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ASPECTS OF THE FINE STRUCTURE OF THE GALL BLADDER EPITHELIUM OF THE MOUSE

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INTRODUCTION

The epithelium of the gall bladder, extensively studied with the light microscope, has received relatively little attention from electron microscopists. In the past, the controversial points have been the presence or absence of a brush border, the existence of secretory material, mucous or otherwise, and the ability of the epithelium to absorb substances other than water and inorganic salts from the bile. Of these, only the question of the brush border has been settled by electron microscopic observations of the gall bladder of the laboratory mouse (Dalton, Kahler & Lloyd, 1951; Yamada, 1955). Yamada (1955) described the fine structure of the gall-bladder epithelium as a typical columnar epithelium rather than as a functional lining membrane, although he commented on the possibility of absorption by pinocytosis.

The information available on the absorption of various foreign substances from the gall bladder (Winkenwerder, 1930) and its unquestioned function, in most animals, of concentrating the bile (Schmidt & Ivy, 1937) suggests that there may be some ultrastructural features with a bearing on these activities. There is no doubt that some mammals possess typical goblet cells in the gall bladder (Harding, 1931) but none has been described in the mouse, and there is no definite information on mucous secretion. The electron microscope should provide information on this aspect.

This work is a report on the features of the fine structure of the mouse gall-bladder epithelium with a possible bearing on the secretory and absorptive activities.

MATERIAL AND METHODS

The gall bladders from apparently healthy mice of known age and sex were removed, under Nembutal or ether anaesthesia, and immediately cut into small pieces and immersed in 1% buffered, isotonic osmium tetroxide solution (Zetterqvist, 1956). Fixation was allowed to continue for 30 min. and the tissue then washed, dehydrated in a graded alcohol series, and impregnated with methyl and butyl methacrylates. Each fragment was finally placed in a gelatine capsule of partially polymerized methacrylate mixture and incubated overnight at 60° C. It was essential to limit the fixation to 30 min. for optimum preservation and to use prepolymerized methacrylate to reduce the incidence of embedding damage which was particularly prone to affect one cytoplasmic component.

Thick (2 μ) and thin sections, exhibiting silver interference colours, were cut on a Porter–Blum microtome. Thick sections were mounted on albumen-coated slides and, after the methacrylate had been removed, were either dried and mounted in

'DPX' for phase contrast microscopy or stained. The staining methods used to show metachromasia due to acid mucopolysaccharides or, after sulphation, neutral mucopolysaccharides, were the periodic acid-Schiff method and aqueous toluidine blue, either alone or following sulphuric acid treatment (Kramer & Windrum, 1953). The periodic acid-Schiff method has become almost standard procedure after osmium fixation and methacrylate embedding (Revel, Napolitano & Fawcett, 1960; Karrer, 1961; Lever, Jeacock & Young, 1961). In this work, diastase and saliva failed to prevent the PAS staining of glycogen either in the suspected deposits in the gall bladder or in the known deposits in liver prepared by the same techniques. Karrer (1961) was equally unsuccessful in this procedure after osmium fixation though Lever *et al.* (1961) extracted glycogen from osmium-fixed diabetic pancreas with diastase. Several other staining methods were tried but were rejected after use on other tissues prepared by the same means. Among them were Best's carmine and Mayer's mucicarmine which were not a success on liver and colon, respectively.

Thin sections for electron microscopy, mounted on celloidin-coated copper grids, were routinely stained with lead acetate and ammonia vapour (Dalton & Zeigel, 1960) and examined and photographed at magnifications of up to 10,000 times on the Philips microscope EM 75B.

RESULTS

Observations with the light microscope

Unstained sections examined by phase microscopy are generally employed for localization purposes. In the sections of the gall bladder, folded low columnar epithelium lines the lumen and small capillaries closely underly the basement membrane. Some of the epithelial cells contain supranuclear groups of 'phase-bright' droplets with occasional dark granules in the rest of the cytoplasm.

Sections stained with the periodic acid-Schiff technique show a faintly positive reaction in the basement membrane, intercellular spaces of the epithelium, and apical border. Within the cells the most striking feature is a supranuclear cap of bright red material (Pl. 5, fig. 17); although absent from many cells it is, when present, found also at the base. The PAS reaction is positive in a diffuse band below the apical border in nearly all cells and between this band and the nucleus, occupying the same position as the 'phase-bright' droplets, are numerous PAS-positive droplets (Pl. 5, fig. 18). Treatment with saliva or diastase has no effect on the PAS staining reaction. Toluidine blue staining produces no metachromasia in gall-bladder epithelium. After sulphuric acid treatment, however, the structures which stain with PAS react metachromatically with toluidine blue, with the exception of the supranuclear cap (Pl. 5, fig. 16). These findings are similar to those of Sulkin (1955) in neurones. The 'phase-bright' PAS-positive droplets stain metachromatically and often have an annulate appearance with more deeply stained periphery. They thus resemble the ring-figures described by Ferner (1949) and Seeliger (1937).

Observations with the electron microscope

The general features of the gall-bladder epithelium of the mouse in electron micrographs have been described by Yamada (1955). The epithelium consists of low columnar cells (Pl. 1, fig. 1), 10 μ in height, differing from each other only in detail.

The close apposition of the capillaries to the epithelial cells is more strikingly shown than with the light microscope (Pl. 1, fig. 1; Pl. 3, fig. 5). Each epithelial cell has a brush border and basal nucleus. The apical cytoplasm contains only small (1200 Å.) granules and vesicles, and apart from some specific constituents described below, the remaining cytoplasm has typical mitochondria, sparse endoplasmic reticulum, and free Palade particles. The Golgi apparatus is found lateral to the upper pole of the nucleus and has no unusual characteristics (Pl. 2, fig. 4). Lipid droplets occasionally occur in small groups at the bases of the cells.

