THE FINE STRUCTURE OF THE HEPATOPANCREAS OF *CARCINUS MAENAS* (L.) (DECAPODA BRACHYURA)

BY

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INTRODUCTION

The hepatopancreas of the Decapoda is a large lobed organ filling much of the body cavity. Each lobule consists of a series of blind-ending tubules opening into larger ducts which finally open into the midgut. The lumen of each tubule is bordered by a single layer of epithelial cells, external to which is a discontinuous layer of contractile cells arranged as a net. The functions of the hepatopancreas are secretion of digestive enzymes, absorption and storage of fat, glycogen and calcium. The epithelial cells have four clearly distinguishable types of appearance, and much of the work of earlier investigators was directed to discovering the function of each cell-type and the sequence of transformation of one cell-type into another.

Much of the basic work on the histology of the hepatopancreas of the Decapoda was done between 1880 and 1910, notably by Frenzel (1884), Guieysse (1907) and Apáthy & Farkas (1906). The first two authors misinterpreted the functions and interrelationships of the various cell-forms, but Apáthy & Farkas were much nearer the truth. Jacobs (1928) published a comprehensive paper on the cytology of secretion formation in the hepatopancreas of *Astacus leptodactylus* Eschscholtz, in which he made a careful study of the changes in various cell inclusions (nuclei, nucleoli, secretion granules, parasomes, Golgi apparatus and mitochondria) at different phases of the secretory cycle. These observations together with the work of Hirsch & Jacobs (1928, 1930) who counted the number of cells of each type at various intervals after feeding previously starved *Astacus,* led to a picture of the sequence of events during this type of secretion (holocrine) which accounted satisfactorily for the facts.

However, histological work on the Brachyura has been much less extensive. The transitions of cell-type during secretion were studied by Van Weel (1955)

in a crab (*"Atya"*) [1]) and by Reddy (1937, 1938) in *Parathelphusa.* It appears that in these two crabs at least secretion is not holocrine but merocrine, i.e. the cell is not destroyed in the process of secretion but renews its contents. This must necessarily involve a sequence of events rather different from that described by Hirsch & Jacobs, and there is presumably a slower rate of mitosis, since only "worn-out" cells need be replaced.

It was hoped that the electron microscope would elucidate the nature of the "parasome", which was studied by Vigier (1901) and Guieysse (1907) and has also been described by Jacobs (1928) in *Astacus* and Van Weel (1955) in a crab [1]). Its presence is not constant, but has been said to depend on the physiological state of the cell. There may be one or more in a cell, usually lying distal to the nucleus. It consists of a "body" (usually acidophilic) lying in a vacuole and is considered by Jacobs to arise from material (possibly nucleolar) extruded from the nucleus. Sometimes it has a "stalk" attaching it to the nucleus.

MATERIALS AND METHODS

Crabs were obtained from Southampton Water between April and June; only males were used. For electron and phase microscopy pieces of hepatopancreas were fixed initially in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, post-fixed in 2% veronal acetate-buffered OSO_4 and embedded in Epon. Sections of 1 μ were examined by phase-contrast, and ultra-thin sections were stained with lead hydroxide (Millonig, 1961) for 45-60 sec., and examined in an A.E.I. E.M. 6 electron microscope. For light microscope the tissue was fixed in formol-acetic-ethanol and stained with haematoxylin and eosin.

RESULTS

Light and phase-contrast microscopy

The arrangement of the epithelial cells of the blind end of a single hepatopancreas tubule is shown in pl. I fig. 1. We shall use the existing nomenclature for the four epithelial cell-stages (as used by Jacobs (1928) for *Astacus,* and Van Weel (1955) for a crab [1])) so as not to confuse an already complicated situation.

1. Embryonic (E) cells. These are small undifferentiated cells occurring at the distal end of the tubule, the only part where the epithelium is more than one cell thick. They are the only cells in which mitoses are seen.

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[1]) In the title and throughout his paper Van Weel consistently mentioned the animals upon which his research was based as "crabs" or "Brachyura", although he used for them the name *Atya spinipes* Newport (and even cited the junior synonym of that name *Atya moluccensis* De Haan) which stands for a fresh water shrimp. As a mistake in the vernacular name seems less likely than one in the scientific name, and as Van Weel indicated (p. 41 of his paper) the carapace width of his material as up to 45 mm, it is practically certain that his material indeed consisted of crabs belonging to the suborder Brachyura (if they really are "tropical fresh-water crabs" they might be Potamonidae) and not of shrimps belonging to the suborder Macrura (section Caridea, family Atyidae). [Ed.].

2. Fibrillar (F) or "dark" cells. The cytoplasm stains intensely with basic dyes (pl. I fig. 2, pl. II fig. 1).
3. Blasenzellen (B) or extrusion cells. These are characterised by large vacuoles often projecting into the tubular lumen (pl. I fig. 2, pl. II fig. 2).
4. Restzellen (R) or "light" cells. These have a vacuolated cytoplasm when fixed in fat solvents; osmium shows that they contain many lipid droplets (pl. II fig. 1). They are also probably responsible for most of the glycogen and calcium storage. Jacobs calculated that they constituted 75 to 80% of the epithelial cells.

All epithelial cell-stages bear microvilli on their luminal borders, and these can be seen in phase photomicrographs (pl. II fig. 1). The microvilli, material in the secretion vacuole, and the basement membrane react positively when stained by the PAS method, both before and after saliva treatment, which suggests that they contain a neutral mucopolysaccharide or mucoprotein component. The microvilli are stained by alcian blue, which indicates the presence of an acid mucopolysaccharide also. Both microvilli and basement membrane when stained with toluidine blue showed metachromasia which was removed by alcohol.

Electron microscopy

The distinction between these four cell-stages is not always clear cut, but representative examples of each stage will be described.

E-cells (pl. III) at the tip of the tubule are polyhedral, undifferentiated and have a high nuclear : cytoplasmic ratio. They contain small spherical mitochondria with few cristae, a moderate amount of rough endoplasmic reticulum and many single-membrane bounded vacuoles. Golgi zones can be identified, and in the cells furthest from the tip a few electron-dense structures, probably the early secretory product of the differentiating cell, can be seen. Microvilli are present on the border adjacent to the lumen.

The basophilia of the F-cells (pls. IV-VI) is accounted for by the presence of abundant rough endoplasmic reticulum, arranged in such a manner as would account for the fibrillar appearance seen in the light microscope by previous workers. They are tall columnar epithelial cells with basally situated nuclei. Golgi zones are not easily identifiable. The spaces between the endoplasmic reticulum appear to develop into secretory vacuoles (pl. V, pl. VI fig. 2) in which myelin figures can be discerned in the larger examples.

The B-cell, characterised by a single large vacuole, is considered to be essentially an F-cell in which the small secretory vacuoles have coalesced (pl. VII fig. 1) to form a single large vacuole occupying a large proportion of the cell volume, leaving a small rim of cytoplasm and a compressed basal nucleus. The contents of the vacuole have obviously been affected by solvents used during dehydration and embedding, but the presence of myelin figures suggests a phospholipid component.

The R-cells (pls. IV, V, pl. VII fig. 2) are characterised by lipid droplets, which are osmiophilic to a varying extent and which because of their composition often appear chattered in thin sections. The cells also contain single-membrane bounded vacuoles, which may be structures from which lipids of a different composition have been extracted during embedding (pl. IV, pl. VII fig. 2). Another characteristic feature of these cells is the presence of calcium. When present in large amounts it becomes chipped out during sectioning, thus leaving holes in the section (pl. V). However, small particles can be seen (pl. VII fig. 2, pl. VIII fig. 1) to be composed of concentric laminations of electron-dense material often more densely compacted at the centre and periphery. When first recognisable each calcium particle is smaller than a mitochondrion and apparently enclosed within a small single-membrane bounded space in the cytoplasm. It grows probably by the accretion of soluble calcium salts from the cytoplasm. A third storage material, glycogen, is not always visible, but is shown in pl. VIII fig. 2 at the apex of a cell. The nuclei of R-cells are in a position similar to those of the F- and B-cells.

