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*With 5 plates and 3 figures in the text*



# A proposed site of fluid secretion in the salivary gland of the ixodid tick *Dermacentor andersoni*

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SUMMARY

The histology and ultrastructure of the salivary gland in *Dermacentor andersoni* are presented with particular emphasis on those aspects relating to fluid secretion.

We suggest that the group III acinus contributes most of the fluid portion of the saliva (i.e. water and small molecules) and that the main cell-type involved is what we name the 'water-cell'.

The granule-cells possibly secrete the cement by which the tick secures its mouthparts to the host, and the 'vacuolar cell' possibly produces a protein-rich secretion. The function of the group I acinus remains obscure.

## INTRODUCTION

The salivary glands of the female tick, *Dermacentor andersoni*, play a prominent role in ionic and water regulation during the feeding period (Kaufman & Phillips, 1973*a*). Salivation could be triggered *in vitro* by bathing the glands in a nutrient medium containing low concentrations of certain catecholamines (adrenaline, noradrenaline and dopamine); *in vitro* glands were, by contrast, insensitive to pilocarpine and acetylcholine (Kaufman & Phillips, 1973*b*). We suggested that fluid secretion depends on the transport of chloride and sodium (Kaufman & Phillips, 1973*c*). The present paper examines the morphology and ultrastructure of the salivary gland with regard to fluid secretion.

## MATERIALS AND METHODS

Observations were made on glands from female ticks which had been actively feeding for 6 days and which weighed 200–350 mg; such ticks are here referred to as 'partially fed'. Salivary glands were dissected out in fixative (6% glutaraldehyde in 1/15 M Sorensen's phosphate buffer (pH 7·4) with 4% sucrose added). Small pieces of glands were washed for 15 min in buffer solution and postfixed in buffered osmium (1%) solution. Tissues were rapidly dehydrated in a series of ethanols ending with propylene oxide, infiltrated with 'Epon 812' for 8 h and embedded. Thin sections were cut on a 'LKB Ultrotome' and picked up on uncoated copper

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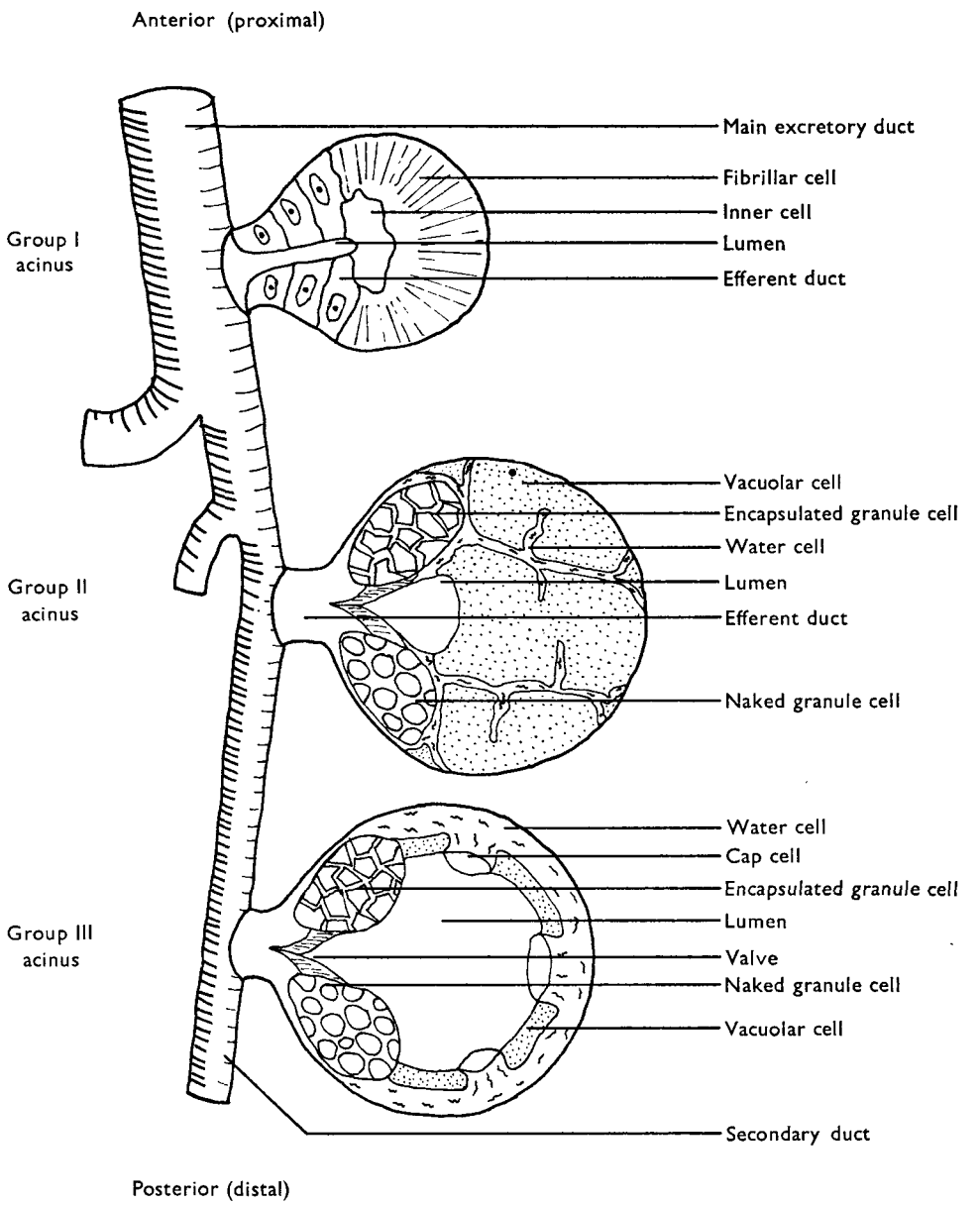


Fig. 1. Diagram to illustrate the relationship between the three acinar groups, ducts and cell-types seen under the light microscope. Acini and ducts are not drawn to scale. Notice that group I acini join the main excretory duct, whereas the others join secondary ducts. Vacuolar cells are more prominent than water cells in group II acini; the reverse holds true in group III acini and lumina of the latter are larger than those of group II acini.

grids. Sections were stained for 15 min in uranyl acetate (saturated solution in 70% ethanol) and for 30 min in lead citrate (Reynolds, 1963). Thicker sections (1 $\mu$m) were cut, and stained in alkaline toluidine blue for light microscopy (Pease, 1964).