Brush border. The brush border of the gall-bladder epithelial cells has been described by Dalton, *et al.*, (1951) and by Yamada (1955). The present material supports their principal findings and it suffices to emphasize certain features. The microvilli are more irregular than those of the alimentary tract, both in length and spacing (Pl. 1, fig. 1; Pl. 2, figs. 2, 3). They have a maximum width of 1000 Å. and two or more occasionally arise from the same base (Pl. 2, fig. 3). Invaginations may be seen between the bases of two microvilli (Pl. 2, fig. 3) and in some cases are in continuity with a small apical granule or vesicle.

Intercellular spaces. The intercellular spaces are closed apically by a typical terminal bar. Below the terminal bar, the lateral cell membranes are separated by a 100 Å. translucent layer which opens nearer the base to form a broad irregular space containing convoluted cell processes. The intercellular space is continuous with that between the plasma membrane and basement membrane (Pl. 1, fig. 1; Pl. 3, fig. 5). The complex spaces are very constant in methacrylate embedded material from the mouse gall bladder. Although Hampton (1960) showed that the intercellular spaces in methacrylate embedded liver are almost eliminated by epoxy resin embedding, it is difficult to believe there is no structural basis for the appearance in the gall bladder.

Small apical granules. In many of the epithelial cells, an apical band of cytoplasm contains only ground substance and occasional free RNP granules; in others, however, a variable number of granules and vesicles occupies this zone (Pl. 2, fig. 2). Each has a single outer membrane and a diameter of 1200 Å. Both the vesicles and the granules often lie very close to the plasma membrane in the vicinity of small invaginations between the microvilli. As Pl. 2, fig. 2, shows, however, there may be many granules in cells with poorly developed microvilli.

Large dense cytoplasmic bodies. In deeper parts of the cell the small dense granules are replaced by larger bodies lying at random among the other cytoplasmic constituents. There appear to be at least two types of dense granule in this material, referred to as types *A* and *B*. Each is a circumscribed granule, usually with no visible outer membrane. The bodies are irregularly shaped, up to 1 μ across, with a matrix of variable density including granular patches and vacuoles (Pl. 5, fig. 14). Those nearest the apical region (Pl. 2, fig. 2) resemble the small apical granules in all but size.

Type *A* granules are particularly liable to polymerization damage after methacrylate embedding resembling in this respect component 1 in Zetterqvist's study of the small intestine (1956). In type *B* granules, a circular body with a dense matrix with barely distinguishable vesicular elements, is enclosed by a definite outer membrane separated from the matrix by a narrow translucent space (Pl. 3, figs. 6,

7). The matrix varies in density (Pl. 3, fig. 8). Type *B* granules are found in the supranuclear region, characteristically adjoining the pale granular area of cytoplasm described later and often close to a group of pale cytoplasmic droplets. The type *B* granules are very similar in appearance to granules of the mucous cells of the tracheal epithelium, described in the rat by Rhodin & Dalhamn (1956) and also to be found in the mouse (personal observations). No structural relationship between type *B* granules and any other cytoplasmic component has been found.

There are occasionally very opaque irregular granules appearing almost black on micrographs (Pl. 5, fig. 15) with patchy, vacuolated, and sometimes crystalline contents, occurring in groups in the epithelial cells. They are regarded as pigment granules.

Pale cytoplasmic droplets. Many epithelial cells in this material have characteristic droplets in the supranuclear cytoplasm. Pl. 4, figs. 9 and 10, show one form in which they occur. A thin circular membrane encloses fine granular material similar to the surrounding cytoplasm with darker, sometimes vesicular, elements just inside the membrane. The bodies formed in this way are often found near the Golgi apparatus and round the periphery of the juxtanuclear pale granular cytoplasm. There is no evidence that either arrangement is more than fortuitous. It appears likely that these structures are identical with the 'ring-figures' of Yamada (1955).

In other cells, clusters of droplets with a distinct structure are found in groups near the apex (Pl. 4, figs. 11, 12). Each has a smooth single layered outer membrane enclosing an electron translucent matrix with the centre condensed as a fine granular cloud. Peripherally there are small vesicular elements. The most striking feature of the droplets is the frequent presence of myeloid figures as small dense arcuate lamellar formations attached to the outer surface or lying free in the matrix. The pale droplets are often closely surrounded by granular endoplasmic reticulum but have no direct connexion with it. They also occur close to the pale granular cytoplasm in the supranuclear area. The pale droplets illustrated (Pl. 4, figs. 9–12) are sufficiently similar to be regarded as different forms of the same structure. Apparently related to the pale droplets, structures of the same size with a single outer membrane occur with the matrix almost completely filled with myeloid elements so that the pale background is practically eliminated. This type of formation is rare and occurs in the groups of the pale droplets. When stained sections of the same area are compared with the micrographs, it can be seen that the pale cytoplasmic droplets correspond exactly in distribution with the PAS-positive droplets which stain metachromatically after sulphation. They are therefore taken to be the same structures.

Pale granular juxtanuclear cytoplasm. Several references have already been made to this localized area of cytoplasm found in many of the epithelial cells. In most cases the area forms a supranuclear cap (Pl. 5, fig. 13) though it may occur at the base of the cell as well. The nuclear membrane commonly forms one boundary, and though the other sides are not membrane bound they are closely limited by surrounding granular endoplasmic reticulum, mitochondria, and pale droplets. No cytoplasmic component is ever seen within the area. The ground substance consists of ill-defined homogeneous granules of 300–500 Å. diameter and is clearly differentiated from the surrounding cytoplasm with no reticular or vesicular matter.

An area of cytoplasm like the one found here was described by Revel *et al.* (1960) in a variety of tissues known to be rich in glycogen and was shown by Lever *et al.* (1961) to correspond to a glycogen deposit in the diabetic pancreas. In the present material comparison of the thin sections with thick stained sections of the same area shows that the pale granular area is in the same position as the deeply PAS staining nuclear cap. Although the deposit is diastase fast, unlike the other PAS-positive areas it does not stain metachromatically after sulphation. The combination of the staining reaction and the ultrastructural appearance strongly suggests that the area is in fact glycogen.