Cells with bundles of contractile fibrils, probably smooth muscle and bearing a certain similarity to the myoepithelial cells of mammalian salivary glands, are present external to the tubular basement membrane (pl. IX fig. 1). Cytoplasmic processes appear to run in all directions around the tubules of epithelial cells. A stellate morphology seems to be the most likely, but so far we have been unable to demonstrate this satisfactorily. Bundles of muscle fibrils run in several directions within an individual cell in the part nearest to the basement membrane (pl. IX fig. 2). Much of the cell volume apart from the nucleus is occupied by large intra-cytoplasmic vacuoles probably containing lipid (pl. IX figs. 1, 2).

The basement membrane (pl. IX, pl. X fig. 1) is split, and by far the thicker component is placed between the epithelial cell and the myoepithelial cell. A very thin layer of basement membrane seems to cover the myoepithelial cells. There appears to be no endothelial cell lining to the sinusoids, and the wall is either myoepithelial cell or the apparently naked basement membrane of the tubular epithelial cells (pl. X fig. 1). The basement membrane itself appears to be approximately 5,000 Å thick and to be composed of two layers. The inner more electron-dense layer is 1,000-2,000 Å thick. The outer, which is possibly composed of compacted fibrillar material, is 3,000-4,000 Å thick.

Crab haematology is a confused subject and we attempt no more than a statement that clumps of nucleated granule-containing cells are found in the sinusoids (pl. X). An interesting observation is that a discontinuous layer of what may be precipitated material surrounds the whole clump, outside the plasma membranes (pl. X fig. 2). Its presence within invaginations or pores in the plasma membrane suggests that it may have been extruded from the cells. Other features of these cells are clusters of ribosomes round the nucleus, and numerous vacuoles, many of which contain myelin figures.

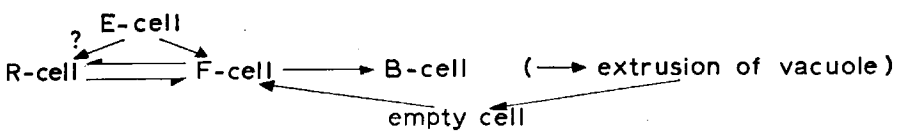
DISCUSSION

The appearances in *Carcinus* agree with the conclusion of Jacobs (1928) for *Astacus* that the sequence of epithelial cell stages is: E-cell → F-cell → B-cell.

All intermediate stages can be seen between embryonic and fibrillar cells, with increasing amounts of endoplasmic reticulum. Then vacuoles increase in number and size between the membranes of the endoplasmic reticulum, and finally coalesce to occupy practically the whole cell, as the endoplasmic reticulum is reduced to a rim round the 'ripe' vacuole. We have never observed in electron microscope sections a vacuole in the act of being extruded, though this can be seen occasionally in the light microscope. Neither have we been able to identify a cell immediately after extrusion has taken place, though pl. VI fig 1 could show one such cell. We are fairly certain that the sequence which Van Weel (1955) proposed for a crab (see footnote, p. 57), i.e. E-cell → R-cell → B-cell, does not take place in *Carcinus*. We have found no evidence that the R-cell could be the immediate precursor of the B-cell. The "typical" R-cell is deficient in endoplasmic reticulum and contains storage materials and vacuoles of irregular shapes (pl. VII fig. 2), different from the smoother-outlined vacuoles formed among the endoplasmic reticulum of an F-cell.

However, the position of the R-cell in the sequence still presents a problem. Hirsch & Jacobs (1930) concluded that for statistical reasons some F-cells, at the proximal end of the tubule, must be derived from R-cells there, as the mitotic rate at the blind end was insufficient to account for increased production of the F- and B-cell stages when starved animals were fed. They found a second centre of mitotic activity in the dorsal coecum, which, they thought, could be the source of these R-cells. So their proposal for the sequence at the proximal end was: E-cell → R-cell → F-cell → B-cell.

Hirsch & Jacobs were dealing with *Astacus* in which the secretion is holocrine. In *Carcinus,* however, in which secretion is probably merocrine, the problem of replacement of B-cells should be less acute if they survive extrusion of the vacuole and produce another one. However there could be situations in which the concentration of F-cells is not high enough to produce the number of B-cells needed, and there could be a reversible equilibrium between R-cells and F-cells whose direction depends on the crab's state of nutrition. We have seen what could be intermediate stages between R- and F-cells, e.g. F-cells with some storage materials (pl. VI fig. 1) and R-cells with rather large amounts of endoplasmic reticulum (pl. V). We hope that a more conclusive answer to this problem will come from autoradiographic experiments now in progress, but meanwhile we put forward the following scheme for *Carcinus*:



It would be interesting to know what is the switch mechanism which causes one cell to specialise in absorption and storage and its neighbour to devote itself to protein-synthesis.

The presence of apparently identical microvilli at each cell-stage suggests that they are all capable of fluid transfer to and from the lumen. Van Weel (1955) found that in a crab (see footnote, p. 57) iron and fat were absorbed primarily into the R-cells, but that the other cell-stages were capable of some absorption. He concluded that the fat droplets in the R-cells were the result of resynthesis, as he saw no phagocytosis of fat droplets. We found no evidence of this in *Carcinus* either.

We found no special cell-inclusions corresponding to the "parasome" seen in the light microscope by earlier workers. This could have been because they do not occur in *Carcinus* or because the cells we examined were not in the right physiological state. Jacobs (1928) found them in *Astacus* in E-cells and "young" F-cells as well as in R-cells, and considered that in the former two cases they were involved in serration vacuole formation. In his crabs (see footnote, p. 57) Van Weel found that they were present in E-cells, absent in F-cells and rare in R-cells. We examined material from *Astacus* hepatopancreas in the electron-microscope but could find nothing identifiable as a "parasome". The only explanation which has occurred to us is that early stages of secretion vacuole formation in F-cells, which are always found distally to the nucleus (pl. V), could give an appearance in the light microscope identical with the bodies in question.

Fat is a very constant feature, especially of the R-cells, and is not reduced in amount until the crab has been starved for several months. Munn (unpublished) found that in male crabs the total lipid was 47 to 77 mg per g fresh hepatopancreas; there was a seasonal variation, and the highest figure for male crabs taken from Southampton Water was obtained in July. These high concentrations of fat make the hepatopancreas a difficult tissue to deal with histologically.

In contrast, the amounts of glycogen and calcium show very wide variation. Glycogen concentration depends possibly on the crab's state of nutrition and on the time of year (Munn, unpublished), and may also be related to sex and stage of moult cycle (Baumberger & Dill, 1928; Renaud, 1949). It is interesting to notice the proximity of glycogen to parallel cisternae of endoplasmic reticulum at the periphery of the cell, an association noted by Wright (1963) in the intestinal cells of the parasitic nematode *Capillaria hepatica.* Porter (1961) has pointed out the frequent association of smooth endoplasmic reticulum with glycogen deposits in vertebrates.

The amount of calcium, also, depends on the stage of the moult cycle. Robertson (1937) followed the calcium and phosphorous content of hepatopancreas through the moult cycle of *Carcinus maenas,* and found that the content of both elements was highest just before the moult, when the Ca : P weight ratio was 1.5. Twelve hours after the moult the ratio had fallen to 0.8, which is consistent with mobilisation of the stored calcium for the hardening of the shell.

It is interesting that no precipitated calcium is shown in the electron micrographs inside mitochondria. De Luca & Engstrom (1961) and Vasington & Murphy (1962) showed that isolated rat kidney mitochondria accumulate calcium ions with a simultaneous uptake of phosphate. The uptake is respiration-dependent and requires the presence of ATP; it takes place also in isolated liver, heart and brain mitochondria. Greenawalt, Rossi & Lehninger (1964) show electron micrographs of isolated rat liver mitochondria which have accumulated Ca^{2+} and HPO_4^{2-} ions; they contain 'dense bodies' which are possibly precipitated calcium phosphate. However, it appears that in vivo calcium ions accumulate inside mitochondria only under pathological conditions, e.g. liver poisoning by carbon tetrachloride (Thiers, Reynolds & Vallee, 1960) and hyper-vitaminosis D. And in the case of *Carcinus,* although calcium must be present in high concentration in the cytoplasm of the hepatopancreas, it precipitates outside rather than inside the mitochondria. If a homogenate of hepatopancreas is centrifuged at low centrifugal force (950 g/min.), the calcium-containing material is visible as a white sediment at the bottom of the centrifuge tube, and appears under the light microscope as highly refractile 'spherules'. The calcium: inorganic phosphorus molar ratio in this fraction is 1.35, which is consistent with the material being mainly calcium phosphate, $Ca_3(PO_4)_2$ in which the ratio is 1.5. Additional evidence that the material is calcium phosphate is that in histological sections (fixed in formaldehyde or glutaraldehyde) it stains orange-red with alizarin red S (Dahl, 1952) which indicates calcium, and black with von Kossa's silver nitrate method (Pearse, 1960), which indicates phosphates or carbonates.

