ALVEOLAR ULTRASTRUCTURAL AND IMMUNO-HISTOCHEMICAL STUDIES OF FEMALE IXODID LONE STAR TICK *AMBLYOMMA AMERICANUM* SALIVARY GLANDS

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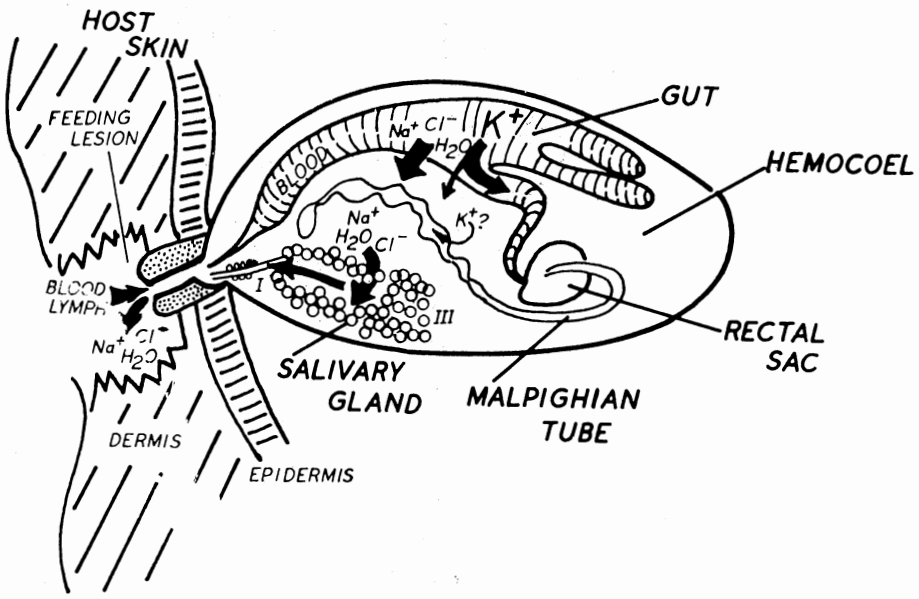
CHAPTER I

FUNCTION AND MORPHOLOGY OF IXODID SALIVARY GLANDS

Introduction

The salivary glands appear to be of great significance in solving the problem of osmoregulation in the ixodid tick. The glands act as excretory organs in removing excess fluids which is obtained during the ingestion of blood meal (Sutton and Arthur, 1963; Balashov, 1965; Arthur, 1965, 1970; Siefert et al., 1968; Snow, 1970; Sauer and Hair, 1972; Koch et al., 1974). The water and ions (Na^+, Cl^- and K^+) are moved across the gut epithelium into the haemolymph, and are reinjected back into the host via the glands (Hsu and Sauer, 1975; Araman, 1972; Guenther and Sauer, 1978) (Fig. 1). Concentrations of Na^+ and Cl^- are somewhat higher in the saliva of feeding ticks than are Na^+ and Cl^- concentrations in the hemolymph (Guenther and Sauer, 1976; Hsu and Sauer, 1975; Kaufman and Phillips, 1973a; Tatchell, 1969). Therefore, movements of Na^+ and Cl^- across epithelia of the salivary gland alveoli probably generate the necessary osmotic force to produce the fluid portion of the saliva. The glands are also important to non-feeding ticks (those not attached to a host and thus not ingesting a blood

Figure 1. Diagram showing a longitutional section of a feeding tick and detailing the movement of fluid and ions; the bloodmeal is first concentrated in the gut by removal of much of the fluid and ionic content. Ecesss fluid and ions are then absorbed by the salivary glands and expelled back into the host. The salivary glands of hard ticks serve as their major osmoregulatory organs. (Diagram) courtesy of Glen R. Needham, Dept. of Ent., Ohio State University.


HOST
SKIN
FEEDING
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BLOOD
LYMPH
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GUT
HEMOCOEL
RECTAL
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GLAND
MALPIGHIAN
TUBE
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III
Na+ Cl− H2O
K+
K+?
Na+ H2O Cl−
Cl− Na+ H2O

meal) to maintain internal water balance. They do this by sorbing water from unsaturated air thereby resolving the potential problem of dessication (Knulle, 1966; Lees, 1946; Hafez et al., 1970; Sauer and Hair, 1971; Rudolph and Knulle, 1974; McMullen et al., 1976). It appears that a secretion of the salivary glands might be intimately involved in this ability (Rudolph and Knulle, 1974, 1978; McMullen et al., 1976).

Gregson (1973) in describing the salivary glands of the ixodid tick Dermacentor andersoni likened them to a cluster of grapes extending about two-thirds the length of the tick. The glands are paired, located in the anteriolateral portion of the body cavity and consist of a lobular mass of alveoli (acini) connected directly or indirectly to the main salivary duct or its secondary branches by short alveolar ducts. The paired main salivary ducts extend through the capitulum to open into the salivarium, a blind chamber dorsal to the pharynx and leading to the buccal cavity. Most recent investigators believe the glands in ixodid females to be composed of three types of alveoli (I, II, III). A cell first labelled "water cell" in the Type III and possibly Type II alveoli were thought to secrete the bulk of the fluid in engorging females (Meredith and Kaufman, 1973; Binnington, 1978; Diehl, personal communication; Megaw and Beadle, 1979). Fawcett et al. (1981a,b) have recently discarded use of "water" cell, stating that no histological data exist to justify calling it a "water" cell as Meredith

and Kaufman (1973) had done and has shown that this alveolar area in feeding females is in fact derived from 2 separate cell types, enlarging abluminal interstitial cells (AbI) and "f" granular cells in Type III alveoli.

The Type I alveoli are confined to the anterior third of the gland. In Amblyomma americanum, Type I alveoli have been seen attached only to the main duct of the gland. Megaw and Beadle (1979) state that in Boophilus microplus Type I alveoli contain pyramidal cells with extensive infoldings of the basal membrane. The Type II alveoli contain three different granule secretory cells "a", "b" and "c". Two types of granular cells "d" and "e" are found in the Type III alveoli according to Megaw and Beadle (1979). During feeding there is great change in Type II and Type III alveoli. Granules in the "a", "d" and "e" cells are secreted during the early stages of feeding at a time corresponding to cement secretion. The "b" and "c" cells were observed to be active throughout feeding. As the "a", "d" and "e" cells regress following secretion of their products, adjacent epithelial cells which appeared non-functional in the unfed ticks enlarged and developed mitochondria and membranes. This area appeared similar to the "water cells" described by Meredith and Kaufman (1973) and is likely the menbranous labyrinth studied by Fawcett et al. (1981a,b). The membranous labyrinth is most obvious in the Type III alveoli and similar but less pronounced changes occur in Type II alveoli (Meredith and Kaufman, 1973; Fawcett et al.,

1981a,b). Fluid secretion, which occurs during feeding, was thought by some to be a consequence of the development of these cells and this is reflected by the progressive increase in the secretory capability of *in vitro* glands, Kaufman (1976). Diehl (personal communication) thought he saw similar phenomenon in *Amblyomma hebreum*. In the unfed females, epithelial cells (abluminal interstitial cells (AbI) according to Fawcett et al., 1981a,b) were small with no apparent invaginations and few mitochondria. The cells increase in size during the first 2-3 days of feeding and later develop a tremendous membranous labyrinth with associated mitochondria which parallels the development and secretory ability of the glands. In an agrasid tick *Ornithodoros monbata*, Diehl found that AbI cells were present, but observed little development of the cells which correspond to low salivary fluid secretion observed in argasid ticks. In addition, the AbI cells in feeding males of the ixodid ticks *A. hebreum* are poorly developed. This also corresponds with the lower secretory abilities seen in male salivary glands.

There appears to be general speculative agreement among most investigators concerning the function and morphology of the Type I alveolus. For example, Megaw (personal communication) and others believe the pyramidal cells of the Type I alveoli may secrete a hygroscopic substance capable of sorbing water vapor from atmospheres with relative humidities as low as 85% (Rudolph and Knulle, 1974, 1978; McMullen

et al., 1976). Investigators (Binnington, 1978; Megaw, 1976) differ however, in their interpretations of the cellular morphology of the Type II and Type III alveoli even in the same species, B. microplus. Megaw as studied earlier described two "a" cells which are adjacent to the valve and found only three granular cell types (a, b, c) in the Type II alveolus. The Type II alveolus according to Binnington (1971) contains 14-15 granular cells of 6 types, "a", "b", "c_{1-4}", and interstitial epithelial cells all of which are surrounded by a central lumen which opens via a cuticular valve into a lobular duct. Binnington (1978) descriped 16-17 cells of three types, "d", "e", "f" in Type III alveoli. In common with cell "a" of the Type II alveolus, cell "d" is adjacent to the valvular duct and contains granular subunits, granules staining deeply with acid dyes, presumably due to a high content of basic protein (Binnington, 1978). Adjacent to cell "d" is a cell (type "e") containing larger granules which stain less intensely with acid dyes. As with "a" cells, "d" and "e" cells are packed with granules in the unfed adult and lose most of their granules after 72 hours of feeding (Binnington, 1978). Interstitial epithelial cells are more apparent in these alveoli than in glands of younger female ticks according to Binnington (1978). Their cytoplasm is divided by a membrane into two zones, apical and basal, the latter extending over a high proportion of the basal surface of the alveolus (Binnington, 1978). Although Meredith and Kaufman (1973)

and Megaw (1976) considered the apical zone to be a separate cell type in D. andersoni, and B. microplus respectively, no nucleus was seen in this cell as viewed in thick sections by Binnington, (1978). Binnington's (1978) histochemical studies have served to define possible functions of the alveolar cells, in particular cells, "a", "d" and "c" which are candidates for the secretion of cement precursors.

Binnington (1978) suggested that since the ticks that Meredith and Kaufman (1973) studied had fed for 6 days, their vacuolar cells may be depleted granular cells. Binnington (1978) agreed with Meredith and Kaufman's (1973) suggestion that the "water cells" in D. andersoni, or the equivalent epithelial cells in B. microplus (Binnington, 1978), or the membranous labyrinth descirbed by Fawcett et al. (1980a,b) excrete the bulk of fluid during engorgement.

Control of Fluid Secretion and Intracellular Second Messengers

Neural rather than hormonal factors are believed to control salivary fluid secretion (Kaufman and Phillips, 1973a). This hypothesis is supported by evidence which indicates that catecholamines rather than "factors" in tick haemolymph stimulate chloride uptake and fluid secretion by isolated salivary glands (Kaufman and Phillips, 1973b; Sauer et al., 1974; Kaufman 1976). This was further substantiated with the discovery of a nerve close to the basal surface of the

Group III acinus in Dermacentor andersoni (Meredith and Kaufman, 1973). Binnington (1978), in studying Boophilus microplus, reports the presence of two major innervations of the salivary gland, the papal nerve and the pedal nerve located distally and proximally, respectively. Axons have also been observed in close association with agranular and granular acini in Amblyomma americanum, Dermacentor andersoni and Argas arboreus (Coons and Roshdy, 1973; Roshdy and Coons, 1974). Nerves have been observed to connect the synganglion (brain) with individual alveoli of the salivary glands (Obenchain and Oliver, 1976). The discovery of noradrenaline and dopamine in high concentrations in both the synganglion and salivary glands further suggested nervous control of the glands. The neurotransmitter is likely a catecholamine or catecholamine-like substance (Megaw and Robertson, 1974). Recent experiments have further suggested cholinergic nerve synapse involvement in glanular control at sites distant from the salivary glands themselves (Kaufman, 1976; Teel et al., 1978), which likely explains why parasympathomimetic drugs (Howell, 1966; Tatchell, 1967; Purnell et al., 1969; Barker et al., 1973) and catecholamines (Kaufman and Phillips, 1973b; Hsu and Sauer, 1975), both are able to stimulate salivary secretion when injected into detached and partially engorged ticks.

Kaufman (1977) found that salivary glands (in vitro) from Dermacentor andersoni are sensitive to analogues of phenylethylamine in the following descending order

of potency: dopamine > adrenaline = noradrenaline > isoproterenol = phenylephrine > norphenylephrine > phenylethylamine > tyramine > DOPA > octopamine.

Tick salivary gland control is apparently multifaceted. There has been considerable research devoted to ascertain the nature of the primary stimulus. Uncertainty as to the factor(s) which initiates the secretion process and maintenance of ion and water balance during feeding still persists, however. In Rhodnius prolixus (L.) stretch receptors in the abdominal wall are sensitive to distention. When a bloodmeal is taken these receptors stimulate neurosecretory cells in the fused mesothoracic mass to release diuretic hormone from nearby neurohormonal organs (Maddrell, 1966). Diuretic hormone causes the Malpighian tubules to initiate diuresis (Maddrell, 1964a). Previous investigations in our lab have focused on control of the salivary glands, the primary stimulus and resultant increase in fluid secretion. It is quite possible that many events are transpiring subsequent to the primary stimulus and prior to fluid secretion. Although pharmacological characteristics have been ascertained, only limited data is available concerning intracellular events which are responsible for salivation.

Recent investigations have demonstrated that catecholamines such as dopamine increase gland cyclic AMP (cAMP) (Sauer et al., 1979) presumably by stimulating an adenylate cyclase that converts ATP to cAMP. Stimulation of fluid secretion by glands in vitro with low concentrations of

dopamine are enhanced by the addition of theophylline (Sauer et al., 1979). Theophylline is a known inhibitor of phosphodiesterase (PDE) which catabolizes cAMP to 5' AMP. Exogenous cAMP and theophylline cause glands to secrete (Sauer et al., 1976) while bathed in TC 199/MOPS support medium (Sauer et al., 1979). It was shown that theophylline had the capability for allowing continued secretion if the gland had been previously exposed to a catecholamine (Needham and Sauer, 1975). Needham and Sauer (1979) believe that the above results demonstrated that a primary catecholaminergic stimulus may induce cAMP formation within the water cells of Type II and Type III alveoli which are involved in some way in controlling fluid secretion. In support of this hypothesis, Schmidt et al. (1981) have found that the concentrations of dopamine required to stimulate fluid secretion half-maximally is the same as that required to stimulate gland adenylate cyclase half-maximally.

Intracellular Localization of Biologically Important Molecules

In 1852, G. G. Stokes demonstrated that fluorescent substances actually generated new light internally while being irradiated from an outside source and that the color of the emitted light did not need to be contained in the incident beam (Goldman, 1961). The fluorescence phenomena involves a large portion of the electromagnetic spectrum. The wavelengths range from about 5×10^{-3} to 8×10^{3} A and

includes substances such as elementary gases to organic compounds (Goldman, 1961). Fluorescence results from the excitation caused by any visible or ultraviolet radiation that is absorbed (Goldman and Carver, 1961). During the time of absorption and emission the excited molecules lose a certain amount of energy due to collision as they return to the ground state by emission of radiation. Emitted photons will have less energy and therefore longer wavelengths, than excited photons. Typically, the emission spectrum will adjoin the long wavelength end of the absorption spectrum, i.e. orange fluorescence will be stimulated by green-yellow light, yellow by blue-green and green by violet-blue (Goldman and Carver, 1961).

General concepts and uses of fluorescent microscopic techniques were developed in the 1950's permitting the localization of biochemicals by ultraviolet absorption and by chemically specific staining reactions. One of these techniques is the use of fluorescent antibodies. An overview of this technique is as follows: Serum proteins are labelled with fluorescent markers (FITC) by means of firm chemical bonds to produce fluorescent solutions in which the biological activity is essentially unaltered. If the application of conjugated serum (FITC bound to an immunoglobulin, IgG) containing antibodies is made on a smear or section of tissue containing homologous antigen, antibody will be precipitated and fixed in place. Unreacted and non-antibody proteins are then washed away and the preparation is

examined under a fluorescence microscope. Location of antibody deposition is made visible by the fluorescence produced against the dark background of non-antigen-containing materials. This procedure is a cytochemical staining method with the idea that antigens are capable of forming insoluble reaction products with antibodies. Basically, fluorescence microscopes are conventional microscopes with color filters added on the far side of the object to provide proper excitation light. There are other matching filters on the near side permitting observation of fluorescence.

The first marker used by Coons and his associates was B-anthracene, introduced into antipneumococcus Type III serum by reacting the latter with B-anthryl isocyanate (Coons et al., 1942). Coons was interested in demonstrating antigens in tissue sections which, however, themselves showed blue fluorescence under ultraviolet illumination. As a result, he began using a green fluorescent dye, fluorescein. He used an isocyanate derivative for coupling purposes (Coons et al., 1942). Coons et al., 1951; Coons and Kaplan, 1950; Hill et al., 1950; Kaplan et al., 1950 describe localization of injected soluble antigens and of injected rickettsial and viral agents in tissues of experimental animals. As a result of these papers, the methodology of the fluorescent antibody technique had been incompletely worked out. Antisera could now be used as a specific cytochemical stain for antigens after subsequent conjugation of fluorescein with serum proteins.

Additionally, these investigators showed that fluorescent antisera are effective for staining soluble as well as particulate antigens. Some of the more basic technical steps, now being routinely used in fluorescent antibody procedures, were worked out; paraffin embedding for some antigens, bright-field illumination, arc light source, ultra-violet filter system, conventional fixation, frozen sections cut in a cryostat and fixed in ethanol, providing specific controls to determine fluorescent specificity and reduction of non-specific staining by treating conjugates with tissue homogenates (Coons et al., 1951; Coons and Kaplan, 1950; Hill et al., 1950; Kaplan et al., 1950).

Due to technical problems inherent in this technique there were few papers written after this time. One of the main problems was that fluorescein isocyanate, the labelling compound, was not commercially available, requiring a long and involved synthesis reaction. Despite this apparent set-back, Marshall (1951) described the localization of a natural protein (adrenocorticotropic hormone) in normal tissue (hog pituitary). This paper is the first experiment showing that fluorescent antisera were specific enough to distinguish differences among native antigens comprising normal tissues, and that so called contaminated antibodies resulting from impure immunizing antigens, could be absorbed out from labelled antiserum without affecting the specific staining capacity of the serum. Marshall (1951) introduced several technical changes such as: 1.) the freeze-drying of

tissues followed by paraffin embedding for the preservation of antigenicity, 2.) the use of a dark-field substage condenser so as to improve the fluorescence image, 3.) and fixation of sections in methanol.

Mellors et al. (1955) explained a more involved technique which allowed in vivo localization of injected antibodies to native antigens (Mellors et al., 1955a; Mellors et al., 1955b). An example of an in vivo technique is the use of unlabelled antibody (Ab) against rat kidney antigen (Ag_1) prepared in rabbits. Antibody (Ab_2) against rabbit serum (Ag_2) is prepared in chickens and labelled with fluorescein. Unlabelled rabbit antiserum (Ab_1) (without fluorescein) was inoculated into rats which were killed after suitable incubation. Sections of rat kidney were then exposed to labelled antirabbit (Ab_2) obtained from the chickens. Thus rabbit serum has a dual function, first as an antibody and then as an antigen for producing antirabbit (Ab_2) which is developed in chickens. This modification (Mellors, Arias-Stella, Siegel and Pressman, 1955a; Mellors, Siegel and Pressman, 1955b), permitted in vivo studies of antigen-antibody reactions and applications to certain diseases which had previously only been studied employing the in vitro technique.

The original cyclic nucleotide immunohistochemical studies were performed on cryostat sections of rat salivary gland (Wedner et al., 1972). Steiner et al. (1976), showed a marked increase in localized fluorescence in rats

injected with isoproterenol in vivo. This increase in fluorescence (related to an increase in cAMP) could be localized from the basal portion of the acinar cells to the basket cells directly beneath them.

Fluorescent Antibody Tissue Sectioning Procedures

The histochemical procedure is performed on 8 μ thick cryostat sections of unfixed frozen tissue. The tissue is first embedded in O. C. T.^{R} (optimal cutting-temperature compound) and frozen via liquid CO_2 which is under pressure. Steiner et al. (1976), mentions various ways to freeze tissue. They suggest acetone and dry ice. Bloom et al. (1973), however, prefer freeze-dried sections because of excellent histological detail.

After the embedded tissue (salivary gland) has been frozen and the sections mounted on 20 x 20 mm cover slips, sections must be designated either experimentals or controls. If designated controls, it must then be decided whether the section will be an FITC-Ab control, in which case it will only be treated with FITC or whether it will be an Anti-cAMP control in which case it will only be treated with Anti-cAMP. This technique is designated the "Indirect" or "Sandwich" method.

Theory of Indirect Method

The substance actually rendered fluorescent is not the antigen under consideration, as in the "direct" method, but rather an intermediate material. This method was first described in detail by Weller and Coons 1954. This indirect technique takes advantage of the fact that antibody molecules ($Antibody_1$) made to a particular antigen (Ag) are themselves capable of serving as antigens ($Antibody_1$) and later used in the production of another antibody (Ab_2) but this time produced in another animal. This antiglobulin (Ab_2) is then labelled with fluorescein. Thus, antiserum that has been prepared in a goat against rabbit globulin and then labelled with fluorescein, will react with rabbit antibody even when the latter is combined in an antigen-antibody complex, such as is the case with the cells of the alveoli of the salivary glands. By this means, antigen may be rendered fluorescent even though the primary antiserum is itself not labelled.

CHAPTER II

ALVEOLAR STRUCTURE OF THE SALIVARY GLANDS OF THE LONESTAR TICK *AMBLYOMMA AMERICANUM* (L.): UNFED FEMALES

Introduction

In recent years, several investigations have been concerned with the morphology of ixodid tick salivary glands (Till, 1961; Chinery, 1965; Balashov, 1965, 1968; Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973; Binnington, 1978; Megaw and Beadly, 1979). The results of these studies indicate that the salivary glands of unfed female ixodid ticks consist of a pair of branched grape-like clusters of alveoli running about two-thirds the length of the tick. The paired main ducts extend through the capitulum and open into the salivarium, a blind chamber dorsal to the pharynx and leading to the buccal cavity. The glands are composed of three types of alveoli: two granular and one agranular. The agranular alveoli (Type I) are located in the anterior region of each gland directly attached to the main salivary duct and are without bicuspid valves. Prominent cellular features include pyramidal cells

with numerous basal membrane infoldings and closely associated mitochondria (Coons and Roshdy, 1973). Rudolph and Knulle (1974, 1978) have suggested that these alveoli may secrete hygroscopic material that is important in enabling the tick to absorb water from unsaturated air.

Various functions including the secretion of biologically active agents, toxins, anticoagulants, and cement to anchor the mouthparts to the host are some probable functions of these alveoli (Sauer, 1977). It has been proposed, depending on the investigator or methods employed, that a variety of granular cell types are present (Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973; Binnington, 1978). Despite extensive investigation there is considerable disagreement on numbers, types and detailed structure of cells, particularly granular cells and the existence of cells such as the cap cells, more recently named adluminal interstitial cell (AdI) (Fawcett et al., 1981a,b). Thus I have examined in detail the ultrastructure of the glands of female Amblyomma americanum (L.). The present study describes the structure of glands from unfed females.

Methods and Materials

Salivary glands from female Amblyomma americanum (L.), unfed (postnymphal ticks which had not yet taken a bloodmeal as an adult) adults, were dissected and fixed in 2% cacodylate buffered glutaraldehyde for 2 h (pH 7.4) at room temperature before being withdrawn with a transfer

pipette. The salivary glands were then rinsed for 1 h in cacodylate buffer (pH 7.4) at room temperature; post-fixed in 2% osmium tetroxide, rinsed in deionized water for 1 1/2 hours and stained en bloc with 0.5% uranyl acetate at room temperature overnight. Tissue was then rinsed in deionized water and dehydrated in a graded series of ethyl alcohol before embedding in Polybed ^{R} from Polysciences (Warrington, Pa.). Initially, 1 μm thick sections were obtained from the polymerized blocks using a Sorvall MT-2 ultramicrotome and stained with Mallory's trichrome for light microscopy. Sections were observed and photographed using a Zeiss photomicroscope with Panatomic X film (Kodak). Alveoli for electron microscopy were selected after viewing these sections. Thin semi-serial sections (70-90 nm) were obtained with a Sorvall MT-2 ultramicrotome using diamond knives. Sections were maintained in order of section but were not placed on slot grids. Therefore, only every third or fourth section was examined and photographed. Sections prepared for electron microscopy were stained with methanolic uranyl acetate and lead citrate (Venable and Coggeshall, 1965), observed and photographed with a Philips EM200 electron microscope.

Results

The determination of the number of alveolar types in the salivary glands of A. americanum was made after careful examination by light microscopy of numerous thick sections stained with Mallory's trichrome and examination of

TEM thin sections stained with uranyl acetate and lead citrate. A distinct alveolar type (I) (diameter of approximately 50 μm) was found directly attached to the anterior to middle portion of the main duct. Another (Type II) was posterior to the Type I alveoli, had a diameter of approximately 50-70 μm, extended the length of the gland and was attached to secondary branches of the gland. A third type (Type III) was attached to the most peripheral and posterior secondary branches of the gland and had a diameter of approximately 60-90 μm.

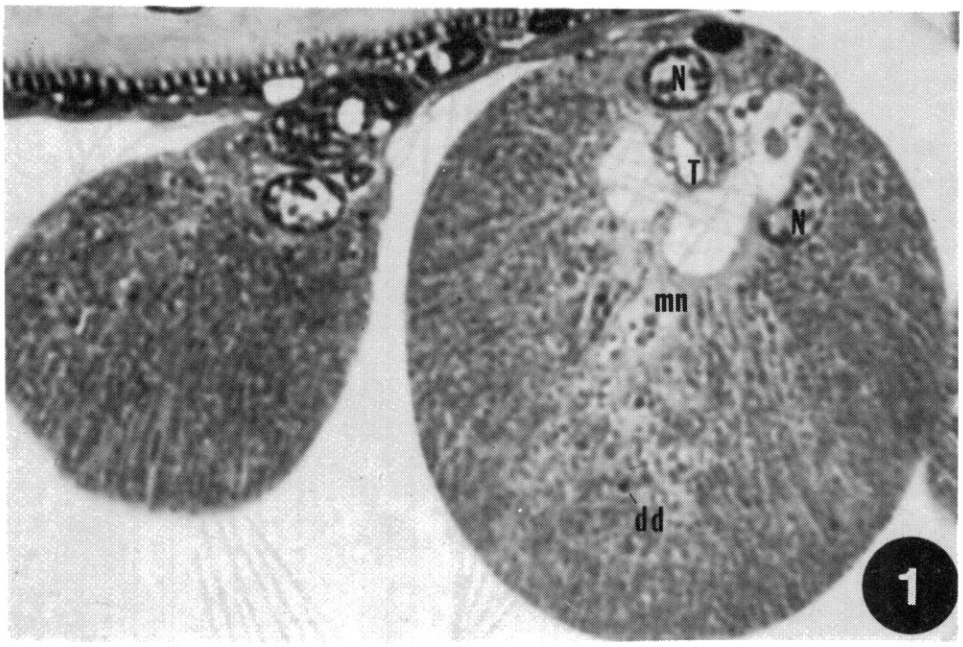
Light Microscopy

Type I Alveolus. At the light microscopic level, the agranular alveolus, appeared to possess, peripherally, a group of cells with a complex membranous network with large nuclei near the apical surfaces (Fig. 1). Typically, we saw 2 to 3 nuclei in a Mallory trichrome stained "thick" section (Fig. 1). Widely distributed in these cells were dark droplets of variable size that stained with Mallory's trichrome and Oil Red O, suggesting the presence of lipids.

The cell in the center of the alveolus failed to react thoroughly with Mallory's trichrome with the exception of the cell's nucleus and densely stained, small cellular particles. The pale centrally oriented large cell appeared to be juxtaposed to the apical side of each membranous cell and closely resembled a similar cell described by Meredith and Kaufman (1973) as an "inner" cell and by Megaw and Beadle

Figure 1. Light microscopic photograph of a Type I alveolus. The basal protion of this alveolus is characterized by a complex membranous network, (mn). Distributed throughout this membraneous network are numerous dark droplets, (dd). At the apical end of this membranous network 2 nuclei (N) can be seen. T- tubule. 1300x

Figure 2. Light microscopic photographs of a Type II alveolus. This alveolar type is composed of heavily granulated cells (DG) as well as and other granular cells (G) which are found distal to the valve. With the exception of nuclei (N) no apparent organelles were seen in the paired dense granular cells (DG). Between these paired dense granular cells is the lumen (L) of the alveolus and the arms of the valve (va) through which alveolar contents pass to the duct. A single nucleus (N) located anterior to the valve and adjacent to the dense granular cells can be seen within the clear cell (C). 1300x.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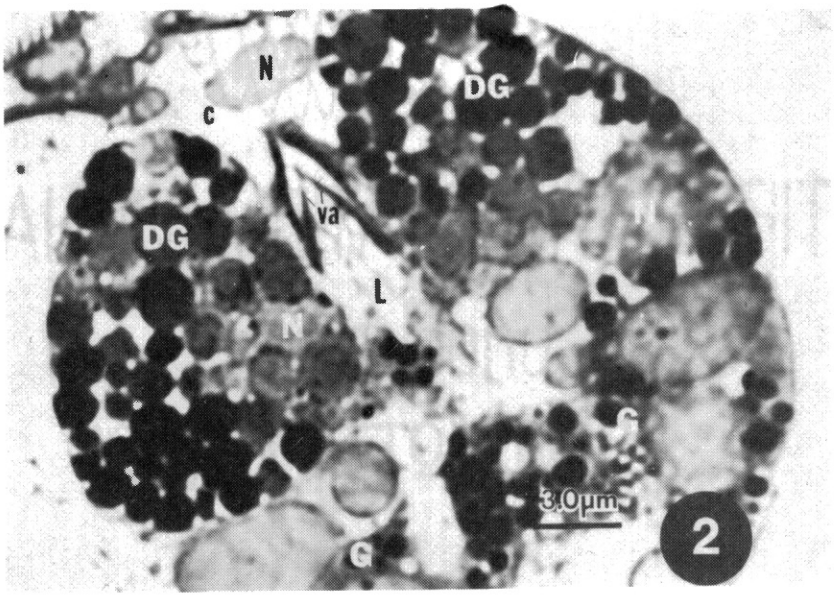
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DG
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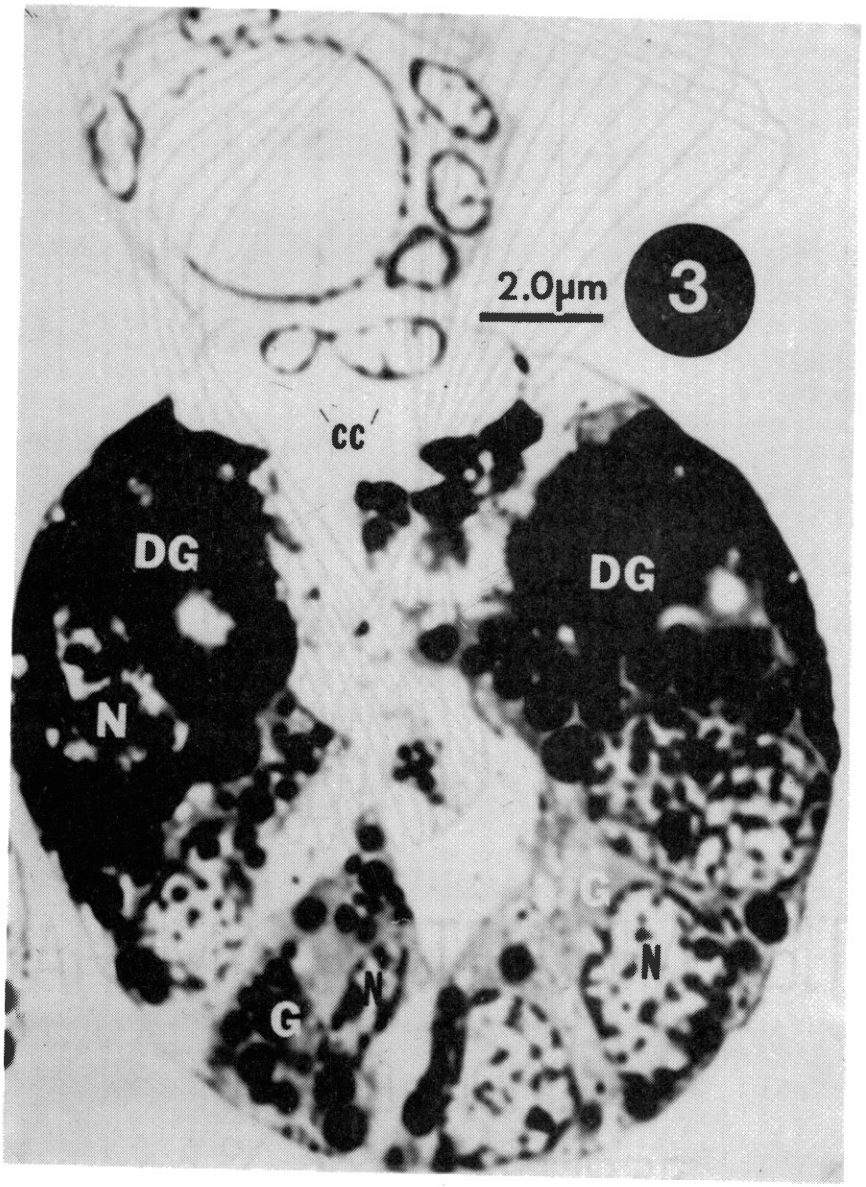
2

(1979) as a "central" cell. We refer to it as the "central" cell in A. americanum.

Type II Alveolus. This alveolar type is composed of heavily granulated cells (Fig. 2). Two cells resembling each other and present on either side of the valve contained dense granules. No additional organelles, except the nucleus were visible in the trichrome-stained thick section. A "clear" cell with a large nucleus was seen anterior to the paired dense granular cells and appeared to envelope the alveolar duct. The duct has two slender valvular arms directed towards the basal portion of the alveolus (Fig. 2). Closely associated with the valvular arms was a faintly visible membrane enveloping an agranular cell along the outer perimeter of the lumen and preventing the dense granular cells from making direct contact with the lumen (Fig. 2). This cell did not appear to be continuous around the lumen, however, adjacent sections revealed a second clear agranular cell nucleus at this same location but in a different geometric plane (Fig. 3). Five other granular cells were seen with smaller, less darkly stained granules and rather large nuclei (Fig. 3). Other nuclei could be seen near the lumen and adjacent to granular cells (Fig. 2).

Type III Alveolus. There were fewer granular cells in Type III alveoli than were seen in Type II alveoli. Figure 4 is a section of a Type III alveolus illustrating a densely granulated cell adjacent to the valve. However,

Figure 3. Light microscopic photograph of a Type II alveolus. This section reveals a second clear cell (cc) and its associated nucleus. Five other granular cells (G) each with a nucleus (N) are present posterior to the previously described dense granular cells (DS). 1000x.


2.0µm
3
CC
DG
DG
N
N
G
G

seen in Type II alveoli. Instead a less darkly stained cell is seen on the opposite side of the valve in Type III alveoli. The faintly visible membranous-bound agranular cell seen lining the alveolar lumen in Type II alveoli was also faintly visible in Type III alveoli. As in Type II alveoli, Type III alveoli had two clear agranular cells in close proximity to the duct-valve region with fairly large nuclei. In addition, and anterior to these cells, were two clear agranular cells next to the duct (Fig. 4). The remainder of the granular cells contain fewer granules. Adjacent to and between the granular cells were thin agranular cells extending from the basal lamina to an agranular cell lining the lumen. These kinds of cells were also visible in Type II alveoli.

Electron Microscopy

Type I Alveolus. At the ultrastructural level, at least four distinct cell types could be seen and are named: pyramidal, central, peritubular and constrictor (Fig. 5). The most conspicuous cell was located on the basal surface of the alveolus and was characterized by the presence of an elaborate labyrinth of membranes with numerous basal infoldings (Fig. 6) and closely associated mitochrondria. These are likely the complex membranous cells seen with light microscopy (compare to Fig. 1). Non-membrane bound material resembling lipid-like droplets were quite abundant and were dispersed throughout the cell (Fig. 7). Similar cells found

Figure 4. Light microscopic photograph of a Type III alveolus illustrating one densely granulated cell (DG) adjacent to the valve (v). On the opposite side of the valve from the densely granulated cell (DG) is another type of granular cell (G). Four additional cells (G) with fewer granules however are also present. Adjacent to and between these granular cells (G) is a thin agranular cell (tgc) that appears to extend from the lumen (L) to the basal lamina. Anterior and juxtaposed to the valve (v) are four clear agranular cells (cc) with their respective nuclei. N- nucleus. 1000x.

