**Zeitschrift:** Acta Tropica
**Herausgeber:** Schweizerisches Tropeninstitut (Basel)
**Band:** 22 (1965)
**Heft:** 4

**Artikel:** Studies on the various glands of the tick "Haemaphysalis spinigera" Neumann 1897. Part III
**Autor:** Chinery, W.A.
**DOI:** https://doi.org/10.5169/seals-311275

National Institute of Health and Medical Research, Accra, Ghana

# Studies on the Various Glands of the Tick *Haemaphysalis spinigera* Neumann 1897.*

By W. A. CHINERY.

## Part III. The Salivary Glands.

### Introduction.

Ticks are of importance not only as parasites of man and animals but also as vectors of arthropod-borne diseases. Apart from the fact that the salivary glands constitute the major route through which pathogens are transmitted to susceptible hosts, they are believed to be the source of paralytic toxins, ROSS (1926), GREGSON (1960), cytolysins, ARTHUR (1962), haemolysins PAVLOVSKY & CHODUKIN (1928, 1929), LAVOIPIERRE & RIEK (1955), as well as toxins which induce other febrile conditions (sweating sickness) in susceptible hosts, NEITZ (1955, 1956, 1959). *Haemaphysalis spinigera* in particular is a species of interest, comparatively recently being the most important vector of Kyasanur forest disease discovered in India in 1957. Recently, VARMA & SMITH (1961) have shown that nymphs and adults of this tick are capable of experimentally transmitting Langat virus isolated by SMITH (1956), from *Ixodes granulatus* between laboratory rodents.

Histological methods e.g. LA MOTTE (1960), have not been instructive in revealing virus infection in vectors nor changes in infected tissues attributable to virus. However, before other newer techniques now available such as electron microscopy, could be carried out as a fresh approach to the problem of virus-vector relationship at cell level as suggested by BERTRAM & BIRD (1961), it is of importance that the histological and histochemical changes occurring in the salivary glands of the tick during the various physiological phases in its life-cycle be well understood as a basis to the further interpretation by other techniques now available. Histological results have therefore been amplified by histochemical methods to elicit the nature of cell components.

### Materials and Methods

see Part I, p. 235-236

### Results.

*Unfed Adults.*

*Morphology.*

The salivary glands of *Haemaphysalis spinigera* consists of a pair of racemose organs lying on either side of the body. Each gland has a main salivary duct which divides posteriorly into a

* This work was carried out in the Department of Entomology, London School of Hygiene and Tropical Medicine, as part of thesis accepted by the University of London for Ph.D. degree.

pair of subsidiary ducts. Both the main duct and its subsidiaries give rise to secondary ducts. The alveoli open directly by their lobular ducts either directly into the main salivary duct and its branches or indirectly through efferent ducts.

*Unfed Females.*

*Histology.*

*Salivary Ducts.* The main salivary duct (m.s.d. Fig. 1) of the unfed female measures about 44 $\mu$ in diameter and consists of a thick layer of cuticle overlaid by a layer of flattened epithelium. The subsidiary and the secondary ducts have similar histological

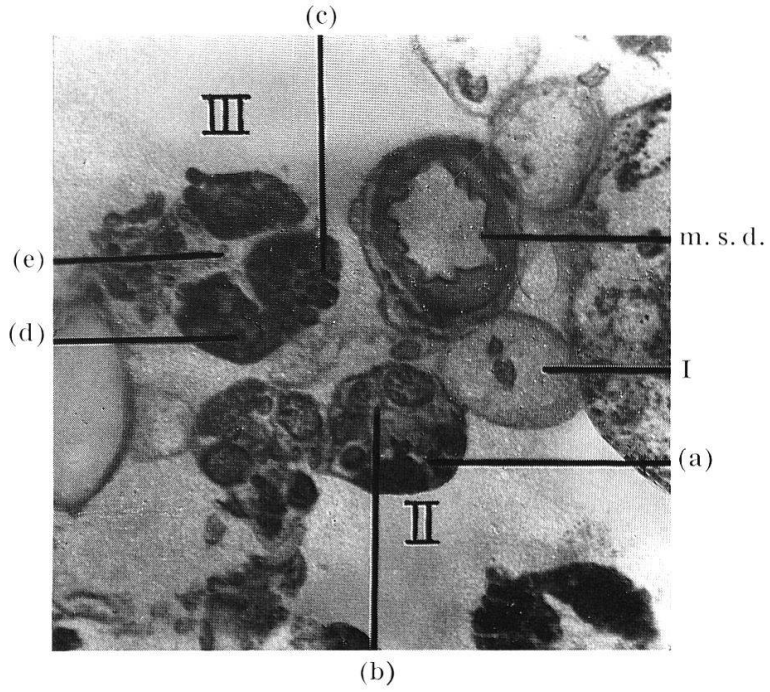


*Fig. 1.* Transverse section through the salivary glands of an unfed female showing types I, II, and III alveoli. ×600 (formol-sublimate fixation; Mallory's triple stain). m.s.d.: main salivary duct.

structure although of finer properties. The cuticular lining of the ducts extends up to the opening of the lobular duct into the lumen of the alveoli at which point it forms a valve-like structure (v. 1. Figs. 9, 12, 14), similar to that observed by NORDENSKIÖLD (1905) and TILL (1961).

*Salivary Alveoli.* Three main types of alveoli were recognised in the unfed female; one of which, type I, was the smallest, non-granular, and localized in the anterior region of the salivary glands whilst two others, types II and III, the larger granule secreting alveoli, form the major part of the gland. These alveoli are named according to the system of TILL (1959, 1961).

*Type I Non-granular Alveoli.* These were named the glands of venin by BONNET (1907) on account of their resemblance to the venom glands of snakes, and Pyramidenzellen by SAMSON (1909). They measure 37-39 $\mu$ in diameter and are in close opposition to the main salivary duct extending for a short distance along the two subsidiary ducts. They open directly into the main salivary duct through their short lobular ducts, especially along its dorsal and mesial sides, though occasionally on all sides (Fig. 1). No individual cell boundaries could be made out in these alveoli although there are three or four nuclei lying in the cytoplasm. The

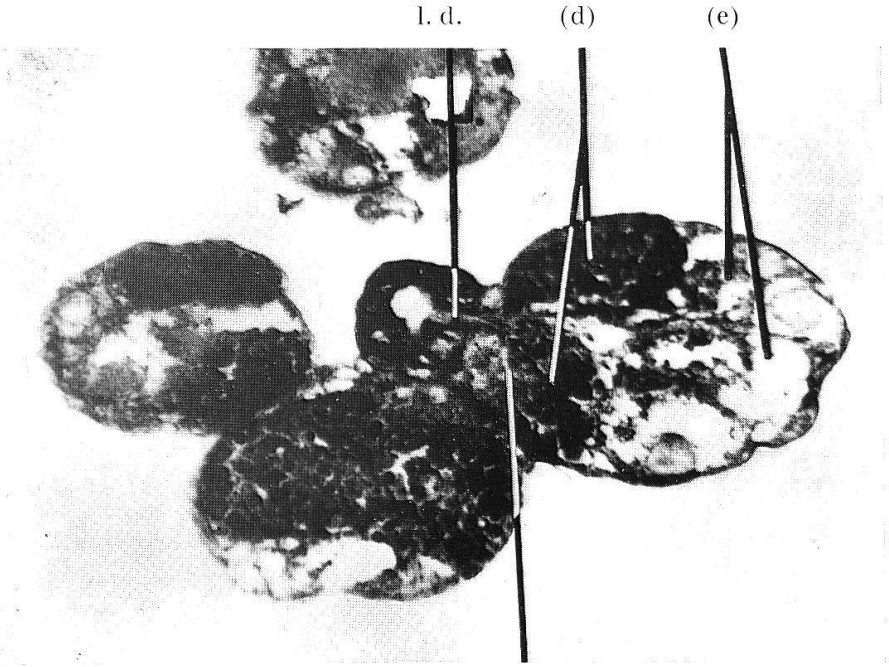


*Fig. 2.* Transverse section through the salivary glands of an unfed female showing type III alveoli. ×600 (formol-sublimate fixation; Mallory's triple stain). l.d.: lobular duct.

cytoplasm of these cells which is confined to the peripheral region is fibrillar, and among the fibrils is a finely granular material.

*Type II Granule Secreting Alveoli.* These granule secreting alveoli consist of about 10-12 large cells surrounding a small lumen which communicates with a small lobular duct (Fig. 2). These alveoli are more numerous in the anterior region of the gland extending posteriorly for about two thirds the length of the gland. They discharge either into the main salivary duct and its main subsidiaries or into the proximal half of the secondary ducts. Each alveolus measures about 37 $\mu$ in diameter and consists of two main types of cells designated types (a) and (b) (Fig. 2).

*Type (a) Cells.* They are one or two in number and are confined to the bases of the lobular ducts. They contain closely packed coarse granules in between which is visible the basophilic cytoplasm. These granules stain purplish red in Mallory's triple stain and bright pink in haematoxylin-eosin. The nuclei measure about 8 $\mu$ in diameter and stain bright red in Mallory's triple stain and blue in haematoxylin-eosin.

*Type (b) Cells.* The rest of the alveolus is composed of this type of cell. In the newly emerged females the cytoplasm is finely granular and stains blue in Mallory's triple stain and light bluish to pink in haematoxylin-eosin. In starved females these cells have a scanty cytoplasm consisting of a few fibrils and granules. The nuclei measure 10-11 $\mu$.

*Type III Granule Secreting Alveoli.* These are the most numerous especially in the posterior part of the gland. They are fewer in the anterior part where they are confined mainly to the distal portion of the secondary salivary ducts (Fig. 2). They measure 40-50 $\mu$ in diameter and are composed of 12-14 cells of three different kinds and designated (c), (d) and (e).