Myoepithelial cells have been described surrounding the acini of salivary, lachrymal and Harderian glands (Chiquoine, 1958; Leeson, 1960; Scott & Pease, 1959; Woodhouse & Rhodin, 1963). The cells described in this paper differ in that the contractile fibrils occupy a far smaller proportion of the cell volume; the remainder is occupied by vacuoles of varying size which may represent readily accessible stores of nutriment. The muscle fibrils are in the part of the cell nearest to the basement membrane (pl. IX fig. 1), where they can contract effectively without disturbing the vacuoles which are placed more superficially.

The cells seen in the sinusoids may be "eosinophil lymphocytes" (Balss, 1944). On the basis of this work we cannot ascribe any function to the granules. In Decapoda these cells have been stated to be phagocytic (Kollman, 1908). Thus the granules may contain hydrolytic enzymes as has been asserted for the granules of mammalian neutrophils and eosinophils (Hirsch & Cohn, 1964). But Kollmann also attributed a nutritive function to the granules, and found that both the number of granules per cell and the number of granular cells were reduced on starvation.

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ZUSAMMENFASSUNG

Die Struktur der Epithelzellen der Hepatopankreasdrüse von *Carcinus maenas* wurde mittels Licht-, Phasen- und Elektronenmikroskopie untersucht. Die Drüse resorbiert, bewahrt und sezerniert die Verdauungsenzyme. Vier Zellarten lassen sich unterscheiden (vergl. die Figuren): die undifferenzierten embryonalen Zellen (E) an der Spitze jedes Drüsenschlauches, die Fibrillenzellen (F), die sich durch Anwendung basischer Farbstoffe intensiv färben lassen und das Endoplasmaretikulum enthalten, die Blasenzellen (B), die eine große Sekretvakuole enthalten, und die Restzellen (R), in welchen sich der größte Teil des Fett-, Kalzium- und Glykogenvorrates befindet. Der Feinbau jeder Zellart wird beschrieben; er stimmt mit der Entwicklungsfolge E $\rightarrow$ F $\rightarrow$ B überein. Die Lage der R-Zelle bleibt unsicher. Es wird ein Gleichgewicht F$\leftrightarrow$R vermutet, dessen Richtung von dem Nahrungszustand des Tieres abhängig ist. Die Epithelzellen tragen Mikrovilli an inren Lumensäumen, und an der Außenseite ihrer Grundmembranen findet man eine unterbrochene Schicht von zusammenziehbaren Zellen, die den Myoepithelzellen der Säugetiere einigermaßen ähnlich sind. Die Sinusse zwischen den Drüsenschläuchen sind durch diese zusammenziehbaren Zellen und eine dichte Grundmembran gesäumt; ein Endothelfutter ist nicht vorhanden. Schließlich wird eine Zellart beschrieben, die Vakuolen und Granula enthält und in diesen Sinussen in Klumpen vorhanden ist.

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EXPLANATION OF THE PLATES

Pl. I fig. 1 and pl. II are phase-contrast photomicrographs of glutaraldehyde-osmium fixed, Epon embedded tissues. Pl. I fig. 2 is of a paraffin embedded, haematoxylin and eosin stained tissue, photographed by conventional light micrography. Pls. III to X are electron micrographs.

Plate I

Fig. 1. Tip of one tubule of *Carcinus* hepatopancreas. The single layer of columnar epithelium standing on the basement membrane can be discerned, but the lumen is obscured. ($\times$ 500 approx.).

Fig. 2. Two F-cells, which appear dark owing to basophilia. The two large vacuoles (lower right) are at the apices of B-cells. Note the nuclei situated about 2/3 of the way down the cells ($\times$ 2,000 approx.).

Plate II

Fig. 1. A dark F-cell (F) is flanked by two R-cells (R_1, R_2) in which osmiophilic lipid droplets can be clearly seen. At the apex of cell R_1 microvilli are visible ($\times$ 2,000 approx.).

Fig. 2. Apical portion of two B-cells with prominent secretion vacuoles (V) below which the osmiophilic lipid droplets of adjacent R-cells can be seen. The secretion vacuoles appear to be ripe for discharge into the tubular lumen (Lu) ($\times$ 2,000 approx.).