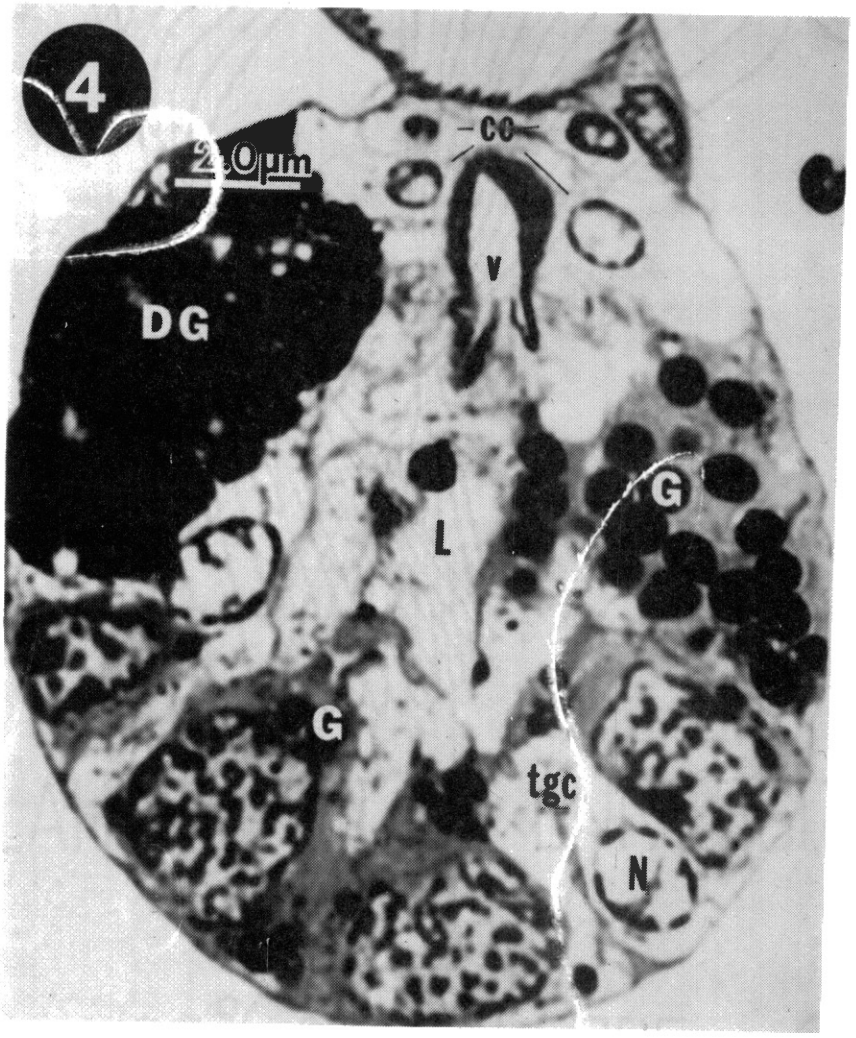

4
20μm
cc
v
DG
L
G
G
tgc
N

Figure 5. Diagram of section from the Type I alveolus showing the various cells and their structures.

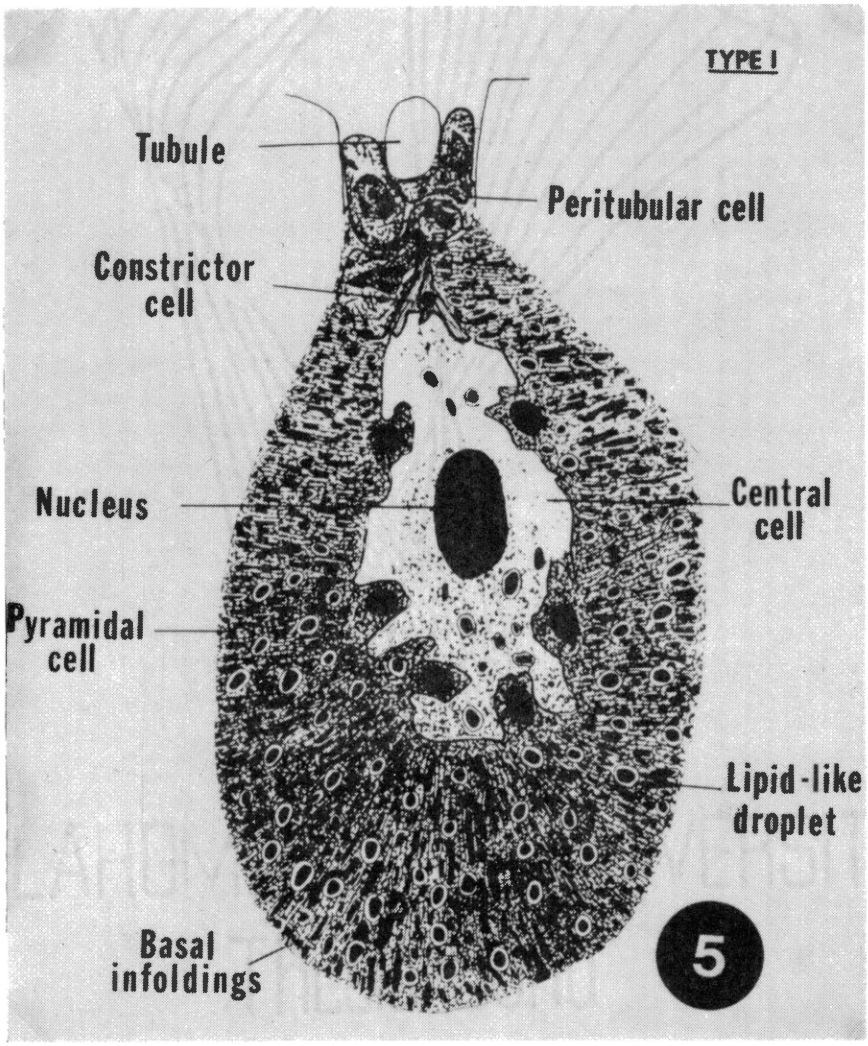
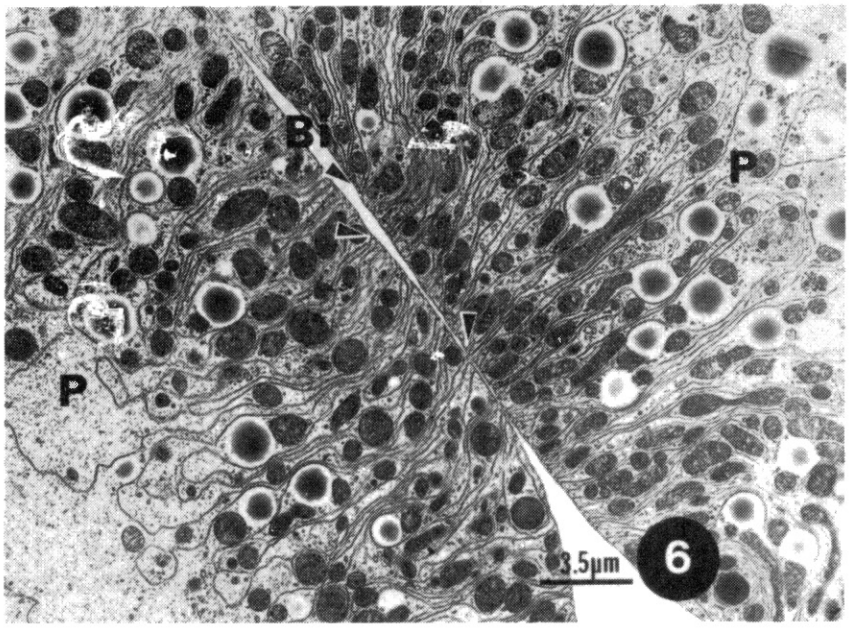
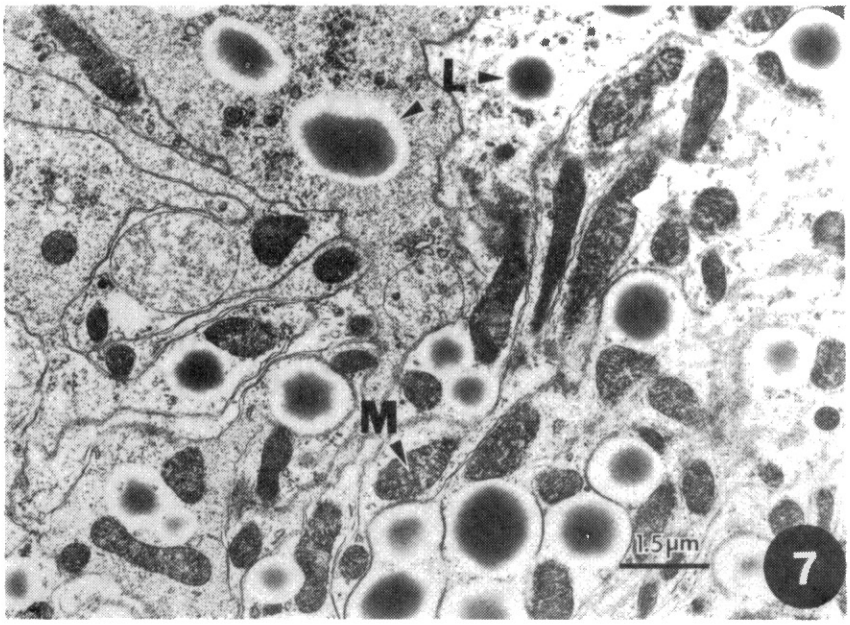

TYPE I
Tubule
Peritubular cell
Constrictor cell
Nucleus
Central cell
Pyramidal cell
Lipid-like droplet
Basal infoldings
5

Figure 6. Electron micrograph of two Type I alveoli opposed to one another illustrate the numerous basal infoldings (Bi) as indicated by arrows. These structures are on the basal portion of the pyramidal cells (P). 3800x.

Figure 7. Electron micrograph revealing the numerous intracellular lipid-like droplets (L) and mitochondria within the pyramidal cell. 9000x.


B
P
P
3.5µm
6

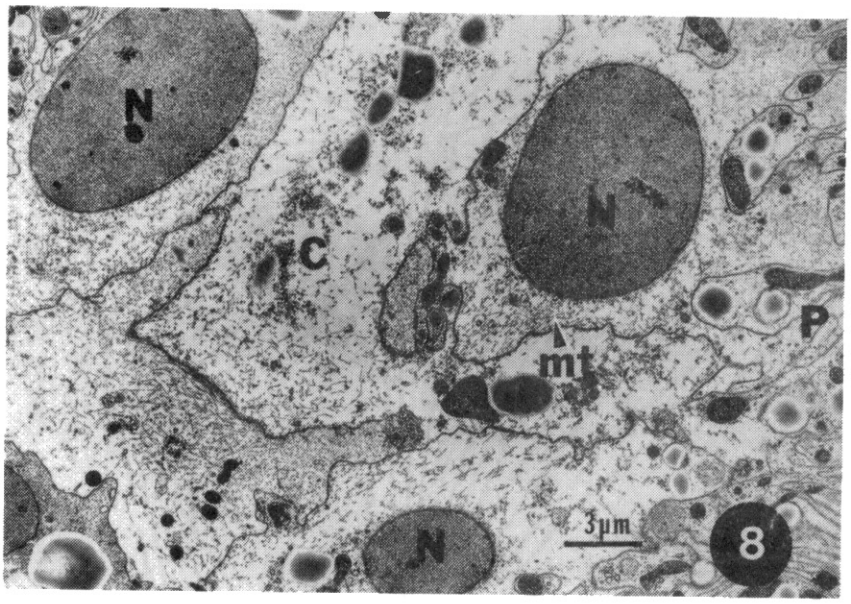

L
M
1.5µm
7

in Type I alveoli of other species of ixodid ticks have been only one dense granular cell was present in contrast to that called pyramidal cells by Megaw and Beadle, 1979; and Binnington, 1978. We shall refer to them as pyramidal cells in Amblyomma americanum as well. The nuclei of these cells were found on the apical side of the cell (Fig. 8). The apical end of the pyramidal cells, where the nucleus was found, lacked membranous organelles, but contained many microtubules and free ribosomes (Fig. 8). An occasional mitochondrion or lipid-like droplet was present, however. There appeared to betheir relatively small number of cellular organelles. We believe these cells should be called "peritubular" cells. An occasional mitochondrion was found in the cytoplasm of these cells. The chromatin pattern within the relatively large cell was similar to that seen in nuclei of other cells of this alveolar type (Fig. 10).

Microfilamentous structures were abundant in another cell type found cutting-off the "central" cell from the lumen of the tubule. Henceforth, we refer to this as the constrictor cell (Fig. 11a). Various organelles such as mitochrondria, microfilaments and nucleus, can be found in this cell (Fig. 11b). Upon closer examination of additional sections, one observes an invagination through the middle of the constrictor cell protruding into the central cell and in close association with both the constrictor and central cells (Fig. 11c). Numerous microtubular structures were within its borders. Nexus junctions were located on either

Figure 8. Electron micrograph showing the apical region of the pyramidal cell (P) with a nucleus (N), and microtubules (mt). The apical region of the prymaidal cells border the central cell (C). 3610x.

Figure 9. Electron micrograph of the Type I's central cell (C) with nucleus (N), and lipid-like droplet (L) and mitochondrion (m). The cytoplasm is electronlucent. 4560x.


N
N
C
P
mt
N
3µm
8

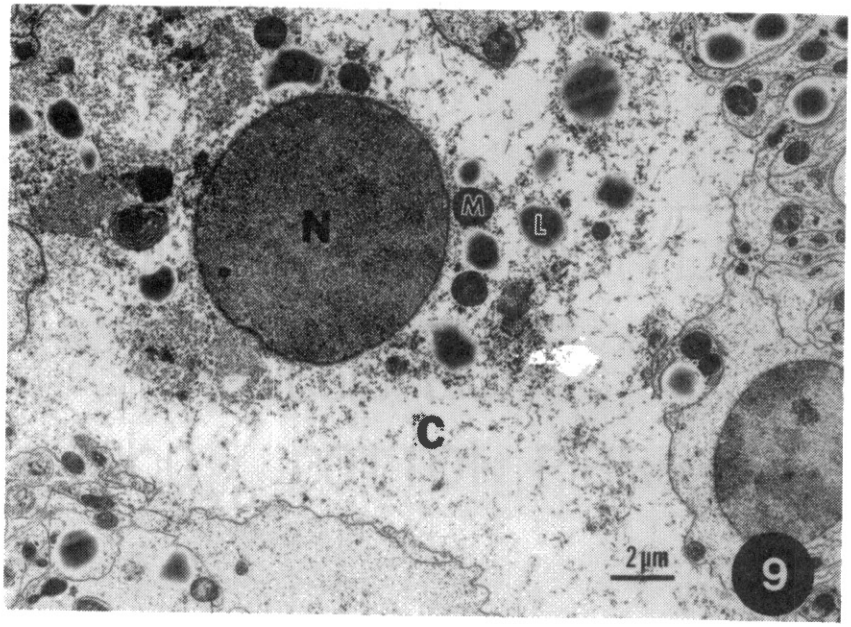
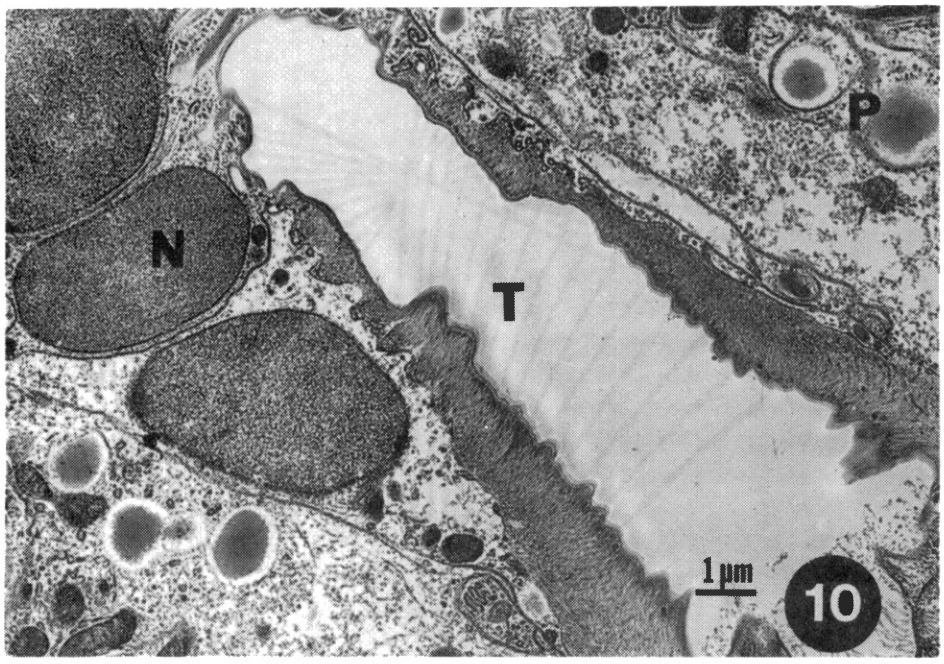

N
M
L
C
2µm
9

Figure 10. An electron micrograph showing the tubular lumen of the Type I alveolus, three small lightly stained peritubular cells (Pt) and their nuclei surrounding the tubule and adjacent to the pyramidal cells (P). 7800x.

Figure 11a. An electron micrograph of the "constrictor cell (Cc) with numerous filaments and positioning between the Type I tubule (T) and the central cell (C). The pyramidal cell (P) is adjacent to the constrictor cell. 11800x.


N
T
P
1µm
10

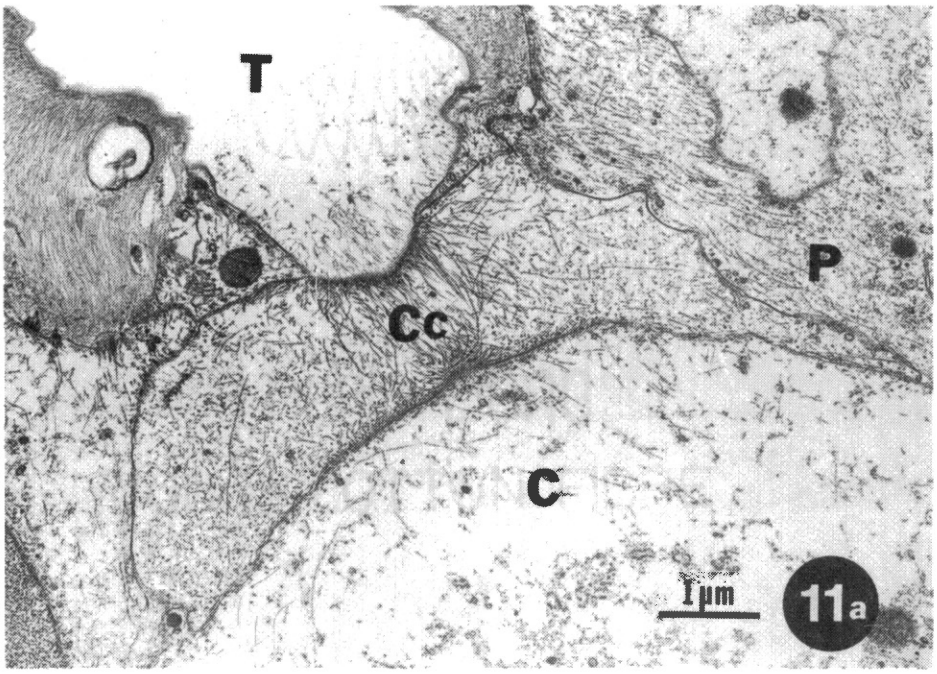
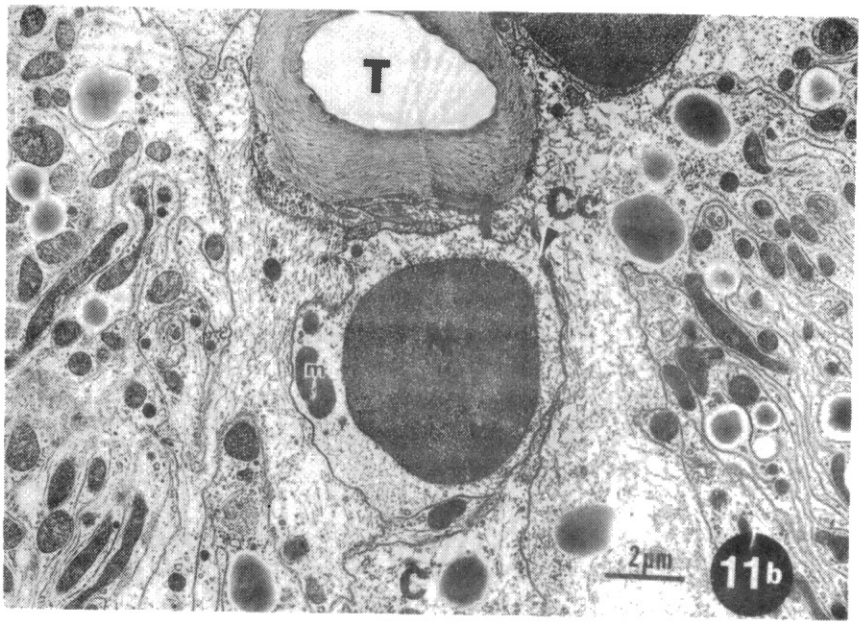
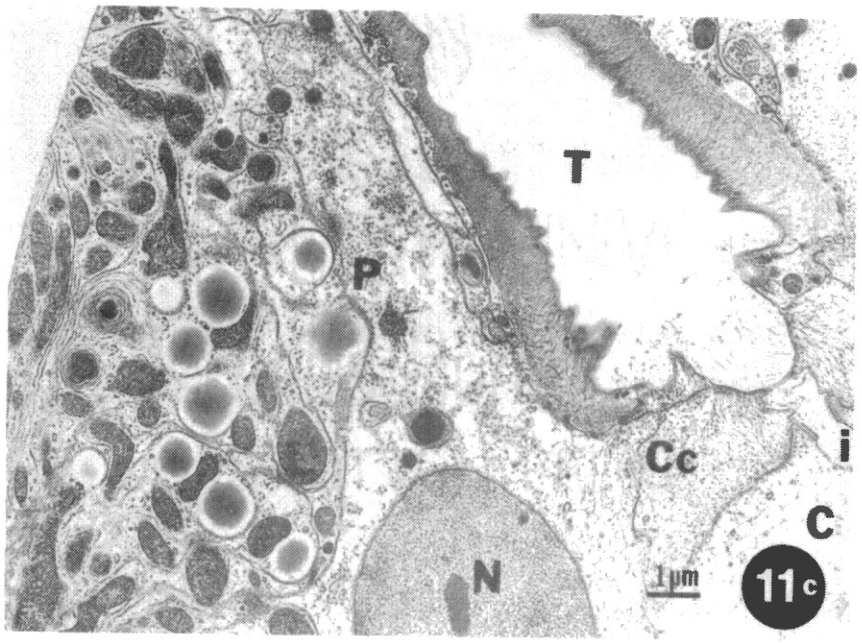

T
P
Cc
C
1µm
11a

Figure 11b. Electron micrograph of a section of the Type I alveolus showing a nucleus (N) and mitochondrion (m) associated with the constrictor cell (Cc) as it interrupts contact between the Tubule (T) and Central cell (C). 5700x.

Figure 11c. Electron micrograph which reveals an invagination (i) through the constrictor cell (Cc) which allows for communication between the tubule (T) lumen and the central cell (C). Nucleus-(N) pyramidal cell-(P). 7800x.


T
Cc
m
C
2µm
11b

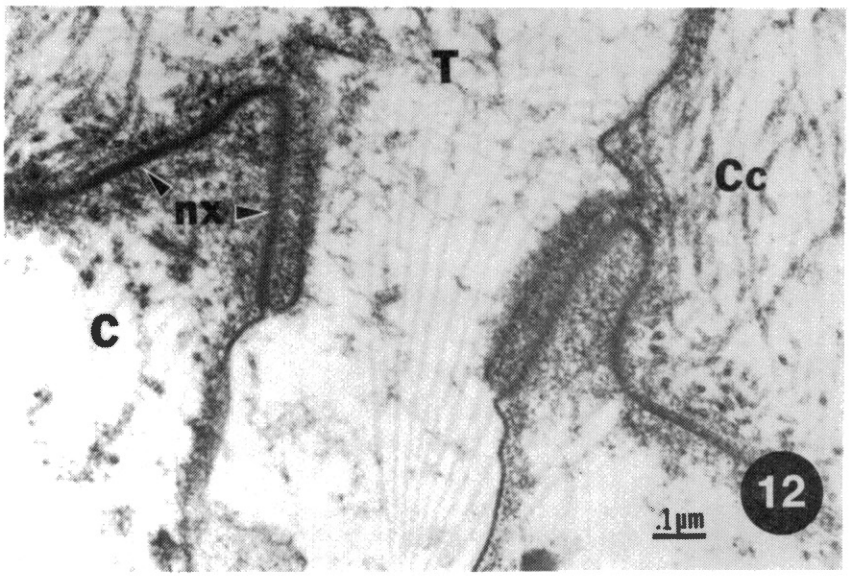

T
P
Cc
i
C
N
1µm
11c

side of the invagination as it is found in close association with the central cell (Fig. 12).

Type II Alveolus. Figure 13 summarizes diagrammatically the morphology of the Type II alveolus. By studying semi-serial sections, we observed a pair of complex granular cells, i.e. cells which exhibit a very tight, non-uniform, compact, membrane-bound arrangement of granular subunits within the cell. These complex granular cells were located near the neck region of the alveolus in the vicinity of the valve. These were likely the heavily granulated cells seen with light microscopy (compare to Fig. 4). It appeared that the cells extended from near the lumen to the basal lamina of the alveolus (Fig. 14). Cellular organelles such as mitochrondria, endoplasmic reticulum and Golgi were not seen but large nuclei were present (Fig. 15). A second, granular cell type found was characterized by the presence of darkly stained, simple granules, i.e. without subunits (Fig. 16). An abundance of rough endoplasmic reticulum was present in addition to some mitochondria and a large, well-stained nucleus. Cell boundaries for all granular cells were obvious not only for their distinct cell membranes, but also for their accentuation and separation by clear, non-granular, thin membranous epithelial cells. These cells extended from the basal lamina toward the lumen where they contacted another type of agranular cell (Fig. 16). The latter cell is analogous to the cap cell (Meredith and Kaufman,

Figure 12. An electron micrograph showing the invagination (seen in Figure 11c) passing through the constrictor cell (Cc) and its relation to the tubule (T). Nexus junctions (nx) are seen making contact with the central cell (C). 79,300x.

Figure 13. A diagram of a Type II alveolus based on a compilation of semi-serial sections.


T
nx
Cc
c
.1µm
12

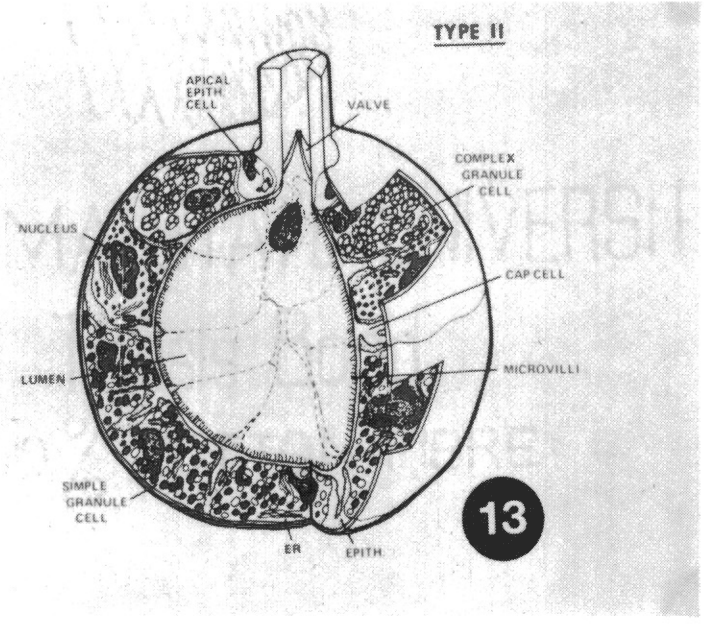
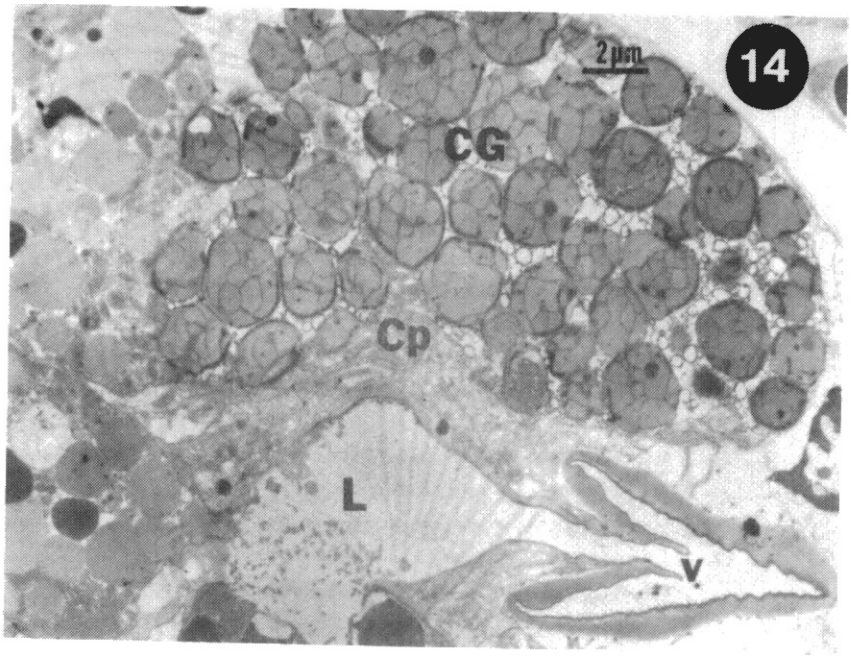
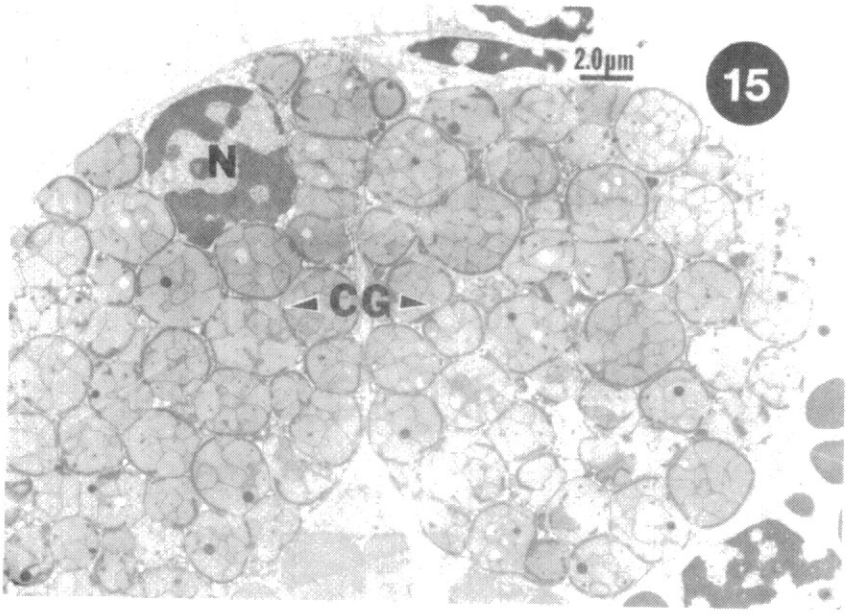

TYPE II
APICAL EPITH CELL
VALVE
COMPLEX GRANULE CELL
NUCLEUS
CAP CELL
LUMEN
MICROVILLI
SIMPLE GRANULE CELL
ER
EPITH.
13

Figure 14. An electron micrograph of a Type II alveolus showing the extent of the complex granular cell (CG) with closely accociated cap cell (Cp) and close association of the cap cell (Cp) with the arms of the valve (v). L=lumen. 4750x.

Figure 15. Characteristic section of a Type II alveolus showing complex granular cells (CG), (i.e. granules with subunits). Few organelles with the exception of the nucleus (N) are seen. 3900x.


2 µm
14
CG
Cp
L
V


2.0µm
15
N
CG

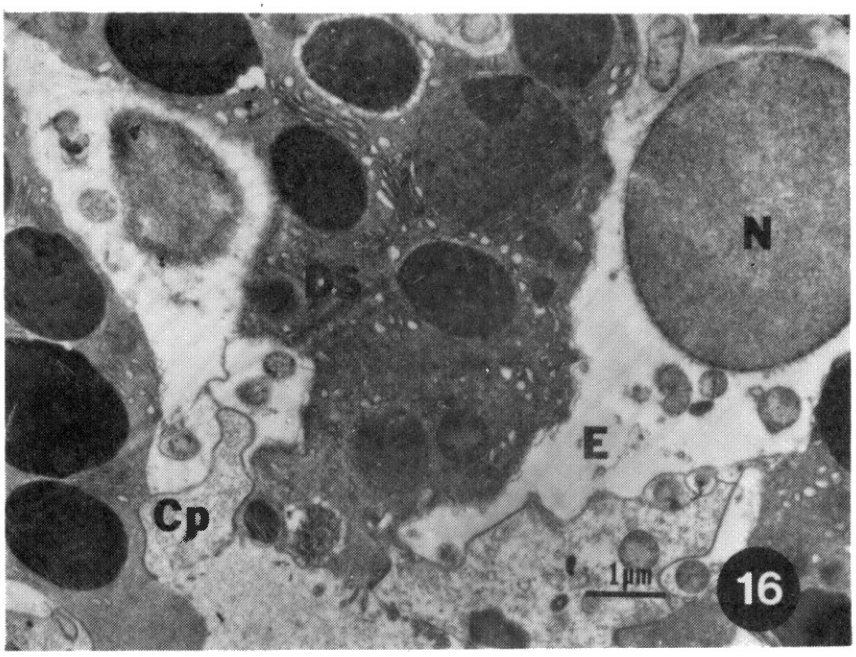
1973; Megaw and Beadle, 1979) or adluminal interstitial cell (AdI) (Fawcett et al., 1981a,b) described by others. We will refer to it as the cap (AdI) cell. The cap (AdI) cell is likely the faintly visible agranular cell seen along the perimeter of the alveolar lumen at the light microscopic level (see Fig. 2).

Additional epithelial cells (3 to 4 in number) with large nuclei were located anterior to the complex granular cells and adjacent to the valve (Fig. 17) that were also faintly visible at the light microscopic level (see Fig. 2). A fourth cell type was seen that contained lightly stained granules with an occasional darkly stained granule (Fig. 18). Mitochondria as well as endoplasmic reticulum were seen interspersed between granules. Three lightly stained granular cells were adjacent to one another and separated by an epithelial cell and an extension of the cap (AdI) cell as seen in Fig. 18. The presence of two complex granular cells characteristic of the Type II alveolus and a darkly stained simple granular cell can also be seen in Figure 18.

The cap (AdI) cell was myoepithelial-like because of very conspicuous and numerous microtubules and microfilaments (Fig. 19a and b). Nerve cell axons were in initimate contact with these cells (Fig. 19b). The cap (AdI) cell had an extensive network of finger-like projections radiating out from the lumen and towards the basal end of the alveolus where it then made contact with thin epithelial cells which extended the width of the alveolus to the basement membrane

Figure 16. An electron micrograph showing the close association between the cap cell (Cp) and the darkly staining (os), simple granular and epithelial (E) cells. The epithelial cells extend from the cap cell (Cp) to the basal lamina of the alveolus. N-nucleus. 11250x.

Figure 17. An electron micrograph showing epithelial cells (E) with their respective nuclei (N) anterior and adjaceant to the valve (V). 7650x.


