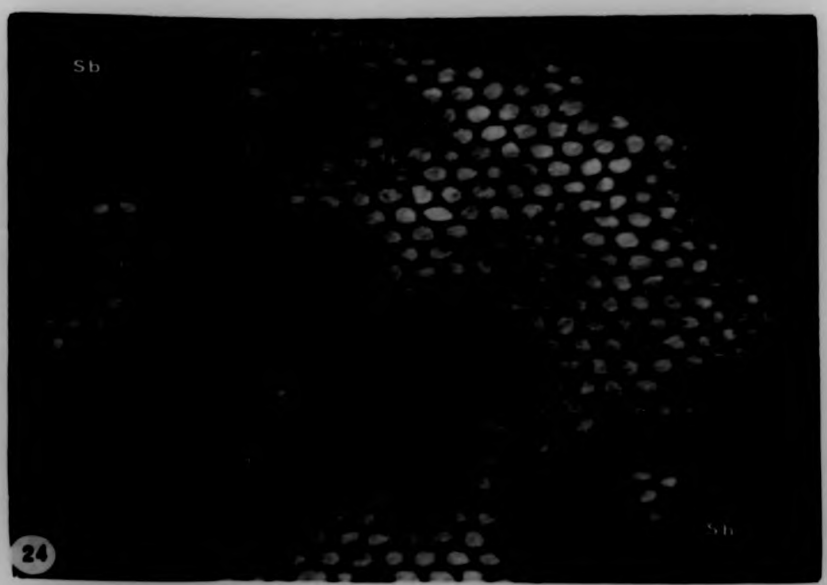
Fig. 23. Electron micrograph of a portion of type II alveolus. Note that the valvular canal (VC) is surrounded by two large curving epithelial cells, containing Golgi bodies (Gb) giving rise to small vesicles (small arrows) and connected with the neighbouring cells by means of macula adherans (MA) and septate desmosomes (SD). Large arrows point to microtubules. AL, alveolar lumen; M, mitochondria; MV, microvilli.

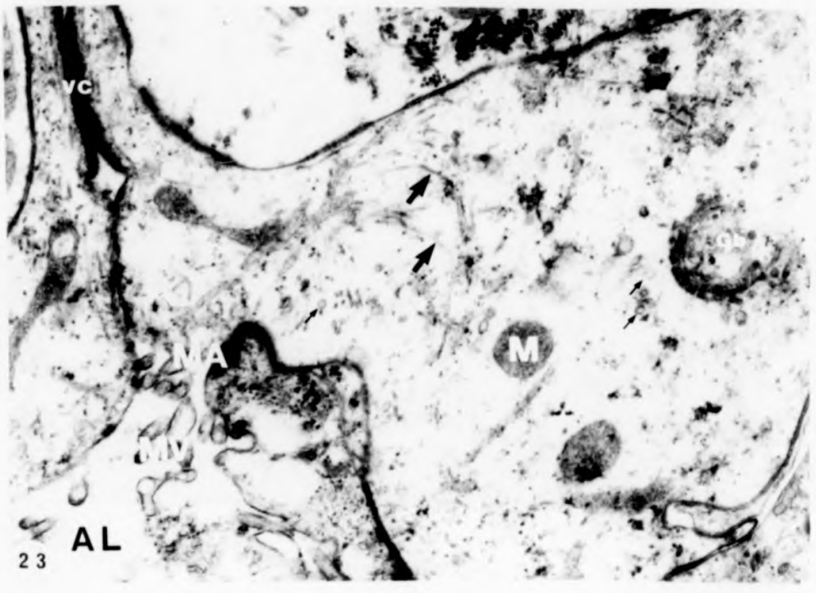
x 32.850

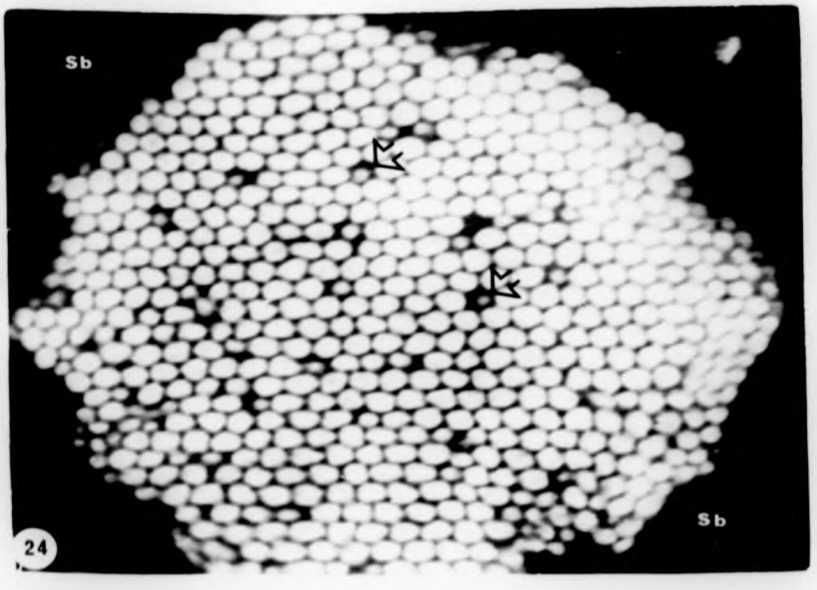
Fig. 24. High magnification micrograph of a granule of type a cell in alveolus II showing the pentagonal and hexagonal lattice matrix with peripheral area occupied by a membrano-bound subunit (sb). Note that some of these lattice vacuoles are more dense than the rest (arrows).

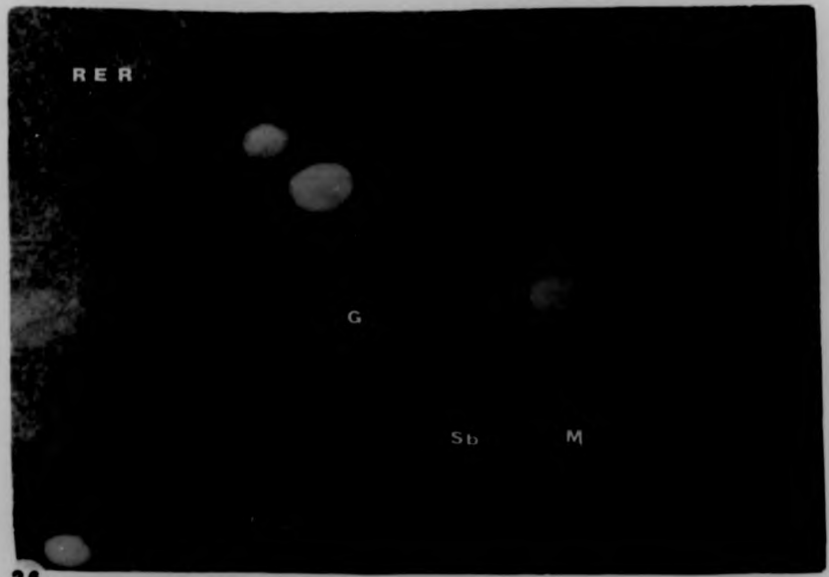
x 92.570


VC
Gb
MA
SD
M
MV
AL
23


Sb
Sb
24


VC
M
AL
23


Sb
Sb
24



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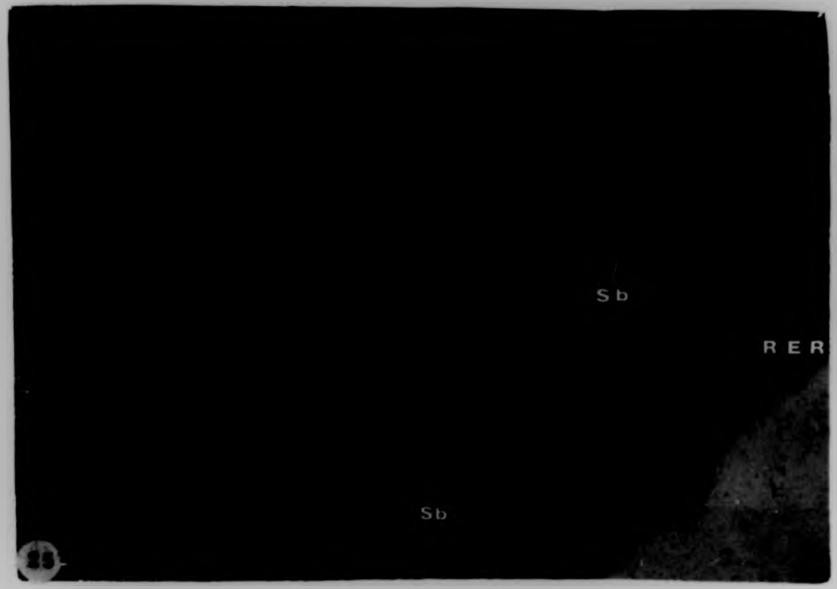
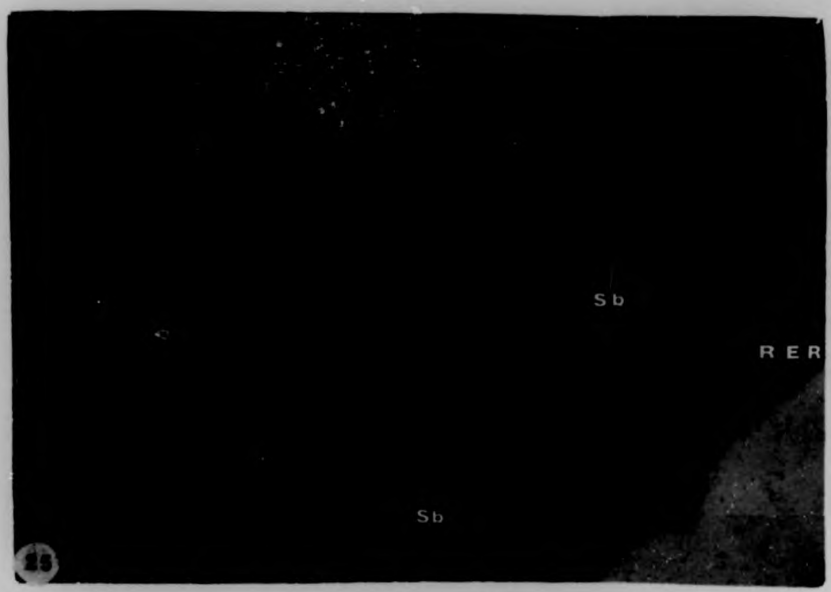

Sb
RER
Sb

Fig. 25. Electron micrograph of a portion of type a cell showing the membrane-bound granules. Note that some subunits (sb) are denser than the rest. BM, basement membrane; RER, rough endoplasmic reticulum.

x 35.500

Fig. 26. Ultrastructural histochemical micrograph of a portion of type a cell after treatment with PA-TCH for polysaccharides. The reaction is moderately positive. Note the isolated subunit which is embedded in the cytoplasm (arrow). G, secretory granules; M, mitochondria. Other lettering as Fig. 25.

x 32.850



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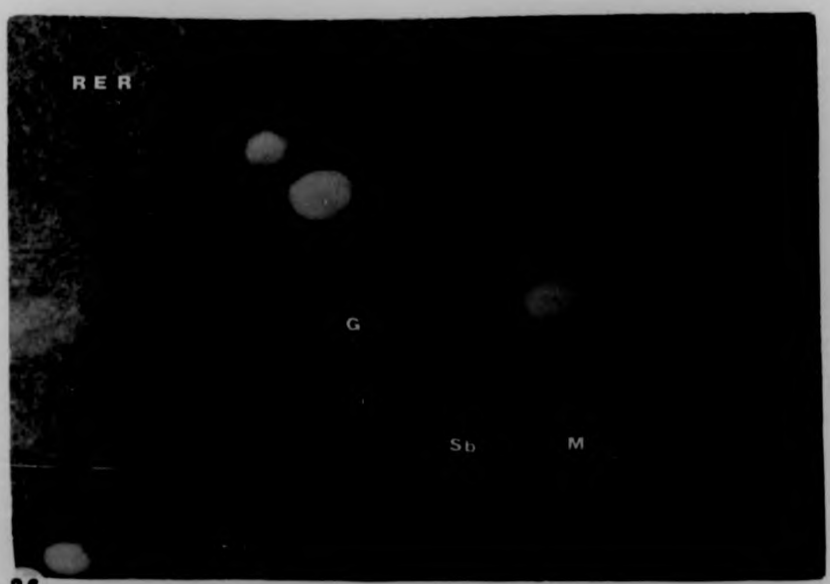

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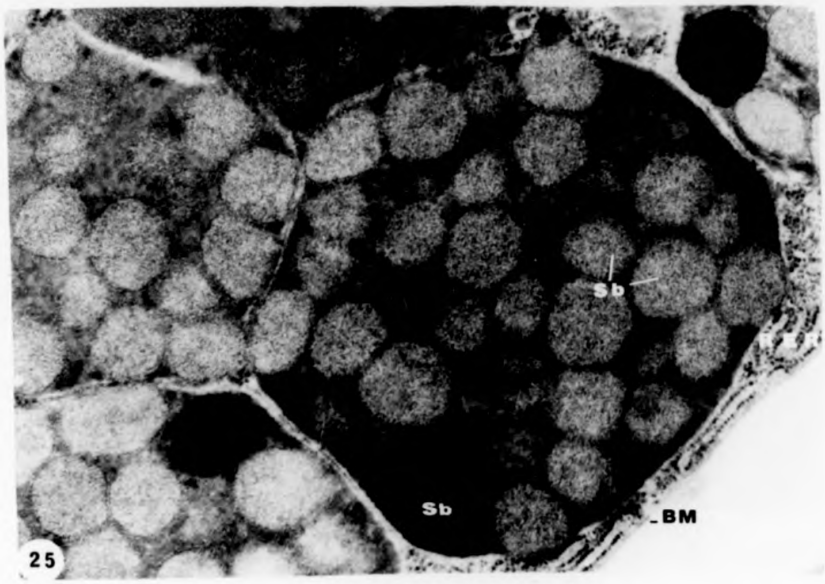

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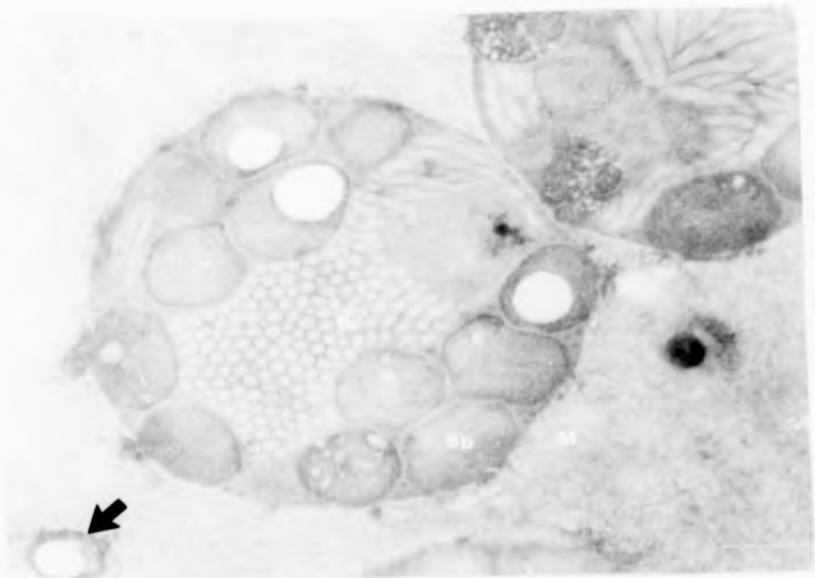


26

Fig. 27. The same as in Fig. 26 but after treatment with pronase enzyme for proteins. Note that the content of the subunits has been completely digested by the enzyme while the lattice matrix of the secretory granules is not affected

x 9.300

Fig. 28. Electron micrograph showing types b and c cells of alveolus II in unfed adult H. leachii salivary glands. Note the position of type c cell between the sides and apical regions of type b cells. AL, alveolar lumen; ax, axon; d, type d cells (narrow interstitial epithelial cells); G, secretory granules; M, mitochondria; MV, microvilli; N, nucleus; RER, rough endoplasmic reticulum. Long arrow points to short, narrow cisternae of rough endoplasmic reticulum. Thick arrows point to intercellular junctions between types b and d cells and between types b and c cells.

x 9.800

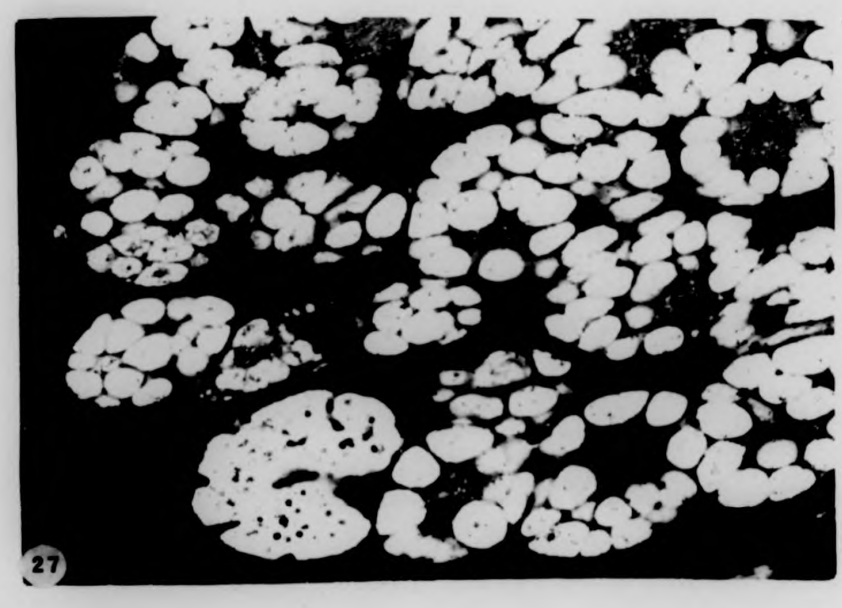
21
28

Fig. 27. The same as in Fig. 26 but after treatment with pronase enzyme for proteins. Note that the content of the subunits has been completely digested by the enzyme while the lattice matrix of the secretory granules is not affected

x 9.300

Fig. 28. Electron micrograph showing types b and c cells of alveolus II in unfed adult H. leachii salivary glands. Note the position of type c cell between the sides and apical regions of type b cells. AL, alveolar lumen; ax, axon; d, type d cells (narrow interstitial epithelial cells); G, secretory granules; M, mitochondria; MV, microvilli; N, nucleus; RER, rough endoplasmic reticulum. Long arrow points to short, narrow cisternae of rough endoplasmic reticulum. Thick arrows point to intercellular junctions between types b and d cells and between types b and c cells.

x 9.800



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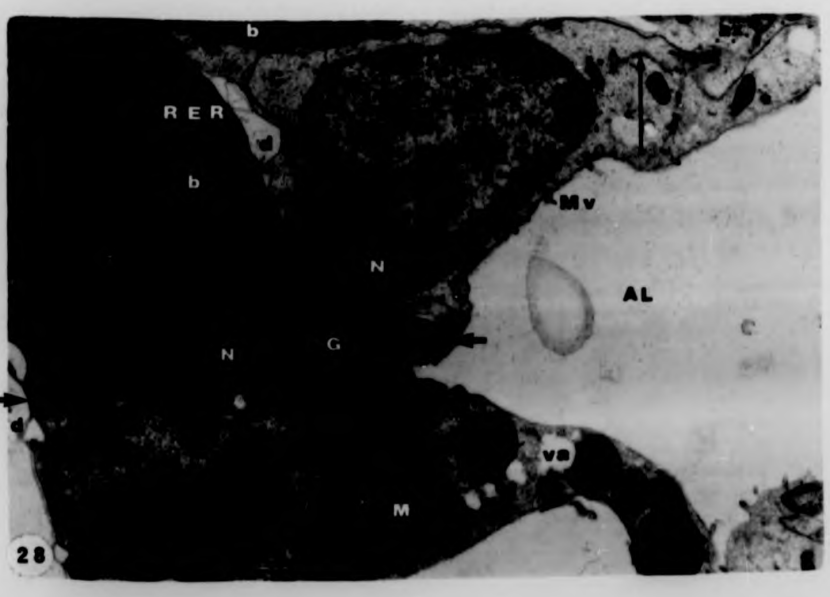


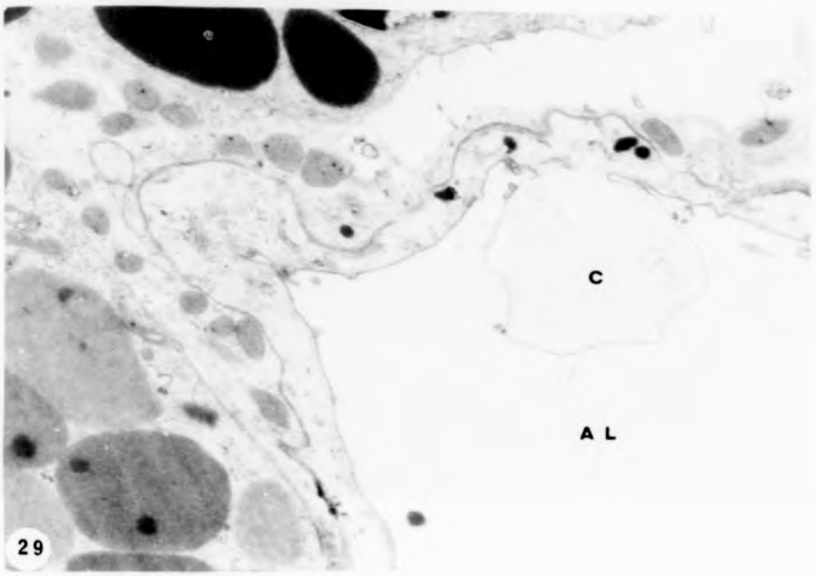
28

Fig. 29. Electron micrograph of a portion of type c cell showing a swollen portion of the cell membrane into the lumen.

x 13.500

Fig. 30 Electron micrograph of type III alveolus of unfed adult H. leachii salivary glands. e - i, types e - i cells; AL, alveolar lumen; G, secretory granules. Note the axon which is embedded between the cap cells, the interstitial epithelial cells and type f cells.

x 10.500



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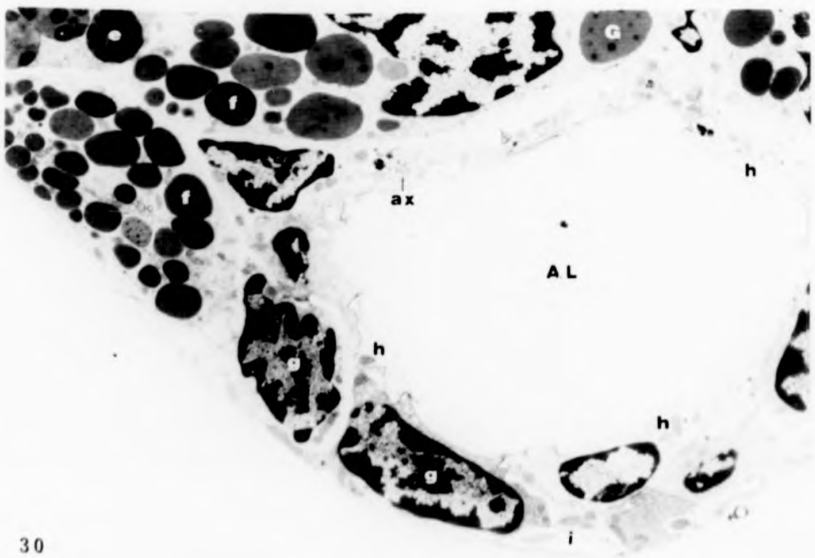


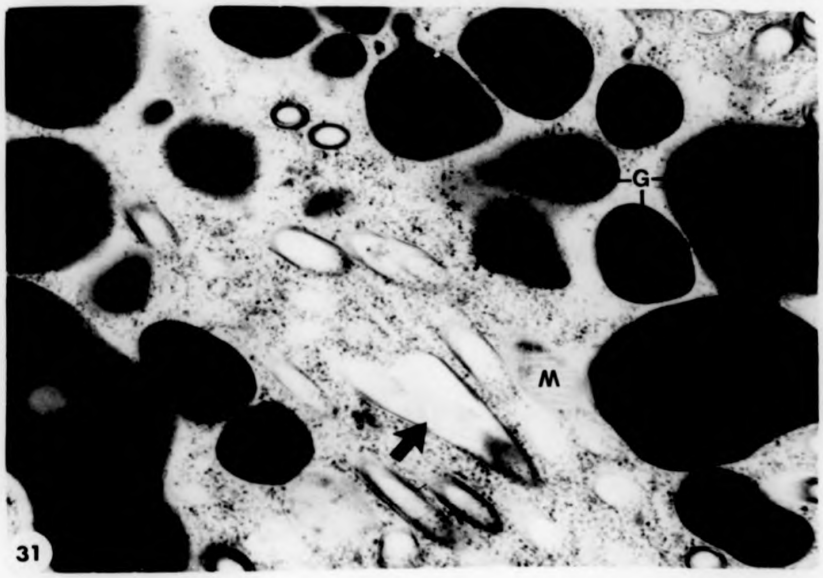
30

Fig. 31. Electron micrograph of a portion of type f cells in alveolus III showing many macrotubules with electron dense double membranes embedded in the cytoplasm. Some of these macrotubules enclose electron-lucent structures with fine membranes (arrow); G, secretory granules; M, mitochondrion.

x 34.500

Fig. 32. Electron micrograph showing types g and h cells of type III alveoli. Note the junction between types g and h cells (cap cells) which border the alveolar lumen (AL) and between types g and i cells (interstitial epithelial cells). BM, basement membrane; MV, microvilli; Tr, trachea.

x 11.480



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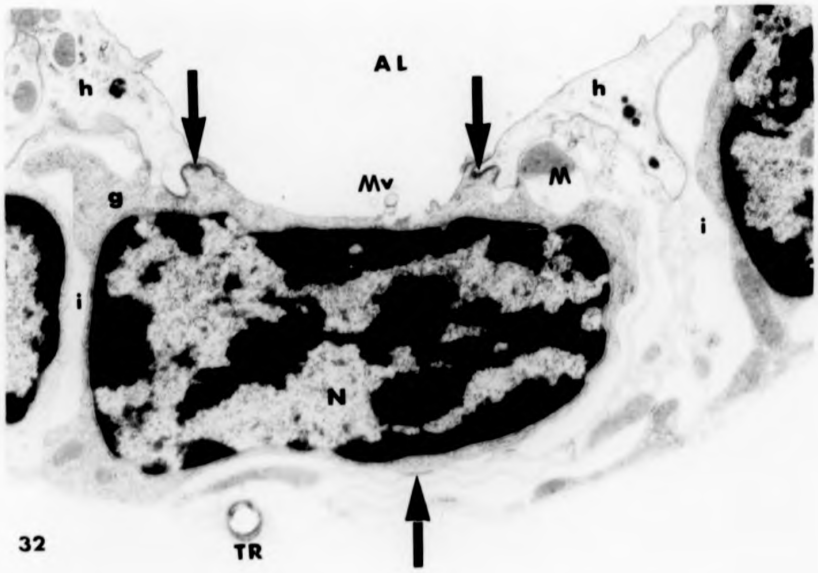


32

Fig. 33. Electron micrograph of the lumen of type III alveolus showing the crowded microvilli projecting from the apical plasma membranes of types g and i cells. Note the junction between the apical sides of both types of cell. MA, macula adherans; N, nucleus; SD, septate desmosomes.

x 27.600

Fig. 34. Electron micrograph of type IV alveolus of unfed adult H. leachii salivary glands. AL, alveolar lumen; G, secretory granules; N, nucleus; j - 1, types j - 1 cells.

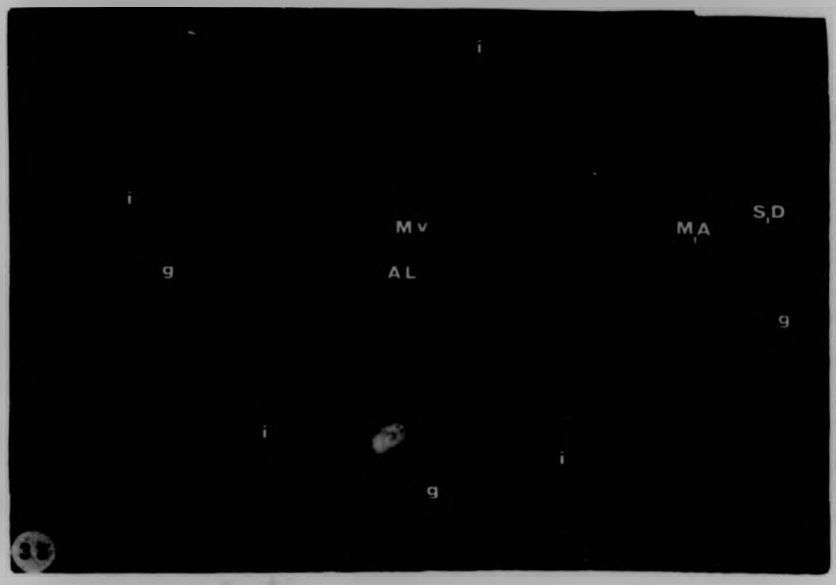
x 4.275

Fig. 33. Electron micrograph of the lumen of type III alveolus showing the crowded microvilli projecting from the apical plasma membranes of types g and i cells. Note the junction between the apical sides of both types of cell. MA, macula adherans; N, nucleus; SD, septate desmosomes.

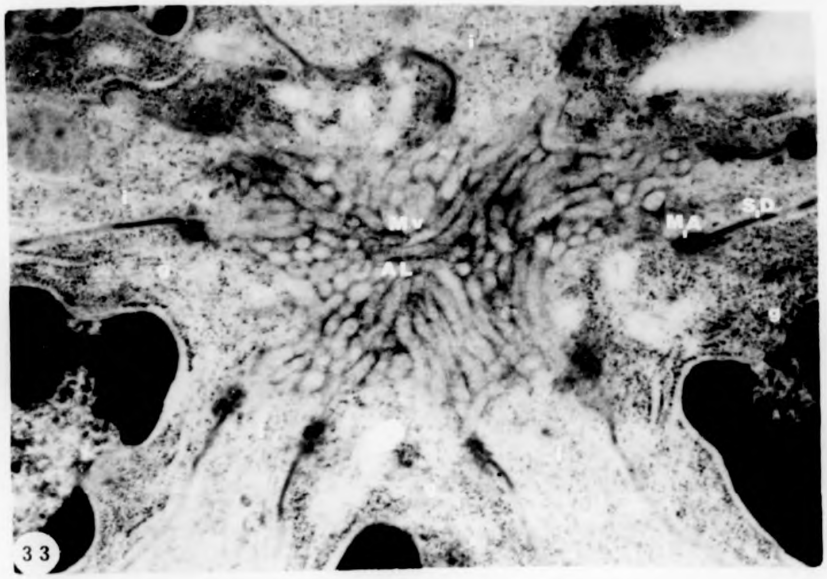
x 27.600

Fig. 34. Electron micrograph of type IV alveolus of unfed adult H. leachii salivary glands. AL, alveolar lumen; G, secretory granules; N, nucleus; j - l, types j - l cells.

x 4.275


i
i
Mv
M,A
S,D
g
AL
g
i
i
g


AL


MV
MA
S.D.
33

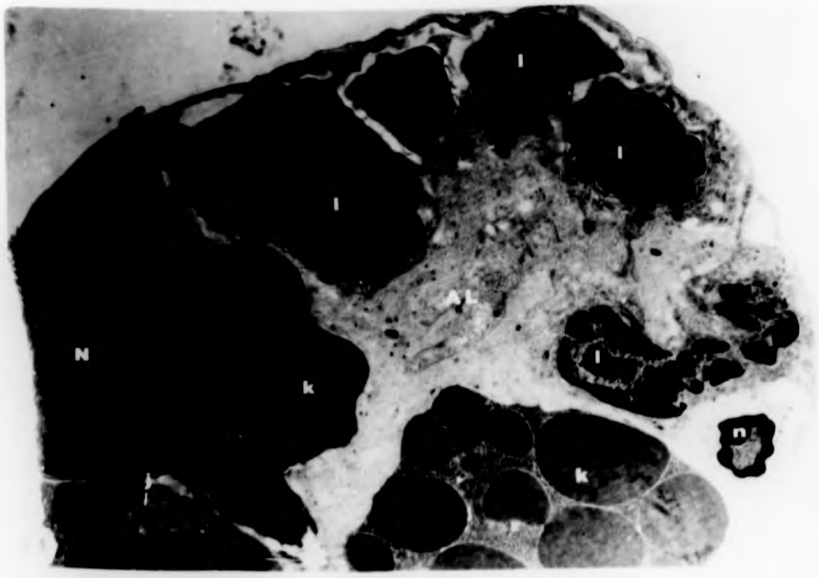
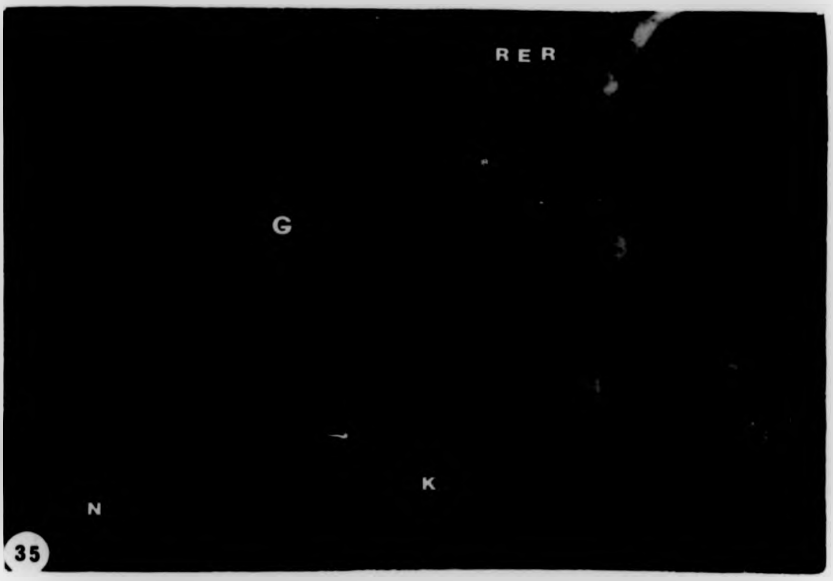

I
I
I
N
k
k
Pl

Fig. 35. High magnification micrograph of a portion of type k cells showing reticular substructure of the secretory granules (G). M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum.

x 29.180

Fig. 36. The same as Fig. 35 but whorls of rough endoplasmic reticulum (RER) show a beaded appearance due to alternate distended and non-distended regions. Note some cisternae contain small intercisternal granules (thin arrow), while others are fully packed (thick arrows). BM, basement membrane.

x 29.570



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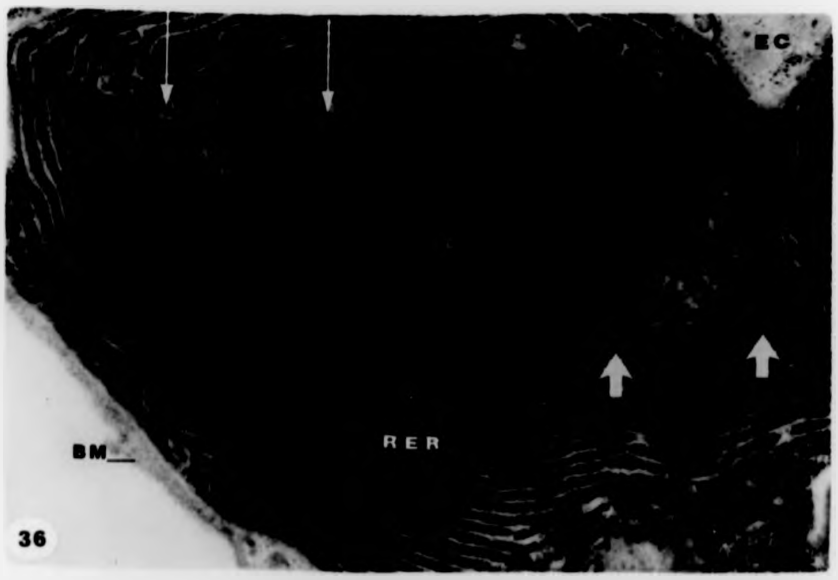


36

Fig. 37. Ultrastructural histochemical micrograph of type k cells (k) after treatment with pronase enzyme for protein detection. The granules (G) are partially digested. Compare the colour intensity of the granules in this micrograph with that of Fig. 35.
n, type n cells (interstitial epithelial cells);
N, nucleus; RER, rough endoplasmic reticulum.

x 33.890

Fig. 38. Electron micrograph of type 1 cells in alveolus IV. M, mitochondria; N, nucleus;
n, type n cells (the interstitial epithelial cells).