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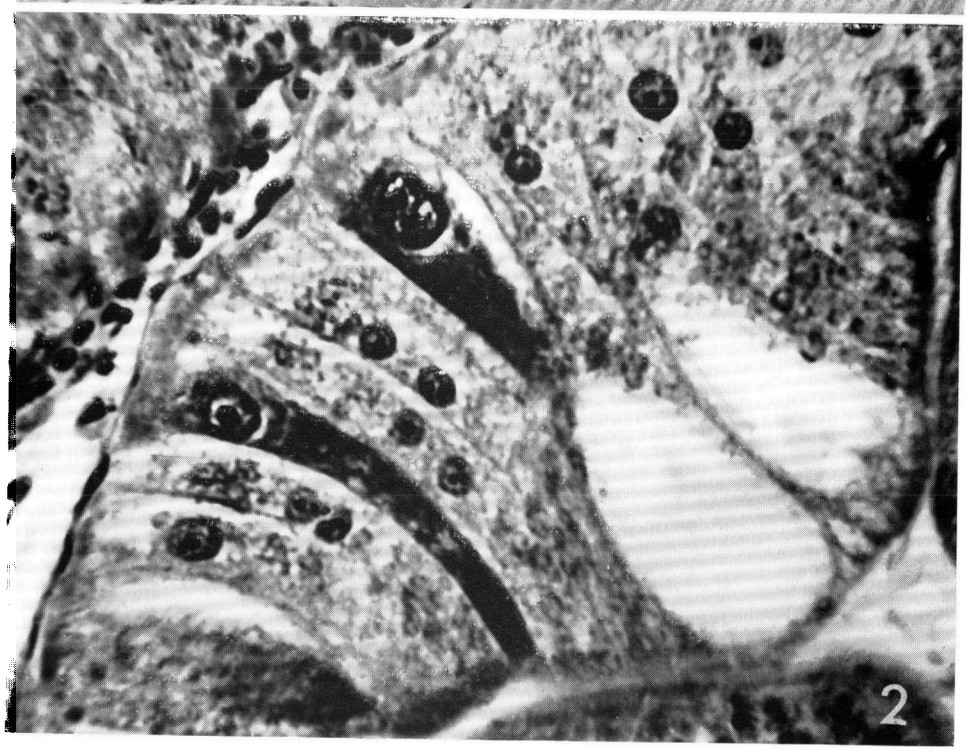
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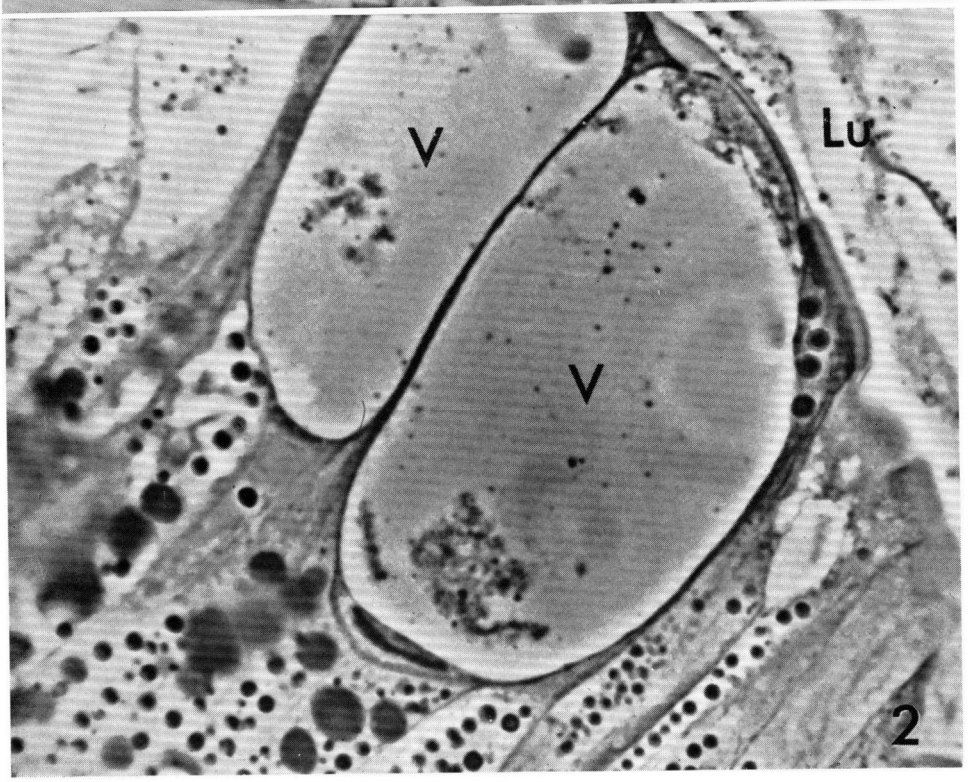