## RESULTS

### *General organization of the salivary gland*

The two salivary glands are prominent organs lying along the lateral portion of the body cavity. A gland comprises many acini, each acinus consisting of a layer of cells surrounding a lumen (Fig. 1). The lumen opens through a valve to a short efferent duct. Numerous efferent ducts drain into the secondary ducts which eventually anastomose to form the single main excretory duct. The acini can be classified into three groups on the basis of size and structure – groups I, II and III acini which, in partially fed females, measure $33 \pm 3$, $112 \pm 3$ and $125 \pm 15$ $\mu$m in diameter respectively (mean and S.E.). The group I acini are relatively few in number and are concentrated in the proximal region of the gland. The group I acinus does not have a valve separating the lumen from the efferent duct and the efferent ducts usually connect directly to the main excretory duct (see also Till, 1961; Chinery, 1965). Group II acini are most numerous in the proximal to mid-region of the gland, while group III acini are most numerous in the distal half of the gland.

### *Morphology and ultrastructure of the salivary gland*

All measurements are reported as the mean and S.E.

#### *Ducts*

The lumen of the main excretory duct is $44 \pm 3$ $\mu$m in diameter and the thickness of the surrounding cell layer is $15 \pm 1$ $\mu$m. Two striking features of the wall of the main excretory duct are the relatively thick (approaching 1 $\mu$m) basement membrane on the haemolymph (basal) side and the thick cuticle (3–4 $\mu$m) on the luminal (apical) side (Pl. 1 A). The secondary and efferent ducts are similar to, but smaller than, the main excretory duct. The cells composing the duct epithelium are characterized by prominent bundles of parallel microtubules orientated from the apical to basal cell borders (Pl. 1 E). The apical plasma membrane is microvillate, while the basal membrane is relatively straight. The lateral membranes are long, tortuous and frequently joined by septate desmosomes (Pl. 1 D). The cells contain large numbers of free ribosomes and many mitochondria. The cuticular lining of the duct comprises an outer epicuticle and a thick, non-lamellate endocuticle. In longitudinal section one recognizes a helical ridge on the inner edge of the endocuticle. This ridge is covered by a thin layer of densely staining material (Pl. 1 E).

#### *Group I acinus*

This acinus is composed of two fibrillar cells surrounding a very lightly stained inner cell (Pl. 1 A). The lumen appears as a small aperture (or in longitudinal section (Pl. 1 A) as a narrow groove) in the inner cell and so does not seem to be in

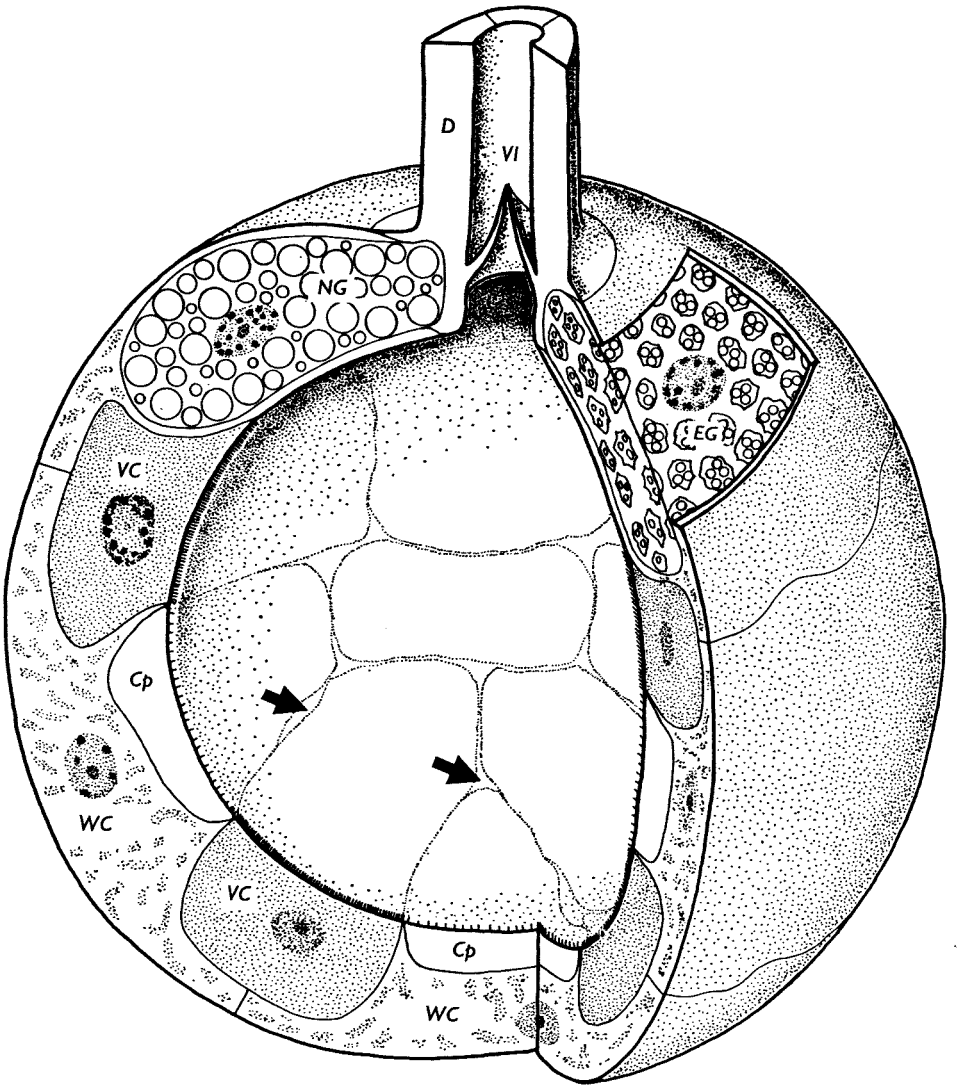


Fig. 2. A diagrammatic reconstruction of the group III acinus showing the spatial relationships among the five cell types. Duct (*D*), valve (*Vl*), naked-granule cell (*NG*), cap cell (*Cp*), encapsulated-granule cell (*EG*), vacuolar cell (*VC*), water cell (*WC*). Although for the most part the water cell is completely cut off from the acinar lumen by means of adjacent cap and vacuolar cells, there may be a few regions where the membranes of the latter cells are not closely apposed (arrows; cf. Pl. 2B). The actual extent of these potential corridors is not known. Notice that the haemolymph surface of the acinus is completely covered by extensions of water cell.

direct contact with the fibrillar cell. The fibrillar cell is characterized by elaborate infoldings of the basal membrane that extend almost to the apical surface of the cell (Pl. 1B). Mitochondria are abundant between these infoldings. Lateral plasma membranes are difficult to locate with certainty. Occasionally we have seen axon-like profiles embedded in the fibrillar cells (Pl. 1C). These profiles are surrounded

by invaginations of the plasma membrane of the fibrillar cell and contain electron-dense granules approaching 750 Å in diameter. The cytoplasm of the inner cell stains lightly as it contains only a nucleus, occasional mitochondria and a few fibres.

