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The ultrastructure of the gall-bladder epithelium of the dog

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INTRODUCTION

The gall-bladder is known to have the function of altering the composition of the bile as the result of absorptive and secretory activity, yet little attention appears to have been paid to the ultrastructure of this organ.

The first investigation at the ultrastructural level seems to be that of Dalton, Kahler, Striebich & Lloyd (1950), but their results, partly as a result of lack of knowledge of the problems involved in preparing material for electron microscopy, added little to what was already known from light microscopy studies. The investigations of Yamada (1955) on the gall-bladder of the mouse marked a considerable advance over this earlier study and demonstrated many specializations which could be related to functional activity. Yamada drew particular attention to the microvilli of this organ, to 'pinocytotic-like structures' in the apical cytoplasm, and to dense absorption granules, mitochondria, Golgi membranes, endoplasmic reticulum, ring figures and lipoid masses. Recently Hayward (1962) also studied the fine structure of mouse gall-bladder epithelium. Among other specializations, he noted the presence of pronounced intercellular spaces, dense cytoplasmic granules and large vesicles filled with a pale granular material. The granules he considered to be related to absorption and the large vesicles to the secretion of mucus.

There are considerable species differences in the structure of the epithelium of the gall-bladder (Pfühl, 1932) and also in its functional specialization. The present investigation was performed on dogs, in which the gall-bladder behaves in a manner similar to that of man, at least as far as the transport and accumulation of cholesterol are concerned, and on which many investigations into the pathology of the gall-bladder have been undertaken.

MATERIALS AND METHODS

Three male and three female healthy adult dogs were used. The animals were fed on a normal high-protein diet and were free to drink at all times. Anaesthesia was induced by intraperitoneal Nembutal and the gall-bladder exposed. It was drained of its bile by means of a hypodermic syringe, and 1 % ice-cold buffered osmium tetroxide (Palade, 1952) was injected into the lumen. The viscus was then removed and blocks of tissue less than 1 mm. on a face were cut in the presence of the fixative. Fixation was allowed to continue in the refrigerator at 5° C. for 30 min., following which the tissues were dehydrated in graded methyl alcohol–water mixtures and embedded in methyl methacrylate. Polymerization was carried out

with benzol peroxide at 60° C. and sections cut with glass knives on a Porter–Blum microtome (Porter & Blum, 1953). The sections were examined in a Siemens Elmiskop I electron microscope.

RESULTS

Low-power electron micrographs show the epithelium to consist of tall columnar cells resting on a basement membrane which separates them from the underlying lamina propria. Two main types of cell can be recognized and these can be readily distinguished by the difference in the electron density of their cytoplasm (Pl. 1, fig. 1). Cells with a light cytoplasm are by far the most common; those with a dense cytoplasm occur singly or in groups of two or three and are also distinguished by their narrowness and elongated nuclei. The dark cells described here probably correspond to the 'crayon-like cells' described by Mori (1938), Pfühl (1932) and Ishikawa (1950) but no cells corresponding to the 'cask-like' cells described by Seeliger (1937) can be detected with the electron microscope. Unless otherwise stated the description which follows is applicable to both types of cell.

Apical border of the columnar cell

The apical surfaces of the columnar cells are slightly convex from side to side and carry numerous finger-like microvilli which in longitudinal sections appear orientated at right angles to the surface. Their number per unit length of cell surface is fairly constant and in shape they show considerable regularity. The microvilli measure 0·65 by 0·1 μ and are surrounded by a continuation of plasma membrane that sometimes invaginates the apical cytoplasm. No projections or invaginations of the plasma membrane occur along the length of the microvilli (Pl. 2, fig. 2). In well-fixed material their contents are not appreciably different in electron density from that of the cytoplasmic matrix; but in specimens from which the cytoplasm has been leached during fixation, dense cores, presumably more resistant to leaching than other cellular contents, extend into the apical cytoplasm in a manner similar to those found in some other types of cell. Structures similar to the terminal web of the columnar cell of the small intestine are, however, absent.

Blunt pseudopodia-like structures occasionally project into the lumen of the viscus and these are also surrounded by a continuation of the apical plasma membrane (Pl. 1, fig. 1). These projections contain a substance similar to the cytoplasmic matrix of the rest of the cell, but rarely contain organelles other than membrane bound vesicles similar to those present elsewhere in the apical cytoplasm. Appearances suggest that the projections may be nipped off from the parent cell and this may account for the large free circular profiles bounded by plasma membranes which are sometimes seen within the lumen of the gall-bladder.

Lateral plasma membrane

At the apices of the columnar cells the opposed plasma membranes are more adielectronic than elsewhere and the adjacent cytoplasmic matrix also shows an increased density (Pl. 2, fig. 2). At deeper levels, but usually within the superficial one-third of the cell, other specializations of the plasma membrane are found and these along with those at the apices of the cells are interpreted as desmosomes and

terminal bars. In addition to these specializations, the apposed membranes of adjacent cells are thrown into frequent convolutions of varying complexity, particularly along the luminal one third of the cell. In these convolutions the plasma membranes rarely show any increase in the normal 100 Å. spacing between them. In the basal half to two-thirds, however, the intercellular spaces frequently show considerable dilatations filled with a material of low electron density. Numerous projections from the adjacent cells extend into these spaces and carry with them a continuation of the cytoplasmic matrix. These projections are variable in size and shape and frequently branch (Pl. 3, fig. 3). They are covered by a continuation of the plasma membrane the electron density of which varies, a phenomenon probably dependent on the plane of section. Although the intercellular dilatations are more marked towards the base of the cell their contents do not come into direct contact with the basement membrane for the bases of adjacent cells send out lateral extensions which meet and intervene between the space and the underlying basement membrane. In this region there are no special mechanisms of attachment of adjacent plasma membranes as in the terminal third of the cell, suggesting that this role is accomplished by the basement membrane (Pl. 4, fig. 4).

Basal surface and basement membrane

Apart from the lateral extensions or 'foot processes' which may be extremely thin and intervene between the intercellular dilatations and the basement membrane, the base of the columnar cell shows no specialization. Occasional indentations of the plasma membrane and underlying basement membrane are seen.