N
E
Cp
1µm
16

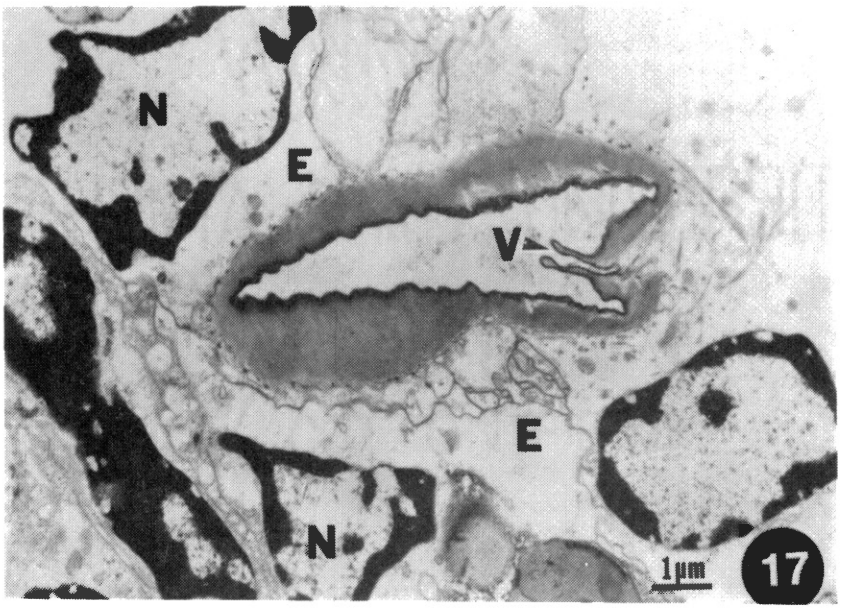
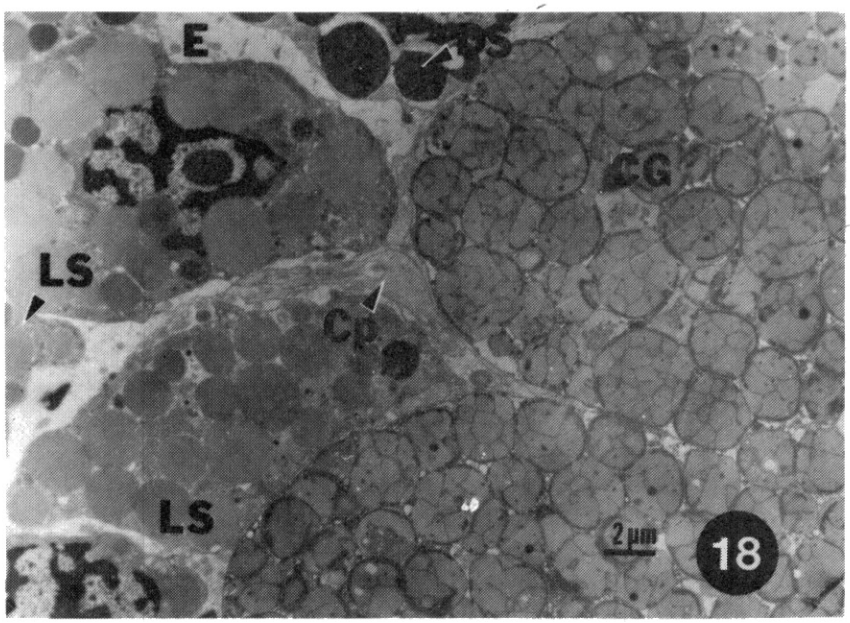

N
E
V
E
N
1µm
17

Figure 18. Electron micrograph of a section from a Type II alveolus showing two complex granular cells (CG) (i.e. granules with subunits) and adjacent darkly staining (DS) and lightly staining (LS) granular cells. The granular cells are separated by membranous epithelial cells (E) (Cp)-cap (AdI) cell 3800x.

Figure 19a. An electron micrograph of a Type II alveolus showing the cap (AdI) cell (Cp) with its microfilaments (mf), single nucleus (N) and its association with the arms of arms of the valve (V). Also present are complex granular cells (CG) and simple, darkly stained granular cells (DS). 5890x.


E
PS
CG
LS
Cp
LS
2µm
18

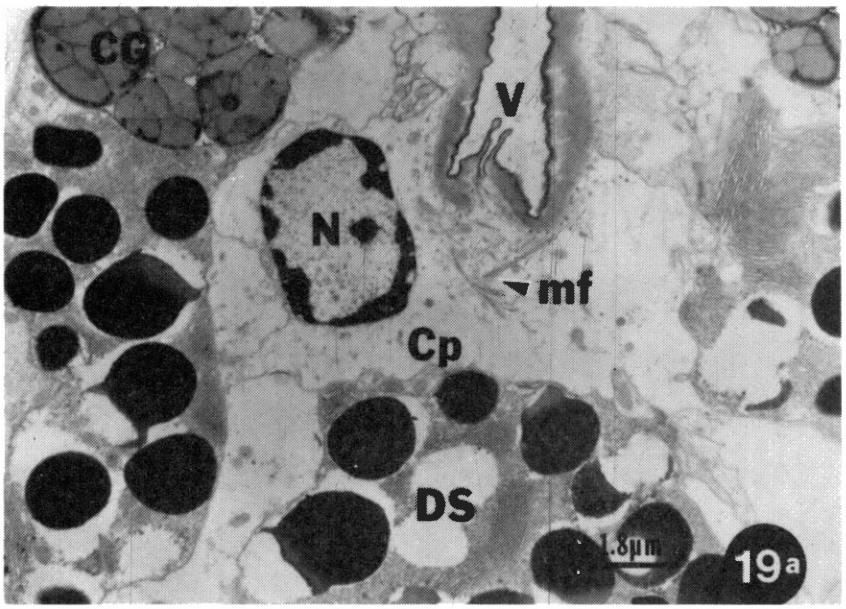
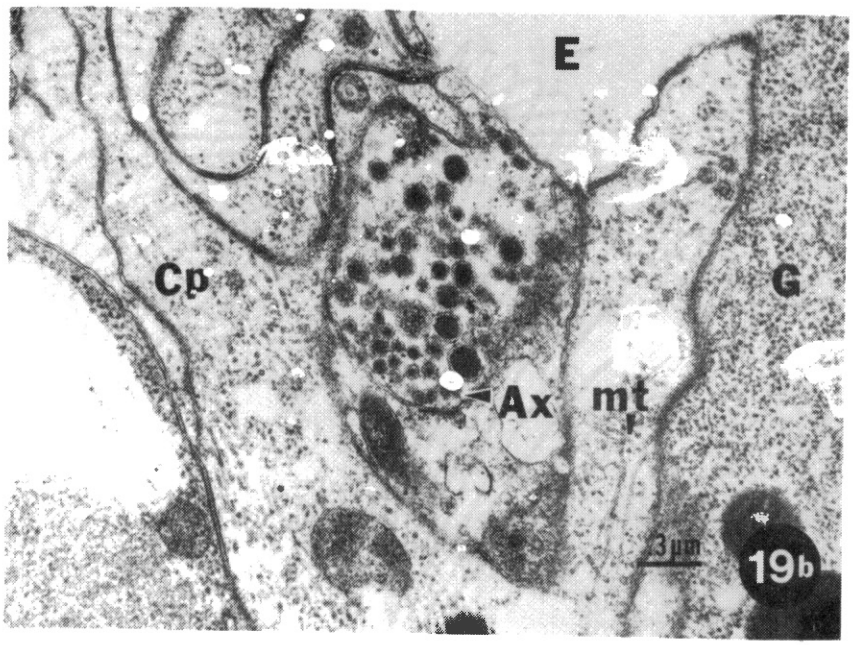
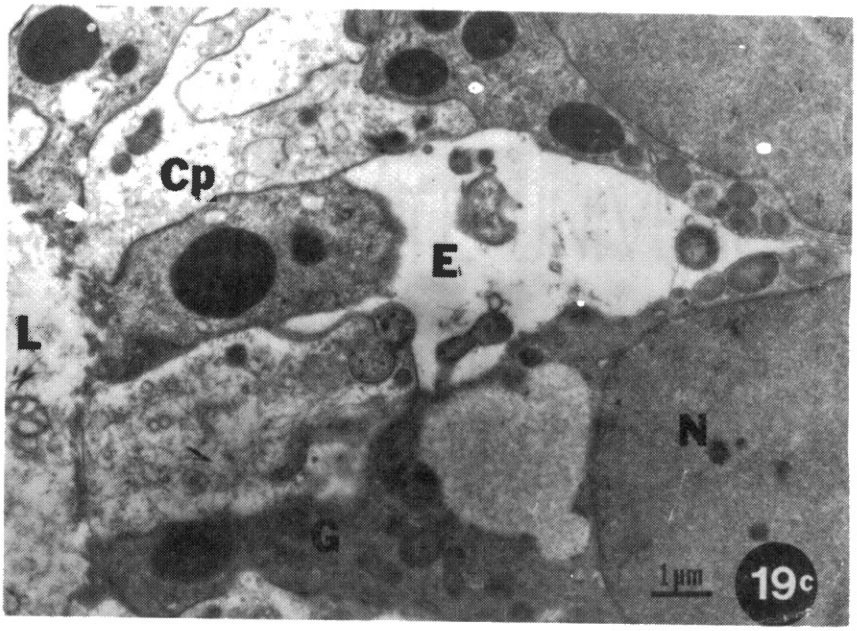

CG
V
N
mf
Cp
DS
1.8µm
19a

Figure 19b. Electron micrograph of a nerve axon (Ax) lying in close association with both the cap cell (Cp), and an epithelial cell (E). Microtubules (mt) are apparent within the cap cell (Cp). G-granular cell. 35000x.

Figure 19c. An electron micrograph of a Type II alveolus showing the cap cells' (Cp) finger-like extentions radiating from the lumen (L) towards the basal lamina. This micrograph shows an alternating pattern of the cap cell (Cp) with granular cells (G) and a close association of the epithelial cell (E) with the cap cell (Cp) extending from the lumen to the basal lamina. Nucleus-(N). 9000x.


E
Cp
G
Ax
mt
3 µm
19b


Cp
E
L
N
G
1 µm
19c

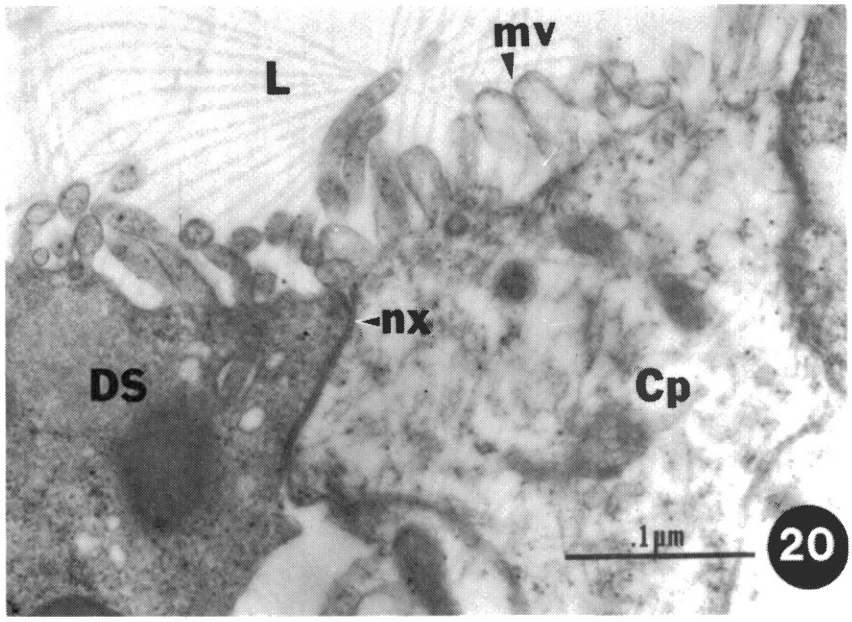
(Fig. 19c). The thin epithelial cells are likely the abluminal interstitial cells (AbI) as described by Fawcett et al. (1981a,b). Numerous microvilli were found on the apical side of the cap (AdI) cell (Fig. 20). Nexus junctions were seen making contact between the cap (AdI) cell and adjacent granular cells (Fig. 20).

Type III Alveolus. The cap (AdI) cell was also present in the Type III alveoli and after careful inspection of numerous thin-sections, we concluded that the cell was continuous about the lumen, but interrupted periodically by granular cells making contact with the alveolar lumen (Fig. 21). The suggestion of only one cell was supported by our finding of only one nucleus within the border of the cell found in both Types II and III alveoli (Fig. 22).

Only one complex granular cell adjacent to the valve was seen in Type III alveoli in contrast to that seen in Type II alveoli (Fig. 23). This agrees with the finding of only one densely granulated cell adjacent to the valve with light microscopy (compare to Fig. 5). This cell was also characterized by its very tight, non-uniform, compact, membrane-bound arrangement of granule subunits within the cell (Fig. 24). Another granular cell type seen was a darkly stained, simple granular cell (Fig. 24). This cell type was similar to that found in the Type II alveolus. Endoplasmic reticulum and mitochondria were numerous in this cell type with some Golgi apparatus also apparent. The Type

Figure 20. Electron micrograph showing a nexus junction (nx), as indicated by an arrow, between a simple, darkly stained granular cell (DS) and the cap cell (Cp). The cap cell is seen to possess numerous long microvilli (mv). Lumen (L). 28900x.

Figure 21. An electron micrograph showing the cap cell (Cp), bordering the apical side of the complex granular cell (CG), a simple, darkly staining (DS) and simple, lightly staining granular cell (LS). Granular cell products (P) can be seen entering the the lumen (L). 5890x.


mv
L
nx
DS
Cp
.1 µm
20

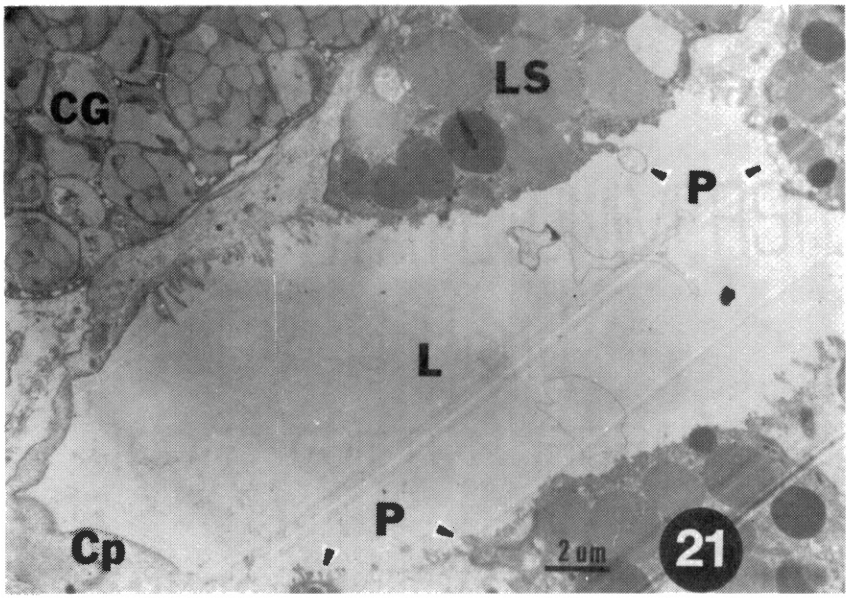
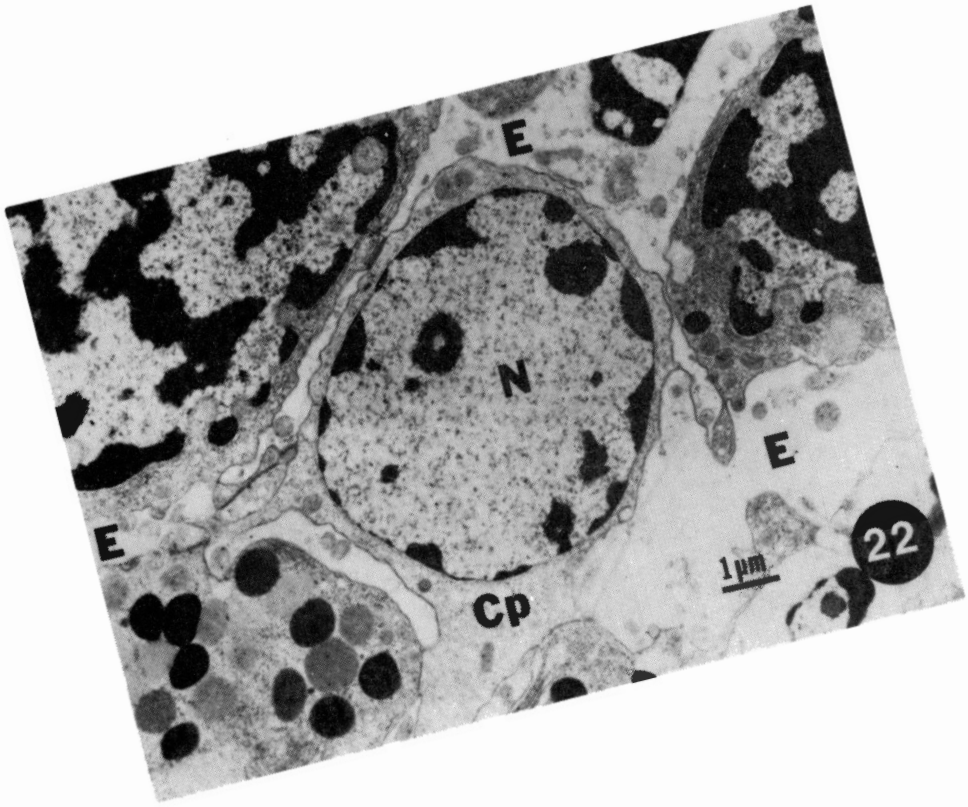

CG
LS
P
L
P
Cp
2 um
21

Figure 22. Electron micrograph of the cap cell (Cp) nucleus (N). The cap cell (Cp) interdigitates between granular cells and is in close association with epithelial cells (E). 9000x.

Figure 23. Diagram of the Type III alveolus compiled from numerous semi-serial thin sections.


E
N
E
E
Cp
1 µm
22

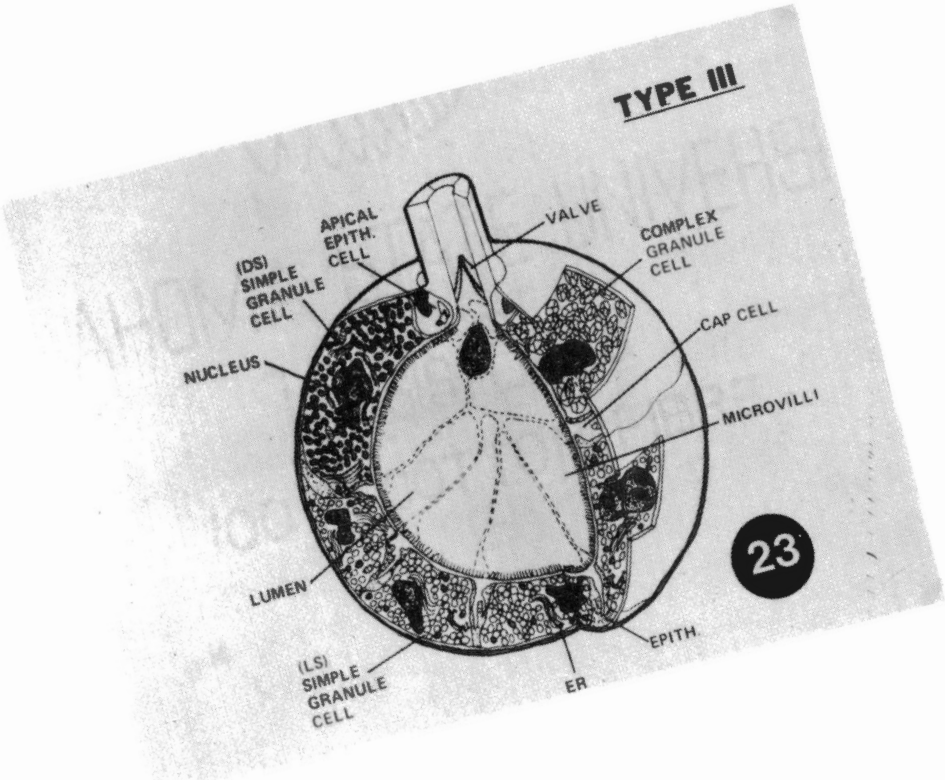
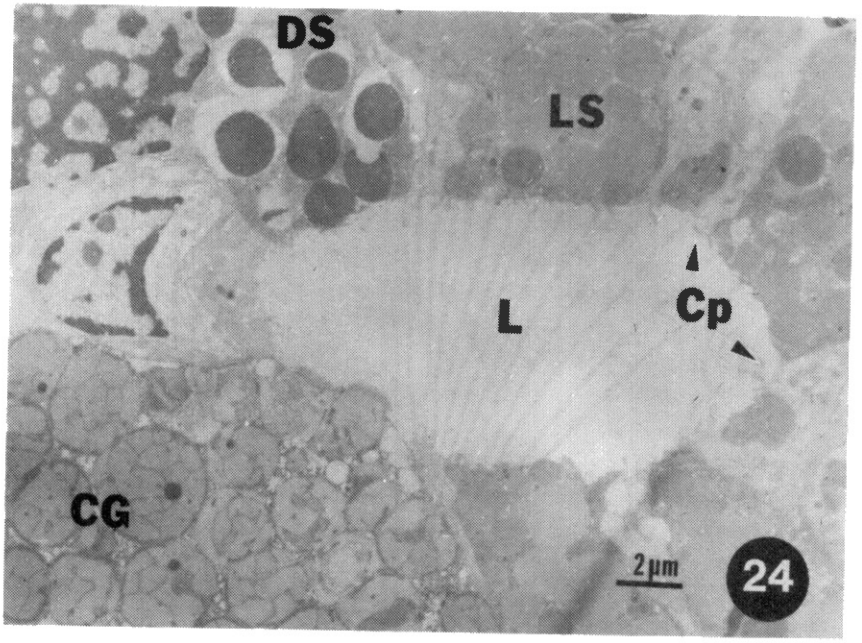
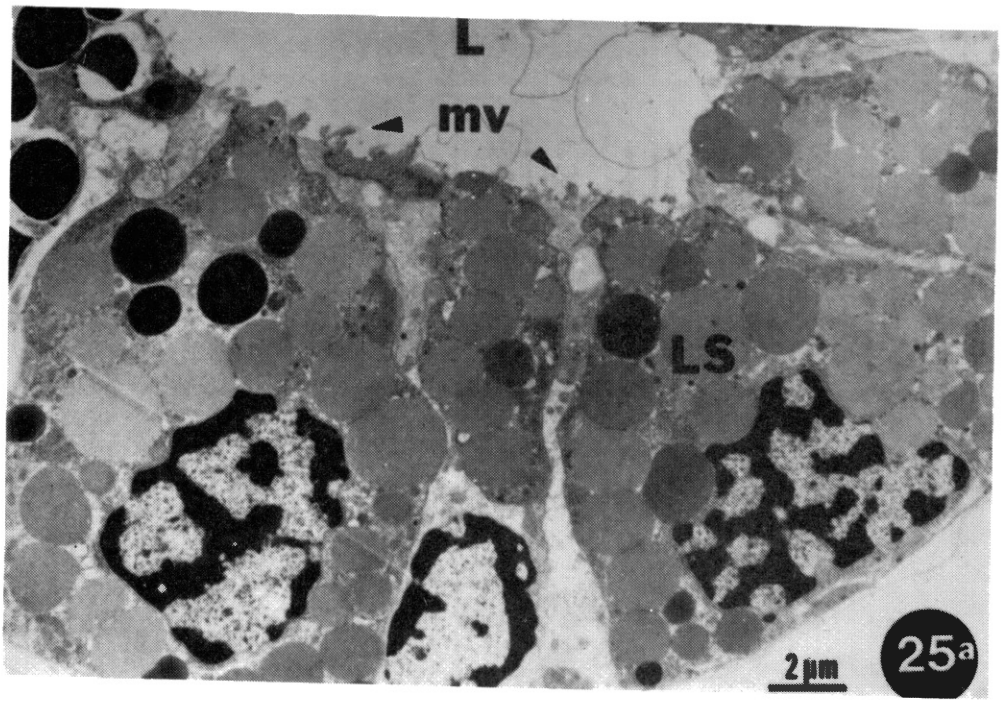

TYPE III
APICAL EPITH. CELL
VALVE
(DS) SIMPLE GRANULE CELL
COMPLEX GRANULE CELL
CAP CELL
NUCLEUS
MICROVILLI
LUMEN
EPITH.
(LS) SIMPLE GRANULE CELL
ER
23

Figure 24. An electron micrograph showing a section from the Type III alveolus. The three types of granular cells seen; complex granular (CG), simple, darkly stained (DS) and the simple, lightly stained granular cells (LS). Each granular cell extends to and makes contact with the lumen (L). 4940x.

Figure 25a. Electron micrograph featuring the luminal side of the cap cell (Cc) and its microvilli (mv) at arrows. Portions of granules from, simple, lightly stained granular cells (LS) can be seen being released into the lumen (L). 4750x.

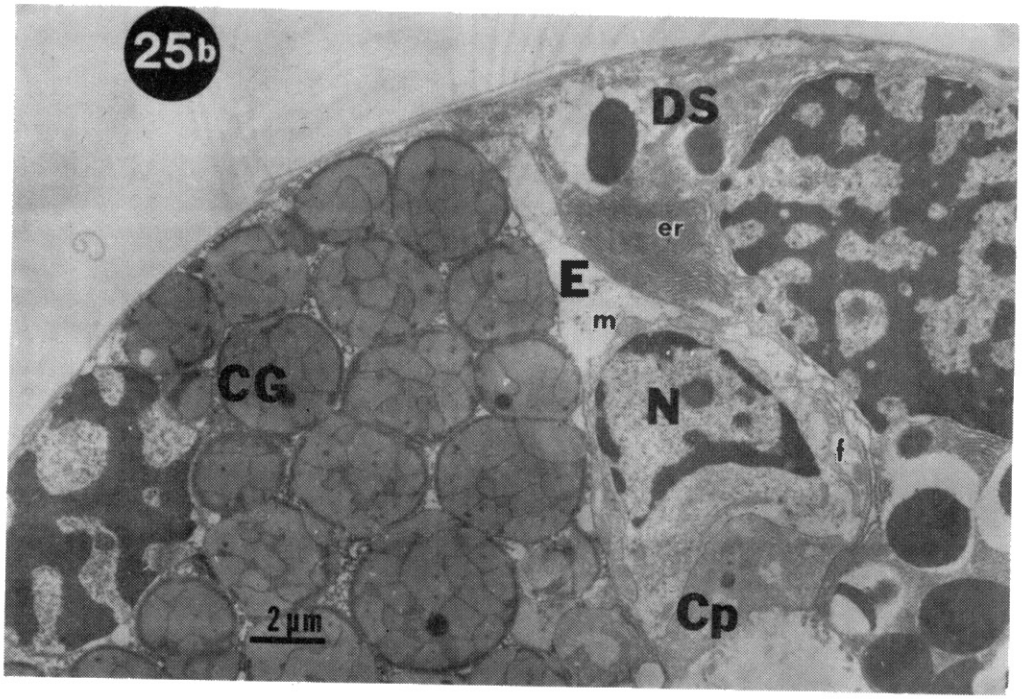

DS
LS
L
Cp
CG
2µm
24


L
mv
LS
2µm
25a

III alveolus was comprised of 4 rather than 3 lightly stained, simple granular cells as seen in Type II alveoli. The granules in this cell type were in a characteristically compact arrangement with numerous endoplasmic reticulum, and few mitochondria. Interdigitating the inter-cellular spaces of adjacent granular cells were the finger-like extensions of the cap (AdI) cell (also seen in the Type II alveolus) which enveloped the luminal region of the alveolus separating much of the granular containing cells' apical surfaces from the lumen (Fig. 24). Although not shown, the cap (AdI) cell could be seen making contact with the valvular arms. The basal end of the cap (AdI) cell extensions also came into association with the basally oriented epithelial (AbI) cells which were located consistently between the basal regions of the granular cells. These are likely the thin agranular cells seen with light microscopy (compare to Fig. 4) extending from the basal lamina to an agranular cell cap (AdI) cell lining the lumen. Gap or nexus junctions were also present between adjacent agranular and cap (AdI) cells.

The luminal side of the cap (AdI) cell possessed numerous short microvilli (Fig. 25a). As in the Type II alveoli, the cap (AdI) cell contained extensive amounts of microfilamentous material. Anterior to the valve there was an agranular epithelial cell containing some mitochondria and membrane fragments (Fig. 25b).

Figure 25b. Electron micrograph of a section from a Type III alveolus showing an agranular epithelial cell (E) anterior to the valve region. Mitochondria (m) and membrane fragments (f) and a rather large nucleus (N) can be seen. x5130.


25b
DS
er
E
m
CG
N
f
Cp
2 µm

Discussion

The present examination of the morphology of the salivary glands of female Amblyomma americanum utilizing both light and electron microscopy verifies previous findings of others in other species of ixodid ticks in addition to providing new insights into female salivary gland structure.

Numerous investigators have observed agranular (Type I) alveoli directly attached to the middle of the anterior protion of the main duct in both ixodid ticks (Balashov, 1968; Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973) and argasid ticks (Balashov, 1968; Guirgis, 1971; Roshdy, 1972; Roshdy and Coons, 1975). The ultrastructural appearance of the pyramidal cells, with their tortuous network of membranes, appears to be similar in both families. This alveolar type undergoes only slight change following tick attachment (Binnington, 1978; Megaw and Beadle, 1979) and is postulated to function in some way in the tick's absorption, if indeed it is proven that Type I alveoli are essential to the process of tick vapor absorption. An examination of Type I alveoli in hydrated, dessicated, and dessicated and subsequently rehydrated ticks is warranted.

The finding of two granular-secreting alveolar types in female A. americanum is not unlike that seen in other species (Till, 1961; Chinery, 1965; Balashov, 1968; Meredith and Kaufman, 1973; Binnington, 1978). The primary feature

of granular Type II alveoli is the concentric arrangement of seven granular cells about a central lumen interrupted by a cuticular valve that perhaps helps control movement of material from the aveolar lumen to its duct. A major feature previously unreported is the finding of only one cap (AdI) cell winding its way around the inner surface of the section. This may explain why other investigators have had difficulty finding nuclei for what was thought to be more than a single cell (Megaw and Beadle, 1979; Meredith and Kaufman, 1973). Furthermore, the presence of apparent contractile elements in the cell might suggest a myoepithelial-like function for the cap (AdI) cell to help "propel" secretions from the alveolar lumen. It is conceivable that the cap (AdI) cell may be involved in both the release of fluid and granular secretory material. Often, one sees regions in the cap (AdI) cell through which granular cell contents, granules, and granular cell cytoplasm protrude (Fig. 21). Semi-serial thin sections indicate a "pinching-off" of this cellular material by the surrounding cap (AdI) cell lements into the alveolar lumen. We suggest that contraction of the cap (AdI) cell might induce a higher luminal pressure, thus forcing luminal contents towards the valve region and force contraction of the cap (AdI) cell in the region of the valvular arms allowing the arms to separate and release luminal pressure and luminal contents. The close association of nerve axons and apparent synaptic like regions near and through the cap (AdI) cell further suggest

for the presence of six rather than seven an important regulatory function for the cell.

The Type III alveolus is similar to the Type II alveolus, except granular cells. Also, the same concentric arrangement of granular cells about a centrally located lumen and an apparent cuticle-lined valve, extending from the basal lamina to the lumen, interrupting these contiguous cells is seen. Other features such as the cap (AdI) cell, nexus junctions between cap (AdI) cell and granular cells, and valve cap (AdI) cell associations are morphologically and histologically similar to Type II alveoli.

The most recent published papers of ixodid female alveolar structure and types of granular cells in Types II and III alveoli are those of Binnington (1978) and Megaw and Beadle (1979) who described the structure of glands from B. microplus. These two studies differ considerably in their interpretation of cell types present. Binnington (1978) used histochemical staining methods with light microscopy and Megaw and Beadle (1979) used electron microscopy. Our results, based on a study of the ixodid female Amblyomma americanum, concur with those of Megaw and Beadle (1979) in finding two complex granular cells in Type II alveoli and only one in Type III. Binnington (1978) described two granular cells with subunits in Type III alveoli and only one granular cell in Type II. Binnington (1978) described a larger number of granular cell types (six) in Type II alveoli based mostly on the intensity of granular reactions to

PAS and 5-bromo-indoxyl acetate reagents and the morphology of the cells' cytoplasm. As did Megaw and Beadle (1979), we observed two types of simple granular cells in Type II alveoli but two rather than one in Type III alveoli. Binnington (1978) described three different granular cell types in Type III alveoli.

As a consequence of these differences in interpretation of kinds of cells present and a lack of obvious and unequivocal criteria with which to assign function to cell type, we find it less confusing to avoid assigning lower case letters to granular cell types, as has been the practice in the past, and instead, refer to Types II and III granular cells as either complex (i.e. with subunits) or simple (light or dark) as viewed at the ultrastructural level following en bloc staining with 0.5% uranyl acetate.

CHAPTER III

THE EFFECT OF ATTACHMENT, FEEDING, AND MATING ON THE ALVEOLAR MORPHOLOGY OF THE SALIVARY GLANDS OF THE LONE STAR TICK AMBLYOMMA AMERICANUM (L.)

Introduction

Salivary gland morphology of ixodid ticks has been studied by many investigators in recent years (Till, 1961; Chinery, 1965; Balashov, 1965, 1968; Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973; Binnington, 1978; Megaw and Beadle, 1979; Fawcett et al., 1981a,b; Krolak et al., 1981). The glands consist of grape-like clusters of alveoli running about two-thirds the length of the tick, are paired and the alveoli are attached directly or indirectly to the main salivary duct or its secondary branches by short alveolar ducts (Gregson, 1973). Three alveolar types are present in the female; one of which is agranular in appearance as viewed by light and electron microscopy. This type is designated Type I and is located anteriorly to the other alveoli and is attached directly to the main duct of the gland. Type I alveoli consist of peripheral pyramidal cells with numerous basal membrane

infoldings and closely associated mitochondria (Coons and Roshdy, 1973). It has been suggested that these alveoli might secrete hygroscopic material (Rudolph and Knulle, 1974, 1978) which enables the tick to sorb water from unsaturated air (Lees, 1946; Sauer and Hair, 1971; Rudolph and Knulle, 1974; McMullen et al., 1976). Previous studies have suggested that Type I alveoli undergo little change during feeding (Binnington, 1978; Megaw and Beadle, 1979).

Types II and III alveoli are granular as viewed by light and electron microscopy and are thought to be essential to the feeding process. Most genera of slow-feeding ixodid ticks secrete a cement substance that helps anchor the mouthparts of the tick to the host during feeding (Cowdry and Danks, 1933; Foggie, 1959; Gregson, 1960; Moorhouse and Tatchell, 1966; Moorhouse, 1969; Balashov, 1972; Chinery, 1973). Chinery (1973) suggested that the cement material from the salivary glands of Haemaphysalis spinigera is proteinaceous and derived from cells in Type II and III alveoli. Other biologically active substances have been found in ixodid salivary gland secretions including toxins, anticoagulants and prostaglandins (Sauer, 1977) which likely play key roles in the feeding process (Chinery, 1981; Chinery and Ayitey-Smith, 1977).

Changes in alveolar morphology during tick feeding have been investigated by Meredith and Kaufman (1973), Binnington (1978), Megaw and Beadle (1979) and Fawcett et al. (1981a,b). Generally, all investigators report few

changes in Type I alveoli the as female progressed through feeding. However, Type II and III alveoli undergo marked changes. Granular material seen in glands of unfed females is no longer observed in the alveoli of females in later stages of feeding. Alveoli increase in size, total protein and specific proteins are synthesized while others are secreted or changed to other substances while the tick is feeding (McSwain et al., 1981). The most conspicuous change is the appearance of an enormous labyrinth of membranes associated with secretion of excess fluid back to the host following concentration of the bloodmeal by the feeding tick. Fawcett et al. (1981a,b) have shown that these membranes are derived from two types of cells in Types II and III alveoli during progression of the tick through the feeding cycle. The purpose of the present study was to investigate morphological changes in salivary glands of feeding female Amblyomma americanum (L.).

After the female lone star tick A. americanum (L.) attaches to a host, it undergoes a slow phase of feeding lasting 8-14 days with an increase of weight from 4-300 mg while feeding on sheep, followed by a rapid phase of feeding lasting 12-24 hours, during which time the weight increases to approximately 300-800 mg. The female will not feed further and increase in weight greater than approximately 35 mg in the absence of mating (McSwain et al., in press). Therefore, glands obtained from female ticks 24 hr post-infestation on sheep, glands from females weighing 100-300

mg and glands from females weighing > 500 mg were compared. The 24 hr females were unmated. The morphology of glands in unfed A. americanum females has been previously reported (Krolak et al., 1981).

Methods and Materials

Unfed male and female ticks Amblyomma americanum (L.) were maintained at 27-28°C and 90-100% RH under a 13 hour light/24 hours photoperiod period to infestation of sheep. In the rearing of engorged females, unfed adults were placed in compartments consisting of a piece of orthopedic stocking approximately 6 inches in diameter and 10 inches deep located laterally to the dorsal medline of the thoracic and lumbar regions of the sheep. The area enclosed by the compartment was shorn to facilitate rapid equal numbers (20) of male and female A. americanum in each compartment and allowed to feed on the host. Males were included because females will not feed to repletion until mating has occured. Salivary glands were forcibly removed from partially fed female ticks by the methods of Sauer et al. (1974).