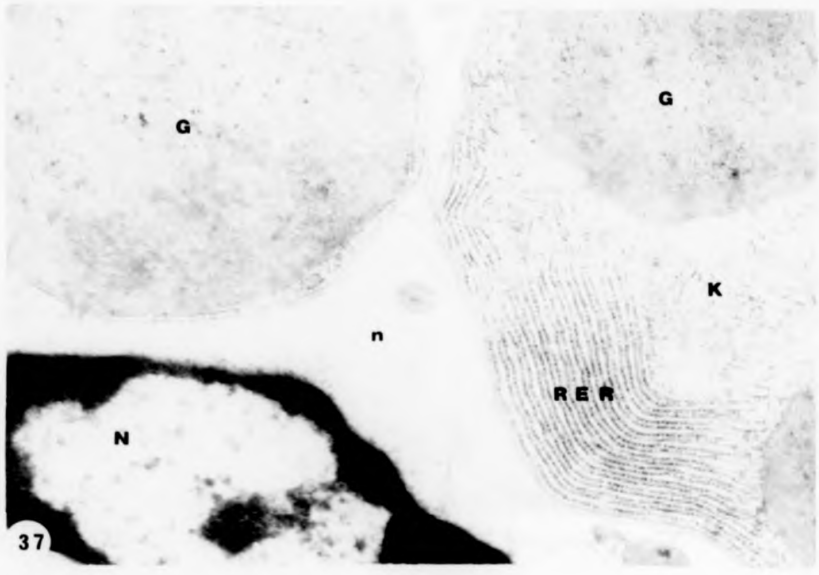
x 28.100

Fig. 37. Ultrastructural histochemical micrograph of type k cells (k) after treatment with pronase enzyme for protein detection. The granules (G) are partially digested. Compare the colour intensity of the granules in this micrograph with that of Fig. 35. n, type n cells (interstitial epithelial cells); N, nucleus; RER, rough endoplasmic reticulum.

x 33.890

Fig. 38. Electron micrograph of type 1 cells in alveolus IV. M, mitochondria; N, nucleus; n, type n cells (the interstitial epithelial cells).

x 28.100



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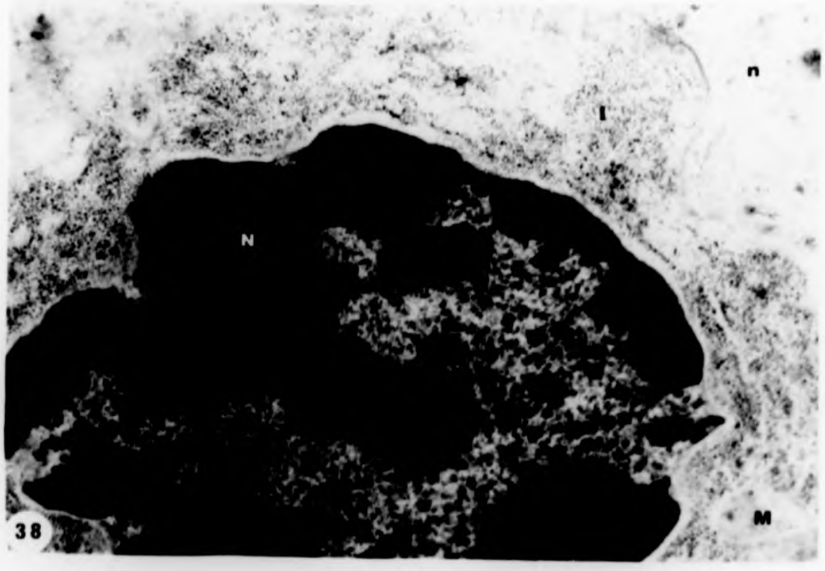

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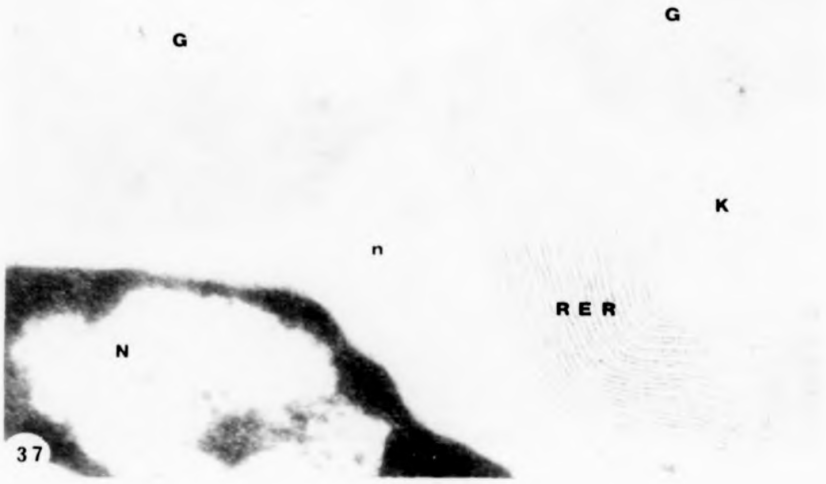

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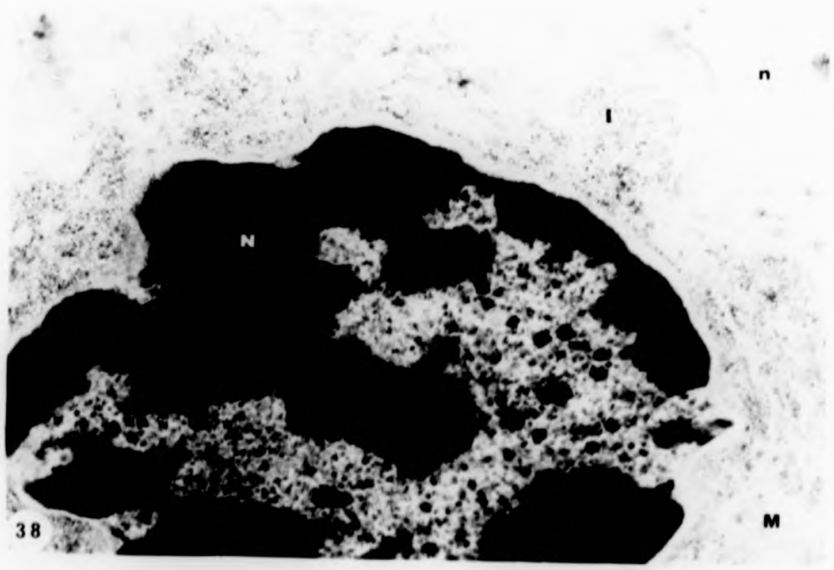
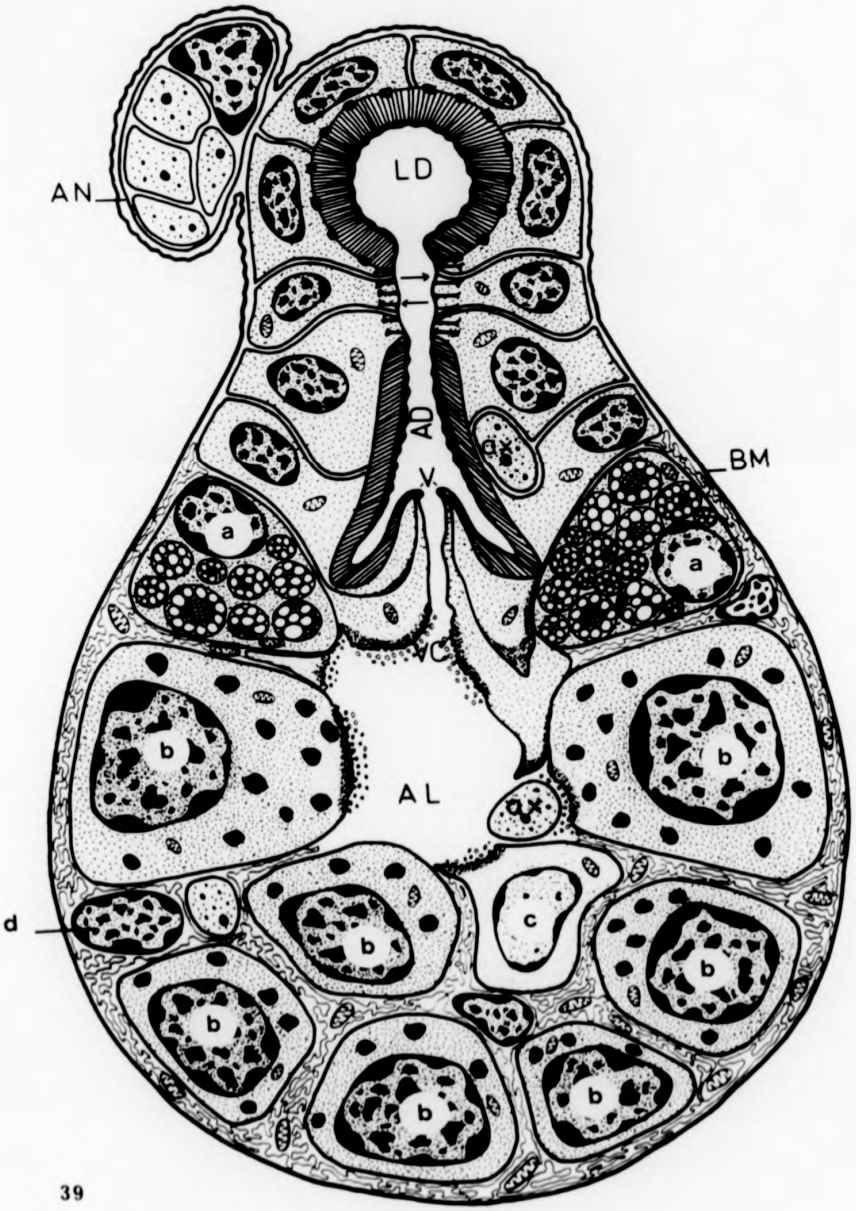
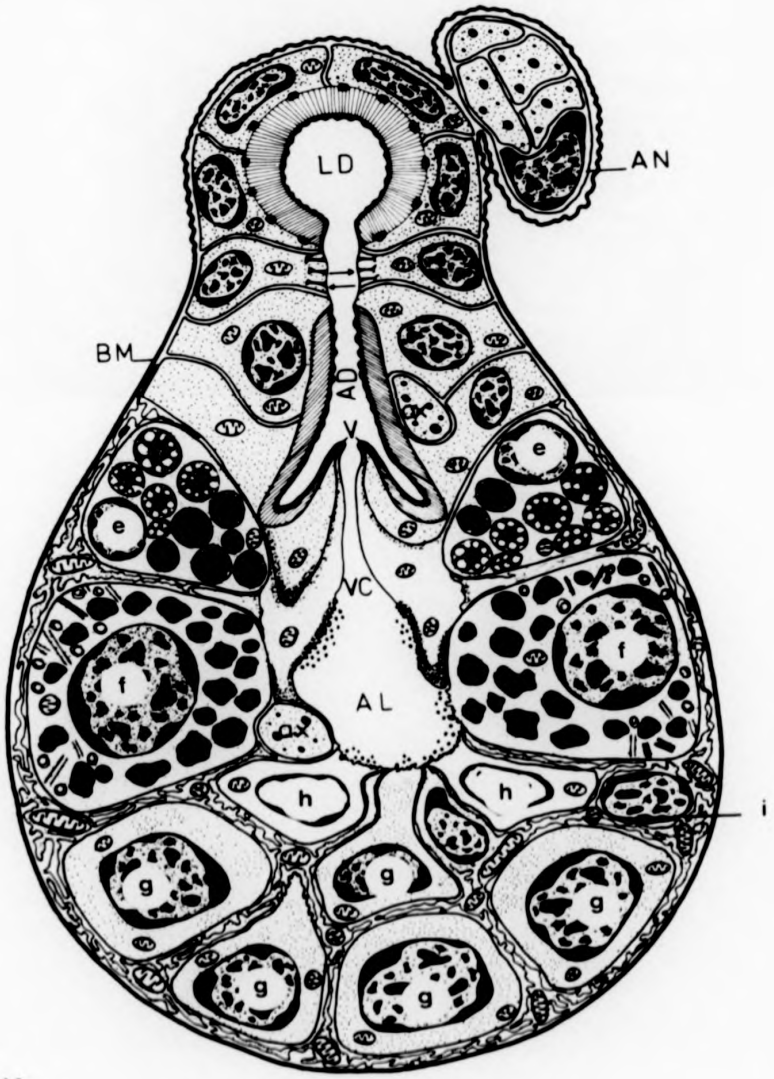


38

Figs. 39, 40, 41. Reconstructed line drawing of types II, III and IV alveoli in unfed adult H. leachii salivary glands at the electron microscopic level. a - n, types a - n cells; AD, alveolar duct; AL, alveolar lumen; AN, alveolar nerve; ax, axon; BM, basement membrane; LD, lobular duct; VC, valvular canal. Arrows indicate area of flexibility.


AN
LD
AD
V
BM
a
a
VC
b
b
AL
ax
d
c
b
b
b
b
b
b


LD
AN
BM
AD
V
VC
AL
e
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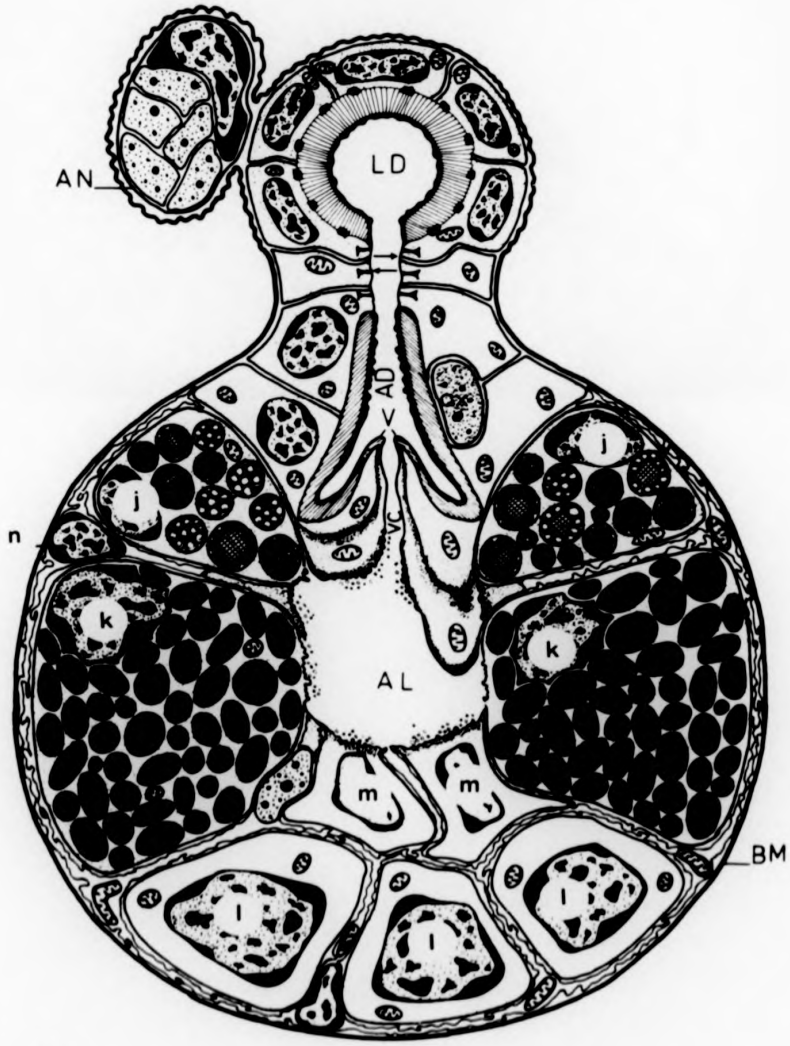


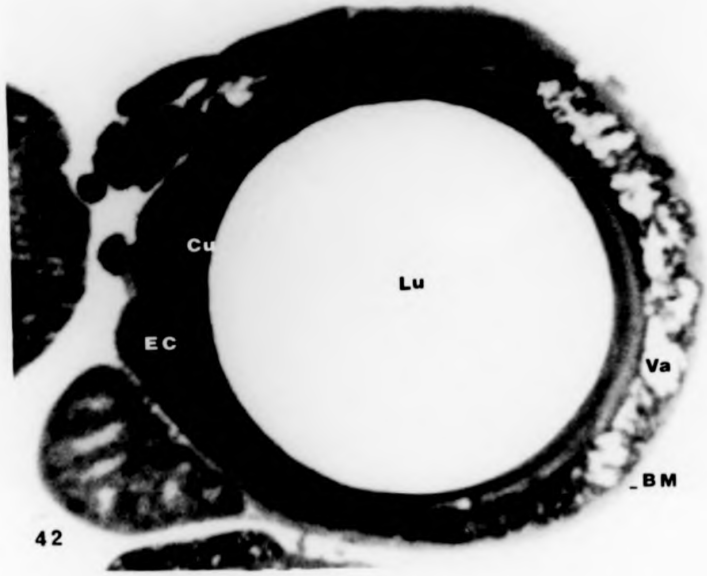
41

Fig. 42. Light micrograph of the main salivary duct on the fifth day after attachment showing that the lumen (Lu) is greatly distended and the cuticular lining (Cu) lacks the folds of the inner region. Both the epithelial layer (EC) and the cuticular lining become thinner, while vacuoles (Va) start to appear in the epithelial layer.

x 4.250

Fig. 43. Light micrograph of semithin section of type I alveoli on the fifth day after attachment to show that the fibrillar cytoplasm lacks the parallel pattern found in unfed females and contain many dark granules (white arrows). Note the increase in irregularity of the alveolar basement membrane. MSD, main salivary duct.

x 4.250



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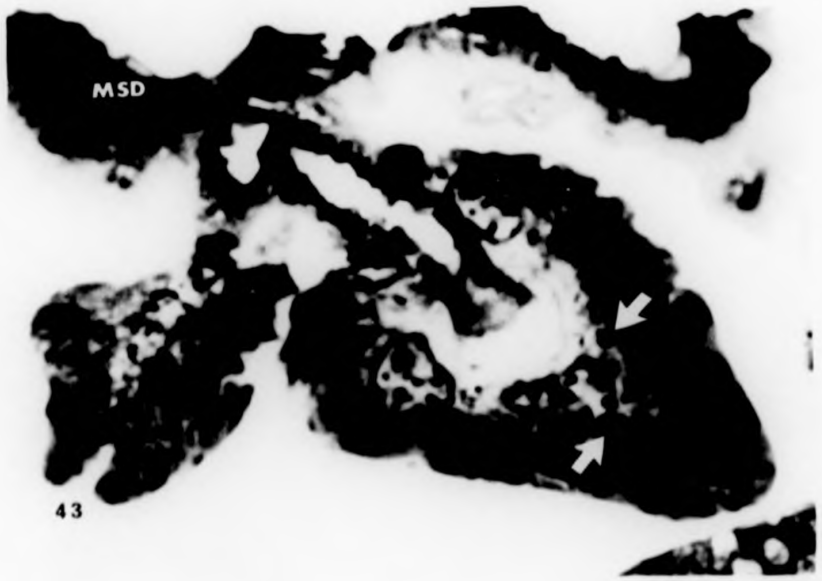

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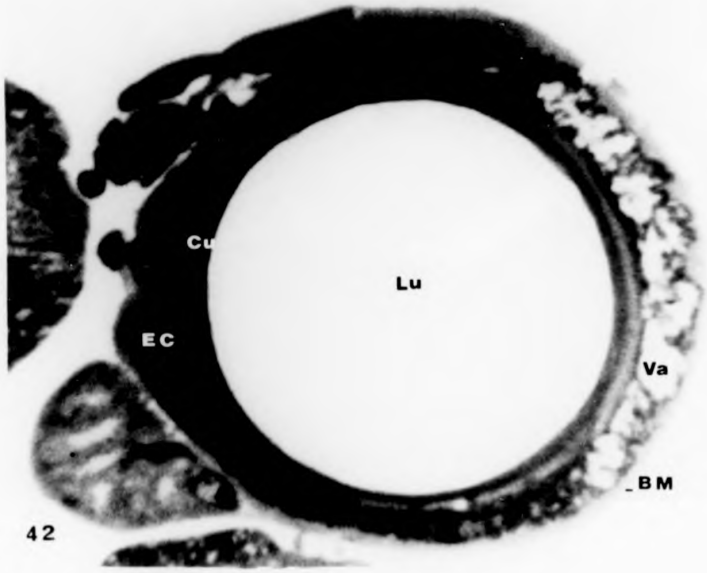


42

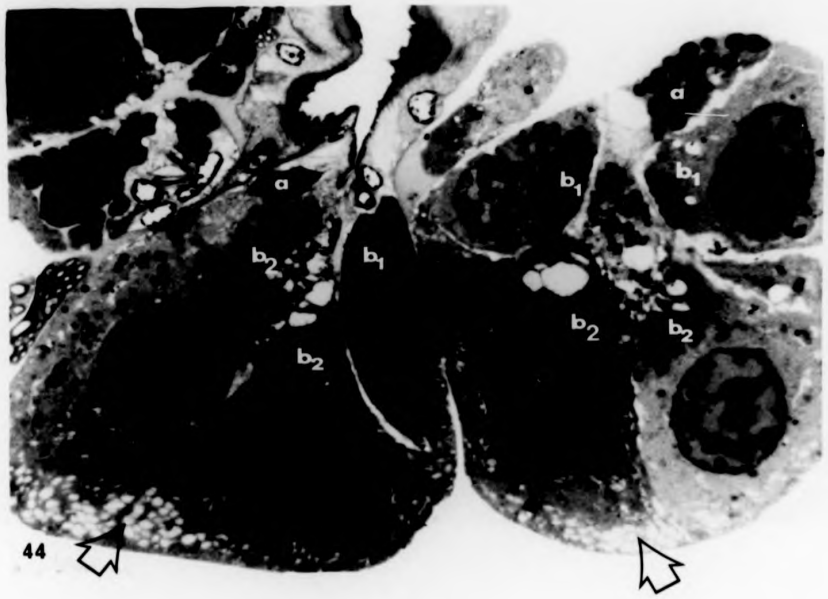
43

Fig. 44. Light micrograph of type II alveoli on the third day after attachment showing the great reduction of type a cells (a) and the new sub-types of b cells: b_1 cells (b_1) and b_2 cells (b_2). Note the new different secretion in the apical regions and in the corners of the basal region of sub-type b_2 cells (arrows).

x 2.500

Fig. 45. The same as Fig. 44 but on the fifth day after attachment. Letterings as in Fig. 44.

x 2.500



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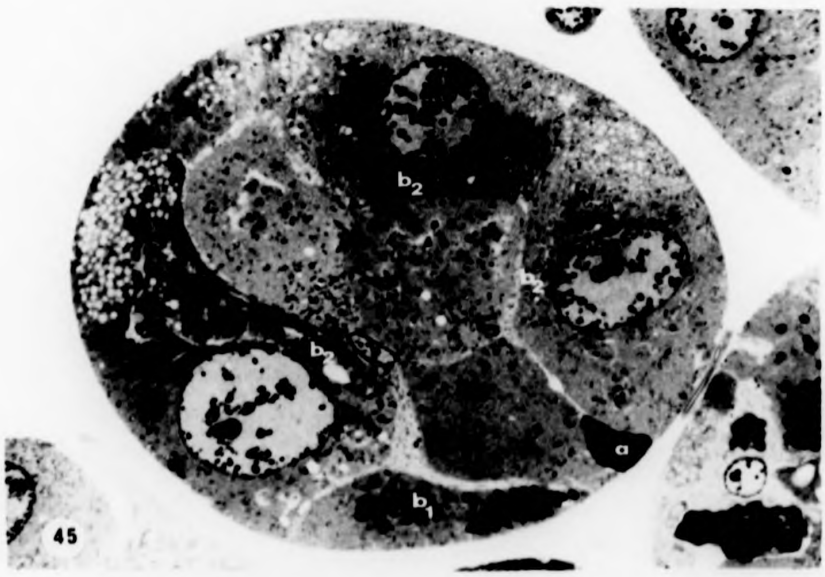

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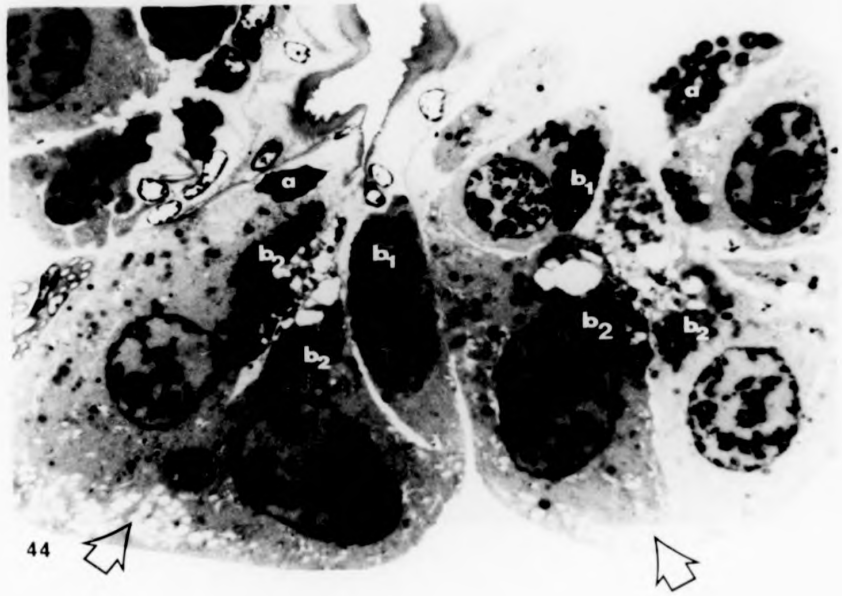

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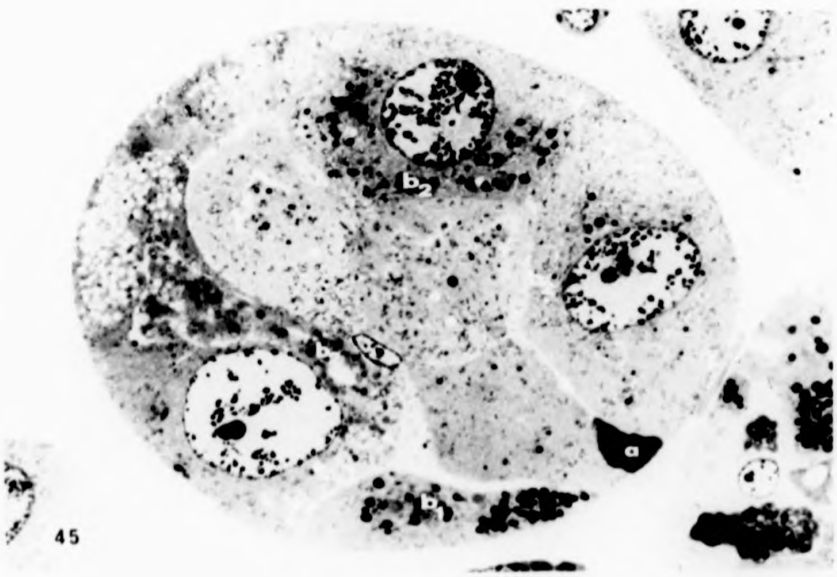


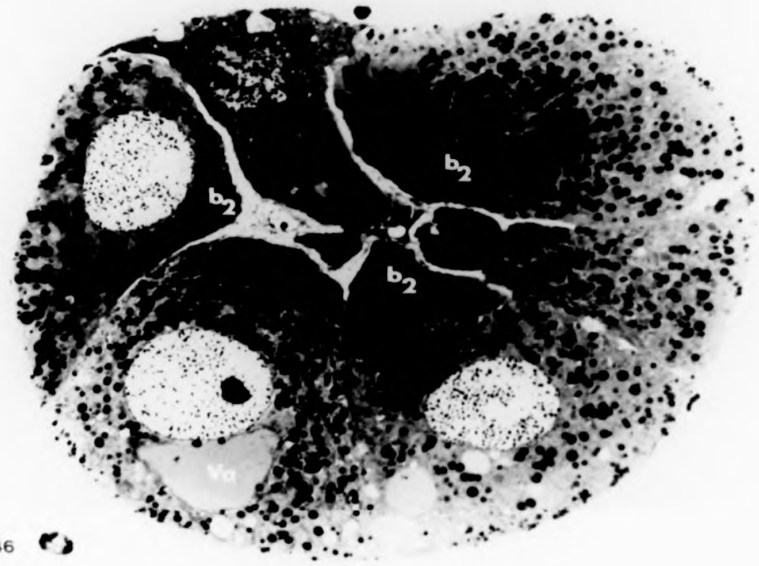
45

Fig. 46. The same as Fig. 45 but on the seventh day after H. leachii female attachment. Note the size of sub-type b_2 cells and their fine spherical secretory granules which accumulate in the apical region of the cells as well as some scattered granules and vacuoles (Va) in the basal regions. The nuclei (N) become hypertrophid with dark and compact nucleoli (n).

x 2.800

Fig. 47. Light micrograph of semithin section of type III alveolus on the third day after H. leachii female attachment to show that types e and f cells (e and f) are reduced in size and have lost most of their secretory granules, while type g cells (g) are enlarged in size and possess fine granules (arrows). Lu, lumen; v, valve-like structure.

x 2.800



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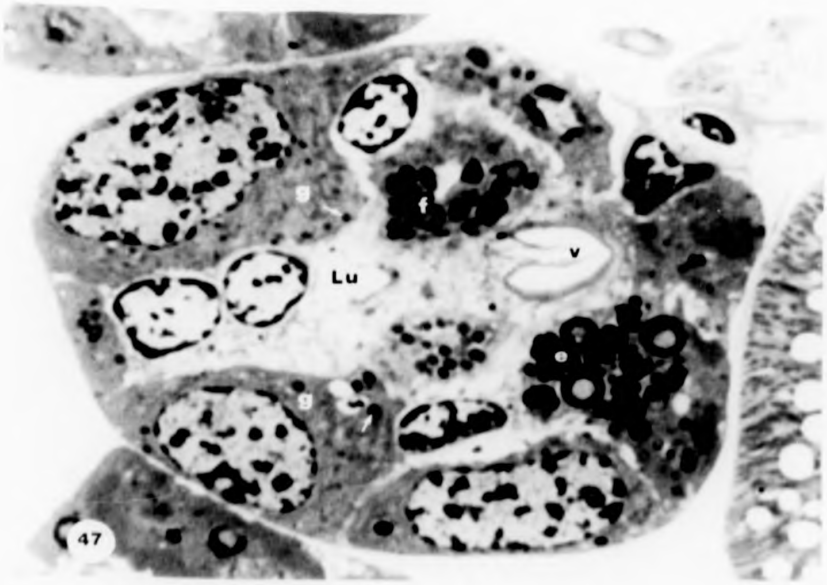

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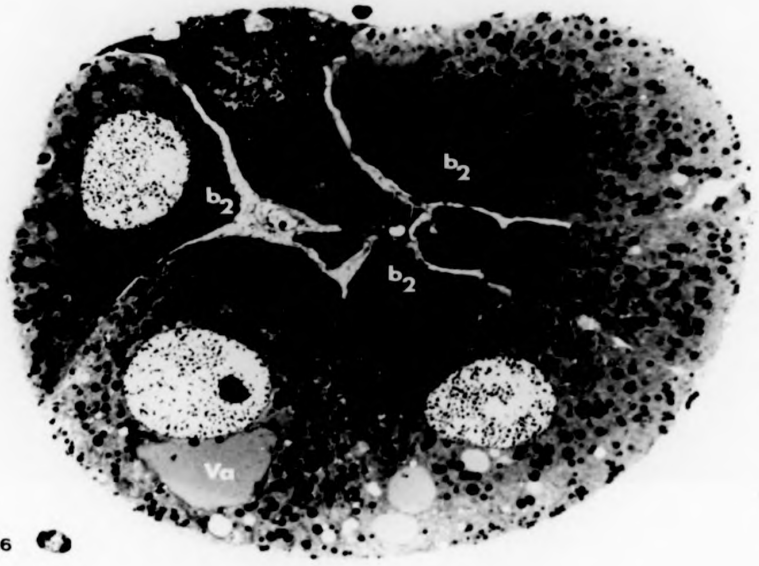

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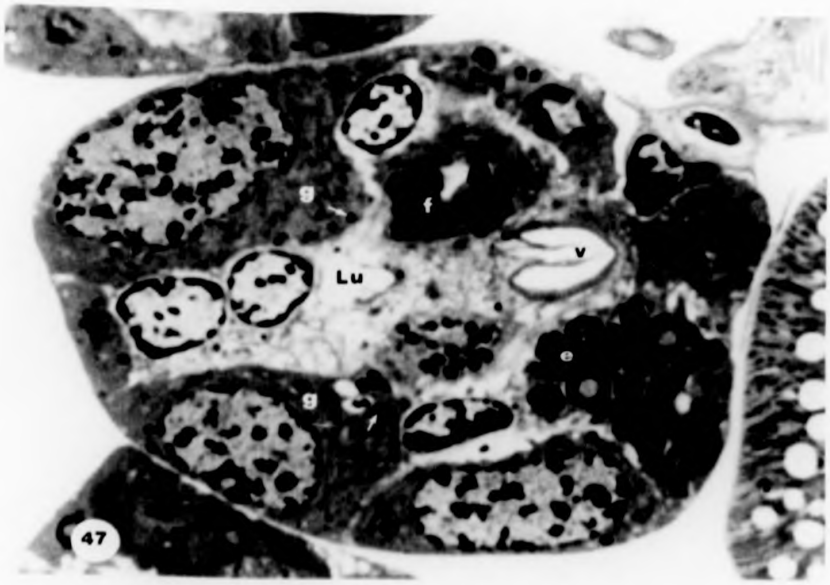


47

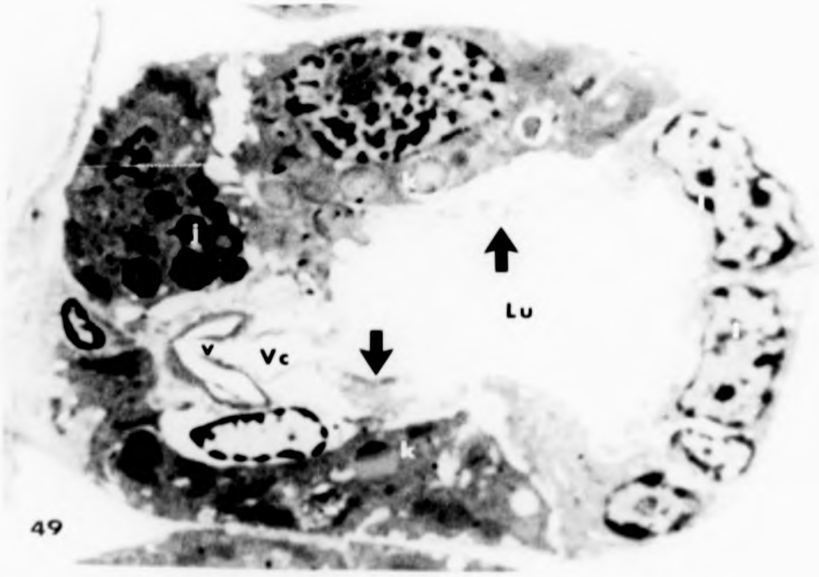
Fig. 48. The same as in Fig. 47 but here on the seventh day after attachment. This micrograph shows the complete reduction of all the alveolar cell types. In this section type i cells (i) appear as fine fibrils and dark small granules. Note that a portion of the basal region of type g cells is found in the bottom of the alveolus (arrow). Other letterings as in Fig. 47.

x 2.250

Fig. 49. Light micrograph of type IV alveolus on the first day after H. leachii female attachment. Letterings: j, k and l represent types j, k and l cells; Lu, distended lumen; v, valve-like structure; vc, valvular canal. Arrows indicate the extensions of the cap cells to touch the valve-like structures.

x 3.000

48



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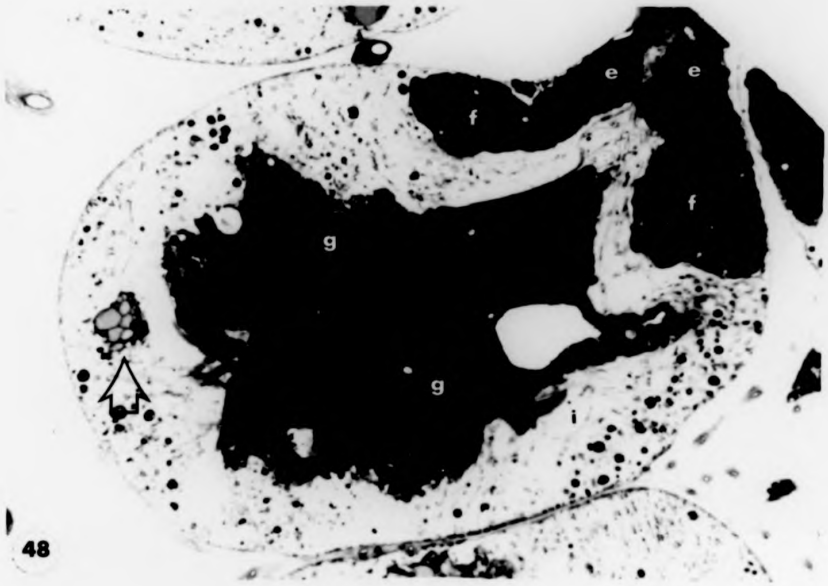

e
e
f
f
g
g
i
48

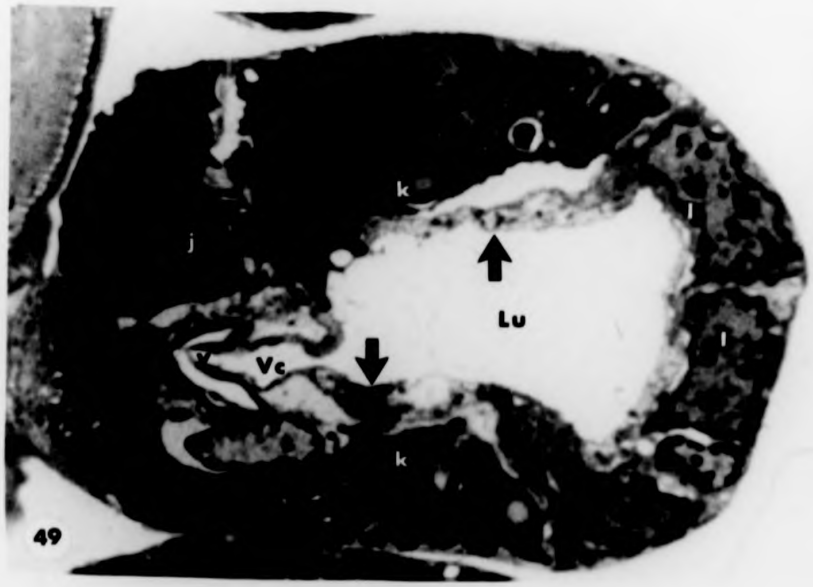

k
l
j
Lu
l
Vc
k
49

Fig. 50. The same as Fig. 49 but on the fifth day after attachment. n is the interstitial epithelial cells. Also note that the surface of type 1 cells is highly irregular. Arrows point to fine secretory granules in type 1 cells.

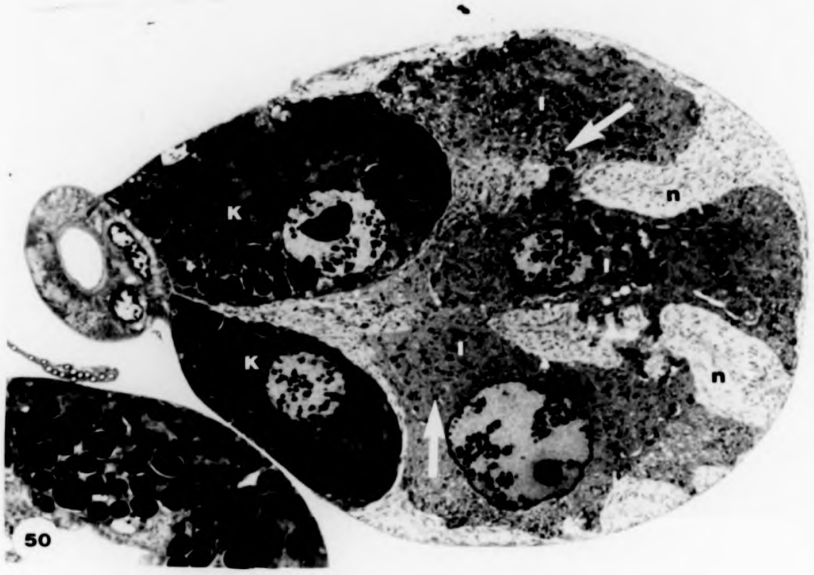
x 2.000

Figs. 51, 52, 53. Light micrographs of semithin sections of types II, III and IV alveoli on the fifth day after H. leachii male attachment. Note that all types of cell do not show any remarkable change comparable to that in the female.