The basement membrane consists of a homogeneous layer approximately 400 Å. in width. It is closely applied to the base of the columnar cells and extends in an uninterrupted fashion from one cell to the next (Pl. 4, fig. 4). No fibrillar component is recognizable in it although its external surface is often loosely associated with collagen fibrils. Capillaries are frequently found in close relationship. These are surrounded by a basement membrane somewhat thinner than that at the bases of the epithelial cells. The capillary endothelial cells have a structure similar to that described for capillaries in other sites (Moore & Ruska, 1957; Bennett, Luft & Hampton, 1959; Wissig, 1960) and contain vesicles within the cytoplasm and attached to the limiting plasma membranes. Pores are present in the more attenuated segments of the endothelial cells; some appear to have membranes stretching across them, but such appearances are considered by the present authors and others (see Wissig, 1960) to be due to the plane of section through the tissue. The spaces between the epithelium, the capillaries and the cells of the lamina propria are filled by a substance that, at the levels of resolution used in the present investigation, appears amorphous and probably represents a gel-like interstitial ground substance (Pl. 3, fig. 3).

Light cells

Cytoplasm. The light cells comprise the majority of the columnar cells and have a pale cytoplasm of uniform electron density (Pl. 1, fig. 1).

The cytoplasm underlying the apical cell membrane, and extending for approximately 3·0 μ, contains vesicles but is devoid of organelles. This zone probably corresponds to the deeper portion of the cuticular zone recognized by Ferner (1949)

in light microscopic studies and to similar zones observed in electron microscopy studies of columnar cells by other workers (Palay & Karlin, 1959*a*; Wissig, 1960). The vesicles found in this region are of two types: small, round or oval structures of approximately 0·065 μ diameter, and larger objects having a diameter of approximately 0·25 μ (Pl. 2, fig. 2). The smaller vesicles are always completely surrounded by a smooth membrane and contain a substance of greater electron density than the surrounding cytoplasm. Although these vesicles extend up to the apical plasma membrane and are on occasion closely associated with invaginations of this membrane at the bases of microvilli, they are usually most numerous some little distance from the apex of the cell. The larger vesicles, on the other hand, seldom show a complete encirclement by membrane and contain a material of low electron density. These structures are sometimes in immediate contact with the plasma membrane, but have not been seen in communication with the lumen of the viscus. Their membranes carry no particles on their external surfaces.

Endoplasmic reticulum. Elements of the granular endoplasmic reticulum, in the form of vesicles, cisternae and tubules, are found throughout the cytoplasm with the exception of the subapical region already mentioned (Pl. 2, fig. 2). The limiting membranes are 70 Å. wide and carry particles approximately 150 Å. in diameter on their external surfaces. Many of the vesicular components in the subapical and supranuclear regions resemble the small vesicles of the apical zone in size and density, differing only in the presence of attached granules on the membranes of the former. These similarities are highly suggestive of a continuity between the two types of vesicle and likewise in the Golgi zone, appearances suggest that there may be a continuity between the vesicles of the endoplasmic reticulum and those of the Golgi apparatus.

In contrast to the small vesicular element of the endoplasmic reticulum, the cisternae and tubules show varying degrees of dilatation in different cells and contain a material of low electron density. They are found also in the basal zone of the cell where they may lie close to the lateral plasma membrane or more rarely appear to be attached to it.

Small granules having a diameter of 150 Å. are found throughout the cytoplasm. These are arranged singly or in the form of rosettes and are morphologically indistinguishable from particles of RNP found in cells of other tissues (Palade, 1955; Palay & Palade, 1955).

Golgi apparatus. The Golgi apparatus is a relatively conspicuous structure situated in the supranuclear zone (Pl. 4, fig. 5). It consists of three elements, namely, small vesicles filled with an electron dense material, smooth-surfaced double-layered membranes, and large vacuoles surrounded by smooth membranes and containing a material of low electron density. The large vacuoles closely resemble those in the apical and subapical zones of the cell and their close association with the double-layered membranes suggests that they are derived from these.

Mitochondria. The columnar cells of the gall-bladder contain many filamentous mitochondria (Pl. 2, fig. 2). They are of considerable length and occasionally have clubbed extremities; at the base of the cells branching forms are sometimes seen. The mitochondria are absent in the apical zone of the cytoplasm though in sections cut obliquely they appear to encroach into this region. They are, however, usually

highly concentrated in the infranuclear region of the cell where they may encroach on the basal plasma membrane (Pl. 3, fig. 3). As in cells from many other regions elements of the endoplasmic reticulum are often associated with the mitochondria. In structure the columnar cell mitochondrion conforms to the general description of these organelles, having double walls, and cristae which lie mainly at right angles to its longitudinal axis.

Dense bodies. The cytoplasm of the columnar cells contains additional bodies of different electron density, shape and distribution.

(1) Lipoid bodies. These structures have the characteristic appearance of lipoid masses in other cells. They are variable in size and grossly irregular in outline. Their density varies in a banded manner suggesting that the section at these regions is not of uniform thickness. They are sharply demarcated from surrounding structures though a limiting membrane has not been detected. The lipoid bodies are seen both in a supranuclear and an infranuclear position; they are absent from the apical cytoplasm and the intercellular clefts (Pl. 1, fig. 1; Pl. 5, fig. 6).

(2) Lysosomes. In almost all columnar cells of the dog's gall-bladder oval electron dense bodies having smooth outlines occur in the subapical and supranuclear cytoplasm. These bodies are similar to the lysosomes found in the parenchymal cells of the liver (see Novikoff, 1961). They are of two main types as judged by their size and homogeneity of contents (Pl. 5, fig. 6). The first range in size from 0·8 by 0·4 μ to 0·7 by 0·5 μ and have a matrix of moderate and uniform density. They are surrounded by a distinct 'unit' membrane which is devoid of adherent particles on its external surface. The matrix contains extremely dense particles of 50–60 Å. in diameter. These particles which are considered to be ferritin because of their size and shape are usually arranged singly and are uniformly distributed throughout the lysosome. Similar ferritin particles occur free in the cytoplasmic matrix, particularly in cells containing numerous lysosomes; they do not occur within membranous vesicles or in association with mitochondria.

The second type of lysosome is much more variable in size than the first, ranging from 1·2 by 0·6 μ to 0·35 by 0·25 μ (Pl. 5, fig. 6). The matrix lacks the uniformity seen in the first type and usually contains dense bodies ranging in size from 200 to 1000 Å or more. Ferritin particles are rarely seen in these lysosomes and, possibly because of the plane of section, bounding membranes are not so clearly delineated. Some of these bodies contain oval or circular less dense areas whose significance is difficult to establish. In addition to these two well-defined varieties of lysosome occasional bodies which may represent intermediate types are seen; they contain ferritin as well as larger dense structures and are surrounded by definite unit membranes.