DISCUSSION

The fine structure of the epithelium of the gall bladder should give some indication of its functions with regard to absorption and secretion. There is considerable species variation in the microscopic structure of the gall bladder and the findings recorded here are therefore not necessarily universally applicable.

The observations on the brush border support those of other authors. The presence of invaginations between the microvilli connecting with small superficial granules provides a morphological basis for pinocytosis. Indeed, it has been suggested that the mere presence of a brush border is a specialization to this end (Holter, 1959). Pinocytosis would provide a means for fat absorption by the epithelium, long known to occur under experimental conditions (Mentzer, 1925), similar to that in the small intestine (Palay & Karlin, 1959). The resorption of other substances, either experimentally introduced (Winkenwerder, 1930) or naturally present in the bile would be facilitated. It should be noted, however, that pinocytosis alone cannot assist in the concentration of the bile, shown repeatedly to be the chief activity of the gall bladder (Rous & McMaster, 1921; Halpert, Thomson & Martin, 1958; Schmidt & Ivy, 1937). The fluid ingested from the lumen by this means is at least equal to the bile in solid content, and if any of the biliary constituents is absorbed on to the plasma membrane as other solids are (Holter, 1959), the ingested fluid would be more concentrated than the bile.

The small superficial granules are considered to be the result of pinocytosis. The fact that the number of superficial granules varies from cell to cell seems to support the views of Policard (1914) and of Aschoff (quoted by Pfühl, 1932) that only a small proportion of the cells is actively absorbing at any one time. The type *A* granules nearest the apex are very similar in appearance, except for their size, to the small granules and may well result from their amalgamation and partial extraction. It is not clear whether the remaining type *A* granules are of the same origin, but the presence of some with denser contents makes it seem possible. The very dense pigment granules could be the end product of the amalgamation-absorption process. Granules of this type are seen to increase in some tissues with age (Fawcett & Burgos, 1960; Duncan, Nall & Morales, 1960) and in the gall bladder could increase in number as the individual cells increase in age and accumulate more 'waste products'.

Concentration of the bile must involve transfer of large quantities of water and inorganic salts across the epithelium. Epithelia noted for their water transport often have complex convoluted basal plasma membranes with which mitochondria are

commonly associated (Pease, 1956) and this is usually considered to be a device for an increase in surface area. No such pattern emerges in the gall-bladder epithelium. There are, however, extensive spaces between the lateral margins and at the bases of the cells, which even if they are accentuated by embedding artefact, must be the basis for considerable increase in cytoplasmic-extracellular interface. The close apposition of the capillaries to the epithelial cells and their basal spaces should also facilitate transfer of water to the blood stream and lymphatics. The picture found in the mouse gall-bladder epithelium is remarkably similar to that in the colon of the same animal (Hayward & Johnston, 1961). There is, of course, a similarity in function in the two organs.

No goblet cells were observed in the gall-bladder epithelium in the mouse. The pale cytoplasmic droplets have the appearance, at first sight, of mucous droplets, but they differ from those of the alimentary tract. There is a considerable amount of dense material in the matrix, no tendency to fuse together or form a goblet, and no apparent relationship to the Golgi apparatus. The comparison of the thin and stained sections from the same area shows that the pale droplets correspond to structures which stain positively with PAS and metachromatically with toluidine blue after, but not before, sulphation. The results point to the presence of a neutral mucopolysaccharide or non-sulphated mucoprotein (Kramer & Windrum, 1953; Sulkin, 1955). PAS-positive structures with the same distribution were described by Yamada (1959) in the golden hamster and were thought to represent a glycoprotein-containing mucus.

There is a suggestion in the literature that, in some species at least, the mucus of the gall bladder is unusual, and several authors reported difficulty in staining it by standard methods. Ferner (1949) and Pfühl (1932) reported that the mucicarmine staining in human material was poor and Jennings (1958) found only traces of mucicarmine staining in the mouse and the rabbit. Harding (1931), however, dismissed all statements regarding the absence of mucus as based on bad fixation. With the exception of the rabbit, all the material he examined, i.e. human, ungulate, guinea-pig, and cat contained typical mucous goblet cells with excellent mucicarmine staining. Two early biochemists (Hammersten (quoted by Winkenwerder, 1930) and Wohlgren (quoted by Policard & Santy, 1914)) are reported as claiming that gall-bladder mucus was not typical in its composition, but referred to the substance as a pseudomucin or nucleo-albumen. There is no sound backing for these statements. Wallraff & Dietrich (1957) showed histochemically that gall-bladder mucus in man contains a glycoprotein containing both acid and neutral mucopolysaccharides. The gall-bladder mucus of the cat becomes radioactive after the administration of ^{35}S (Jennings, 1958) but that of the mouse does not, further suggesting that there may be a species difference in the mucus and confirming the deduction that the droplets in the mouse contain a non-sulphated mucoprotein or neutral mucopolysaccharide. The presence of a non-sulphated mucoprotein suggests an affinity with salivary gland mucus and Brunner's gland secretion (Pearse, 1960) but the granules in the acinar cells of these glands differ ultrastructurally from the pale droplets of the gall-bladder epithelium (Scott & Pease, 1959; Moe, 1960).

A further point of interest is the presence of elements resembling small lamellated myeloid bodies in the pale droplets. Schulz & de Paola (1958) described large cyto-

somes filled with myeloid figures in the developing mucous cells of the gills in axolotl. From their observations they concluded that the lamellated or 'delta-cytomembranes' played a part in mucopolysaccharide production in general and mucus formation in particular. The myeloid figures in the present material are on far smaller scale and no such conclusions can be drawn. They emphasize, however, that if the droplets represent mucus it may be of a different variety from that of the alimentary tract.