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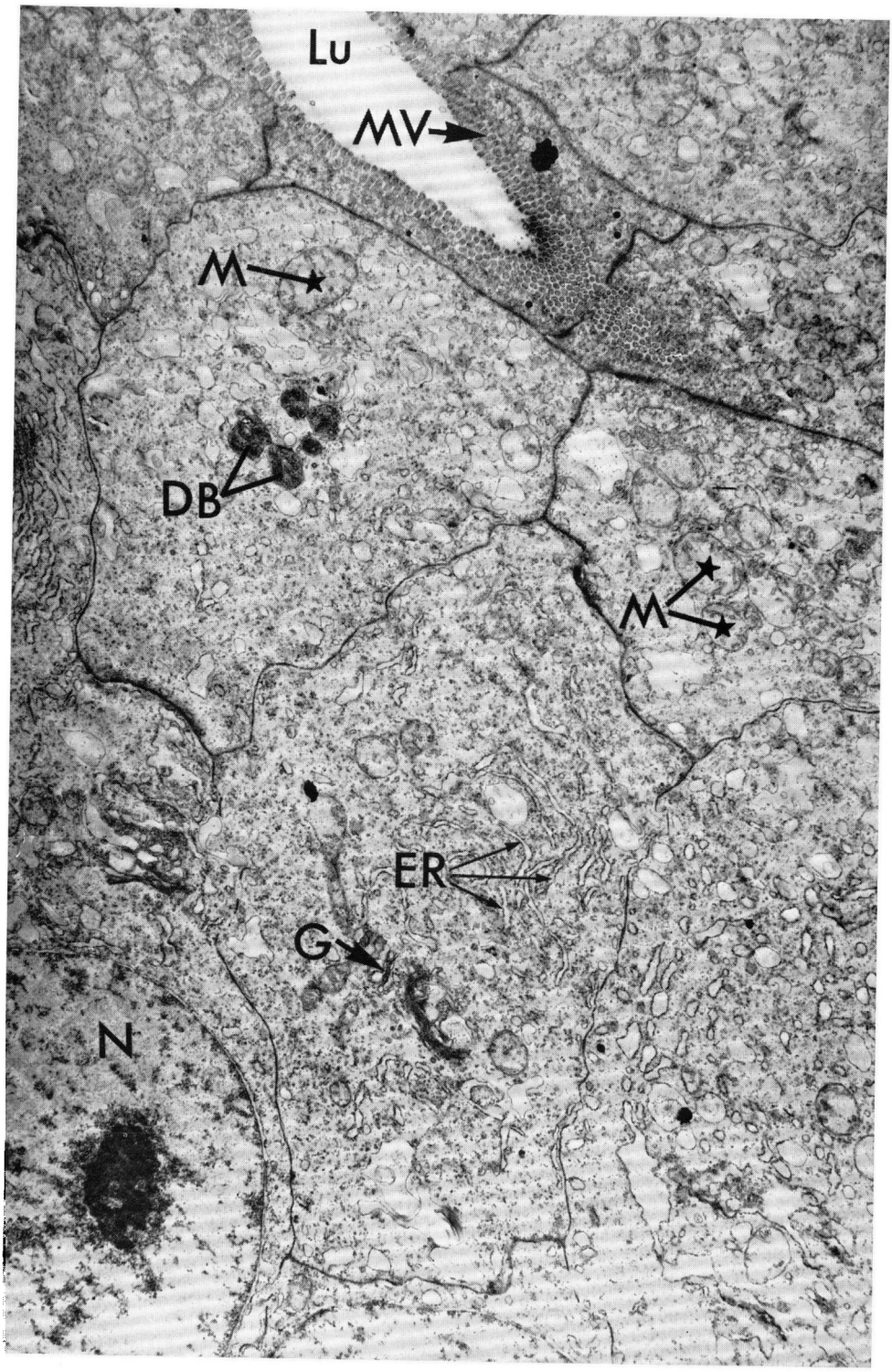
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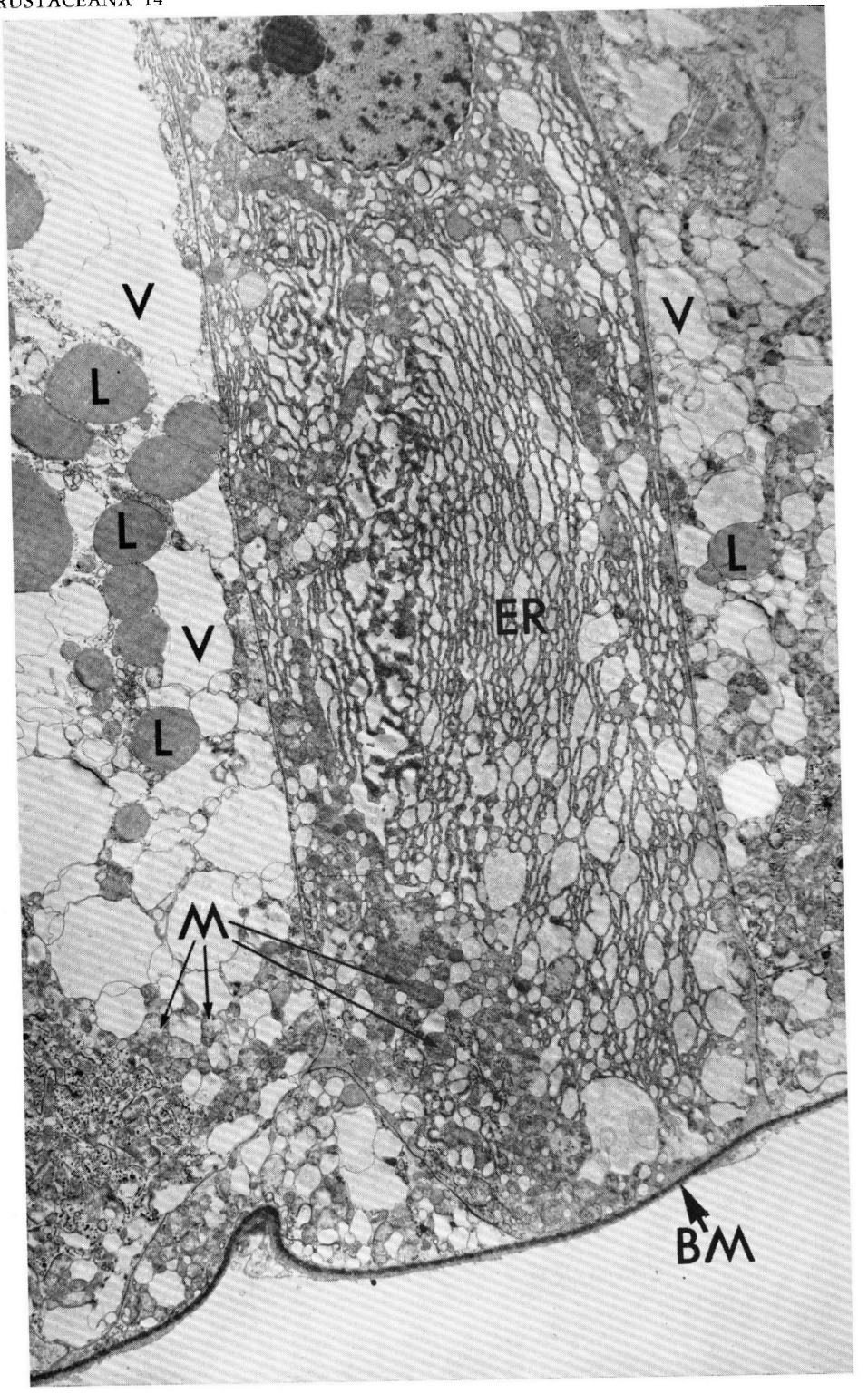
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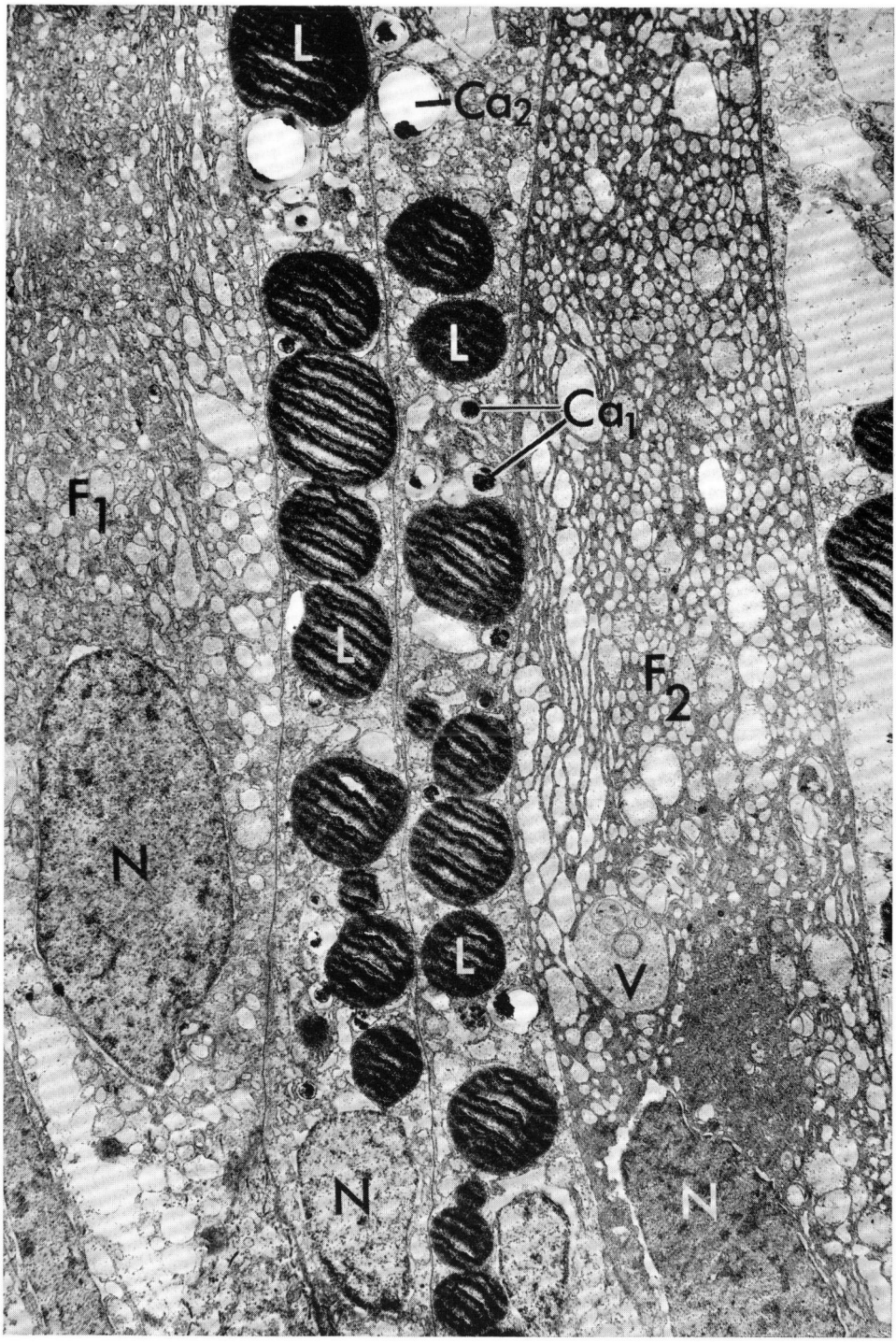