Multivesicular bodies. Circular or oval bodies bounded by smooth membranes are sometimes seen near the Golgi apparatus. They measure approximately 0·5 μ in diameter and contain a homogeneous material of low electron density. Within these structures varying numbers of vesicles measuring about 0·05 μ in diameter and arranged in a random fashion can be seen; they are bounded by membranes which sometimes appear to be continuous with the smooth membranes enclosing the whole body. These structures are probably similar to the multivesicular bodies found in many other cells but so far their significance is not understood (see Novikoff, 1961).

Nucleus

The nucleus is situated in the basal half of the columnar cell (Pl. 6, fig. 7). It is surrounded by a nuclear membrane that occasionally shows pores. The membrane is double layered and the outer layer has particles on its external surface and is sometimes continuous with the endoplasmic reticulum of the cytoplasm. The nucleoplasm, apart from a structure representing the nucleolus, is uniform in appearance and has a density similar to that of the cytoplasm.

Dark cells

As already mentioned the cytoplasm of the dark cells is very electron dense, and even when extremely thin sections are examined it is difficult to distinguish cellular details within it. The density is uniform throughout the cell and its processes (Pl. 6, fig. 7).

Lipoid bodies and lysosomes similar to those in the light cells can be seen but mitochondria are not detectable in any position within the cell (Pl. 6, fig. 7). Profiles of the endoplasmic reticulum are rare and there is no evidence of either a Golgi apparatus or of smooth-walled vesicles.

The nuclei of these cells are also extremely electron dense and little detail can be detected within them. They are considerably more elongated than in the light cells and structures resembling the nucleolus are not seen.

DISCUSSION

Electron microscopy of the epithelium of the dog's gall-bladder demonstrates at least two distinct types of cell which have been designated light and dark cells. The dark cells probably correspond to the crayon-like cells of light microscopy (Yamada, 1959). Mori (1938) regarded these cells as representing a regressive phase in the development of the epithelium but Ishikawa (1950) supported Pfühl's (1932) view that they have decreased functional activity produced by lateral compression. A study of the ultrastructure of these cells certainly indicates that their metabolic, synthetic and secretory activities are decreased. There is no evidence to support the concept that these changes are due to lateral compression or that they represent regressive phenomena.

The ultrastructure of the light cells presents a picture of considerable complexity suggesting a functional versatility much greater than is usually attributed to the gall-bladder.

The free surfaces of the cells carry numerous well-defined microvilli which are similar to those on the columnar cells of the small intestine (Zetterqvist, 1956; Palay & Karlin, 1959*a*). Although the presence of microvilli obviously increases the surface area which is presented to the contents of a viscus there is no evidence that they function in the same way in different cells or that they either aid or hinder transport into or out of the cell. Yamada (1955) found that in the mouse gall-bladder the microvilli have a dense tip from which a corona of delicate filaments radiates. He also found small pit-like depressions along the cell membrane of the microvilli and similar but larger structures in relation to the cell membrane between the bases

of the microvilli. In the dog pit-like depressions are sometimes seen between the bases of the microvilli but the other specializations seen in the mouse are absent.

The dilated intercellular spaces between the basal regions of the cells form a striking feature. Similar spaces have been observed in the gall-bladder of the mouse (Hayward, 1962) and between the columnar cells of the colon (Wyburn, 1961). Their functional significance, however, is far from clear. Palay & Karlin (1959*b*) have shown that in the intestine they form pathways in fat absorption and in the present study vesicles are seen attached to and near the bounding plasma membranes suggesting by analogy that their contents are in the process of being poured into the space. These vesicles, however, contain a material of low electron density similar to the contents of the space, and in no way resembling the lipoid material seen either in the intestine or within the cytoplasm of the columnar cells under consideration. Nevertheless, it must be remembered that the failure to identify the contents of this space does not make it any less significant and possibly implicates it in the transport of water and salts from the bile. It may be carrying the analogy too far, but the complexity of the intercellular spaces, and the concentration of the mitochondria in the bases of the epithelial cells, is in some ways reminiscent of the structure of the cells of the proximal and distal convoluted tubules in the kidney where water transport is an active process (Ruska, Moore & Weinstock, 1957; Rhodin, 1958).

The apical cytoplasm contains vesicles of two distinct types, and these have also been noted in the gall-bladder of the mouse (Hayward, 1962). Appearances suggest that the smaller of these may be formed by pinocytosis at the bases of the microvilli, though actual communication between a vesicle and an invagination of the apical cell membrane is rarely seen. Palay & Karlin (1959*b*) have considered the dynamics of pinocytosis in fat absorption in the small intestine and have shown that the presence of only seven vesicles, each with a diameter of 60 mμ in the apical cytoplasm, could account for the transport of a volume of one-third of that of the cell during 1 hr. The number of small vesicles in the columnar cells of the gall-bladder would certainly indicate that this cell also is capable of rapidly transporting substances from the lumen of the viscus.

The nature of the contents of the small vesicles is open to speculation. The gall-bladder is known to concentrate its contained bile by the absorption of water and salts. In the dog, and probably in all carnivores, it is also capable of absorbing cholesterol and cholesterol esters from the bile and this is demonstrable histochemically (Blaisdell & Chandler, 1927; Nylander, 1961). The electron density of the contents of the small vesicles, although not approaching that of the triglycerides and long-chain fatty acids seen in Palay & Karlin's study, is compatible with the transport by these vesicles of cholesterol into the cell. Once the cholesterol has been engulfed by pinocytotic activity at the apex of the cell its further transport towards the Golgi zone might be facilitated by the small vesicles of the endoplasmic reticulum which contain an electron dense material.

The larger less dense vesicles of the apical zone are always associated with smooth membranes. The membranes may completely encircle the vesicle but usually only a portion is clearly seen, a finding also noted by Yamada (1955) in the mouse gall-bladder and one which is probably due to the plane of sectioning. These vesicles can be traced to the Golgi zone where it appears they arise by dilatation of the cisternae

of the Golgi apparatus. It seems that their contents represent a secretion elaborated by this apparatus, a not unexpected finding in view of the histochemical evidence put forward by McMinn & Kugler (1961) for such activity in the bile duct of several species. Morphological studies give little indication of the type of secretion which is formed, but its density and general appearance is similar to that of mucus (Palay, 1958; Kurosumi, 1961).