*Type (c) Cell.* Confined to the bases of the lobular ducts are one or two cells which are very similar to the type (a) cell of type II alveoli.

*Type (d) Cells.* Situated adjacent to the (c) cells are a group of 5-6 cells filled with granules which are slightly larger than those of (a) and (c) cell types, and are also less compact. In between the granules the basophilic cytoplasm shows up quite prominently especially in Carnoy-fixed tissues. These granules stain cherry red in Mallory's triple stain and pink in haematoxylin-eosin. The type (a) cells of type II alveoli and the types (c) and (d) cells of the type III alveoli correspond to NORDENSKIÖLD's Funduszellen.

*Type (e) Cells.* In the fundus of each type III alveolus are 5-8 wedge-shaped cells with scanty cytoplasm. They take a faintly blue to pink in haematoxylin-eosin stain and light blue colour in Mallory's triple stain (Fig. 2). They resemble the type (b) cells of the type II alveolus and probably they constitute the Funduszellen of NORDENSKIÖLD (1905, 1908) and VITZTHUM (1943). According to VON KÜNSSBERG (1911) Funduszellen are absent in *Ornithodoros moubata*. This opinion is shared by ROBINSON & DAVIDSON (1913) in *Argas persicus*.

*Histochemistry.*

Histological stains being non-specific, histochemical stains were employed to elicit the nature of cell components. The result of this histochemical study is given in Table 1. In the triple stain of

TABLE 1.

*Histochemical Results on Salivary Glands. Unfed Adults.*

| Methods | | Type I | Type II (a) | Type II (b) | Type III (c) | Type III (d) | Type III (e) |
|---|---|---|---|---|---|---|---|
| *A* | *Polysaccharides* | | | | | | |
| | P.A.S. | + | — | ++ | — | — | ± |
| Glycogen | Diastase/P.A.S. | — | — | — | — | — | — |
| | Best's Carmine | — | — | — | — | — | — |
| Galactogen | – Pectinase/P.A.S. | — | — | — | — | — | — |
| Mucoproteins | Pepsin/P.A.S., Trypsin/P.A.S. | — | — | — | — | — | — |
| Glycoproteins | Bismarck Brown-y | — | — | — | — | — | — |
| | Metachromasia | — | — | — | — | — | — |
| Glycolipids | Methanol | — | — | — | — | — | — |
| | Chloroform/P.A.S. | — | — | — | — | — | — |
| Acid Mucopolysaccharides | Alcian Blue | — | — | — | — | — | — |
| | Colloidal Iron | — | — | — | — | — | — |
| Orthochromasia | – Toluidine Blue | ± | — | + | — | — | + |
| *B* | *Proteins* | | | | | | |
| Basic Proteins | Mercuric-Bromo-Phenol-Blue | + | +++ | ± | +++ | +++ | ± |
| | Naphthol Yellow-S | + | +++ | ± | +++ | +++ | ± |
| Tryptophane | – D.M.A.B. Nitrite | — | ++ | — | ++ | ± | — |
| Tyrosine | – Millon's Reagent | — | ± | — | ± | ++ | — |
| Arginine | – Sakagukyi's Reaction | — | — | — | — | — | — |
| - SS - GPS. | – P.F.A.A.B. Method | + | + | + | + | — | ± |
| $\alpha$ Amino Acids | – Ninhydrin-Schiff | — | — | — | — | — | — |
| *C* | *Lipids* | | | | | | |
| Sudan Black B. | | + | — | — | — | — | — |
| Regaud's Haematoxylin | | ++ | ++ | — | ++ | — | — |
| *D* | *Nucleic Acids* | | | | | | |
| R.N.A. Methyl Green Pyronin-y | | ± | — | + | — | — | — |
| R.N.A. Methyl Green Toluidine Blue | | ± | — | + | — | — | — |

(a), (b), (c), (d), (e) indicates types of cells. ± sign indicates a faint positive reaction. The number of plus signs indicates the relative intensity of staining.

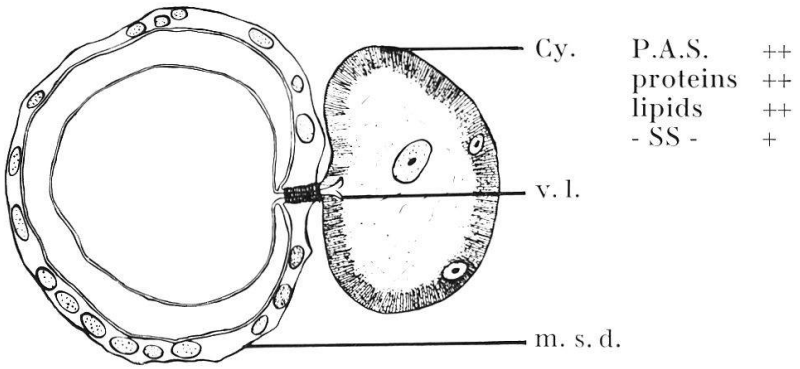


*Fig. 3.* Drawing of a section through type I alveolus of an unfed female to show the histochemical nature of this alveolus, cy.: cytoplasm, v.l.: valve-like structure, m.s.d.: main salivary duct.

HIMES and MORIBER the type I alveoli gave positive results for both proteins and polysaccharide groupings (Fig. 3). In the types II and III alveoli, the cytoplasm of the types (b) and (e) cells respectively stained P.A.S. positive, the type (e) cells only faintly so. The type (a), (c) and (d) cells stained for proteins only (Figs. 4 and 5). There was no reduction in the intensity of P.A.S. staining in all the three types of alveoli following diastase and pectinase digestion indicating that P.A.S. staining is not due to glycogen and galactogen respectively. Best's Carmine test also gave negative results. On treatment with pepsin or trypsin prior to P.A.S. staining, there

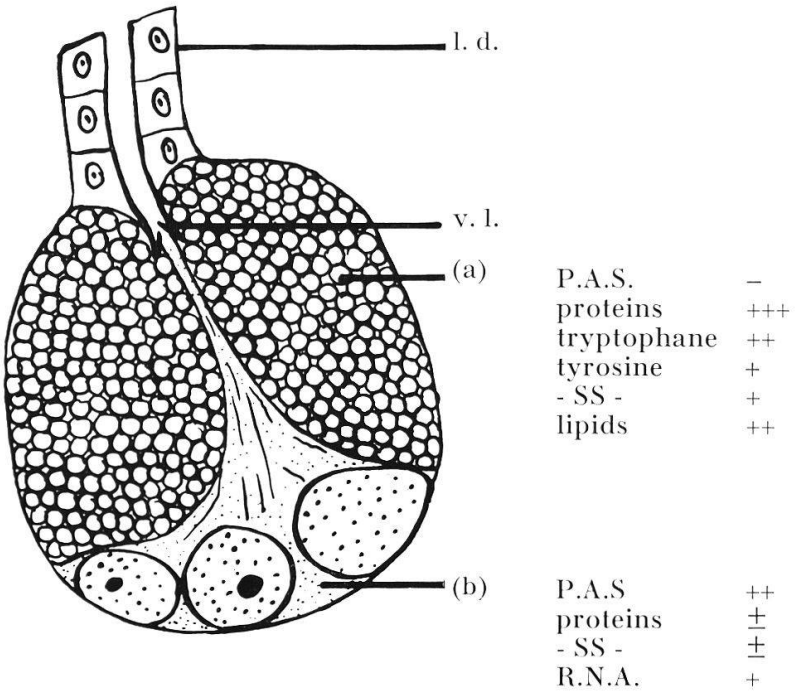


*Fig. 4.* Drawing of a section through type II alveolus of an unfed female to show the histochemical nature of the cells of this alveolus. l.d.: lobular duct, v.l.: valve-like structure.

was no diminution in P.A.S. staining intensity. This together with failure to stain with Bismarck-brown-y or non metachromasia with toluidine blue might indicate that, the P.A.S. staining in the three types of alveoli is neither due to mucoproteins nor glycoproteins. In the type I alveoli, treatment with methanol-chloroform considerably reduces intensity of P.A.S. staining and coupled with moderately positive staining for lipids it could be concluded that the P.A.S. positive reaction in the type I alveoli might be due to the presence of some phospholipids. However in the types (b) and (e) cells, failure of prolonged period of methanol-chloroform extraction procedure to reduce the intensity of the P.A.S. reaction indicates, the P.A.S. staining is neither due to phospholipids nor glycolipids.