*Group III acinus*

Surrounding the region where the efferent duct joins the acinus there are usually two encapsulated-granule cells and five naked-granule cells (Fig. 2). The remainder of the acinus is composed of three cell types: cap cells, water cells and vacuolar cells (see Fig. 2 and Pl. 2A). The complete haemolymph surface of the acinus is defined by a layer of water cells and the lumen of the acinus is surrounded by a layer of five or six vacuolar cells alternating with an equal number of cap cells.

*Naked-granule cell*

Thick sections show that the naked-granule cell is filled with dark spherical granules approaching 6 $\mu$m in diameter (Pl. 5A). In electron micrographs the granules appear as light-staining oval bodies and the cytoplasm is filled with whorls of granular endoplasmic reticulum (ER) (Pl. 5B).

*Encapsulated-granule cell*

Under the light microscope (not clear in Pl. 5A) the encapsulated-granule cell appears to be packed with irregular-shaped bodies. In the electron micrograph (Pl. 5D) these bodies appear as membrane-bound capsules containing darkly stained granules.

*Vacuolar cell*

Under the light microscope (Pl. 2A, B) the vacuolar cell stains densely, has an apical brush border and contains vacuoles. In electron micrographs (Pl. 3A, Pl. 4) the cytoplasm has an extensive granular ER showing distended profiles. The apical plasma membrane is thrown into short, irregular microvilli. The lateral and basal plasma membranes interdigitate with the lateral membranes of the water cell in an intercellular space.

*Water cell*

The water cell stains lightly with toluidine blue (Pl. 2); prominent intercellular spaces appear between adjacent water and vacuolar cells (Pl. 2A). In electron micrographs (Pl. 3C; Pl. 4) the basal and lateral plasma membranes are seen to be extensively infolded, forming a complex network of canaliculi throughout the cytoplasm. Numerous, large mitochondria are closely associated with these canaliculi. The lateral plasma membranes of adjacent water and vacuolar cells interdigitate extensively; by contrast, contiguous membranes of water and cap cells form less tortuous interdigitations (Pl. 3C).

*Cap cell*

The cap cell is small, irregularly shaped and derives its name from its position, by covering the apical surface of the water cell. Both the cytoplasm and nucleus stain lightly with toluidine blue (Pl. 2C). In electron micrographs (Pl. 3C, D) the cytoplasm is sparse, containing only a few mitochondria and ribosomes. The apical plasma membrane bears a few microvilli. The lateral plasma membranes of the cap cell join those of the adjacent vacuolar cells over short distances by means of septate desmosomes (Pl. 3B). Bundles of parallel microtubules are especially abundant in the region of this juncture.

*Group II acinus*

The group II acinus is organized similarly to the group III acinus. As in the latter, encapsulated-granule and naked-granule cells occur around the efferent duct. Vacuolar cells make up most of the remainder of the acinus, however, and the water cells are diminutive (Pl. 5A), thus suggesting that water transport is only a minor function of the group II acinus.

*Naked- and encapsulated-granule cells*

These cells are similar to the corresponding cells in the group III acinus.

*Vacuolar cell*

The vacuolar cells are the largest cells of the acinus (Pl. 5A); they stain densely and contain small vacuoles. In the electron micrograph (Pl. 5C) the cell contains vacuoles, some mitochondria and many ribosomes. The basal region (Pl. 5E) contains distended profiles of granular ER filled with a homogeneous material.

*Water cell*

Under the light microscope (Pl. 5A) the water cell appears only as a stroma surrounding the other cells. In the electron micrograph (Pl. 5E) the cell appears as cytoplasmic projections containing free ribosomes and mitochondria associated with the canaliculi.

*Cap cell*

This cell has not been seen in the light microscope, but in the electron micrographs it possesses the same characteristics as its sister cell in the group III acinus.

## DISCUSSION

The salivary glands of several ticks are believed to secrete such varied substances as: (1) a cement which secures the mouthparts to the host (Moorehouse, 1969); (2) anticoagulants (Nuttall & Strickland, 1908; Ross, 1926; Gregson, 1960); (3) a paralytic toxin (Ross, 1926; Gregson, 1957) which may be synthesized by the gland; and (4) a watery fluid (Tatchell, 1967; Kaufman & Phillips, 1973*a*, *b*, *c*). Therefore it is not surprising that the salivary gland comprises more than one cell type. Unfortunately this makes the task of assigning definite functions difficult.

*Fluid secretion*

The following circumstantial evidence suggests that group III acini secrete most of the watery fluid:

(1) The structure of the water cell (cf. Pl. 3C; Pl. 4) is characteristic of the cells in other fluid-transporting epithelia (Berridge & Oschman, 1972). Except for the fibrillar cell (see below), the other cell types that we find in the salivary gland do not possess those features associated with fluid transport.

(2) The total haemolymph-facing surface of the group III acinus is enveloped by basal extensions of the water cell (Fig. 2). This feature maximizes the basal membrane exposed to the haemolymph from which the fluid is secreted.

(3) The distal portion of the salivary gland is composed mostly of group III acini. This is a reasonable location for the fluid-secretory tissue since secretory products of proximal acini are then washed along the ducts into the buccal cavity.

(4) In argasid ticks the salivary glands do not secrete much fluid (Kaufman, 1971) and possess salivary acini composed only of granule-containing cells (Till, 1961; Chinery, 1965; Dzhafarov, 1965). There do not seem to be any fluid-secreting cells.

It is relevant here to consider more carefully the fibrillar cell of the group I acinus since Kirkland (1971) and Balashov (1972) have proposed that the group I acini of a number of ixodid ticks may be responsible for water secretion. Certainly the ultrastructural features of the fibrillar cell (cf. Pl. 1B) suggest that this is a very active cell. However, group I acini are not numerous, exist only in the proximal part of the gland and possess very small lumina. However, Kirkland (1971) and Balashov (1972) do not describe cell types similar to the water cell which we have seen in *D. andersoni*. We feel, therefore, that the water cell of the group III acinus is a better candidate for secreting the bulk of fluid than is the fibrillar cell of the group I acinus, at least in the case of *D. andersoni*. The fibrillar cell may be involved in the secretion of another material.

Although the cap cell would appear to effect morphological isolation of the water cell from the lumen, it need not form much hindrance to fluid flow from the water cell to the acinar lumen. Secreted fluid could enter the lumen via the septate desmosomes joining adjacent cap and vacuolar cells (Pl. 3B), or it may even pass through the cap cell itself. Finally, in some thick sections we have noticed that the cap cell appears absent, or virtually so, in limited areas (compare Pl. 2B, C); if so, the water cell's apex reaches the lumen in these regions.