Although secretion vesicles are often in contact with the apical cell membrane communication between them and the lumen of the viscus has not been seen. Sometimes the apex of the cell looks distended but in spite of this the microvilli show no tendency to flattening. It is tempting to infer that the blunt pseudopodia-like projections occasionally present may be involved in the elaboration of secretion as is sometimes claimed in the thyroid gland (Braunsteiner, Fellinger & Pakesch, 1953), eccrine and apocrine sweat glands (Kitamura, 1958), and in the epithelium of bile ducts (Kurosumi, 1961). The projections in the dog's gall-bladder epithelium usually contain secretory vesicles and cytoplasm of a density similar to that in the apex of the cell, but organelles such as mitochondria and endoplasmic reticulum are absent. Microvilli are not present on the surface of the projections, indicating that they have either been incorporated into the projections or that their genesis is unrelated to the microvilli. The latter possibility seems the more probable since the microvilli immediately adjacent have a normal size and shape, a finding which would not be expected if microvilli were incorporated as the result of a dilatation of the apical region of the cell. That the blunt projections are at least involved in the extrusion of secretion seems likely, since membrane-bound structures and profiles are found free within the lumen of the viscus. If the secretion is extruded in this manner the gall-bladder can be included as another site of apocrine secretion.

The large masses of lipoid material probably represent accumulated cholesterol or cholesterol esters in the columnar cells of the gall-bladder. Nylander (1961) fed dogs for prolonged periods with cholesterol and found histochemical evidence of an increased accumulation of sudanophilic material in the epithelium of the intrahepatic bile ducts and gall-bladder. He believed that prolonged feeding with cholesterol resulted in a raising of the bile lipid and that if this level was maintained the absorptive powers of the gall-bladder epithelium might be overtaxed with consequent accumulation within the cells. Evidence has already been presented inferring that the lipoid material is brought within the cell by pinocytotic activity and then transferred by way of the endoplasmic reticulum to the region of the Golgi apparatus. During its course to the Golgi zone the lipid is not arranged in discrete droplets as in the small intestine (see Fig. 5, Palay & Karlin, 1959*b*) but completely fills the small vesicles of the endoplasmic reticulum. The physical or chemical factors responsible for the maintenance of discrete droplets in the small intestine may not be present in the gall-bladder. Absence of these factors may further be responsible for the irregular massing of lipid in the Golgi zone. The apparent absence of a limiting membrane to the lipoid masses is an unexpected and not readily explained phenomenon. Similar masses devoid of limiting membranes are seen in many other cells and have been considered to 'represent either absorbed fat resynthesis *in situ* or fat that has escaped from the membrane-limited channels' (Palay & Karlin, 1959*b*).

The presence of lysosomes within the columnar cells of the gall-bladder is not a surprising finding in view of their presence in the parenchymal cells of the liver (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955). These bodies have been intensively investigated from a biochemical and ultrastructure point of view (see Novikoff, 1961, for a critical review and list of references). Ultra-structural investigations have clearly delineated them from mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, and lipid droplets. They have shown them to be often closely associated with the Golgi apparatus, to be limited by a smooth 'unit membrane' and to have a homogeneous matrix which frequently contains ferritin granules. Biochemical investigations have revealed the presence of a wide range of acid hydrolases including acid phosphatase, cathepsin, acid deoxyribonuclease and acid ribonuclease. In addition to the lysosomes of typical appearance, other bodies have been found in the cells of the epithelium and for lack of a better understanding these have also been classified as lysosomes. Essner & Novikoff (1960) have described bodies of a similar appearance in human liver cells and have suggested that they contain lipofuscin in addition to acid phosphatase and non-specific esterase. Referring to 'lipofuscin granules' these authors consider them to be altered lysosomes in which oxidized cephalin and other materials have accumulated (Novikoff, 1961). The intermediate type of lysosome referred to earlier may represent the changing state mentioned by Essner & Novikoff.

The present study adds nothing to our understanding of the function of lysosomes which de Duve (1959) believes to be 'organelles of intracellular digestion'. Nor does it help to answer any of the many intriguing questions which Novikoff has raised regarding the origin of these bodies, but if it may be assumed that they contain the same range of enzymes as are found in other sites then there is further evidence for biochemical activity in this epithelium.

The association of ferritin granules with lysosomes has been noted by several workers in many different sites in the body (Muir & Goldberg, 1961*a*, *b*; Richter, 1959, and others). Farquhar & Palade (1960) studied the uptake of ferritin in renal epithelium of normal and nephrotic rats and showed that the ferritin particles are picked up by the epithelium in pinocytotic vacuoles which convey them to larger vacuoles where they are transformed into dense bodies by progressive condensation. Although the epithelial cells of the gall-bladder appear to contain ferritin, transport of this substance through the cell has not been observed.

SUMMARY

1. Electron microscopic studies of gall-bladder epithelium in the dog demonstrate two types of cell, dark and light.

2. Dark cells have an electron dense cytoplasm and nucleoplasm and few organelles.

3. All cells carry well developed microvilli and occasionally blunt pseudopodia. The latter are thought to be involved in secretion.

4. The light cells show secretory and pinocytotic vesicles which are considered to be involved in the transport of mucus and cholesterol.

5. The intercellular spaces between the bases of the cells show dilation and are thought to be involved in the transport of water and salts.

6. The light cells contain endoplasmic reticulum, a large Golgi apparatus and, particularly towards their bases, many filamentous mitochondria.