According to BRACHET (1957), FICQ (1955), the intensity of staining with mercuric-bromo-phenol blue is strongly modified when R.N.A. is removed by ribonuclease treatment. Consequently control slides were treated with ribonuclease, prior to staining with mercuric-bromo-phenol blue, and only cells retaining the blue colour after ribonuclease treatment were taken as containing basic proteins. The (a), (c) and (d) cells stained with the same intensity for basic proteins which in the case of the type (d) cells is removable by pepsin digestion indicating that it might be an "unmodified" protein. In the case of the types (a) and (c) cells the

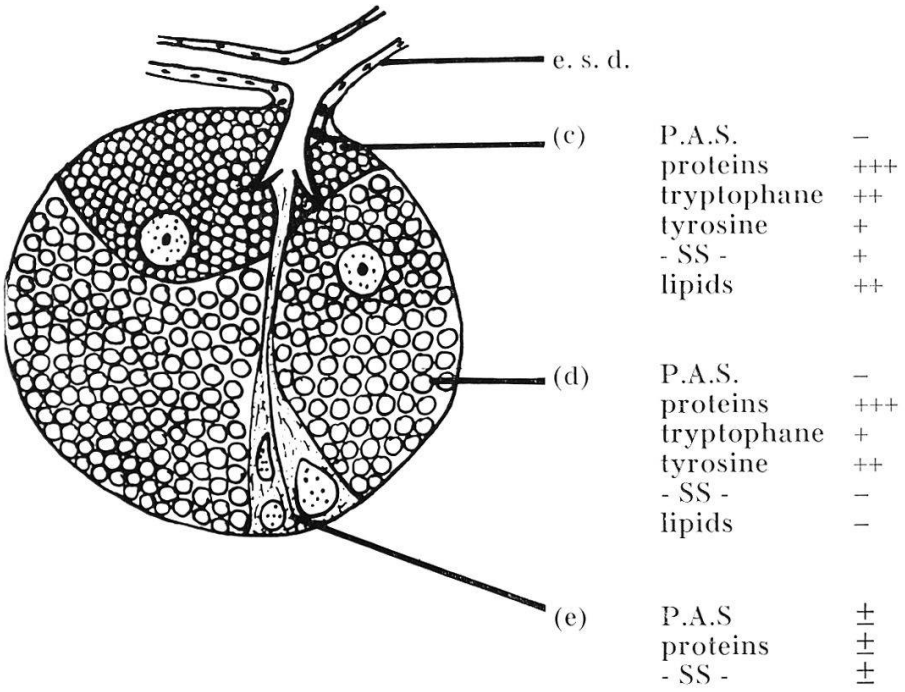


*Fig. 5.* Drawing of a section through type III alveolus of an unfed female to show histochemical nature of the cells of this alveolus. l.d.: lobular duct. v.l.: valve-like structure, e.s.d.: efferent salivary duct.

basic protein is only partially removed after prolonged pepsin digestion indicating, that the lipid present in these cells might possibly be linked to the protein. The (a) and (c) cells contain some tryptophane, whilst the type (d) cells are rich in tyrosine groups.

*Feeding Females.*

*Histology.*

*Type I Alveoli.* There is no appreciable increase in the size of these alveoli and neither is there any marked alteration in their histological appearance. They measure about 42.5 $\mu$ in diameter.

*Type II Alveoli.* Histological changes in these alveoli, are visible after 24 hours of attachment. They start to increase in size, becoming the largest alveoli in fully fed ticks, in which they measure about 122 $\mu$ (Fig. 5). Apparently, the 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 is highly basophilic in haematoxylin-eosin. In contrast, the type (b) cells increase enormously in size without any replication, however, among the cells (Fig. 6). The nuclei increase

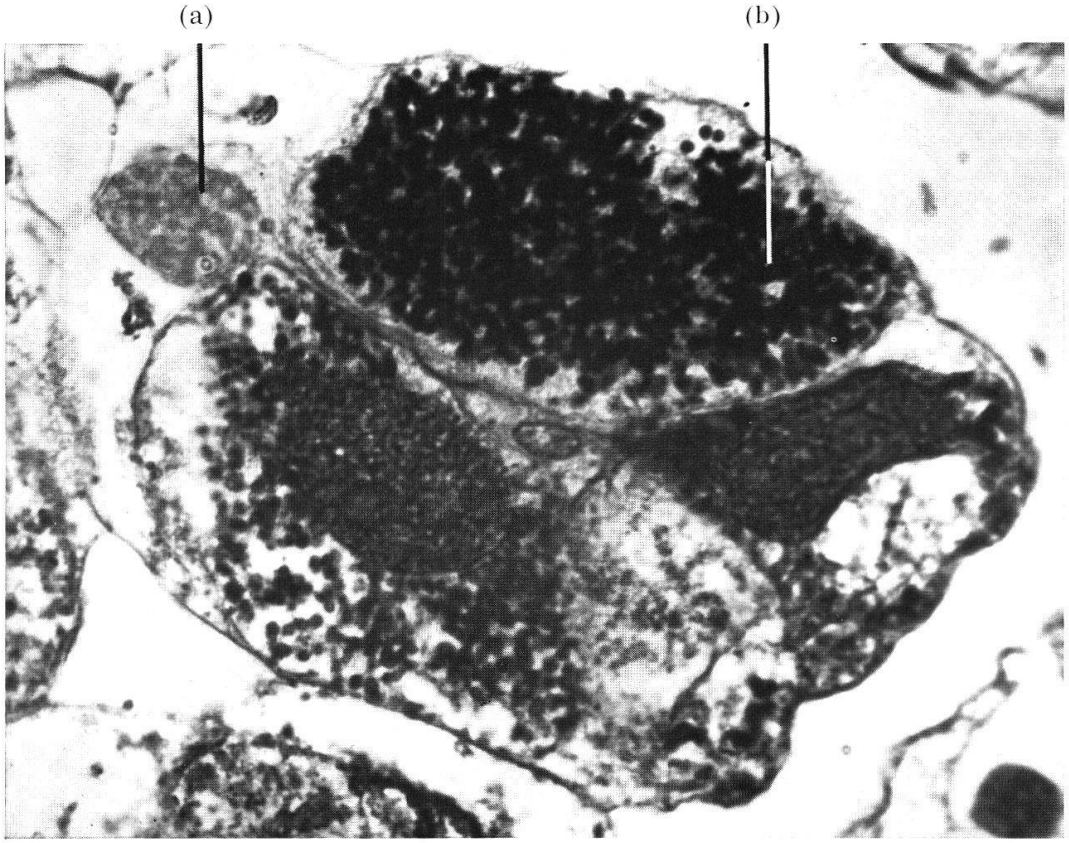


*Fig. 6.* Transverse section through type II alveolus of a feeding female. ×1500 (formol-sublimate fixation; Mallory's triple stain).

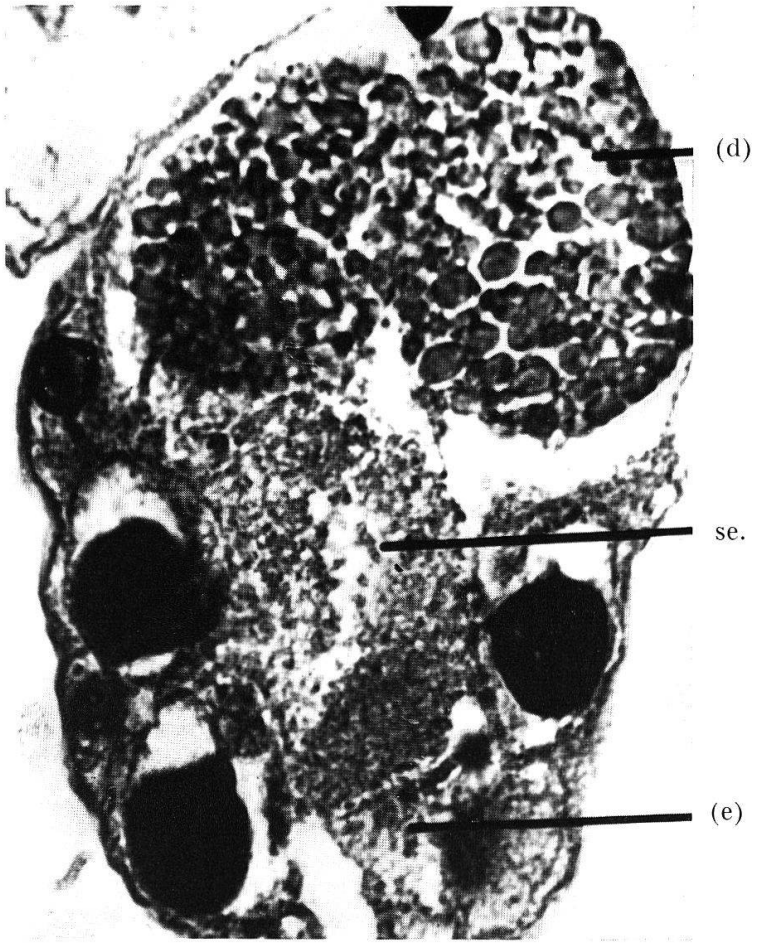


*Fig. 7.* Transverse section through the type III alveolus of a female which is almost fully fed. ×1500 (formol-sublimate fixation; Mallory's triple stain). se.: secretion.

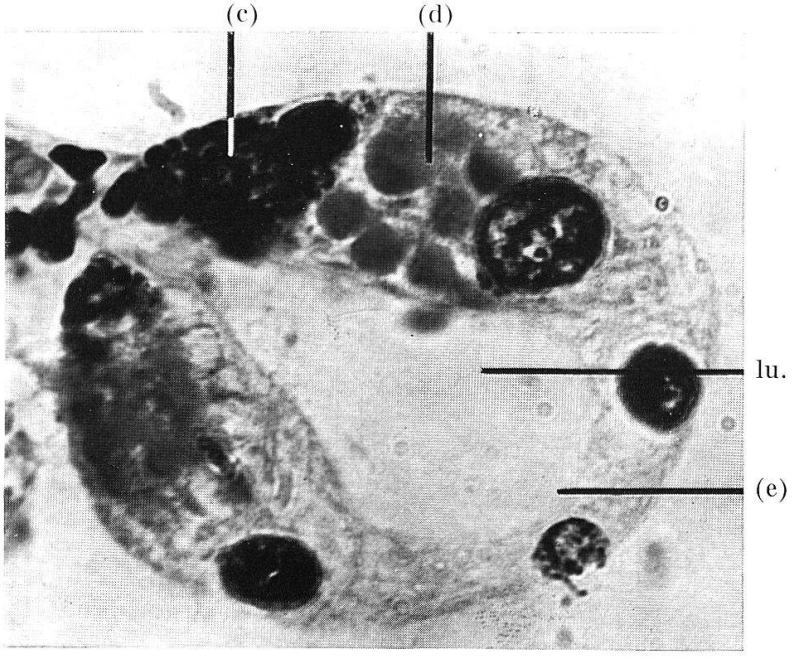


*Fig. 8.* Transverse section through the type III alveolus of a feeding female. ×600 (formol-sublimate fixation; Mallory's triple stain). lu.: lumen.

markedly in size measuring about 27-29 $\mu$ in diameter, and the chromatin shows up quite prominently, whilst in the centre or towards one side is a blue staining nucleolus. The cytoplasm becomes fibrillar and in between there fibrils is a finely granular material which forms the ground cytoplasm. In addition there are secretory granules of various sizes. In some of the cells these secretory granules are very numerous and closely packed whilst in others they are few and scattered (Fig. 6). These cells stain deep blue in haematoxylin-eosin and light blue in Mallory's triple stain. These granules increase in number as the tick continues to feed and apparently they are constantly released into the lumen of the alveolus as salivary secretion. In ticks which have just completed engorgement, they are quite numerous. After the tick has dropped off, these alveoli begin to shrink and after oviposition they are almost degenerate.