Fig. 51 x 2.800

" 52 x 2.000

" 53 x 2.250



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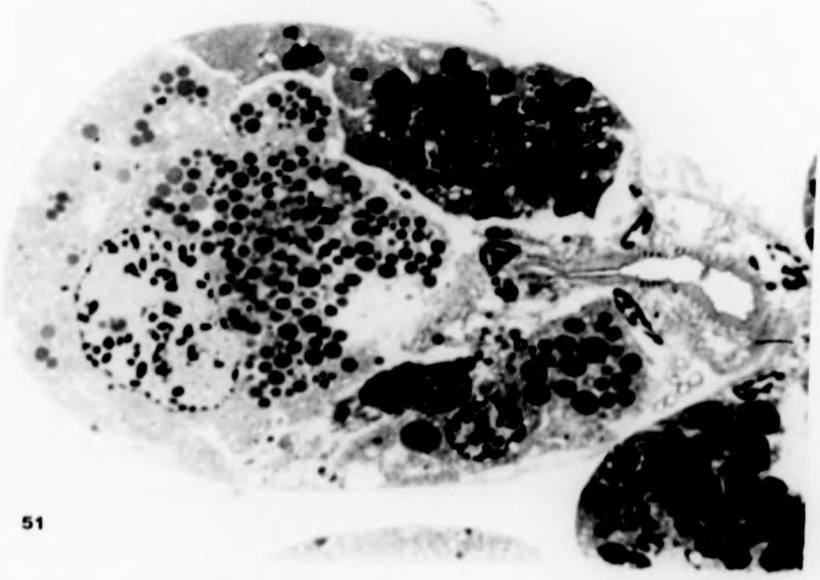

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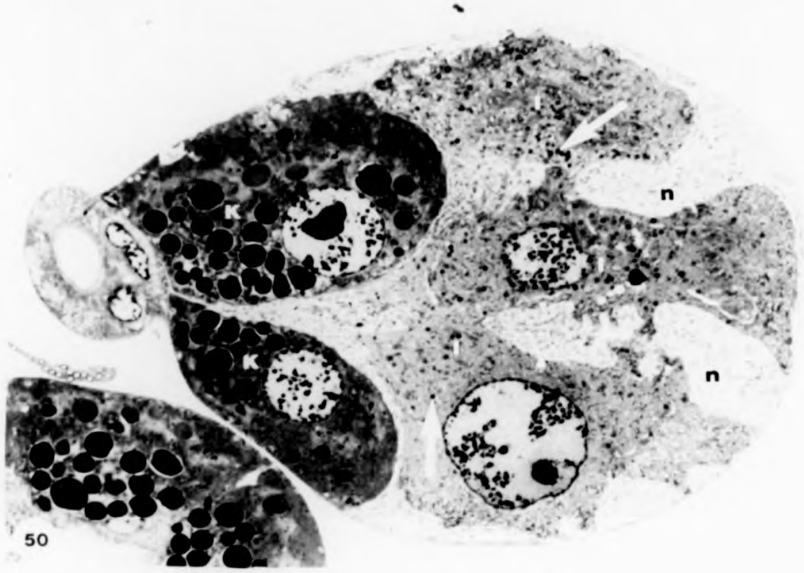

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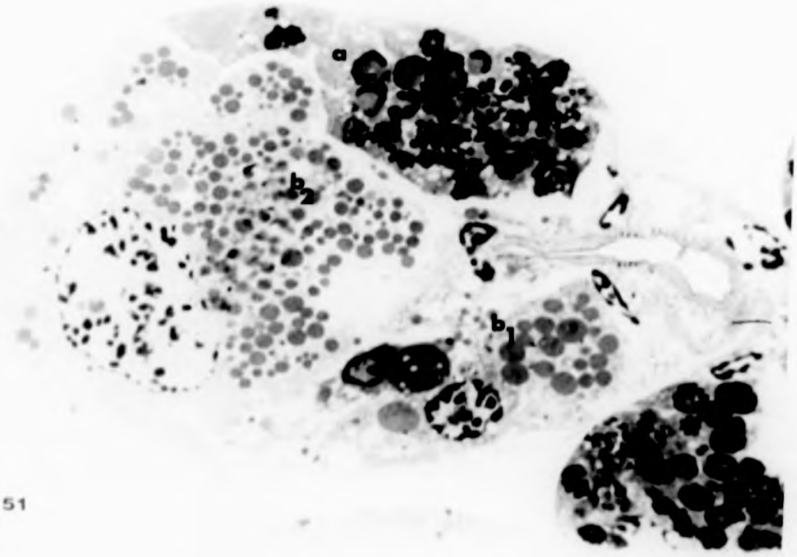

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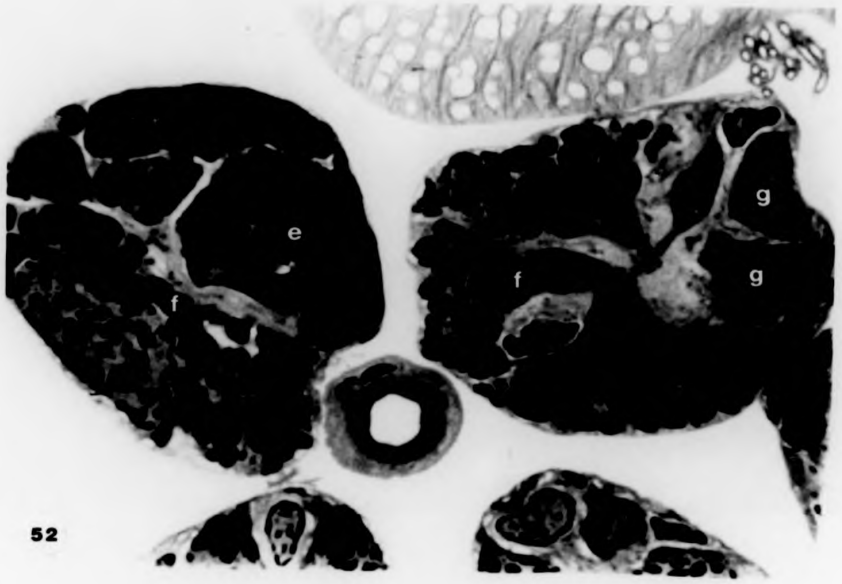
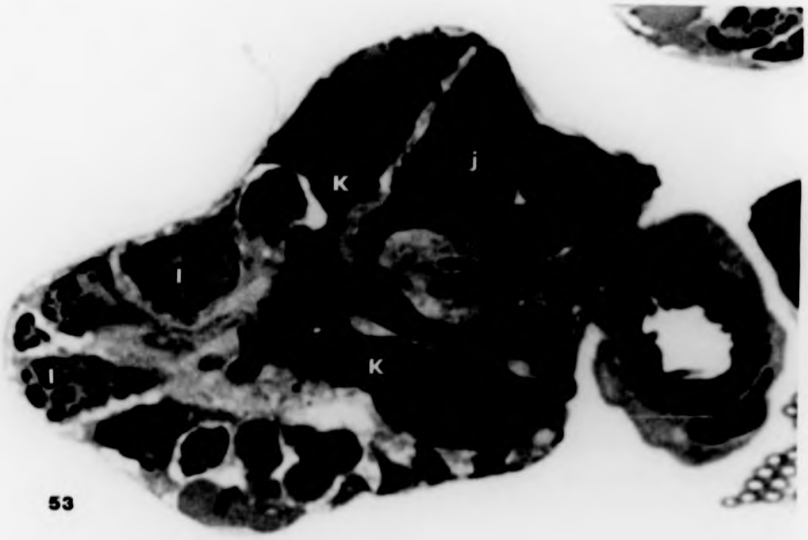


52



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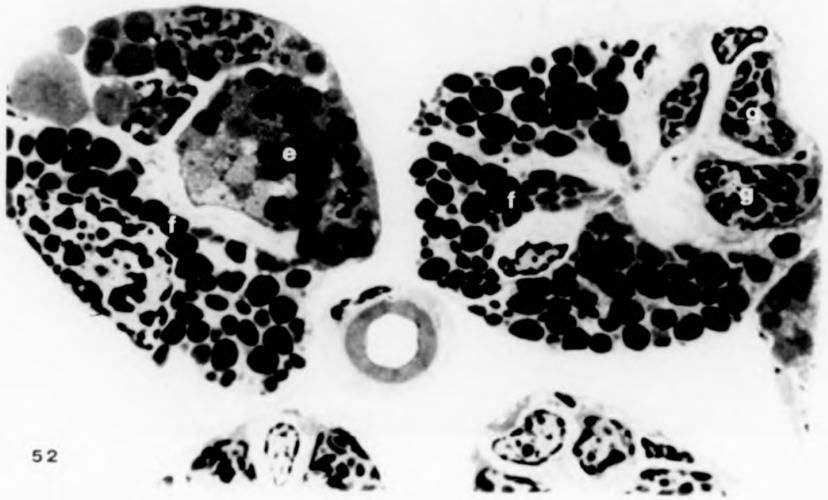

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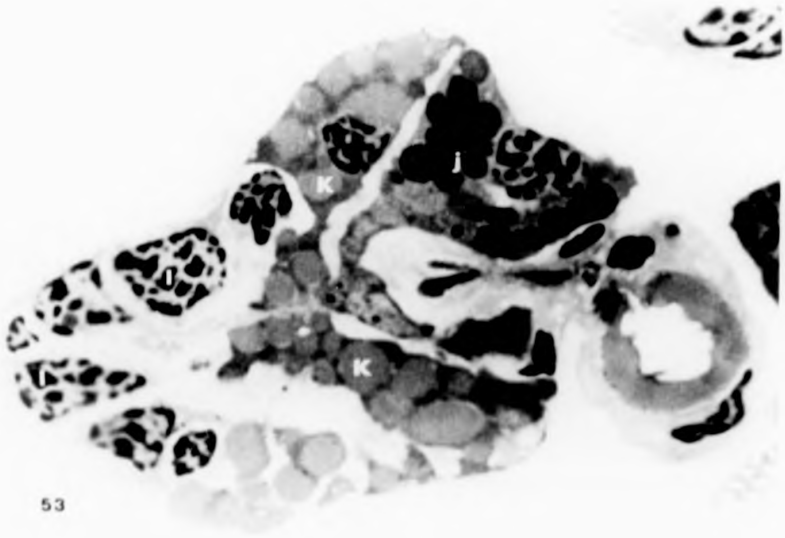

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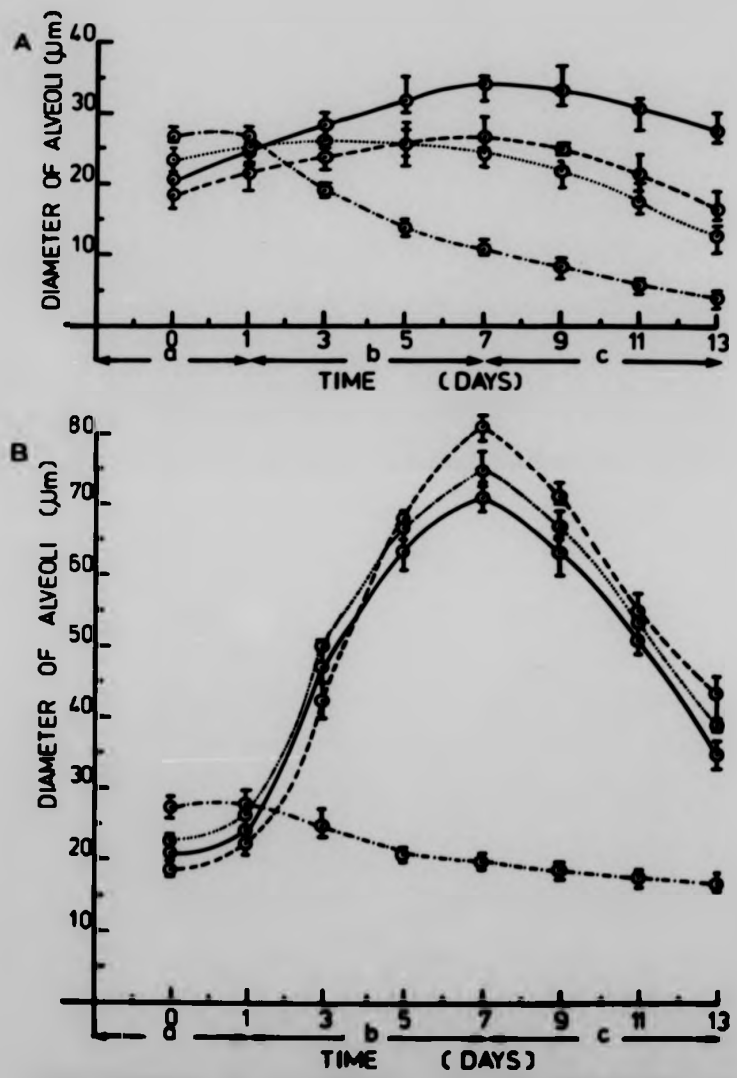
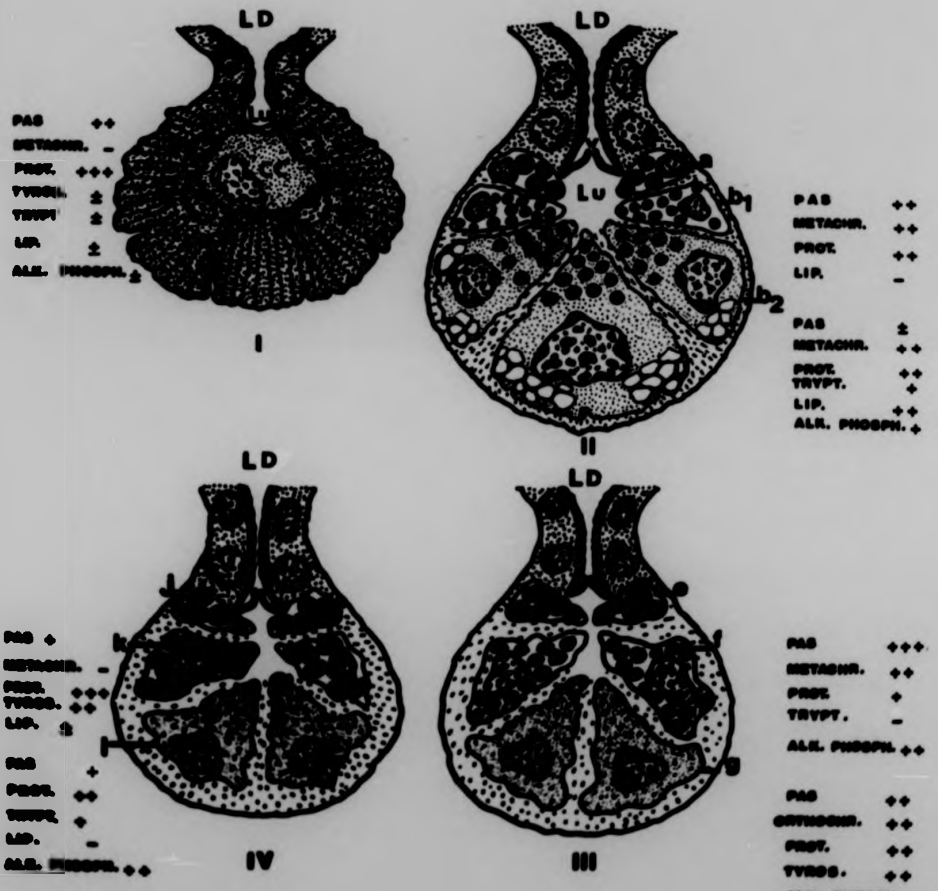


FIG.54 EFFFECT OF BLOODMEAL ON THE DIAMETER OF FOUR TYPES OF ALVEOLI OF MALE (A) AND (B) FEMALE H. LEACHII SALIVARY GLANDS DURING:
a. PREFEEDING PERIOD b. FEEDING PERIOD.
c POSTFEEDING PERIOD.
TYPE I ALVEOLI TYPE II ALVEOLI
" III " " IV "

Fig. 55. Drawing of types I, II, III and IV alveoli of the salivary glands of female H. leachii on the fifth day after attachment to show the histochemical nature of the cells of these alveoli. cc, central cell; LD, lobular duct; Lu, alveolar lumen; v, valve-like structure.


LD
LD
Lu
PAS ++
METACHR. –
PROT. +++
TYROS. ±
TRYPT. ±
LIP. ±
ALK. PHOSPH. ±
I
PAS ++
METACHR. ++
PROT. ++
LIP. –
PAS ±
METACHR. ++
PROT. TRYPT. ++ +
LIP. ++
ALK. PHOSPH. +
II
LD
LD
PAS +
METACHR. –
PROT. +++
TYROS. ++
LIP. ±
PAS +
PROT. ++
TYROS. +
LIP. –
ALK. PHOSPH. ++
IV
PAS +++
METACHR. ++
PROT. +
TRYPT. –
ALK. PHOSPH. ++
PAS ++
METACHR. ++
PROT. ++
TYROS. ++
ALK. PHOSPH. ++
III

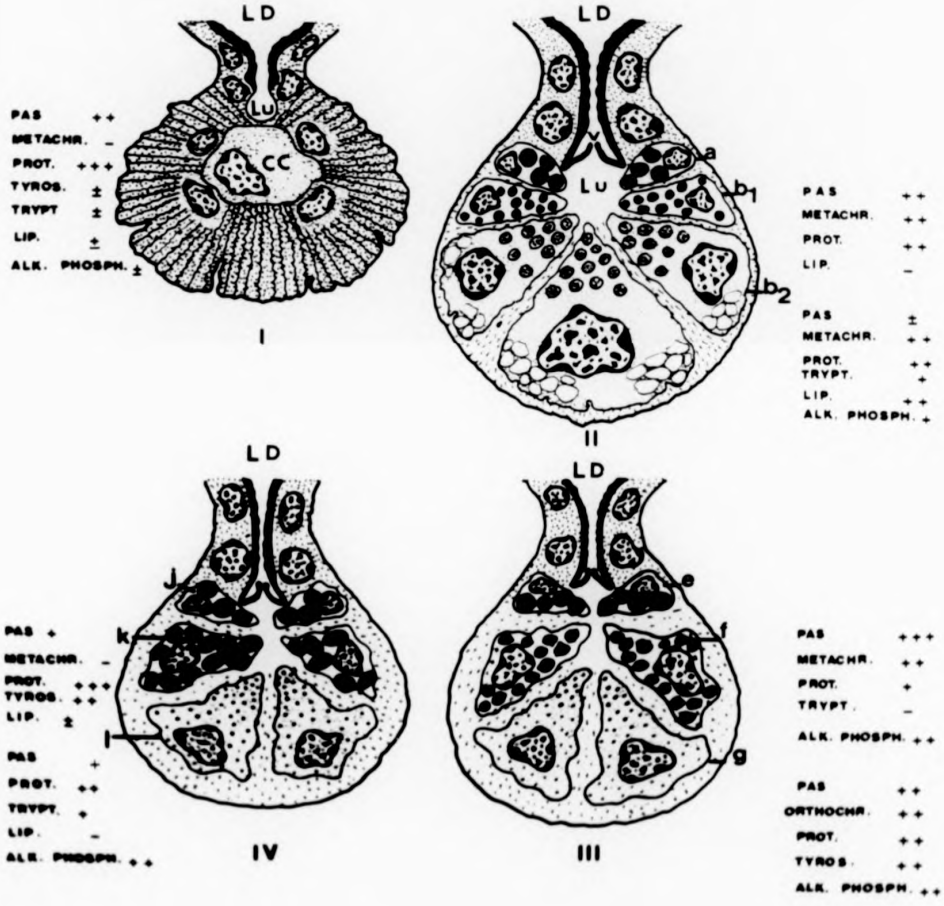
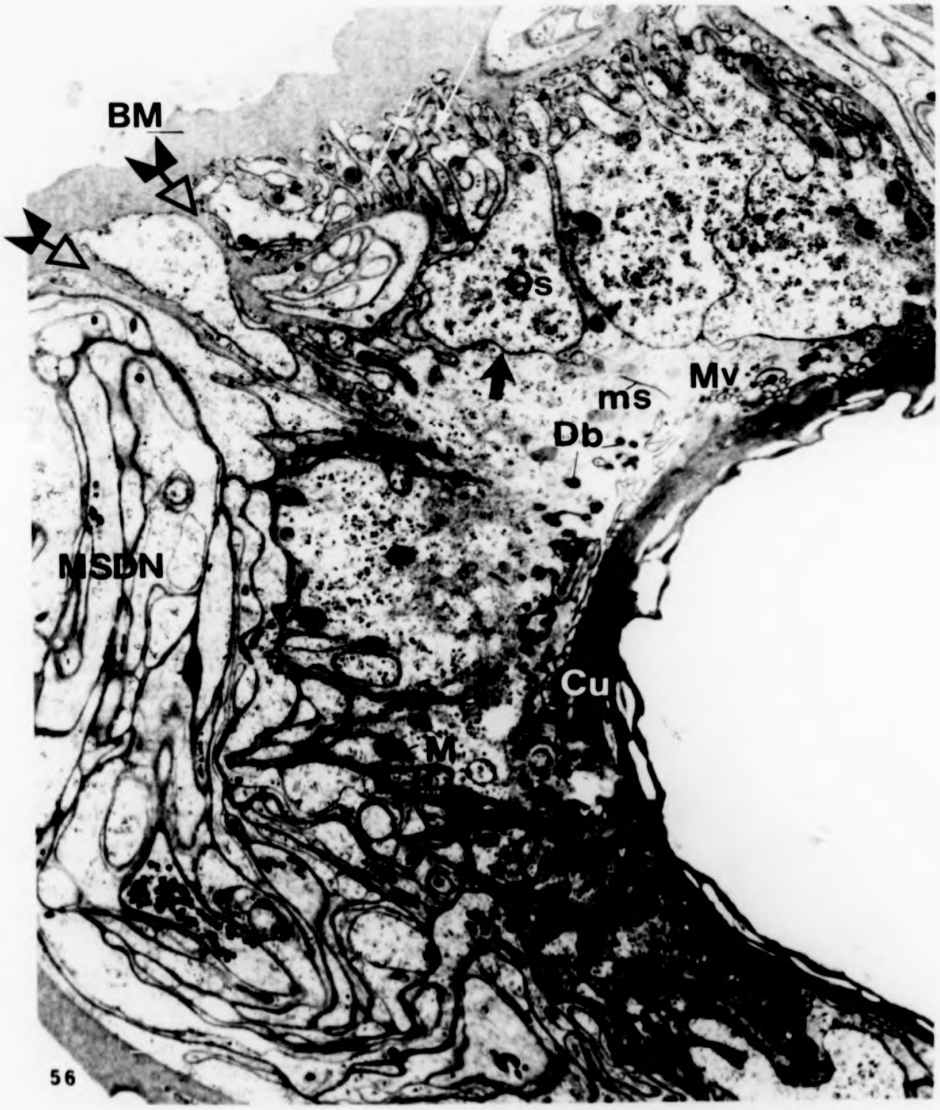

LD
Lu
CC
PAS ++
METACHR. –
PROT. +++
TYROS. ±
TRYPT ±
LIP. ±
ALK. PHOSPH. ±
I
LD
Lu
a
b1
b2
PAS ++
METACHR. ++
PROT. ++
LIP. –
PAS ±
METACHR. ++
PROT. ++
TRYPT. +
LIP. ++
ALK. PHOSPH. +
II
LD
J
k
l
PAS +
METACHR. –
PROT. +++
TYROS. ++
LIP. ±
PAS +
PROT. ++
TRYPT. +
LIP. –
ALK. PHOSPH. ++
IV
LD
e
f
g
PAS +++
METACHR. ++
PROT. +
TRYPT. –
ALK. PHOSPH. ++
PAS ++
ORTHOCHR. ++
PROT. ++
TYROS. ++
ALK. PHOSPH. ++
III

Fig. 56. Electron micrograph of the main salivary duct on the fifth day after H. leachii female attachment showing that the cytoplasm of the epithelial cells possesses large areas of an osmiophilic aggregates (Os) surrounded by infoldings of the basal cell membrane (thick arrow). The long arrows point to short infoldings of the plasma membrane, while the large arrows point to the extensions of the basement membrane (BM) inbetween these infoldings. Note that the cuticular lining (Cu) is thinner, fenestrated and irregular. The axons of the main duct nerve (MSDN) have lost most of their neurosecretory vesicles. Db, dense bodies; Lu, main duct lumen; M, mitochondria; ms, membrane-like structures; MV, microvilli.

x 7.200



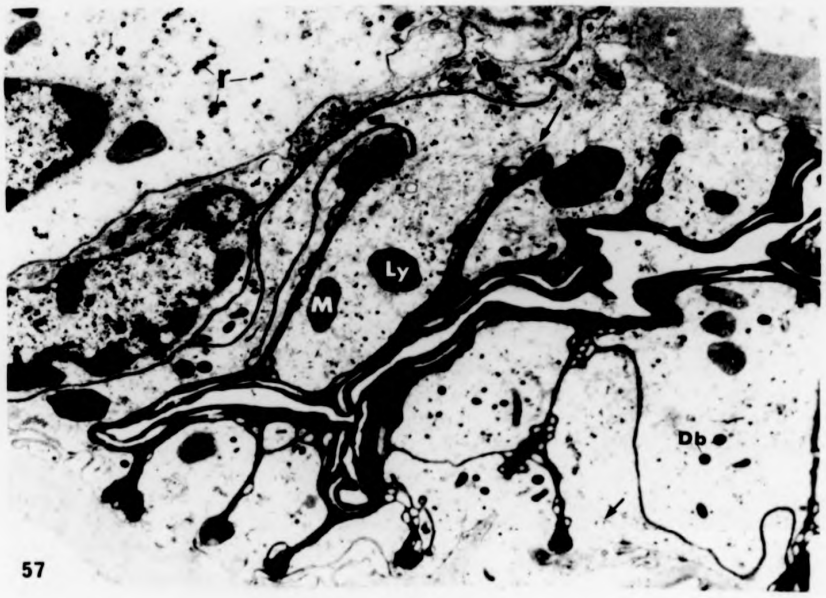
56

Fig. 57. As Fig. 56 but at the beginning of oviposition, showing that the peithelial layer is shrunken and the cytoplasm contains a few lysosomes (Ly) and aggregates of ribosomes (r). Arrow points to microtubules. Note that the cuticular lining is completely collapsed and its lumen is very narrow. Db, dense bodies; M, mitochondria.

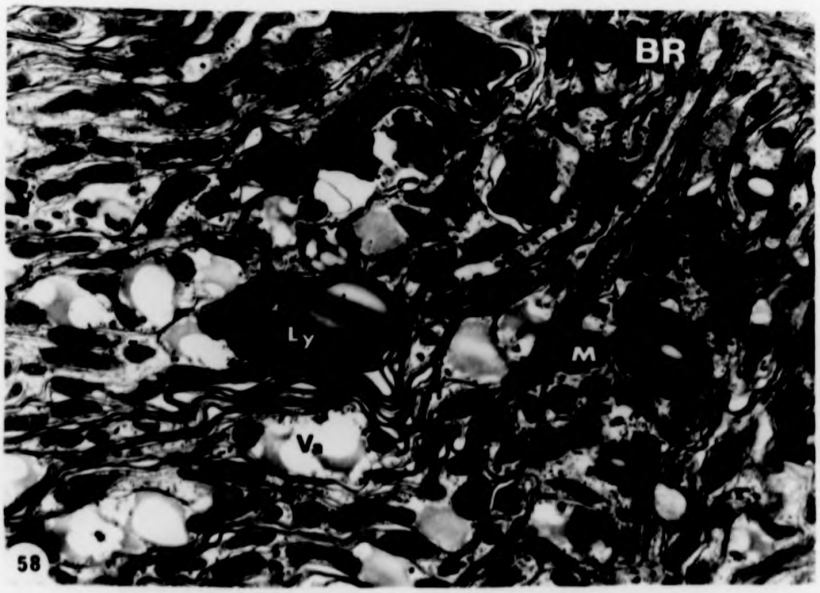
x 13.500

Fig. 58. Electron micrograph of a portion of type I alveolus on the third day after H. leachii female attachment showing the basal region (BR) of the alveolus contains some lysosome-like structures (Ly) of variable size and with moderately dense vacuoles. The nonmembrane-bound vacuoles (va) have lost most of their contents. M, mitochondria.

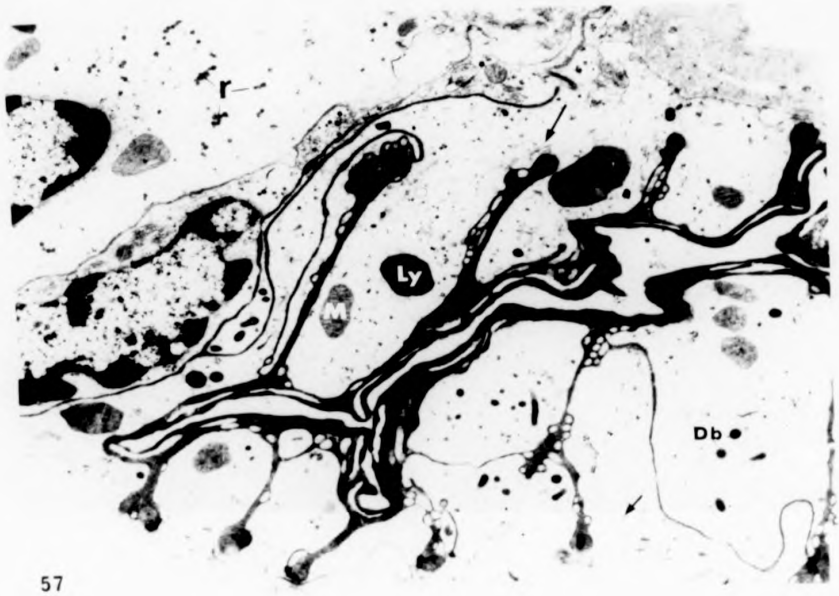
x 12.275



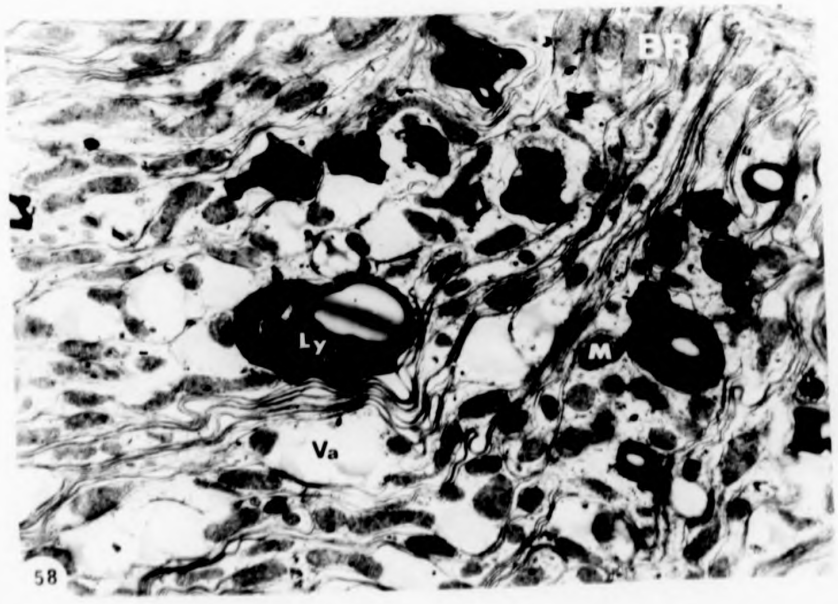
57



58



57



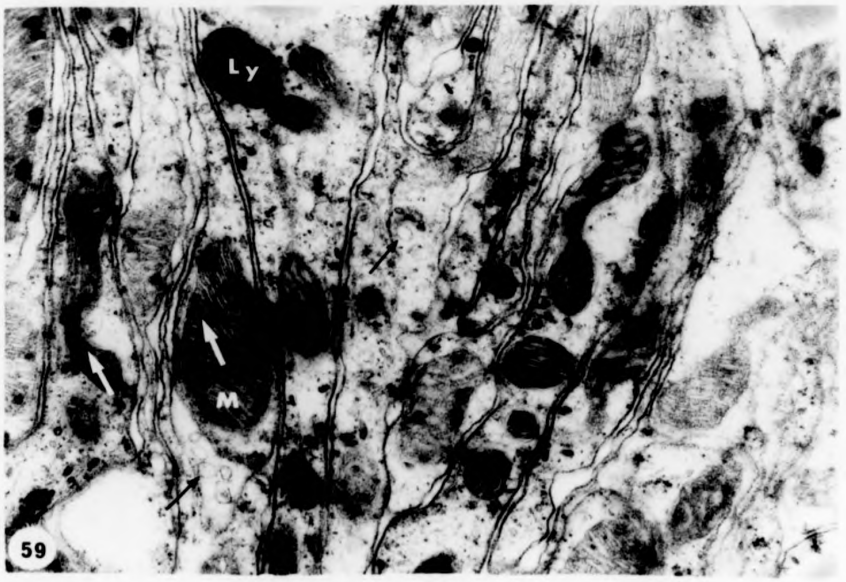
58

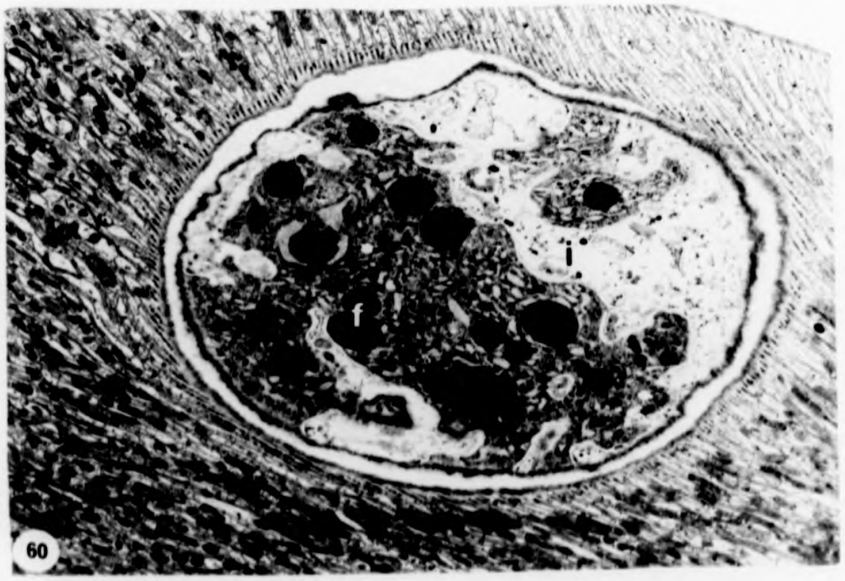
Fig. 59. The same as Fig. 58, but this micrograph shows small, membrane-bound vacuoles (small, black arros) with low dense interiors which are embedded in the cytoplasm. The mitochondria (M) possess low dense gaps between their cristae, and their matrix contains small dense granules (white arrows).

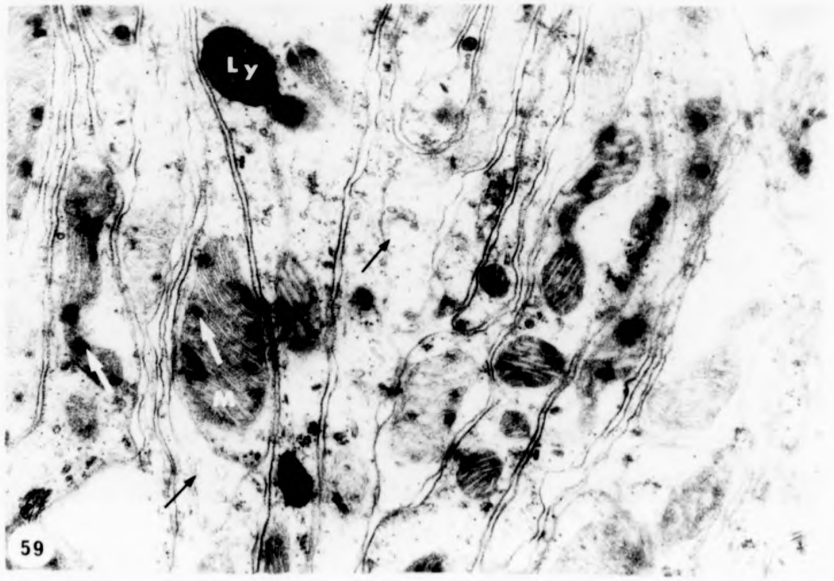
x 28.100

Fig. 60. The same as Fig. 58 but here type I alveolus encloses a portion of types f and i cells of type III alveolus as a result of the great irregularity of type I alveolus.

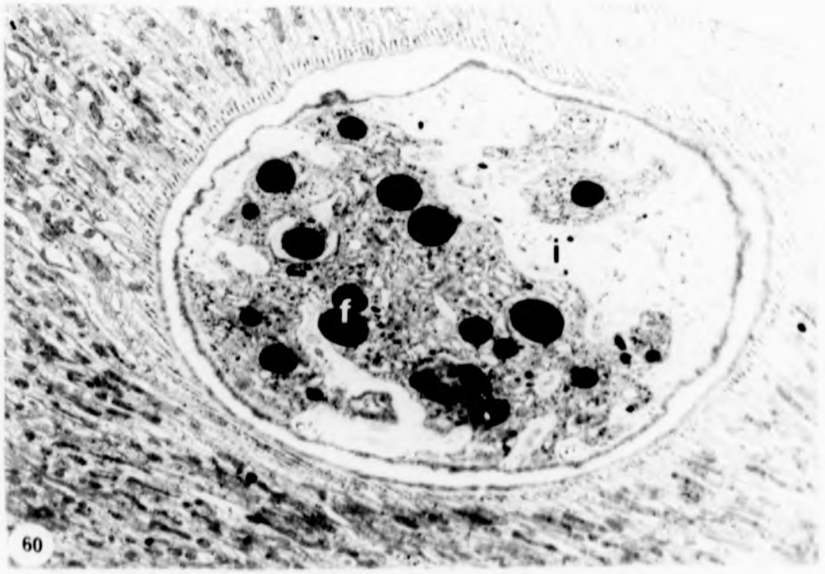
x 5.000


Ly
M
59


i
f
60



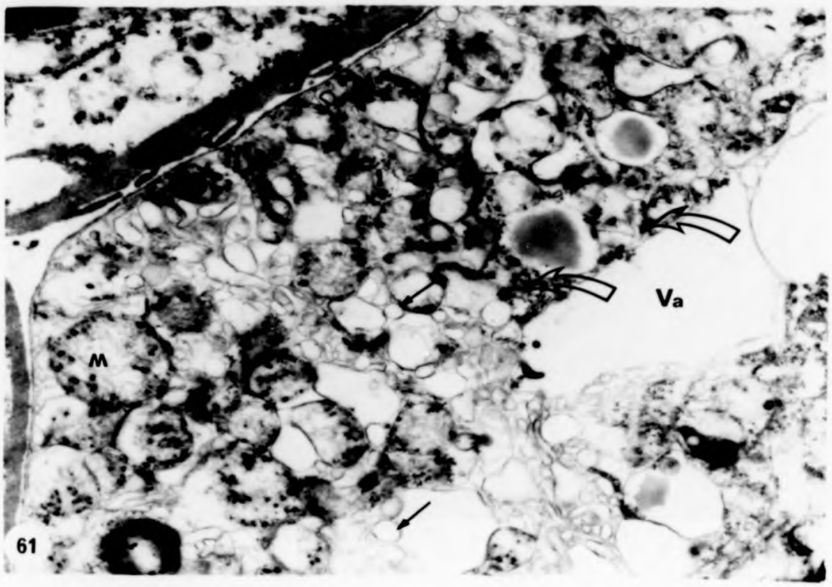
59

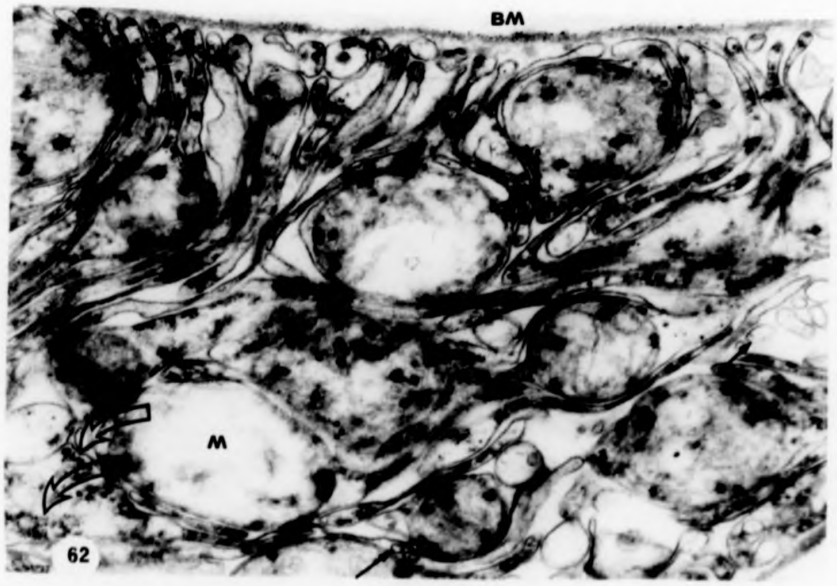


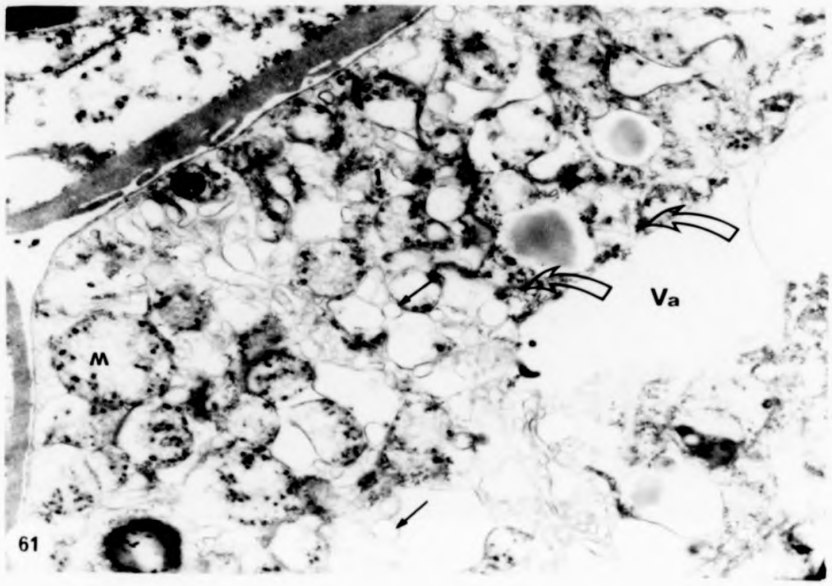
60

Figs. 61, 62. Electron micrographs of portions of type I alveolus on the fifth day after H. leachii female attachment to show the osmiophilic aggregates (empty arrows), large membrane-bound vacuoles in the middle of the alveolus (Fig. 61) as a result of the great distension of the infoldings in the intermediate and apical regions of the alveolus. Note that many infoldings (especially in Fig. 61) lack their normal appearance and contain small membrane-bound vacuoles with low dense interior (small arrows). Some mitochondria (M) are greatly distended with relatively few cristae in a homogeneous matrix that contains mitochondrial granules (Fig. 62). BM, basement membrane.