7. Lipoid bodies, interpreted as cholesterol, are found in supra-nuclear and infra-nuclear positions.

8. Lysosomes are frequently found and usually contain ferritin.

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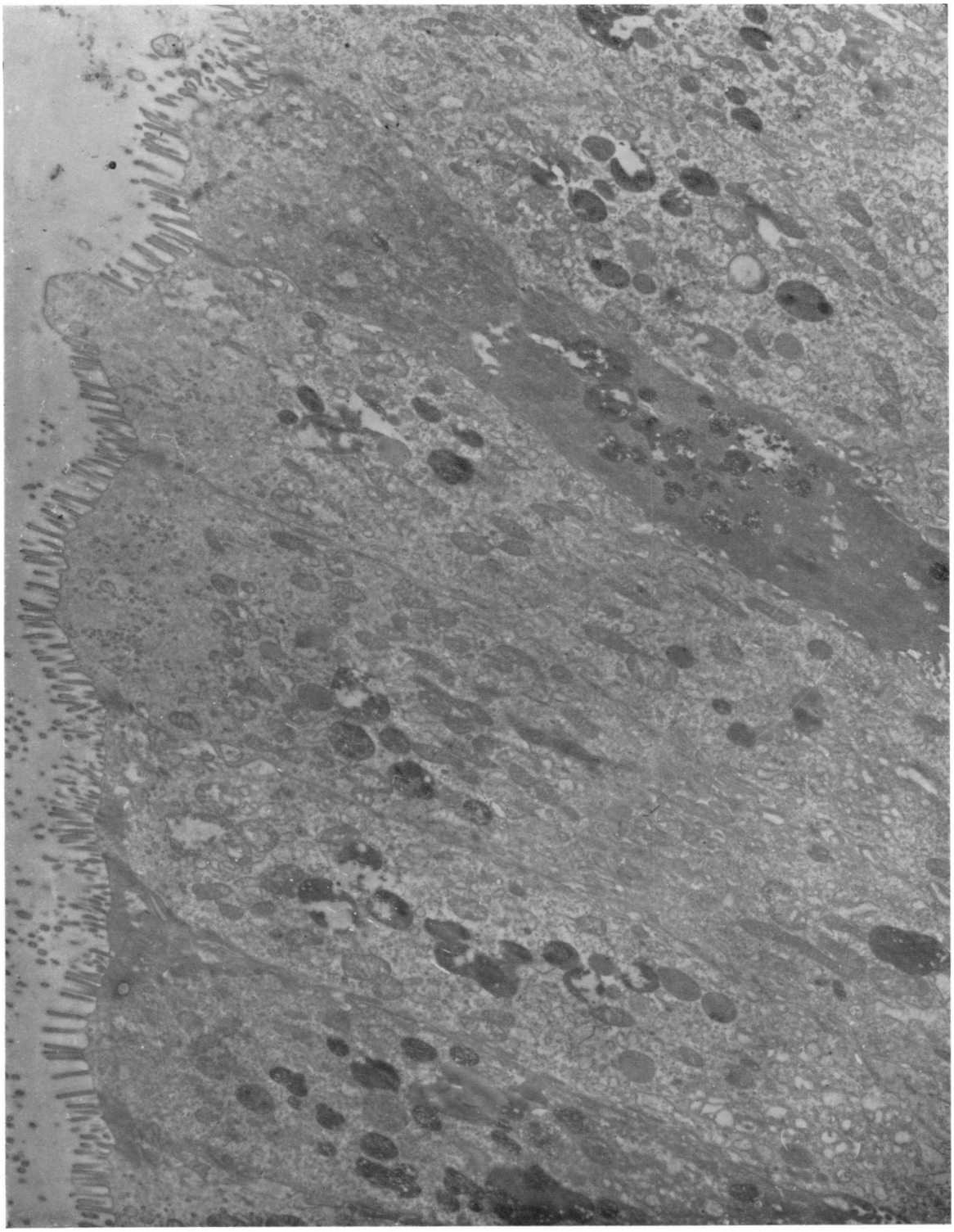
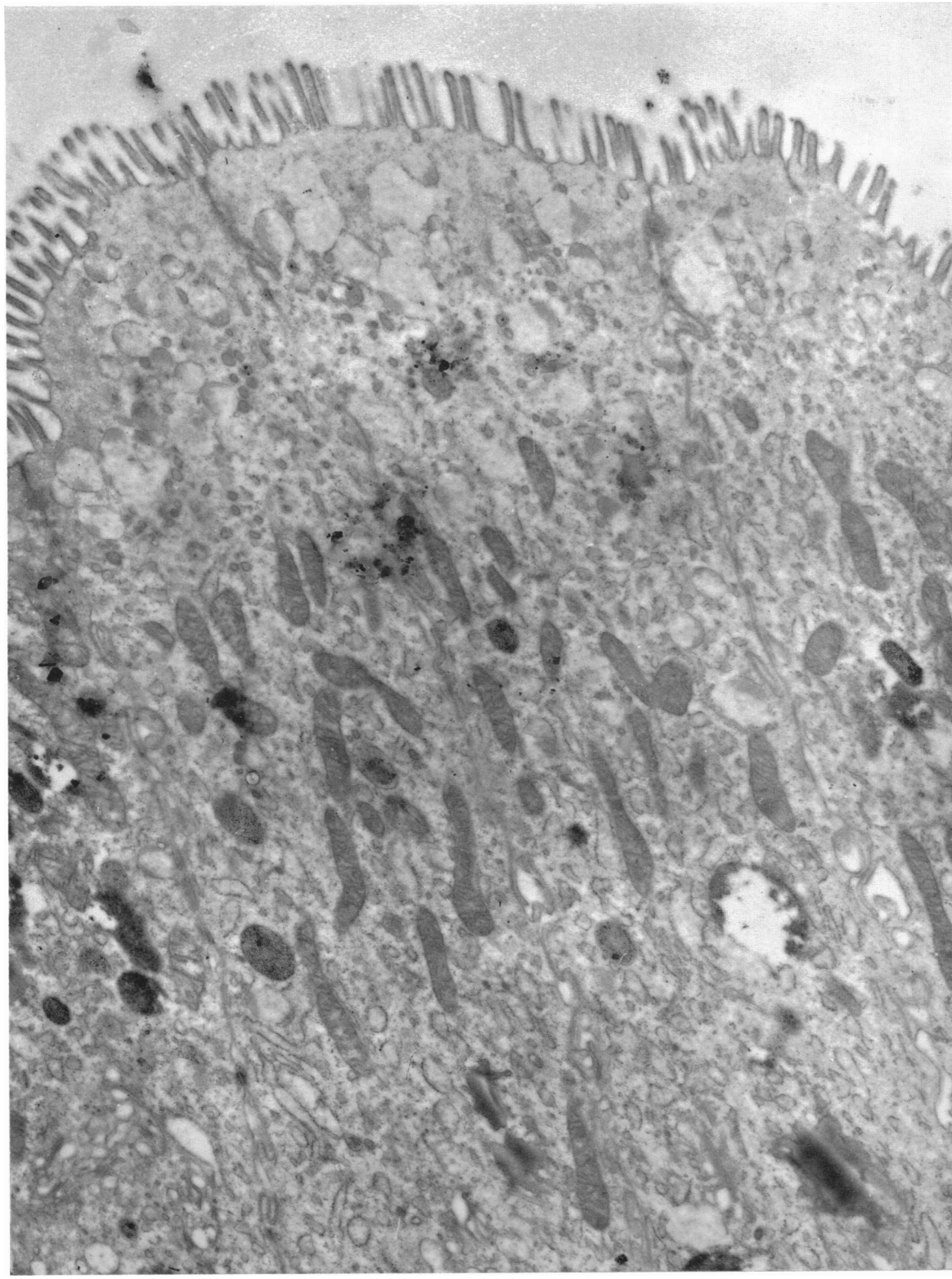
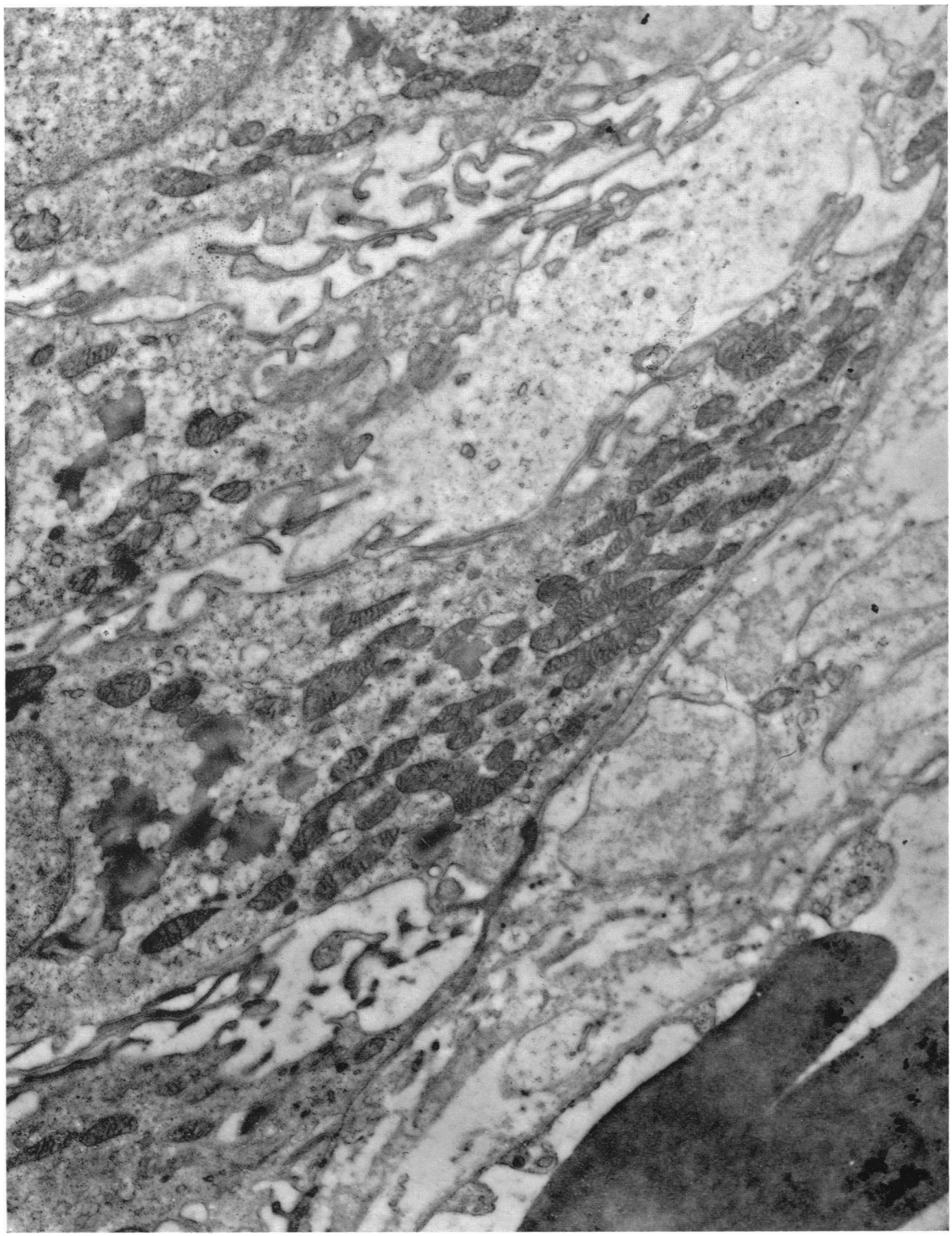