Salivary glands from unfed adult females, Ambylomma americanum, (L.) were fixed in 2% cacodylate buffered glutaraldehyde for 2 hours (pH 7.4) at room temperature before being withdrawn with a transfer pipette. The salivary glands were then rinsed for 1 hour in cacodylate buffer (pH 7.4) at room temperature; post-fixed in 2% osmium

tetroxide, rinsed in deionized water for 1 1/2 hours and en bloc stained with 0.5% uranyl acetate at room temperature overnight. Tissue was then rinsed in deionized water and dehydrated in a graded series of ethyl alcohol before embedding in Polybed ^{R} from Polysciences. Initially, 1 μm thick sections were obtained from the polymerized blocks using the Sorvall MT-2 ultramicrotome and stained with Mallory's trichrome for light microscopy. Sections were observed and photographed using a Zeiss photomicroscope with Panatomic X film (Kodak). Alveoli for electron microscopy were selected after viewing these sections. Thin semi-serial sections (70-90 nm) were obtained with a Sorvall MT-2 ultramicrotome using diamond knives. Sections were maintained in order of section but were not placed on slot grids. Therefore, only every third or fourth section was examined and photographed. Sections prepared for electron microscopy were stained with methanolic uranyl acetate and lead citrate (Venable and Coggeshall, 1965), observed and photographed with a Philips EM200 electron microscope.

The determination of the number and location of alveolar types in the salivary gland of A. americanum was made after careful examination of numerous light level thick sections stained with Mallory's trichrome and electron microscopic level thin sections stained with uranyl acetate and lead citrate. A distinct alveolar type (I) (diameter of approximately 50 μm) is found directly attached to the anterior to middle portion of the main duct.

Another (Type II) is posterior to the Type I alveoli and has a diameter of approximately 50-70 μm and extends the length of the gland and is attached to secondary branches of the gland. A third type has a diameter of approximately 50-70 μm and extends the length of the gland and is attached to secondary branches of the gland. A third type (Type III) is attached to the most peripheral and posterior secondary branches of the gland and has a diameter of approximately 60-90 μm. Staining frozen sections with Oil Red O suggested the presence of lipid in Type I alveoli.

Results

Light Microscopy

24 Hours

Type I. At this stage of tick feeding and as seen at the light microscopic level, Type I alveoli exhibited an accumulation of numerous opaque droplets in the central region of the alveolus (Fig. A) that stained positive for Oil Red O syggesting the presence of lipid. Lipid in this region of the alveolus contrasts with presence of lipid-like droplets in more peripheral parts of pyramidal cells in unfed female ticks (Krolak et al., 1981). Numerous peripheral clear, circular areas at about the same location of where lipid droplets were seen in unfed females (Krolak et al., 1981) did not stain with Oil Red O.

A pale, centrally located larger region of the alveolus, proximal to the duct and adjacent to the accumulated lipid-like droplets, may be the central cell as described by Megaw and Beadle (1979) and the "inner" cell described by Meredith and Kaufman (1973). We designated this the "central" cell in unfed A. americanum (Krolak et al., 1981).

Type II. Type II alveoli were characterized by heavily granulated cells. Two cells on either side of the alveolar valve held a dense arrangement of granules (Fig. B). Other than a nucleus, few organelles were observed following staining with Mallory's trichrome. Similar cells were seen at the level of light microscopy in unfed females (Krolak et al., 1981). Four-five darkly stained granular cells were found throughout the cell (Fig. B).

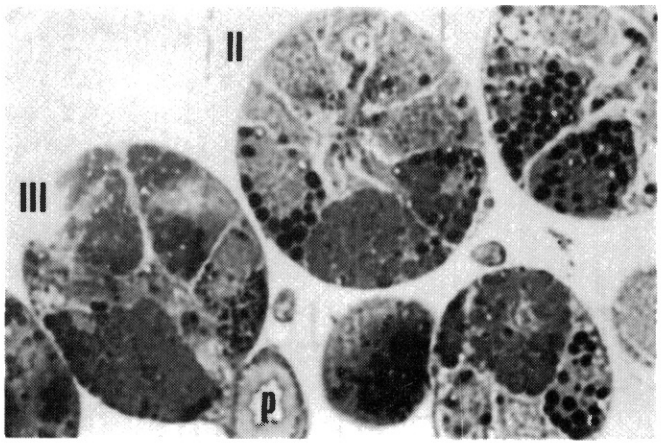
Clear, non-granular cells extending from the center of the alveolus peripherally were located between the granular cells. Similar but smaller cells were observed in Type II alveoli of unfed females (Krolak et al., 1981).

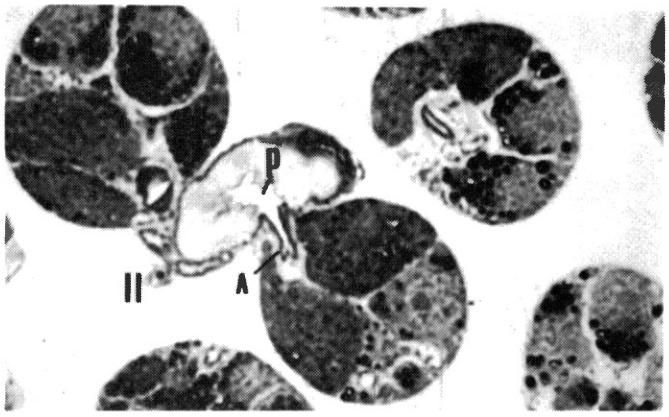
Type III. One heavily granulated cell proximal to the duct and adjacent to the alveolar valve and similar to that seen in the unfed female (Krolak et al., 1981) (Fig. C) was observed. A darkly stained granular cell was observed on the opposite side of the valve. Three-four additional darkly stained granular cells seen in Type III alveoli.

Figure A. Light micrograph of Type I alveoli from 24 hr. feeding female ticks. Numerous clear, circular regions characterize the cellular area of Type I (I) alveoli. Lipid-like droplets (ld) are seen in a central region of the alveolus. x288.

Figure B. Light micrograph of Type II (II) alveoli from 24 hr. feeding female ticks. This alveolar type is characterized by two dense granular cells adjacent to the valve (v). The valve of the alveolus can be seen to open into an associated duct (d). x468.

Figure C. Light micrograph of Type III (III) alveolus from 24 hr. feeding female ticks. The presence of a large densely staining granular cell adjacent to darkly stained granular cell are found next to the valve. d - duct of the salivary gland. x468.


II
III
p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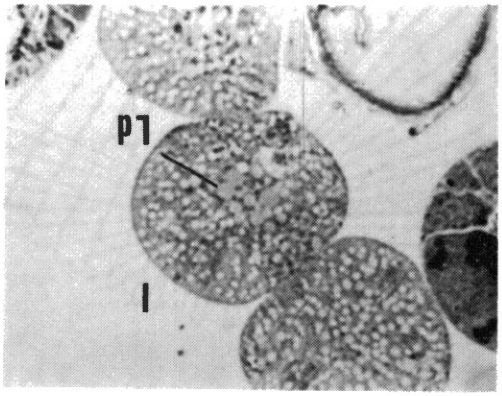
A


pT
I

Clear, non-granular cells, extending from the center of the alveolus peripherally and similar to that seen in Type II alveoli, separated the 4-5 granular cells (Fig. C). Similar but smaller agranular cells were seen in Type III alveoli of unfed females (Krolak et al., 1981).

100-300 mg

Type I. By this stage of female feeding, Type I alveoli exhibited small, clear vacuolar areas throughout most the alveolus (Fig. D). Reaction to Oil Red O staining in these areas was slight. A central cell with conspicious nucleus few cellular elements was observed (Fig. D). A small region void of cellular organelles was observed next to the tubular lumen. The cell in this region of Type I alveoli in unfed females is the constrictor cell (Fig. D) (Krolak et al., 1981).

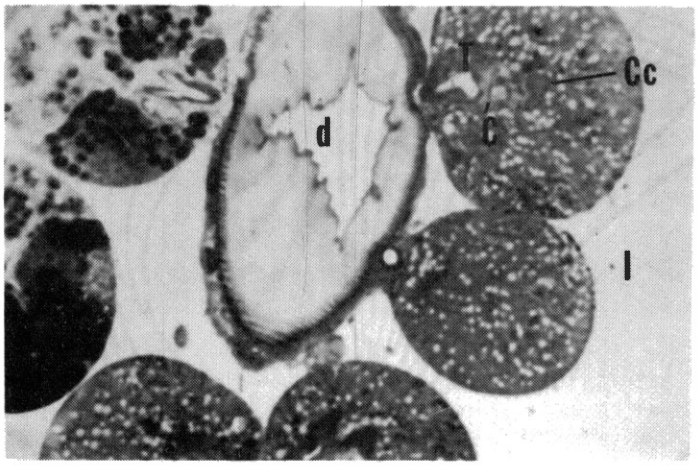
Type II. The cell type with the dense arrangement of granules similar to that seen in the 24 hr feeding tick was observed in salivary gland alveoli from feeding female ticks weighing between 100-300 mg (Fig. E). Three-four darkly stained granular cells were also seen (Fig. E).

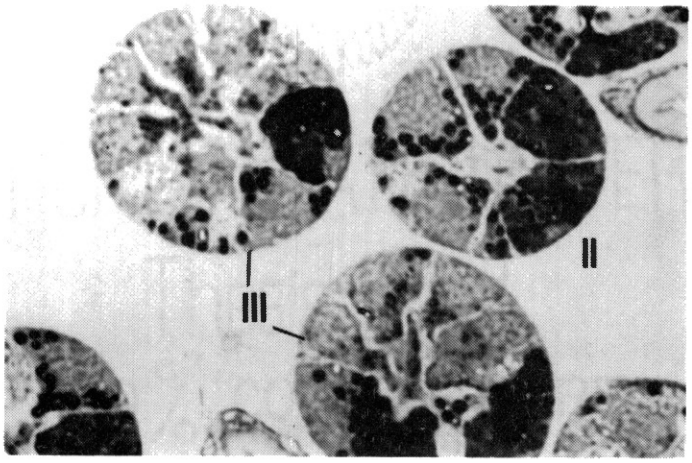
Enlarged clear, non-granular cells radiating basally from the center of the alveolus between granular cells continued to be seen. (Compare Fig. E with Fig. B).

Type III. One cell with densely arranged granules was still seen in Type III alveoli of salivary glands of

Figure D. Light micrograph of a Type I (I) alveolus from feeding female ticks weighing between 100-300 mg shows a centrally located nucleus encompassed by a relatively small region void of cellular elements. This nucleus is thought to be the central cell nucleus (Cc). Proximal to the tubules (T) is another clear region thought to be the constrictor cell (C). d = duct of the salivary gland. x468.

Figure E. Light micrograph of Type II (II) and Type III (III) alveoli from feeding female ticks weighing between 100-300 mg showing a Type II (II) alveolus with two densely arranged granular cells adjacent to the valve. Three to four additional granular cells can be seen. Type III (III) alveoli with the a single, dense granular cell near the valve of one alveolus while the other (at the lower end of the photomicrograph), exhibits a darkly stained granular cell in juxtaposition to a dense granular cell. x468.


Cc
d
I


II
III

feeding female ticks weighing between 100-300 mg (Fig. E). The alveolar arrangement of cells was quite similar to that observed and described in Type III alveoli of 24 hr feeding female ticks except that agranular cells separating granular cells were larger (Compare Fig. C and E).

$\geq$ 500 mg

Type I. The clear, circular vacuolar areas observed in Type I alveoli of 24 hr and 100-300 mg feeding females (Compare Fig. F with Figs. A and D) were reduced in size in Type I alveoli of feeding females weighing greater than 500 mg. Dark droplets resembling those seen in Type I of unfed female ticks (Krolak et al., 1981) but not found in Type I alveoli of lighter feeding ticks were seen.

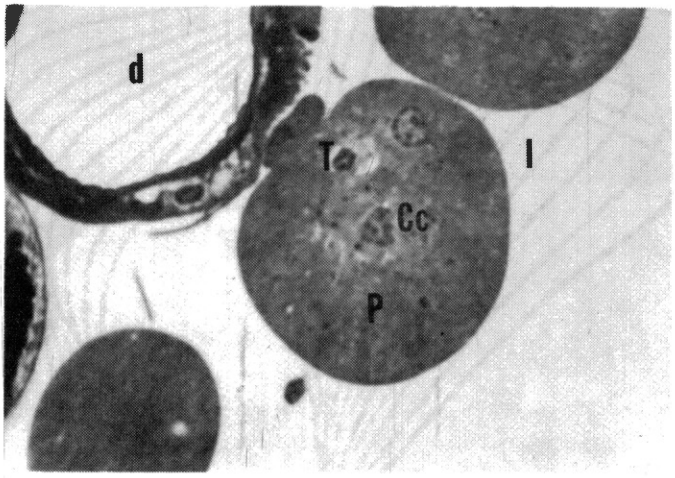
A central cell with nucleus surrounded by a small clear cellular region was observed (Fig. F) in addition to more peripheral membranous pyramidal cells. A clear region proximal to the gland duct and resembling that seen in feeding female ticks weighing between 100-300 mg (Fig. D) and that in Type I alveoli of unfed female ticks (Krolak et al., 1981) was observed. A more peripheral nucleus (Fig. F) probably associated with a pyramidal cell was also seen.

Type II. Five-six nuclei from degranulated cells were seen in a section of a Type II alveolus from a feeding female weighing more than 500 mg (Fig. G). Portions of cells protruded into the alveolar lumen and their basal regions appeared to have lost cellular integrity (Fig. G).

Figure F. Light micrograph of Type I alveoli from feeding female ticks weighing greater than 500 mg. A reduced clear, circular area was seen near the pyramidal (P) cell. A centrally located nucleus surrounded by a small clear cellular region is likely the central cell (Cc). A small portion of alveolar tubule (T) can be seen in the alveolar neck attached to the duct (d). x468.

Figure G. Light micrograph of Type II and Type III alveoli of feeding ticks weighing greater than 500 mg. At least 5-6 nuclei (n) were seen in some alveoli whose cells appeared to have lost some cellular integrity. The lumen of the alveolus was enlarged.

The Lumen (L) of Type III alveoli was greatly enlarged and the size of cells much reduced. The valve (v) can be seen closely associated with the gland duct (d). x468.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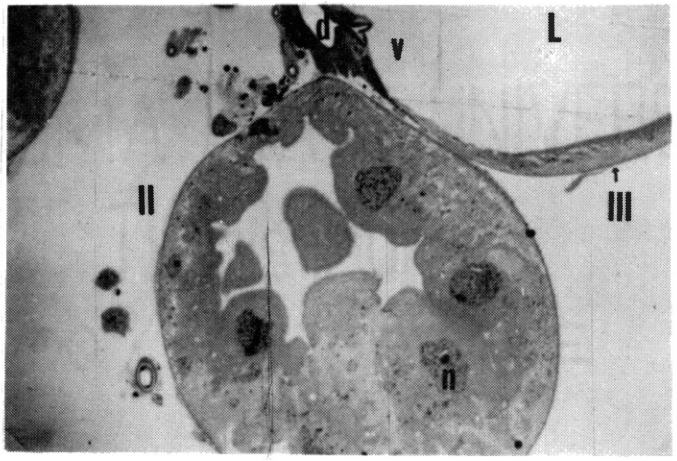
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Type III. The size of cells in some Type III alveoli, were markedly reduced and the alveolar lumen greatly expanded in glands from ticks weighing more than 500 mg. Overall the appearance was that of thin bands of cells surrounding a very large alveolar lumen (Fig. G).

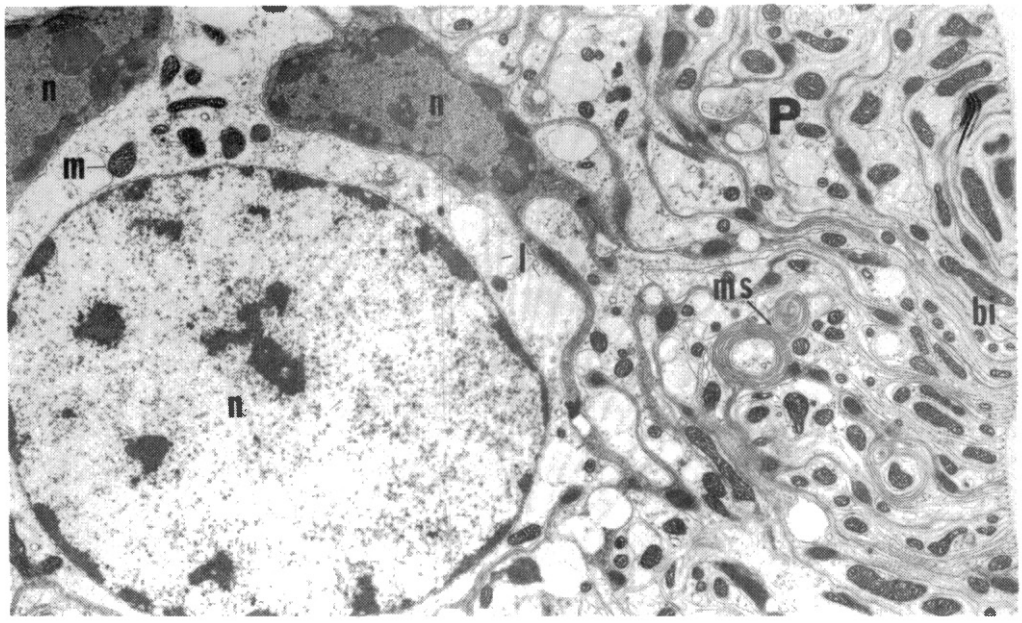
Electron Microscopy

24 Hours

Type I. Type I alveoli consist of 5-7 peripheral pyramidal cells, one oval-shaped, clear central cell, a constrictor cell separating the "central" cell from the alveolar tubule lumen and several peritubular cells as seen at the ultrastructural level in unfed females (Krolak et al., 1981). The number and kinds of cells in ticks having fed 24 hr was the same as that seen in unfed ticks. As in unfed females, the pyramidal cells possessed numerous basal infoldings with closely associated mitochondria (Fig. 1). Numerous swirling membranes not seen in alvoli of unfed ticks were seen (Fig. 2). Lipid-like droplets distributed abundantly throughout the pyramidal cells in the unfed female were absent. The lipid-like material appeared to coalesce in the central cell (Fig. 2) and an occasional lipid-like droplet was visible within the borders of the constrictor cell (Fig. 3). Upon closer examination a considerable number of lipid-like droplets were seen within the cytoplasm of the central cell (Fig. 3). Extensions of the

Figure 1. Electron micrograph of a Type I alveolus showing numerous basal infoldings (Bi) and closely associated mitochondria (m) in pyramidal cells (P). Nuclei (n) of pyramidal cells. Adjacent to the central cell and its heterochromatic nucleus exhibited a darkly stained heterochromatic pattern. Numerous lipid-like droplets and membrane swirls were also seen in the pyramidal cells. x5868.

Figure 2. Electron micrograph of a section from a Type I alveolus of a 24 hr feeding female tick. The pyramidal cells (P) had numerous basal infoldings (bi) and membrane swirls (ms). Lipid droplets (l) were seen to coalesce (cl) in the central cell. The constrictor cell (Cc) with mitochondria (m) and microfilaments (mf) separated the tubular lumen (T) from the central cell. x 4238.


n
n
m
P
j
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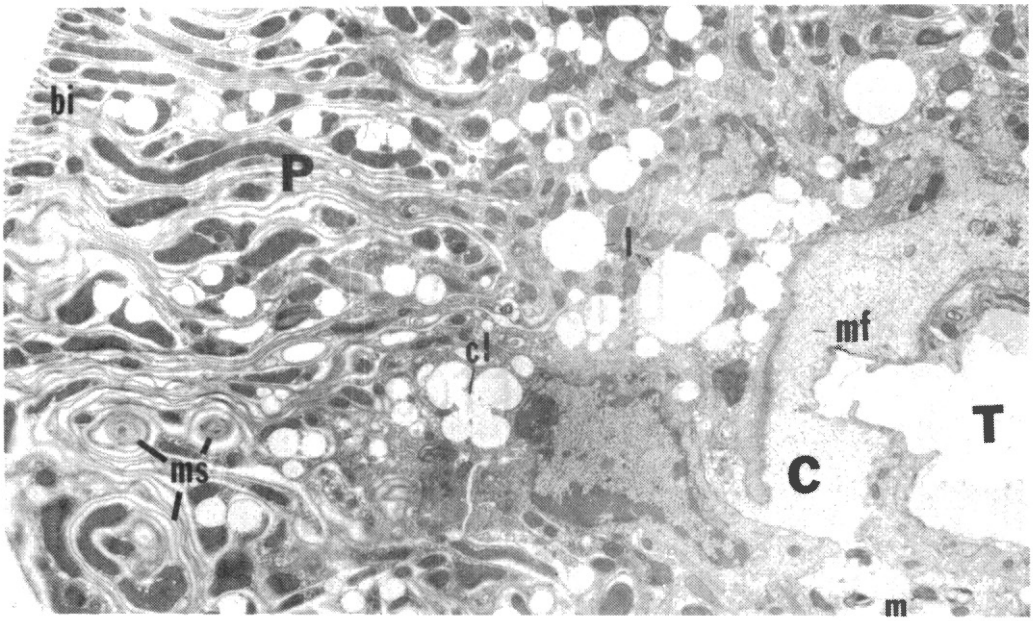
n


bi
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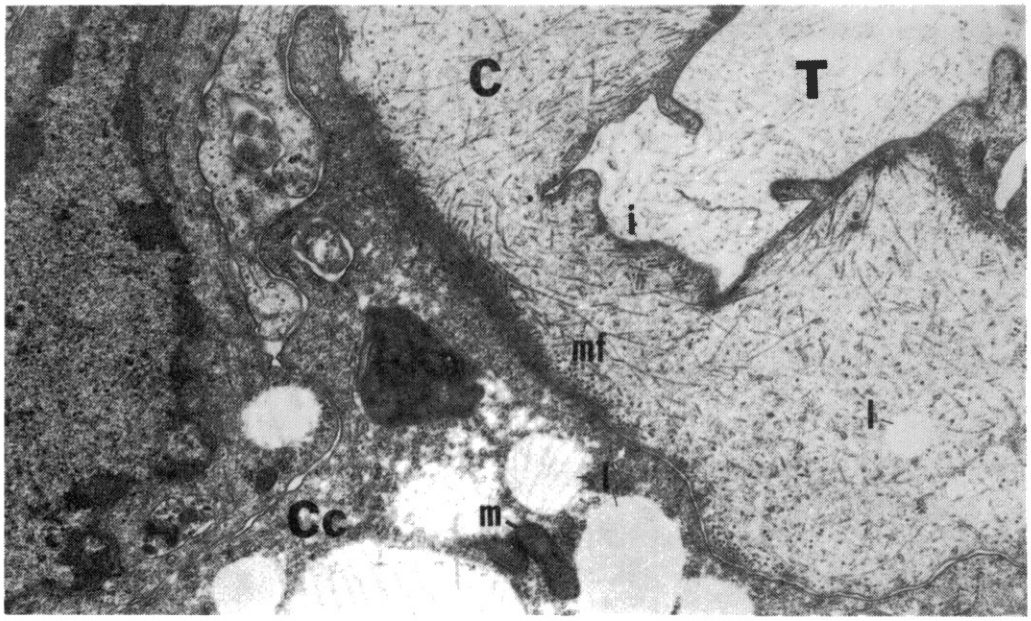
central cell radiated from the center of the alveolus to the basal lamina in close association with the tortuous membrane network of pyramidal cells. The central cell was larger and more extensive than that seen in unfed females with more numerous mitochondria. The cytoplasm of the central cell also appeared more consistent with that of the surrounding pyramidal cells and more dense than that previously seen in Type I alveoli of unfed females. The nuclei of the central and pyramidal cells possessed a heterochromatic pattern as opposed to a euchromatic pattern seen in unfed females.

A constrictor cell with numerous microfilamentous structures was seen cutting off the central cell from the lumen of the tubule (Fig. 3). Its structure included mitochondria, microfilaments and an invagination of the tubule lumen passing through the constrictor cell (Fig. 3). In passing through the "constrictor" cell, membranous structures not seen in unfed females (Krolak et al., 1981) were seen protruding from the wall of this invagination into the lumen of the tubule (Figs. 2 and 3). Four-five peritubular cells surrounded the short, cuticle-lined tubule connecting the alveolus to adjacent ducts. An occasional mitochondrion was found in the cytoplasm of these cells. The appearance of these cells was much the same as that seen in unfed females (Fig. 2).

Glands were also observed in ticks which had fed for 48 and 72 hours on sheep. In general the morphology of

Figure 3. Electron micrograph of a Type I alveolus of a 24 hr feeding female tick showing an invagination (i) of the tubular (T) lumen through the constrictor cell (Cc). Lipid-like droplets (l) were seen in both the constrictor cell and the central cell (C). Microfilaments (mf) were apparent in the constrictor cell (Cc). Mitochondria (m) were visible in the central cell. x16,137.

Figure 4. Electron micrograph of a Type II alveolus of a 24 hr feeding female tick showing two complex granular cells (CG) on either side of the alveolar valve (V). A nerve axon (ax) was seen in close association to one of the granular cells. Spatial openings ( ) could also be seen in the complex granular cells. A cap (AdI) cell was seen in close proximity to the alveolar valve. x4238.


C
T
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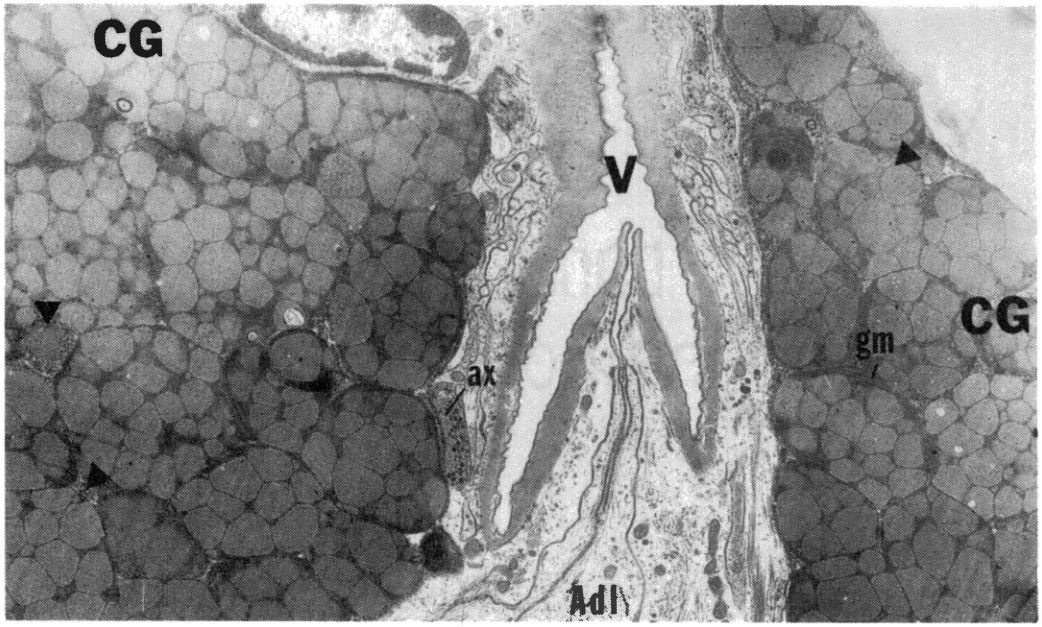

CG
V
CG
gm
ax
Adl

alveoli resembled that in 24 hr females except that lipid-like droplets in Type I alveoli were absent suggesting a possible role for this material in the initial phases of tick feeding (Figs. 11 and 12).

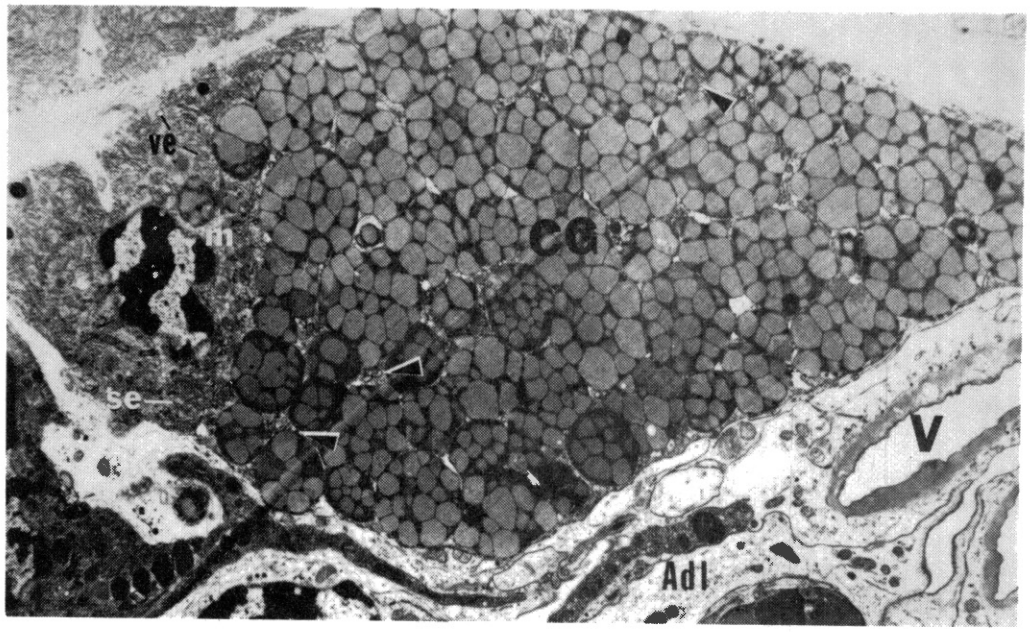
Type II. At the ultrastructural level Type II alveoli of unfed females consist of 2 complex granular cells near the alveolar valve, dark and lightly staining simple granular cells, one cap (AdI) cell, epithelial (AbI) cells between adjacent granular cells and agranular cells about the region of the alveolar valve (Fawcett et al., 1981a,b; Krolak et al., 1981).

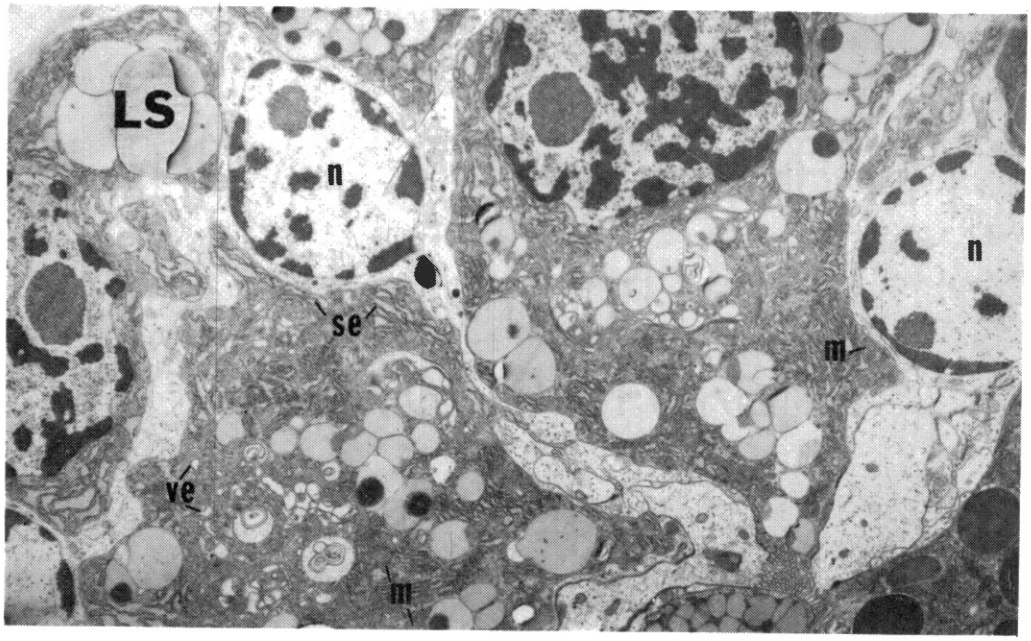
The two complex granular cells located on either side of the valve in Type II alveoli from ticks at this stage of feeding included a common membrane surrounding an arrangement of granular subunits with spaces between granules (Fig. 4). The granular subunits were not seen in similar cells of unfed ticks. Membranous elements, vesicular structures and glycogen-like granules were seen within spaces (Fig. 5). Swollen endoplasmic reticulum, (due to proteinaceous material within the cisternae of the reticulum), mitochondria and vesicles were seen in a region of cell void of complex granules (Fig. 5).

The three lightly stained, simple granular cells contained an abundance of swollen endoplasmic reticulum, mitochondria and some vesicles (Fig. 6). In the two darkly stained, simple granular cells microtubules and endoplasmic reticulum were found near the apical region of the granule

Figure 5. An electron micrograph of a complex granular cell (CG) in a Type II alveolus of a 24 hr feeding female tick showing spatial openings (arrows). Vesicles (ve) void of granules, mitochondrdia (m) and numerous swollen endoplasmic reticular cell were seen. The adluminal interestitial cell (AdI) was also seen extending from the alveolar valve (v). x3083.

Figure 6. An electron micrograph of a lightly staining, simple granular cell (LS) in a Type II alveolus of a feeding female tick. The lightly staining, simple granular cells were seen to possess numerous swollen endoplasmic reticulum (se), vesicles (ve) and mitochondria (m). N= nucleus. x3083.


ve
m
cc
se
V
Adl


LS
n
n
se
m
ve
m

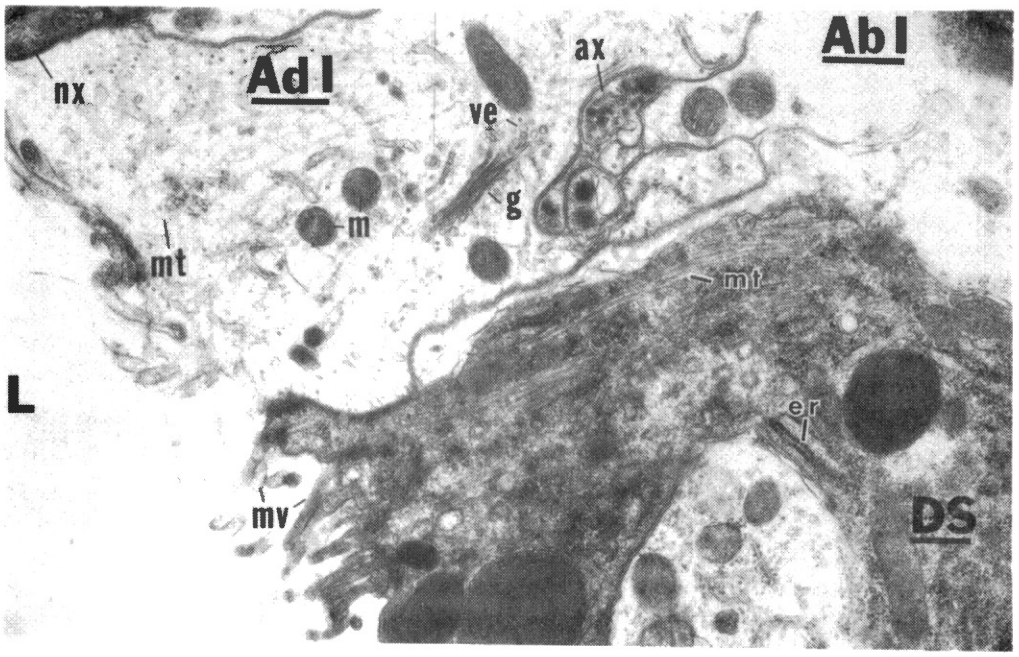
cells (Fig. 7). The cap (AdI) cell was seen in unfed females apical to the clear epithelial cell and between two granular cells as it made contact with the lumen of the alveolus (Krolak et al., 1981) (Fig. 7). As in unfed females, the cap (AdI) cell in 24 hr ticks was seen to contain Golgi complexes, mitochondria, microtubules and vesicles. Microvilli projected into the lumen from this cell (Fig. 7). Nexus junctions were seen connecting granular and (AdI) cells near their apical surfaces (Fig. 7).