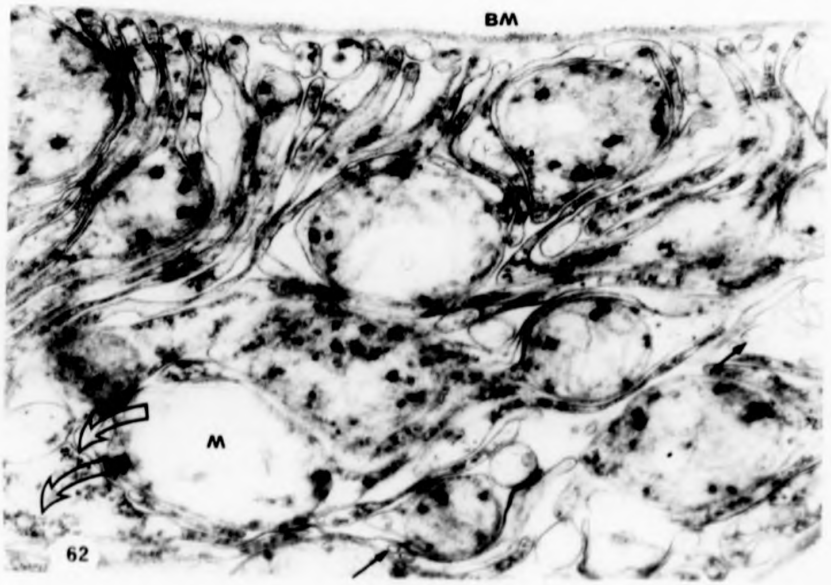
x 26.285


Va
M
61


BM
M
62



61



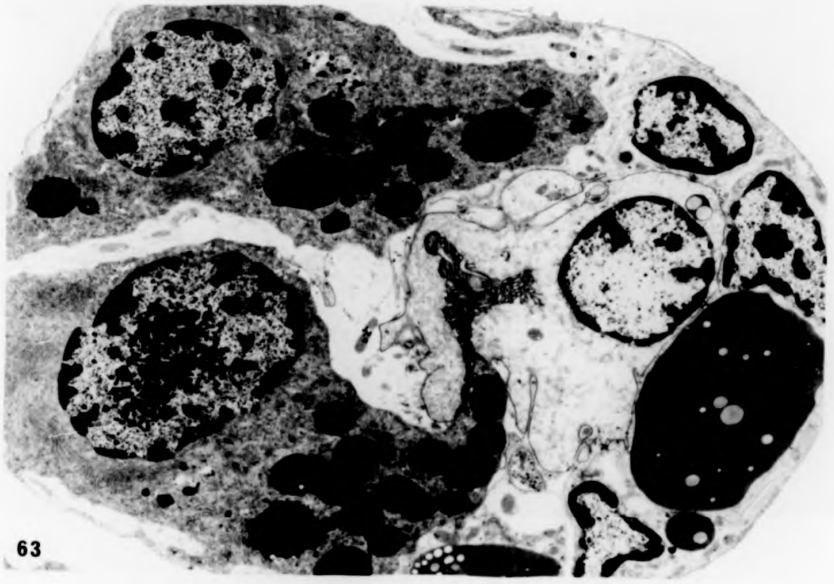
62

Fig. 63. Electron micrograph of unknown type of granule-secreting alveolus on the first day after attachment with autolized fundus cells. This probably represents an earlier abnormal alveolar degredation.

x 4.630

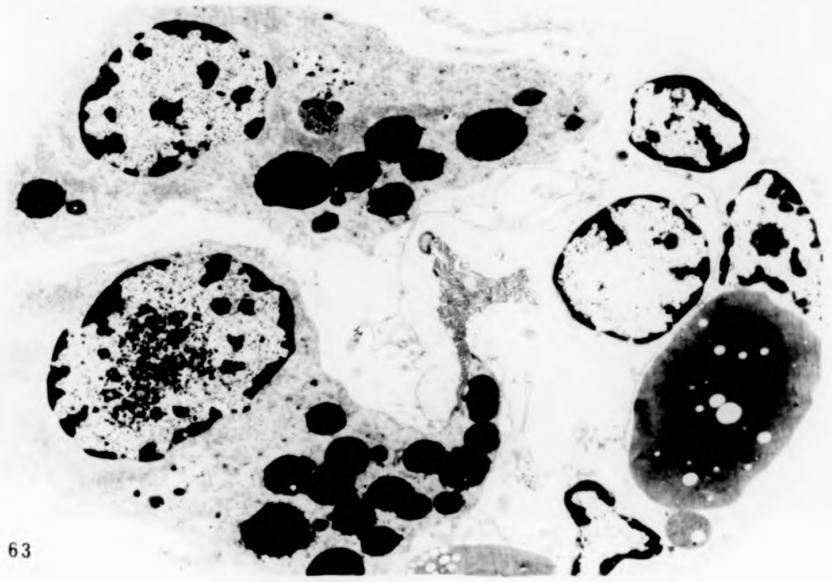
Fig. 64. Electron micrograph of type a cells of type II alveolus on the first day after attachment showing the fusion of the limiting membrane (small arrow) of the subunits (sb). Gb, Golgi bodies; M, mitochondria; RER, rough endoplasmic reticulum. The long arrows point to small vesicles in the Golgi region.

x 31.940

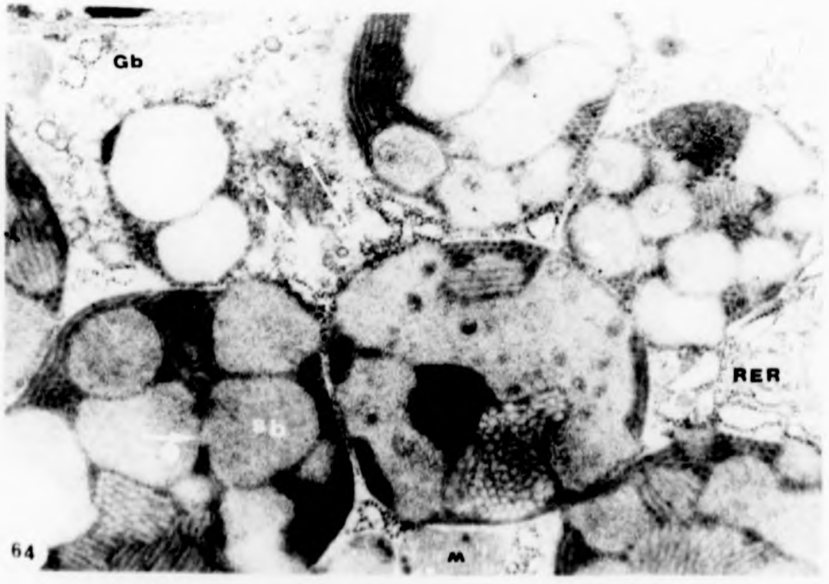


63

64



63



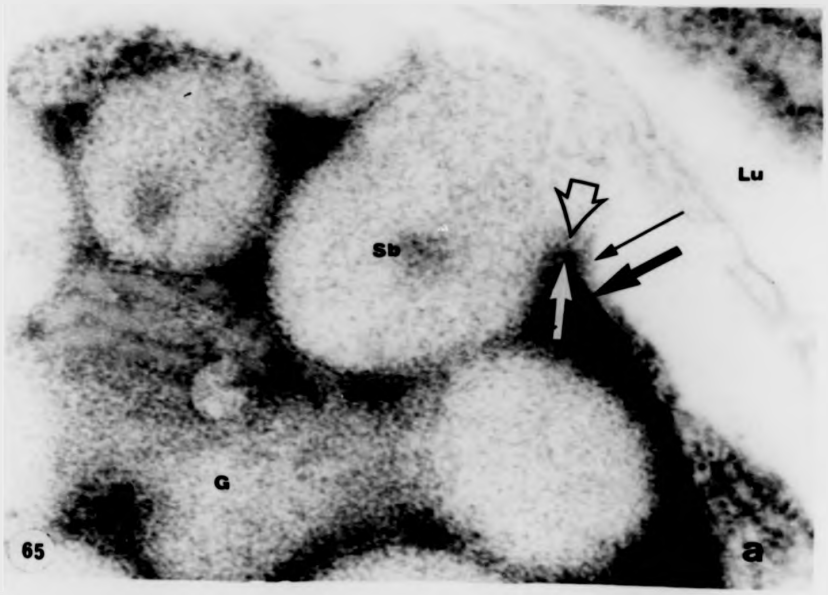
64

Fig. 65. The same as Fig. 64 but on the third day after attachment. This micrograph shows the subunits (sb) of type a cell granules (G) during release of their contents into the alveolar lumen (Lu). Thick arrow indicates the limiting membrane of the large granule, thin arrow points to the plasma membrane of type a cell and white arrow indicates the fusion of the limiting membrane of both the subunit and the large granule. The opened arrow points to the fusion of the three membranes together.

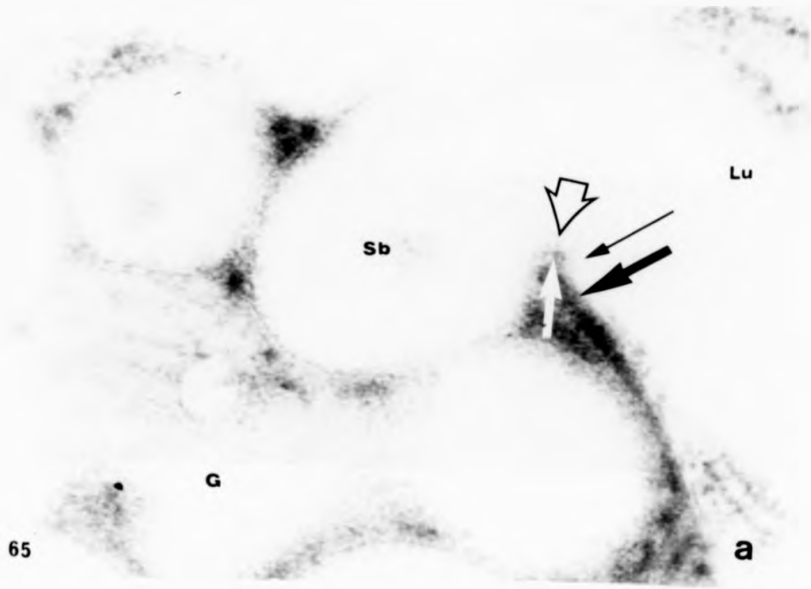
x 86.400

Fig. 66. The same as Fig. 64 but here the isolated subunit (sb) is completely pinched off into the alveolar lumen (Lu) (i.e. macroapocrine secretion) as an individual cytoplasmically bounded subunit.

x 86.400


Lu
Sb
G
65
a


Lu
a
Sb
66


Lu
Sb
G
65
a

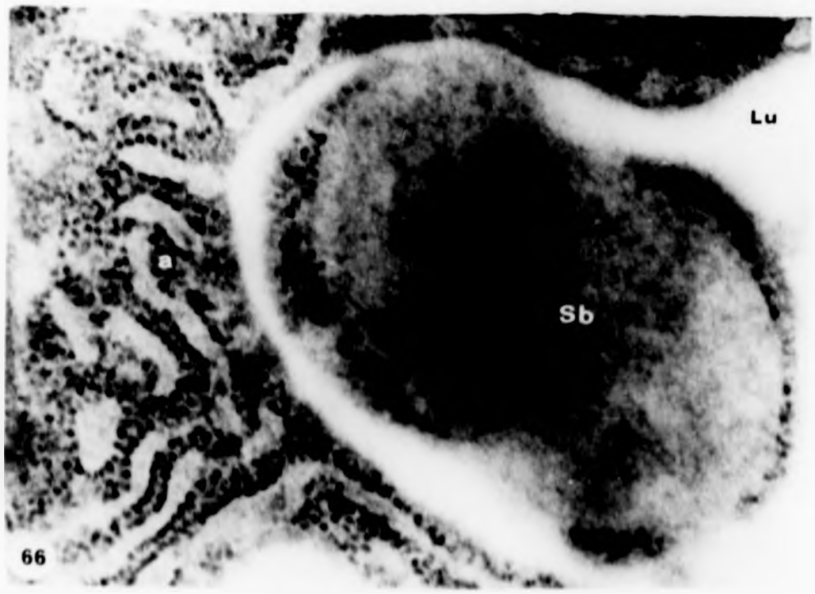

Lu
Sb
66

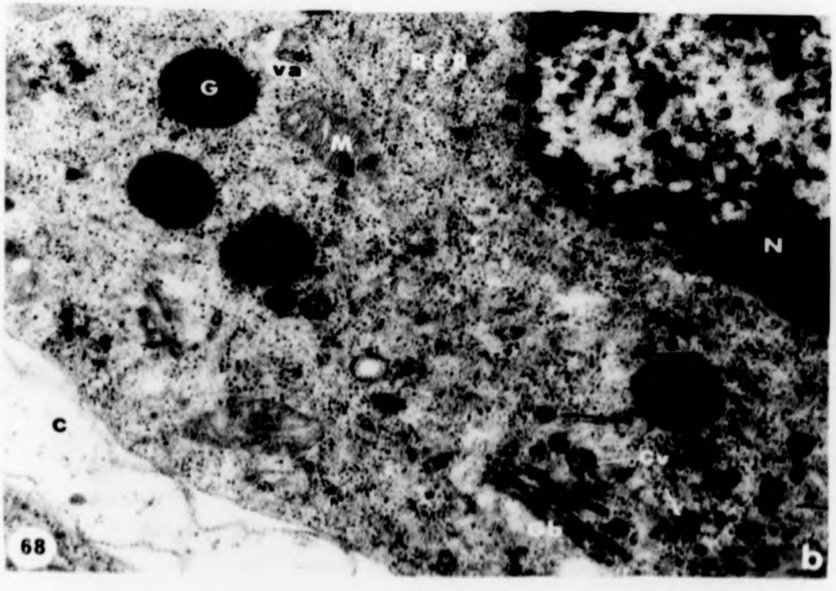
Fig. 67. Spheroidal moderately dense inclusion (thick arrow) is found in the middle of the nucleus of type a cell on the third day after attachment. This inclusion is readily distinguished from the other components of the nucleoplasm. Note that some isolated distended portions of the rough endoplasmic reticulum may contain dark osmiophilic granules (empty arrows).

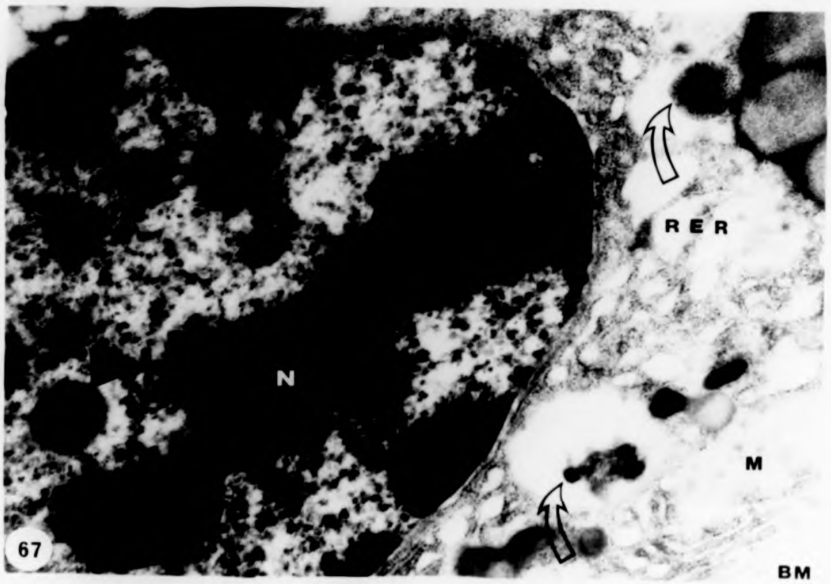
x 25.100

Fig. 68. High magnification of type b cells on the first day after attachment to show that their cytoplasm contains parallel stack arrays of Golgi bodies (Gb) which are surrounded by many free ribosomes (r) and numerous cisternae of rough endoplasmic reticulum (RER). Note that small vacuoles of variable size and density are found in the Golgi region. c, type c cells; cv, condensing vacuoles; G, secretory granules; Va, vacuoles.

x 34.500


RER
N
M
BM
67


G
va
M
N
C
68
b


RER
N
M
67
BM

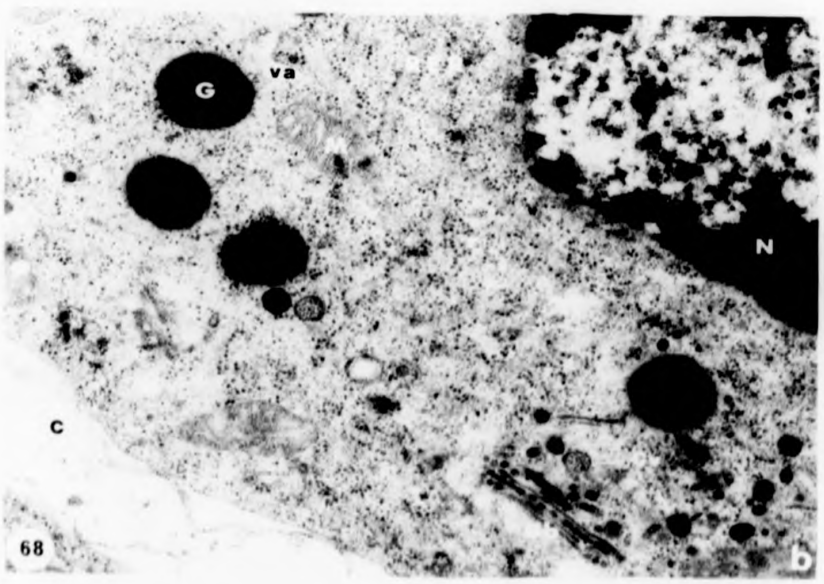
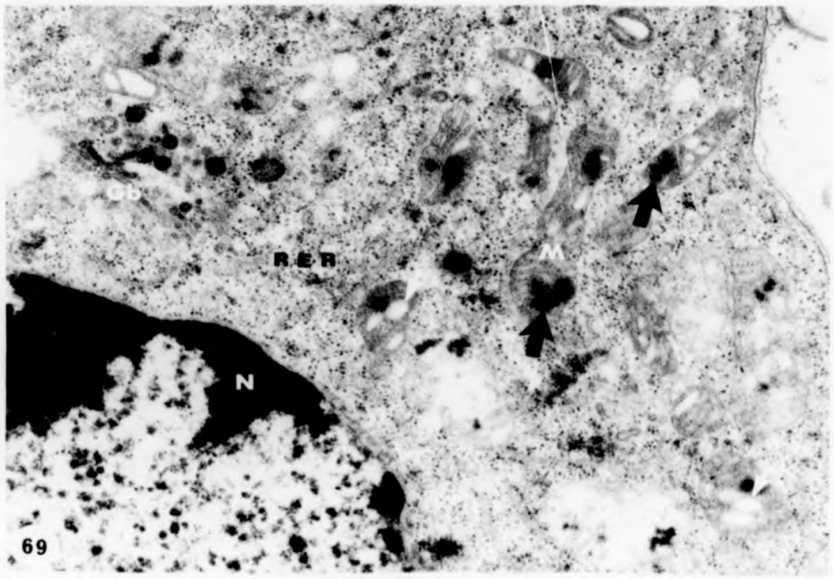

G
va
N
C
68

Fig. 69. The same as Fig. 68 but here showing the basal region contains mitochondria (M) with few, small dense granules (black arrow) in their matrix. Small low dense gaps (white arrows) can be recognised inbetween their parallel cristae. Other letterings as in Fig. 68.

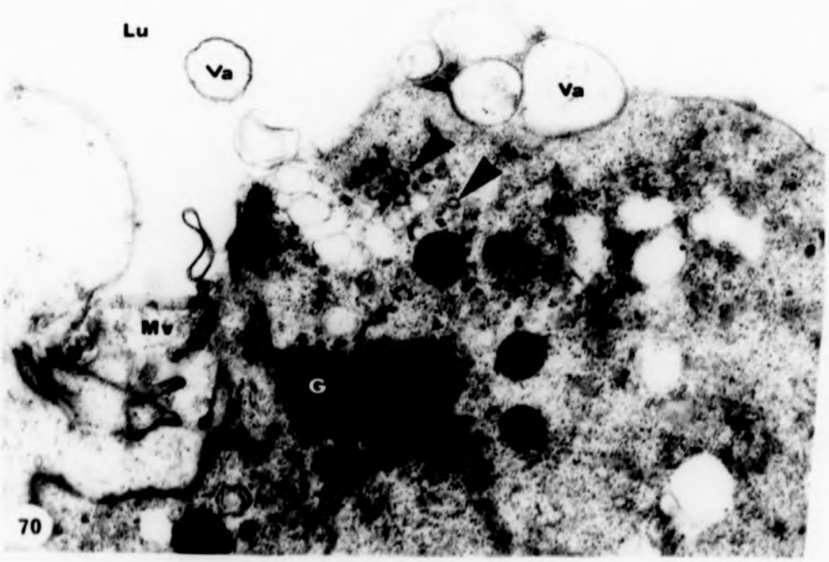
x 32.200

Fig. 70. The same as Fig. 68 but another b cell showing a few completely dense granules (G) are found in the apical region of the cells. Some membrane-bound empty vacuoles (va) are attached to the apical plasma membrane while some others are embedded in the cytoplasmic matrix or in the alveolar lumen (Lu). Note some small vesicles (arrowheads) are embedded in the apical cytoplasmic region. MV, microvilli.

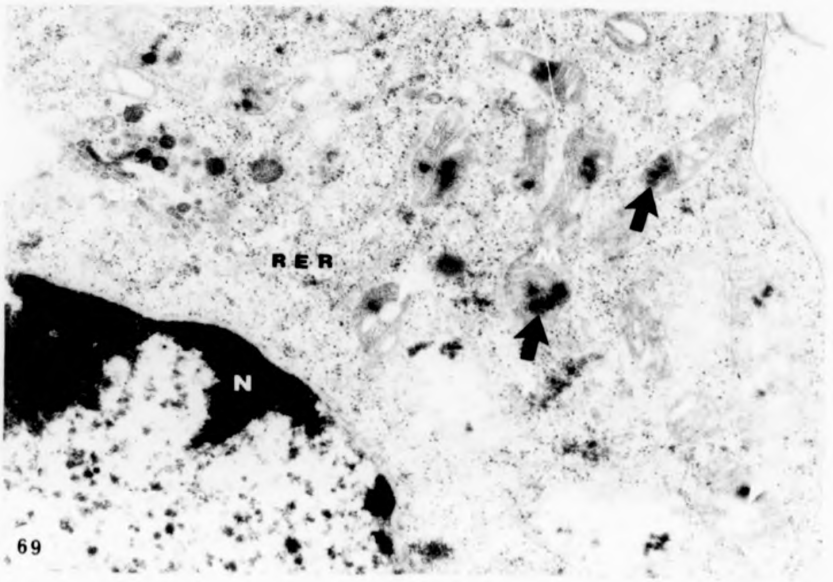
x 28.100



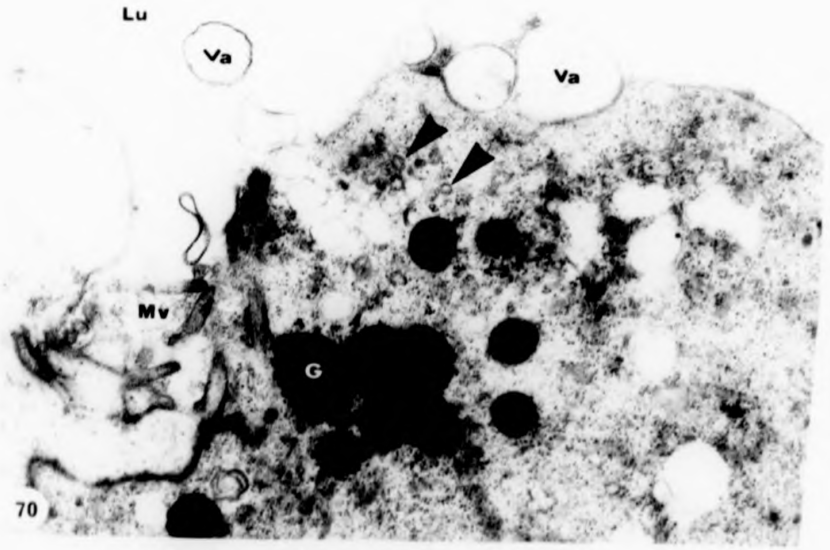
69



70



69



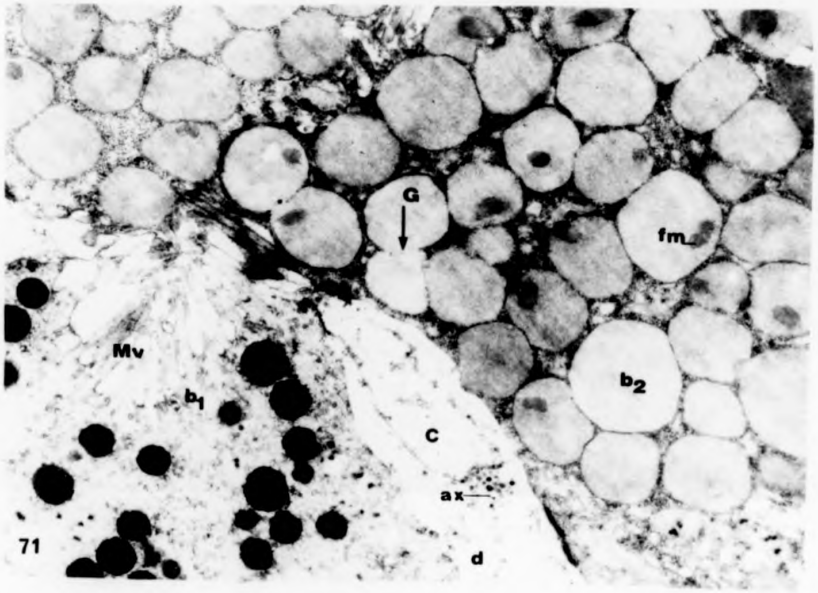
70

Fig. 71. Electron micrograph of the apical regions of some sub-types b_1 and b_2 cells on the third day after H. leachii female attachment. Note the new granules (G) of sub-type b_2 are different in size and density from those of sub-type b_1 (which still contain the original dense granules). Arrow indicates the fusion of twc granules of sub-type b_2 cells via their limiting membrane before releasing their content. ax, axon; c, type c cells; d, type d cells; fm, filamentous material; Mv, microvilli.

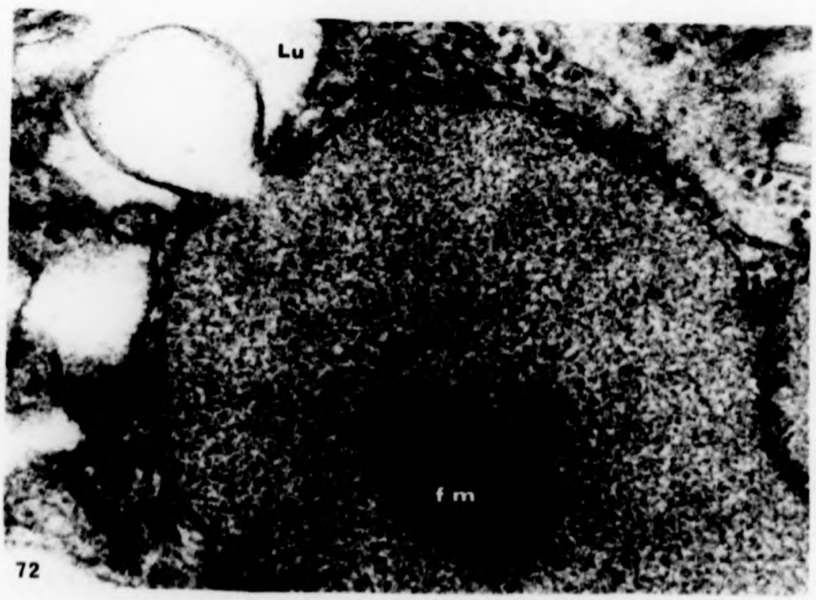
x 9.925

Fig. 72. High magnification of a new granule (G) of sub-type b_2 cells on the third day after attachment containing an aggregate of a filamentous material (fm) in its matrix. Lu, lumen.

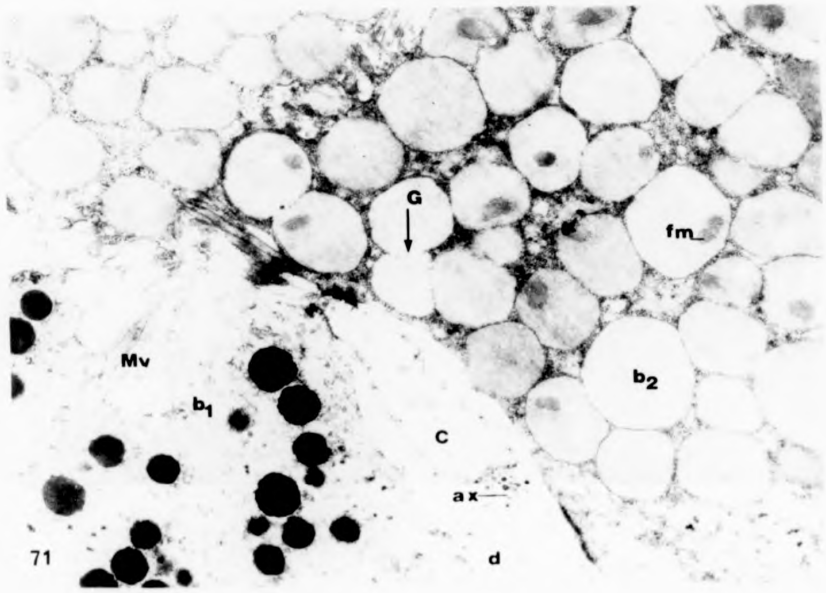
x 83.520



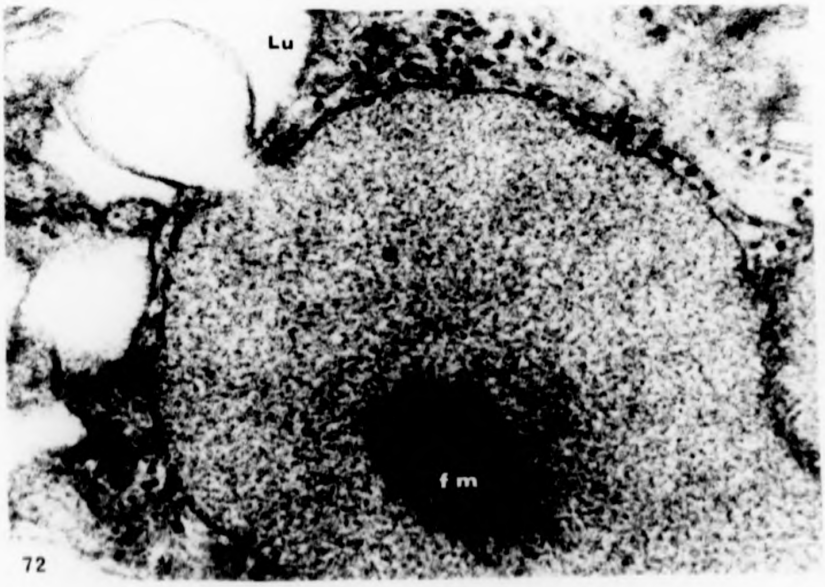
71



72



71



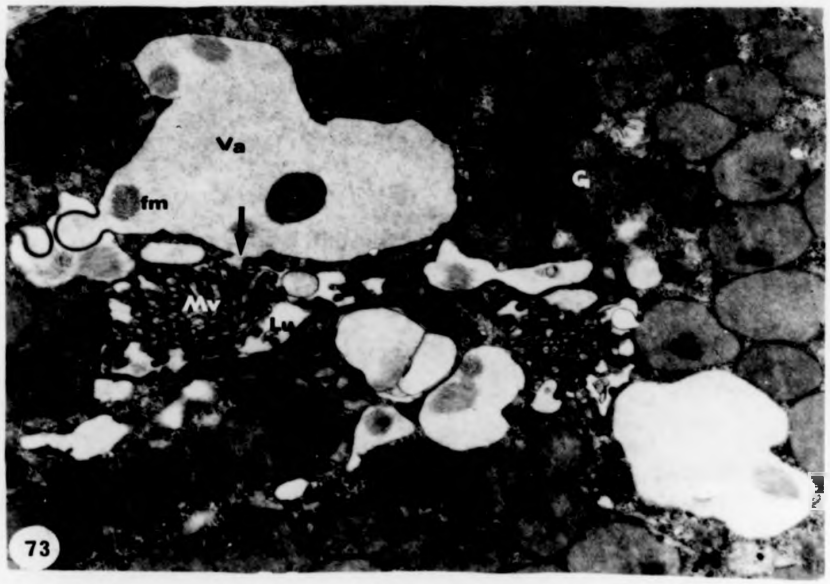
72

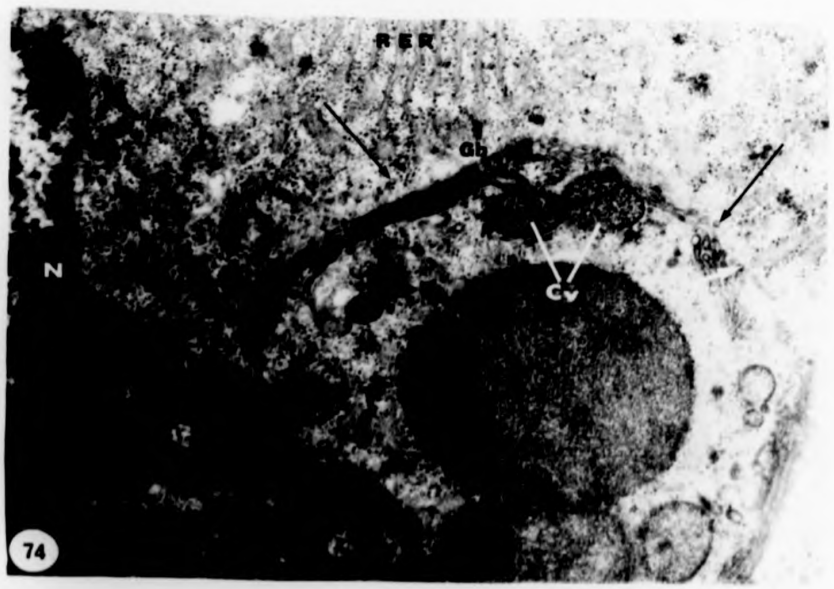
Fig. 73. The middle region of sub-type b_2 cell on the third day after attachment to show the large vacuoles (Va) underlying the plasma cell membrane. These vacuoles contain some filamentous material (fm). Arrow indicates a narrow channel connecting the large vacuole with the microvilli (MV) and ultimately with the alveolar lumen (Lu). G, secretory granules.