*Type III Alveoli.* These alveoli increase appreciably in size measuring about 92 $\mu$ in diameter (Fig. 7), but do not attain the ultimate size of the type II alveoli (Fig. 7).

*Type (c) Cells.* There is no apparent change in size of histological appearance of these cells (Fig. 7).

*Type (d) Cells.* Like the (c) cells these cells do not exhibit any appreciable increase in size but the granules increase slightly in size and towards the last stages of feeding they break down into a finely granular homogeneous material which collects in the greatly enlarged lumen (Fig. 7).

*Type (e) Cells.* These cells show a marked increase in size, beginning from about 24 hours after attachment when they begin to show up quite distinctly (Fig. 8), and in ticks which are near the final stages of feeding they constitute more than half the size of the alveolus (Fig. 7). These cells which were originally wedge-shaped become flattened as the lumen of the alveolus widens, becoming rectangular in shape. They contain a finely granular homogeneous highly eosinophilic material.

*Histochemistry.*

The histochemical results on the salivary glands in feeding females is given in Table 2.

*Type I Alveoli.* There is marked increase in the intensity of P.A.S. staining as well as increase in protein material as revealed by staining with naphthol yellow and mercuric-bromo-phenol blue. The intensity of P.A.S. staining is markedly reduced after treatment with methanol-chloroform. This increase in P.A.S. staining might probably be due to the presence of some phospholipid.

TABLE 2.
*Histochemical Results on Salivary Glands. Fed Adults.*

| Methods | | Females | | | | | | Males | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | Type II (a) | Type II (b) | Type III (c) | Type III (d) | Type III (e) | Type II (a) | Type II (b) | Type II (f) | Type IV (g) | Type IV (h) |
| *A* | *Polysaccharides* | | | | | | | | | | | |
| | P.A.S | ++ | — | +++ | — | — | ++ | — | +++ | ++ | ++ | — |
| Glycogen | Diastase/P.A.S. | — | — | — | — | — | — | — | — | — | — | — |
| | Best's Carmine | — | — | — | — | — | — | — | — | — | — | — |
| Galactogen | – Pectinase/P.A.S. | — | — | — | — | — | — | — | — | — | — | — |
| Mucoproteins | Pepsin/P.A.S. | — | — | — | — | — | — | — | — | — | — | — |
| Glycoproteins | Trypsin/P.A.S. | — | — | — | — | — | — | — | — | — | — | — |
| | Bismarck Brown-y | — | — | — | — | — | — | — | — | — | — | — |
| | Metachromasia | — | — | — | — | — | — | — | — | — | — | — |
| Glycolipids | Methanol | — | — | — | — | — | — | — | — | — | — | — |
| | Chloroform/P.A.S. | — | — | — | — | — | — | — | — | — | — | — |
| Acid Mucopolysaccharides | Alcian Blue | — | — | — | — | — | — | — | — | — | — | — |
| | Colloidal Iron | — | — | — | — | — | — | — | — | — | — | — |
| Orthochromasia | – Toluidine Blue | + | — | +++ | — | — | ++ | — | +++ | — | — | — |
| *B* | *Proteins* | | | | | | | | | | | |
| Basic Proteins | Mercuric-Bromo-Phenol-Blue | ++ | +++ | ± | +++ | +++ | ± | +++ | ± | ++ | ++ | +++ |
| | Naphthol Yellow-S | ++ | +++ | ++ | +++ | +++ | + | +++ | ++ | ++ | ++ | +++ |
| Tryptophane | – D.M.A.B. Nitrite | ± | ++ | — | ++ | + | — | ++ | + | + | + | ++ |
| Tyrosine | – Millon's Reagent | ± | + | — | + | +++ | — | + | — | ± | ± | + |
| Arginine | – Sakagukyi's Reaction | — | — | — | — | — | — | — | — | — | — | — |
| - SS - GPS. | – P.F.A.A.B. Method | — | + | ++ | + | — | + | + | ++ | — | — | + |
| α Amino Acids | – Ninhydrin-Schiff | + | — | — | — | — | — | — | — | — | — | — |
| *C* | *Lipids* | | | | | | | | | | | |
| Sudan Black B. | | ++ | — | — | — | — | — | — | — | — | — | — |
| Regaud's Haematoxylin | | ++ | ++ | — | ++ | — | — | ++ | — | — | — | ++ |
| *D* | *Nucleic Acids* | | | | | | | | | | | |
| R.N.A. Methyl Green Pyronin-y | | — | ± | +++ | ± | + | + | | ++ | + | ± | — |
| R.N.A. Methyl Green Toluidine Blue | | — | ± | +++ | ± | + | + | | ++ | + | ± | — |

(a), (b), (c), (d), (e), (f), (g), (h) indicates types of cells. ± sign indicates a faint positive reaction. The number of plus signs indicates the relative intensity of staining.

Further support for this supposition lies in the fact that after osmium fixation and Sudan black B staining there is also a marked increase in staining removable by methanol-chloroform treatment. Staining with Regaud's haematoxylin after formol-sublimate fixation, BHARRADWAJ & LOVE (1959) also gave positive results for lipids.

*Granule Secreting Alveoli.* The increase in basophilia of the cytoplasm especially of the type (b) cells and to a less extent the type (e) cells, observed after haematoxylin-eosin staining was shown to be due mainly to R.N.A. There is also increase in the intensity of P.A.S. staining of the ground cytoplasm of the type (b) and (e) cells, and also in between the granules of the type (d) cells. The secretory granules which have begun to accumulate in the type (b) cells stain very intensely with P.A.S. Only a few P.A.S. positive secretory granules formed in the type (e) cells during the early stages of feeding. They appear in moderate amounts in the last stages of engorgement scattered in a uniformly finely granular cytoplasm which stains positive for proteins as well as with P.A.S. (Fig. 9). The secretory granules of the type (b) and (e) cells stain quite intensely with naphthol-yellow-s and mercuric-bromo-phenol blue stains for basic proteins. Treatment with pepsin or trypsin prior to P.A.S. staining failed to give any diminution in the staining intensity of the secretory granules of the types (b) and (e) cells. The same treatment failed to reduce mercuric-bromo-phenol blue

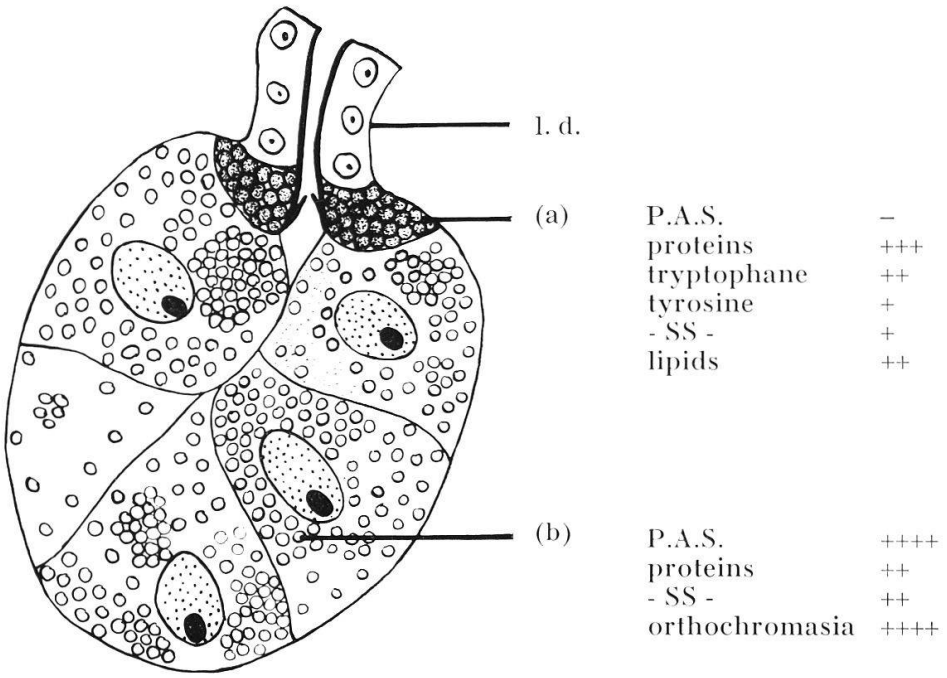


*Fig. 9.* Drawing of a transverse section through a type II alveolus of a feeding female to show the histochemical nature of the cells. l.d.: lobular duct.

or naphthol-yellow-s staining of these secretory granules. Negative results with Bismarck brown-y stain and non-metachromasia with toluidine blue suggests that the P.A.S. staining of the secretory granules of the type (b) and (e) cells is not due to mucoproteins or glycoproteins. Failure to prevent P.A.S. staining after methanol-chloroform extraction procedure suggests that the P.A.S. positive material, is due neither to glycolipids nor phospholipids. From the above analyses it is reasonable to conclude that the P.A.S. staining of the secretory granules of the types (b) and (e) cells is explicable by one of the following four interpretations:

(1) Neutral mucopolysaccharide protein complex in which the protein is not removable by pepsin or trypsin.

(2) A protein firmly linked with a polysaccharide.

(3) A low sulphated mucopolysaccharide and protein that is resistant to pepsin and trypsin digestion.