The iso-osmotic transport of fluid across certain epithelia is now generally explained in terms of the standing osmotic gradient hypothesis as developed from the double membrane hypothesis (of Curran and his colleagues) by Diamond & Bossert (1967). The mathematical considerations of Diamond & Bossert suggest how long, closed-end canaliculi (such as inter- and intracellular spaces etc.) could develop a standing gradient of decreasing osmolarity from the blind end to the open end (Fig. 3). Their model requires that the canaliculi be closed at the compartment from which fluid is absorbed and open at the compartment towards which it is transported. Their hypothesis has gained increasing popularity because

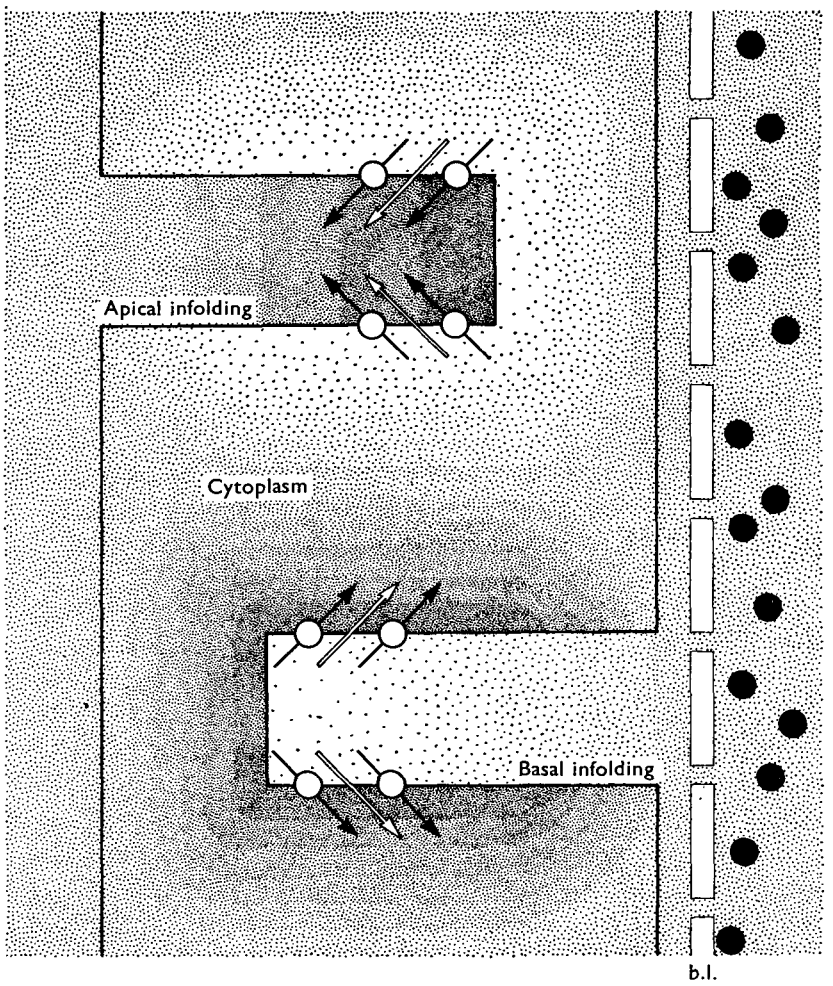


Fig. 3. A highly schematic representation of how the standing-gradient hypothesis could explain water-to-solute coupling in the water cell of the group III acinus. The basal lamina (*b.l.*) is generally assumed to filter out only large molecules such as proteins (circles at right of diagram), but not smaller solutes such as ions (stippling). The density of the stippling denotes the degree of ion concentration in any region. (The pattern of stippling is intended not to represent known profiles of ion density, but merely to indicate schematically how the model is believed to work.) The ion pumps are believed to exist in the membranes of the infoldings and the direction of pumping is denoted by the solid arrows. The active pumping of solute out of the basal infoldings initially creates an area of low solute concentration in the channel, and high concentration in the cell. Water enters the cell down an osmotic gradient (open arrows) and haemolymph water moves down the channel to take its place. When the steady state is reached the result is a standing gradient in a 'backwards' channel (see text). Other ion pumps initially create a high solute concentration in the apical infoldings (solid arrows) and water leaves the cell in response to this gradient (open arrows). The resulting hydrostatic pressure in the channel forces fluid to flow toward the lumen and the steady state again reflects a standing gradient down a 'forwards' channel. Thus fluid appears to flow between two equi-osmolar solutions, but in reality it is flowing along local osmotic gradients within the cell (cf. Diamond & Bossert, 1967, 1968; Berridge & Oschman, 1969).

the ultrastructure of numerous transporting epithelia conforms well to the geometrical requirements of the model. In addition, it has recently been shown in the cockroach (Wall, Oschman & Schmidt-Nielsen, 1970) that the fluid in the intercellular spaces of the rectal pads is consistently more concentrated than the fluid in the rectal lumen proper; this is direct experimental evidence that the model for local osmosis may apply in at least some biological systems. Some secretory epithelia possess so-called 'backwards' channels, namely, ones which are open at the surface from which fluid is transported. Diamond & Bossert (1968) have accommodated such epithelia in a model which is in most respects similar to the original one.

Water-to-solute coupling in the water cell of the group III acinus could be explained in terms of the standing gradient hypothesis (Fig. 3), since the cell possesses tortuous canaliculi originating as basal and lateral infoldings of the plasma membrane. The basal infoldings could constitute 'forwards' and the lateral ones 'backwards' channels. Mitochondria are in close association with the infoldings an arrangement which is generally believed to bring the energy stores of the cell near to the ion pumps which are responsible for active transport.

The standing-gradient hypothesis does not allow for the secreted fluid to be hypo-osmotic to the haemolymph, yet such is the case with the saliva emerging from the main excretory duct of the salivary gland of *D. andersoni* (Kaufman & Phillips, 1973*c*). Reabsorption of solute from the primary secretion might occur somewhere in the salivary gland. The elegant micropuncture experiments of Martinez, Holzgreve & Frick (1966), Young & Schögel (1966) and Mangos, Braun & Hamann (1966) on rat salivary glands demonstrated that the fluid in the intercalated ducts (just proximal to the acinus itself) was essentially an ultrafiltrate of plasma. This fluid becomes hypo-osmotic only on passing through the striated and excretory ducts. The tubular salivary gland of the blowfly, *Calliphora*, is regionally specialized into secretory and reabsorptive regions (Oschman & Berridge, 1970). Although one would suppose that reabsorption of solute in *D. andersoni* occurs in the duct system, one cannot rule out the possibility that other regions (e.g. the vacuolar cells) participate in this process. The saliva of another ixodid tick, *Boophilus microplus*, is hyperosmotic to the haemolymph (Tatchell, 1969), so solute reabsorption from the primary secretion may not be a general phenomenon in ixodid ticks. It would be interesting to compare the ultrastructure of the duct epithelium from *Dermacentor* and *Boophilus*.