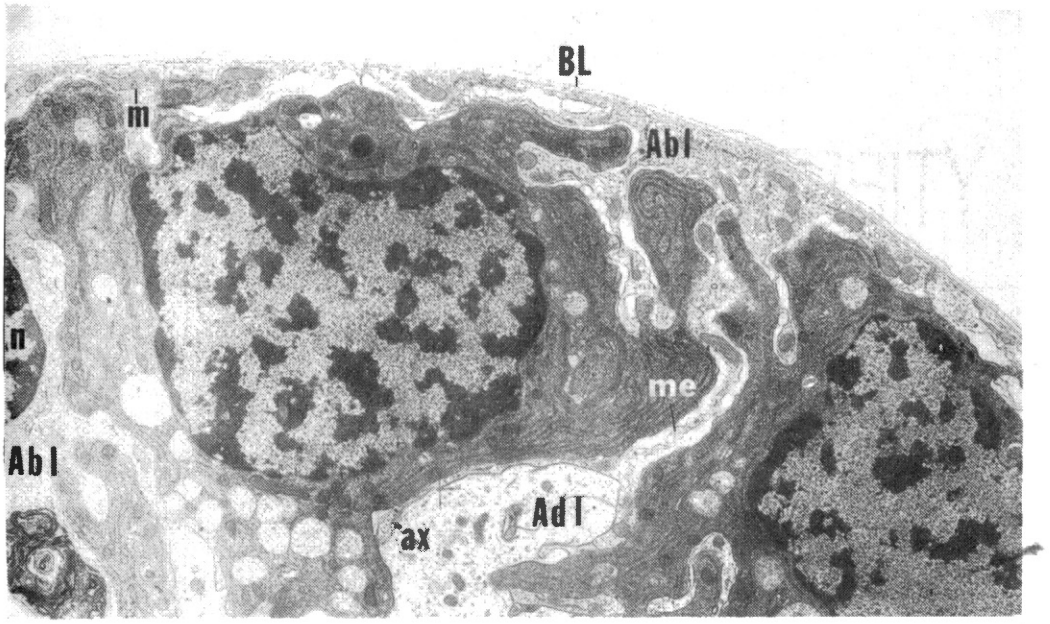
The clear abluminal interstitial (AbI) cells located peripheral to the cap (AdI) cell, contained numerous membranous elements, mitochondria and individual nuclei (Fig. 8). In some instances, and similar to that seen in unfed females, AbI cells extended around the basal region of granular cells disrupting its apparent continuation between the basal lamina of the alveolus and the granular cells (Fig. 8). The AbI cells in 24 hour feeding ticks were larger than that seen in unfed ticks. Nerve axons were observed in close associations with the cap (AdI) cell and AbI cells (Fig. 7).

Type III. In unfed females, the Type III alveolus consists of a complex granular cell near the alveolar valve, light and darkly staining, simple granular cells, one cap (AdI) cell, between adjacent granular cells and agranular cells surrounding the valve (Krolak et al., 1981).

Figure 7. Electron micrograph of a darkly staining, simple granular cell (DS), in a Type II alveolus of feeding female tick, bordering the cap (AdI) via a nexus junction (nx). The adluminal interstitial (AdI) cell was seen to possess a Golgi complex (g), mitochondria (m), and numerous apical microtubules. The darkly staining, simple granular cell (DS) possessed endoplasmic reticula (er) microvilli (mv). A nerve axon (ax) could be seen in close association with both the cap (AdI) and abluminal interstitial (AbI) cells. x16,137.

Figure 8. Electron micrograph of a Type II alveolus from a 24 hr feeding female tick showing the abluminal interstitial (AbI) cells with numerous membranous elements (me) and mitochondria (m) extend around the basal region of the lightly staining, granular cell near the basal lamina (BL) of the alveolus. ax-nerve axon. AdI cap or adluminal interstitial cell. x4238.


nx
Ad I
ve
ax
Ab I
m
g
mt
mt
L
er
mv
DS


BL
m
Ab I
n
me
Ab I
Ad I
ax

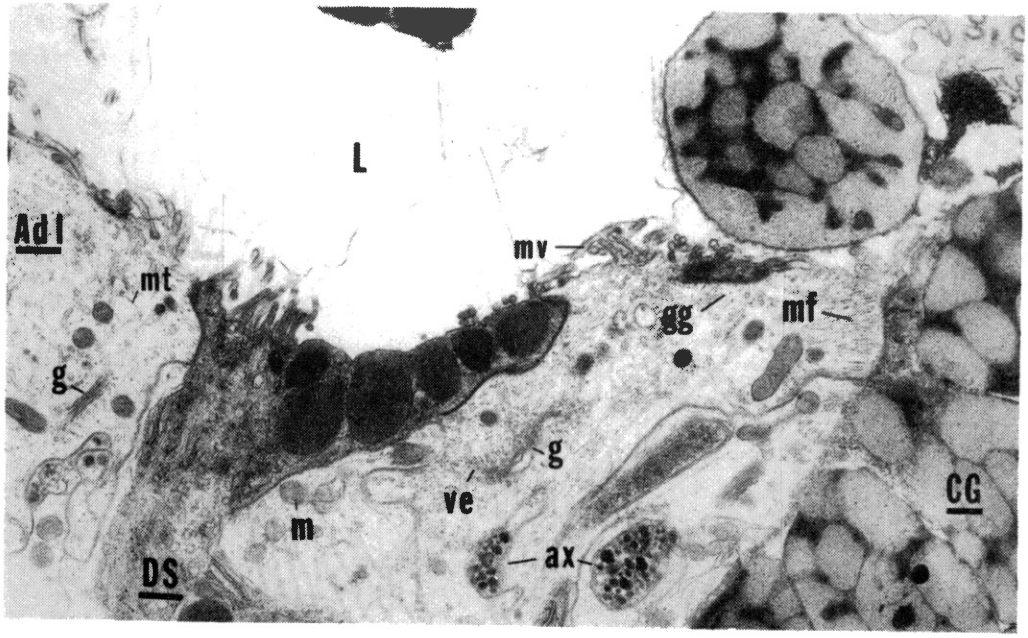
The cap (AdI) cell had microfilamentous structures, mitochondria, microtubules, a Golgi complex, vesicles and numerous glycogen granules (Fig. 9). Numerous microvilli extended from the apical region of the cap (AdI) cell into the lumen of the alveolus. An AbI cell adjacent to the perpherial region of the cap (AdI) cell extended to the basal lamina of the alveolus. Nerve axons were seen closely associated with cap (AdI) and AbI cells (Fig. 9). The AbI cells contained numerous membranous structures, mitochondria, empty vesicles, glycogen granules, nuclei and were located on the basal side separating granular cells from the basal lamina of the alveolus (Fig. 9). As in unfed females, only one complex granular cell was seen in Type III alveoli of ticks at this stage of feeding. The cell was adjacent to the valve and contained a common membrane surrounding an arrangement of granular subunits. The compact arrangement of granules seen in unfed females was not apparent in the 24 hour feeding tick (Fig. 10a) with membranous elements seen between granules. Other granules in the complex granular cell appeared to be in the process of lysis. Other regions contained swollen endoplasmic reticulum and mitochondria (Fig. 10a).

The darkly staining, simple granular cells were similar to that found in Type II alveoli with endoplasmic reticulum and numerous mitochondria. The endoplasmic reticulum was swollen suggesting increased protein synthesis by the

Figure 9. An electron micrograph of Type III alveolus feeding female tick showing a darkly staining, simple granular cell (DS) with numerous microtubules (mt) vesicles (ve) and glycogen granules (gg). The darkly staining, simple granular cell (DS) could be seen juxtaposed to the cap (AdI) cell. Both cell types possessed microvilli (mv) protruding into the lumen (L). The cap (AdI) possessed mitochrondria (m), a Golgi complex (g) and numerous microfilaments (mf). A complex granular cell (CG) was also seen. x7335.

Figure 10a. An electron micrograph of a Type III alveolus from a 24 hr feeding female tick showing numerous spatial openings among granules in the complex granular cell (CG). Membranous elements (me) were seen in these spatial openings. Granular subunits (enveloped within a common granular membrane (gm)) were in the process of being lysed (lg). Mitochondria (m) were also observed. x4238.

Figure 10b. An electron micrograph showing a lightly staining, simple granular cell (LS) of a Type III alveolus. The lightly staining, simple granular cell were seen to possess numerous swollen endoplasmic reticula (se), mitochondria (m) and empty vesicles (ev). Occasional granular membranes (gm) were seen as the remnants of lysed granules. x4238.


L
Ad I
mt
mv
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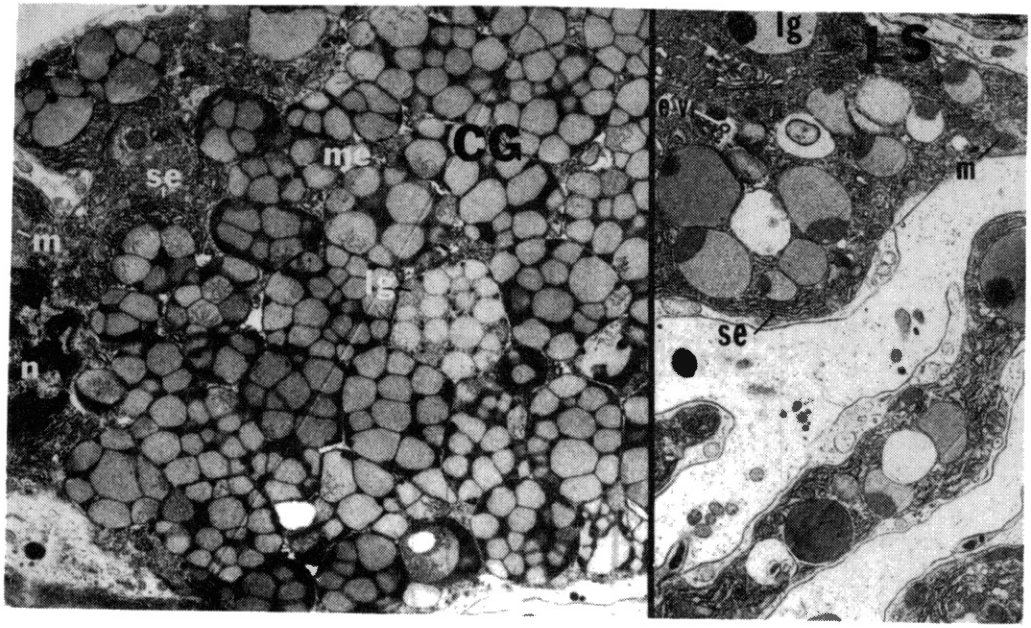
mf
g
g
ve
m
ax
DS
CG


CG
se
me
m
lg
n
lg
LS
ev
m
se

cell.

Granules in the four lightly staining, simple granular cells were in different stages of lysis ranging from homogenously stained granules to granules that stained light and dark and granules void of material except for rememants of membranes (Fig. 10b). Numerous empty vesicles, mitochondria and swollen endoplasmic reticulum were also within these cells. The nuclei of all cells had a characteristic heterochromatic pattern.

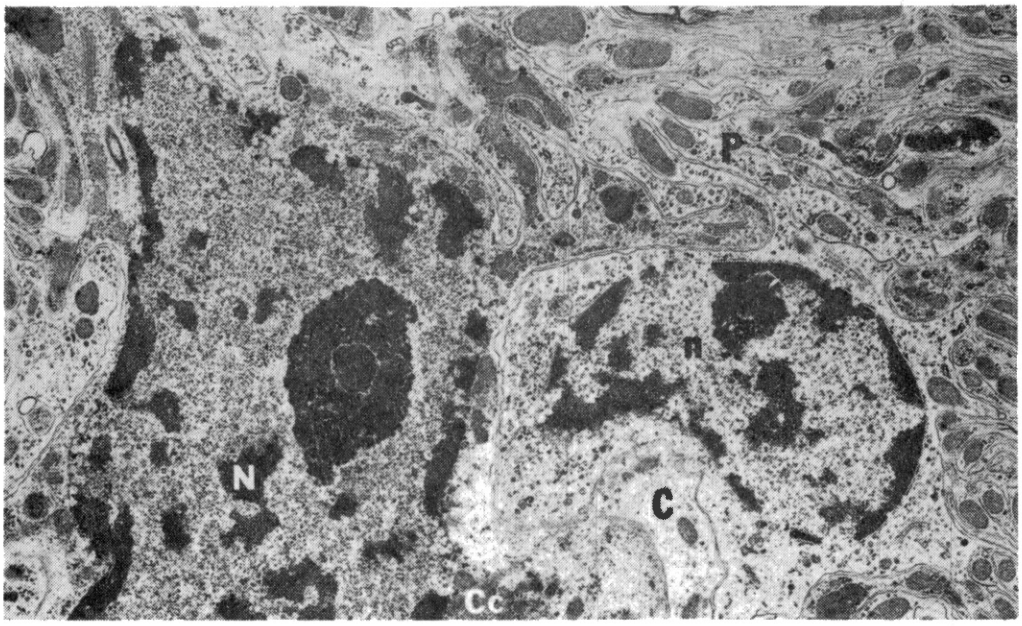
100-300 mg

Type I. The morphology of Type I alveoli in female ticks weighing 100 to 300 mg was different from that seen in the same cells of Type I alveoli in other stages of tick feeding. The membranous network of pyramidal cells was less distinct in relationship to its surrounding cytoplasm. Basal membrane infoldings with closely associated mitochondria were less identifiable (Fig. 13) and swirling membranes more numerous (Fig. 14). Numerous glycogen-like granules and mitochondria were embedded in the cytoplasm of the central cell. The "constrictor" cell nucleus was still present near the central cell nucleus and peritubular cell nuclei were conspicuous but their cell areas reduced (Fig. 15).

Type II. The differences between Type II alveoli in ticks having fed for 24 hours and 100-300 mg females were marked. A large membranous labyrinth with closely

Figure 11. An electron micrograph of a Type I alveolus from a 48 hr feeding female tick. A nucleus (n) in the apical region of a pyramidal cell (P) was adjacent to the large heterochromatic nucleus (n) of the central cell (c). Lipid-like droplets were rarely seen in Type I alveoli of 48 hr feeding ticks. The constrictor cell can be seen on either side of the central cell. x5868.

Figure 12. An electron micrograph of a Type I alveolus from a 72 hr feeding female tick. The constrictor cell (C) was seen to partially extend around the nucleus (N) of the central cell (c). Nuclei (n) were seen at the apices of pyramidal cells (P) adjacent to the centrally located central cell. A portion of the cuticle-lined tubule can be seen. x5868.


P
n
N
C
Cc

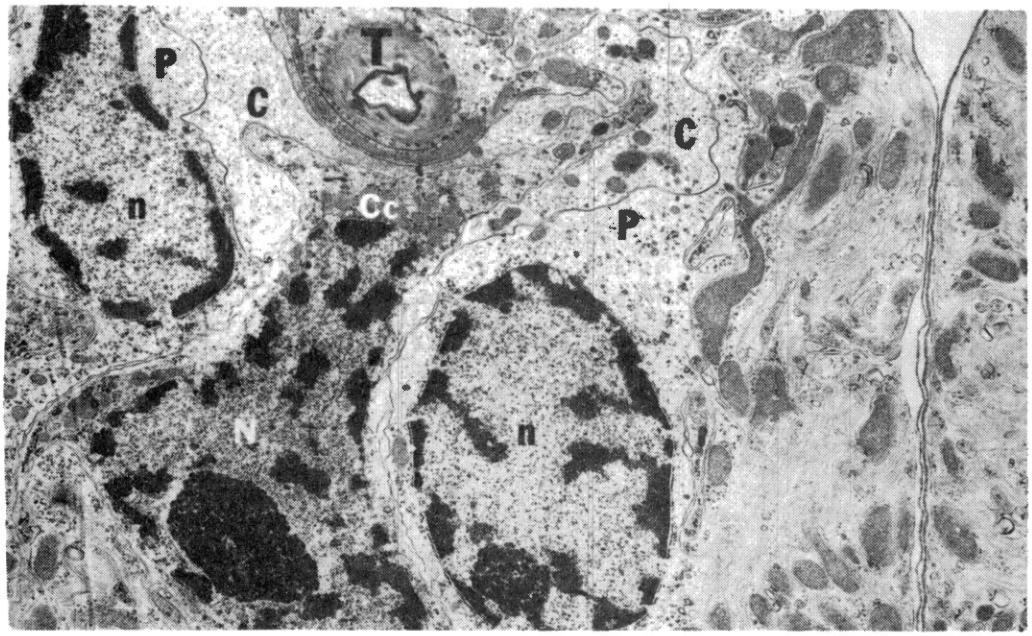
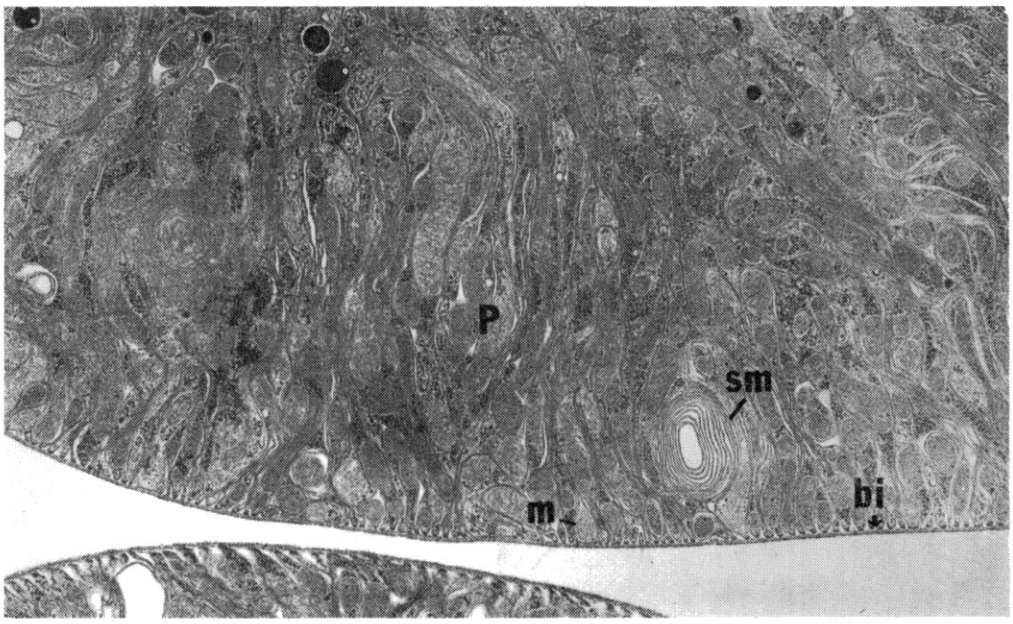

P
T
C
C
n
Cc
P
N
n

Figure 13. Electron micrograph of a Type I alveolus in a 100-300 mg feeding female tick. The pyramidal cell (P) containing basal infoldings (bi) with closely associated mitochondria (m) and swirling membranes (sm). x4238.

Figure 14. An electron micrograph showing a section of the Type I alveolus in a 100-300 mg. feeding female tick. A nucleus (N), glycogen granules (gg) and mitochondria (m) were seen within the borders of the central cell (Cc). p= pyramidal cell. x4238.


P
sm
m
bi

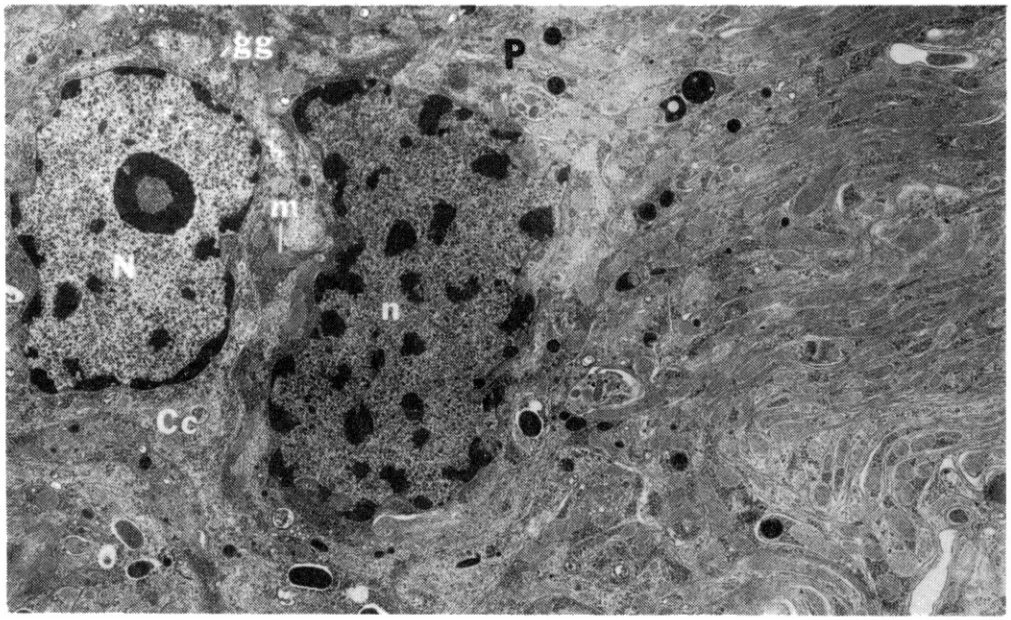
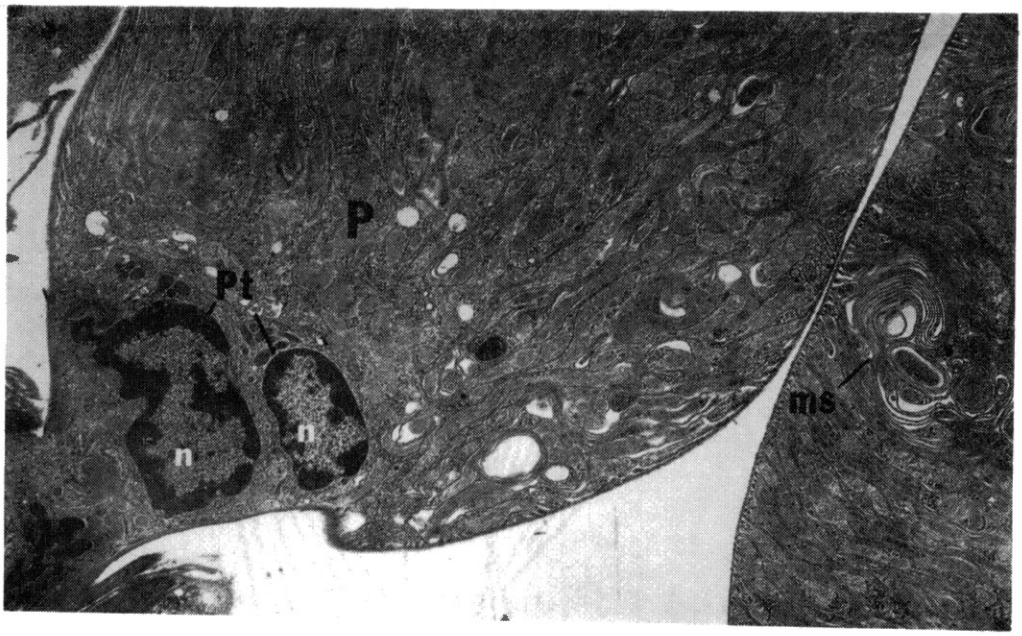
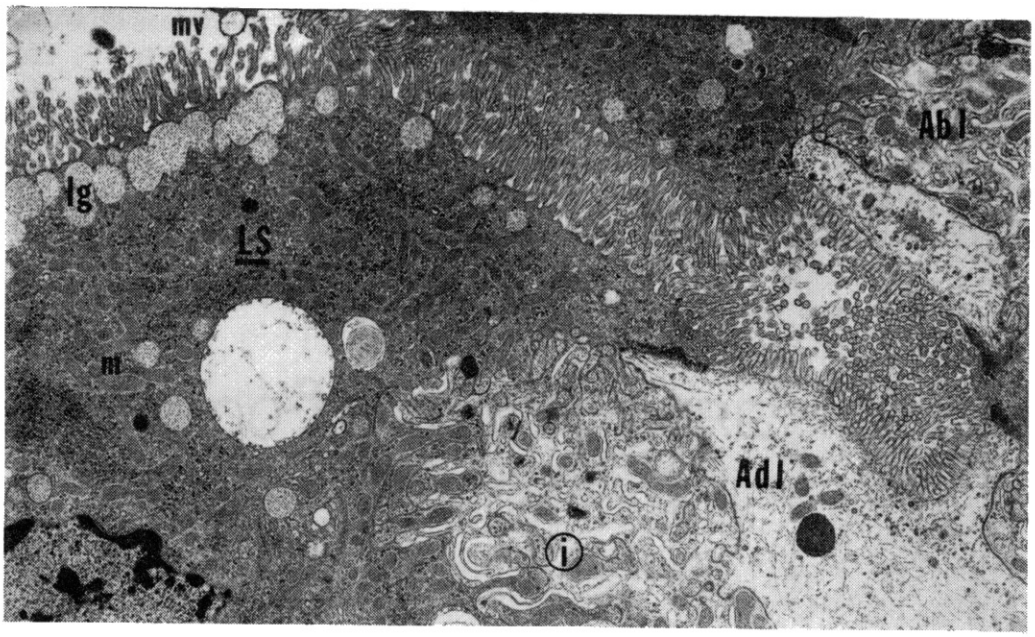

gg
P
m
N
n
Cc

Figure 15. An electron micrograph of a Type I alveolus On feeding female ticks weighing between 100-300 mg showing peritubular (Pt) cell nuclei (n) proximal to the salivary gland duct. Membrane swirls (ms) were also observed in the pyramidal cells. x4238.

Figure 16a. Electron micrograph of a Type II alveolus from a feeding female tick weighing between 100-300 mg showing interdigiting (i) lightly staining, simple granular (LS) cells and abluminal interstitial (AbI) cells. Many lysed granules (lg) were found in the lightly staining, simple granular cells (LS) of this stage of feeding. Numerous mitochondria (m) were seen in both the lightly staining, simple granular cell and the adjacent cap (AdI) cell There was an abundance of microvilli (mv) found on the apical side of both cap (AdI) and (LS) cells. x4238.


P
Pt
n
n
ms


mv
Abl
lg
LS
m
Adl
i

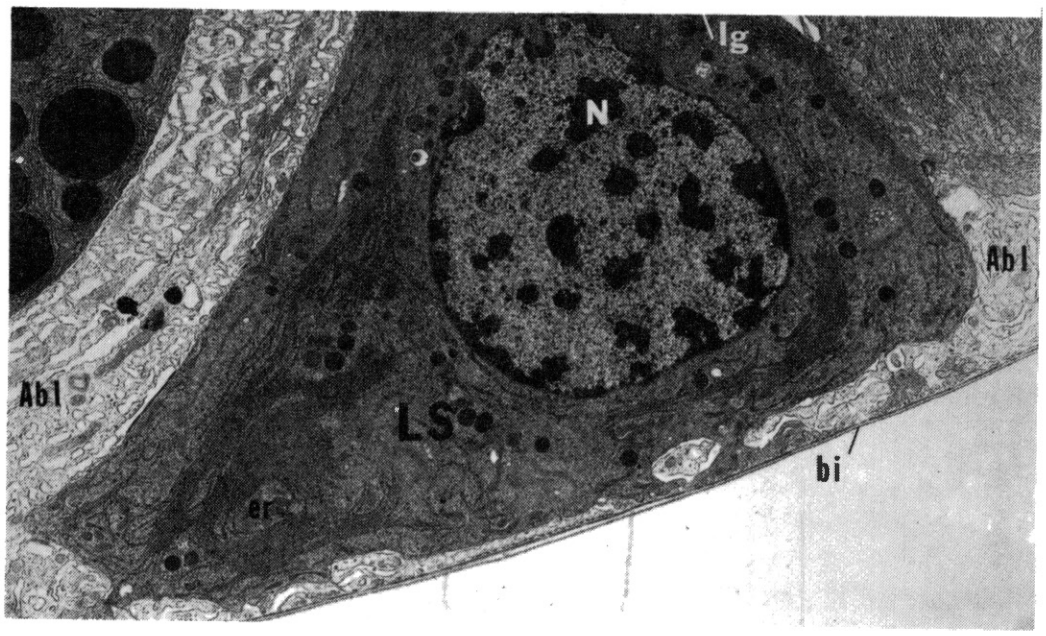
associated mitochondria and first described as "water" cells by Meredith and Kaufman (1973) was seen. Because of its location in relation to granular cells and the cap (AdI) cell, its derivation, at least in part, from abluminal interstitial (AbI) cells seems certain. Fawcett et al. (1981a,b) have recently drawn attention to the dual origin of these structures, namely (AbI) cells and projections of former granule containing cells. In support of these observations, interdigitating membranes originating from two cells in the membranous labyrinth were identified (Fig. 16a). The origin of membranes from one of these cells is a depleted lightly staining, simple granular cell and the other is an AbI cell. The depleted, lightly staining agranular cell is likely the vacuolar cell as described by Meredith and Kaufman (1973) and Megaw and Beadle (1979).

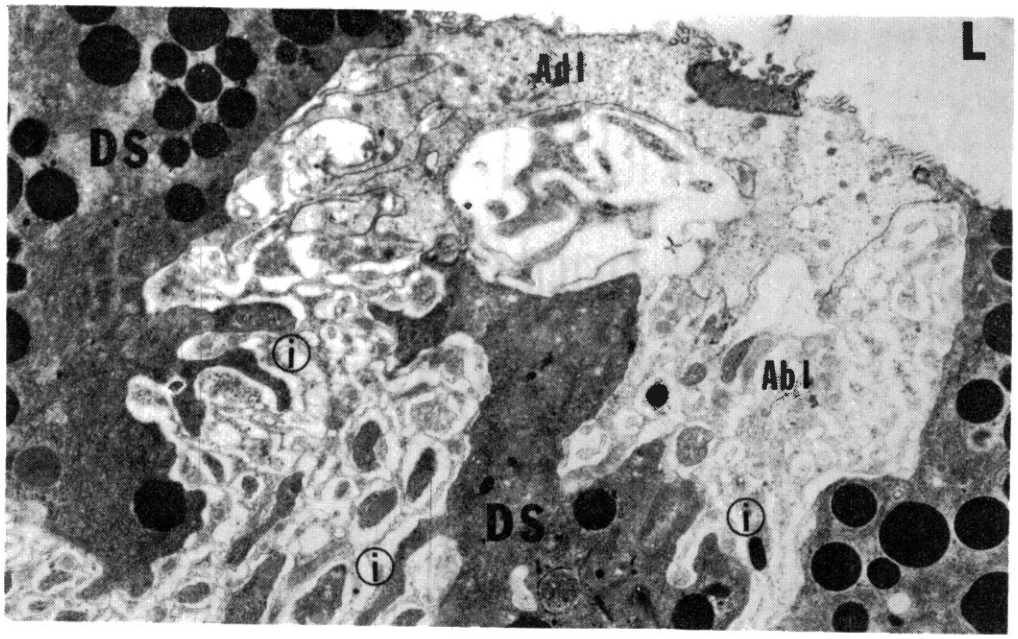
The membranous elements extend peripherally from the basal region of the cap (AdI) cell between lightly and darkly stained, simple granular cells to the basal lamina of the alveolus and in some locations completely envelopes granular cells (Figs 16b and 16c). The membranous region does not make direct contact with the lumen in regions of the cap (AdI) cell (Fig. 16a).

A nerve axon was found at the basal region of the cap (AdI) cell, near membranes of an AbI cell (Fig. 17). Numerous empty vesicles, ribosomes, mitochondria, and glycogen granules were seen in the cytoplasm of adjacent lightly staining, simple granular cells which interdigitated with

Figure 16b. Electron micrograph of the Type II alveolus from a 100-300 mg feeding female tick showing the membranous elements within the abluminal interstitial cells (AbI) extending around the basal region of the lightly staining, simple granular cells (LS) close to the basal lamina (Bl) of the alveolus. A few lysed granules were seen within the lightly staining, simple granular cell. x4238.

Figure 16c. Electron micrograph of a Type II alveolus from a feeding 100-300 mg female tick showing regions of interdigitations (i) between the abluminal interstitial (AbI) cells and adjacent darkly staining (DS), simple granular cells The cap (AdI) cell can be seen with its basal extensions interdigitating with the abluminal interstitial (AbI) cells. L= alveolar lumen. x4238.


lg
N
Abl
Abl
LS
bi
er


L
Adl
DS
Abl
DS
i
i
i

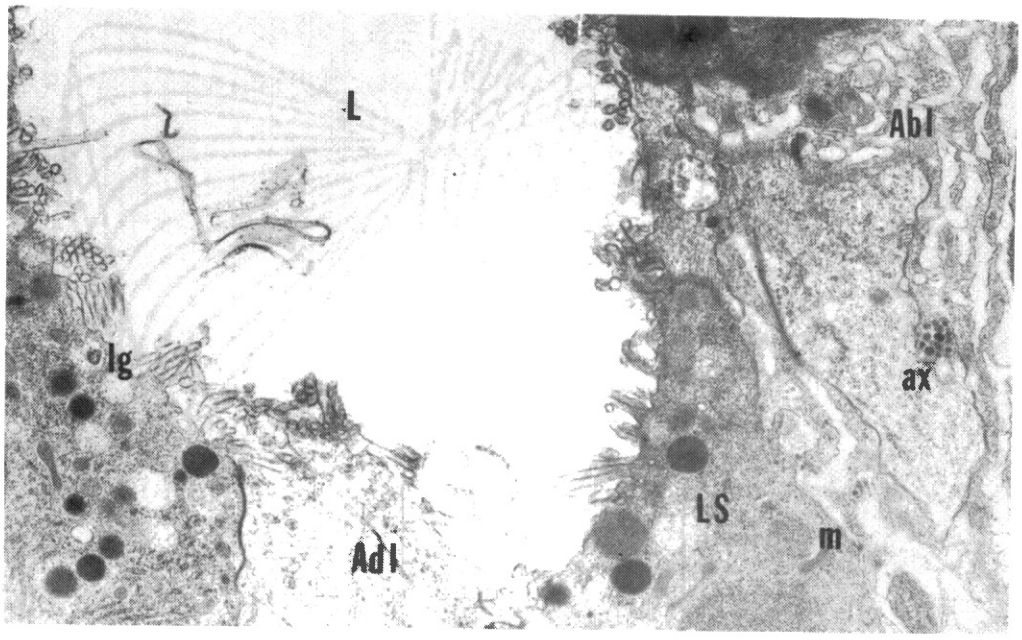
the AbI cells, forming part of the large membranous labyrinth. Regions of swirling membranes, similar to the swirling membranes seen in Type I alveoli, were observed in several locations. The concentric arrangement of membranes increased as the tick continued to feed.

During this period of feeding, concurrent transformations of the lightly stained, simple granular cells were seen. Some granules located in the apical region of this cell appeared to be in final stages of lysis (Fig. 17). Empty vesicles appeared near granules undergoing degradation. Numerous granules were seen on the apical side of lightly stained, simple granule cells and appeared about to be released from between microvilli into the lumen (Fig. 18). In addition to granules, numerous mitochondria, rough endoplasmic reticula, free ribosomes and a few Golgi complexes were observed in these cells and their nuclei possessed a characteristic heterochromatic pattern.

Despite the remarkable changes occurring within the Type II alveolus, the cap (AdI) cell remained firmly associated with the adjacent granular containing and granular depleted cells. The nexus junctions between the apical region of granular cells and cap (AdI) cell remained intact (Fig. 19). Microtubules, mitochondria, ribosomes, vesicles and glycogen deposits were present in this cell. The luminal area of the alveolus increased causing the elongation of the cap (AdI) cell (Fig. 20). At the same time, the finger-like basal extensions of the cap (AdI) cell were

Figure 17. An electron micrograph of a Type II alveolus of a 100-300 mg feeding female tick weighing between showing two alveolar cell types in contact with the alveolar lumen (L) and one cell in close proximity. The lightly staining (LS), simple granular cells in contact with the lumen possessed both intact and lysed granules (lg). The cap (AdI) cell is adjacent to the lightly stained simple granular cells in contact with the lumen, possessing numerous microtubules. A nerve axon (ax) can be seen between interstitial cell (AbI). The AbI cell, in close proximity to the lumen is seen to possess many mitochondria (m). x4238.

Figure 18. Electron micrograph of a Type II alveolus of female weighing 100-300 mg with numerous granules in lightly stained (LS), simple granular cells at its apical surface. mv= microvilli. N-nucleus. x5868.