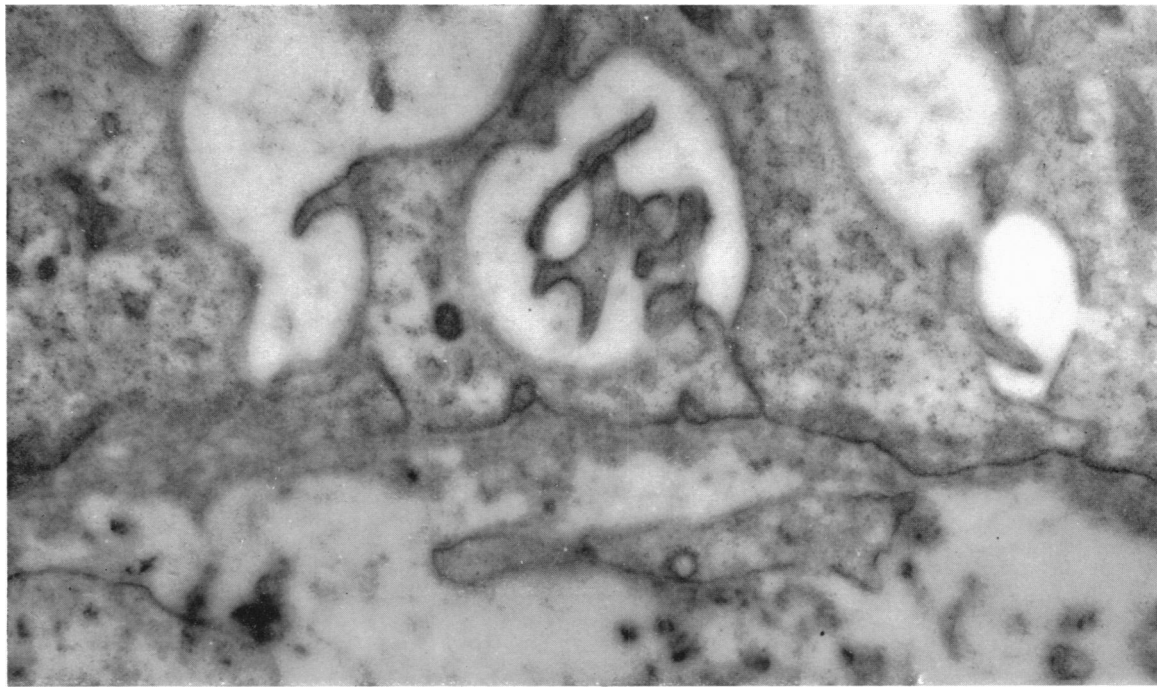
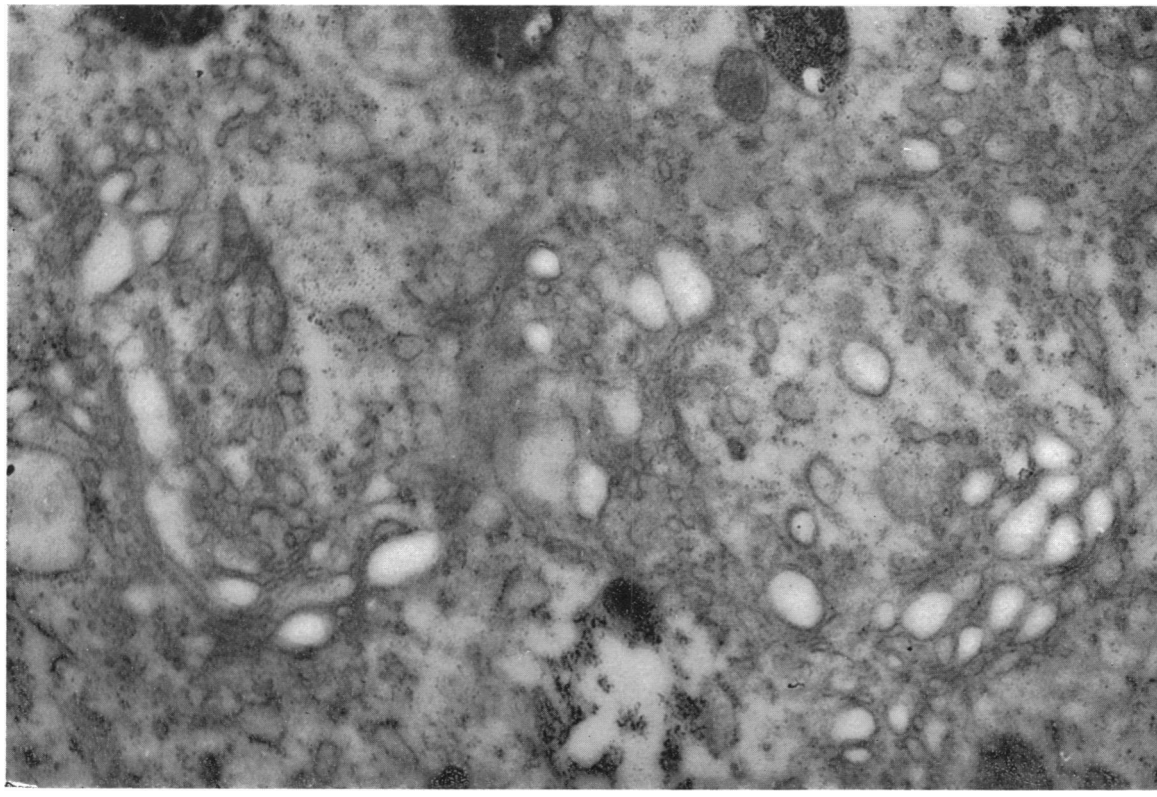