Rhodin & Dalhamn (1956) described granules in the mucous cells of tracheal epithelium which are very similar to those here termed type *B* granules. They showed a numerical relationship between the dense granules and the mucus droplets, and the possibility exists that there may be a similar relationship in the gall bladder. There are insufficient granules to produce statisical results, but they have the same distribution as the pale droplets, and granules with paler contents (Pl. 3, fig. 8) could provide an intermediate stage of development. On the other hand, the pale droplets resembling Yamada's ring figures are often small, whereas the type *B* granules are of constant size. If the droplets in the gall-bladder epithelial cells contain a mucoprotein the relative paucity of endoplasmic reticulum is surprising, though small strands are often seen closely applied to the droplets. The presence of a glycogen deposit is of note, however. Glycogen was described in the normal gall-bladder epithelium by Seeliger (1937), McMinn & Johnson (1957), and Yamada (1959) in the cat, guinea-pig, and golden hamster. Seeliger linked the formation of mucus with the presence of glycogen and the Golgi apparatus. Apart from the close proximity of glycogen and pale droplets, no definite relationship is demonstrable but the presence of a high carbohydrate reserve and apparently poor protein-producing capacity might repay further study.

An alternative and completely different interpretation of the origin of the pale droplets is suggested in a recent review by Novikoff (1961) in which it is shown that PAS-positive droplets or granules with a single outer membrane containing phospholipids and hydrolytic enzymes can be considered as lysosomes. Until information is available on the ultra-microscopic distribution of acid phosphatase in mouse gall-bladder epithelium, the exact nature of the pale droplets must remain uncertain.

It can be seen from a brief electron microscopic study that the gall-bladder epithelial cells have features reflecting their physiological activity. The examination of static material does not, of course, determine the nature of the activity, but provides a basis for further experimental work.

SUMMARY

1. The epithelium of the gall bladder in the mouse has been examined with the electron microscope.

2. Invaginations in the brush border and small granules in the apical cytoplasm provide a basis for possible pinocytotic activity.

3. The well-developed intracellular spaces and closely applied subjacent capillaries are related to the absorption of water and inorganic salts by diffusion.

4. Dense cytoplasmic bodies are divided into two groups. Type *A* are irregularly shaped and variable in form and thought to result from the fusion of the pinocytotic granules. Type *B* are circular with an outer membrane separated from the

matrix by a translucent space and resemble granules in other tissues which have been related to mucous secretion.

5. Pale cytoplasmic droplets, corresponding to PAS-positive droplets which stain metachromatically after sulphation, are found in supranuclear groups. They contain small, apparently myeloid elements. Their possible relationship to mucus is discussed.

6. A pale granular area of cytoplasm found above the nucleus or at the base of the cells is considered to represent glycogen.

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EXPLANATION OF PLATES

Scale line = 1 μ

PLATE 1

Fig. 1. Base of a fold of gall-bladder epithelium of the mouse. The lumen is lined by the microvillous border and the epithelial cells are separated by spaces (*S*) extending to the basement membrane (*BM*). The capillary is closely applied to the epithelium. *Coll*, subepithelial collagen fibres. *G*, dense cytoplasmic granules of type *A*.

PLATE 2

Fig. 2. Apical cytoplasm of epithelial cell. Microvilli are absent over some of the surface and there are many absorption granules (*A*). Deeper in the cell, between the mitochondria (*M*) are type *A* granules (*G*). The small black particles scattered at random are due to contamination during lead staining.

Fig. 3. Brush border and apical cytoplasm. An invagination between two microvilli communicates with a small vesicle (indicated by the arrow). Two microvilli on the right appear to arise from a common base.

Fig. 4. Typical Golgi zone of gall-bladder epithelium lying lateral to the upper pole of the nucleus.

PLATE 3

Fig. 5. Capillary blood vessel underlying gall-bladder epithelium. The epithelial cells (upper left) have prominent spaces at the bases (*S*) with projecting microvillous processes. The attenuated capillary endothelium is interrupted by pores, closed only by a thin membrane (indicated by the arrows). *E*, junction between endothelial cell processes.

Fig. 6. Dense cytoplasmic granules of type *B* lying between the nucleus and brush border. The outer membrane is separated from the matrix by a translucent space.

Fig. 7. Granule of type *B*. *Go*, Golgi apparatus.

Fig. 8. Granules of type *B* lying near the pale granular cytoplasm believed to be glycogen (*Gl*). The upper granule has a paler matrix and should be compared with the structures in Pl. 4, figs. 9 and 10.

PLATE 4

Fig. 9. Pale droplets in supranuclear cytoplasm. The matrix is similar to the surrounding cytoplasm and the droplet is close to a strand of granular endoplasmic reticulum (*ER*).

Fig. 10. Pale cytoplasmic droplets. *G*, granules of type *B*.

Fig. 11. Tangential section of epithelial cell with a group of pale cytoplasmic droplets. Those on the left include dense lamellae.

Fig. 12. Pale cytoplasmic droplets containing granular vesicular and annulate elements. Some are closely invested with granular endoplasmic reticulum.

PLATE 5

Fig. 13. Pale granular area of cytoplasm in the supranuclear region of an epithelial cell; it is surrounded by mitochondria and granular endoplasmic reticulum but has no limiting membrane.