The function of the cap cell is largely a matter for conjecture. It is highly unlikely from the ultrastructure of the cap cell that it is engaged in secretion or absorption. The presence of orientated microtubules near the contiguous lateral membranes of adjacent vacuolar and cap cells suggests that the cap cell may help to hold the acinus together during secretion when the luminal hydrostatic pressure may be considerably high. In 'deflated' acini (acinar lumen collapsed) the apical-basal dimension appears greater than the lateral dimension. But in swollen acini with large lumina, the basal surface of the water cell expands to a far greater extent than the apical surface, possibly due to the restraining influence of the cap cell.

*Secretion of other substances*

We propose that one or both of the granule-cells secretes the cement by which the tick secures its mouthparts to the host skin. Moorehouse (1969) reported that the cement itself and the cement-secreting cells stain strongly with eosin. Although Moorehouse showed no figure denoting the position of these cells in the acinus, Douglas (1943), Till (1961) and Chinery (1965) all reported that the secretory granules of the granule-cells were intensely eosinophilic. Kirkland (1971), using histochemical criteria, also suggested that the large granules of the group II acinus in the nymphal salivary gland of the rabbit tick represented the cement or its antecedent.

The vacuolar cells are also actively engaged in secreting some substance as evidenced by (1) the moderate array of apical microvilli, (2) the generous endowment of dense mitochondria, (3) the distended profiles of granular ER and (4) the vacuoles which probably contain the substance to be secreted. Since granular ER is a characteristic feature of glandular cells which synthesize a protein-rich secretion (Fawcett, 1966), we suggest that the secretory product of this cell may be a protein.

*Control of salivation*

As we think the water cell is responsible for fluid secretion, we want to mention how this secretion may be controlled. Fluid secretion in mammalian salivary glands is usually controlled by parasympathetic nerves (Emmelin, 1964; Krikos, 1966) and in limited cases (e.g. parotid gland of the rabbit) by sympathetic nerves (Fritz, 1971). Salivation in the blowfly, *Calliphora*, on the other hand, is probably controlled hormonally (Oschman & Berridge, 1970). Recent pharmacological investigations suggest 'adrenergic' nervous control of salivation in *Dermacentor* (Kaufman & Phillips, 1973*b*). In the present study we found a nerve close to the basal surface of the group III acinus (Pl. 2D) although we do not know whether such nerves influence fluid secretion or some other function.

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## EXPLANATION OF PLATES

### PLATE 1

A. Light micrograph of a longitudinal section of a group I acinus joining main excretory duct. Note that the inner cell is enveloped by fibrillar cells. Duct cells are characterized by thick cuticle on apical border and thick basement membrane. (× 1880.)

B. Electron micrograph of a section through basal region of a fibrillar cell of a group I acinus. Basal plasma membrane is extensively infolded and is closely associated with mitochondria. (× 32000.)

C. Section of a nerve axon within fibrillar cell of group I acinus. (× 21400.)

D. Electron micrograph of lateral membranes joining two duct cells in region of a septate desmosome. (× 42000.)

E. Electron micrograph of a longitudinal section of a portion of main excretory duct. Note helical ridge in cuticle and tortuous lateral plasma membranes. (× 8100.)

### PLATE 2

A. Light micrograph of a transverse section through a group III acinus to show arrangement of granule, vacuolar, cap and water cell types. Cytological features of two granule cell types is not clear here; refer instead to Pl. 5A since granule cells are similar in groups II and III acini. (× 700.)

B. Light micrograph of a section through wall of a group III acinus in a region where cap cell is not conspicuous. (× 2960.)

C. A section similar to that in Pl. 2B but cap cell is conspicuous containing a large nucleus. (× 1680.)

D. Light micrograph of portions of two water cells showing a nerve approaching basal surface and a water cell nucleus. (× 2360.)

### PLATE 3

A. Electron micrograph of a portion of one of the vacuolar cells seen in Pl. 4. Note swollen profiles of endoplasmic reticulum. (× 35000.)

B. Electron micrograph of septate desmosome joining adjacent cap and vacuolar cells. Note microtubules in cap cell near septate desmosome. (× 35000.)

C. Electron micrograph of a portion of the water cell shown in Pl. 4. Note membrane infoldings with associated mitochondria and the contiguous cap and water cell membranes. (× 35000.)