L
Abl
lg
ax
LS
m
Adl

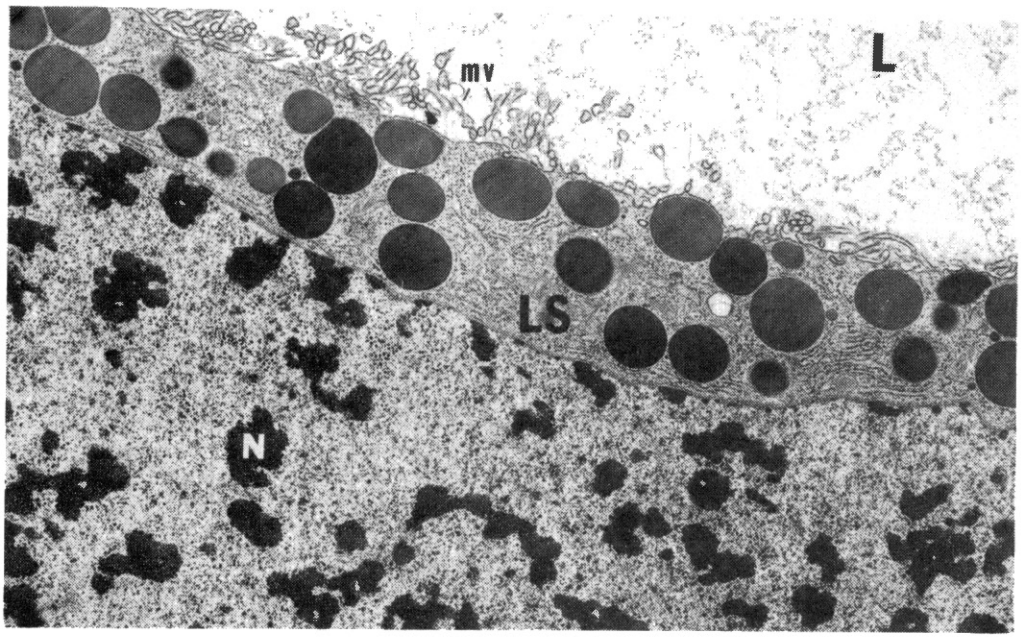
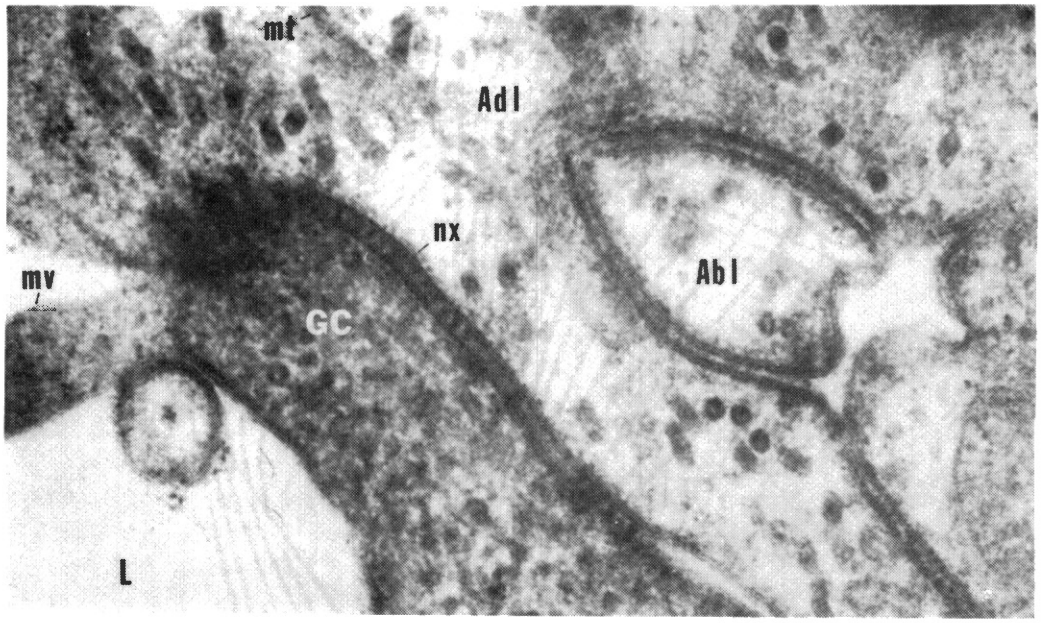

mv
L
LS
N

Figure 19. An electron micrograph showing a nexus junction (nx) at the apical region of a granular cell (GC) and the cap (AdI) cell of a Type II alveolus of a 100-300 mg feeding female tick. mv= microvilli, L-lumen; AbI- abluminal interstitial cell; mt-mitotubules. x66,830.

Figure 20. An electron micrograph showing the elongated, microvillate (mv) cap (AdI) cell as it bordered the lumen (L), in a Type II alveolus of a feeding female tick weighing between 100-300 mg. Both the lightly staining (LS), simple granular cell, depleted of granules, and the abluminal interstitial cell (AbI) exhibit a tremendous membranous labyrinth with numerous mitochondria and vesicles. x5868.


mt
Adl
nx
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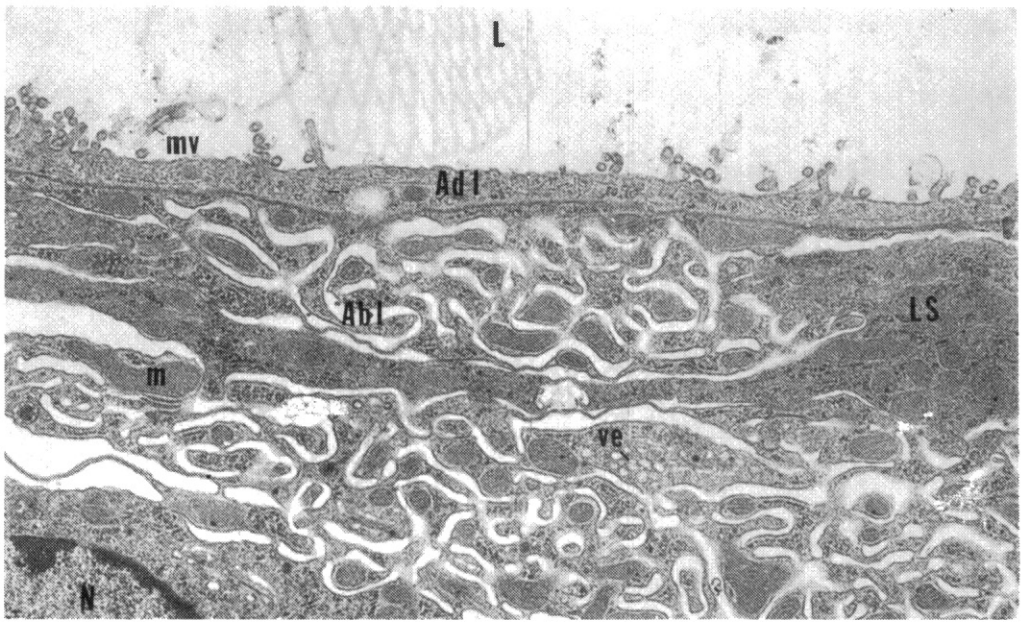
mv
GC
L


L
mv
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ve
N

attenuated. The cap (AdI) cell also possessed a large number of microvilli around the circumference of the lumen.

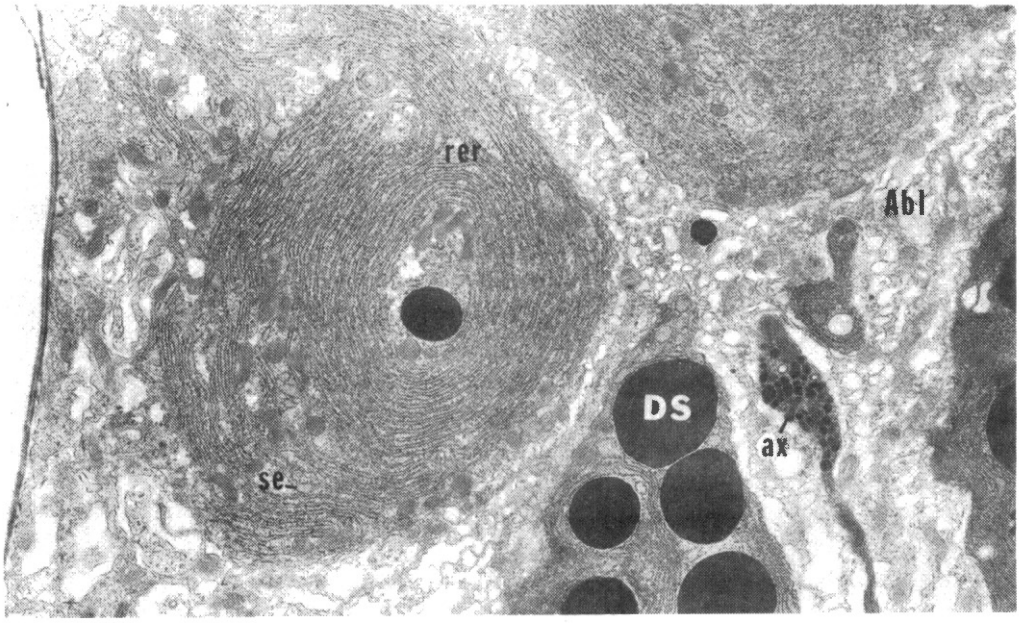
Darkly stained, simple granular cells in Type II alveoli were large. An occasional granule in these cells appeared in the process of being lysed. An extensive network of rough endoplasmic reticulum was present, some of which appeared swollen (Fig. 21). Mitochondria could be seen in basal and apical regions of the cell. Prior to secretion, the granules accumulated at the apical border of the cell (Fig. 22).

The complex granular cells of Type II alveoli were reduced in number of granules and overall area (Fig. 23). The granules were seen undergoing change with regard to subunit structure. In later stages of feeding, the subunits began to coalesce and disappear but mitochondria, empty vesicles and heterochromatic nuclei could be seen (Fig. 23 and 24).

Type III. The overall appearance of Type III alveoli in females of this weight suggested even more cellular lysis and reduction in cell constituents than that seen in Type II alveoli. The lightly stained, simple granular material was mostly secreted (Fig. 25). Mitochondria, a few granules, rough endoplasmic reticula, and some empty vesicles were still present in these cells. Discernible lightly stained, simple granular cells were reduced in size, had numerous microvilli and were found in close association with the lumen. An extensive labyrinth of membranes derived from AbI

Figure 21. Electron micrograph of a darkly staining (DS), simple granular cell as seen in a Type II alveolus of a feeding female tick weighing between 100-300 mg, showing an extensive network of rough endoplasmic reticulum (rer). Some of the rer is swollen (se). A nerve axon (ax) can be seen in close association with the abluminal interstitial cell (AbI). x7335.

Figure 22. Electron micrograph of a darkly staining, simple granular cell (DS), as seen in a Type II alveolus of a feeding female tick weighing between 100-300 mg. Numerous granules can be seen lining, in close association with microvilli (mv), the apical region of a darkly staining (DS), simple granular cell as it borders the lumen (L). AbI-abluminal interstitial cell. m-mitochondria. x5868.


rer
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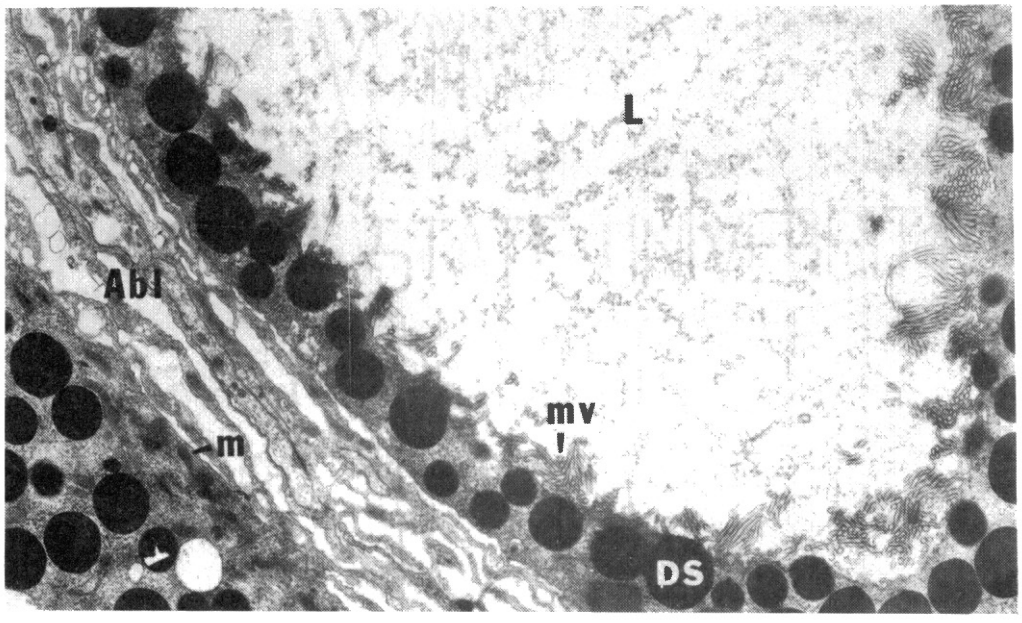
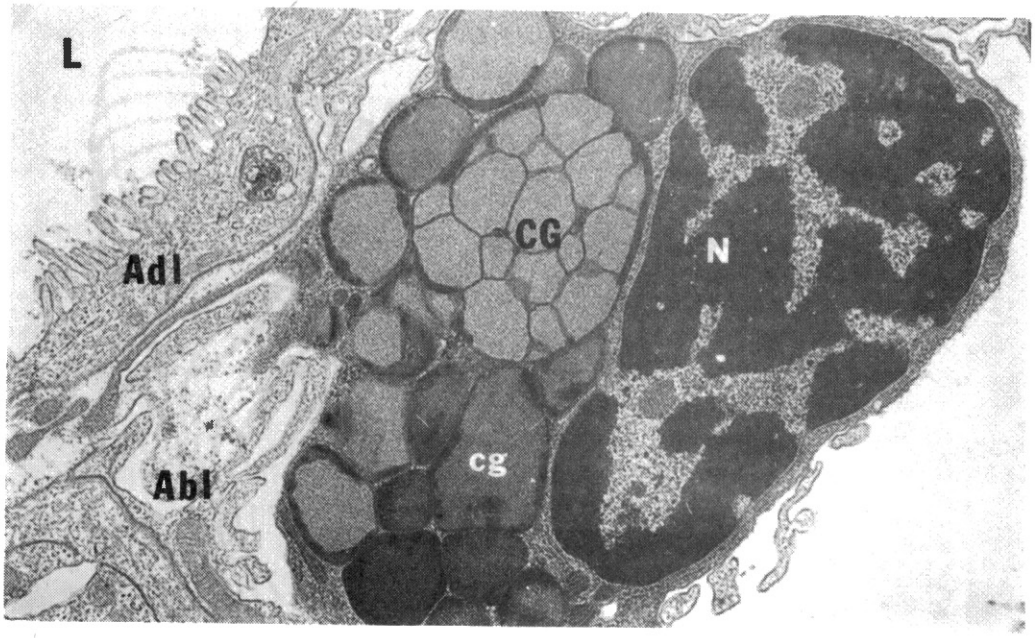

L
Abl
mv
m
DS

Figure 23. Electron micrograph of a complex granular cell (CG) as seen in the Type II alveolus of a feeding female tick weighing between 100-300 mg. The granular subunits had begun to coalesce (cg) at this stage of feeding with some granules undergoing lysis. Both the cap (AdI) and abluminal (AbI) cells can be seen. x5868.

Figure 24. Electron micrograph showing the coalescence and lysis of granular subunits (lcg) in a complex granular cell (CG) of Type II alveolus from feeding female ticks weighing 100-300 mg. Mitochondria (m) and empty vesicles (ev) can be seen in spaces among granular subunits. AbI-abluminal interstitial cell. x9517.


L
Adl
CG
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cg
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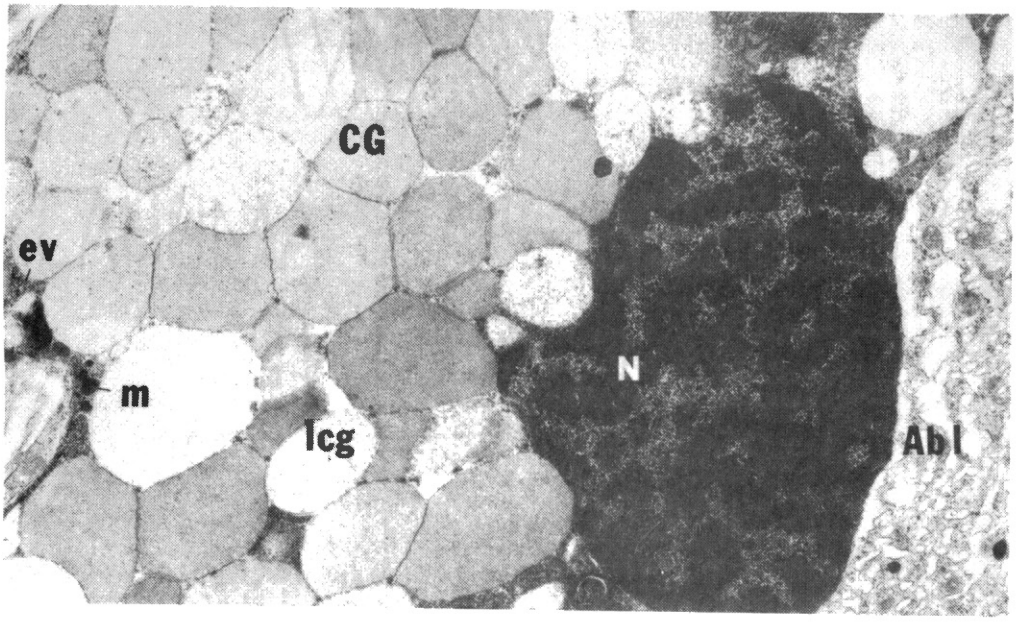
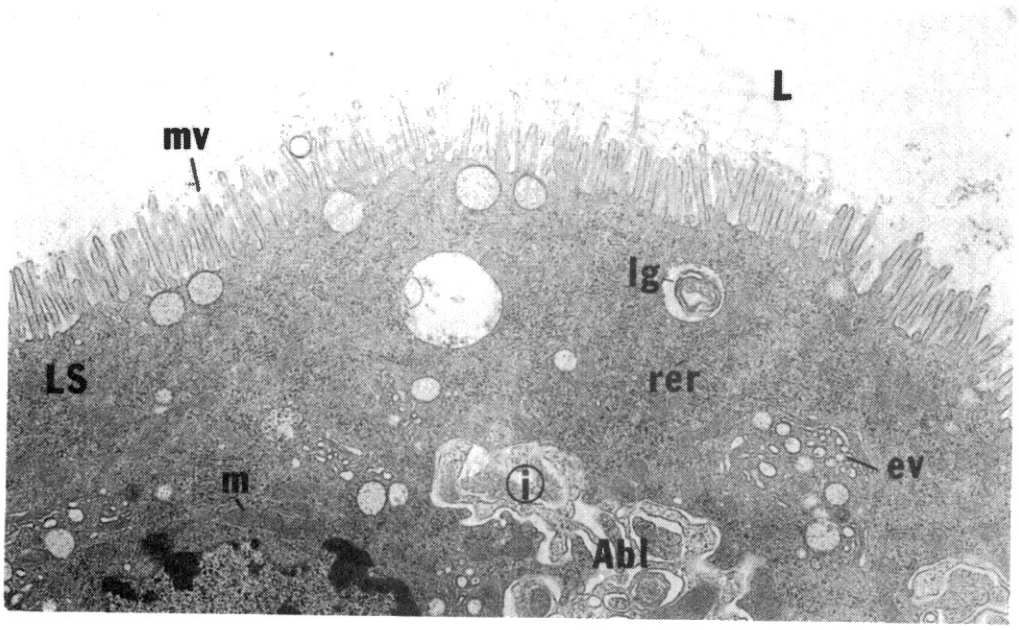

CG
ev
m
lcg
N
Abl

Figure 25. An electron micrograph of a Type III alveolus of a feeding female tick weighing 100-300 mg showing a (LS) simple granular cell depleted of granules and proliferating membranes interdigitating (i) with membranes from an abluminal interstitial (AbI) cell. The lightly staining, simple granular cell (LS) has lysed granules as indicated by remnants of granular membranes, and granules in the apical region in the process of being lysed (lg). Many empty vesicles (ev) from a Golgi complex can also be seen throughout the cell. Mv-microvilli, L-lumen. x5868.

Figure 26. An electron micrograph of a lightly staining (LS), simple granular cell in close association with the cap (AdI) cell via a nexus junction (nx) in a Type III alveolus of a female weighing between 100-300 mg. Near to the cap (AdI) cell are membrane swirls (ms) characteristically seen in the abluminal interstitial (AbI) cells. L-lumen, mv-microvilli. x5868.


L
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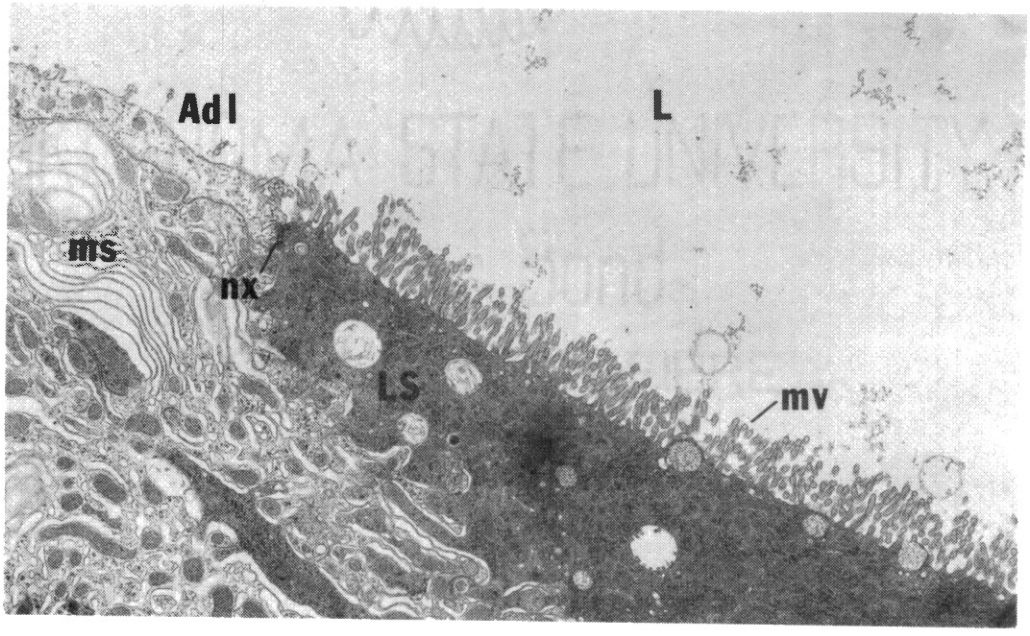
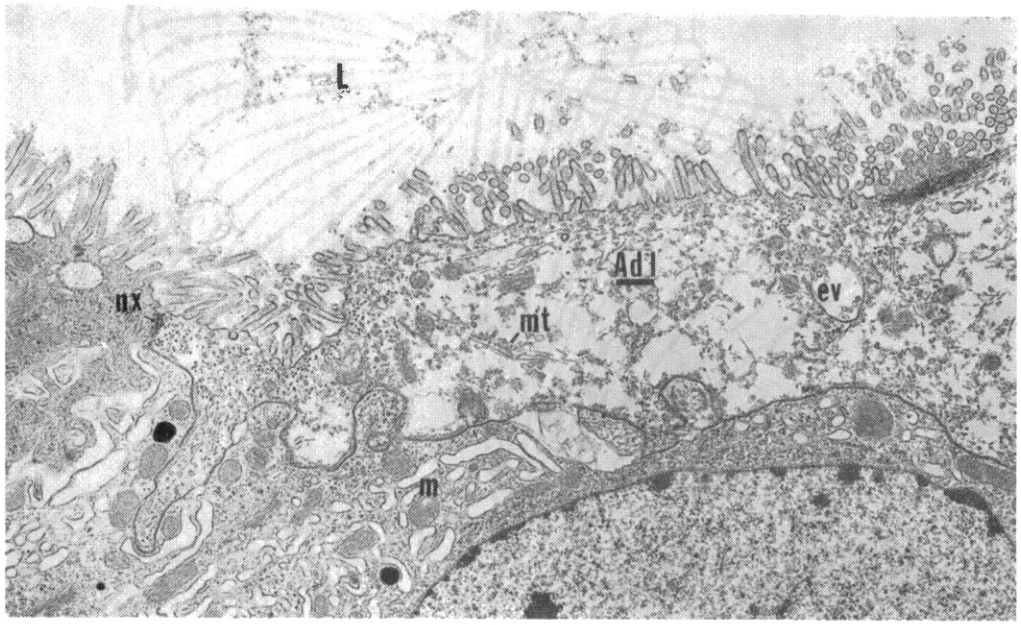

Adl
L
ms
nx
LS
mv

cells and depleted, lightly staining, simple granular cells was also seen forming the surface of the basal lamina of the alveolus (Fig. 25). AbI cells were separated from the lumen by regions of cap (AdI) cells and some granular cells (Fig. 26). Nexus junctions were observed between the apical regions of cap (AdI) cells and granular cells and the cap (AdI) cells appeared less dense (Fig. 27); the latter condition was not observed in the cap (AdI) cell of Type II alveoli. Numerous microtubules, mitochondria and empty vesicles were also seen in the cap (AdI) cell (Fig. 27). Canaliculi, akin to bile canaliculi seen between adjacent parenchymal cells in the vertebrate liver (Matter et al., 1969) were associated with the cap (AdI) cell and some lightly stained, simple granular cells (Fig. 28).

The darkly stained, simple granular cell had not yet undergone extensive morphological changes. Granules were present along with free ribosomes, mitochondria and a small amount of rough endoplasmic reticula (Fig. 29). An apparent reduction in numbers of granules in complex granular cells as compared to the complex granular cell in Type III alveoli of the 24 hour feeding ticks was observed. However, lysis of the complex granules appeared to be underway.

Figure 27. Electron micrograph of a cap (AdI) cell with numerous microtubules (mt) and vesicles (ve) in a Type III alveolus of the. The cap (AdI) cell is in close association with a lightly staining, simple granular cell via a nexus junction (nx) as interdigitates with a nearby abluminal interstitial cell. M-mitochondria. x4238.

Figure 28. An electron micrograph of a Type III alveolus of a female weighing 100-300 mg illustrating an alveolar canaliculus (Can) formed at the basal region of a lightly staining, simple granular cell (LS) with an extension of the cap (AdI) cell. ax-nerve axon. (AbI)- abluminal interstitial cell. x5868.


L
nx
Ad l
mt
ev
m

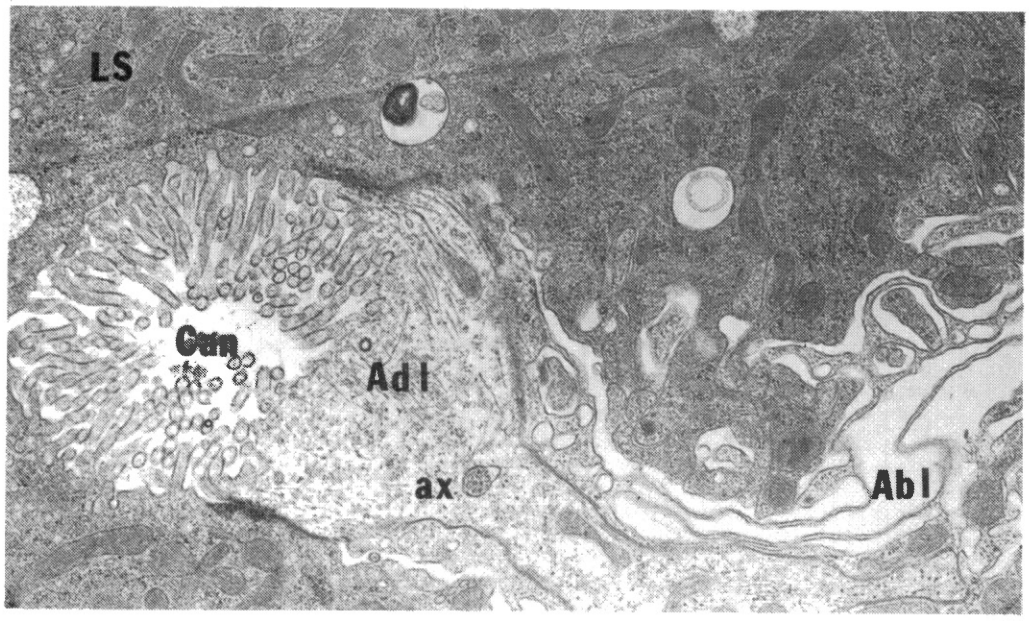
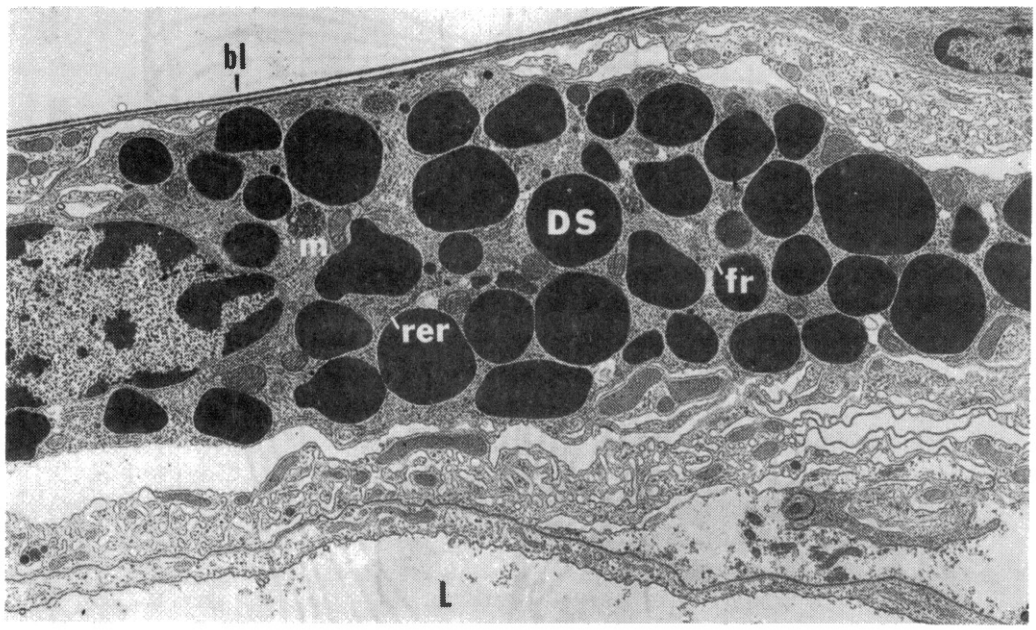
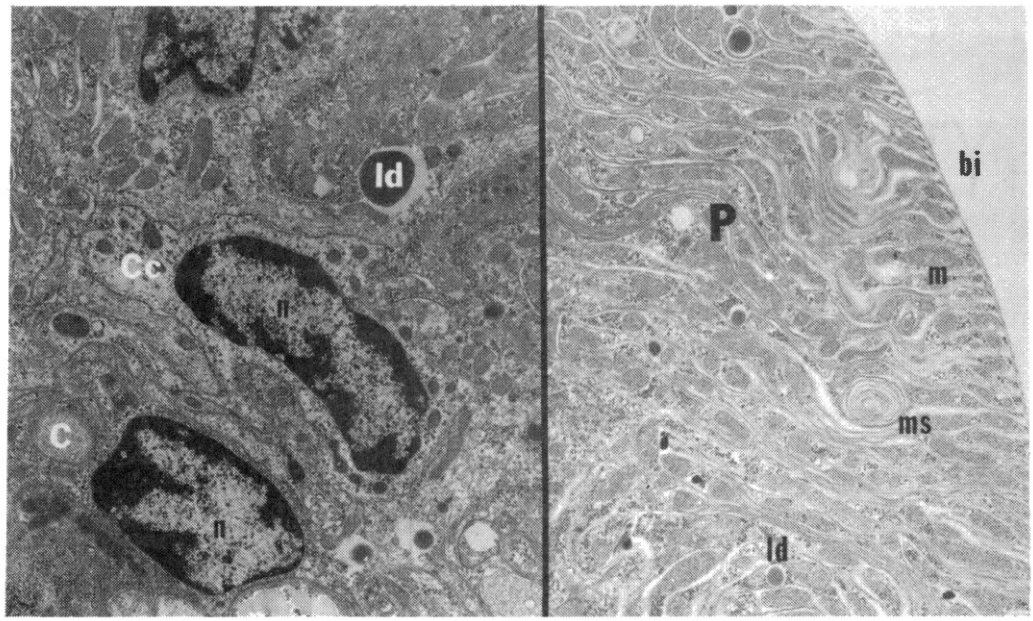

LS
Cun
Ad l
ax
Ab l

Figure 29. Electron micrograph of a Type III darkly staining, simple granular cell (DS) from a feeding female tick weighing between 100-300 mg. An apparent reduction in granular cell size had occurred by this stage of feeding. Free ribosomes (fr), rough endoplasmic reticulum (rer) and mitochondria (m) can also be seen. L - lumen, bl - basal lamina. x4238.

Figure 30a. Electron micrograph of a Type I alveolus of a feeding female tick weighing 500 mg. More than illustrating the constrictor cell (Cc), central cell (c) with nucleus (n) and nearby lipid-like droplet (ld). x4238.

Figure 30b. Electron micrograph of a Type I alveolus from a feeding female tick weighing more than 500 mg. The pyramidal cell (P) had numerous basal infoldings (bi) with closely associated mitochondria (m) and many membrane swirls (ms). An occasional lipid-like droplet (ld) was seen in the peripheral region of the pyramidal cell. x4238.


bl
DS
m
fr
rer
L


ld
Cc
n
C
n
P
bi
m
ms
ld

≥ 500 mg

Type I. Remnants of a constrictor cell were seen adjacent to the central cell nucleus (Fig. 30a) and peritubular cells about the peritubular lumen were still seen.

The membranes of pyramidal cells of Type I alveoli were more condensed in salivary glands of ticks weighing > 500 mg than that seen in Type I alveoli of ticks in earlier phases of feeding.

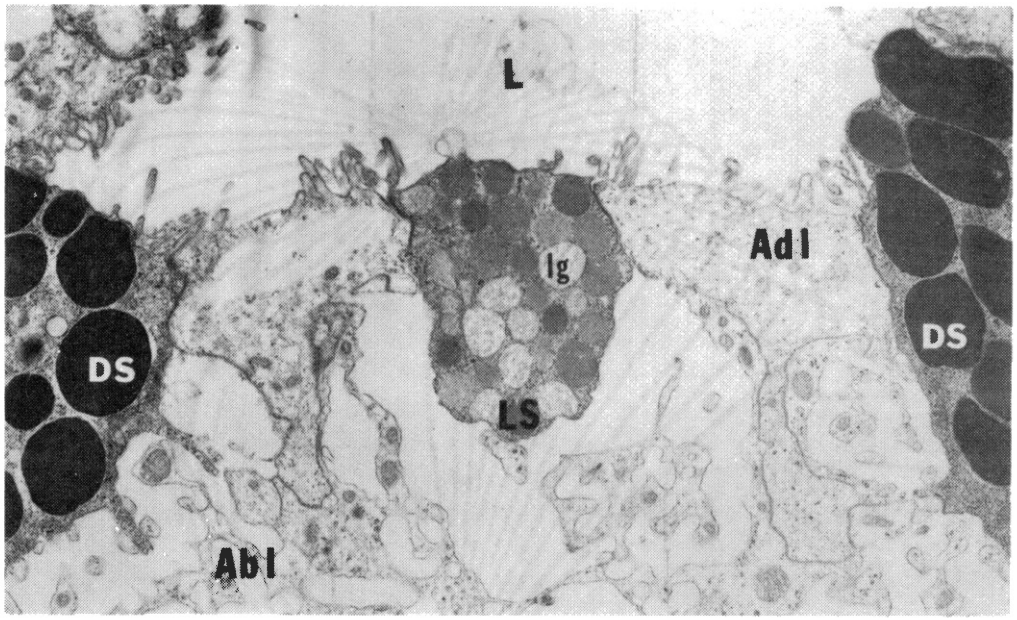
Numerous basal infoldings, closely associated mitochondria and an occasional lipid-like droplet were seen in the pyramidal cells (Fig. 30b). Free ribosomes were quite numerous and nuclei had a heterochromatic pattern.