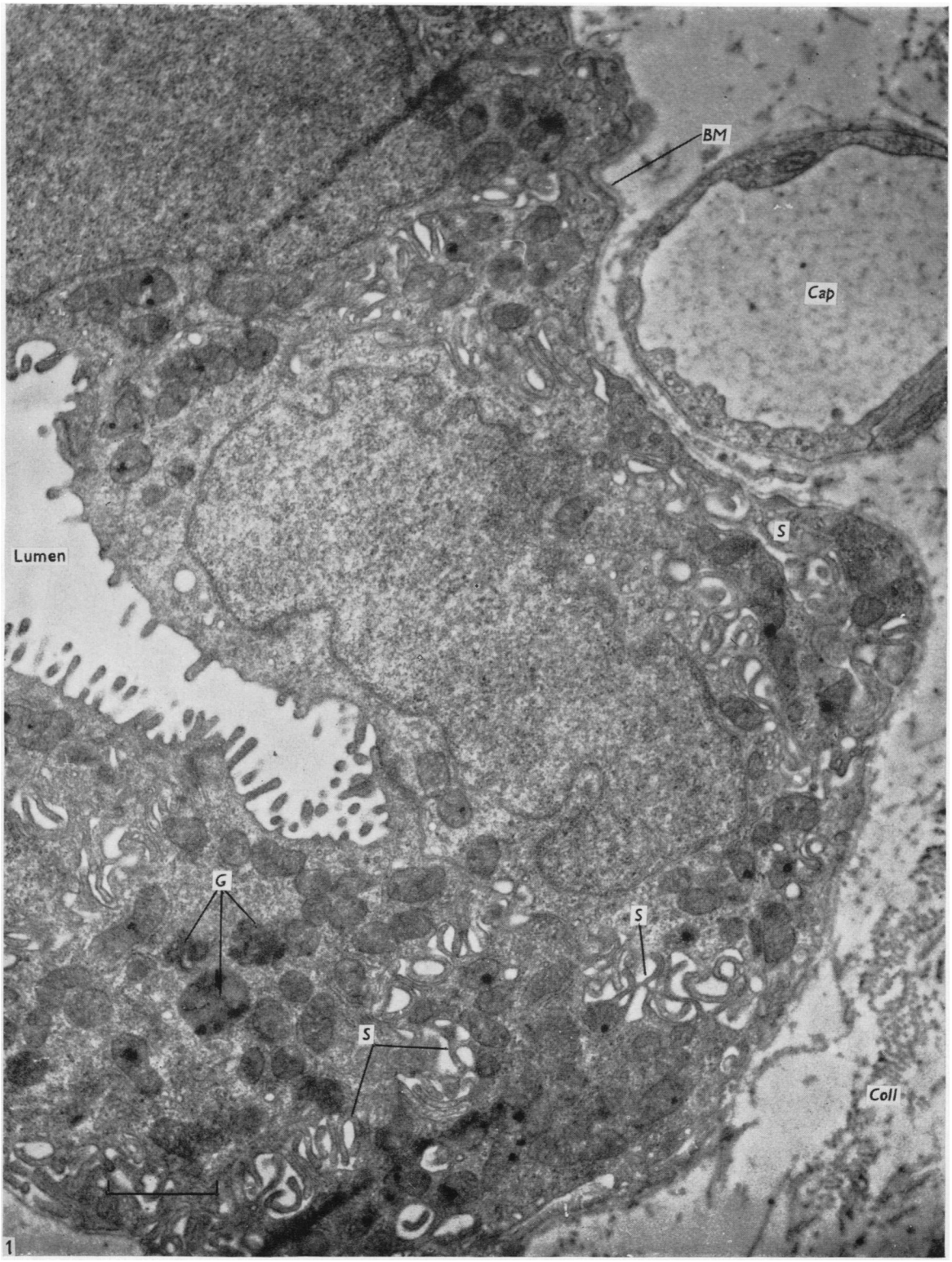
Fig. 14. Type A granules. The outer membrane is not visible and the matrix has vacuolated contents.

Fig. 15. Very dense granules, believed to be pigment, of the epithelial cytoplasm.

Fig. 16. Light micrograph of 2 μ methacrylate section stained with toluidine blue after treatment with sulphuric acid. The epithelial cells contain groups of supranuclear droplets which stain metachromatically under these conditions.

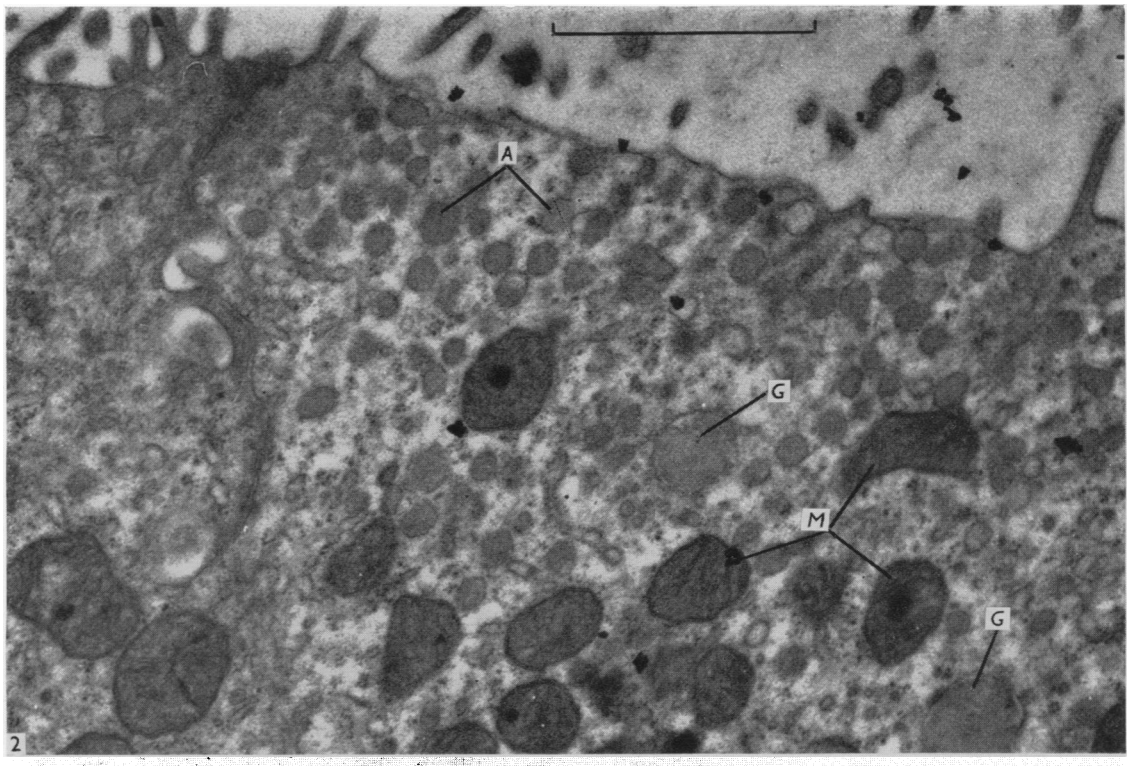
Fig. 17. Light micrograph of 2 μ methacrylate section stained by the PAS method. Some of the cells have a deeply stained supranuclear cap and others have a similar area at the base.