D. Electron micrograph of portion of cap cell shown in Pl. 4. (× 35000.)

### PLATE 4

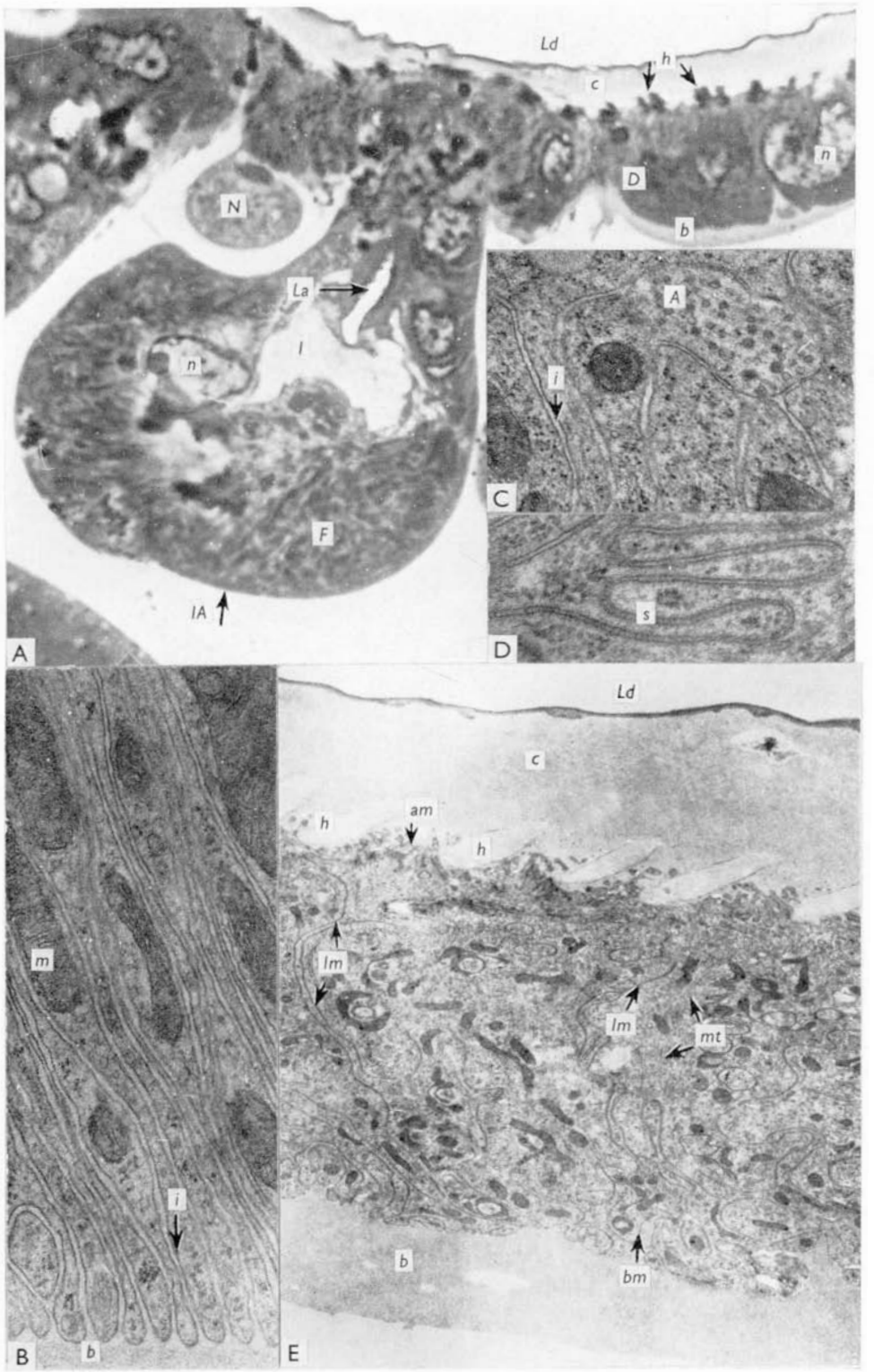
Low-power electron micrograph of a transverse section through wall of a group III acinus to show relationship between water, vacuolar and cap cells. Note the extensively infolded basal and lateral membranes of water cell, relatively close apposition of cap and water cell membranes and short regions in which cap and vacuolar cells join (arrows). Apical membranes of cap and vacuolar cells bear microvilli, and lateral and basal membranes of vacuolar cell interdigitate with lateral membrane of the water cell (asterisks). (× 10000.)

### PLATE 5

A. Light micrograph of a longitudinal section through a group II acinus showing relationship between cell types. Note relatively small lumen compared to that of the group III acinus. (× 600.)

B. Electron micrograph of a portion of a naked granule cell showing a single granule surrounded by cytoplasm packed with granular ER. (× 29000.)