Fig. 1



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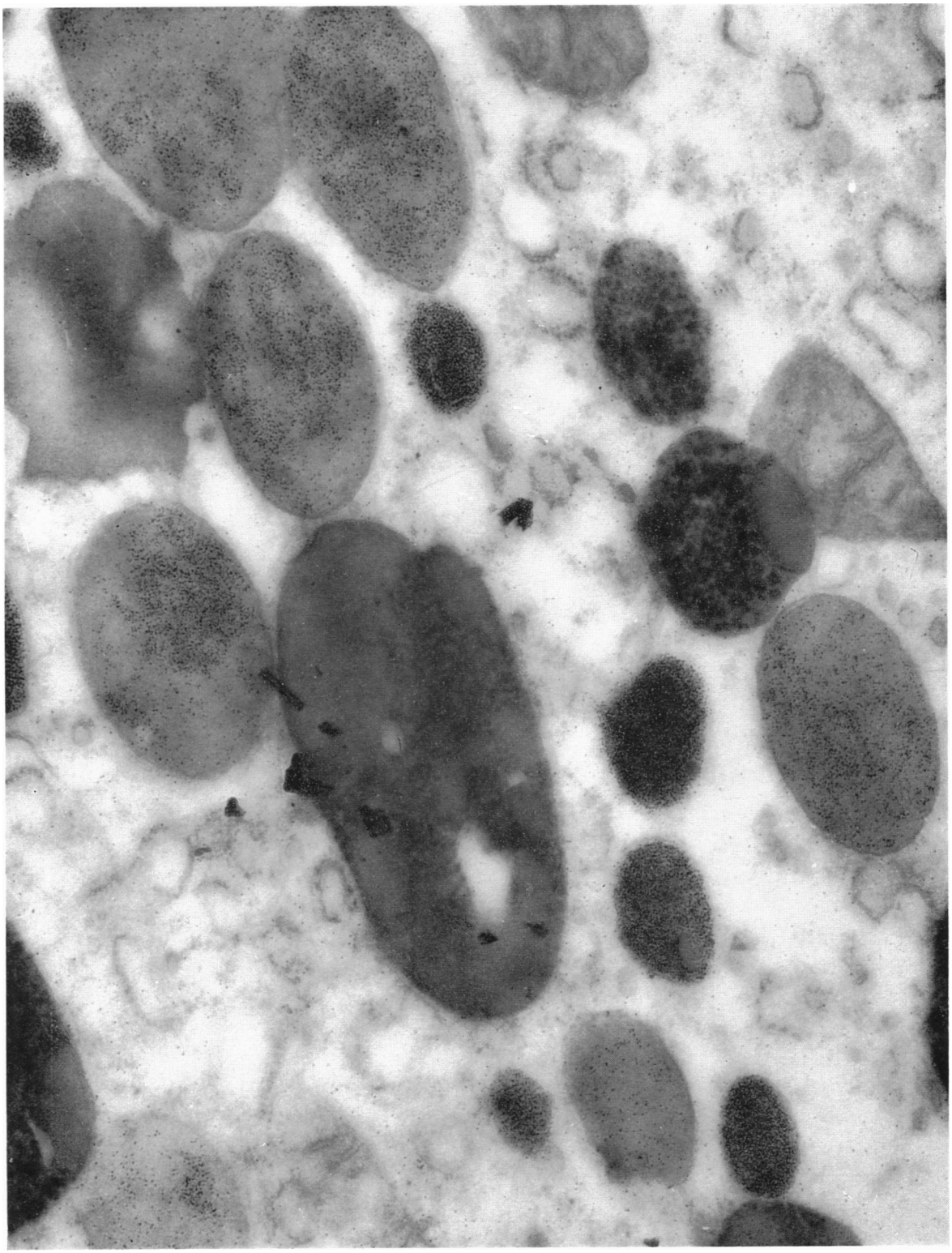