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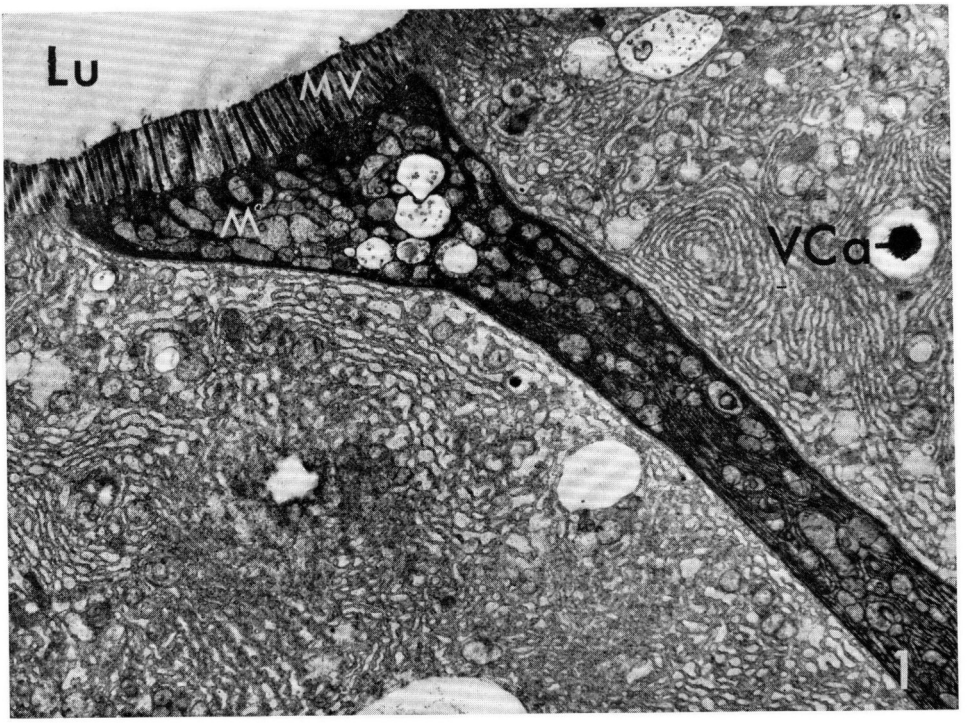
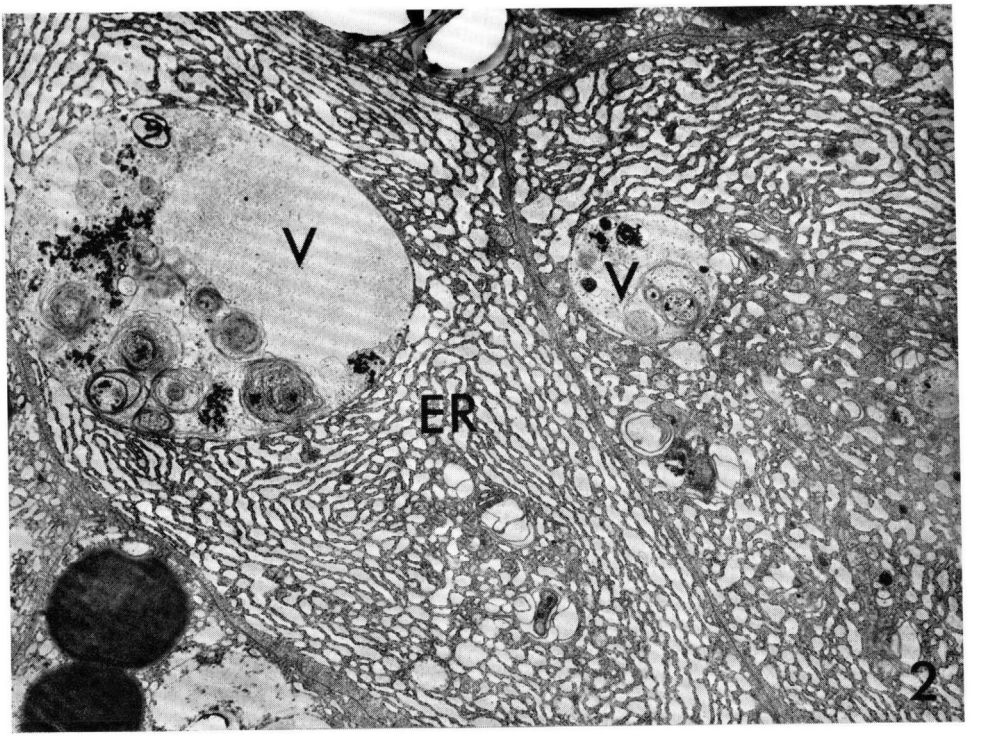
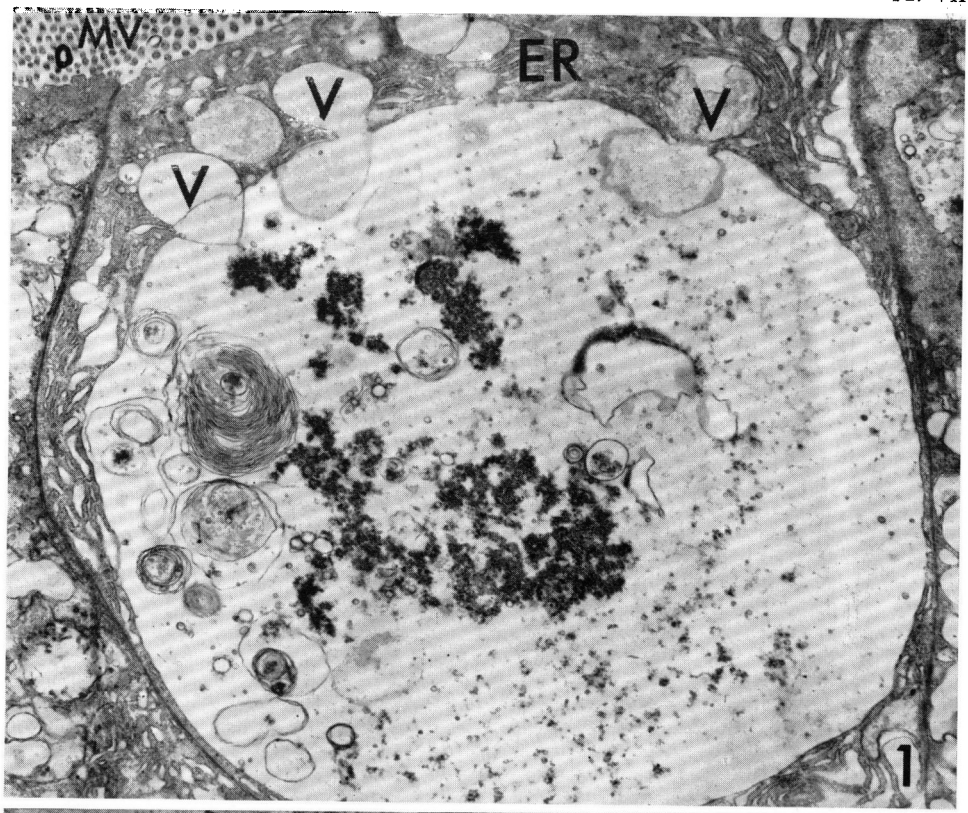
N
L
V
N
N