C. Electron micrograph of a transverse section through a group II acinus. Lumen in this region is largely occluded with microvilli of the bordering vacuolar cell. Vacuoles in these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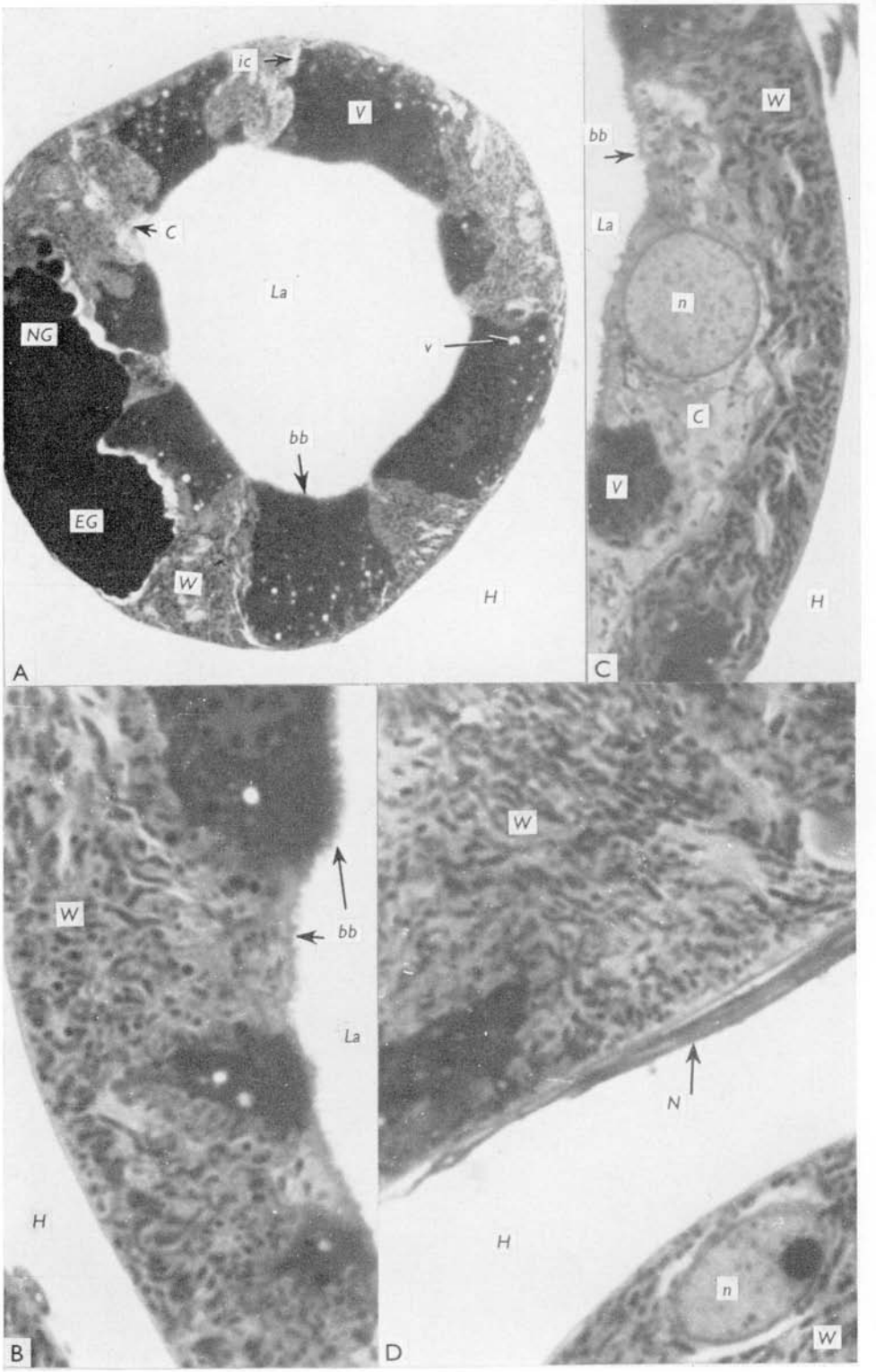
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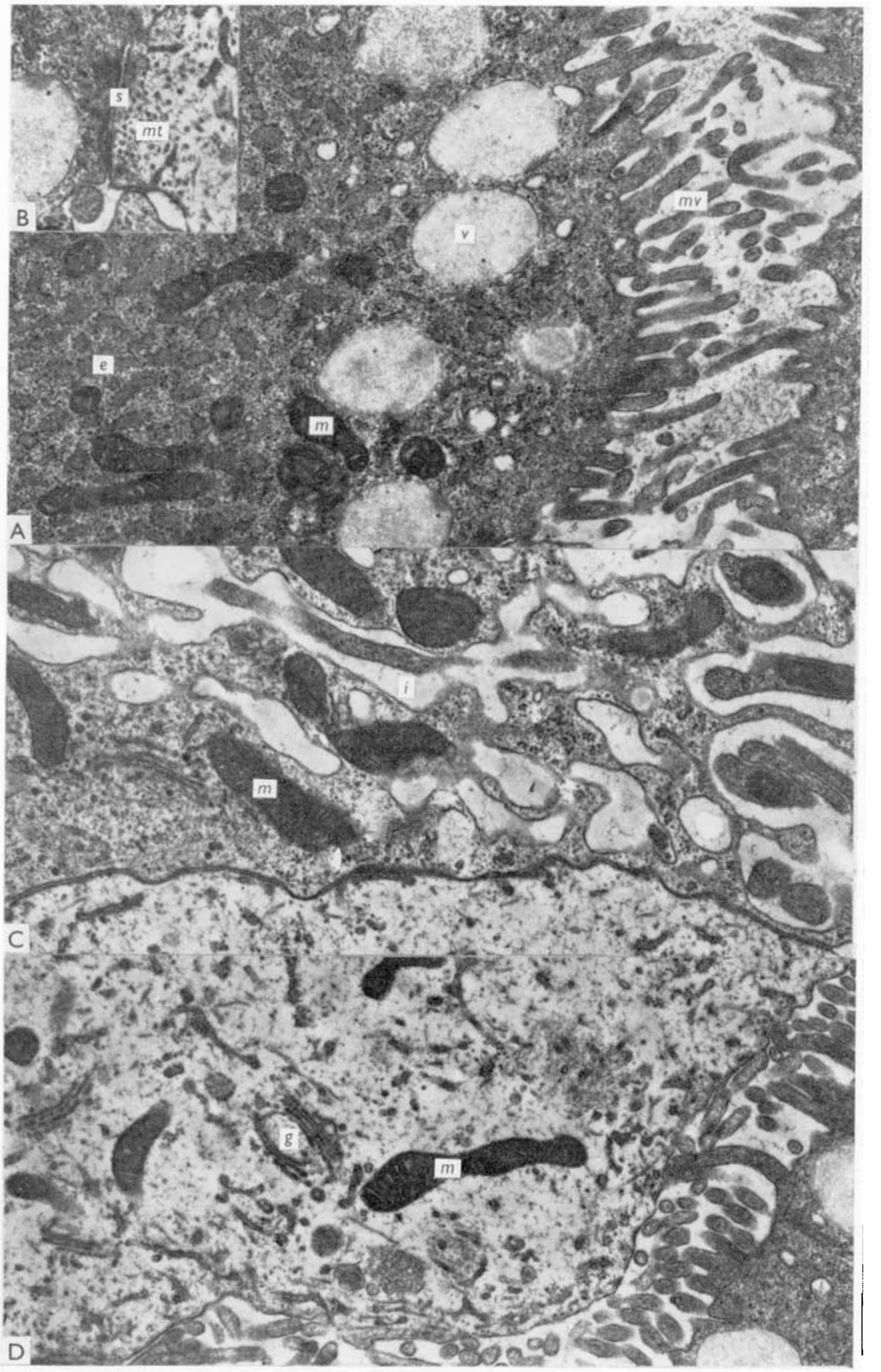