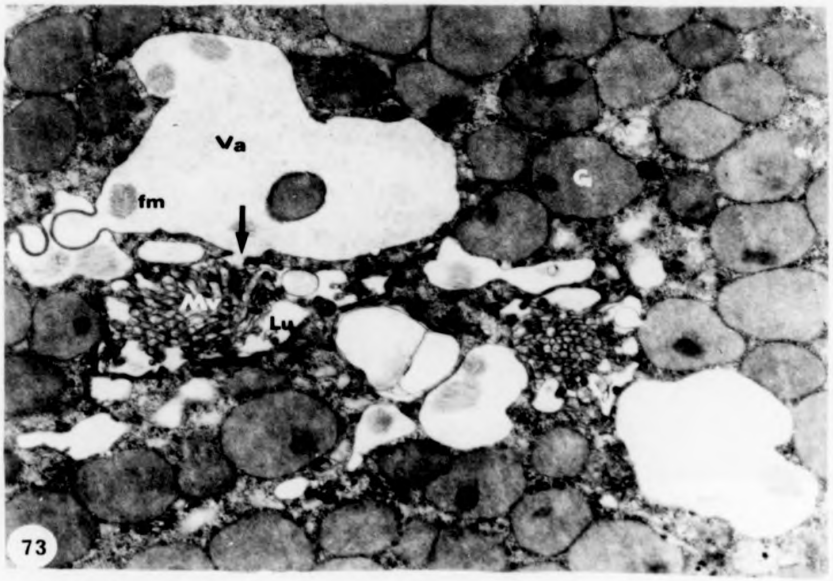
x 10.800

Fig. 74. Electron micrograph of a lateral portion of sub-type b_2 cell on the third day after attachment. The curved arrays of parallel Golgi cisternae (Gb) are often associated with one end of a rough endoplasmic reticulum stack (RER). Small vesicles (black arrows) are found in the Golgi region and appear to carry a synthesised material. These vesicles arise by budding off from areas of the RER which have lost their ribosomes (white arrows). Golgi bodies give rise to small condensing vacuoles (cv) which are converted into mature granules probably by progressive filling and concentration of their content.

x 27.600


Va
fm
Mv
Lu
G
73


RER
Gb
N
Cv
74


Va
fm
Lu
73

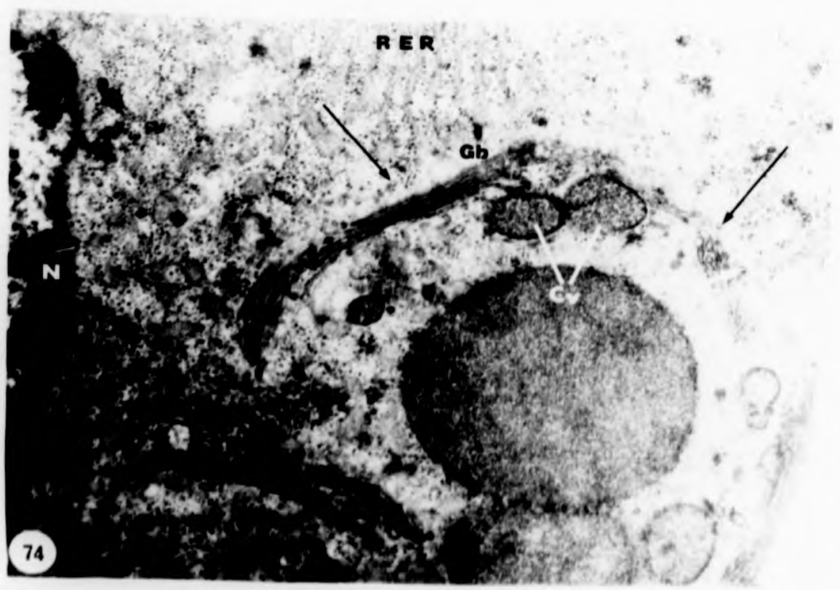
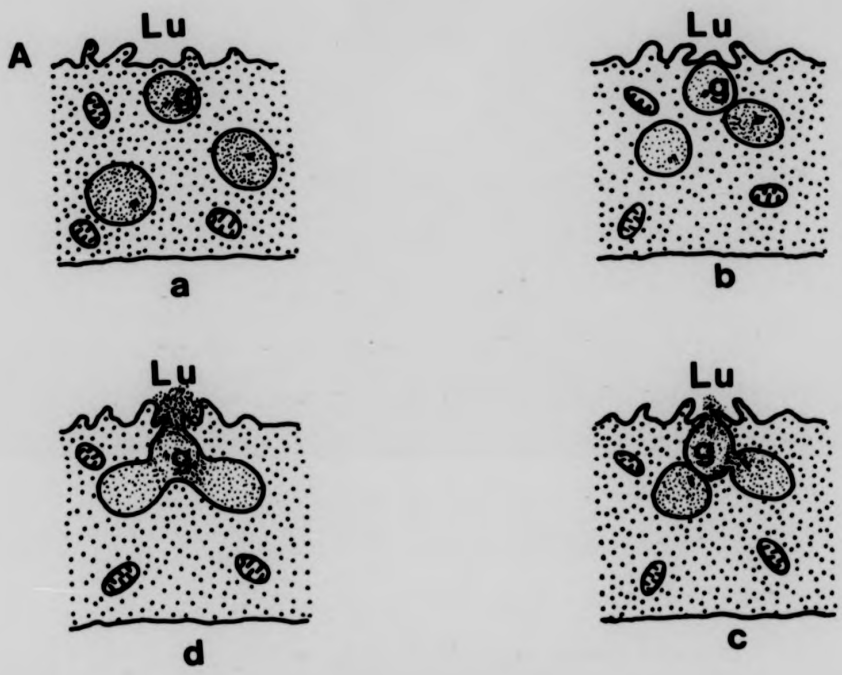

RER
Gb
N
74

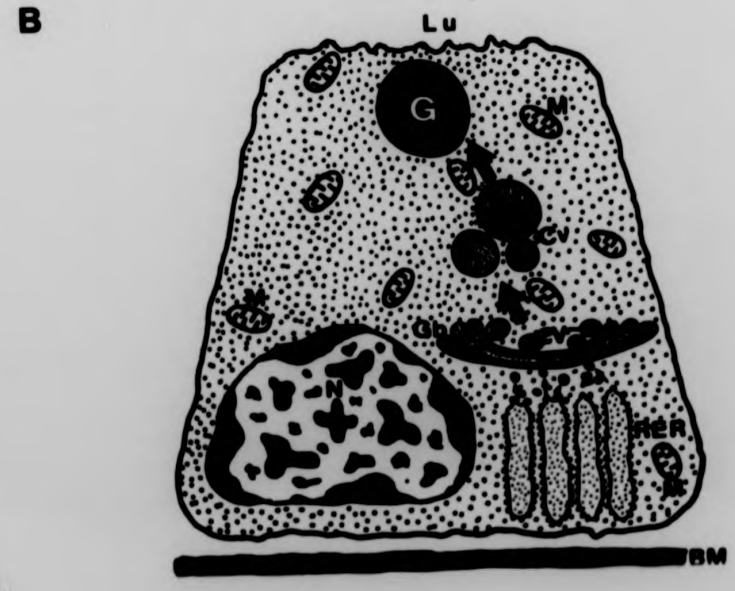
Fig. 75. Diagram of type b_2 cells on the third day after attachment showing:

A) (a-d), the discharging mechanism of the secretory granules (g) and formation of a large vacuole underlying the apical plasma cell membrane. Lu, lumen.

B) The synthesising mechanism of the membrane-bound granules.

BM, basement membrane; cv, condensing vacuoles; G, mature granule; Gb, Golgi bodies; Lu, lumen; M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; s, small vesicles.


A
Lu
a
Lu
b
Lu
d
Lu
c


B
Lu
G
M
CV
GL
N
RER
BM

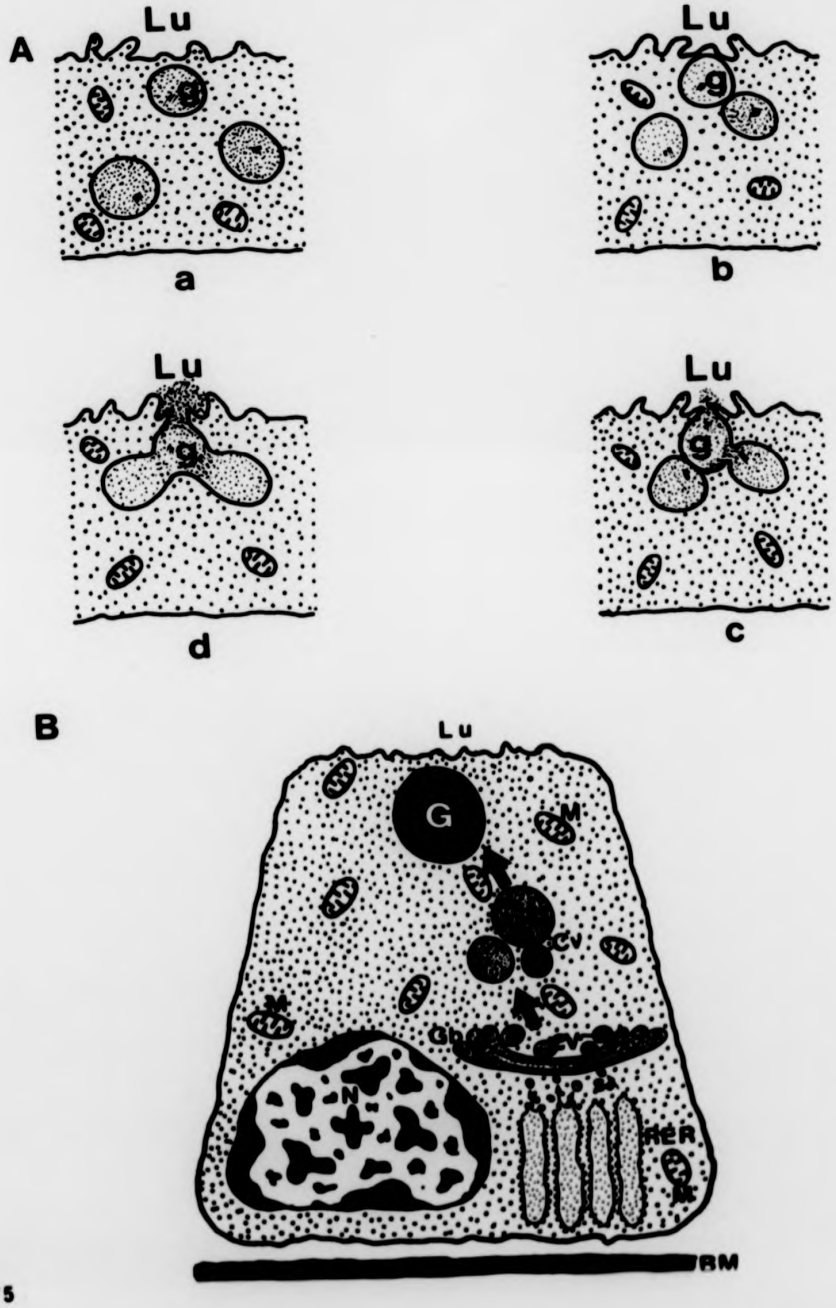
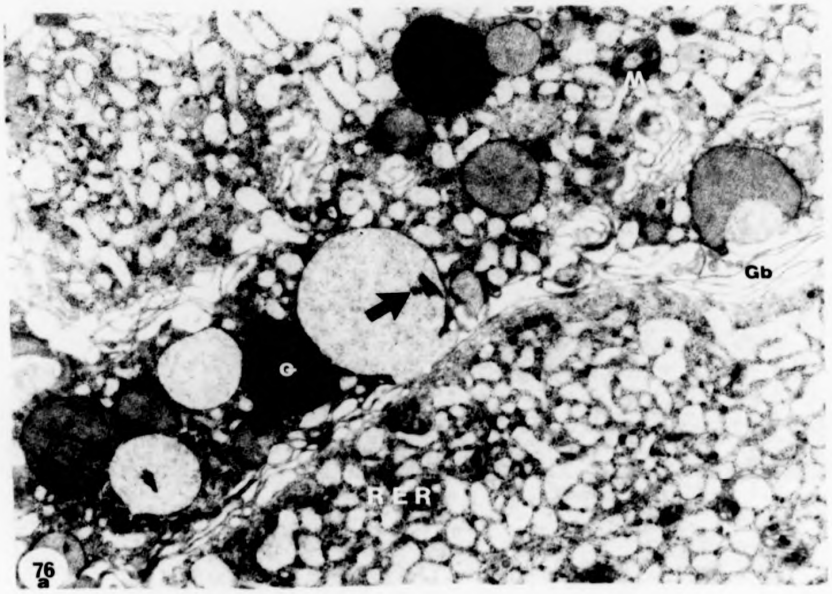

A
Lu
g
a
Lu
g
b
Lu
g
d
Lu
g
c
B
Lu
G
M
CV
GL
N
RER
BM

Fig. 76a. Electron micrograph of sub-type b_2 cell on the fifth day after H. leachii female attachment showing new dark granules (G) in the Golgi region (Gb) in addition to the granules possessing the filamentous material (arrow). Note that some cisternae of rough endoplasmic reticulum (RER) are now distended.

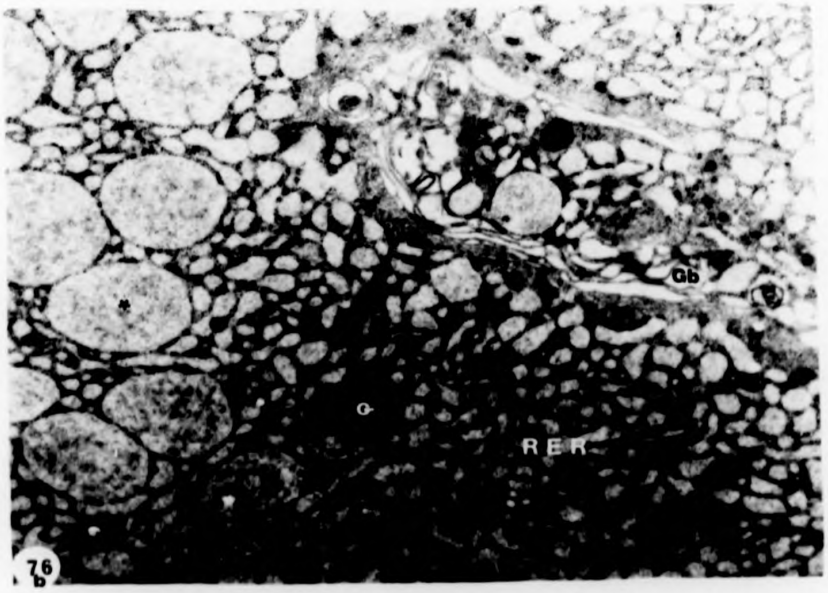
x 10.100

Fig. 76b. The same as Fig. 76a but here the basal region of the same cell, showing distended cisternae of RER (*) while the rest are smaller, irregular in shape and distributed throughout the cytoplasm. Other letterings as in Fig. 76a.

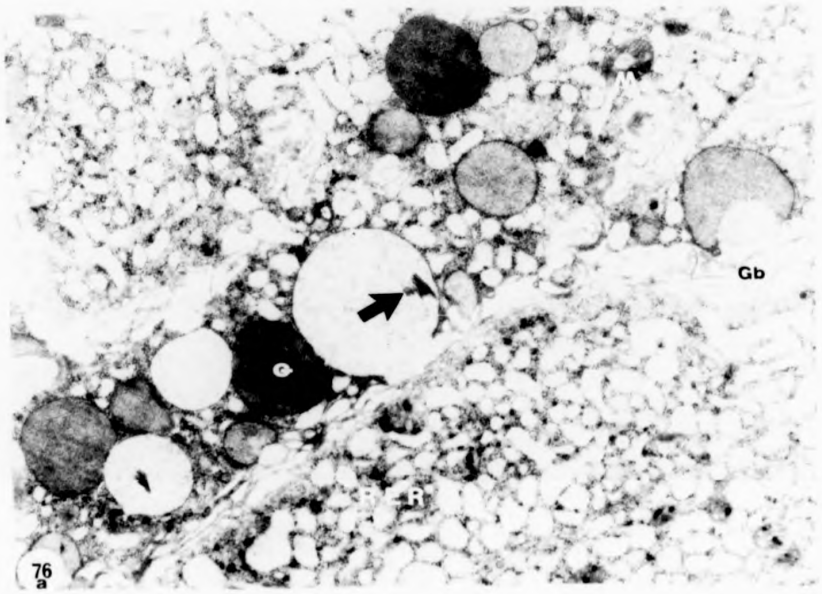
x 9.925



76a



76b


Gb
76
a

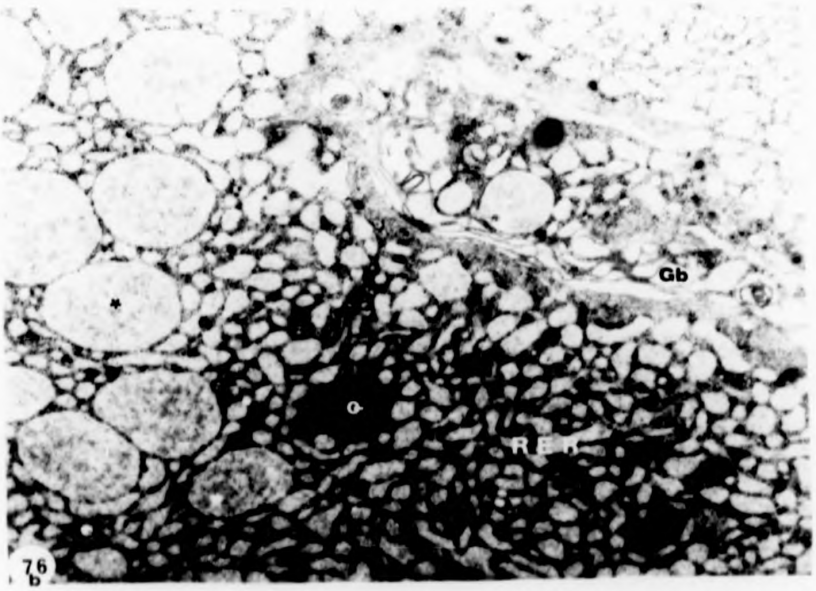
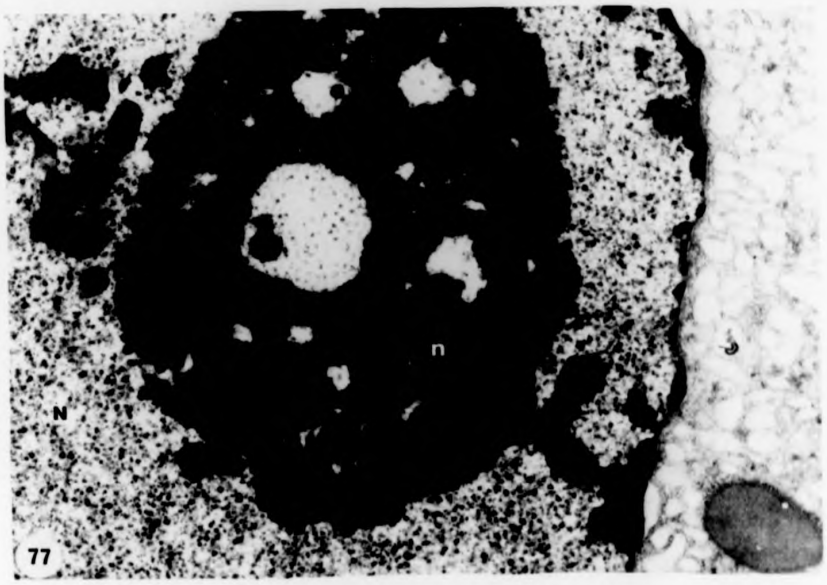

Gb
76
b

Fig. 77. Electron micrograph of a nucleus (N) of sub-type b_2 cell on the fifth day after attachment. Note the large low donse area of the nucleus with scattered patches of chromatin. The nucleolus (n) is compact, granulated and possesses low dense vacuoles of variable size. Compare Fig. 77 with Fig. 21.

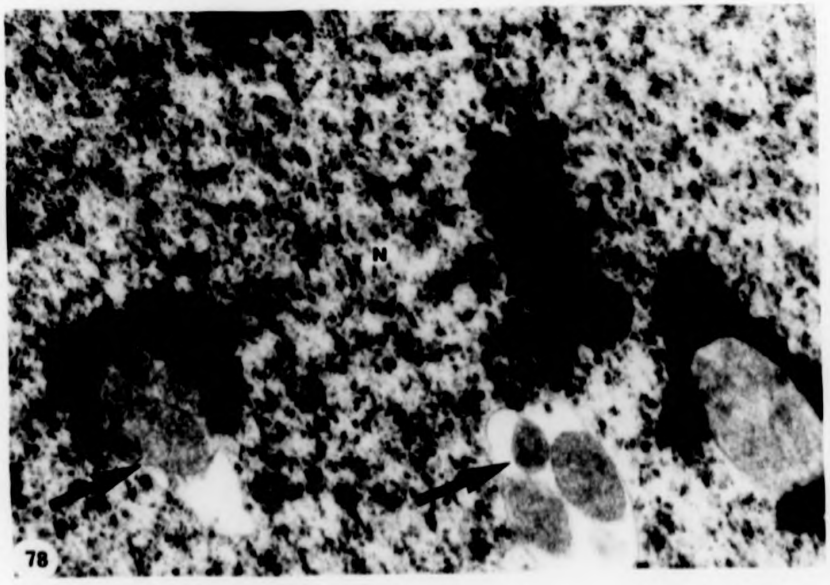
x 11.550

Fig. 78. The same as Fig. 77 but on the seventh day after attachment, showing unknown granules (arrows) enclosed within the nucleus (N).

x 32.850



77



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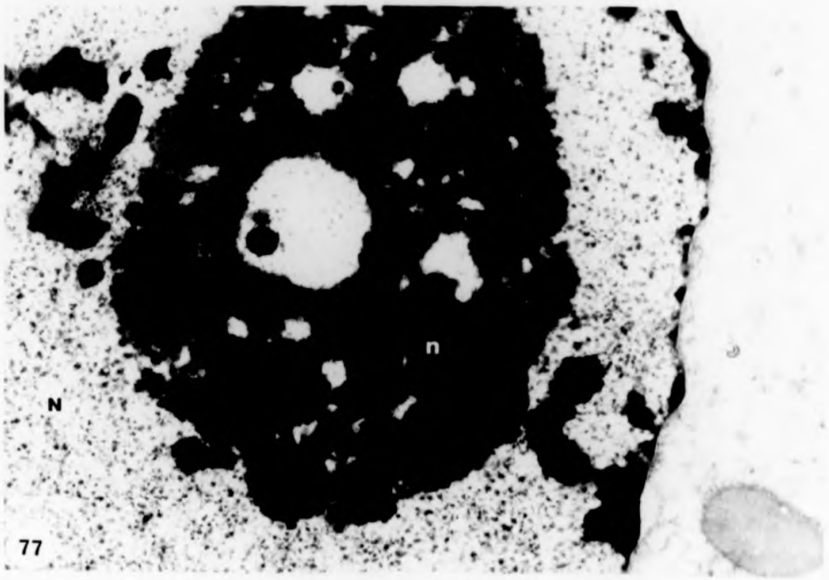
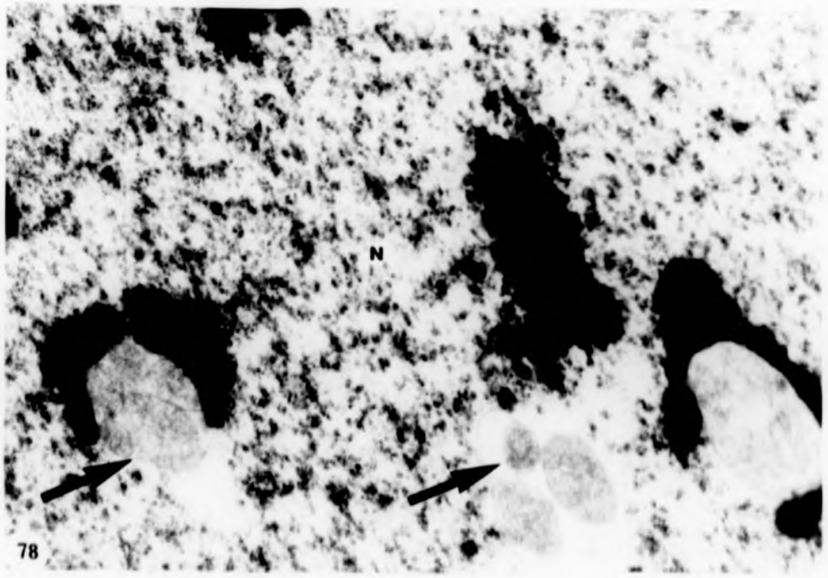


77



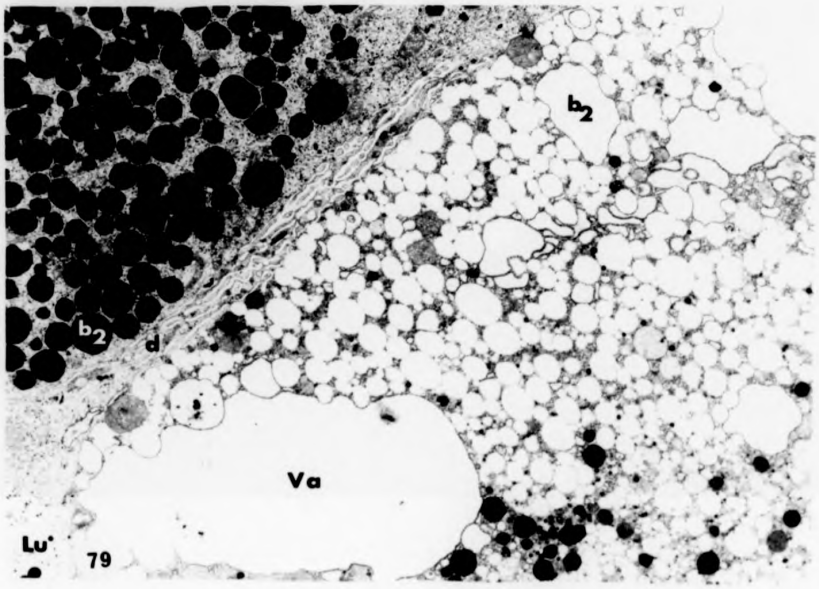
78

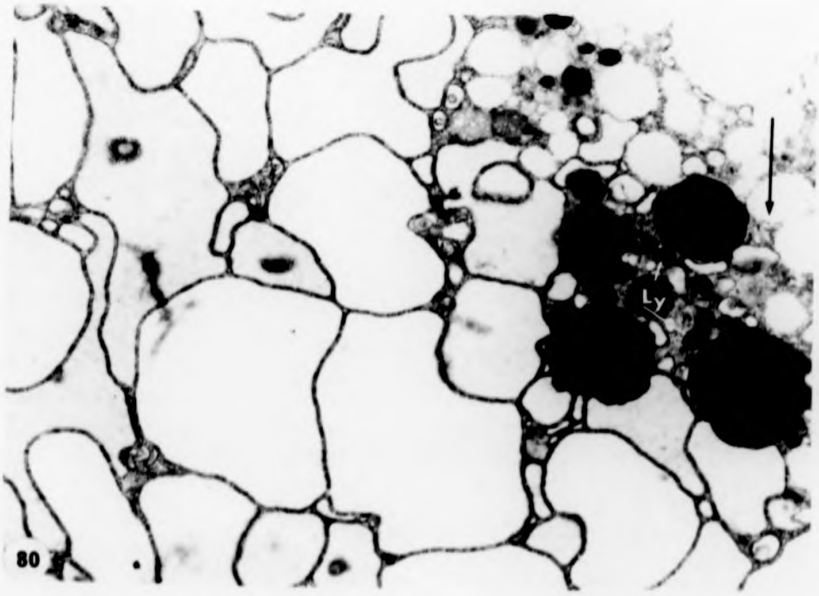
Fig. 79. Electron micrograph of sub-type b_2 cells (b_2) on the seventh day after H. leachii female attachment. Note that the cytoplasm of one cell is packed with dark, spherical, small granules, while the cytoplasm of the other contains few of these granules, especially in the apical region, as well as numerous, low dense membrane-bound vacuoles of variable size occupying the middle and the apical regions of the cell. d, type d cells; Lu, alveolar lumen; Va, vacuoles.

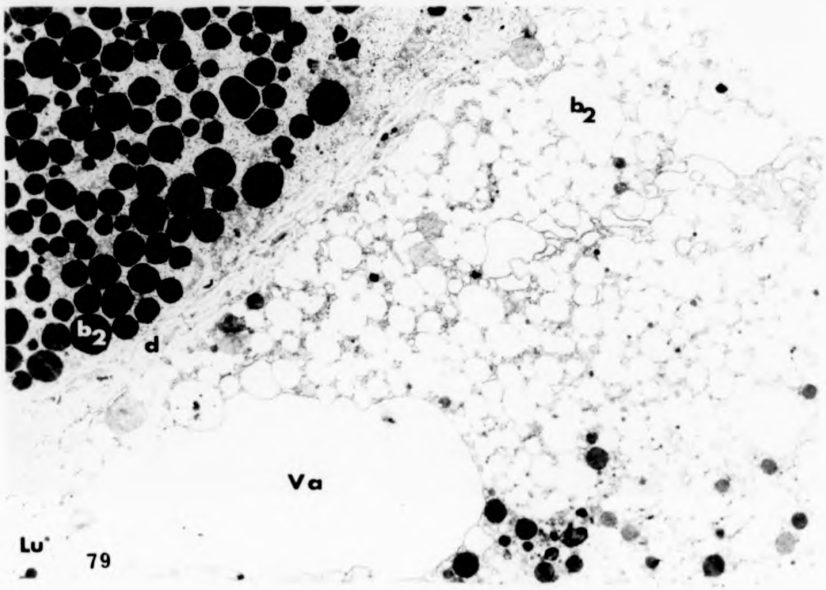
x 4.320

Fig. 80. The same as Fig. 79 but here showing the great distension of the rough endoplasmic reticulum cisternae lying in the basal region of the cell. Note lysosome-like structures (Ly) between these distended cisternae and the low dense vacuoles. The arrow points to the limiting membrane of a low dense vacuole. G, secretory granules.

x 10.500


b2
b2
d
Va
Lu
79


Ly
80


b2
b2
d
Va
Lu
79

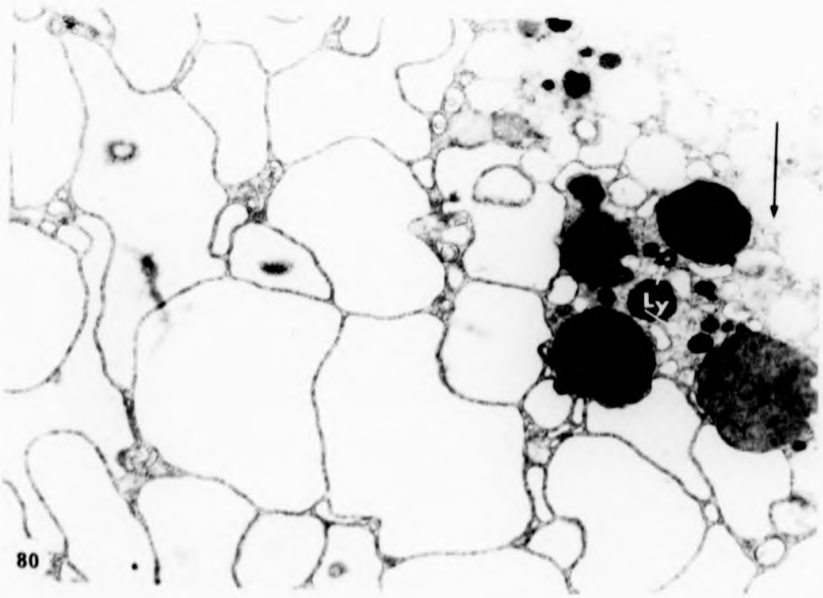
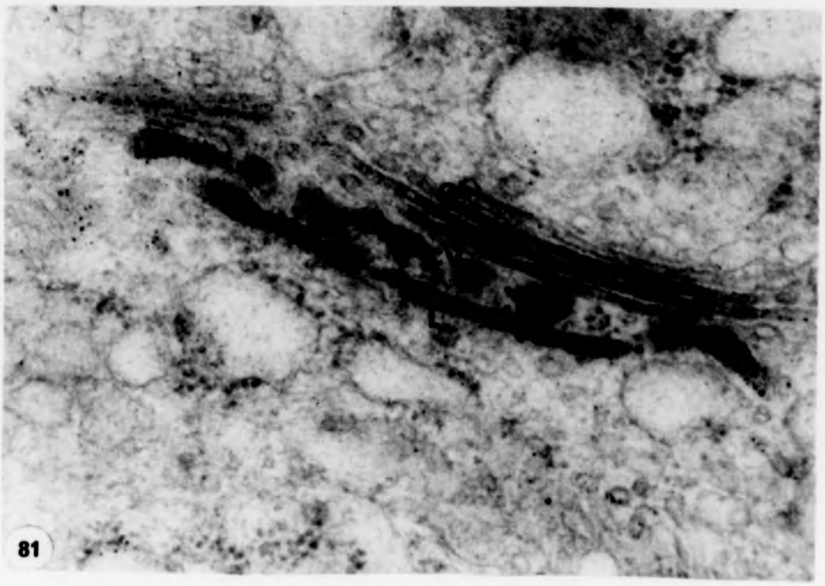

Ly
80

Fig. 81. Ultrastructural histochemical micrograph of a portion of sub-type b_2 cell incubated in acid phosphatase medium on the fifth day after H. leachii female attachment. Note that the Golgi cisternae (Gb) contain only light deposits of reaction product, while some small condensing vacuoles (cv) as well as tubular or lamellae-like structures (L) found in the Golgi region are intensely reactive.

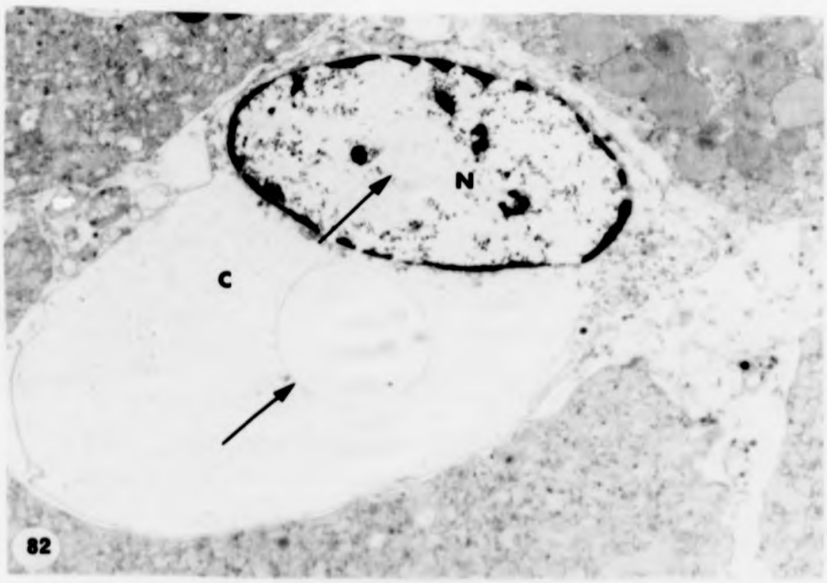
x 102.855

Fig. 82. Electron micrograph of type c cells (c) on the third day after H. leachii female attachment. Note the great distension of the plasma membrane. Note also the low dense membrane-bound vacuoles (arrows) found in the cytoplasmic matrix and in the nucleus (N).

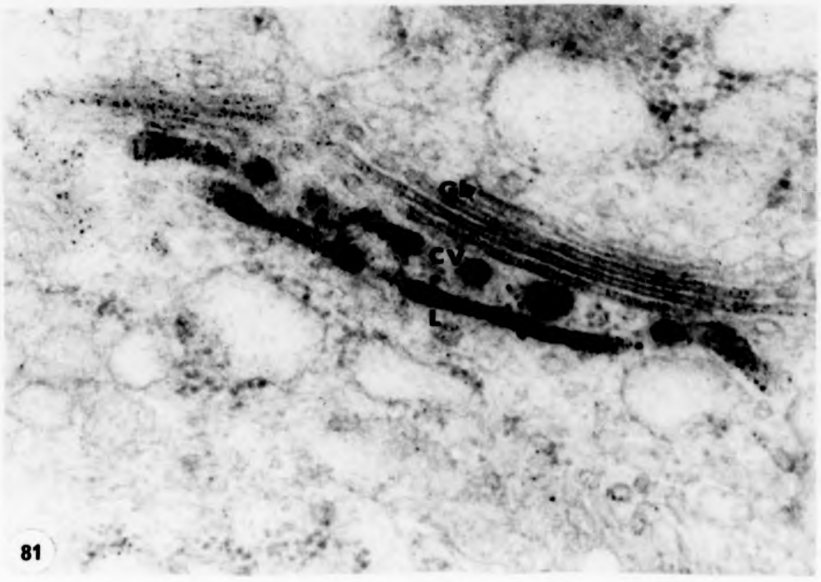
x 6.000



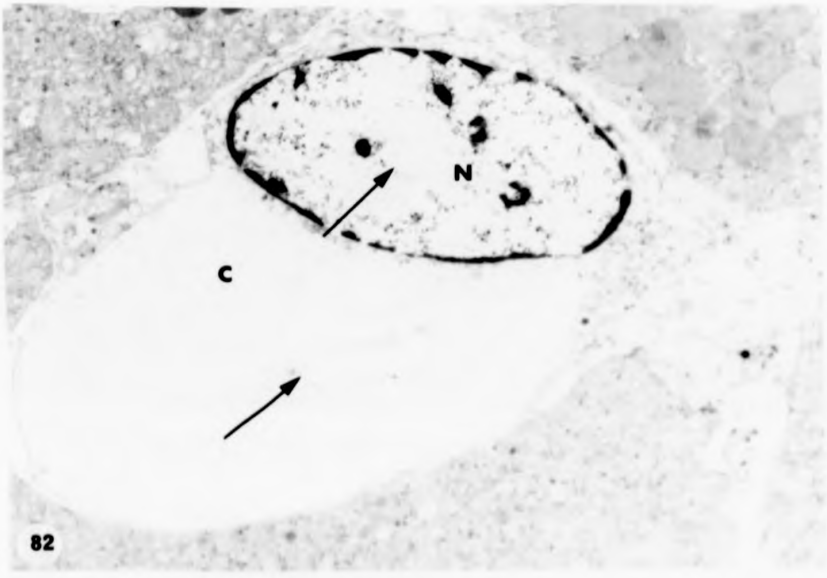
81



82



81



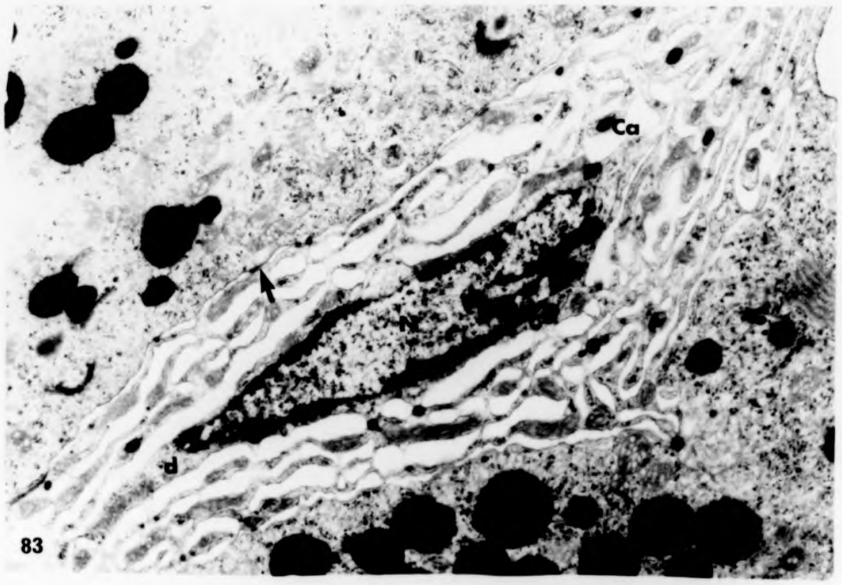
82

Fig. 83. Electron micrograph of type d cells (d) on the seventh day after H. leachii female attachment to show the complexity of the canaliculi (Ca) which surround the alveolar cells and attach with their plasma membranes by means of intercellular junction (arrow). N, nucleus.

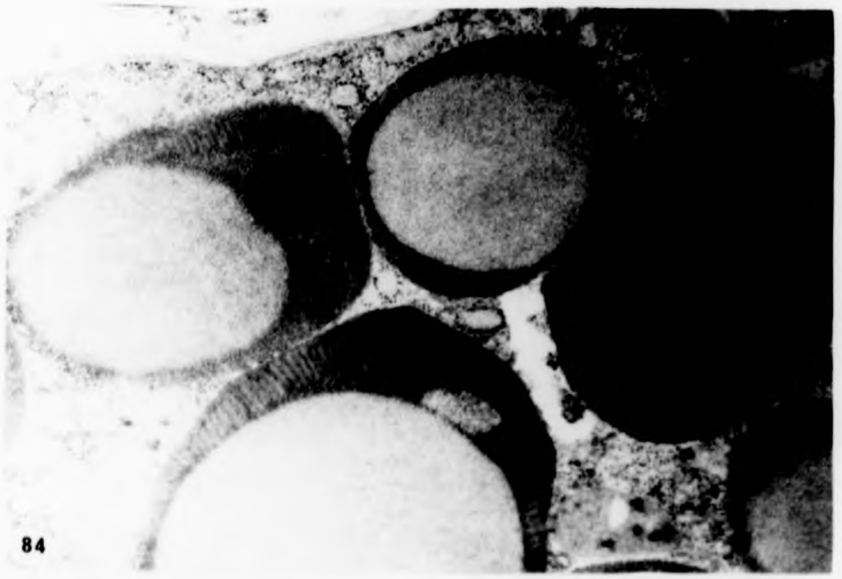
x 14.440

Fig. 84. Electron micrograph of a portion of type e cells on the third day after H. leachii female attachment showing the peculiar shape of the secretory granules. Note that the granules lack the subunits formerly seen in unfed females and are surrounded by an irregular dark lattice which is occasionally thicker on one side than the other. The lattice matrix of some granules contains a dense spherical spot.