(4) That the two are firmly linked together. These secretory granules also contain moderate amounts of disulphide groups. It is of interest to note that neutral mucopolysaccharides from animal

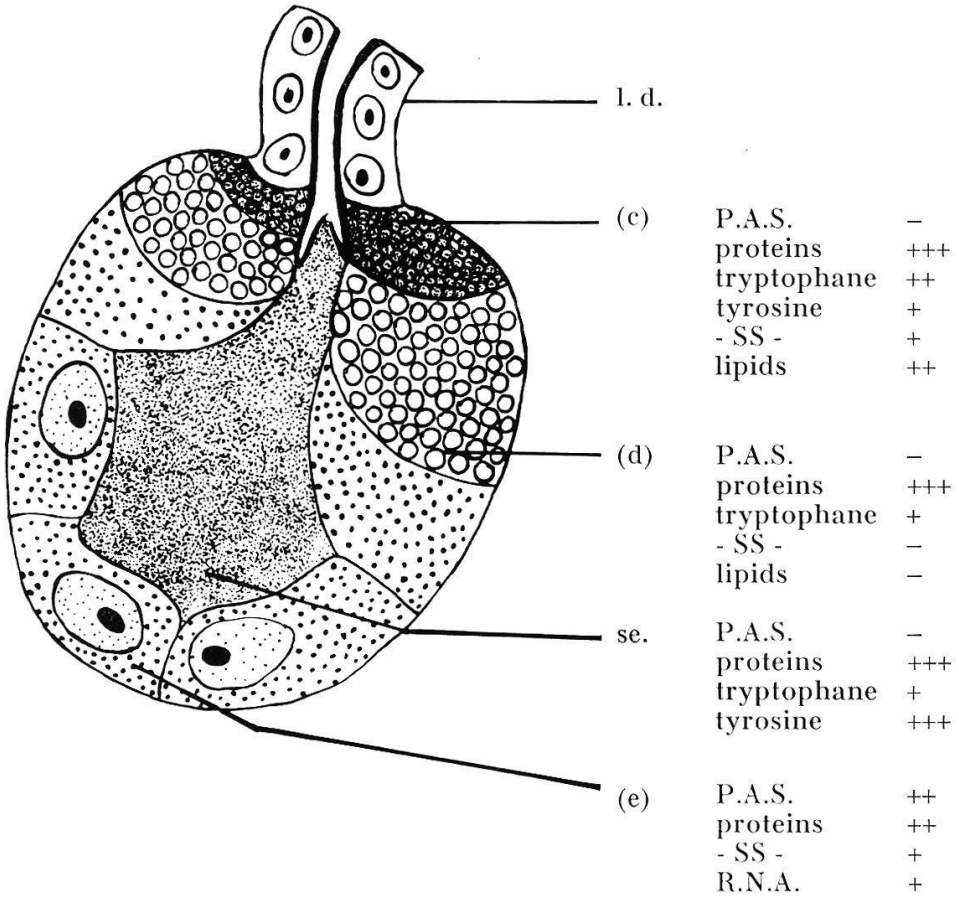


*Fig. 10.* Drawing of a transverse section through a type III alveolus of a partly fed female to show the histochemical nature of the cells. l.d.: lobular duct, se.: secretion.

sources are considered to occur in firm combination with proteins, MEYER & SMYTH (1937), BETTELHEIM-JEVONS (1958). It has been shown that mucopolysaccharides with low sulphate content give a positive P.A.S. reaction and are non-metachromatic, JORPES & GARDELL (1948). On the other hand WALTON & RICKETS (1954) have shown that non metachromatic staining of an acid mucopolysaccharide might be caused by combination of the carbohydrate with protein.

As already shown above, there is no marked histological change in the types (a), (c) and (d) cells during engorgement. Cytochemical techniques did not reveal much difference except for the fact that the ground cytoplasm of the type (c) cells of type III alveoli increases in amount staining intensely with P.A.S. as well as for R.N.A. (Fig. 10). The protein of the type (d) cells has already been shown to contain moderate amount of tyrosine groups whilst the types (a) and (c) cells contain some tryptophane. During the last stages of feeding the widely enlarged lumen of the type III alveoli are filled with highly eosinophillic secretory material which stains quite intensely for tyrosine (see Figs. 7, 10). It is therefore most likely that the source of this secretion is from the type (d) cell granules; and in fact most of these granules were seen to be losing their spherical shape and breaking down.

### *Unfed Males.*

*Histology.*

The three different types of alveoli described in the salivary glands of the female exist in the salivary glands of the male, but as will be shown later, the histological picture of the salivary glands in the feeding male differs to some extent from those of feeding females.

*Histochemistry.*

The histochemical findings in the salivary glands of the male are parallel to those of the females.

### *Feeding Males.*

*Histology.*

In feeding males, the alveoli do not attain the same size as those of the feeding females, and the histological changes which occur in these alveoli are quite similar but a few differences warrant some description. In the female the type II alveoli are

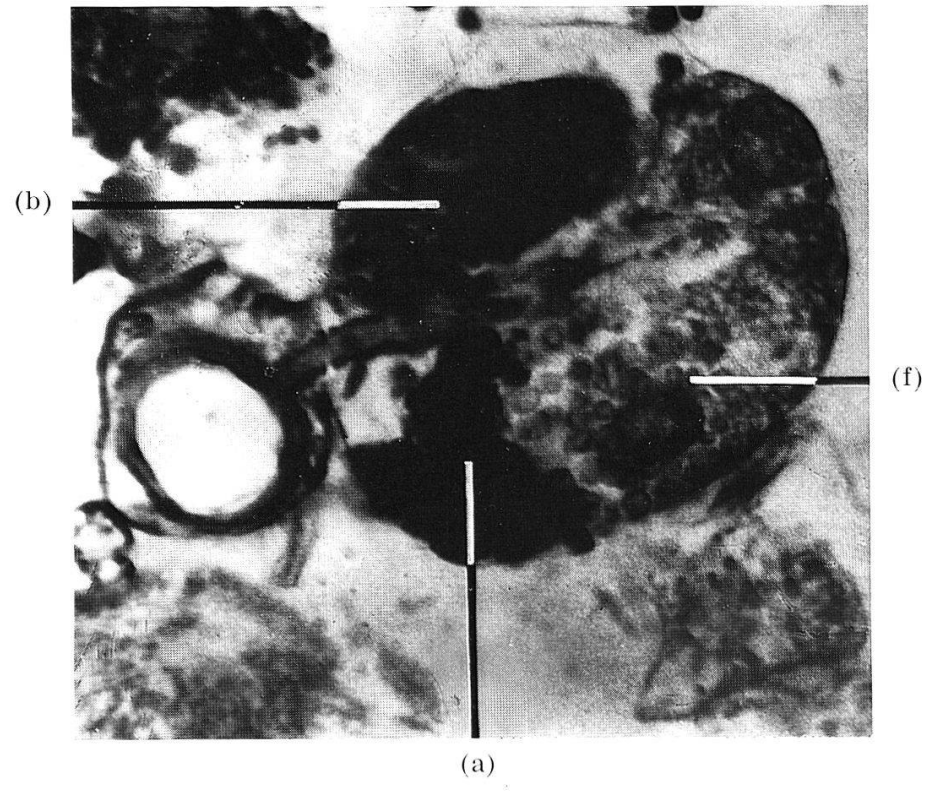


*Fig. 11.* Transverse section through a type II alveolus of a partly fed male. ×1500 (formol-sublimate fixation; Mallory's triple stain).

considerably larger than the type III alveoli, but in the male the type II alveoli are smaller than the type III alveoli. They measure about 44 $\mu$ (Fig. 11). Also the type (e) cells of the type III alveoli remain quite insignificant in contrast to the large rectangular form attained by these cells in late feeding females (Fig. 7), and the secretory granules are very few. In feeding males a new type of cell, the type (f) cell and a completely different alveolus, the type IV alveolus become evident.

*Type (f) Cell.* Lying adjacent to the type (a) cell of the type II alveolus is one cell containing coarse secretory granules which stain yellow in Mallory's triple stain after formol-sublimate fixation (Fig. 11) (f). The granules are less compact than those of the type (a) cells and are more or less evenly distributed. In haematoxylin-eosin, these granules stain pink.

*Type IV Alveolus.* These alveoli which TILL (1959) was able to differentiate with difficulty in the unfed males of *R. appendiculatus* and which are supposed to be made up of one type of cells, could not be seen in any of the unfed males so far examined. However, in feeding males, they are the largest alveoli, composed of two main types of cells (Fig. 12). They measure about 57-58 $\mu$ in diameter.