Fig. 18. Similar preparation to Fig. 17. Between the PAS positive brush border area and the nucleus, numerous droplets stain by this method and correspond to those staining with toluidine blue in Fig. 16.



HAYWARD—FINE STRUCTURE OF GALL BLADDER EPITHELIUM

(*Facing p.* 236)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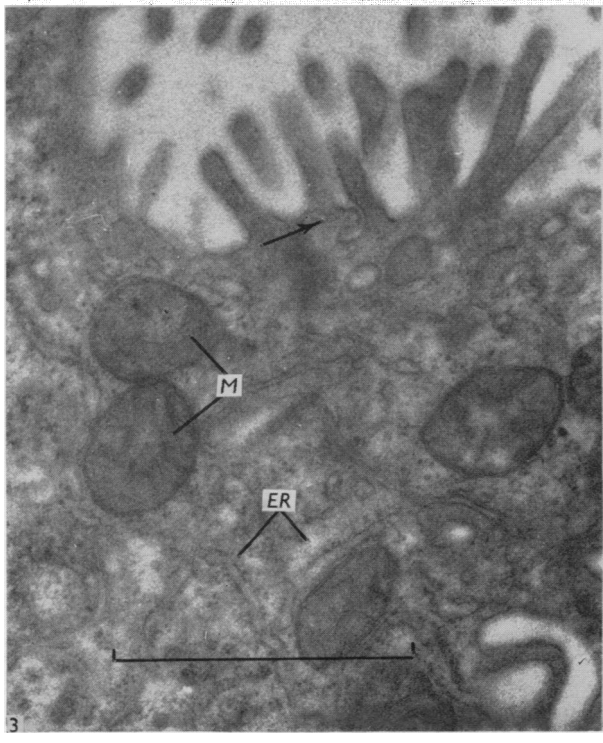
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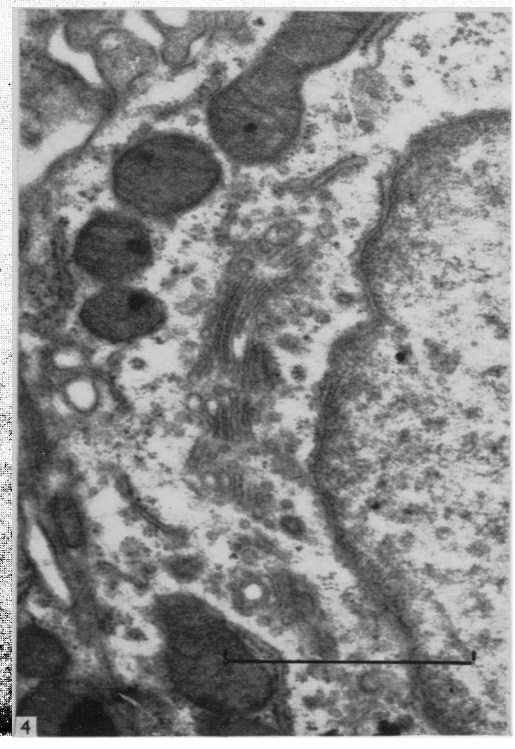