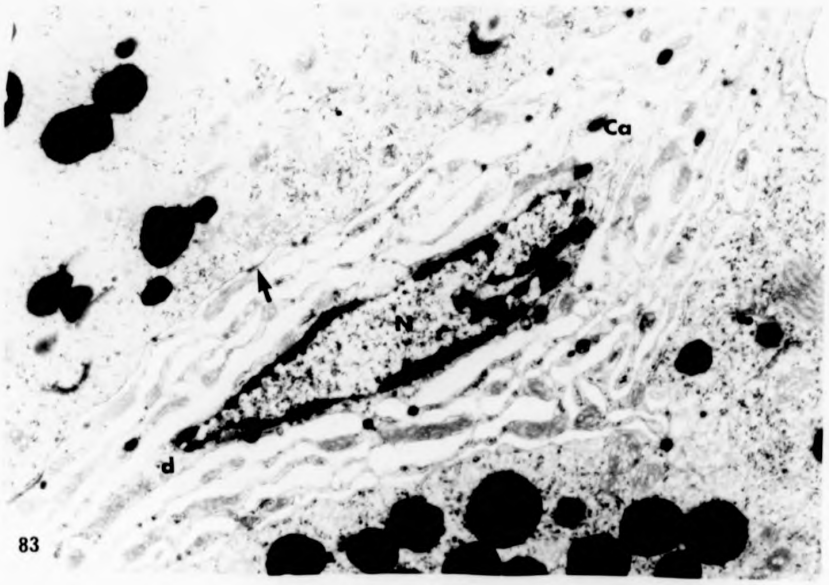
x 29.900



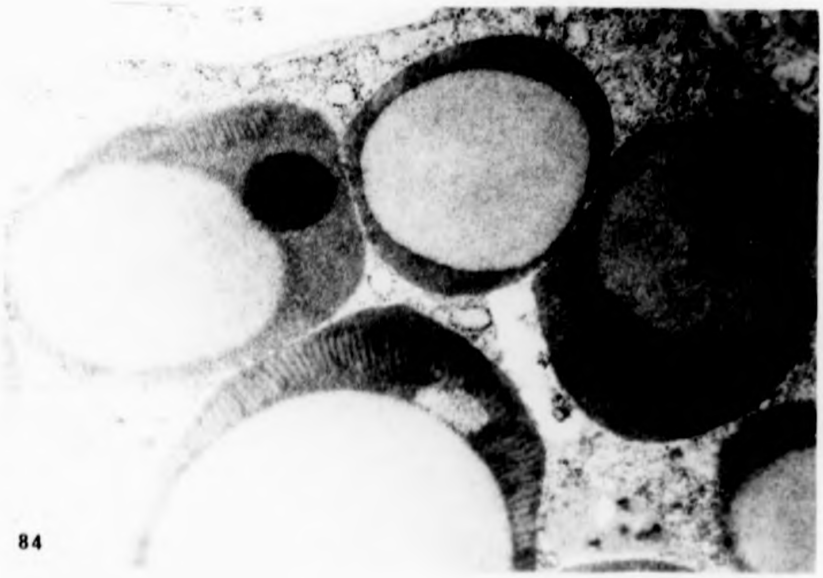
83



84



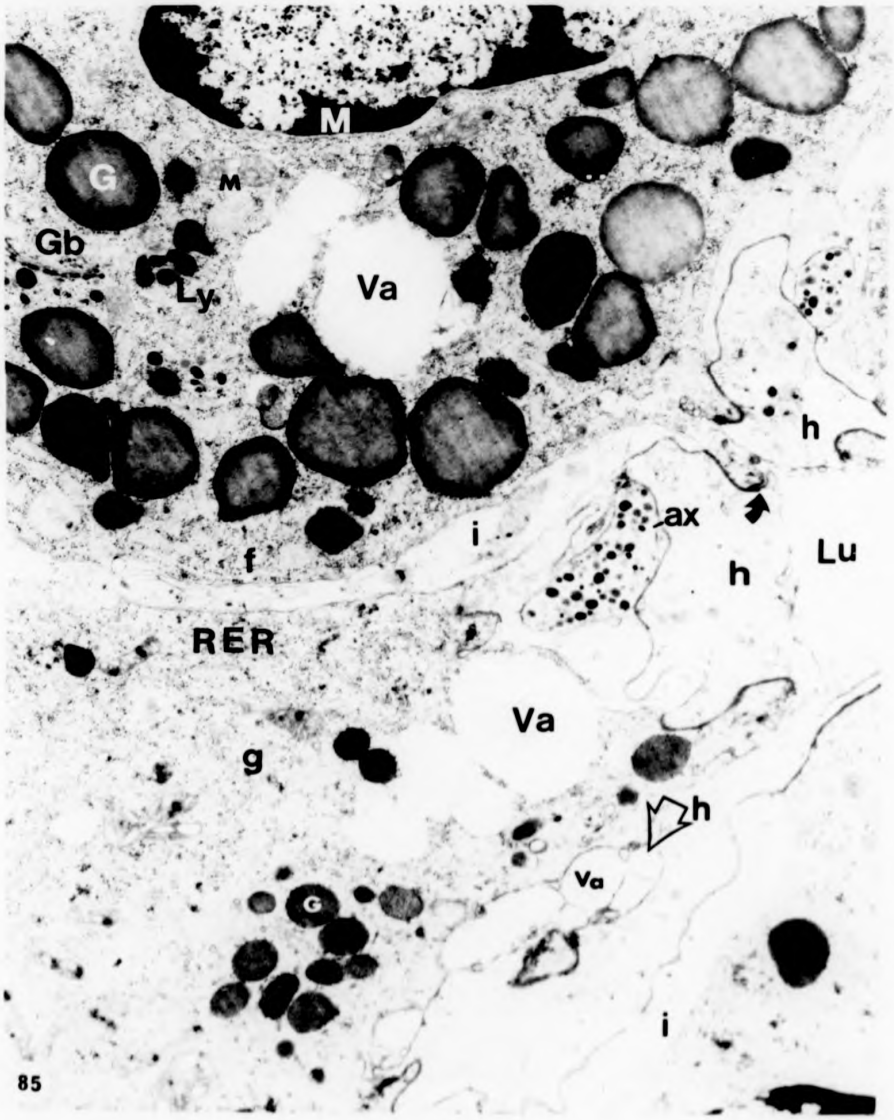
83



84

Fig. 85. Electron micrograph of the apical regions of types f and g cells (f, g) on the first day after H. leachii female attachment. Note the Golgi bodies (Gb) and lysosome-like structures (Ly) in the cytoplasm of f cell. Note also the fine dark granules (G) (not seen in the unfed female) in the apical region of typo g cell as well as membrane-bound vacuoles (Va), some of which are attached to the apical and lateral plasma membranes. Some vacuoles are found between the lateral plasma membranes of type h cells (h) and type g cells (open arrow). Black small arrow points to macula adherans, white arrow points to septate desmosomes between types g and h cells. ax, axon; i, type i cells; Lu, alveolar lumen; M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum.

x 14.330



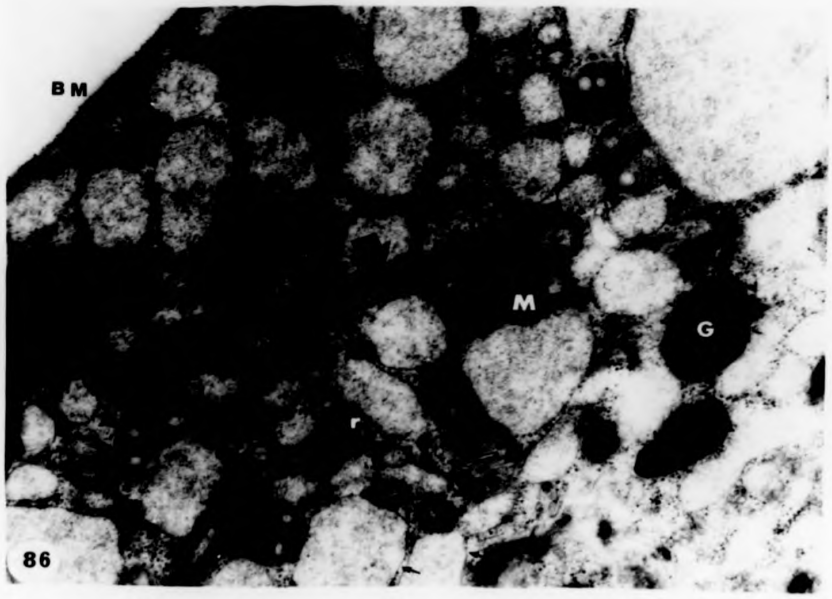
85

Fig. 86. Electron micrograph of the basal region of type f cell on the fifth day after H. leachii female attachment showing the enormously distended cisternae of rough endoplasmic reticulum. Parts of these cisternae have lost their ribosomes (small arrows). BM, basement membrane; r, ribosomes. Other letterings as in Fig. 85. Thick arrow points to the new type of macrotubule which are surrounded by ribosomes on their outer surface.

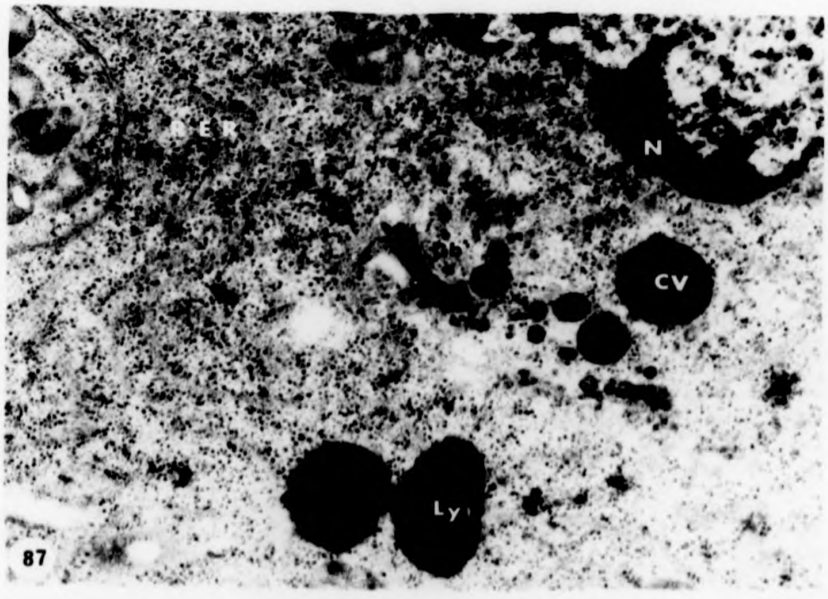
x 38.330

Fig. 87. The basal region of type g cell (g) on the first day after attachment. cv, condensing vacuoles; other letterings as in Fig. 85.

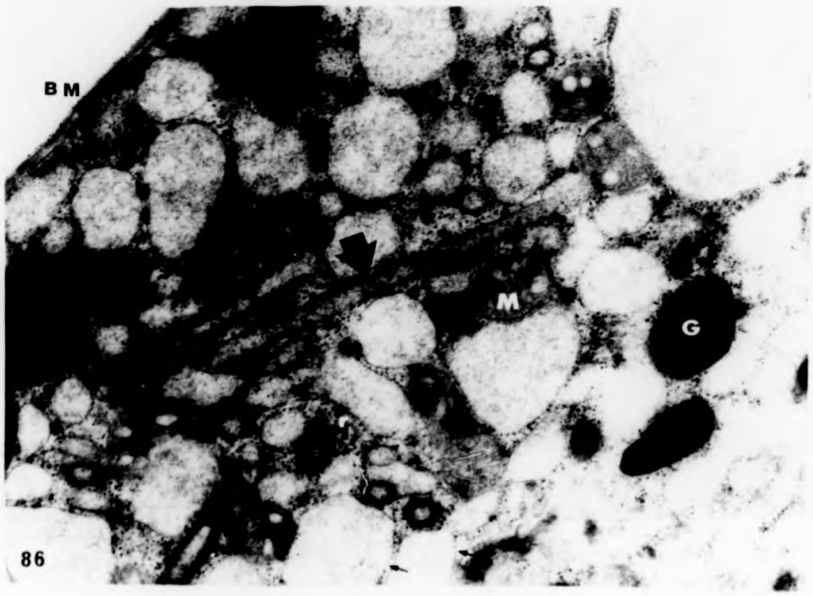
x 34.500



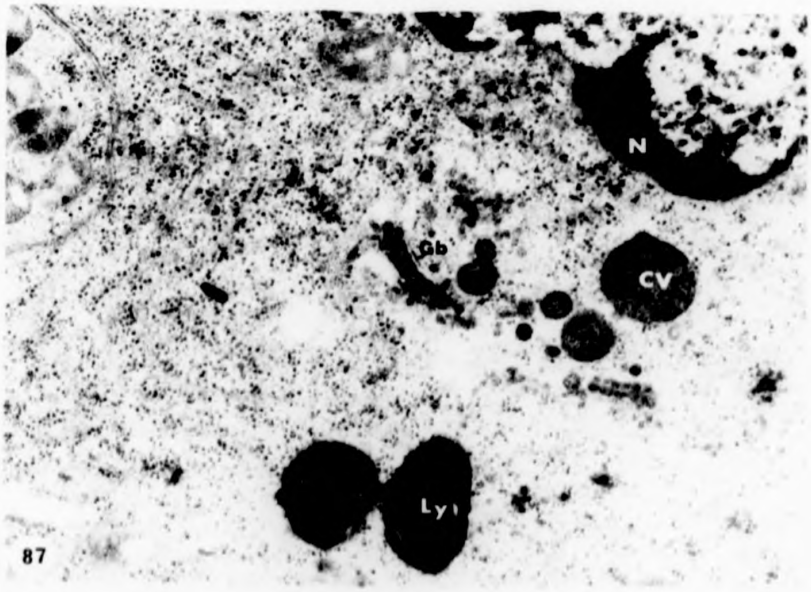
86



87



86



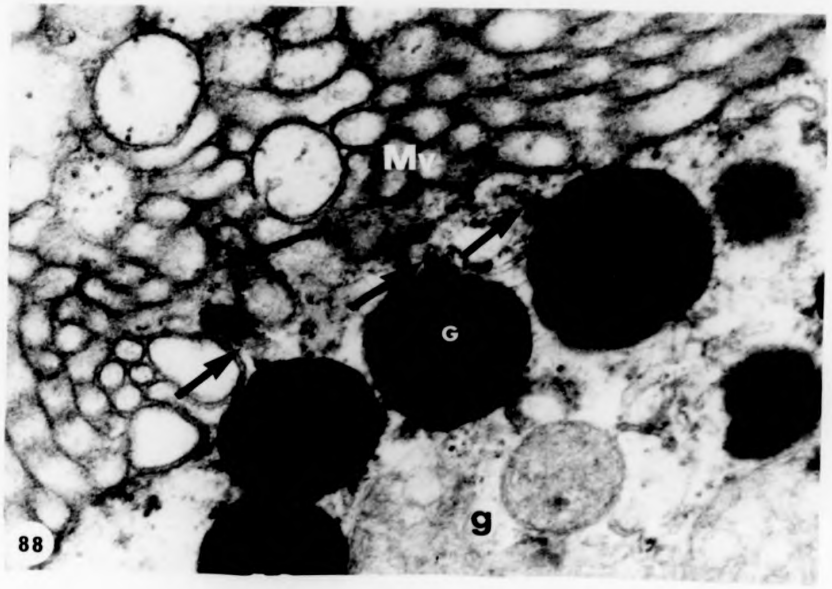
87

Fig. 88. The apical region of type g cells (g) on the third day after attachment to show streaming of the granular content producing a tail (arrows) from their main body. These tails can often be traced into the microvilli (MV) projected from the apical cell membrane.

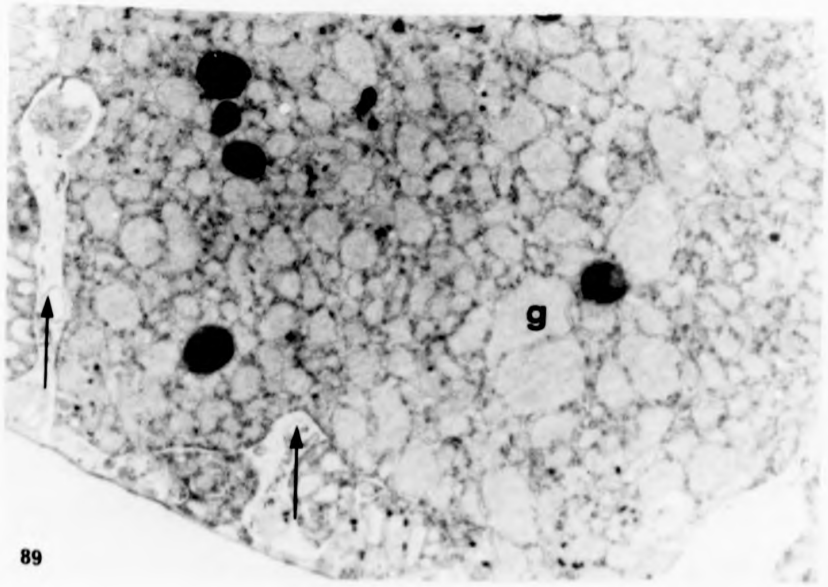
x 55.580

Fig. 89. The same as Fig. 88 but the basal region to show the irregular folds (arrows) of the basal plasma membrane of type g cells (g) which enclose portions of type i cells (the interstitial epithelial cells).

x 10.340



88



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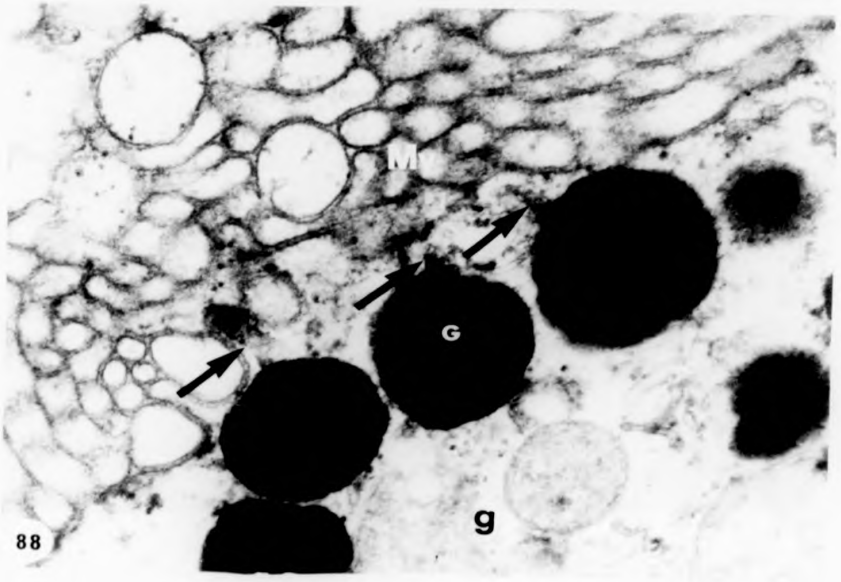
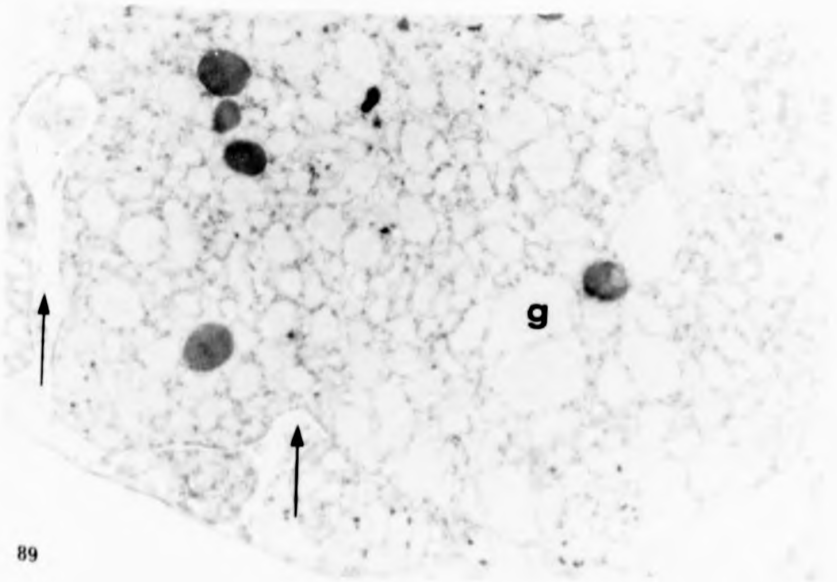


88



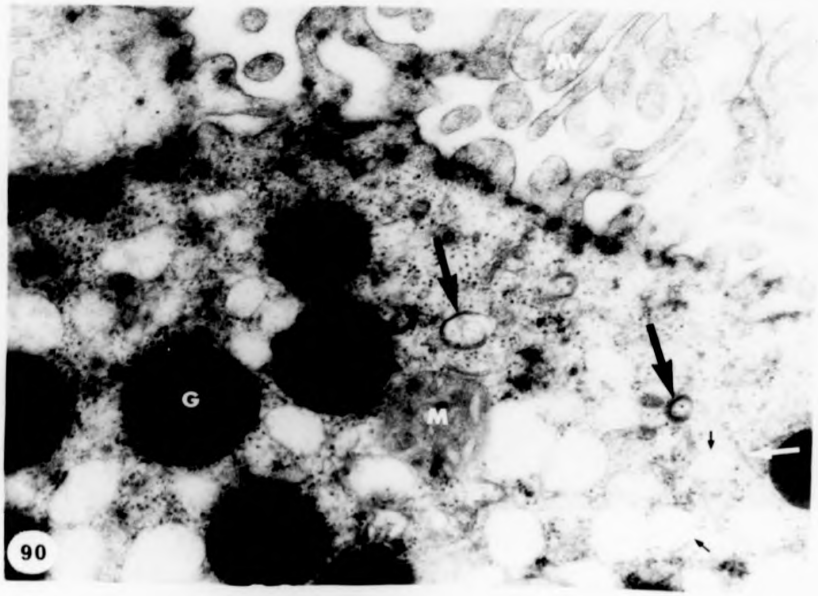
89

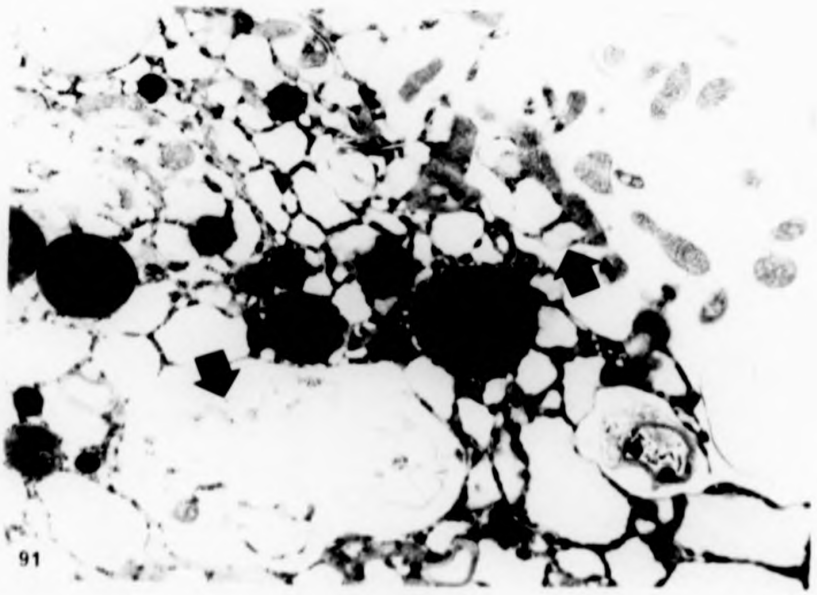
Fig. 90. Electron micrograph of the apical region of type g cells on the fifth day after attachment. White arrow points to microtubules; thick black arrows point to membrane-like structures; small black arrow indicates a portion of rough endoplasmic reticulum which has lost its ribosomes. G, undischarged granules; M, mitochondria with dark small granules in their matrix; MV, microvilli.

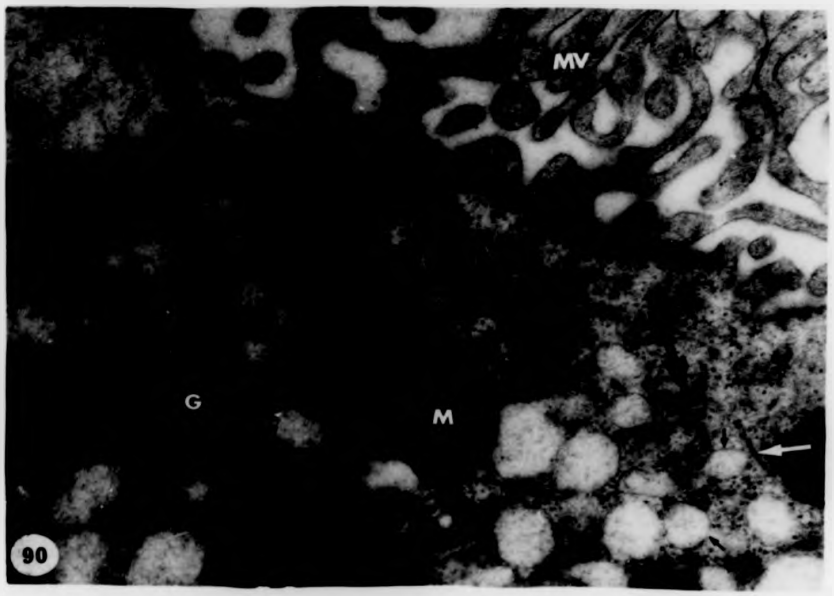
x 36.400

Fig. 91. Electron micrograph of a part of the basal region of type g cell on the seventh day after H. leachii female attachment showing detached cytoplasmic portions with a few, isolated, distended cisternae of rough endoplasmic reticulum, membrane-like structures and mitochondria (arrows).

x 10.260


M
G
M
90


91


MV
G
M
90

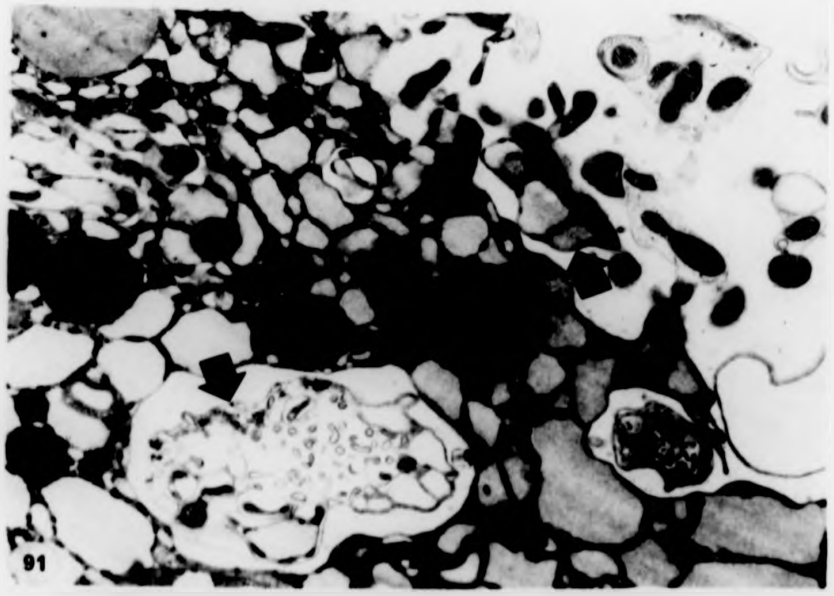
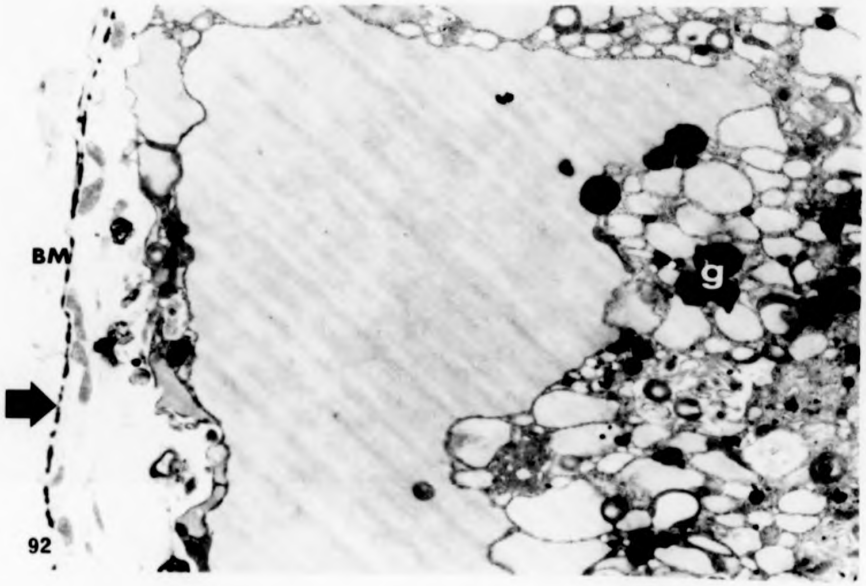

91

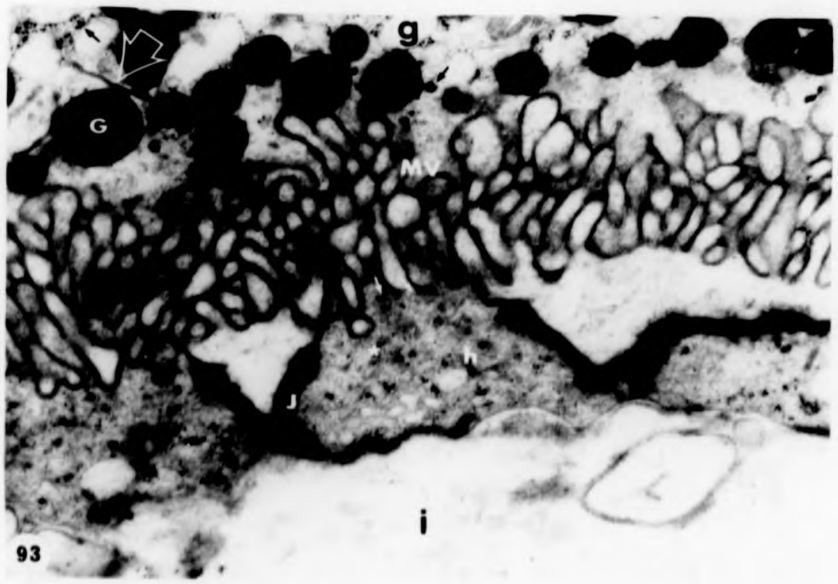
Fig. 92. The same as Fig. 91 but this micrograph shows that the accompanied cisternae of rough endoplasmic reticulum has reached its maximum distension. Arrow points to the dense interrupted line in the basement membrane (BM). g, type g cells.

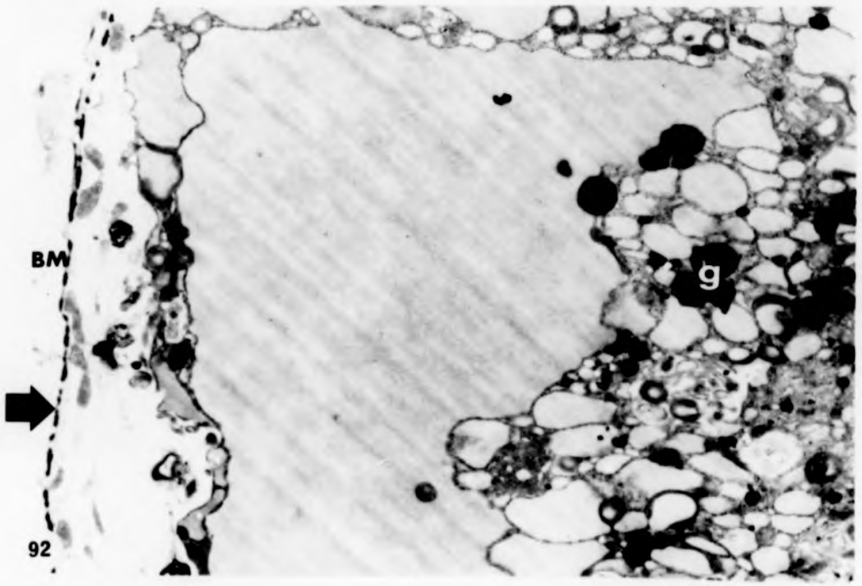
x 4.680

Fig. 93. The same as Figs. 91 and 92 but here the microvilli of type g cell are interdigitating with those of type h cells (h). Small black arrows indicate the aggregates of free ribosomes in the cytoplasmic matrix. (*) indicates the trough-like structure of type h cells. Opened arrow points to membrane-like structures. Small white arrows indicate the streaming of type g cell granular content through the microvilli (MV) into the h cell. G, secretory granules; i, type i cells; J, intercellular junction. Compare Fig. 93 with Fig. 32.

x 28.750


BM
g
92


g
G
MV
J
L
i
93


BM
g
92

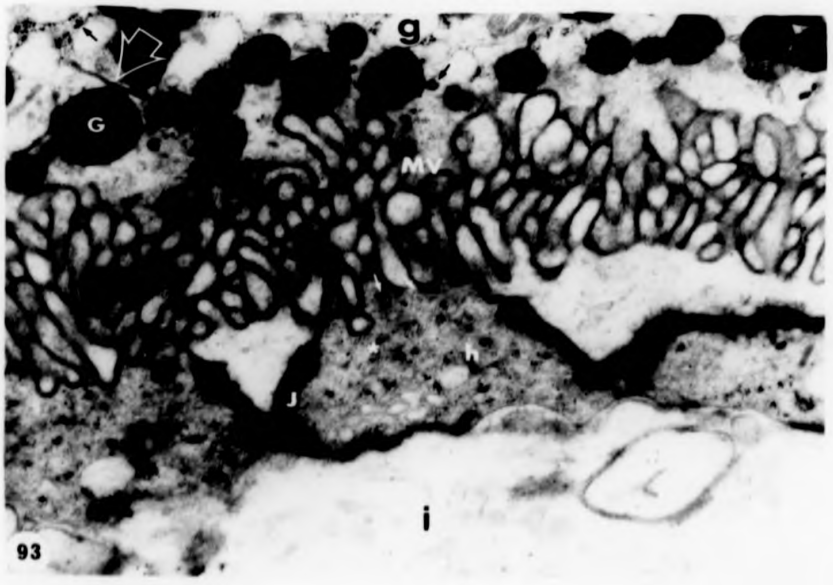
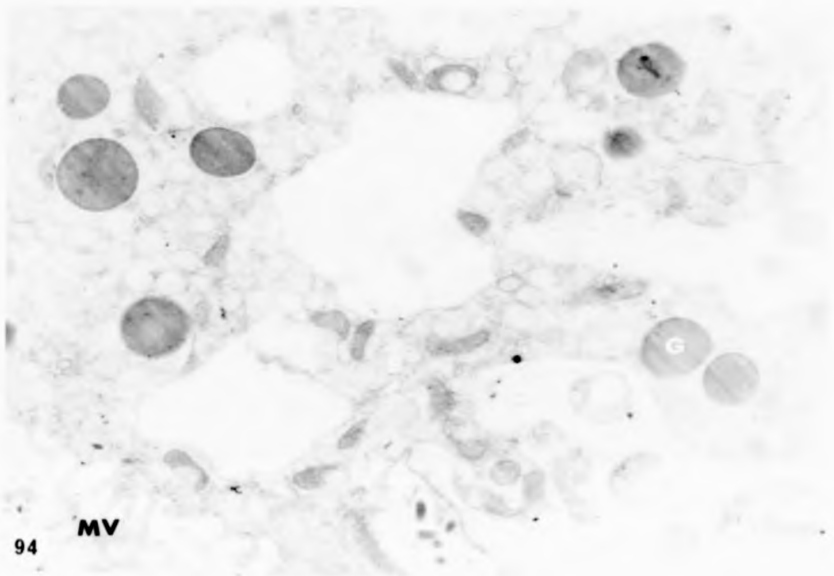

g
G
MV
J
i
93

Fig. 94. Ultrastructural histochemical micrograph of a portion of type g cells (g) on the fifth day after attachment after treatment with PA-TCH. Note that the secretory granules (G) are polysaccharides positive.