Lu
MV
M
VCa
1


V
V
ER
2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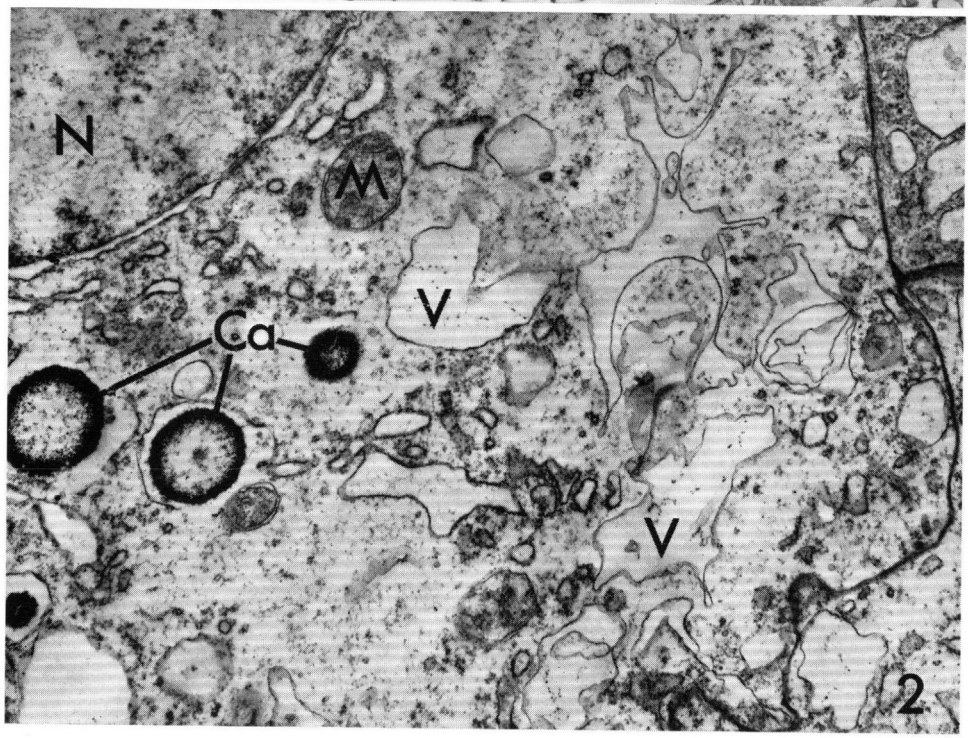
V
ER
V
V
1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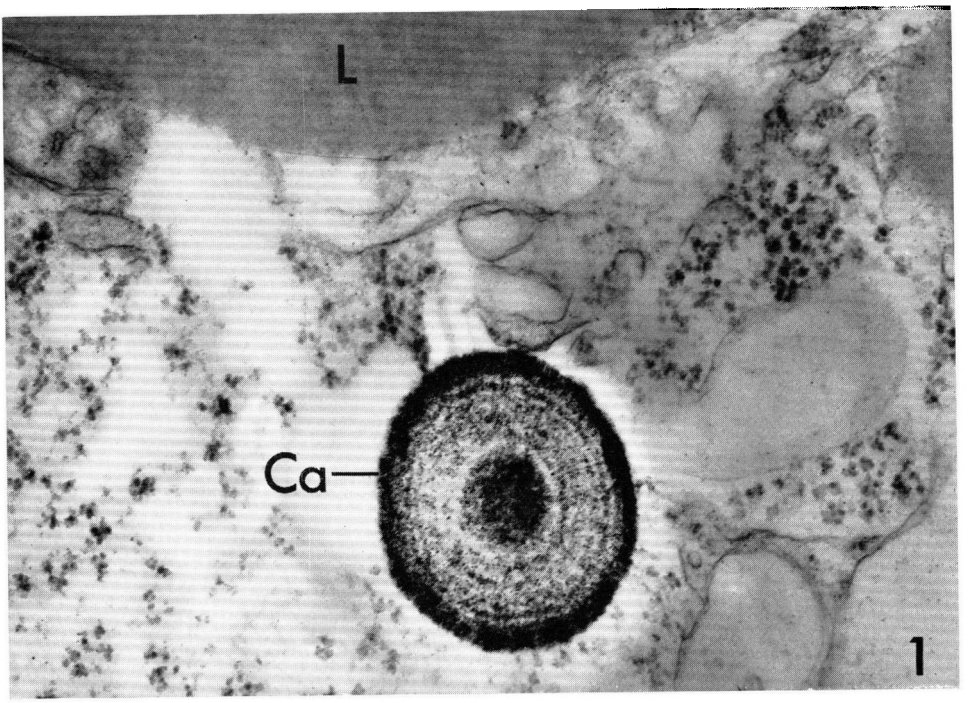
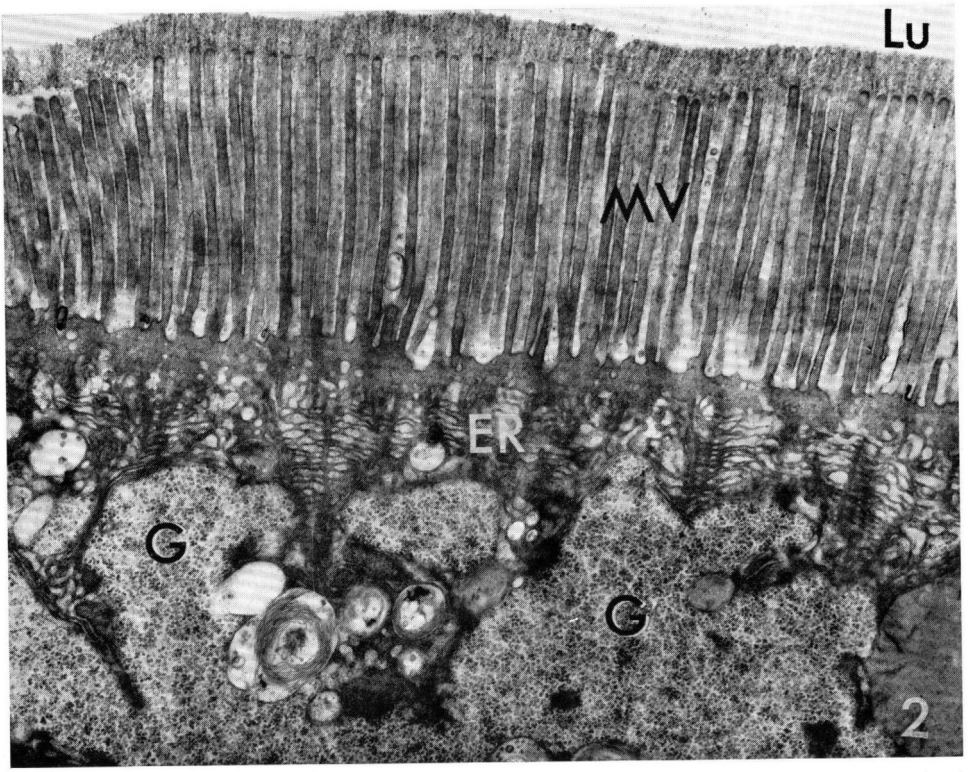
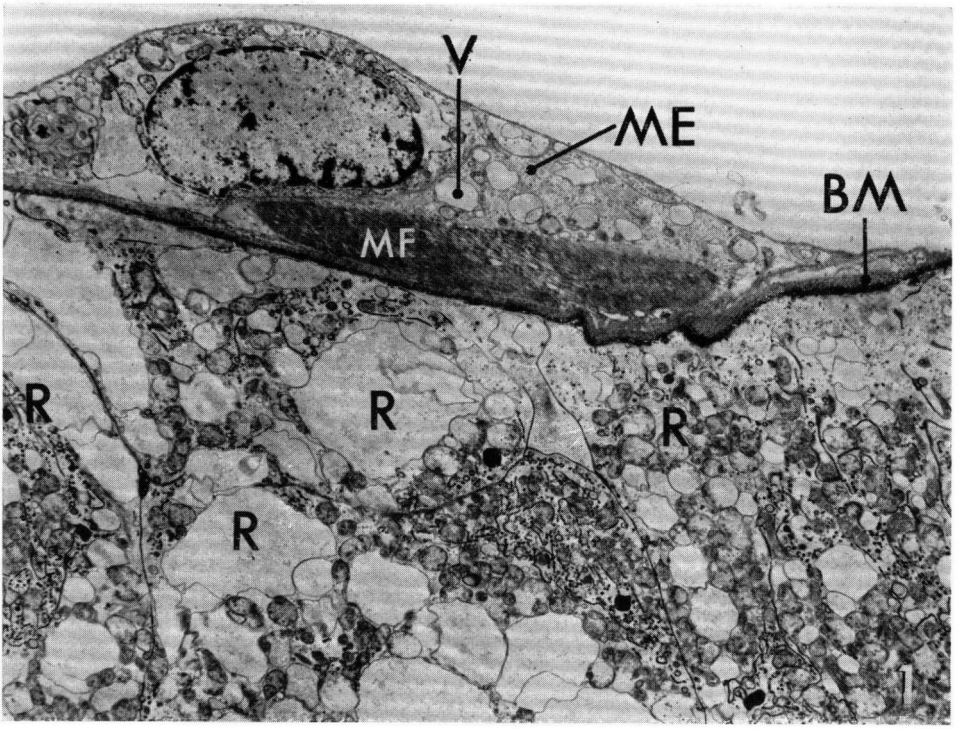
M
V
Ca
V
2


L
Ca
1


Lu
MV
ER
G
G
2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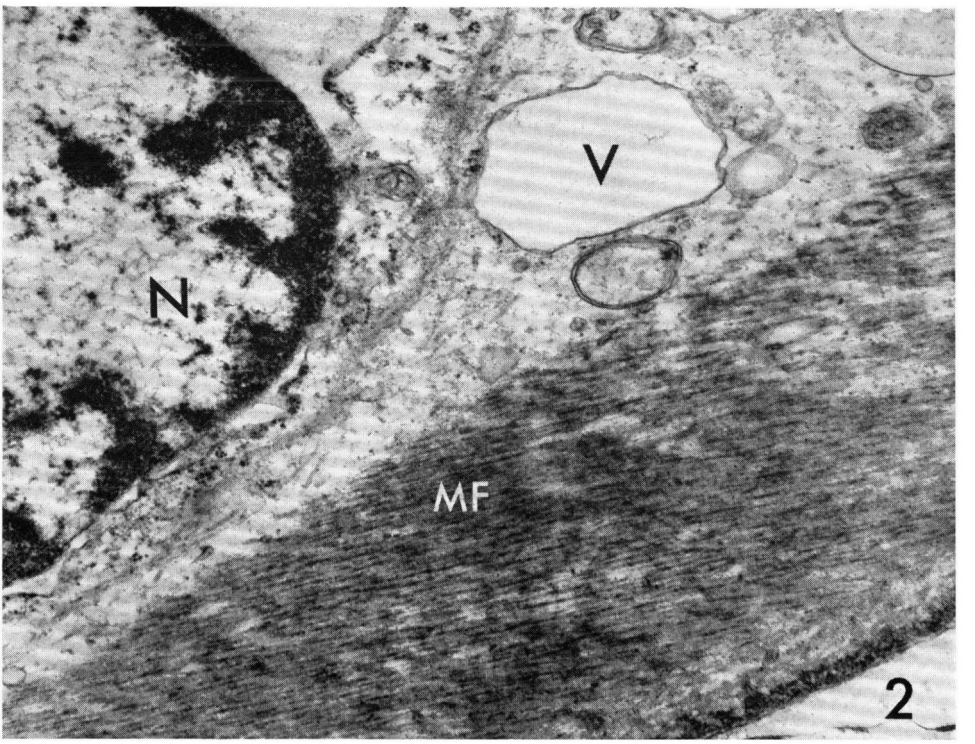
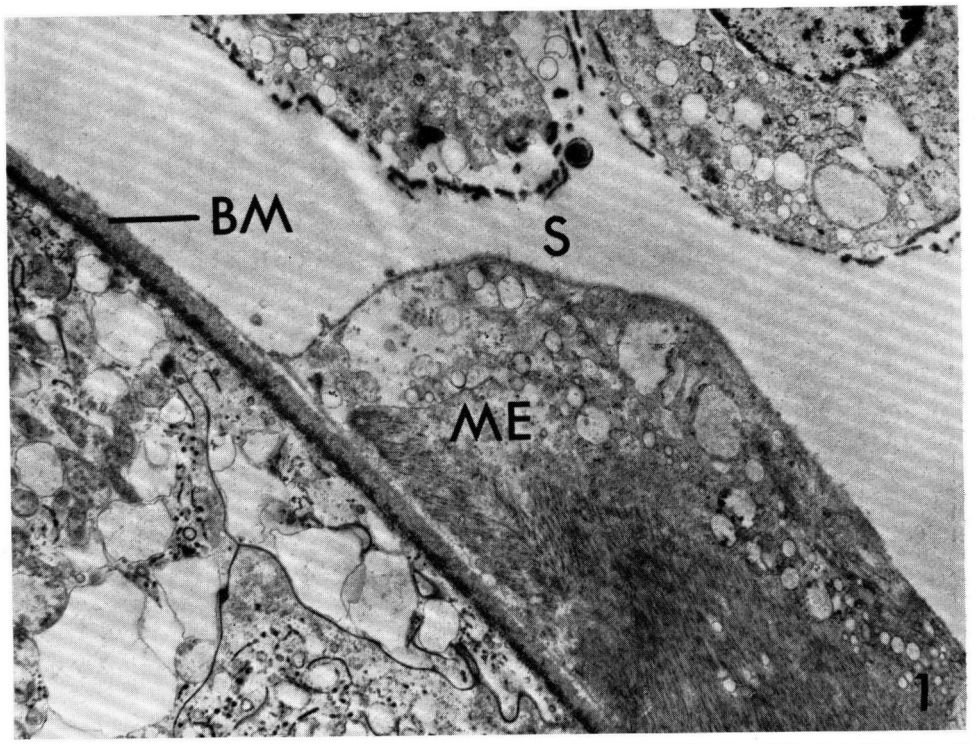
ME
BM
MF
R
R
R
R