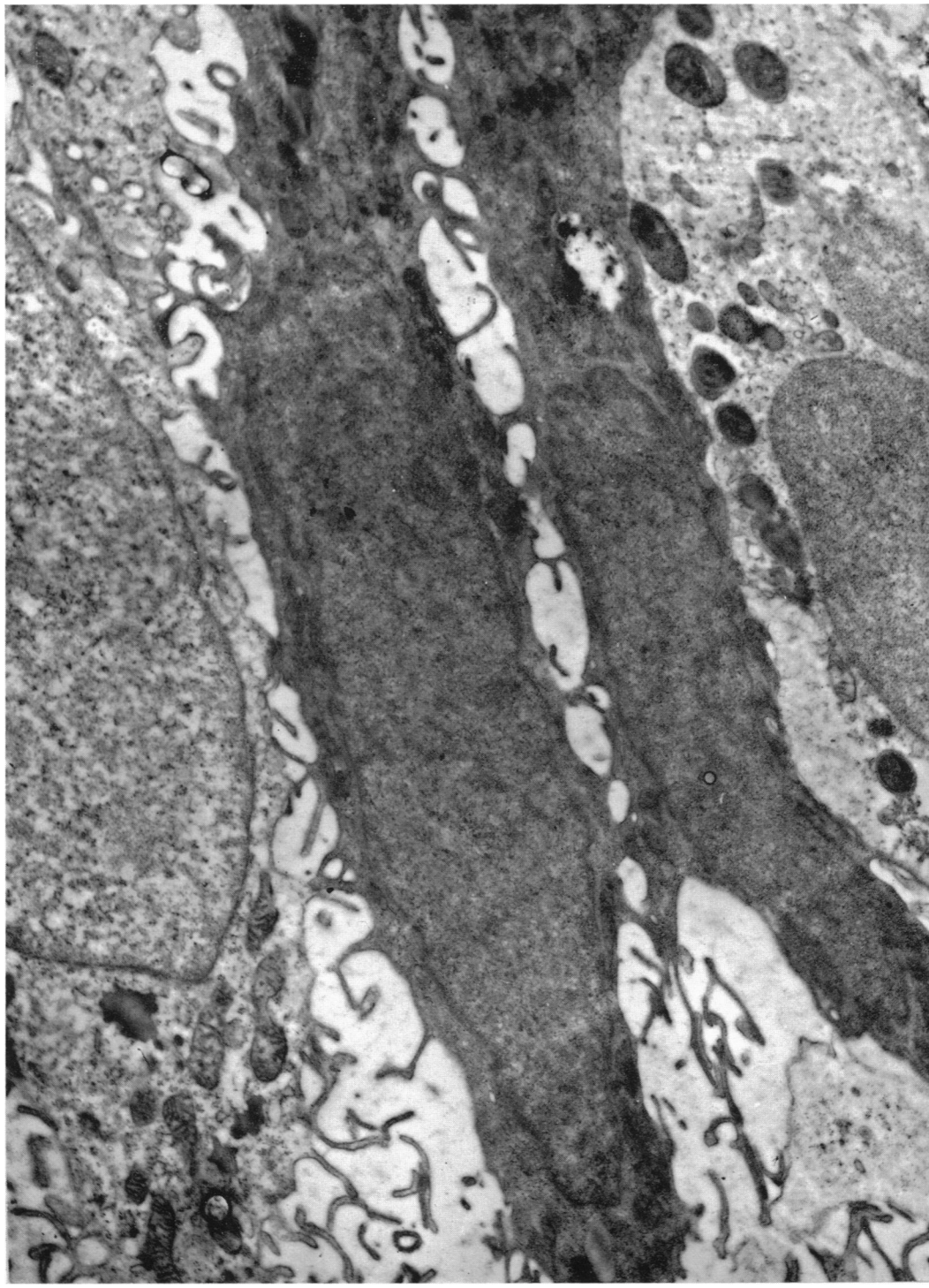

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Apical, subapical and supranuclear zones of columnar cells. A dark cell is seen between the light cells. The cells have numerous microvilli and one shows a blunt pseudopodia-like protrusion. × 8200.

PLATE 2

Fig. 2. Light cells showing microvilli on their free extremities and numerous large secretion granules and small dense pinocytotic vesicles in the apical cytoplasm. The subapical cytoplasm contains many mitochondria, elements of the endoplasmic reticulum, occasional lysosomes and lipoid bodies in addition to secretion granules and small dense vesicles. × 15,700.

PLATE 3

Fig. 3. Bases of columnar cells separated by dilated intercellular spaces. Many mitochondria are present and some lipoid bodies immediately below the nucleus. A capillary is separated from the epithelium by an amorphous substance containing some collagen fibres. × 19,100.

PLATE 4

Fig. 4. Bases of columnar cells resting on a basement membrane. Note how the attachment of adjacent cells prevents the intercellular space extending to the basement membrane. × 30,000.

Fig. 5. Golgi apparatus showing paired membranes, small dense vesicles and large vacuoles. × 32,700.

PLATE 5

Fig. 6. Subapical region of a columnar cell showing several electron dense bodies. An irregular-shaped lipoid mass is seen towards the upper left corner. The remaining bodies represent lysosomes. Some of these have easily defined limiting membranes, and a homogeneous matrix on which small electron dense particles are imposed. Others vary in size, in homogeneity of matrix and in ease with which a limiting membrane can be detected. × 65,000.

PLATE 6

Fig. 7. Section through two dark cells at the level of the nucleus. Note the difference in density of their cytoplasm and nucleoplasm compared with those of adjacent light cells. × 15,100.