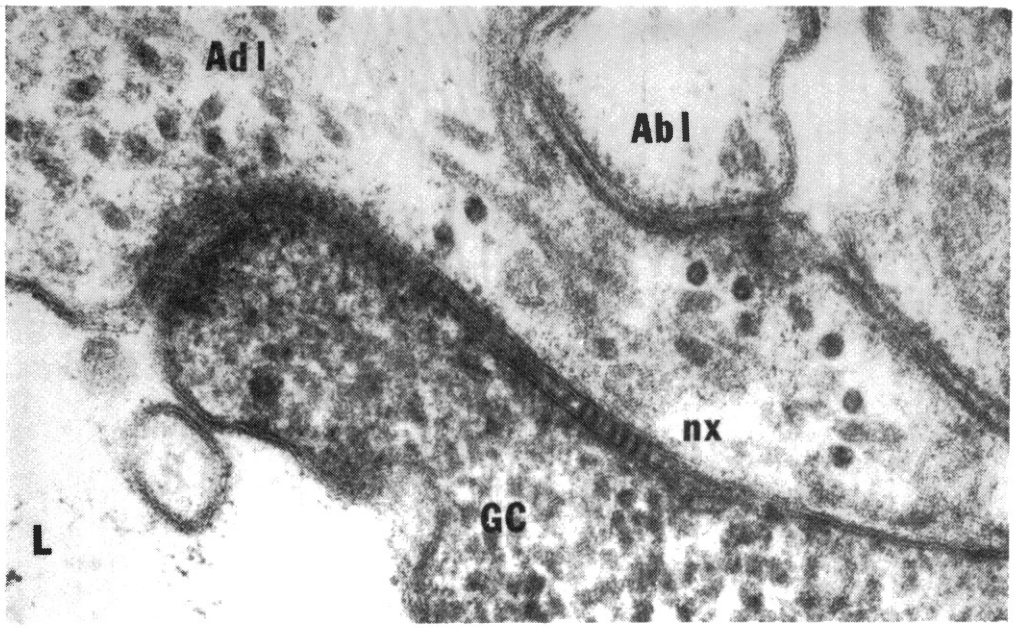
Type II. Type II alveoli as seen at this stage of feeding varied considerably in state of development suggesting that not all alveoli secrete at the same rate during the feeding process. By examining lightly stained, simple granular cells one sees some Type II alveoli with obviously stained, simple granular cells, other alveoli with cells having only a few intact granules and others with partially lysed granules (Fig. 31).

The cap (AdI) cell, remained unchanged in location and cellular morphology as compared to that seen in Type III alveoli of ticks in earlier phases of feeding. Nexus junctions between the cap (AdI) cell and adjacent granular cells were present (Fig. 32). Nerve axons were observed in close association with the cap (AdI) cell and the AbI, depleted

Figure 31. An electron micrograph of a Type II alveolus of a feeding female tick weighing more than 500 mg showing the relationship between the cap (AdI) cell a lightly staining (LS), simple granular cell , two darkly staining (DS), granular cells and the abluminal interstitial cell (AbI). The lightly staining granular cell (LS) was reduced in size with lysed granules (lg). L - lumen. x5868.

Figure 32. An electron micrograph of a Type II alveolus from a female weighing greater than 500 mg showing a nexus junction (nx) between a granular cell (CG) and the cap (AdI) cell and the abluminal interstitial cell (AbI). L - lumen. x66,830.


L
Adl
lg
DS
DS
LS
Abl


Adl
Abl
nx
GC
L

granular cell membranous labyrinth (Fig. 33). Complex granular cells and usually one of the two darkly staining, simple granular cells were still seen intact during this phase of tick gland development. The interdigitations of membranes between juxtaposed depleted granular cells were more numerous and tortuous than those seen in earlier stages of tick feeding (Fig. 34).

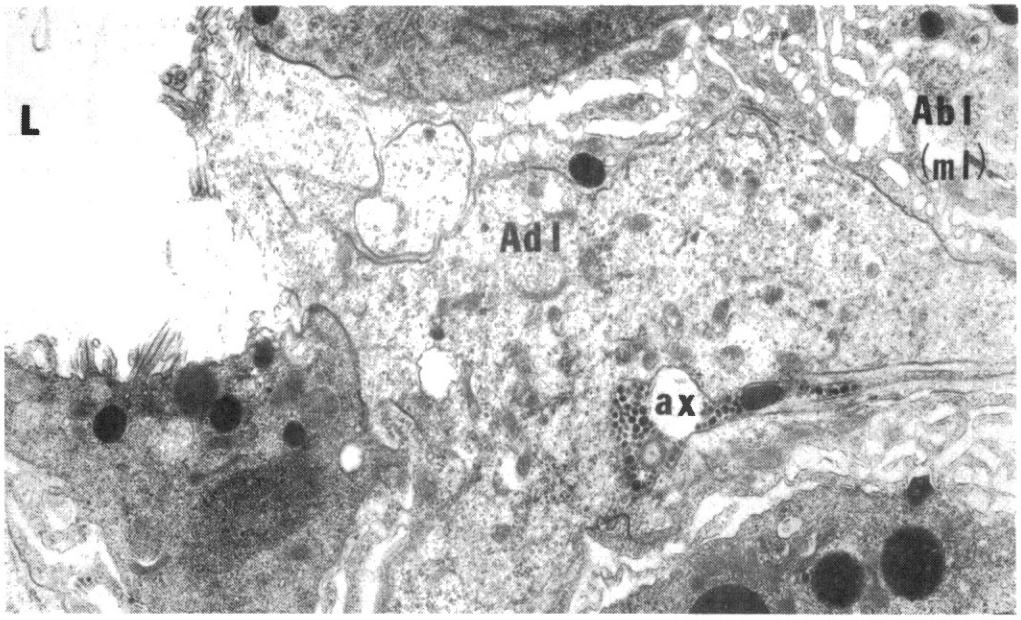
During the final stages of Type II alveolar development the luminal area was greatly enlarged. A few complex and darkly stained, simple granular cell types were part of alveolar morphology along with numerous, tortuous membranes and closely associatd mitochondria. Remnants of former granular cells, now agranular in appearance, were visible, especially near the alveolar lumen. The cap (AdI) cell was much less conspicuous about the lumen (Fig. 35).

Complex granular cells were reduced in size and quantity of granules. Complex granules were distinguishable from adjacent simple granular cells because a few still possessed subunits (Fig. 26). An occasional mitochondrion and some rough endoplasmic reticulum were located about each cell's nucleus (Fig. 36).

The darkly stained, simple granular cells possessed some intact granules. Its cytoplasm became less distinct, being penetrated by membranes from AbI cells or depleted lightly stained granular cells within its borders (Fig. 36). Cellular organelles such as mitochondria and rough endoplasmic reticula were present (Fig. 37). Darkly stained,

Figure 33. An electron micrograph of a Type II alveolus of a female weighing in excess of 500 mg showing a nerve axon (ax) as seen in close association with both the adluminal (AdI) and abluminal (AbI) interstitial cells. At this stage of feeding an extensive membranous labyrinth (ml) originted from lightly staining, simple granular cells and abluminal interstitial cells (AbI). x5868.

Figure 34. Electron micrograph of a Type II alveolus of a female weighing more than 500 mg. A darkly staining (DS), simple granular cell had regions of close association (i) with the swirling membranes (sm) of the abluminal interstitial (AbI) cell The cap (AdI) cell is elongated as it borders the alveolar lumen (L). Some granules of the (DS) cell appeared lysed (lg). x4238.


L
Ab I
(m I)
Ad I
ax

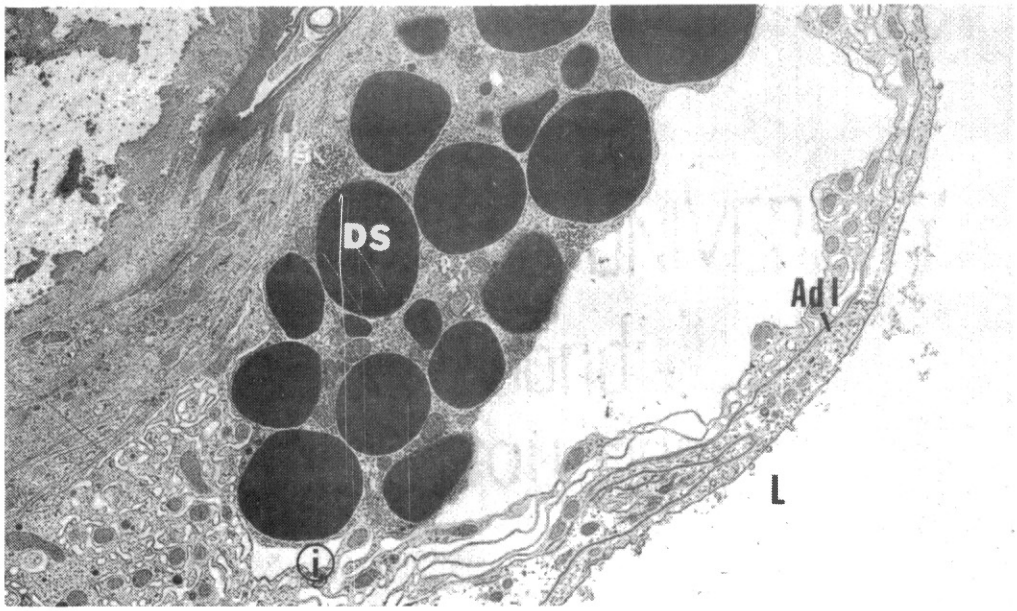
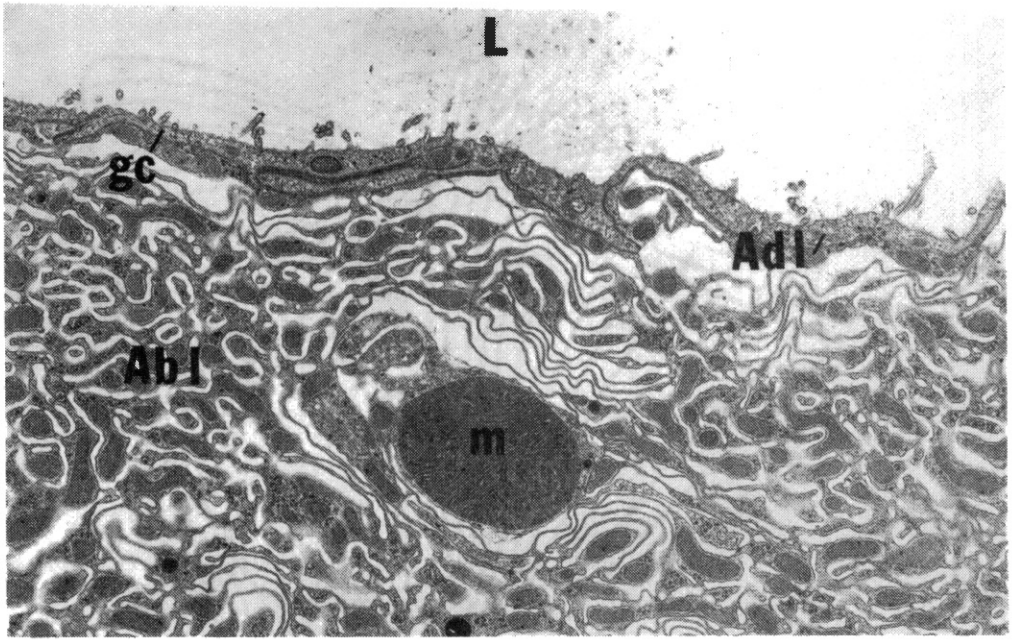

DS
Ad I
L
i

Figure 35. An electron micrograph of a Type II alveolus of a female weighing more than 500 mg showing a more advanced phase of the membranous labyrinth, with numerous mitochondria (m). A much depleted granular cell (gc) and cap (AdI) cell were seen as slender, elongated cells lining the apices of the (AbI) cell. L - lumen. x4238.

Figure 36. Electron micrograph of a Type II alveolus of a female weighing more than 500 mg. The complex granular cell (CG) contained few granules. Granules no longer possessed the subunits but had coalesced (cg) or were lysed. Mitochondria (m) and rough endoplasmic reticulum (rer) were, observed in the cell. The complex granular is completely surrounded by the membranous labyrinth. An adjacent darkly staining, simple granular cell (DS) was apparent. AbI-abluminal interstitial cell. N-nucleus. x5868.


L
gc
Adl
Abl
m

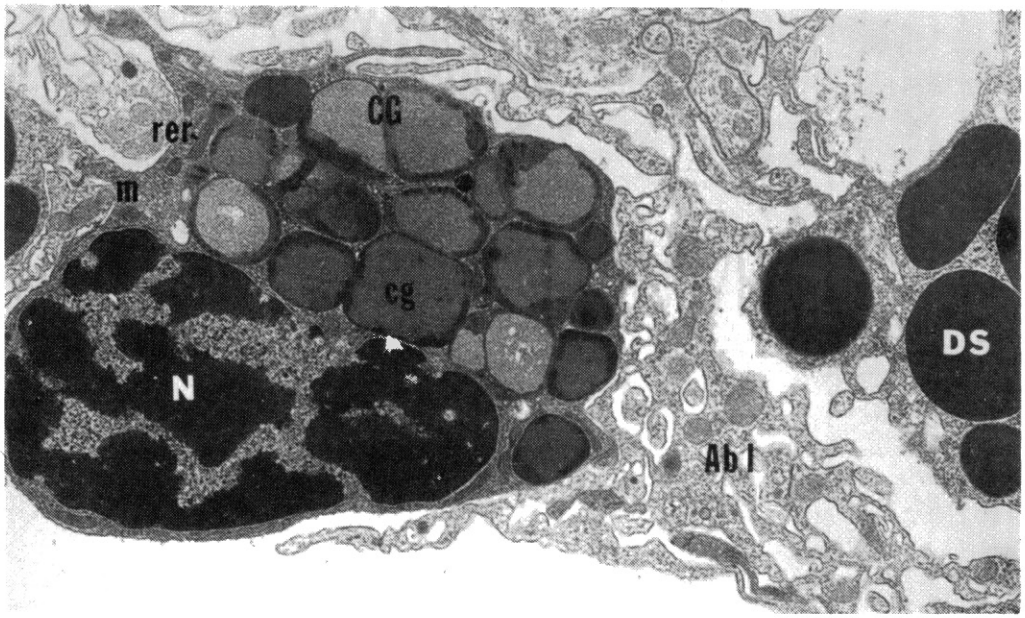
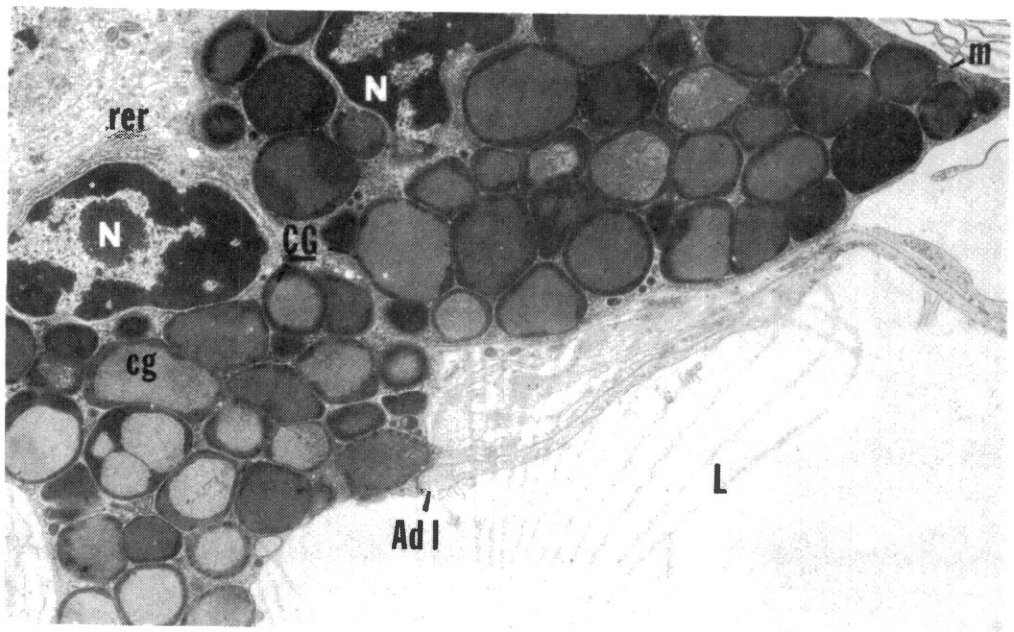

rer
CG
m
cg
N
DS
Abl

Figure 37. An electron micrograph of a Type II alveous of a female weighing more than 500 mg. The two complex granular cells (CG), which are seen to possess a nucleus (N), mitochondria (m) and some rough endoplasmic reticulum (rer), are adjacent to one another and juxtaposed to the elongated, cap (AdI) cell. cg-coalescing granules, L-lumen. x4238.

Figure 38. An electron micrograph of a Type III alveolus of a female weighing more than 500 mg showing the vast membranous labyrinth surrounding remnants of the darkly staining, simple granular cell (DS-gc). A small basal extension of the cap (AdI) cell was observed. In addition, the complex granular cell (CG) had numerous coalescing granules in the final stages of lysis (lcg). m-mitochondria. x4238.


rer
N
m
N
CG
cg
L
Ad I

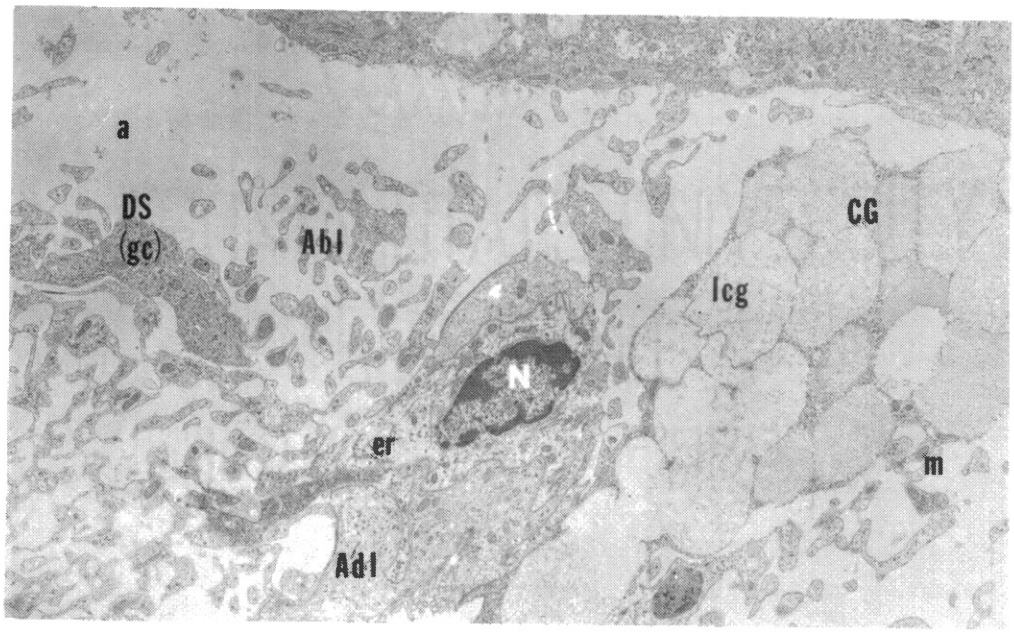
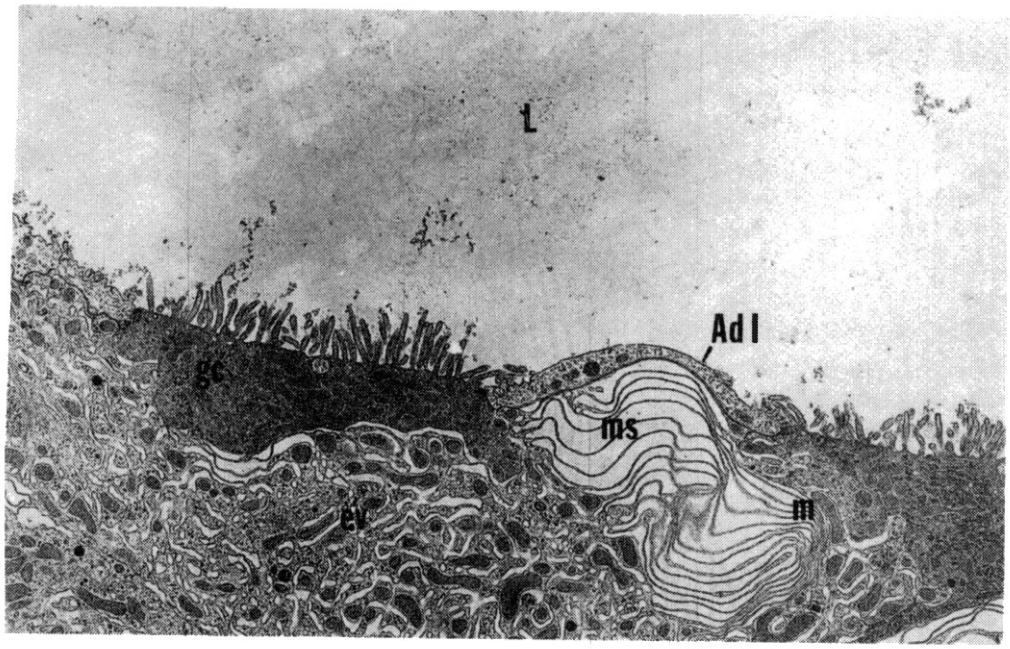

a
DS
(gc)
Ab I
CG
lcg
N
er
m
Ad I

Figure 39. An electron micrograph of a Type III alveolus of a female weighing more than 500 mg with a complex of membranous swirls (ms), with the abluminal interstitial cell (AbI), and distented cap (AdI) cell. In addition to these membranous swirls, empty vesicles (ev) were observed in the membranous labyrinth. Portions of microvillate granular cells (gc) were seen on either side of the (AdI) cell. L-lumen. x4238.


L
Ad I
gc
ms
ev
m

simple granular cells had completely lost their identity as seen in earlier stages of tick feeding and the cellular membranous labyrinth occupied regions of the alveolus previously occupied by the darkly stained, simple granular cells.

Type III. Type III alveoli were mostly a vast network of membranes from (AbI) cells and depleted granular cells in glands of females at this stage of feeding (Fig. 38). Mitochondria, empty vesicles and membrane swirls were observed in its cells (Fig. 39). The cap (AdI) cell appeared to lose some cellular integrity at its apical and basal sides (Fig. 39). Depleted granular cells were characterized by numerous mitochondria, vesicles, free ribosomes and rough endoplasmic reticula.

The darkly stained, simple granular cell and cap (AdI) cells were enveloped by the cellular membranous labyrinth peripherally. On the luminal side, parts of the cap (AdI) cell alternate with a darkly stained, simple granule cell (Fig. 38). Mitochondria as well as vesicles and free ribosomes were seen in the darkly stained, simple granular cells. Type III alveoli can be seen in various stages of development. Some alveoli contained more numerous complex granules with mitochondria and endoplasmic reticula while other Type III alveoli contained completely lysed complex granules (Fig. 38).

Discussion

Type I alveoli

Contrary to other reports (Binnington, 1978; Megaw and Beadle, 1979) in other species of ixodid ticks Type I aveoli in the salivary glands of female A. americanum changed during tick feeding.

The pyramidal cells of 24 hour feeding females had a characteristic membranous labyrinth similar to that seen in Type I alveoli of unfed females (Krolak et al., 1981). However, swirling membranes and a reduction in lipid-like droplets were seen after 24 hr feeding. The central cell was still quite conspicuous but more extensive finger-like cellular extensions interdigitating with the pyramidal cells to the basal alveolar lamina were more apparent than that seen in Type I alveoli of unfed females. Coalesced lipid droplets seen in the pyramidal cells of unfed females were seen in the central cell at this stage of female feeding.

An increase in swirling membranes of the pyramidal cells with an occasional lipid-like droplet were observed in females having reached a weight of 100-300 mg. Pryamidal cells appeared to be larger and the central cell contained only an occasional lipid-like droplet. The constrictor cell was present but reduced in size and three to four peritubular cells were present.

The pyramidal cells had swirling membranes, an occasional lipid-like droplet and an increase in area within Type I alveoli of female ticks weighing 500 mg or greater.

Elongated mitochondria were numerous and in close association with membranes of pyramidal cells and the central cell was peduced in size without lipid-like droplets. The constrictor and peritubular cells were present but reduced in size in the glands of females having reached this advanced stage of feeding

Type II alveoli

As in other species of ixodid females (Binnington, 1978; Megaw and Beadle, 1979; Fawcett et al., 1981a,b) Type II alveoli in A. americanum changed remarkably during tick feeding. The two complex granular cells on either side of the valve (Krolak et al., 1981) had increased in size by 24 hr of feeding. Spatial openings were seen between granules in the complex granular cells in addition to swollen endoplasmic reticula, numerous mitochondria and vesicles during the early stages of tick feeding.

The three lightly stained, simple granular cells showed no apparent changes in early stages of feeding (24 hr) with the exception of swollen endoplasmic reticula, indicating a more active cell, and an increase in mitochondria and vesicles. The abluminal interstitial (AbI) cells exhibited a proliferation of membranes with numerous mitochondria and the initial phases of close association of its membranes with those of proliferating membranes from lightly stained, simple granular cells in early stages of tick feeding.

The cap (AdI) cell changed little in early stages of

female feeding. By later stages of tick feeding (100-300 mg), granular material in complex granular cells had been reduced by secretion or utilization with some coalescence of granular subunits.

By later stages of tick feeding, granules in lightly stained, simple granular cells were reduced and in the final stages of lysis with more mitochondria, rough endoplasmic reticula and free ribosomes seen. An occasional Golgi complex was also seen.

The darkly staining, simple granular cells, revealed granules undergoing lysis and an extensive network of rough endoplasmic reticulum with no apparent increase in mitochondria in later stages of tick feeding.

By later stages of tick feeding, the AbI was characterized by a remarkable increase in size and membranes interdigitating with membranes of lightly stained granular cells.

By the last stage of tick feeding, (500 mg and greater) complex granular cells were reduced in size and quantity of granules present. Granular subunits were coalesced and their subunit appearance lost. Lightly staining, simple granular cells exhibited an almost complete loss of granular material. Canaliculi were observed near lightly staining, simple granular cells. Both the cap (AdI) and lightly staining, simple granular cells had numerous microvilli protruding into the lumen of the canaliculi. The association of membranes originating from lightly staining, simple granular cells and membranes from abluminal interstitial cells

were complex.

Type III alveoli

As in unfed females, Type III alveoli in feeding ticks had one complex granular cell. In early stages of feeding (24 hr) the cell was larger with considerable swollen endoplasmic reticula. Lightly stained, simple granular cells had granules in different stages of lysis with empty vesicles and swollen endoplasmic reticula present. A darkly stained, simple granular cell similar to that seen in unfed female ticks was seen with numerous mitochondria and swollen endoplasmic reticula. The cap (AdI) cell was similar to that seen in the unfed female (Krolak et al., 1981). Abluminal interstitial (AbI) cells, as in Type II alveoli, demonstrated the beginnings of membrane proliferation.

In ticks having fed longer (100-300 mg) the complex granular cell was reduced in size. At this stage, lightly stained, simple granular cells had changed considerably with only a few granules, mitochondria, rough endoplasmic reticula, and some empty vesicles present. Darkly stained, simple granular cells were reduced in size with fewer granules and numerous free ribosomes, mitochondria, and a small amount of rough endoplasmic reticula. The cap (AdI) cell was not changed as compared to that seen in earlier phases of feeding except for an attenuation of the basal extensions of the cell. Membranous elements of abluminal interstitial

(AbI) cells were remarkably extensive throughout the alveolus, interdigitating with closely associated lightly stained, simple granular cells.

In female ticks weighing 500 mg and greater, Type III alveoli showed the presence of one complex granular cell adjacent to the valve of the alveolus. The complex granular cell in ticks in the final stages of feeding (500 mg and greater) was reduced in size but with more granular material than other granular cell types. The cap (AdI) cell appeared to lose some cellular integrity and the enlarged abluminal interstitial (AbI) cell and proliferating membranes from previously lightly stained, simple granular cells constituted the bulk of Type III alveoli at this stage of feeding.

Comparative Interpretation of the Morphology of Female Ixodid Tick Salivary Glands

The Problem of Identifying Cell Types and Numbers

In recent years there have been several investigators have studied the morphology of female salivary glands in various species of ixodid ticks, Boophilus microplus (Binnington, 1978; Megaw and Beadle, 1979), Dermacentor andersoni (Meredith and Kaufman, 1973), Rhipicephalus appendiculatus (Fawcett et al., 1981a,b) and Amblyomma americanum (Krolak et al., 1981). These investigators

concur in finding three alveolar types and the region of the gland where the alveoli are located. Type I in the anterior region of the gland attached to the main duct, Type II in the anterior/mid region of the gland and Type III alveoli located on the peripheral/distal half of the gland.

Confusion has arisen when discussing cell types and their numbers in alveolar Types II and III.

Salivary glands Boophilus microplus were studied by Binnington (1978) and Megaw and Beadle (1979). According to Megaw and Beadle (1979) the two granular cells adjacent to the valve in Type II alveoli contained aggregations of granules containing electron-dense subunits that they designated "a" cells. They found only one complex granular cell in Type III alveoli. I also found two complex granular cells adjacent to the valve in Type II alveoli of A. americanum and one in Type III alveoli. Binington (1978), however, using light microscopy and histological staining methods reported finding only one granular cell with subunits adjacent to the valve and designated the granular cells in this region "a" and "b". Binnington (1978) found two complex granular cells in Type III alveoli.

Fawcett et al. (1981a,b) studied only one granular alveolar type (Type III) in R. appendiculatus accepting Binnington's premise that Type III have 2 complex granular cells. It is uncertain whether or not Fawcett et al. (1981a,b) and Binnington (1978) are describing what Megaw and Beadle (1979) and Krolak et al. (1981) designate as Type

II alveoli. In the absence of detailed micrographs to determine from Merideth and Kaufman's (1973) communication if Type II and III alveoli in Dermacetor andersoni have one or two complex granular cells. Interestingly, Coons and Roshdy (1973) described two complex granular cells in Type II alveoli of salivary glands in Dermacertor variabilis.

It is difficult to explain why some investigators find two complex granular cells (cells with granular subunits) in Type II alveoli and others find only one and the opposite for Type III alveoli. Possibly there are species differences or possibly Type II and III alveoli vary in cellular composition in some species. In our studies, the granular alveolus, located in the anterior/mid region of the gland, that we designated Type II, always contained two complex granular cells adjacent to the valve. However, it is difficult to explain why differences in interpretation of cell types occur even in the same species (Binnington, 1978; Megaw and Beadle, 1979). Possible explanations include differences in techniques, an absence of serial thin sections and physiological differences in ticks.

Binnington (1978) observed a variety of histochemically determined "c" cells, c_1-c_4 in the distal, fundus region of Type II alveoli. Cell type 'c_1' reacted strongly to indoxyl acetate indicating more esterase than that seen in c_2-c_4 cells. Cell type "c_2" had the largest granule diameter and c_4 the smallest. Cell type "c_3" reacted with the greatest intensity to PAS stain. Megaw and Beadle

(1979) using electron microscopy, also reported the presence of "c" cells in the distal region of Type II alveoli. The diameters and staining intensity of "c" cell granules varied but the authors did not subdivide "c" into different types. The results of Binnington (1978) are quite interesting but it seems possible that compartmentalization of products in multidimensional alveolar cells could cause different reaction profiles depending upon the plane of section through which the section was taken across the spherical alveoli. Since the section is planar, one might, when comparing histochemically stained thick sections, conclude the existence of more cell types than actually exist because of localized histochemical reaction substrates. I believe that until additional and more obvious and unequivocal criteria are found for identifying cell types it is less ambiguous to avoid assigning lower case letters to granular cell types and refer to granular cell types as either complex (i.e. with subunits) or simple (light or dark) as viewed at the ultrastructural level following en bloc stainning with 0.5% uranyl acetate.

Possibly the most important developmental changes in the glands are those associated with the tissue becoming competent to secrete large quantities of fluid. We concur with Fawcett et al. (1981b) that the developing membranous labyrinth(most obvious in Type III alveoli) orginates from two cell types, the abluminal interstitial cells and lightly stained, simple granular cells (Krolak et al., 1981). The

latter cell type was designated as an "f" cell by Fawcet et al. (1981b). Nothing is known about the control of these changes but changes appear shortly after tick attachment. The mechanism of fluid transport and the role of individual cells has not been determined. Fawcett et al. (1981b) suggest a mechanism similar to the secretion of fluid by avian salt glands (Ellis et al., 1977) where the degranulated and membranous "f" cell secretes fluid into the alveolar lumen and the abluminal interstitial is involved in reabsorption. The important finding of the present study and that of Fawcett et al. (1981b) is the discovery that one of the cells responsible for creating the membranous labyrinth makes contact with the alveolar lumen. Previously it was felt that adluminal interstitial cell and vacuolar cell separated the membranous labyrinth (originating solely from the abluminal interstitial cell) from the alveolar lumen (Meredith and Kaufman, 1973; Megaw and Beadle, 1979). Further research is required to determine the actual mechanism and routes of fluid transport in these remarkable cells.

CHAPTER IV

IMMUNOHISTOCHEMICAL LOCALIZATION OF ADENOSINE 3':5' - CYCLIC MONOPHOSPHATE IN FEMALE IXODID TICK *AMBYLOMMA AMERICANUM* (L.) SALIVARY GLANDS

Introduction

The salivary glands of female ixodid ticks are involved in several important functions. The glands consist of three types of alveoli each of which contains several different kinds of cells (Krolak et al., 1981). Type I alveoli are thought to possess, in the unfed tick, the ability to absorb water from atmospheres having relative humidities of 75-80% (Rudolph and Knulle, 1974, 1978; McMullen et al., 1976). During tick feeding, alveolar Types II and III exhibit morphologic changes such as overall increase in size, granular depletion in certain cells and formation of an enormous membranous labyrinth derived from abluminal interstitial and depleted granular cells (Fawcett et al., 1981a,b) the latter parallels the increase in rate of tick feeding and rate of excretion of fluid back to the host (Sauer et al., 1979; Krolak et al., 1981). There is good evidence that during

feeding, salivary secretion is controlled by nerves and that the innervation is dopaminergic (Schmidt et al., 1981).

The actions of neurotransmitters such as dopamine may be mediated by cyclic AMP (Robison et al., 1968). As a result of its association with the cell membrane, the primary stimulus activates adenylate cyclase which catalyzes the synthesis of cyclic AMP from adenosine triphosphate (ATP). Elevated intracellular levels of cyclic AMP cause phosphorylation of certain proteins via the mediation of nucleotide-dependent protein kinases; the phosphorylated proteins are ultimately responsible for the biological response of the neurotransmitter (Greengard, 1978). Kaufman (1976) and Sauer et al., (1979) demonstrated that salivary glands of female ixodid ticks can be stimulated to secrete fluid in vitro with exogenous dopamine and cyclic AMP. Futhermore, the level of gland cyclic AMP increases when glands in vito are stimulated by dopamine (Sauer et al., 1979).

Because of its importance to cell function, cyclic AMP has been localized with immunohistochemical techniques in heterogeneous tissues such as rat salivary glands (Guidotti et al., 1971; Wedner et al., 1971); rat testis (MacIndoe, 1977); rat cerebral tissue(Bloom et al., 1972); Gilman and Nirenberg, 1971; Kakuichi and Rall, 1968; Kebabian and Greengard, 1971; Kebabian et al., 1975a,b; McAfee and Greengard, 1972; Siggins et al., 1973); cellular slime mold (Konijn et al., 1968; Pan et al., 1974; Mato and Steiner,

1980); toad bladder (Scott et al., 1974; Goodman et al., 1974) as well as subcellular localization in thyroid follicular cells (Fallon et al., 1974); cells of the fasciculata zone of the adrenal cortex (Fahey, 1967; Whitley et al., 1974); and human neutrophilic granulocytes (Pryzwansky et al., 1981).

More recently, Schmidt et al. (1981) demonstrated that the concentration of dopamine required to stimulate fluid secretion by *in vitro* glands half maximally was about the same as that required to stimulate tissue adenylate cyclase. In view of the probable involvement of cyclic AMP in tick salivary gland function, its localization in alveoli under different physiologic conditions could be important in correlating cell type with function in a multicellular, multifunctional tissue.