V
N
MF
2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S
ME
1

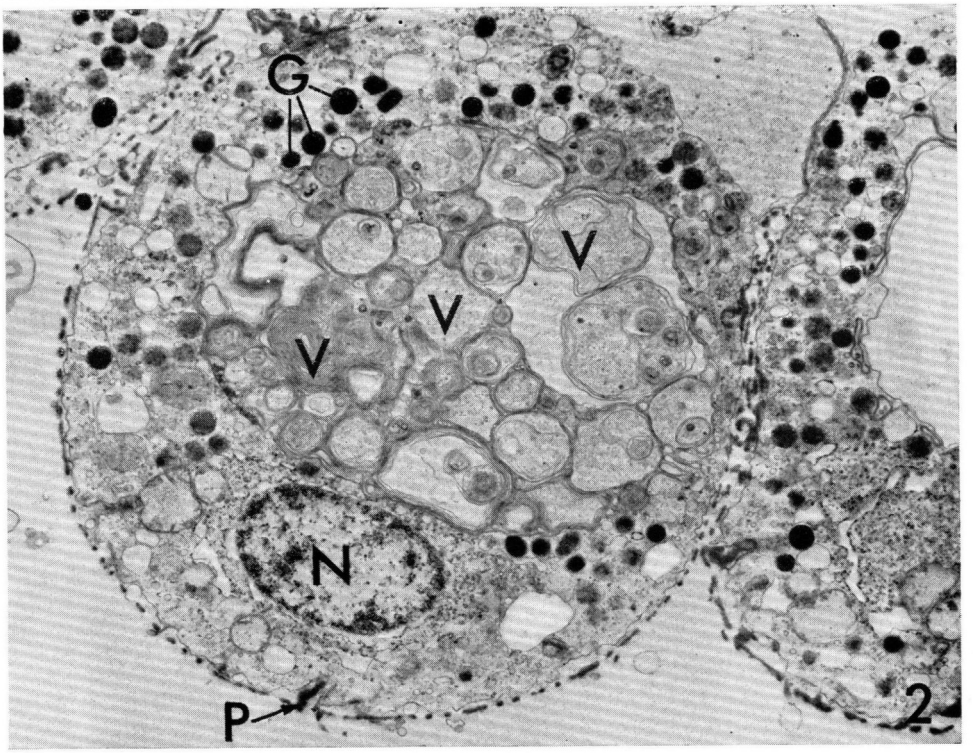
G
V
V
V
N
P
2

Plate III

Tip of tubule showing E-cells with apical microvilli (MV), small spherical mitochondria (M), fairly loose endoplasmic reticulum (ER) and a few electron-dense intravacuolar structures (DB). Lu, lumen of tubule; N, nucleus of E-cell; G, Golgi zone (× 5,500 approx.).

Plate IV

Basal portion of F-cell between two R-cells. Note abundant endoplasmic reticulum (ER) in F-cell, lipid (L) and vacuoles (V) in R-cells. BM, basement membrane; M, mitochondria (× 5,500 approx.).

Plate V

R-cells containing lipid droplets (L) are flanked by F-cells (F_1, F_2). Note the small calcium particles (Ca_1) which when larger are chipped out by sectioning, leaving holes (Ca_2). In cell F_2 a small secretory vacuole (V) is starting to form. N, nucleus (× 5,500 approx.).

Plate VI

Fig. 1. Three F-cells bordering lumen (Lu). The central cell may be extra dark because of the angle at which it is lying, or because it is in a post-secretory phase. M, mitochondria; MV, microvilli; VCa, vacuole containing calcium (× 4,400 approx.).

Fig. 2. Secretory vacuoles (V) in different stages of development in F-cells. Note abundant endoplasmic reticulum (ER) surrounding vacuoles (× 4,400 approx.).

Plate VII

Fig. 1. Secretion vacuole showing pleomorphism of contents. Smaller vacuoles (V) at the periphery are apparently coalescing with the main vacuole, thus increasing its size. The myelin figures probably indicate phospholipid. The nature of the amorphous osmiophilic granular material is not known and it is emphasized that fixation has extracted many substances and possibly altered the appearance of others. Note narrow remaining rim of endoplasmic reticulum (ER). MV, microvilli (× 4,400 approx.).

Fig. 2. Part of an R-cell, showing irregularly shaped vacuoles and calcium deposits, some of them inside vacuoles. Fixation and embedding artefacts probably account for the irregular shape of the vacuoles. Ca, calcium; M, mitochondrion; N, nucleus; V, vacuole (× 16,000 approx.).

Plate VIII

Fig. 1. Calcium spherule, showing its typical structure with concentric lamellae between dense central and peripheral layers. Ca, calcium; L, lipid (× 30,000 approx.).

Fig. 2. Glycogen near lumen of cell. ER, endoplasmic reticulum; G, glycogen; Lu, lumen; MV, microvilli ($\times$ 12,000 approx.).

Plate IX

Fig. 1. A myoepithelial cell (ME) showing the bundle of contractile fibrils (MF) in the deeper part of the cytoplasm; the more superficial area contains vacuoles (V). Underneath the myoepithelial cell is the thick basement membrane (BM) composed of an outer fibrillar and inner compact layer. On the other side of this are the tubular epithelial cells, mostly R-cells (R) in this photograph ($\times$ 5,500 approx.).

Fig. 2. Part of myoepithelial cell shown in Fig. 1, at higher magnification to show detail of contractile fibrils (MF). N, nucleus; V, vacuole ($\times$ 25,000 approx.).

Plate X

Fig. 1. Part of a myoepithelial cell (ME) with double-layered basement membrane (BM) separating it from the tubular epithelial cells. This shows the way in which the sinusoid (S) is lined partly by myoepithelial cells and partly by naked basement membrane. At the top of the photograph are parts of two granule-containing cells ($\times$ 6,600 approx.).

Fig. 2. The plasma membrane of the cells in the sinusoids is covered by a thin layer of amorphous osmiophilic material which is also present in an invagination (P). Note the granules (G) and vacuoles (V), some containing myelin figures. The nature of these cells is uncertain. N, nucleus. ($\times$ 6,600 approx.).

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