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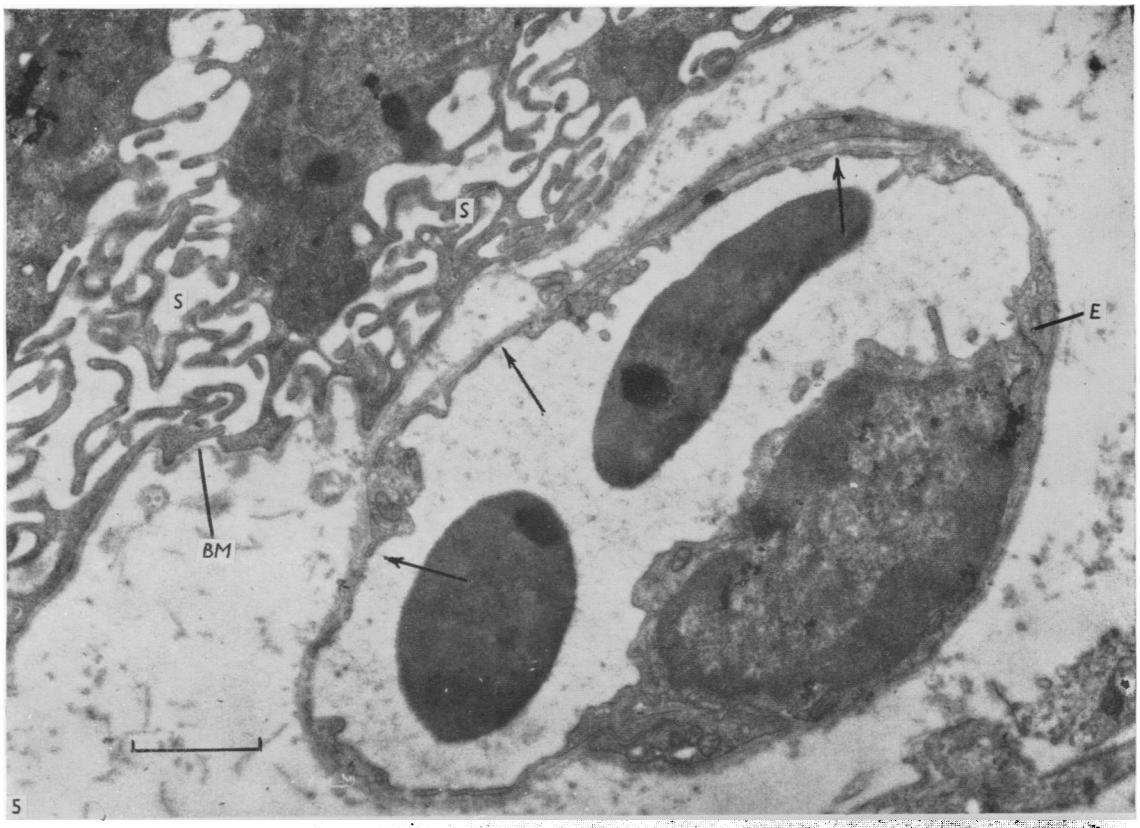
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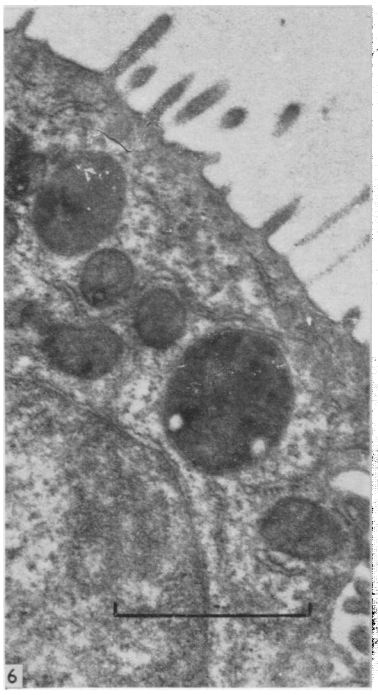
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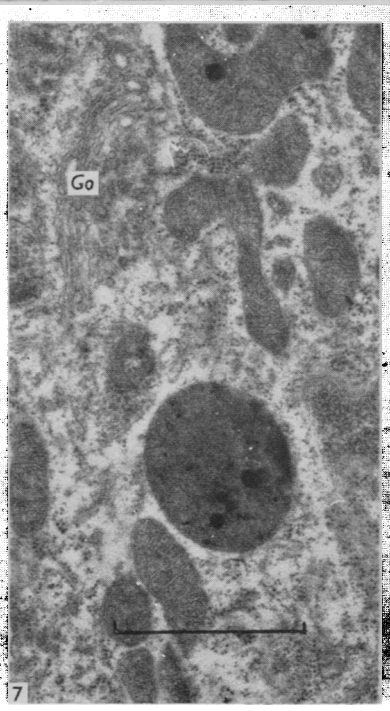
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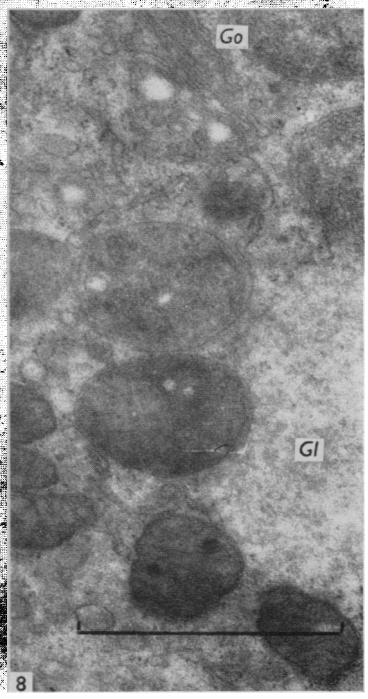
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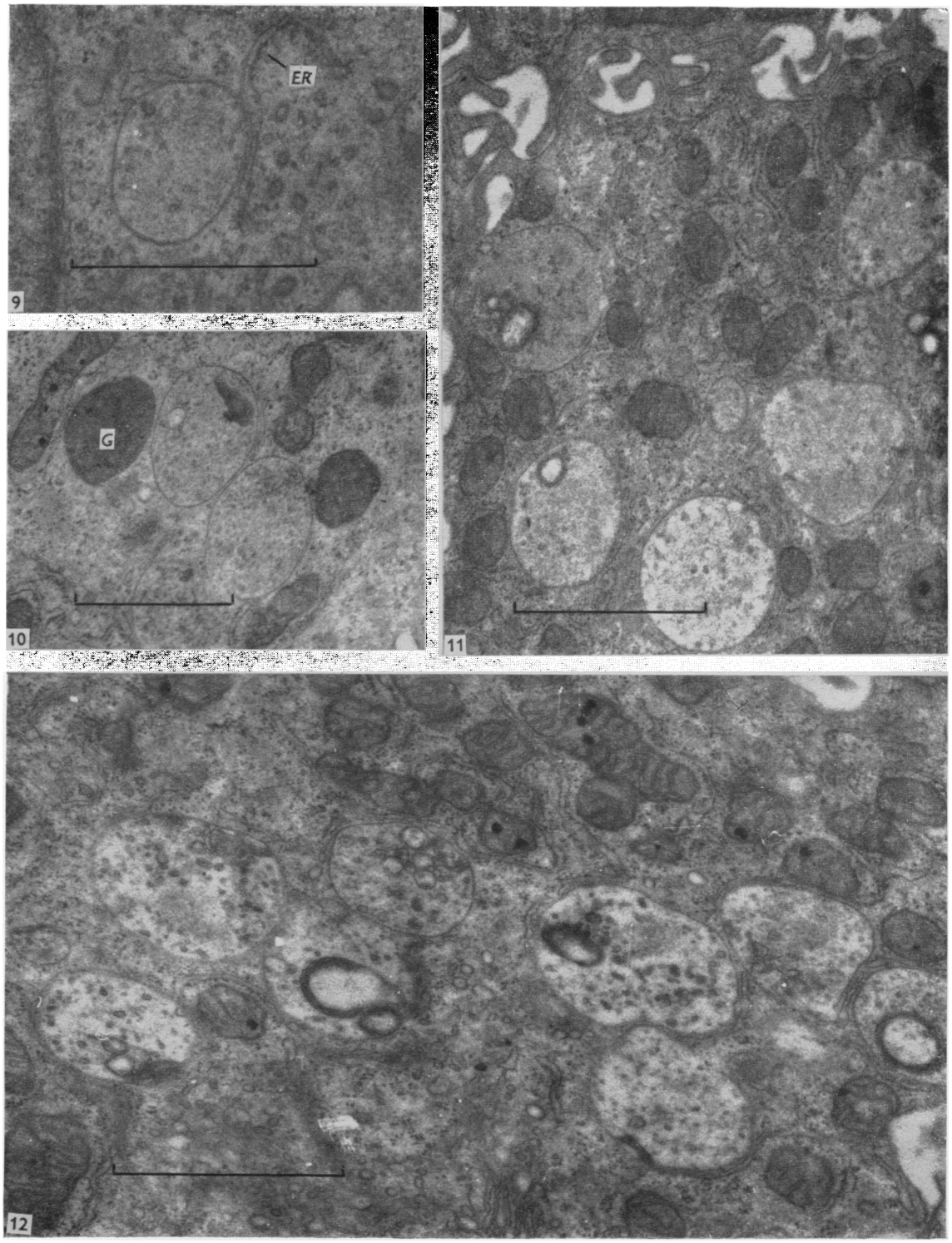
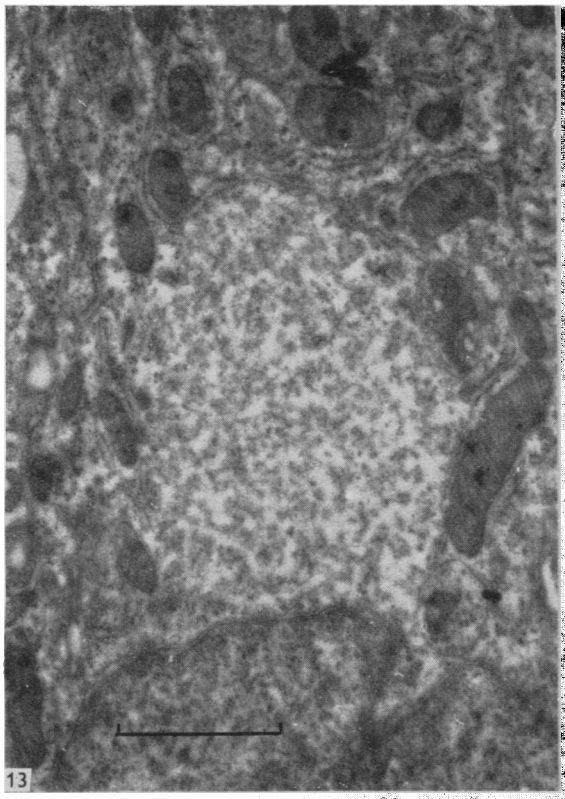