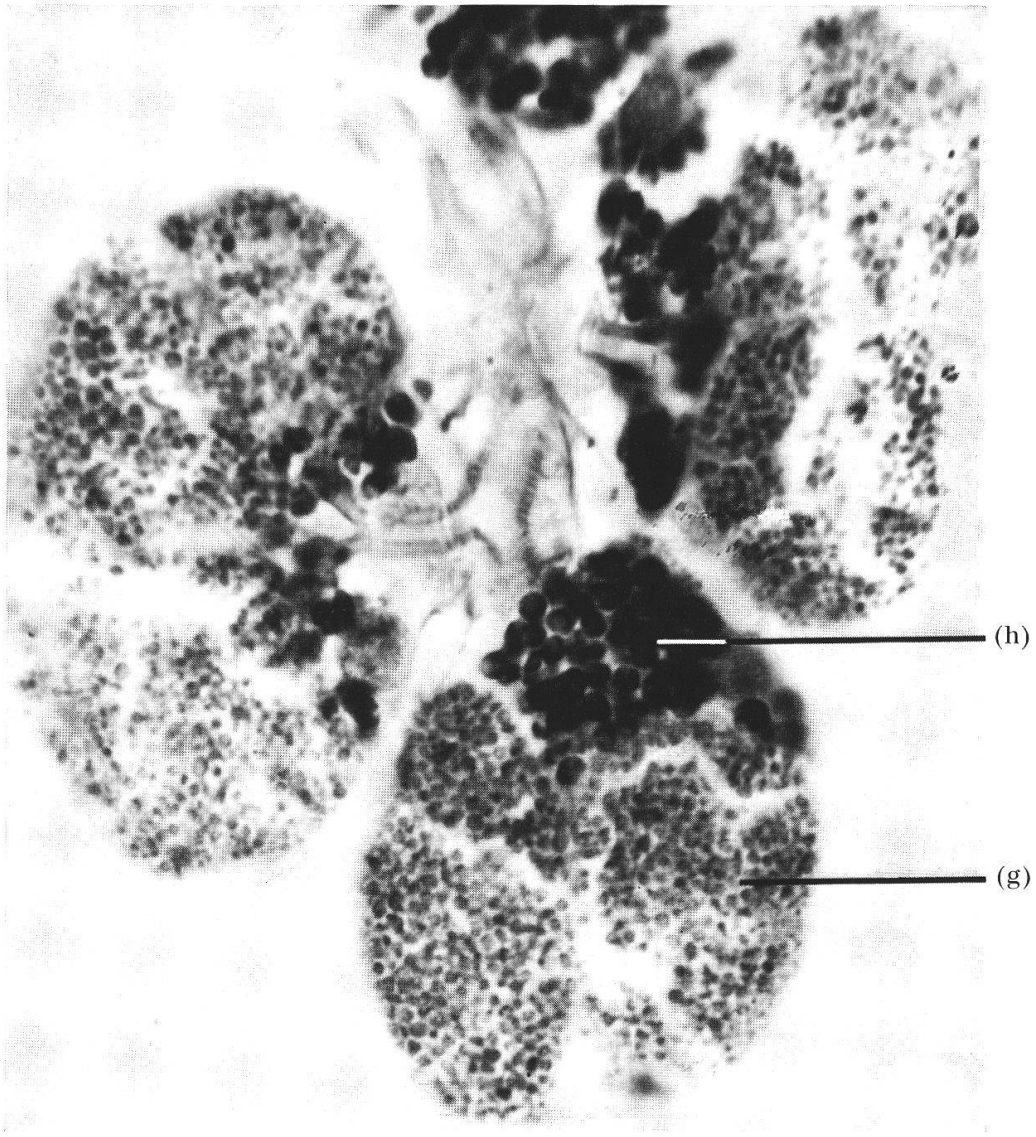


*Fig. 12.* Transverse section through type IV alveoli of a partly fed male. ×1500 (formol-sublimate fixation; Mallory's triple stain).

*Type (h) Cell.* Located at the base of the lobular duct are one or two cells, which are filled with large closely packed granules which stain purple in Mallory's triple stain. From the histological point of view, these cells are similar to the (a) and (c) cells of types II and III alveoli respectively and occupy a similar position in the type IV alveolus (see Fig. 12).

*Type (g) Cell.* The rest of the alveolus consists of four large cells containing evenly distributed finely homogeneous granules which stain purplish-blue in Mallory's triple stain after formol sublimate fixation. Their nuclei which are large, and vesicular lie in close proximity to the base of the alveolus. In haematoxylin-eosin these cells stain bright pink.

*Histochemistry.*

Since the histochemical changes observed in the types I, II and III, alveoli of the male are similar to those in the female (see Table 2), attention will only be given to the (f) cell of type II alveolus and the type IV alveolus.

*The Type (f) Cell.* The granules of the (f) cells stain in the P.A.S. stain but not as intensely as the granules of the type (b) cells of type II alveoli. Naphthol yellow-s and mercuric bromphenol blue stains indicate that these granules contain proteins

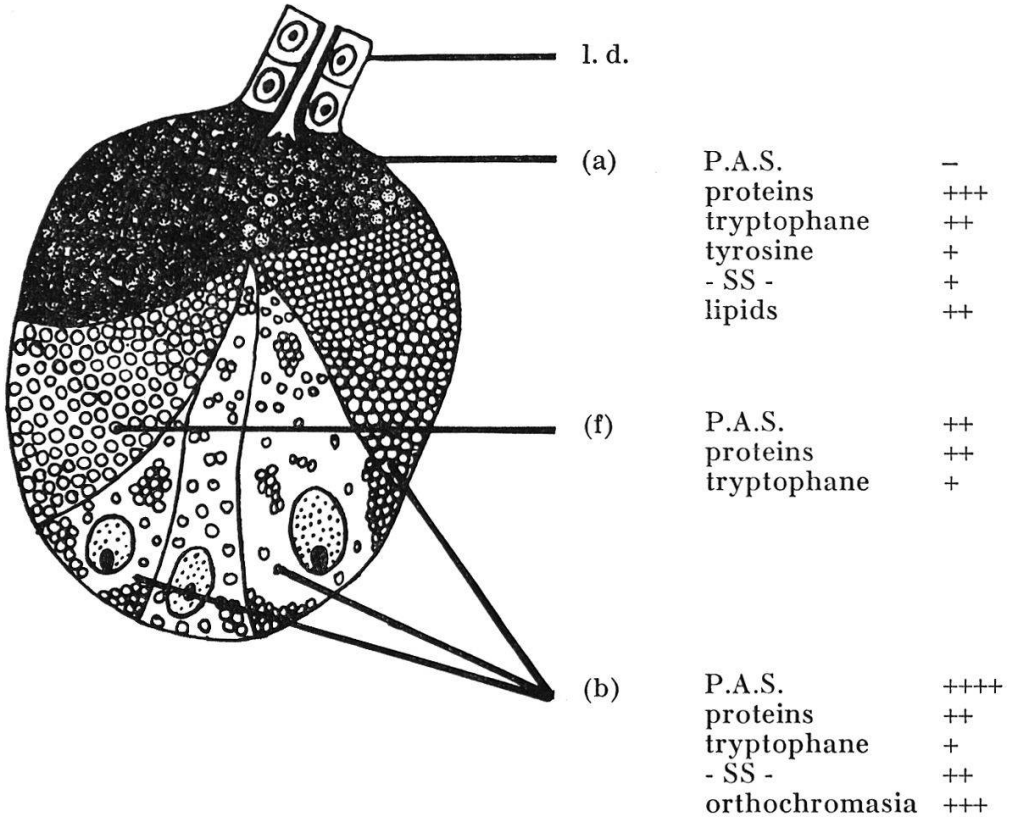


*Fig. 13.* Drawing of a transverse section through a type II alveolus of a partly fed male to show the histochemical nature of the cells. l.d.: lobular duct.

which are either resistant to pepsin and trypsin digestion or else are firmly linked to carbohydrate grouping present. These granules differ from those of the (b) cells in not only being less P.A.S. positive but are non-orthochromatic in contrast to orthochromasia observed in the granules of type (b) cells (see Fig. 13).

*The Type IV Alveolus.*

*Type (h) Cell.* This cell which lies at the base of the lobular duct contains mainly basic proteins which was shown to be associated with tryptophane and some traces of disulphide groups.

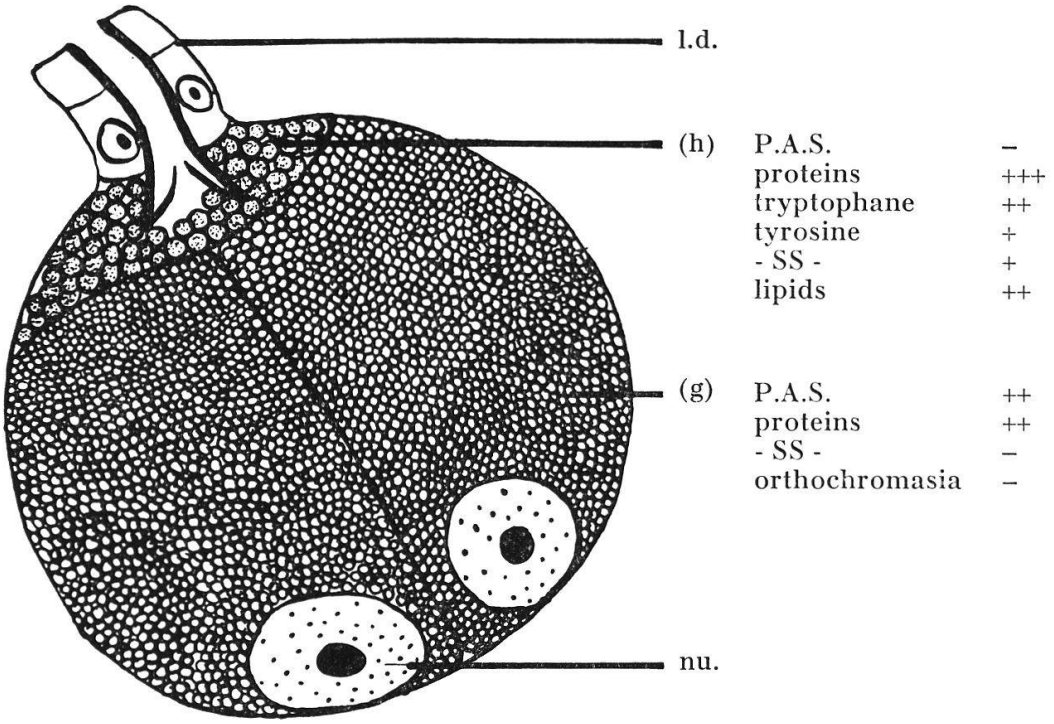


*Fig. 14.* Drawing of a transverse section through a type IV alveolus of a partly fed male to show the histochemical nature of the cells. l.d.: lobular duct, nu.: nucleus.