Materials and Methods

Salivary glands from unfed ticks, ticks that have been attached to sheep for 24 hrs., and from feeding ixodid ticks *Amblyomma americanum* (L.) weighing 100-300 mg and 500 mg were dissected and incubated for 10 sec. in either modified TC-199 (Needham and Sauer, 1979) or modified TC-199 with 10^{-7} M dopamine. Prior to treatment, the anti-cyclic AMP antibody (Sigma Chemical Co.) was prepared by adsorption to cyclic AMP-agarose column (Sigma Chemical Co.) and later eluted off of a G-100 Sephadex column with modified TC-199 pH 7. The salivary gland was then embedded in O.C.T.^{R}

compound on a Slee cryostat chuck and frozen with liquid carbon dioxide.

Frozen 8 μm thick sections were placed on glass coverslips and treated with 100% acetone for 10 min. prior to reactions with antibodies. The sections were initially treated with adenosine 3':5' - cyclic monophosphate antibody for 1 hr, rinsed with TC-199 tissue culture medium (pH 7.0) and treated again for 1 hr with goat anti-rabbit globulin conjugated with FITC (Grand Island Biological Co.) and subsequently rinsed with TC-199 tissue culture medium. Controls, which were used to verify method and antibody specificity included: sections of glands treated with anti-cyclic AMP and excess cyclic AMP; sections treated with only cyclic AMP and sections treated with only dopamine. Sections were viewed and photographed with a Zeiss fluorescent microscope with epi-illumination and Kodachrome 400 film.

Results

The various alveoli and cell types in the salivary glands of female A. americanum have been described by Krolak et al. (1981) after careful examination by light microscopy of numerous thick sections stained with Mallory's trichrome and examination of TEM semi-serial thin sections stained with cyclic uranyl acetate and lead citrate. The following description of where cyclic AMP is located is based upon this account of gland morphology. Examination of the various controls employed revealed no specific

immunohistochemical detection of cyclic AMP in either the non-stimulated or stimulated gland sections.

Localization of Cyclic AMP in Salivary Glands: Unstimulated

Unfed

Type I alveoli in glands of unfed females showed no indication of detectable amounts of cyclic AMP (Fig. 1A). Type II alveoli demonstrated considerable fluorescence in the two complex granular cells adjacent to the alveolar valve (Fig. 1B). Little evidence of significant amounts of cyclic AMP was seen in the remaining alveolar cells (Fig. 1B). The one complex granular cell in Type III alveoli adjacent to the alveolar valve was also brightly fluorescent (Fig. 1C).

Feeding Ticks

24 Hours

The salivary glands already exhibit considerable change in morphology after 24 hours of tick feeding (Krolak et al., unpublished results). Type I alveoli, at this stage of feeding exhibited more fluorescence than that seen in similar alveoli of unfed females (Fig. 2A). Again, considerable cyclic AMP was localized in the two complex granular cells adjacent to the alveolar valve in Type II alveoli (Fig. 2B). Non-fluorescing regions indicated presence of nuclei (Fig.

Figure 1A. Type I alveoli (I). No indication of cyclic AMP. x625.

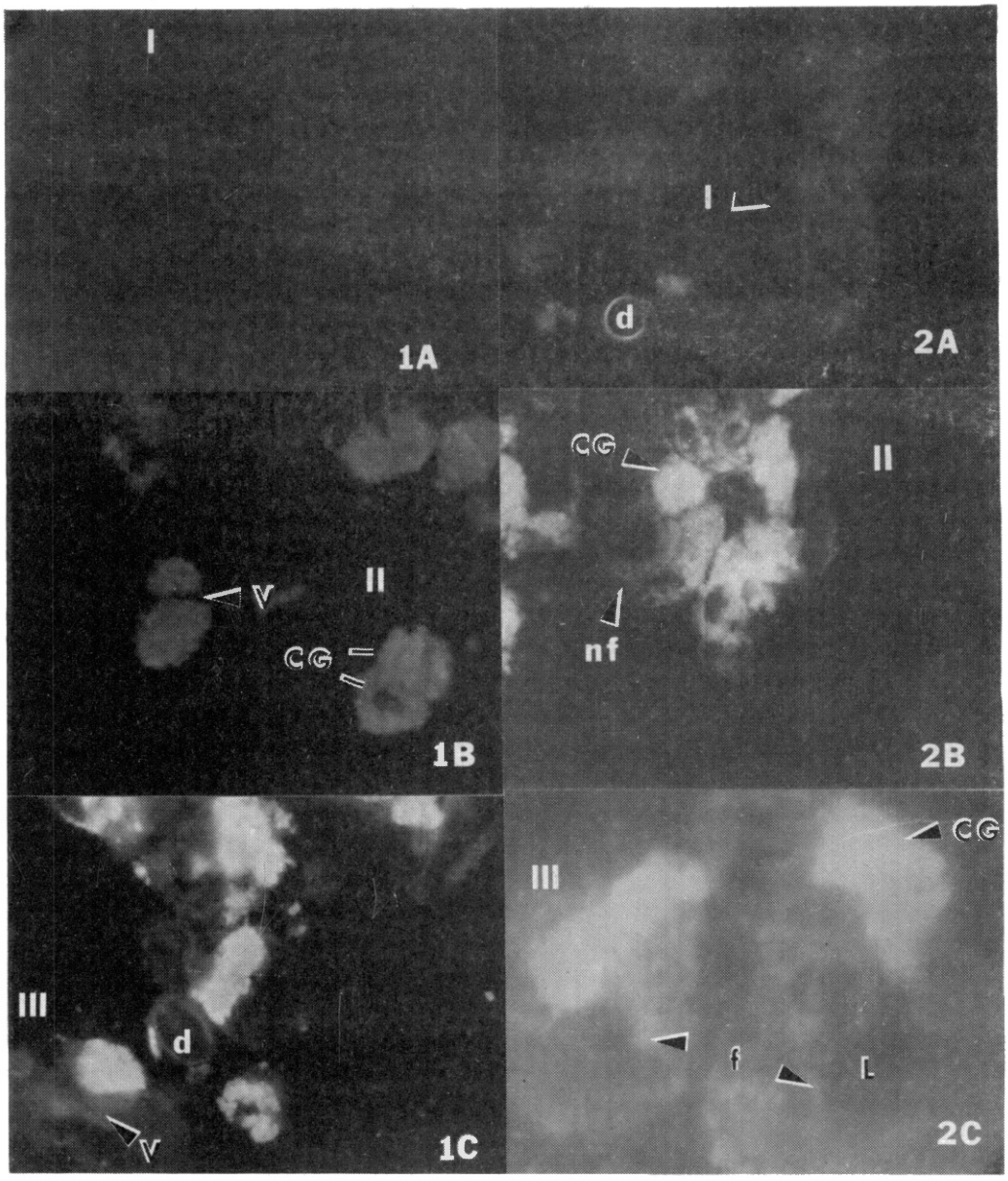
Figure 1B. Type II alveoli (II) demonstrating considerable cyclic AMP in two complex granular cells, designated (CG), adjacent to the region of the alveolar valve (V). Little evidence of cyclic AMP seen in the remaining cells. x625.

Figure 1C. Type III alveoli (III). The one complex granular cell adjcent to the alveolar valve (V) contains considerable cyclic AMP. Portions of the salivary glands' duct (d) can be seen among the alveoli. x625.

Figure 2A. Type I alveoli (I) showing an apparent increase in the level of cyclic AMP over that seen in similar alveoli of unfed females (FIg. 1A). The arrow indicates a Type I alveolus with a crosssection of a salivary gland duct (d) also apparent. x625.

Figure 2B. Type II alveoli (II). Localization of cyclic AMP in the two complex granular cells adjacent to the alveolar valve. CG indicates one of the two fluorescing complex granular cells and the non-fluorescing region (nf) where the remaining alveolar cells are located. x625

Figure 2C. Type III alveoli (III) considerable cyclic AMP was located in the complex granular cell (CG) and some cyclic AMP was apparent in other granular cells of this alveolar type. L=lumen. x1000.


I
1A
I
d
2A
V
II
CG
1B
CG
II
nf
2B
III
d
V
1C
CG
III
f
L
2C

2B). In Type III alveoli there was considerable cyclic AMP associated with the complex granular cell; lesser amounts of cyclic AMP was detected in other granular cells (Fig. 2C). Cyclic AMP was also localized on the luminal side of the cap cell, (Fig. 2C), a cell that surrounds the lumen and possesses myoepithelial cell-like characteristic (Krolak et al., 1981). Recently the cap cell has been renamed adluminal interstitial (AdI) cell by Fawcett et al. (1981a,b).

100-300 mg

After the female lone star tick A. americanum attaches to a host, it undergoes a slow phase of feeding lasting 8-14 days with an increase of weight from 4-300 mg while feeding on sheep, followed by a rapid phase of feeding lasting 12-24 hours, during which time the weight increases to approximately 300-800 mg (McSwain et al., 1981). The salivary glands undergo dramatic changes in morphology during this period of feeding. When salivary glands of feeding ticks that had attained a weight of 100-300 mg were examined for the location of cyclic AMP, Type I alveoli demonstrated moderate fluorescence dispersed evenly throughout the alveoli (Fig. 3A). Bright fluorescence was again visible in the complex granular cells of Type II alveoli (Fig. 3B). There was an enlarged luminal area in Type III alveoli, and fluorescence in the complex granular cell adjacent to the valve and to a lesser extent in other cells

Figure 3A. Type I alveoli (I) showed low level of cyclic AMP dispersed evenly throughout. Additional fluorescence seen in from other alveolar types. x625.

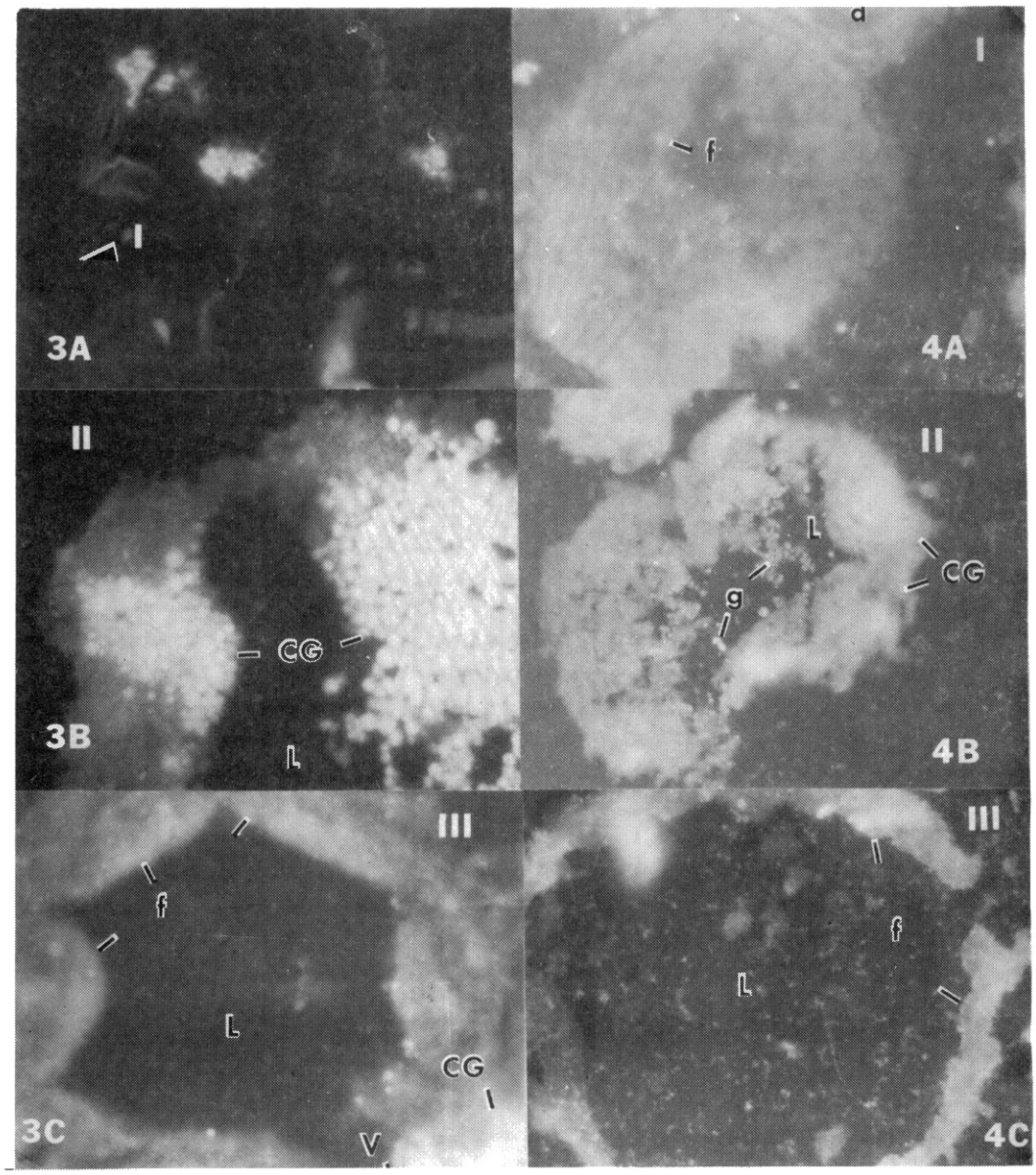
Figure 3B. Type II alveoli (II). Fluorescence located in the complex granular cell (CG). Most of the cyclic AMP localized in this alveolar type was restricted to the complex granular cell and its granules. L=lumen. x1000.

Figure 3C. Type III alveoli (III) cyclic AMP was located in the complex granular cell (CG) which is adjacent to the region of the alveolar valve (V) and to a lesser extent in other granular cells of this alveolar type. F= cyclic AMP specific fluorescence. L=lumen. x1000.

Figure 4A. Type I alveolus (I) showing cyclic AMP dispersed throughout. f= cyclic AMP specific fluorescnece. d= duct. x1000.

Figure 4B. Type II alveolus (II). Cyclic AMP appears to be associated with granules (g) in the lumen (L). Complex granular cells (CG) also exhibit the presence of cyclic AMP. x1000.

Figure 4C. Type III alveolus (III). Cyclic AMP was located in small regions on the luminal side of this alveolus. L=lumen. f=cyclic AMP specific fluorescence. x1000.


3A
I
4A
f
II
3B
CG
L
4B
L
g
CG
III
3C
f
L
CG
V
4C
f
L

throughout the alveolus (Fig. 3C). Some fluorescence was seen in regions of cells bordering the lumen.

$\geq$ 500 mg

Cyclic AMP was distributed throughout Type I alveoli in the salivary glands obtained from ticks weighing in excess of 500 mg (Fig. 4A). Considerable fluorescence was associated with both dispersed and non-dispersed granules in the complex granular cells of Type II alveoli (Fig. 4B). The lumen of the Type III alveolus was greatly enlarged, and the fluorescence associated with granules of its complex granular cell was intense (Fig. 4C). Small areas of fluorescence were visible on the luminal side of the alveolar cells.

Localization of Cyclic AMP in Salivary Glands: Stimulated with 10^{-7} M Dopamine

Unfed

Non-specific fluoresence was well dispersed with a few specific regions of cyclic AMP flourescence in Type I alveoli of salivary glands obtained from unfed ticks following stimulation with 10^{-7} M dopamine (Fig. 5A). Specific fluoresence was seen in the complex and simple granular cells of Type II alveoli (Fig. 5B). The region of non-fluorescence in the Type II alveolus (as indicated by the arrow in Figure 5B) is that of the cells' nucei. In Type

III alveoli, fluoresence is located on the apical and basal side of the granular cells (Fig. 5C).

24 Hours

The fluorescence in Type I alveoli of glands obtained from ticks that had fed for 24 hours and were then stimulatd by dopamine (Fig. 6A) was less intense than that seen in alveoli of unfed ticks (Fig. 5A). Type II alveoli exhibited more areas of fluorescence than that seen in Type II alveoli of non-stimulated salivary glands during the same stage of feeding (Fig. 6B and Fig. 2B). Fluorescence was again seen in the complex granular cells in association with the granular material (Fig. 6B). Thin bands of fluorescence seen in Type III alveoli were localized near the valve region and within nuclei of several granular cells (Fig. 6C). Localization of cyclic AMP was minimal in the apical region of cells bordering the lumen. An enlarged alveolar lumen was observed in both Type II and Type III alveoli following stimulation of glands with dopamine (Compare 6B and 6C with 2B and 2C).

100-300 mg

Dispersed fluorescence was quite evident in Type I alveoli of salivary glands obtained from 100-300 mg females when stimulated with dopamine (Fig. 7A). In cells, other than in complex granular cells of Type III alveoli fluorescence was more intense over the entire alveolus than that seen in

Figure 5A. Type I alveolus (I). Arrows indicated fluorescence specific for cyclic AMP. x625.

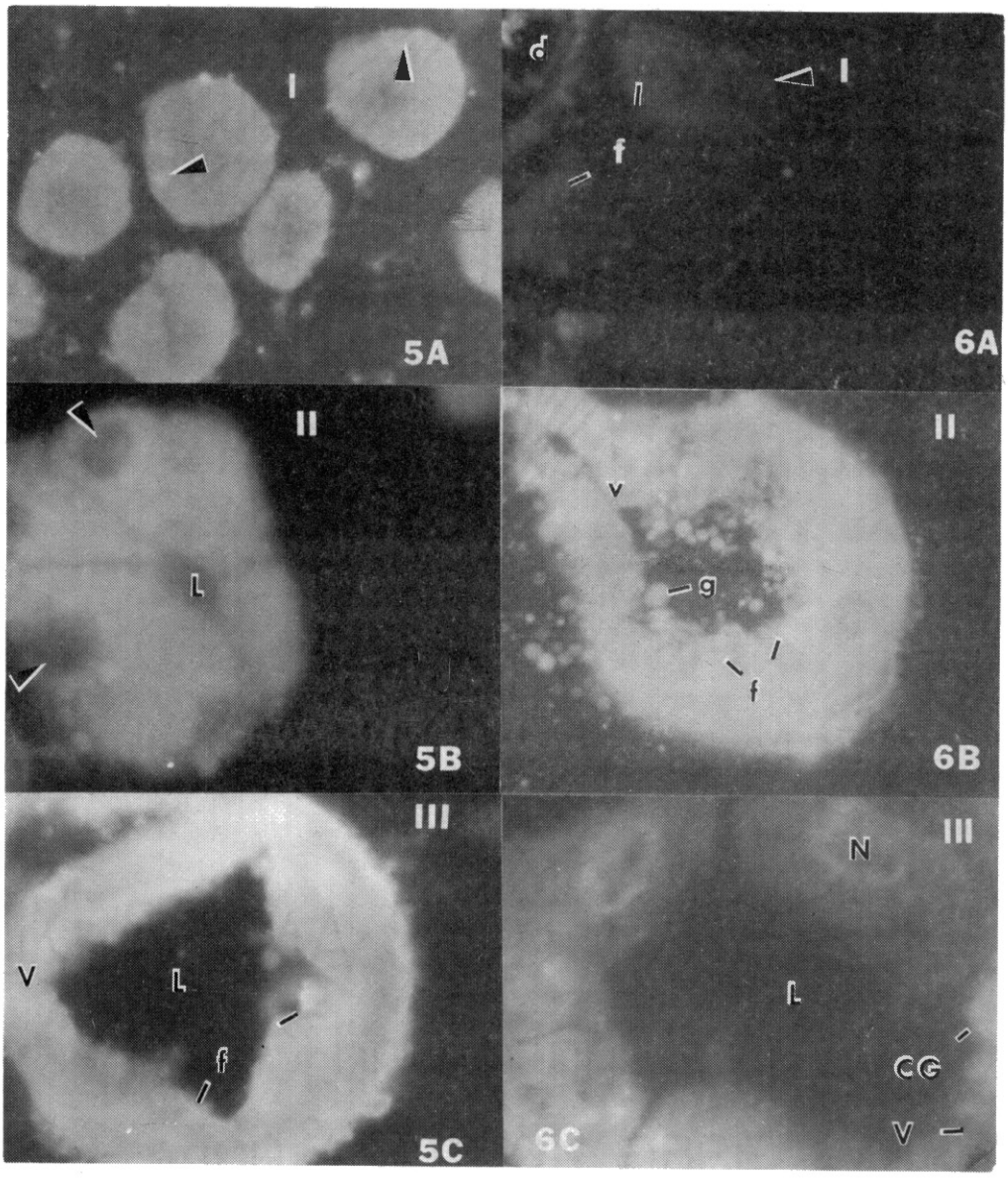
Figure 5B. Type II alveolus (II). Cyclic AMP detected in the complex granular cells. The arrows indicated the location of the cells' nuclei. Cyclic AMP was also located in other granular cells of this alveolar type. Lumen (L). x1000.

Figure 5C. Type III alveolus (III). Cyclic AMP was distributed throughout the cells of its alveolus. Some areas of cyclic AMP specific fluorescence are designated by (f). V= valvular region. L= lumen. x1000.

Figure 6A. Type I alveoli (I). Note that fluorescence was less intense, following stimulation with 10^{-7} M dopamine, than that seen in dopamine stimulated alveoli of prefed ticks (Fig. 5A). f= cyclic AMP specific fluorescence. d= duct. x625.

Figure 6B. Type II alveoli (II). Cyclic AMP was located in the various granular cells and in association with granular material (g) of other cells following stimulation with 10^{-7} M dopamine. f= Cyclic AMP specific fluorescence. v= valve region. x1000.

Figure 6C. Type III alveolus (III). Cyclic AMP was localized near the valve region (v) in the complex granular cell (CG), following stimulation with 10^{-7} M dopamine, as well as in the nuclei (N) of several granular cells. L=lumen. x1000.


I
5A
d
I
f
6A
II
L
5B
II
v
g
f
6B
III
V
L
f
5C
III
N
L
CG
V
6C

glands obtained from ticks of similar weight but which were not stimulated by dopamine (Compare Figs. 7B, to 3B, 3C). Specific fluorescence was visible in the region of the alveolar valve and in association with dispersed granules from Type II alveolar cells following dopamine stimulation (Fig. 7B). Fluorescence was seen in cells adjacent to the lumen (Fig. 7B). Type III alveoli cyclic AMP was localized in cells adjacent to the valve region as well as more intense that that seen in non-stimulated glands obtained from females weighing 100-300 mg (Fig. 3C).

$\geq$ 500 mg

The loss of integrity of salivary gland tissue at this stage of feeding makes the tissue fragile and difficult to section. However, fluorescence in Type I alveoli was dispersed in glands obtained from ticks weighing in excess of 500 mg and then stimulated by dopamine (Fig. 8A). Numerous fluorescent granules were seen dispersed within the luminal area of the Type II alveolus (Fig. 8B). Upon careful examination, the granules were seen to contain fluorescent cytoplasmic and membranous elements around the outer edges of the granules (arrows). All cells in Type III alveoli were fluorescent (Fig. 8C) and fragmented because stimulation with dopamine greatly increases the diameter of the alveolar lumen possibly because of an increase in luminal fluid (Fig. 8C).

Figure 7A. Type I alveolus (I). Note cyclic AMP-specific fluorescence near basal region of alveoli (f). x625.

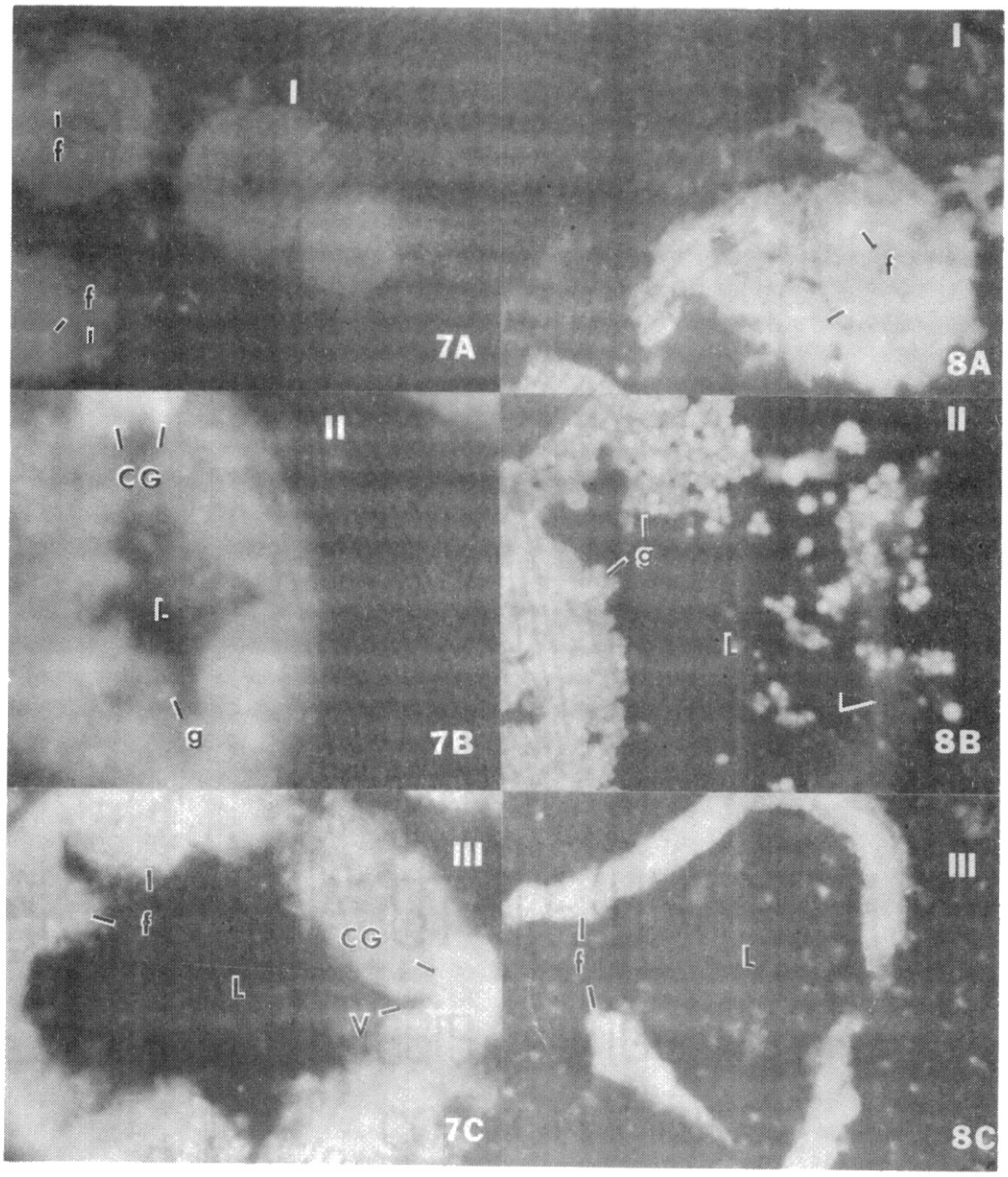
Figure 7B. Type II alveolus (II). Cyclic AMP was seen around the valvular region of the alveolus where the complex granular cells are seen adjacent to the lumen (L) and in association with granules (g). x1000.

Figure 7C. Type III alveoli (III). Cyclic AMP (f) was localized in the complex granular cell (CG) adjacent to the valve region (v) as well as in other alveolar cells. L=lumen. x1000.

Figure 8A. Type I alveoli (I). Dispersed fluorescence exhibiting the presence of cyclic AMP following stimulation with 10^{-7} M dopamine. f= cyclic AMP specific fluorescence. x1000.

Figure 8B. Type II alveolus (II). Type II alveolar cell granules (g) dispersed in the lumen (L) contain cyclic AMP following stimulation with 10^{-7} M dopamine. Arrow indicates remaining portion of the non-granular containing cells of the Type II alveolus. x1000.

Figure 8C. Type III alveolus (III). Cells contain cyclic AMP. Note fragmented appearance of alveolus following stimulation with 10^{-7} M dopamine. L=lumen. f= cyclic AMP specific fluorescence. x1000.


I
f
f
7A
I
f
8A
II
CG
g
7B
II
g
8B
III
f
CG
L
V
7C
III
f
L
8C

Discussion

The increased amount of cAMP found in Type I alveoli following dopamine stimulation suggest that only Type I alveolar cells might be under nervous control, but that Type I alveoli may be physiologically active subsequent to its attachment to a host.

The involvement of cyclic AMP in the complex granular cells may be for the maintenance and/or release of the granular material. Preliminary electron microscopic studies have shown that the granules are still present but at reduced levels within cells during the rapid phase of engorgement, suggesting a gradual secretion of granular material during feeding. This gradual rate of secretion of material from the complex granular cells and continual presence of cyclic AMP in the Type II and Type III alveoli suggests that some factor may be present with an ability to control release of granular material through an increase in cyclic AMP. It is not known if granular secretion by ixodid ticks is controlled by nerves or hormones; however, nerve axons have been seen in close association with complex granular cells (Krolak et al., 1981). The remaining cells within the Type II and Type III alveoli of the non-stimulated glands did not contain detectable levels of cyclic AMP. This may indicate that the release of material from these cells is controlled by factors that do not utilize AMP as a second messenger or that the changes in the cyclic nucleotide are quite

transitory.

Possibly the most significant finding of the present studies is that there is an apparent over-all increase in cyclic AMP seen in all cells of Type II and Type III alveoli following stimulation of glands with dopamine. There is ample evidence that cyclic AMP is a second messenger of the fluid secretory process in ixodid ticks (Sauer et al., 1979). There is also good evidence that the fluid secretory process is controlled by nerves (Sauer, 1977) and that dopamine may be the neurotransmitter at the neuroglandular junction (Schmidt et al., in press). A shortcoming of the present results, however, is the difficulty in precisely localizing cyclic AMP in cells and the membranous labyrinth of Type II and III alveoli that are thought to be involved in the fluid secretory process. We are presently investigating the localization of cyclic AMP at the ultrastructural level after subjecting glands to similar conditions.

The general and dispersed fluorescence, seen in glands stimulated with 10^{-7}M dopamine, may not be entirely specific for cyclic AMP (Figs. 5A, 6A, 7A and 8A) except for regions which are obviously more intense and similar to the degree of flourescence seen in non-stimulating glands. Catecholamines, such as dopamine, possess characteristic fluorescing properties when excited by ultraviolet light, Binnington and Stone (1977). It is possible that the over-all increase in the increase in intensity of diffuse fluorescence observed in glands following incubation of tissue

with dopamine (Figs. 5 through 8) is to some extent a result of treatment with dopamine in addition to an increase in cyclic AMP. There was a small degree of dispersed fluorescence seen in the alveolus when the glands were treated with dopamine alone. Based upon a more intense fluorescence, which the presence of cyclic AMP would elicit, a distinction could be made between the non-stimulated and dopamine stimulated treatments of the glands.

An interesting and obvious effect caused by dopamine was an increase in cyclic AMP on the luminal borders of cells in Type II and Type III alveoli. The localization of cyclic AMP was also seen in the more basal regions of some of these same cells. Interestingly, cyclic AMP was seen to increase within the nuclei of granular cells of Type II and Type III following stimulation of glands with dopamine.

Morphological studies of the unfed lone star tick Amblyomma americanum (Krolak et al., 1981) have shown areas of innervation between nerve cell axons and cells, and Schmidt et al. (in press) has shown the presence of dopamine sensitive adenylate cyclase in the salivary glands of A. americanum suggesting that some of the effects of dopamine are likely to represent physiological events in cells of the alveoli.

CHAPTER V

SUMMARY AND CONCLUSIONS

The ixodid tick salivary glands appear to be of great significance in solving the problem of osmo-regulation. The glands act as excretory organs in the removal of excess fluids while concentrating its bloodmeal. The water and ions are removed across the gut epithelium into the hemolymph and secreted back into the host by way of the salivary glands. The intention of this investigation was to determine the ultrastructural changes which occurred in the three alveolar types during various stages of ixodid tick feeding. In addition, immunohistochemical localization of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in non-stimulated and dopamine stimulated tick salivary glands was performed to determine the possible role of cyclic AMP in alveolar cells during various phases of tick feeding.

As in other species of female ixodid ticks, the salivary glands of the unfed female ixodid lone star tick, Amblyomma americanum (L.) consist of three alveoli, one agranular and two granular. The agranular alveoli (Type I) are directly attached to the anterior portion of the main salivary duct, consist of approximately 13-14 cells, and are without valves. Six peripheral cells have tortuous plasma

membrane infoldings with closely associated mitochondria, an abundance of lipid-like droplets and relatively flat apical surfaces. A relatively large, clear "central" cell occupies most of the alveolar midsection. The lumen of the Type I alveolus appears to be continuous with the alveolar tubular lumen as it passes through the previously undescribed, concentric myoepithelial-like constrictor cell which is an analogus structure to the valve seen in Type II and III alveoli.

Granular alveoli consist of approximately 14-16 cells. Type II granular alveoli have two complex granular cells in close proximity to the cuticular alveolar valve, whereas Type III alveoli have only one. Thin epithelial cells separate adjacent granular cells in both alveolar types and there is only one cap (AdI) cell with myoepithelial-like features lining the alveolar lumen in web-like fashion. The latter may play a significant role in helping propel secretions from alveoli to associated ducts.

Developmental changes in the three alveolar types of salivary glands in the female ixodid tick Ambylomma americanum were studied during various phases of tick feeding.

Lipid-like droplets present in pyramidal cells of Type I alveoli in unfed females (postnymphal ticks which had not yet taken a bloodmeal as an adult) coalesced in the central cell of Type I alveoli during early stages of feeding. Most of the lipid-like droplets were absent from the central cell during later phases of tick feeding except for an occasional

lipid-like droplet seen in the constrictor cell, a cell adjacent to the alveolar tubular lumen, and the pyramidal cells during the final phases of tick feeding.

During early phases of tick feeding there were increases in alveolar cell size. This increase in cell size was seen in both Type II and Type III alveolar cells. Subsequent to this increase in cell size was the secretion of granules primarily from the lightly staining, simple granular cells with an apparent increase in the size of abluminal interstitial cells as well. Later stages of tick feeding showed granules present in complex and darkly stained, simple granular cells but mostly absent from lightly staining, simple granular cells. At this phase of feeding a tremendous membranous labyrinth had formed from depleted lightly staining, simple granular cells and the abluminal interstitial cells.

It is likely that the increased surface area of the membranous labyrinth on the basolateral surfaces of the alveoli might facilitate the transport of fluid while the tick is feeding on the host.

Morphological studies of unfed and feeding female ixodid lone star ticks have not determined if granular secretion from alveolar cells is controlled by nerves or hormones; however, nerve axons have been seen in close association with complex granular cells and at the basal extensions of the cap (AdI) cell where they are found in close association with the abluminal interstitial cells (Krolak

et al., 1981).

There is ample evidence that cyclic AMP is second messenger of the fluid secretory process in ixodid ticks (Sauer et al., 1979). There is also good evidence that the fluid secretory process is controlled by nerves (Sauer, 1977) and that the presence of dopamine may be the neurotransmitter at the neuroglandular junction in the salivary glands of A. americanum (Schmidt et al., in press).

The localization of cyclic 3':5' adenosine monophosphate (cyclic AMP) in the alveoli of female ixodid lone star tick salivary glands of Ambylomma americanum (L.) has been accomplished with an indirect immunofluorescent technique. Cyclic AMP was localized in alveolar cells after incubating whole glands in vitro with and with out 10^{-7} M dopamine.

The presence of cyclic AMP in Type I alveoli of both non-stimulated and stimulated glands suggests that not only might Type I alveolar cells be under nervous control but that this alveolar type might be physiologically active subsequent to its attachment to a host.

The involvement of cyclic AMP in the complex granular cells of Types II and III alveoli, may be for the maintenance and/or release of the granular material. Preliminary electron microscopic studies have shown that the granules are still present but at reduced levels within cells during the rapid phase of engorgement, suggesting a gradual secretion of granular material during feeding. This gradual

rate of secretion of material from the complex granular cells and continual presence of cyclic AMP in the Type II and Type III alveoli suggests that some factor may be present with an ability to control release of granular material through an increase in cyclic AMP.

The remaining cells within the Type II and Type III alveoli of the non-stimulated glands did not contain detectable levels of cyclic AMP. This may indicate that the release of material from these cells is contolled by factors that do not utilize cyclic AMP as a second messenger or the changes in the cyclic nucleotide are quite transitory.

Possibly the most significant finding of the present studies is that there is an apparent over-all increase in cyclic AMP seen in all cells of Type II and Type III alveoli following stimulation of glands with dopamine.

Ultrastructural changes in Types II and III alveolar cells of the feeding female ixodid tick A. americanum (Krolak et al., 1981) have shown areas of innervation between nerve cell axons and cells, and Schmidt et al. (in press) has shown the presence of a dopamine sensitive adenylate cyclase in the salivary glands of A. americanum suggesting that some of the effects of dopamine are likely to represent ultrastructural and physiological events in alveolar cells.

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VITA[2]

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