HAYWARD—FINE STRUCTURE OF GALL BLADDER EPITHELIUM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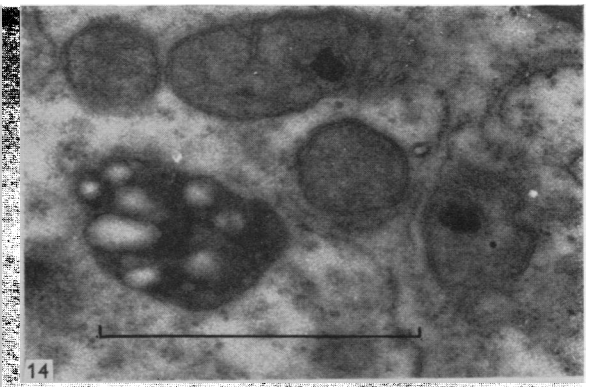
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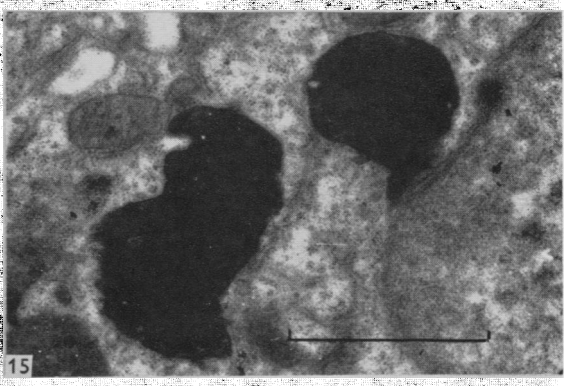
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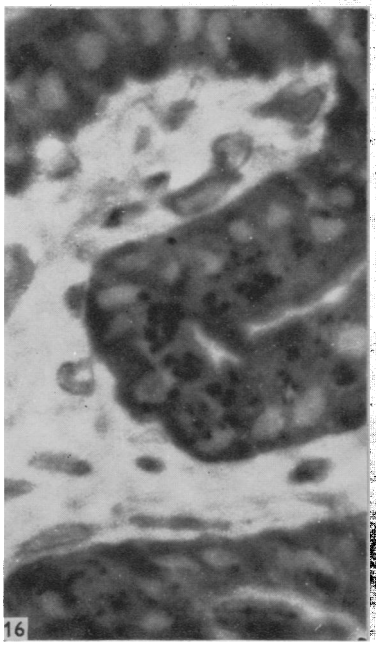
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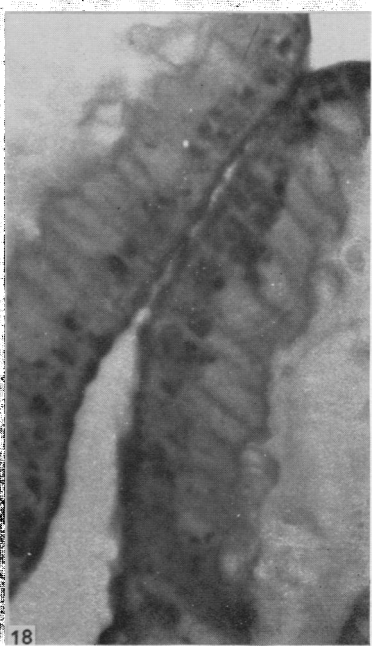
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