*Type (g) Cells.* These form the major part of this alveolus and contain moderately P.A.S. positive granules which contain proteins resistant to pepsin and trypsin digestion. Histochemical tests indicate that they contain some amounts of tryptophane and tyrosine. Like the granules of the (f) cells of the type II alveoli they are non-orthochromatic with toluidine blue (see Fig. 14).

### *Unfed Larvae*

The alveoli of the salivary glands are very few indeed numbering between 11 and 13. The three main types (I-III) of alveoli described in the unfed adults were observed in the salivary glands of unfed larvae. The linear arrangement of the three types of alveoli along the main salivary duct is not constant in *Haemaphysalis spinigera* in contrast to *Rhipicephalus appendiculatus* (TILL, 1961), but varies among individual ticks as can be seen from Table 3.

### *Partly Fed Larvae.*

The histological changes observed in the salivary gland of feeding larvae are similar to those of feeding females, but the salivary glands never become as large as those of the females.

TABLE 3.

*A Table Showing the Variation in the Linear Arrangement of the Salivary Alveoli in the Unfed Larva.*

| Larva A | | Larva B | |
|---|---|---|---|
| Left | Right | Left | Right |
| I | I | I | I |
| III | II | III | III |
| II | III | III | II |
| II | I | I | I |
| III | II | II | II |
| II | II | III | II |
| II | I | II | I |
| II | III | III | III |
| I | II | I | III |
| II | II | II | II |
| III | III | III | II |
| III | III | III | III |

Neither the type IV alveoli nor the type (f) cell of the type II alveolus observed in the feeding males were observed in feeding larvae. This is in agreement with the findings of TILL for *Rhipicephalus appendiculatus*. Of the three types of alveoli the type II is the largest. Histological changes are first noticeable after sixteen hours of attachment. At this stage only a few secretory granules are present in a few of the type (b) cells, but they appear in increasing numbers as the tick continues to feed.

## *Fully Fed Larvae.*

In fully fed larvae the granules are still present in the (a), (c) and (d) cells and to a lesser extent in the (b) and (e) cells. The alveoli are very much compressed by the expanded gut diverticulae.

*Metamorphosing Larvae.*

On the first day after dropping off, the salivary glands are still intact and remain so until the fourth day of the post-feeding period when only very few intact alveoli are seen. The epithelial cells of the salivary duct continue to give rise to a system of branching ducts which terminate in small alveoli consisting of groups of undifferentiated masses of cells. These were first seen on about the fourth day of the metamorphosing period. These round masses of undifferentiated salivary alveoli continue to be formed in increasing numbers with time, but apparently there is no differentiation into types I, II and III alveoli until one day after moulting into the nymph.

*Unfed Nymph.*

In the nymph the salivary gland alveoli are more numerous numbering about forty in each gland. The main salivary duct has divided posteriorly into subsidiary ducts as well as giving rise to a few secondary ducts. The three main types of alveoli can be distinguished in this instar of the tick from the very day of its emergence. The distribution of the alveoli is similar to that of the adult.

*Feeding Nymph.*

The changes observed in feeding nymphs are similar to those described above for the feeding larvae. Up to this stage there is still no difference in the histological appearance of the salivary glands indicative of the difference of sex as in the adult stages.

*Metamorphosing Nymphs.*

One day after dropping off the host, the salivary glands of the engorged nymph are still intact, but by the third day of the post-feeding period they have almost degenerated and disappeared. New alveoli consisting of roundish masses of nuclei continue to be formed as in the larvae during the metamorphosing period. The histological appearance of the alveoli on the 13th day differs from that of the preceeding days. Up to the 12th day, the alveoli consist of spherical masses of nuclei without clearly defined lumen, whilst on the 13th day there is a clearly defined lumen surrounded by a single layer of nuclei. Also at this stage the type I alveoli could be distinguished from the granule secreting (types II and III) alveoli although the latter are devoid of granules at this stage. The granule secreting alveoli are pear-shaped or round,

with more than six nuclei peripherally disposed in a clear staining cytoplasm whilst the type I alveoli are oval in shape with a faintly pink staining peripheral cytoplasm with never more than four nuclei one of which is larger than the rest and lies in the centre of the alveolus. Similar findings were reported by TILL in *R. appendiculatus*. The granules of the (a), (c) and (d) cells appear on the second day after the emergence of the adult.

In the histochemical studies on the larvae and nymph only the triple stain, the P.A.S. stain, the naphthol yellow stain and the R.N.A., D.N.A. stains of KURNICK (1955) and KORSON (1951) were used. The histochemical changes observed were similar to those already described for the adult female.

## Discussion.

The salivary glands of *Haemaphysalis spinigera* are similar in both sexes and follow the general pattern as in other Ixodid ticks in having an anterior main duct which divides into two subsidiary branches, in contrast to those of Argasids with a main salivary duct which runs undivided to the posterior end of the gland, VITZTHUM (1943).

Histologically the type I non-granular alveoli are similar to those of Argasid ticks, but differ in their distribution. Those of *Haemaphysalis spinigera* are confined to the main salivary duct up to the anterior ends of the subsidiary ducts, whilst in Argasids they are concentrated into a tongue-like mass on the mesial side of the main salivary duct, see CHRISTOPHERS (1906), ROBINSON & DAVIDSON (1913), TRUE (1932), BALASHOV (1961), ROSHDY (1961). Previous workers, ALLEN (1905), BONNET (1907), NORDENSKIÖLD (1908), SAMSON (1909), ZEBROWSKI (1926), TRUE (1932), DOUGLAS (1943), and ARTHUR (1957) have distinguished only one type of granular alveolus in addition to the type I non-granular alveolus. WILLIAMS (1905), figured only granular alveoli and made no mention of different types of alveoli. TILL (1959, 1961), working with *Rhipicephalus appendiculatus* was able to differentiate two different types of granule secreting alveoli in the female, namely types II and III and an additional type IV in the male. Further another type of cell (f) was described by her in the type II alveoli of feeding males. This amounts to five types of cells in females and seven types of cells in the males. In the present studies, the writer was able to confirm TILL's observations in addition to describing another cell, the type (h) cell in the type IV alveolus in *Haemaphysalis spinigera* by the use of the same tech-

nique as well as using Mallory's triple stain on formol-dichromate fixed tissue. Since previous workers, ALLEN (1905), ZEBROWSKI (1926), and DOUGLAS (1943), apparently used haematoxylin-eosin in their studies and therefore could not differentiate between types (a), (c) and (d) cells, it is difficult to know which of their description fits into TILL's type II or III alveoli. In the Argasid tick *Ornithodoros moubata* the writer found one type of granule secreting alveolus. Two types of cells were observed in this alveolus after staining with Mallory's triple stain. The first of these lies at the base of the lobular duct and stains purplish red, whilst the other group of cells which constitute the rest of the alveolus contain granules of various sizes which stain blue. These granules stain various shades of pink in haematoxylin-eosin, as was also found by CHRISTOPHERS (1906) in *Ornithodoros savinyi*. He was of the opinion that difference in staining is due to the progression of secretory process. ROBINSON & DAVIDSON (1913) working with *Argas persicus,* observed that "in the same alveolus secretory cells may be found in all stages, from cells in which secretory product is just commencing to accumulate, to the cell which is literally bursting with its secretion". According to TILL (1961), all the cells of the type IV alveolus are similar in *Rhipicephalus appendiculatus*. However working with *Haemaphysalis spinigera,* the writer was able to differentiate an additional type (h) cell in the type IV alveolus, and from its histological appearance and its position it corresponds to either the type (a) cell of type II alveolus, or type (c) cell of type III alveolus. It is of interest to note that on examination of sections of fed males of *Dermacentor andersoni* and *Rhipicephalus sanguineus,* all the cells of the type IV alveoli were the same, thus confirming the findings of TILL (1961), for *Rhipicephalus appendiculatus*. However the writer failed to locate these type IV alveoli in unfed males of *Haemaphysalis spinigera* so far examined. It has been possible to support histological results with histochemical findings, although it has not been possible to give a clear indication of the function of all the different types of cells so far described. As already shown, the granules of the type (a) cell of type II alveolus, types (c) and (d) cells of type III alveolus, and type (h) cells of type IV alveolus contain mainly basic proteins, which in the case of the types (a), (c) and (h) cells, is associated with some tryptophane some lipids and disulphide groups. In the case of the type (d) cells the basic protein is rich in tyrosine and is "unmodified". The types (b) and (e) cell of the types II and III alveoli respectively contain a carbohydrate-protein complex and this could either be

(1) a neutral mucopolysaccharide linked to a protein, or

(2) a less sulphated form of a mucopolysaccharide linked to protein.