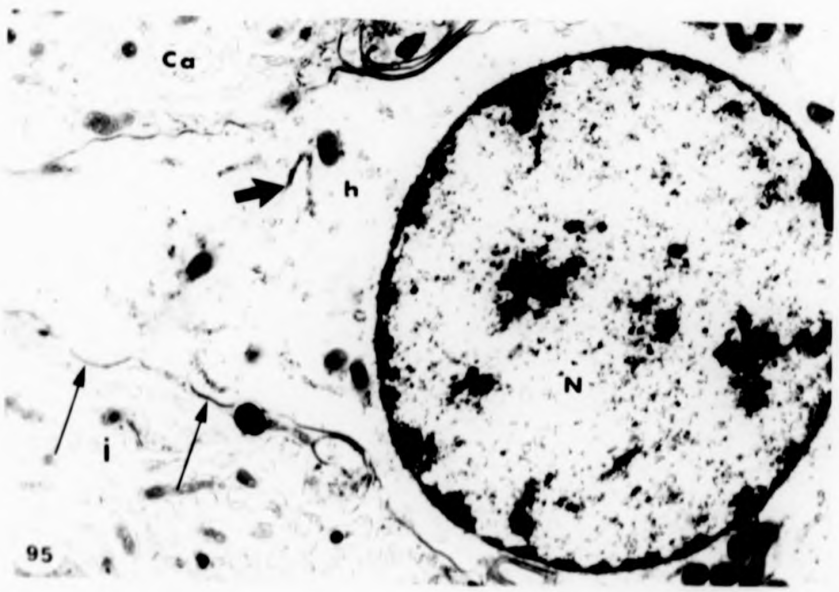
x 10.500

Fig. 95. Electron micrograph of a portion of types h and i cells (h and i) on the seventh day after attachment. Type h cells extend to a distal part of the alveolus to lie between the alveolar cells. In the micrograph, it lies between the canaliculi (Ca) of type i cells (i). Note the intercellular junction between the two cells (long arrows). Thick arrow points to a small narrow isolated cisternae of rough endoplasmic reticulum. Small arrows point to microtubules. N, nucleus.

x 12.600



94



95

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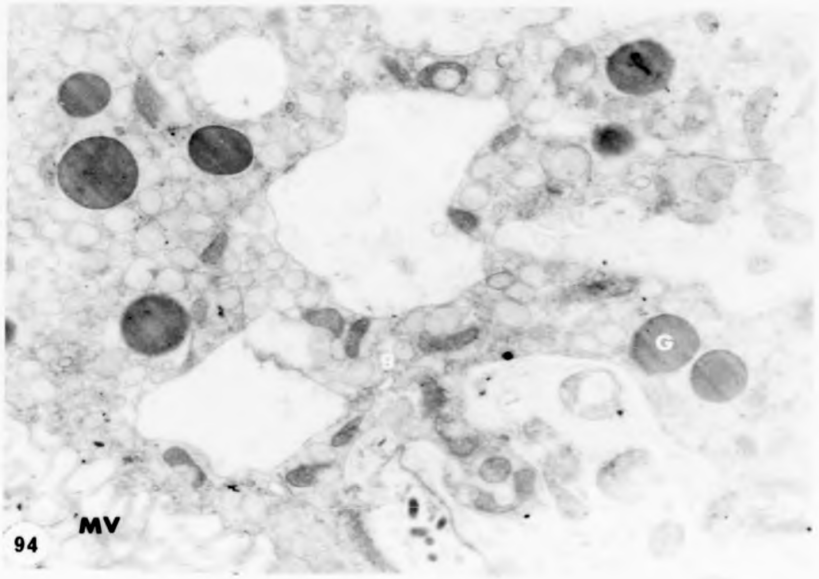
i cells
two cells
cw



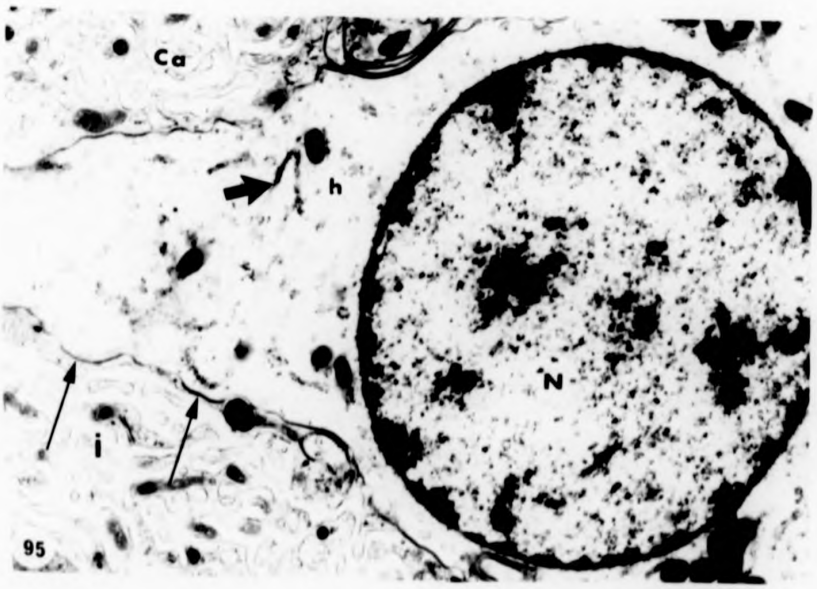
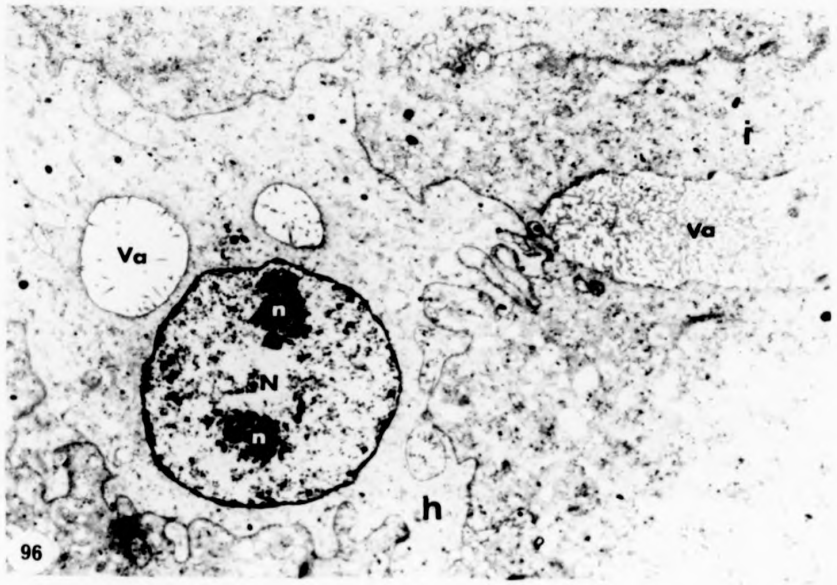


Fig. 96. Types h and i cells (h and i) on the third day after H. leachii female detachment showing the membrane-bound vacuoles (va) with low dense content in both types of cell. Their limiting membrane projects small microvilli into the lumen of these vacuoles which are probably extensions of the alveolar lumen. N, nucleus; n, nucleolus.

x 4.150

Fig. 97. Electron micrograph of a portion of the branched canaliculi of type i cells (i) in the basal region of type III alveolus on the seventh day after H. leachii female attachment. The distal extensions of the canaliculi (Ca) form arm-like structures which are arranged regularly to be very close to the basement membrane (BM) of the alveolus. Note the swollen ends of these arms which contain rounded mitochondria (M). Arrow points to the denser line in the basement membrane of the alveolus. Ly, lysosome engulfing mitochondrial remnant.

x 24.920



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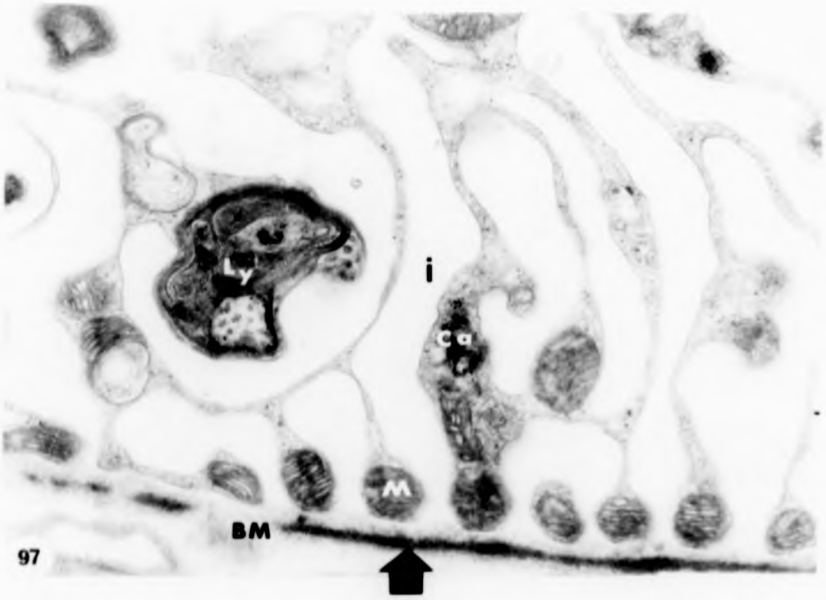

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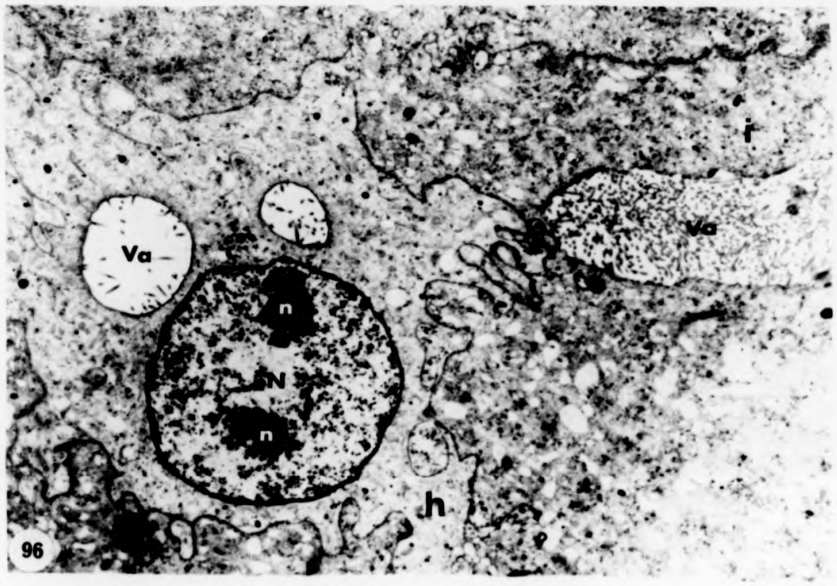

Va
n
N
n
h
f
Va
96

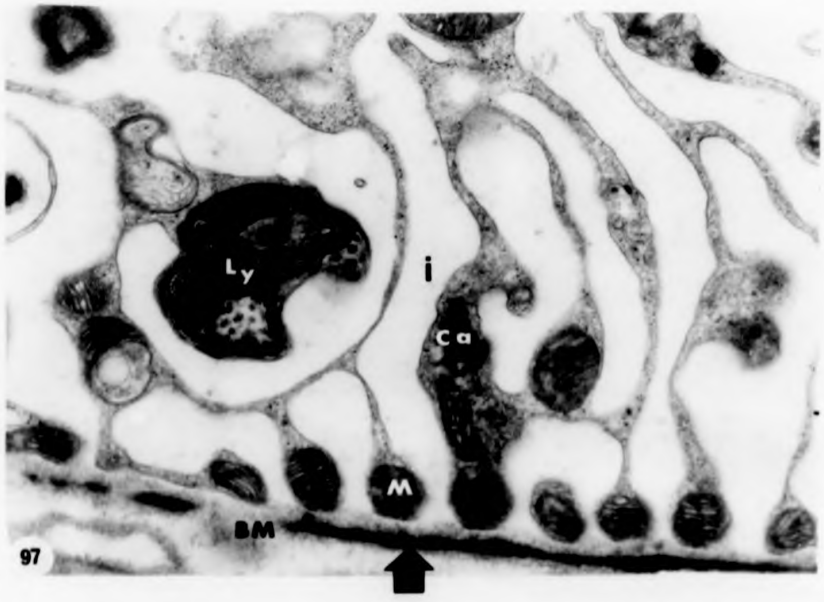

Ly
i
Ca
M
BM
97

Fig. 98. The same as Fig. 97 but this portion is in another alveolus of type III. Note the different shapes of mitochondria, some of which have an unusual morphology. Note also lysosomes engulfing membrane-like structures (long arrow) and small vesicles (openod arrow). Small arrows indicate some partially discharged granules, their limiting membranes are fused with the plasma membranes of the canaliculi. Thick arrow represents the dark line in the basement membrane of the alveolus.

x 16.800

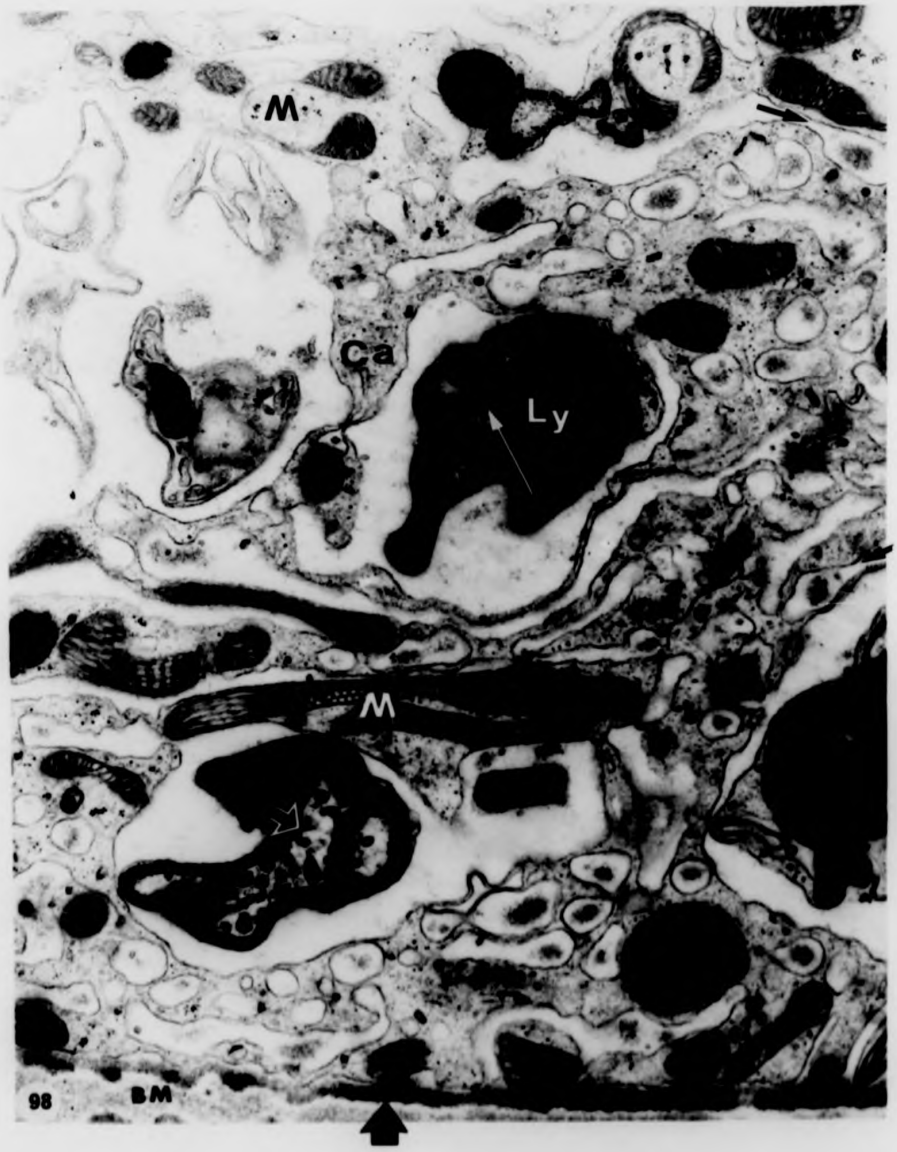
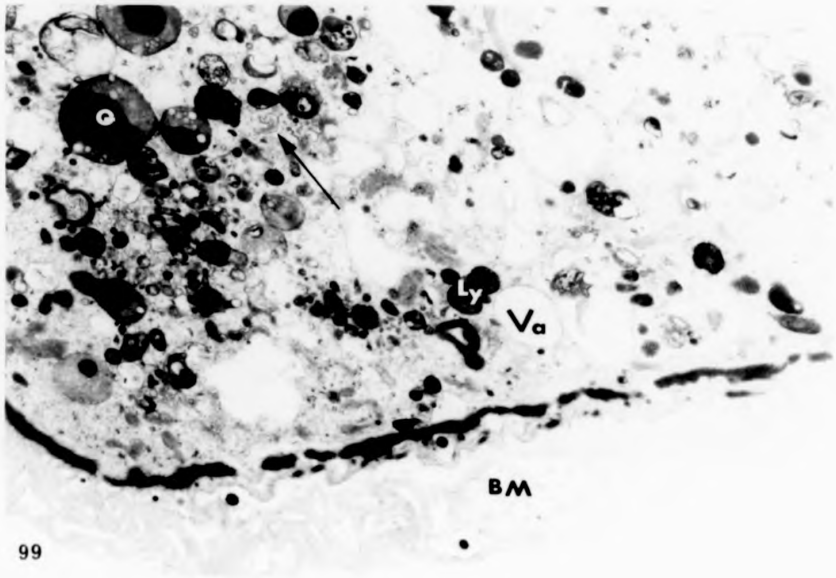

M
Ca
Ly
M
98
BM

Fig. 99. The same as Fig. 98 but on the first day after dropping off from the host. These canaliculi are packed with lysosome-like structures (ly), degenerated and autolized granules (G), many low dense membrane-bound vacuoles (va), some membrane-like structures (arrow). Note the complicated structure of the basement membrane (BM) of the alveolus.

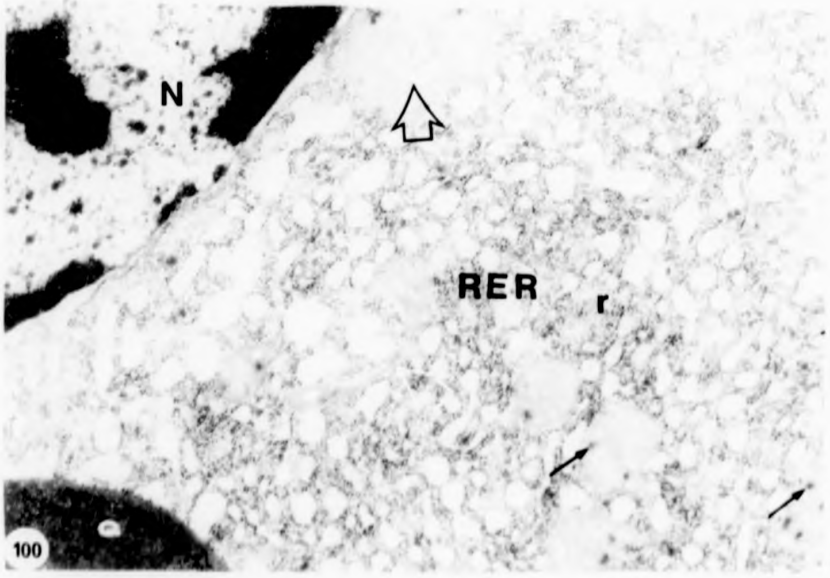
x 9.550

Fig. 100. Electron micrograph of type k cells on the first day after H. leachii female attachment. Note that rough endoplasmic reticulum (RER) consists of isolated cisternae which are distended with low dense intercisternal content. The intervening cytoplasm is filled with free ribosomes (r). Mitochondria (M) are different in shape and possess dense granules in their matrix (small arrows). White open arrow points to dumb-bell shaped mitochondria. Black open arrow points to rounded mitochondria with ribosomes in the middle but separated by membranes, this is probably due to the irregular shape of mitochondria. G, secretory granules; N, nucleus.

x 26.830



99



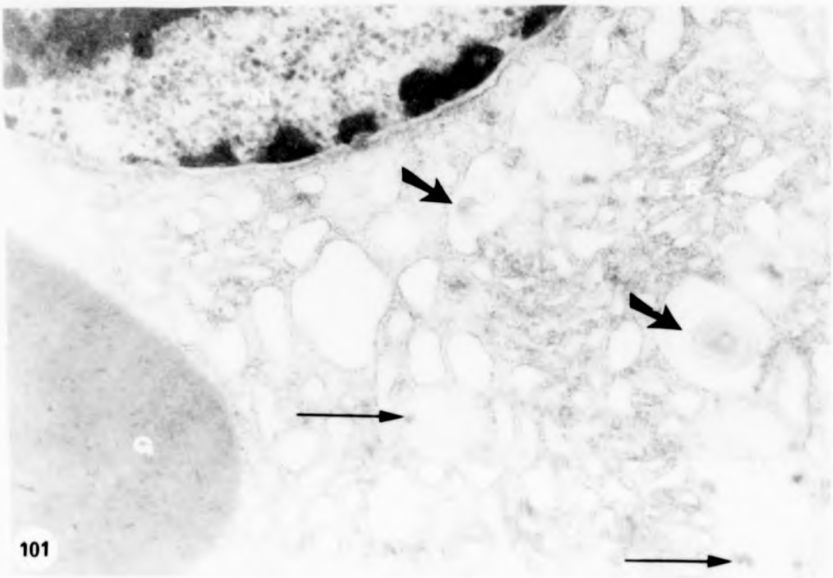
100

Fig. 101. The same as Fig. 100 but on the third day after attachment. Note the further distension of the endoplasmic cisternae (RER) which contains irregular membrane-bound structures (thick arrows). Mitochondria possess dark small granules in their matrix (small arrows) and some ribosomes as in Fig. 100 (opened arrow).

x 26.830

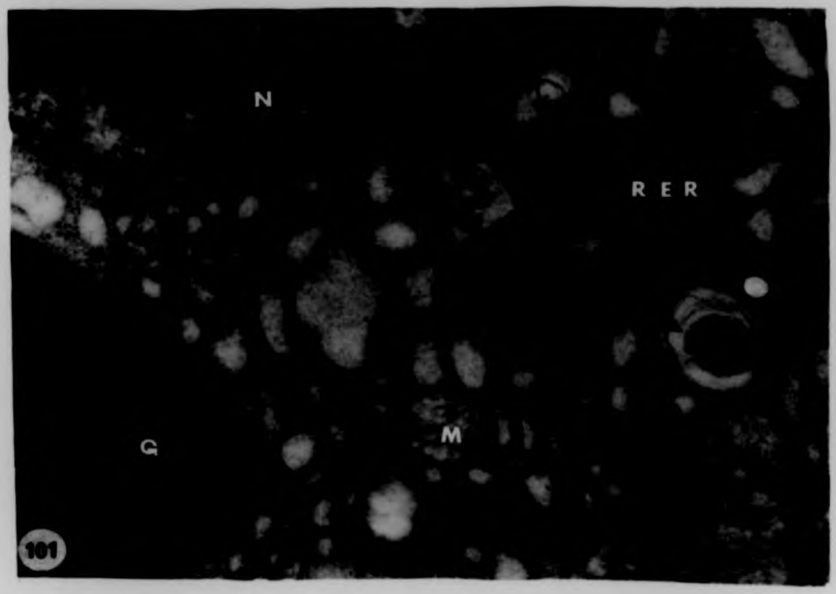
Fig. 102. The same as Fig. 100 but on the fifth day after attachment to show many broken down granules (opened arrows) scattered in the cytoplasm. Black arrows point to the intercellular junctions between types l and m cells. k, l, m and n represent the types of cell. MV, microvilli; N, nucleus.

x 3.960



101

102


N
RER
G
M
101

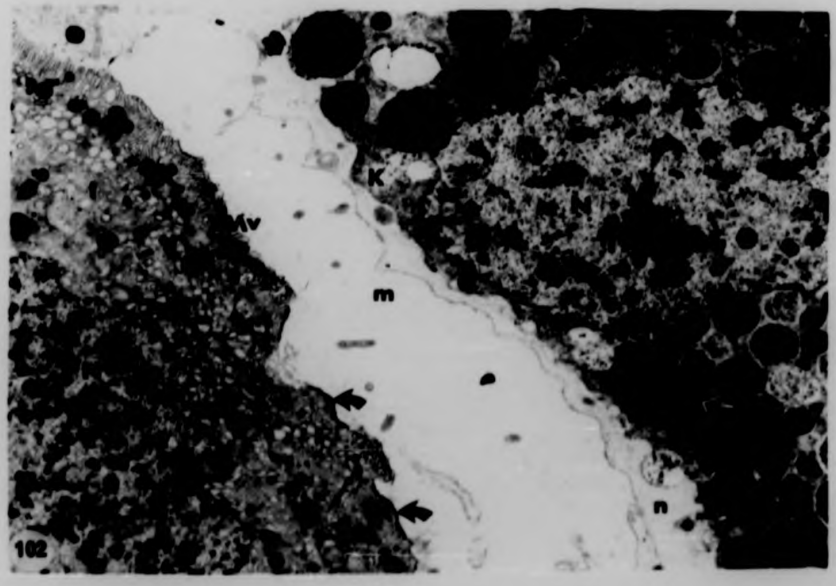
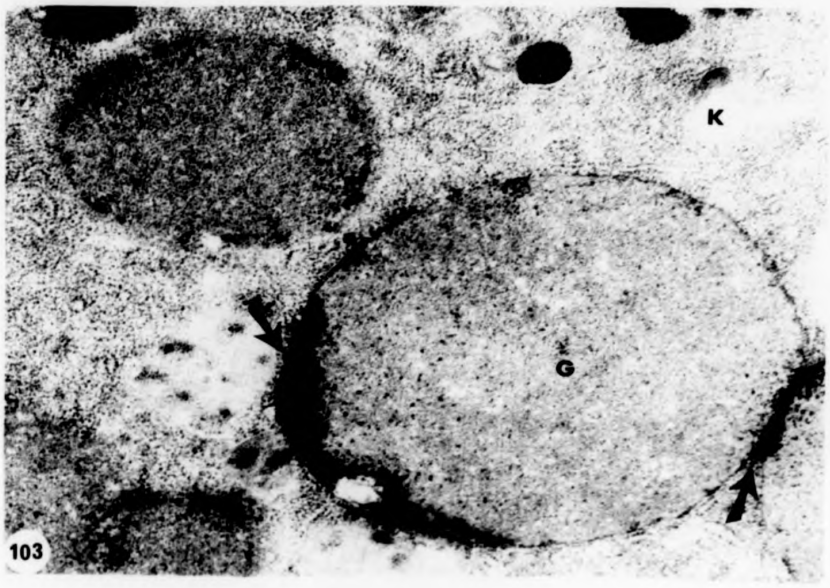

K
Mv
m
n
102

Fig. 103. Ultrastructural histochemical micrograph of a portion of type k cell (k) incubated in acid phosphatase medium on the fifth day after attachment. Note that the peripheral region of some small granules (G) shows intense deposits of reaction products (arrows) indicating the phosphatase activity.

x 37.240

Fig. 104. Electron micrograph of an autolized alveolus on the final stages of H. leachii female oviposition.

x 4.400


K
G
103

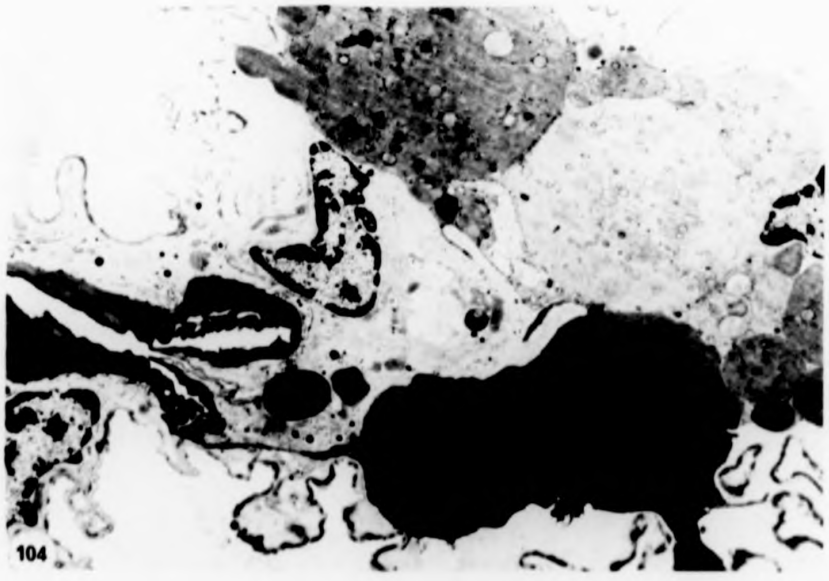
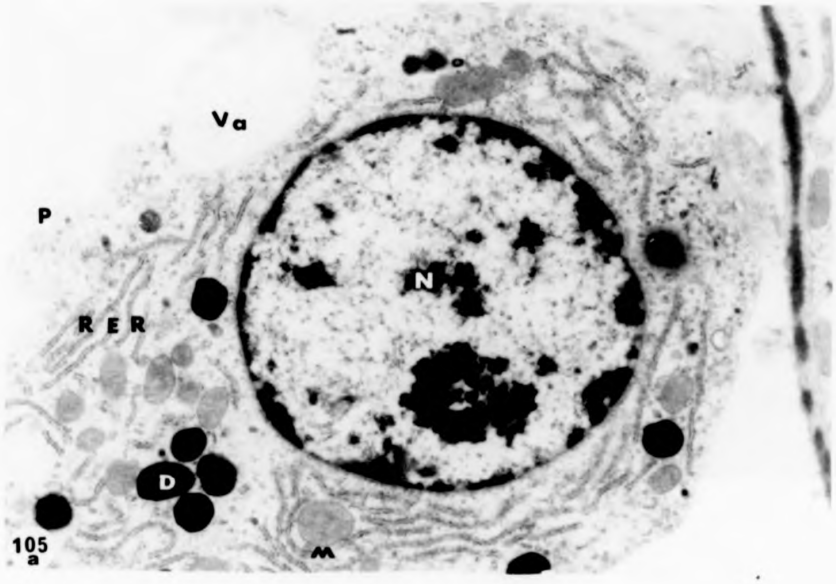

104

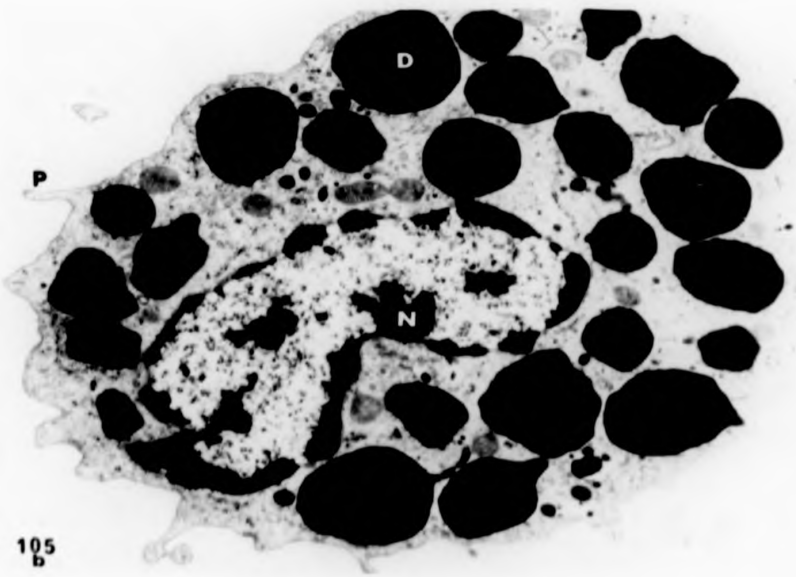
Fig. 105a, b. Electron micrographs of two different shapes and structures of haemocytes at the final stages of feeding. D, dense granules which are probably lysosomes; M, mitochondria; N, nucleus; P, pseudopodia; RER, rough endoplasmic reticulum. Va, Vacuole.

105a x 11.760

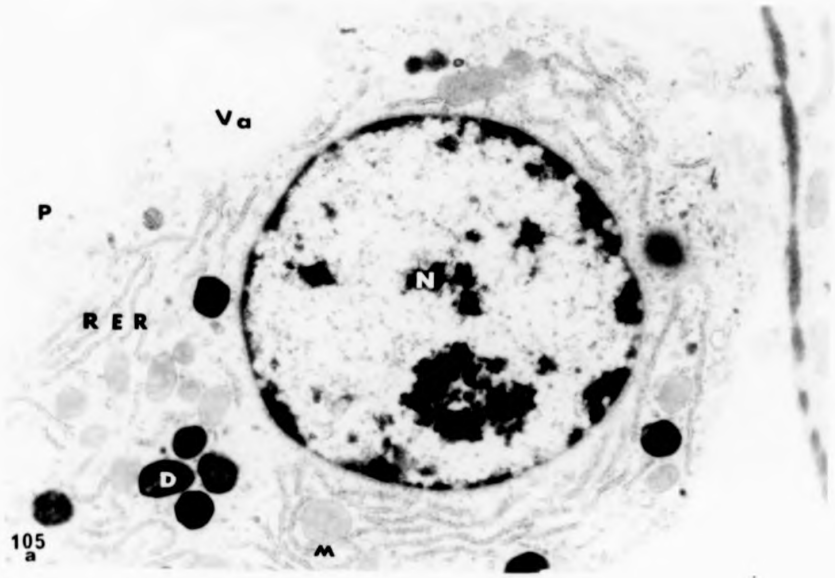
105b x 9.725



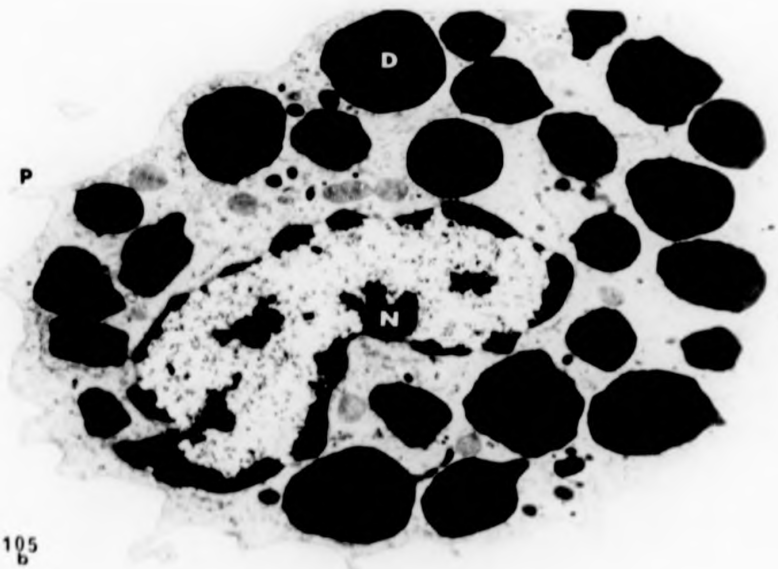
105
a



105
b



105
a



105
b

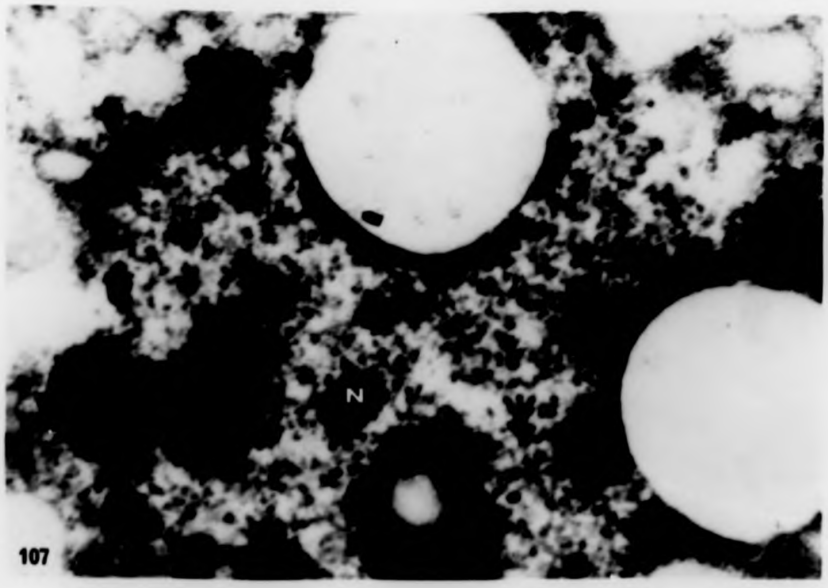
Fig. 106. Electron micrograph of a portion of type II alveolus on the first day after H. leachii male attachment. b_1 and b_2 represent the sub-types b_1 and b_2 cells; c, type c cell (the cap cell); d, type d cells (the interstitial epithelial cells). Arrow points to the membrane-bound vacuoles in the middle of type c cell nucleus. Note that the sub-type b_2 cell granules lack the filamentous material seen in the female.

x 9.880

Fig. 107. High magnification of a nucleus of sub-type b_2 cells on the fifth day after male attachment to show the variable size of vacuoles in the nucleus.

x 25.675.

106



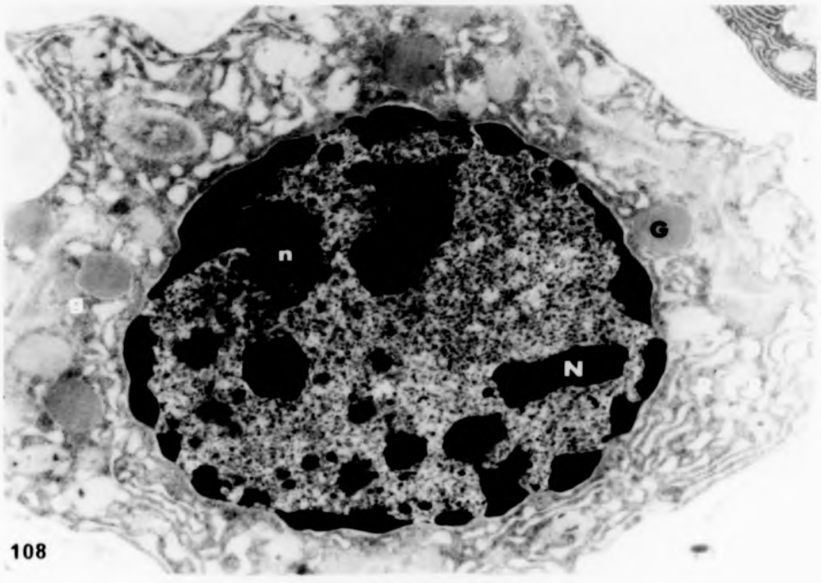
107

Fig. 108. Electron micrograph of type g cells (g) of type III alveolus on the third day after the male attachment to show that these cells contain few moderately and low dense scattered granules (G) and their cytoplasm is packed with distended and nondistended cisternae of endoplasmic reticulum (RER). N, nucleus; n, nucleolus.

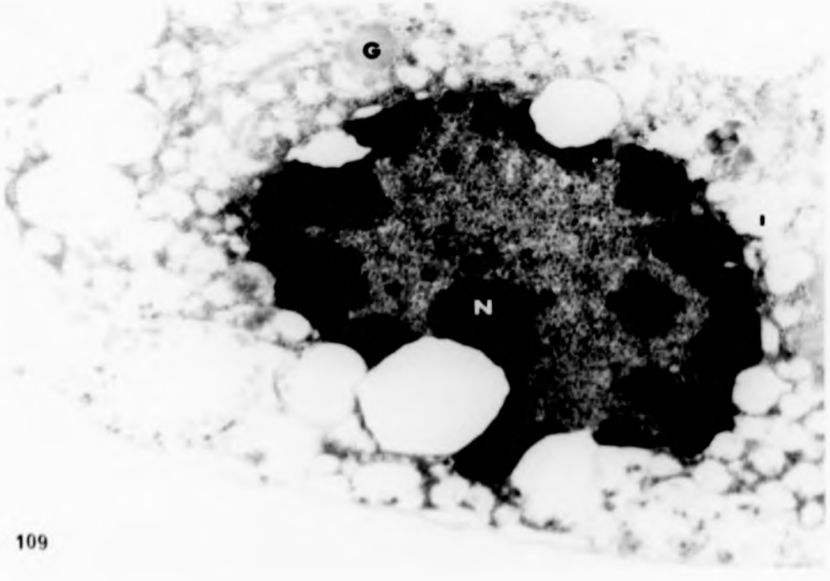
x 9.600

Fig. 109. Electron micrograph of type l cells (l) of type IV alveolus on the fifth day after the male attachment. Note the vacuolated nucleus (N) and the degenerated matrix of the cytoplasm while a few undischarged granules (G) remain in the cell. Lu, lumen.

x 12.845



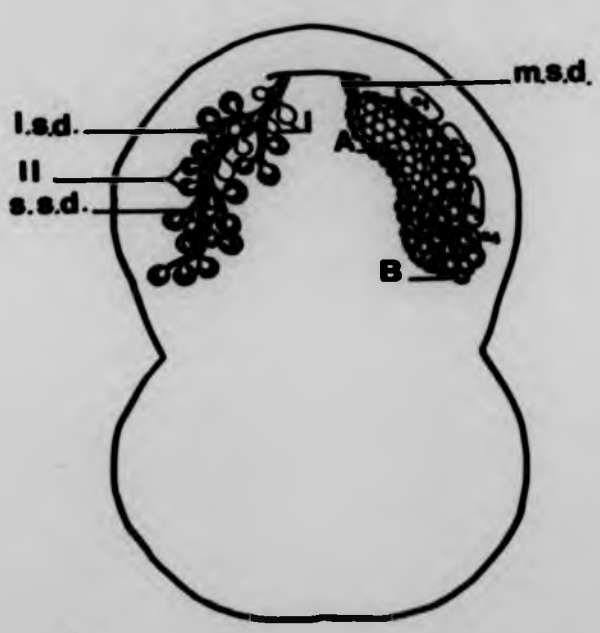
108



109

110 ORNITHODOROS (ORNITHODOROS) MOUBATA

Fig. 111. Ornithodoros moubata, unfed adult. Dorsal view showing the disposition of the salivary glands: c_1-c_4, coxae no. 1 to 4; L.s.d., Lobular salivary duct; I and II, indicate the arrangement of the two different types of alveoli on the main salivary duct. A, nongranular and B granule-secreting alveoli.