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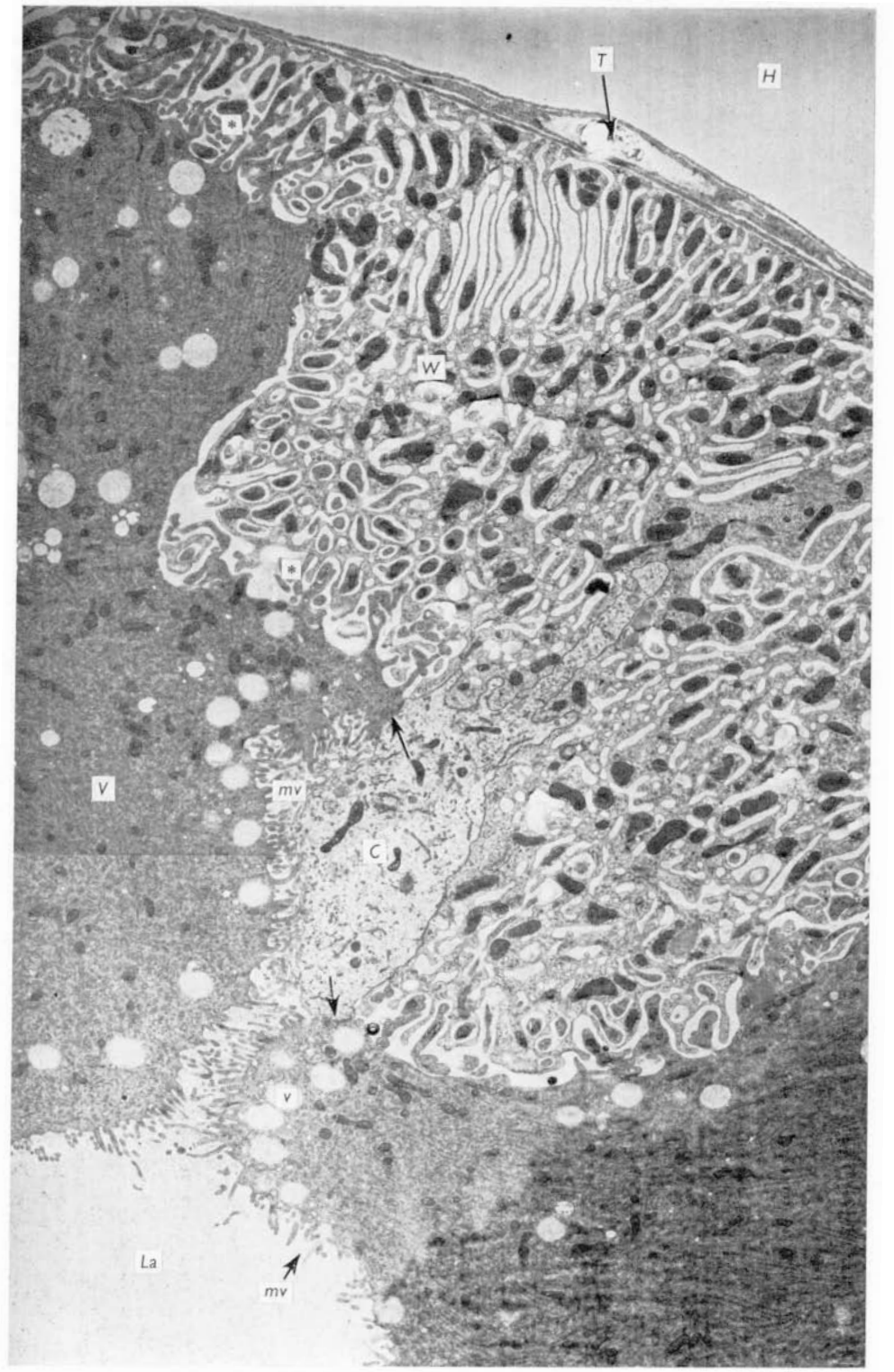
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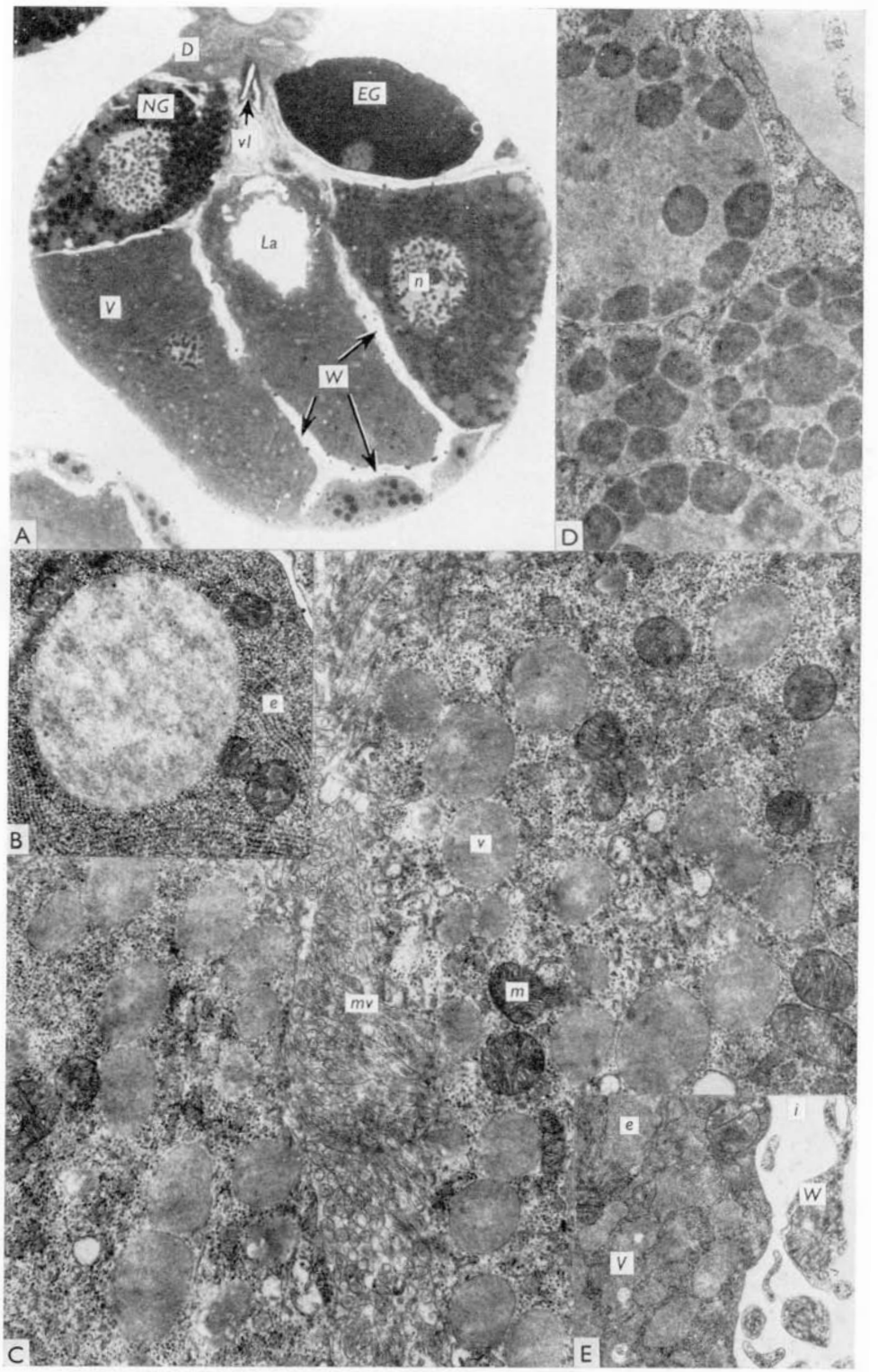


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cells are relatively small and concentrated near the apical border. Mitochondria and lightly stained granules are also apparent. ( × 27 500.)

D. Electron micrograph of part of an encapsulated-granule cell showing portions of several granules. ( × 16 500.)

E. Electron micrograph of a section through basal region of group II acinus showing highly distended profiles of smooth ER as well as a small portion of water cell. ( × 19 000.)

## KEY TO LETTERING OF PLATES

| | | | |
|---|---|---|---|
| *A* | axon | *am* | apical plasma membrane |
| *C* | cap cell | *b* | basement membrane |
| *D* | duct cell | *bb* | brush border |
| *EG* | encapsulated-granule cell | *bm* | basal plasma membrane |
| *F* | fibrillar cell | *c* | cuticle |
| *H* | haemocoele | *e* | endoplasmic reticulum |
| *I* | inner cell | *g* | Golgi apparatus |
| *IA* | group I acinus | *h* | helical ridge |
| *La* | acinar lumen | *i* | infolding of plasma membrane |
| *Ld* | duct lumen | *ic* | intercellular space |
| *N* | nerve | *lm* | lateral plasma membrane |
| *NG* | naked-granule cell | *m* | mitochondrion |
| *T* | trachea | *mt* | microtubules |
| *V* | vacuolar cell | *mv* | microvilli |
| *W* | water cell | *n* | nucleus |
| | | *s* | septate desmosome |
| | | *v* | vacuole |
| | | *vl* | valve |

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