The salivary glands of ticks are possible sources of anticoagulants, cytolysins, and toxins, ARTHUR (1962). GREGSON (1953) working on the feeding process of *Dermacentor andersoni* is of the opinion that the frequent intervals of salivation of the tick is consonant with the theory that tick paralysis is caused by some action of the tick's saliva on the host. SABBATINI (1898, 1899), showed that both male and female ticks of *Ixodes ricinus* contain a substance which retards the coagulation of blood and lymph. According to him this substance annules the action of fibrinogen. *In vitro* parenteral injection of this substance showed similar results. NUTTALL & STRICKLAND (1908) showed that a given quantity of anticoagulant from the salivary glands of a single tick was capable of delaying the clotting of human blood for 45-95 minutes. It has also been reported by GREGSON (1960) that the crushed salivary glands of *Dermacentor andersoni* contains a mild anticoagulant. ROSS (1926) has also expressed this view, and the work of FOGGIE (1959) has confirmed these findings. Haemolysins have been dectected in the salivary secretions of some ticks, but according to PAVLOVSKY & CHODUKIN (1928, 1929), they are weak and slow acting, as has also been found to be the case in *Ornithodoros turicata* females by LAVOIPIERRE & RIEK (1955). Histological evidence suggests to the writer that the type (b) cells of type II alveoli and the type (e) cells of type III alveoli might be the major contributors to the salivary secretion, for they are the cells which show marked changes in size and cell content during engorgement. When the cells of the salivary alveoli are examined in order from the unfed to the fully engorged state it can be observed that these type (b) and (e) of starved ticks are almost devoid of secretory granules whilst in ticks which have attached for about twenty four hours the secretory granules have began to appear and increase in amount as the tick proceeds to engorge. Histochemical evidence suggests that the type (b) cells of type II alveoli and possibly the type (e) cells of the type III alveoli might possibly be the sources of anticoagulant which has been conclusively proved to be present in the salivary glands. Heparin is well known to possess anticoagulant properties, FOSTER (1955). Chemical evidence suggests that heparin is a highly sulphated mucopolysaccharide, FOSTER (1955), HALE (1957). According to FOSTER the high anticoagulant activity of heparin is associated with the degree of sulphation. It is of interest here to point out that many polysaccharides have been sulphated and studied, MEYER et al. (1952); all the compounds formed were found to possess anticoagulant activity,

although much lower than that of heparin. Heparin appears to be firmly bound to protein material in tissues since autolytic or proteolytic methods seem to be necessary to liberate it in soluble form, STACEY (1947). It is significant to note that the secretory granules of the type (b) and (e) cells are P.A.S.-positive and contain proteins which, as histochemical evidence indicates is either resistant to pepsin and trypsin or else firmly linked to the carbohydrate component, as to resist digestion. Heparin has been shown to occur in tissues containing large amounts of mast cells. According to HALE (1957), the presence of three sulphate radicals per monosaccharide unit would block the groups capable of yielding aldehyde with periodic acid. Less sulphated forms of mucopolysaccharide have been shown to be P.A.S.-positive, JORPES & GARDELL (1948), JORPES et al. (1948). The last authors observed that heparin monosulphate was P.A.S.-positive *in vitro*. Some mast cells have been shown to stain with P.A.S., JORPES et al. (1948), WISLOCKI et al. (1949), PEARSE (1949 b), SMITH (1950), LEBLOND (1950), FRIBERG et al. (1951), and MONTAGNA et al. (1954). Thus this latter form of heparin with low sulphate content may be present in them, HALE (1957). Other mast cells fail to stain with P.A.S. stain, HALE (1957); LILLIE (1950) suggests the variation in staining of mast cells which he observed to be caused by differences in the ages of the cells. He has pointed out that those which are P.A.S. negative are metachromatic whilst the P.A.S. positive ones are orthochromatic. However, the secretory granules of the type (b) and (c) cells showed little variation with P.A.S. staining and they were orthochromatic with toluidine blue. Probably the anticoagulant with low sulphate content is present in these cells. If this is so, it is consistent with hard ticks taking long periods to engorge and therefore not requiring a highly sulphated anticoagulant. On the other hand it is possible, that the secretory granules contain the less active form of an anticoagulant which is converted into a more active form when the granules break down and pass into the salivary ducts. The types (a), (c) and (d) cells do not show any appreciable change in size and because of the marked increase in the size of the type (b) and (e) cells they appear relatively smaller (see Figs. 9 and 10). They no doubt have some functional significance, especially when the salivary glands of ticks have been shown to be the source of other potent substances such as cytolysins and toxins. It is also possible that secretions from different types of cells might react with each other. As already noted, the granules of the type (d) cells of the type III alveoli break down and are liberated into the greatly enlarged lumen of the alveoli, during the final stages of

rapid engorgement. This secretion which is mainly protein rich in tyrosine is probably connected with the final rapid engorgement. It is of interest to note that tick paralysis has been associated with the final stages of rapid engorgement, Ross (1926). It is rather surprising that in males which take in small amounts of blood, an additional type of cell, the type (f) cell (see Figs. 11 and 13) and an alveolus, the type IV alveolus (see Figs. 12 and 14) should exist. Histochemical evidence suggests that the type (f) cells belonging to the type II alveoli, and type (g) cells of the type IV alveoli contain a carbohydrate-protein complex which is non-orthochromatic. The function of these cells peculiar to males is not clear.

*Possible Function of the Valve Like Structure.*

All the lobular ducts were found to be provided with a valve-like structure at their point of entry into the lumen of the salivary alveoli. Bertram (1939), working with *Ornithodoros moubata,* suggested that if the wall of the salivary ducts which show spiral thickening, react to increasing pressures in a manner analogous to the tracheal tubes of insects, then during the dilatation of the pharynx, the ducts would be compressed and their lumen reduced or obliterated. Conversely, constriction of the pharynx would cause the ducts to assume their circular outline and since, the buccal cavity would be closed during the constriction of the pharynx salivary fluid would be drawn from the alveoli into the lumen of the ducts. Since, the salivary alveoli, are not provided with any well developed musculature for the pumping of saliva, it is possible that the function of the valve-like structure is to prevent backflow of saliva during the dilatation of the pharynx.

## Acknowledgements.

I wish to express my sincere thanks to Professor D. S. Bertram, Director of the Department of Entomology, under whose guidance this work was carried out for his constructive criticism, constant encouragement and advice. I am indebted to Dr. M. G. R. Varma for a constant supply of ticks and helpful suggestion and to Dr. M. A. Roshdy for his cooperation on histochemical techniques. Finally I wish to express my gratitude to the Government of Ghana for a generous financial support.

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## *Zusammenfassung.*

1. Drei verschiedene Typen von Alveoli konnten in den Speicheldrüsen der ungefütterten Adulten und der unreifen Stadien gefunden werden. Man bezeichnet sie als Typus I, II und III.

2. Die Alveoli vom Typus I sind nicht granulär und sind auf die vordere Hälfte der Drüse beschränkt; sie öffnen sich direkt in den Hauptspeichelgang. Sie enthalten Proteine und ein Lipid, das wahrscheinlich ein Phospholipid ist.

3. Die Alveoli vom Typus II und III sind vielzellig und granulär. Die Alveoli vom Typus II sind zahlreich in den zwei vorderen Dritteln der Drüse und bestehen aus zwei Arten von Zellen, nämlich (a) und (b). Die Alveoli vom Typus III sind am zahlreichsten in den zwei hinteren Dritteln der Drüse und bestehen aus drei Arten von Zellen, (c), (d) und (e).

4. Beim Saugen nehmen die granulären Alveoli beträchtlich an Größe zu infolge des Anwachsens der Zellen (b) und (e). Sekretionsgranula sammeln sich in diesen Zellen an, und histochemische Untersuchungen zeigen, daß diese Granula einen Mucopolysaccharid-Protein-Komplex enthalten, der entweder neutral oder mit niedrigen Sulfatgruppen verbunden ist. Die Zellen (a) und (b) enthalten Granula, welche hauptsächlich aus Proteinen und Lipiden bestehen. Die (d)-Zellen enthalten hauptsächlich basisches Protein.

5. Zusätzliche Arten von Zellen (f), (g) und (h) wurden bei saugenden Männchen beobachtet. Die Art (f)-Zellen kommen in den Alveoli vom Typus II vor, während die (g)- und (h)-Zellen den Alveolus vom Typus IV bilden. Die Sekretionsgranula der (f)- und (g)-Zellen enthalten einen Kohlenhydrat-Protein-Komplex, während diejenige der (h)-Zellen einen Lipid-Protein-Komplex enthalten.

5. Zustäztliche Arten von Zellen (f), (g) und (h) wurden bei saugenden Männchen beobachtet. Die Art (f) Zellen kommen in den Alveoli vom Typus II vor, während die (g) und (h) Zellen den Alveolus vom Typus IV bilden. Die Sekretionsgranula der (f) und (g) Zellen enthalten einen Kohlenhydrat-Protein-Komplex, während diejenige der (h) Zellen einen Lipid-Protein-Komplex enthalten.

6. Nach dem Saugen degenerieren die Speicheldrüsen, neue Alveoli werden aber in den sich entwickelnden Larven und Nymphen gebildet.

## *Résumé.*

1. Trois types différents d'alvéoles des glandes salivaires ont été observés chez l'adulte non nourris de même que chez les stades immatures. Ils furent appelés types I, II et III.

2. Les alvéoles du type I sont non-granuleuses et sont confinées dans la moitié antérieure de la glande. Elles se déversent directement dans le canal

salivaire principal. Elles contiennent des protéines et un lipide qui est probablement phospholipide.

3. Les types alvéolaires I et III sont multicellulaires et granuleux. Les alvéoles du type II sont plus nombreux dans les ⅔ antérieur de la glande et comprennent 2 genres de cellules, (a) et (b).

Les alvéoles du type III sont très nombreuses dans les ⅔ postérieur de la glande et comprennent 3 genres de cellules, (c), (d) et (e).

4. Pendant la nutrition, les alvéoles granuleuses augmentent fortement de taille, particulièrement parce que les cellules (b) et (e) grandissent. Des granules de sécrétion s'accumulent dans ces cellules et des tests histochimiques ont montré que ces granules contiennent un complexe de mucopolysaccharides et de protéines, lequel est plutôt neutre ou associé à des groupes sulfates faibles.

Les cellules (a) et (e) contiennent des granules constitués principalement de protéines et de lipides. Les cellules (d) contiennent surtout du matériel protéinique de base.

5. D'autres genres de cellules (f, g et h) furent observés chez les mâles se gorgeant. Le genre (f) se trouve dans les alvéoles du type II, alors que le genre (g) et (h) constituent les alvéoles du type IV. Les sécrétions granuleuses des cellules (f) et (g) contiennent un complexe d'hydrates de carbonne et de protéines, tandis que les granules des cellules (h) contiennent un complexe de lipides et de protéines.

6. Après engorgement de la tique, les glandes salivaires dégénèrent, mais de nouvelles alvéoles sont formées chez les larves et les nymphes en métamorphose.