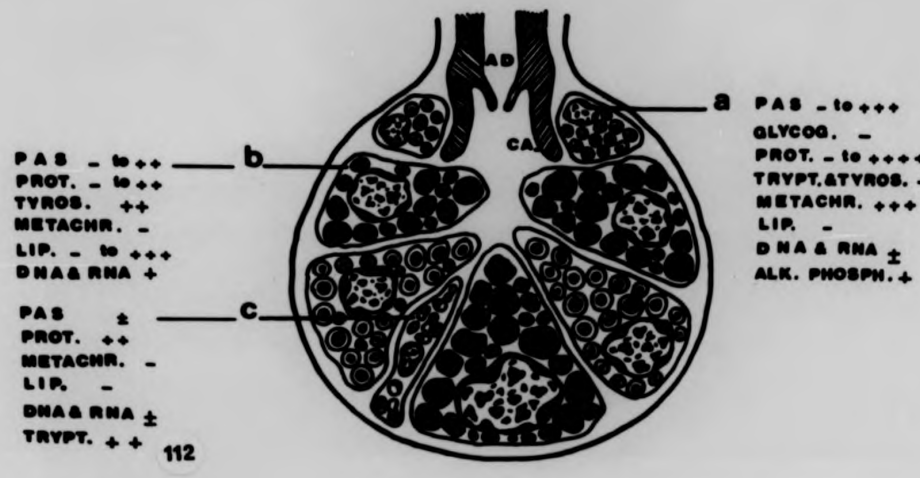


111

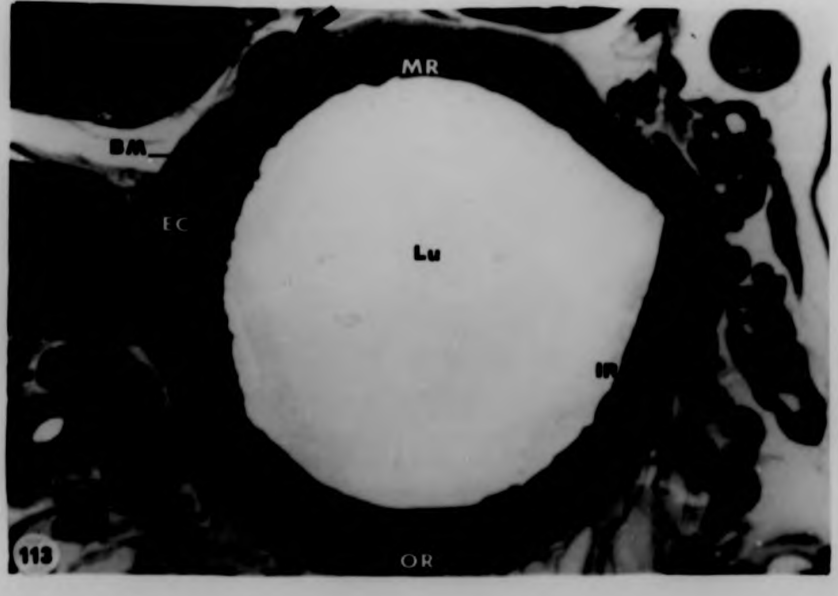
Fig. 112. Drawing of type II alveoli of unfed adult O. moubata salivary glands to show the histochemical nature of the alveolar cells. a, type a cells; AD, alveolar duct; AL, alveolar lumen; b, type b cells; c, type c cells; CA, cuticular arm; V, valve-like structure.

Fig. 113. Epon-embedded section of the main salivary duct showing: BM, basement membrane; EC, epithelial cells; IR, inner cuticular region; Lu, main duct lumen; MR, middle region; OR, outer region; arrow points to a nerve enclosed in the same basement membrane surrounding the epithelial layer.

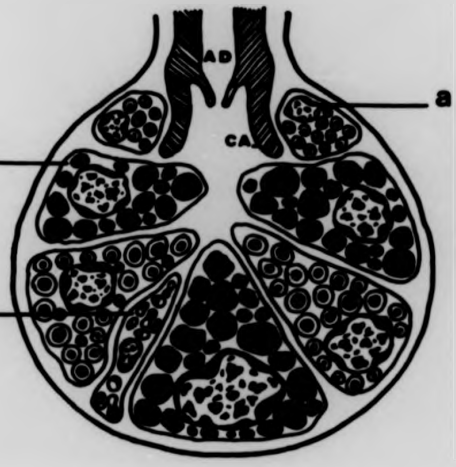
x 5.000


AD
CA
a
PAS - to +++
GLYCOG. -
PROT. - to ++++
TRYPT.&TYROS. -
METACHR. +++
LIP. -
DNA & RNA ±
ALK. PHOSPH. +
b
PAS - to ++
PROT. - to ++
TYROS. ++
METACHR. -
LIP. - to +++
DNA& RNA +
c
PAS ±
PROT. ++
METACHR. -
LIP. -
DNA& RNA ±
TRYPT. + +

112


MR
BM
EC
Lu
IR
OR

113


AD
CA
a
b
c
PAS - to +++
GLYCOG. -
PROT. - to ++++
TRYPT, TYROS. -
METACHR. +++
LIP. -
DNA & RNA ±
ALK. PHOSPH. +
PAS - to ++
PROT. - to ++
TYROS. ++
METACHR. -
LIP. - to +++
DNA & RNA +
PAS ±
PROT. ++
METACHR. -
LIP. -
DNA & RNA ±
TRYPT. + +
112

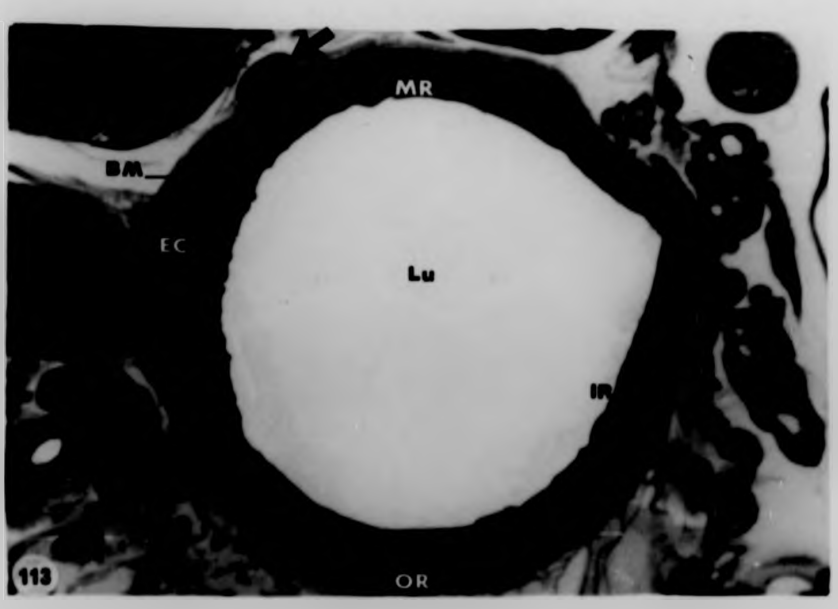
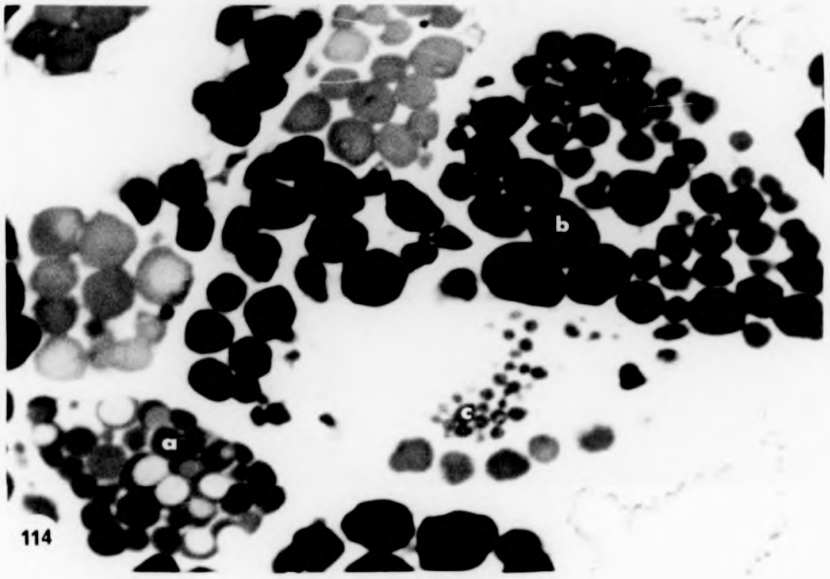

MR
BM
EC
Lu
IR
OR
113

Fig. 114. Light micrograph of unfed adult O. moubata salivary glands showing type II granule-secreting alveoli. a, type a cells; b, type b cells; c, type c cells.

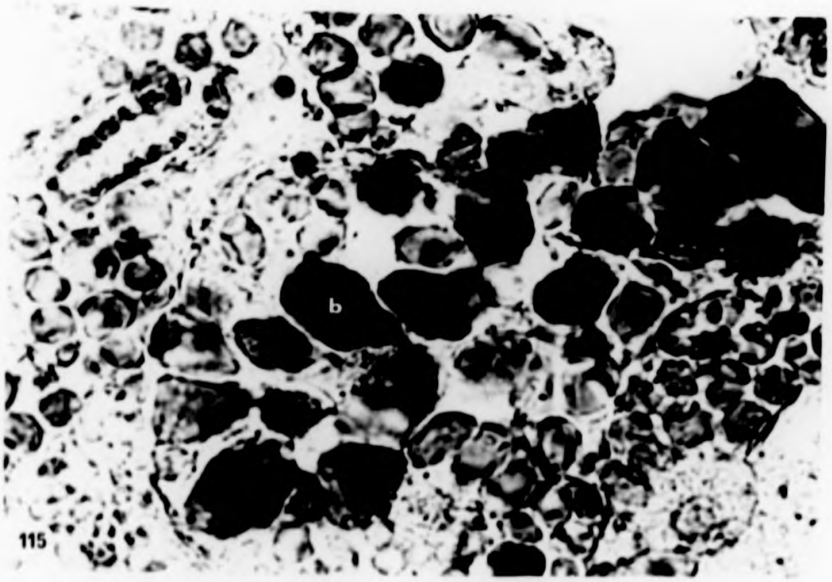
x 4.500

Fig. 115. Paraffin section of type II alveoli stained for lipids with sudan black. As seen here, only the large granules of type b cells are intensively positive, while the rest of the granules are different in intensity.

x 5.500



114



115

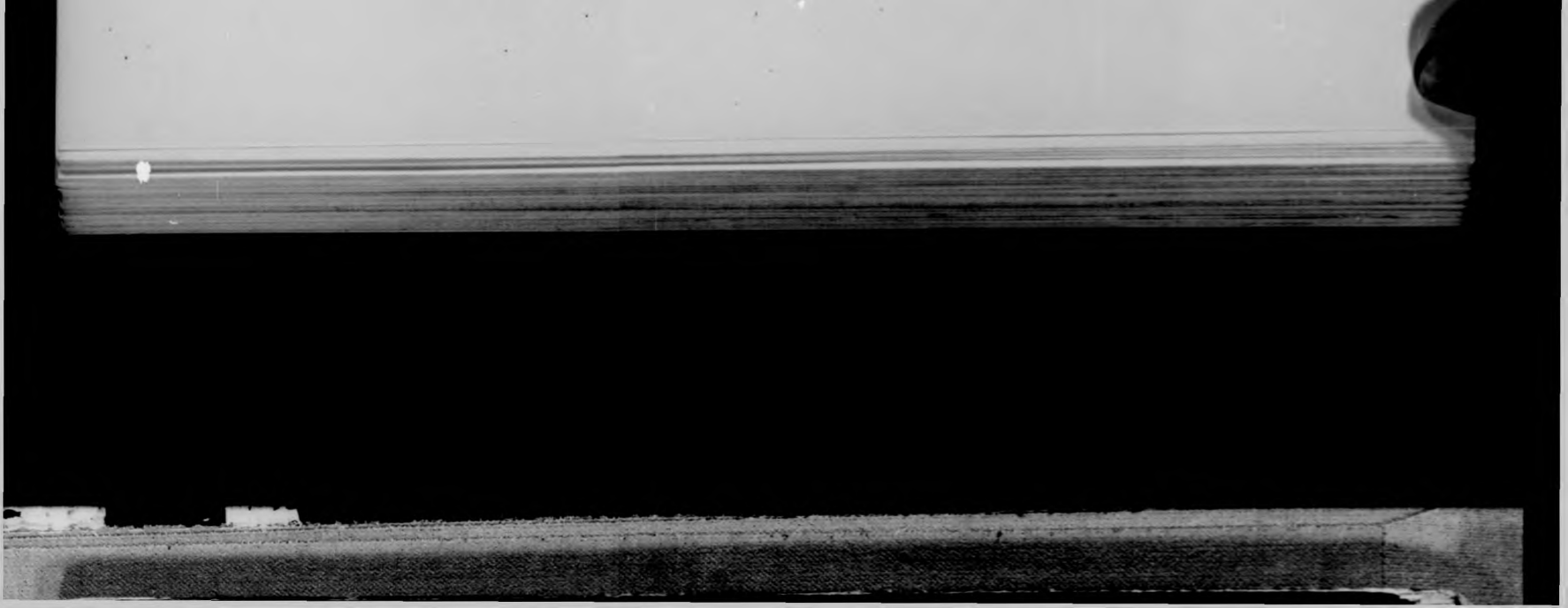


Fig. 116. Electron micrograph of type II alveolus of unfed O. moubata adult showing the extensions (large arrow) of the cap cells (type d cells) (d) touch the valve-like structures (white arrow). a, b, d and e represent the types of cell; AD, alveolar duct; AL, alveolar lumen; CA, cuticular arm; N, nucleus; VC, valvular canal. Two arrows indicate area of flexibility.

x 6.000

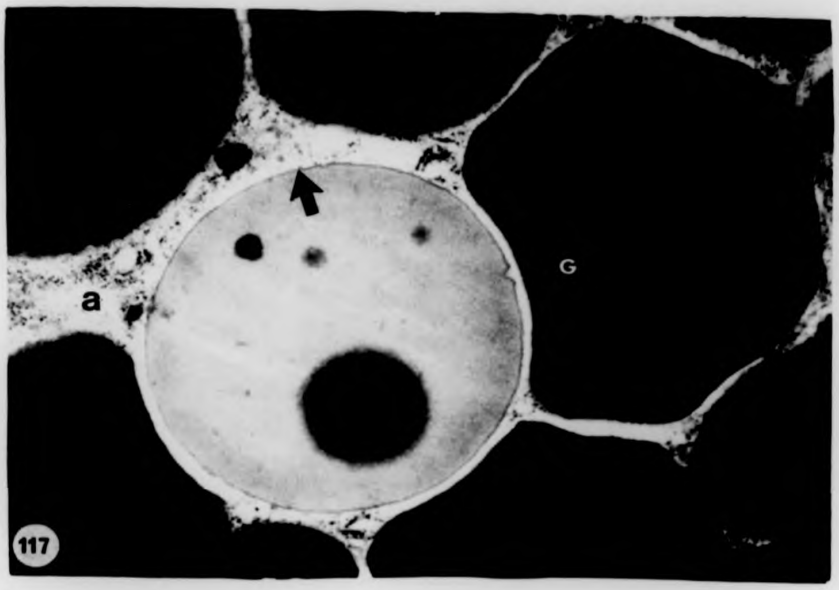

AD
CA
AL
a
N
d
b
e
116

Fig. 117. Electron micrograph of a portion of type a cell (a) showing membrane-bound mature granules (G) and others which possess a small very electron-dense core and a large less dense peripheral zone. Note that the peripheral zone of some granules is electron-lucent and enclosed in a limiting membrane (arrow).

x 26.345

Fig. 118. The same as Fig. 117 but in this micrograph the Golgi cisternae (Gb) are giving rise to small dark vacuoles (black arrows) of the same density as those of the middle cores of the mature granules. In Golgi regions, some of these dark vacuoles are surrounded by lower dense peripheral zones (opened arrow) which represent the condensing vacuoles.

x 38.335


a
G
117

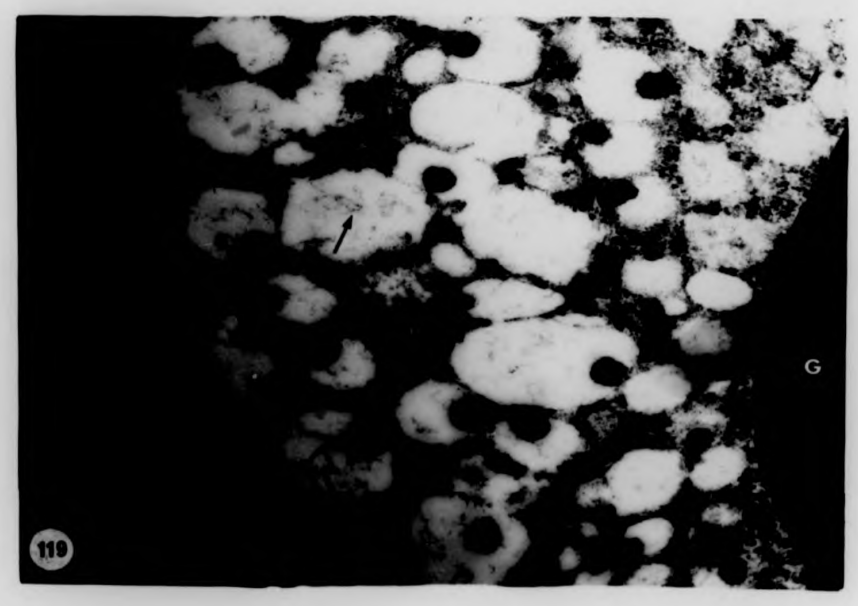

G
Gb
a
118

Fig. 119. This micrograph has been taken in another area of the same cell and on the same grid of Fig. 118 to show that the dark vacuoles are enclosed in electron-lucent vacuole-like structures which possess a material (small arrows) similar in density to that of the peripheral zones of the condensing vacuoles found in the Golgi region (opened arrow, Fig. 118). This material appears to be coming out from the dense core (opened arrows).

x 32.000

Fig. 120. Ultrastructural histochemical micrograph of a portion of type a cell after treatment with PA-TCH for polysaccharides. The reaction is intensely positive for the limiting membranes of the mature granules (arrowed) and moderately positive for the peripheral zone (pz) while the middle core is negative.

x 28.020


G
119

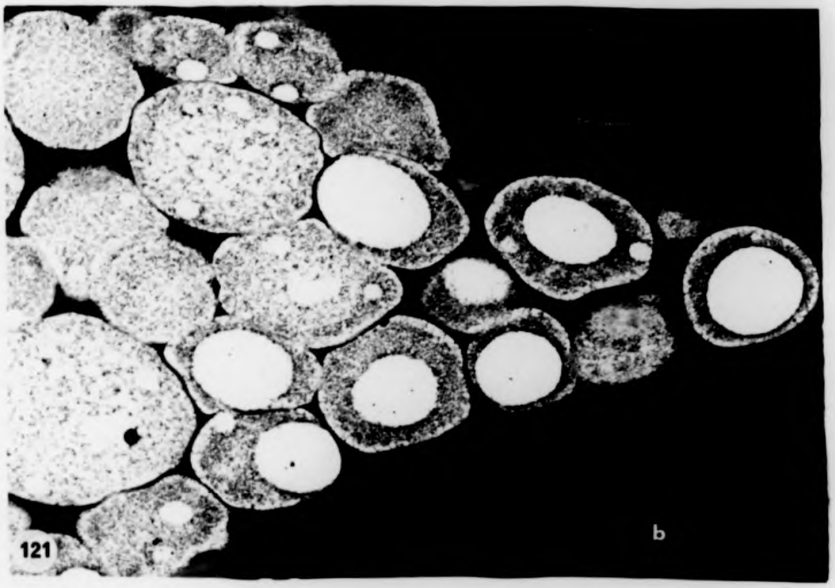

PZ
120

Fig. 121. The same as Fig. 120 but after treatment with pronase enzyme for proteins. Note that the middle ones are completely digested by the enzyme.

x 9.520

Fig. 122. Electron micrograph of a portion of type b cell (b) of unfed O. moubata adult showing the mechanism of the granule formation. Small vesicles (arrows) arise from areas of rough endoplasmic reticulum (RER) which surround the Golgi bodies (Gb). The latter give rise to small condensing vacuoles (cv) which are converted into mature granules probably by progressive filling and concentration of their content. G, mature granule; M, mitochondria.

x 37.635


121
b

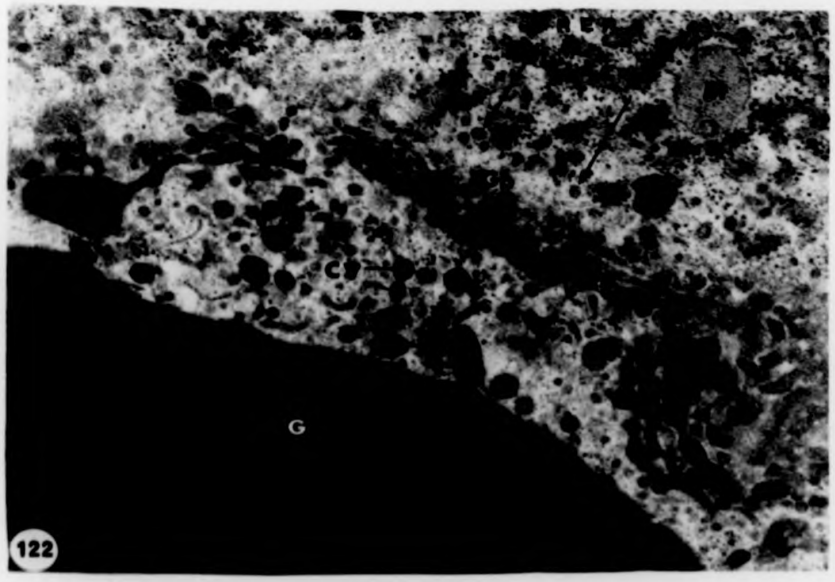

G
122

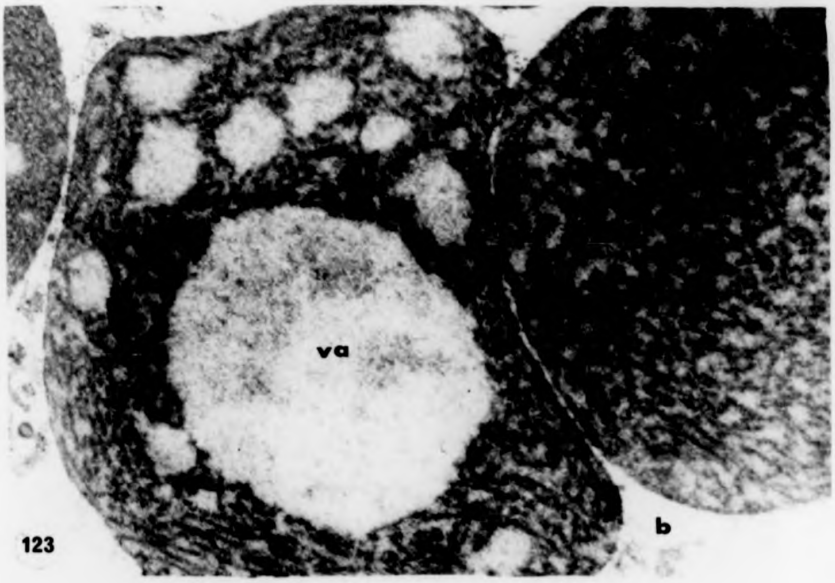
Fig. 123. Type b cell granules with dense fine textured material. va, vacuoles.

x 32, 410

Figs. 124, 125. Type b cells after treatment with PA-TCH for polysaccharide detection. Note that the limiting membrane of the granule is more dense than the granular content Fig. 124. In Fig. 125, the Golgi cisternae (Gb) and the condensing vacuoles (Cv) are polysaccharide positive. G, secretory granule.

Fig. 124 x 32.860

Fig. 125 x 28.750

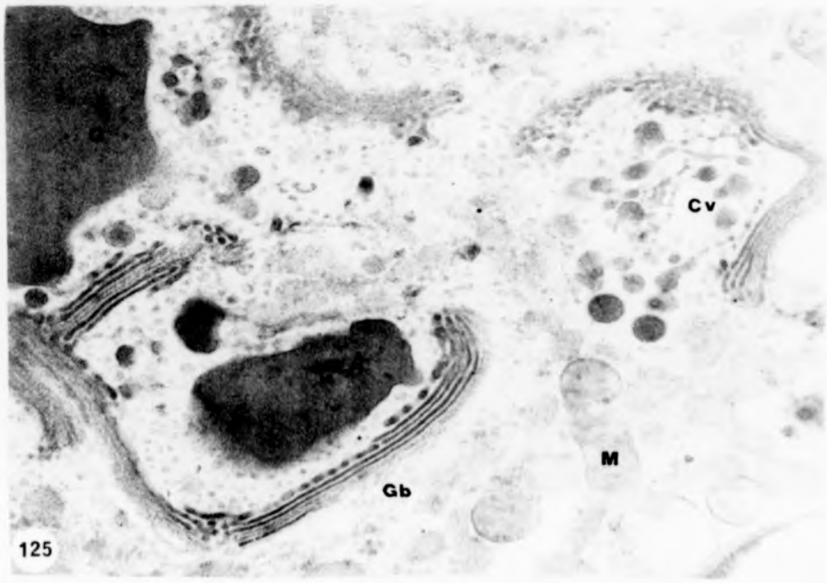


123

124

Fig. 126. Electron micrograph of type c cell of unfed O. moubata adult. Note that the compact ellipsoidal Golgi bodies (Gb) with their multilayered cisternae are surrounded by rough endoplasmic reticulum (RER). Apparently both are involved in the mechanism of the granular formation. e, the interstitial epithelial cells; M, mitochondria; Arrows point to small vesicles arising from areas of RER.

x 27.600


Cv
M
Gb
125

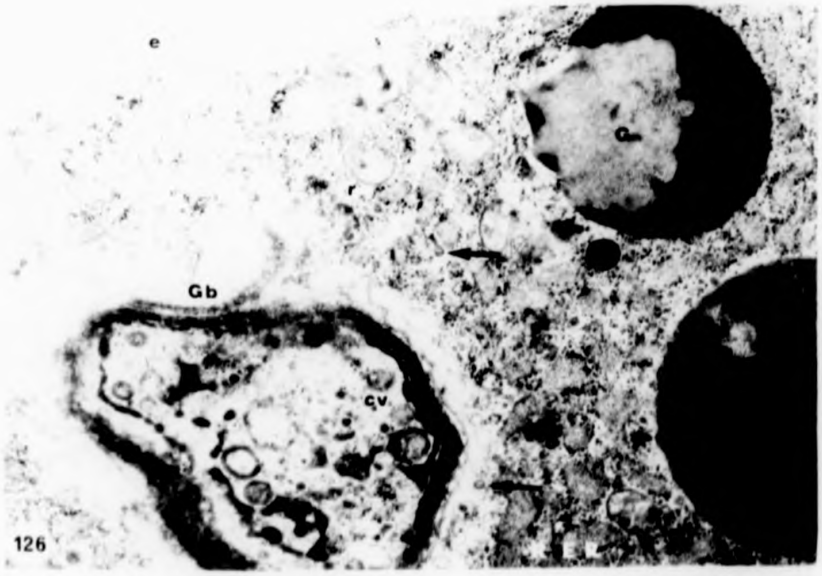
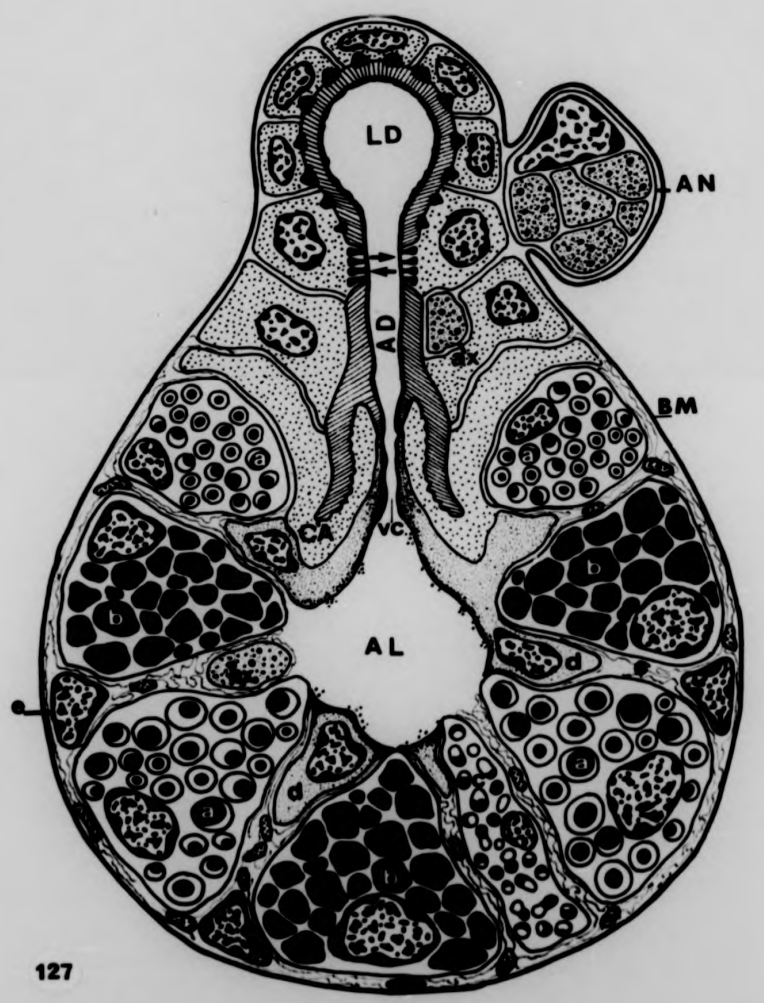

e
r
Gb
Cv
126

Fig. 127. Reconstructed line drawing of type II alvecli in unfed adult O. moubata salivary glands at the electron microscopic level. a - e, types a - e cells; AD, alveolar duct; AL, alveolar lumen; AN, alveolar nerve; ax, axon; BM, basement membrane; LD, lobular duct; VC, valvular canal. Arrows indicate area of flexibility.



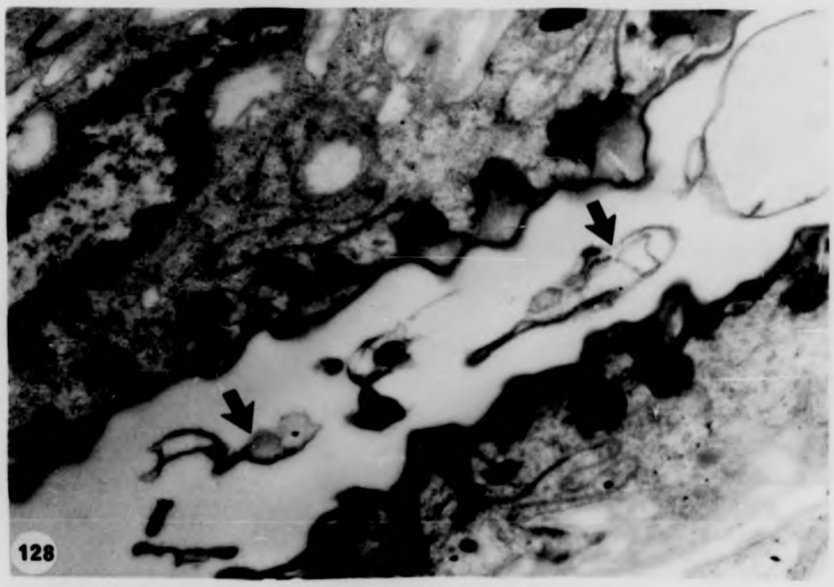
127

Fig. 128. Fine micrograph of female O. moubata at 15 minutes after attachment showing that their secondary duct lumen contains membrane-like structures (arrows) of irregular shape and variable size.

x 15.600

Fig. 129. Electron micrograph of alveolus II at the final stages of adult feeding to show an electron-dense discharged coagulated secretory mass (arrow) streaming from the alveolar lumen (AL) into the alveolar duct (AD), passing through the narrow valvular canal (VC).

x 14.000


128

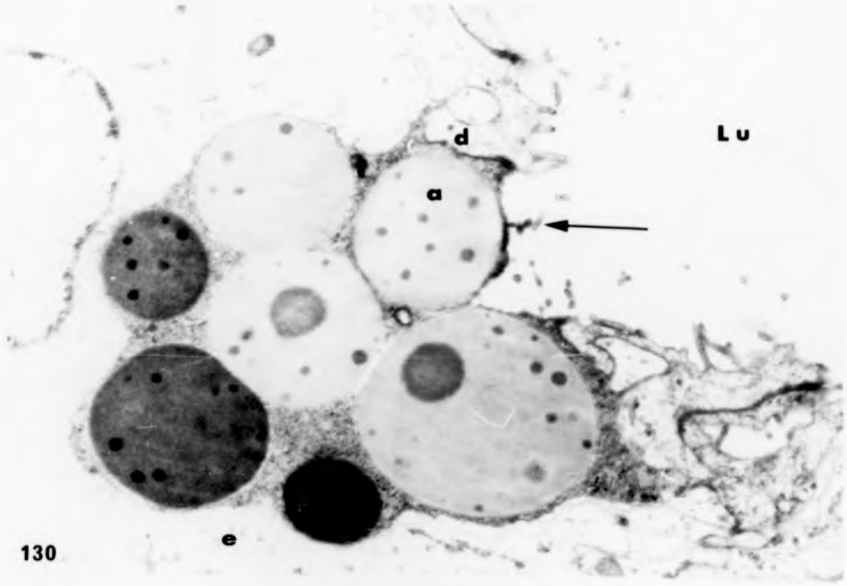

AD
VC
CA
AL
129

Fig. 130. Electron micrograph of type a cell (a) at 20 minutes after O. moubata adult attachment. Note the great reduction in the size of the cell and in the number of the secretory granules. d, the cap cell; e, the interstitial epithelial cells; Lu, alveolar lumen. Arrow points to microvilli which are projected from type a cell surface.

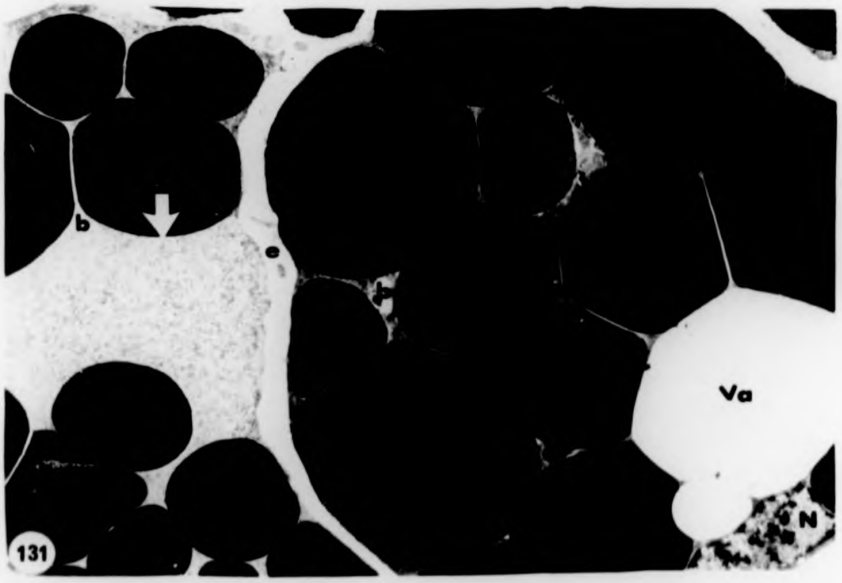
x 19.600

Fig. 131. Fine micrograph of portions of type b cells (b) at 15 minutes after attachment to show that the cytoplasm appears granulated (white arrow) while other cells contain low dense vacuoles (Va. of variable size. e, type e cells; N, nucleus.

x 5.650



130

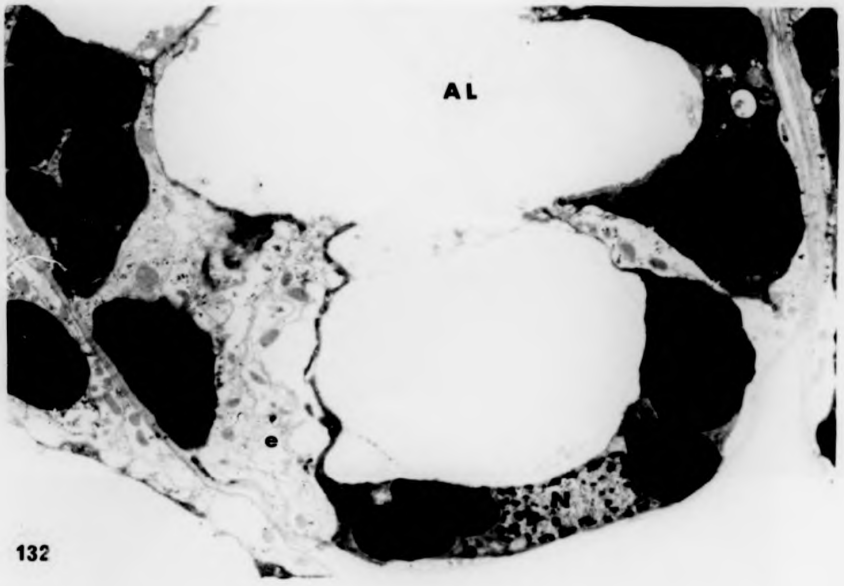


131

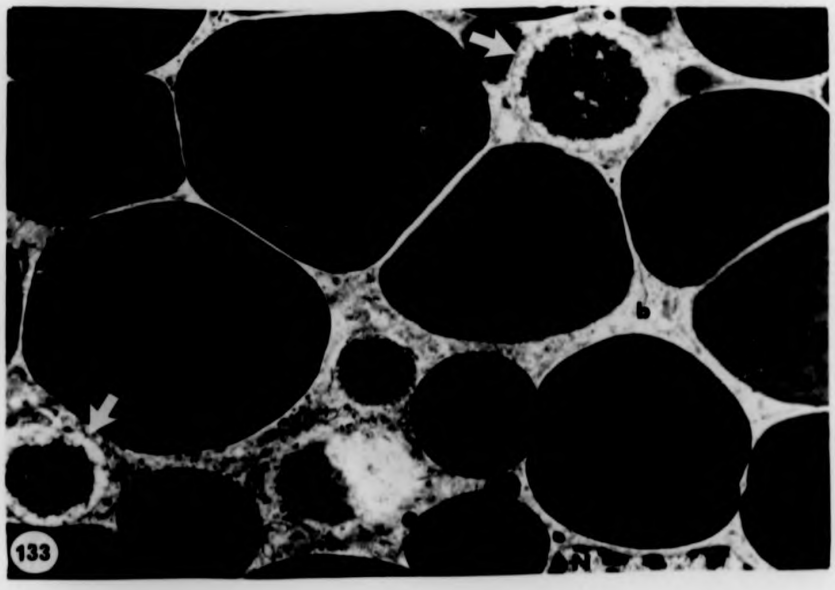
Figs. 132, 133. The same as Fig. 132 but at 2 hours after adult detachment to show that these cells have lost most of their secretory granules and the cell and the nuclei (N) appear flattend according to the plane of the section (Fig. 132). Note the normal condition of the cell in Fig. 133 except its cytoplasm contains a few rounded membrane-bound vacuoles with osmiophilic aggregates in the middle (arrow). They probably represent a degredation granular phase. AL, alveolar lumen. Other letterings as in Fig. 132.

Fig. 133 x 4.000

Fig. 134 x 9.335



132



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