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Fine structure of squamous epithelium and submucosal glands of human oesophagus

TARIQ M. AL YASSIN AND PETER G. TONER

Department of Pathology, Basrah Medical College, Basrah, Iraq, and University Department of Pathology, Glasgow Royal Infirmary

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INTRODUCTION

The human oesophagus has not been fully explored at the ultrastructural level, particularly in regard to its squamous epithelium and its submucosal glands. The present study examines the fine structure of the non-keratinized human oesophageal epithelium and draws comparisons with similar surfaces in the mouth and cervix uteri. The submucosal glands are examined in detail and compared with the mucus-secreting labial salivary glands, to which they bear a histological resemblance.

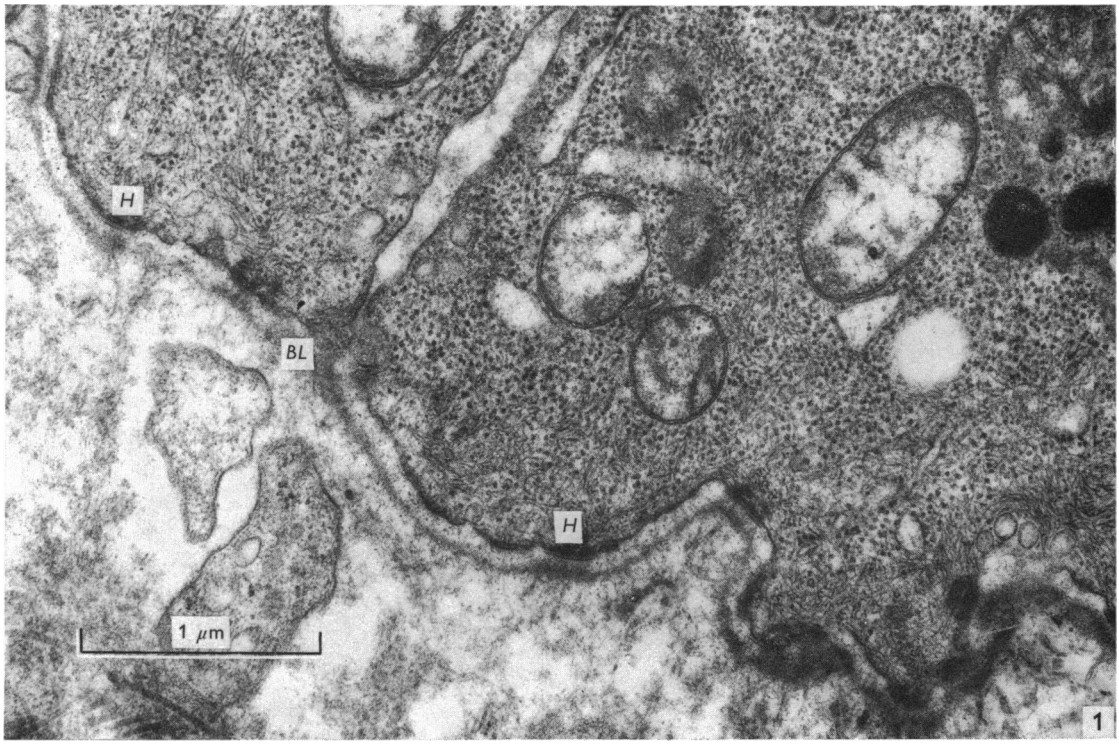
MATERIALS AND METHODS

Twelve specimens of normal human oesophageal mucosa were examined. The specimens were obtained by biopsy or were taken from the normal portions of resected oesophagus removed for therapeutic purposes. In four resection specimens the submucosal glands were identified by longitudinal incision through the mucosa. Blocks of tissue 0·5–1 mm diameter were fixed in 2 % glutaraldehyde in phosphate buffer, washed in buffer, post-fixed in 1 % osmium tetroxide in distilled water, dehydrated in ethanol and embedded in Epon. In addition, small blocks of tissue as described above were processed and stained for carbohydrate components using the periodic acid thiocarbohydrazide silver proteinate technique (Pearse, 1972). Thin sections were cut on an LKB ultratome III, stained with uranyl acetate and lead citrate, and examined on a Philips EM 200. Paraffin-embedded tissues were also prepared from each specimen and submitted to histopathological examination.

RESULTS

Squamous epithelium

The epithelial cells can be assigned to three layers, basal, intermediate and superficial, each with its own characteristic fine structural features. The basal cells are cuboidal or oblong, with centrally placed nuclei which have occasional indentations. The cytoplasm is relatively unspecialized, with moderate numbers of mitochondria, a small Golgi apparatus and some free ribosomes, but with little organized endoplasmic reticulum. Fine bundles of tonofilaments are scattered throughout the cytoplasm and are inserted into the cytoplasmic plates of the desmosomes. Distinct intercellular spaces are crossed by interdigitating folds and projections of cytoplasm.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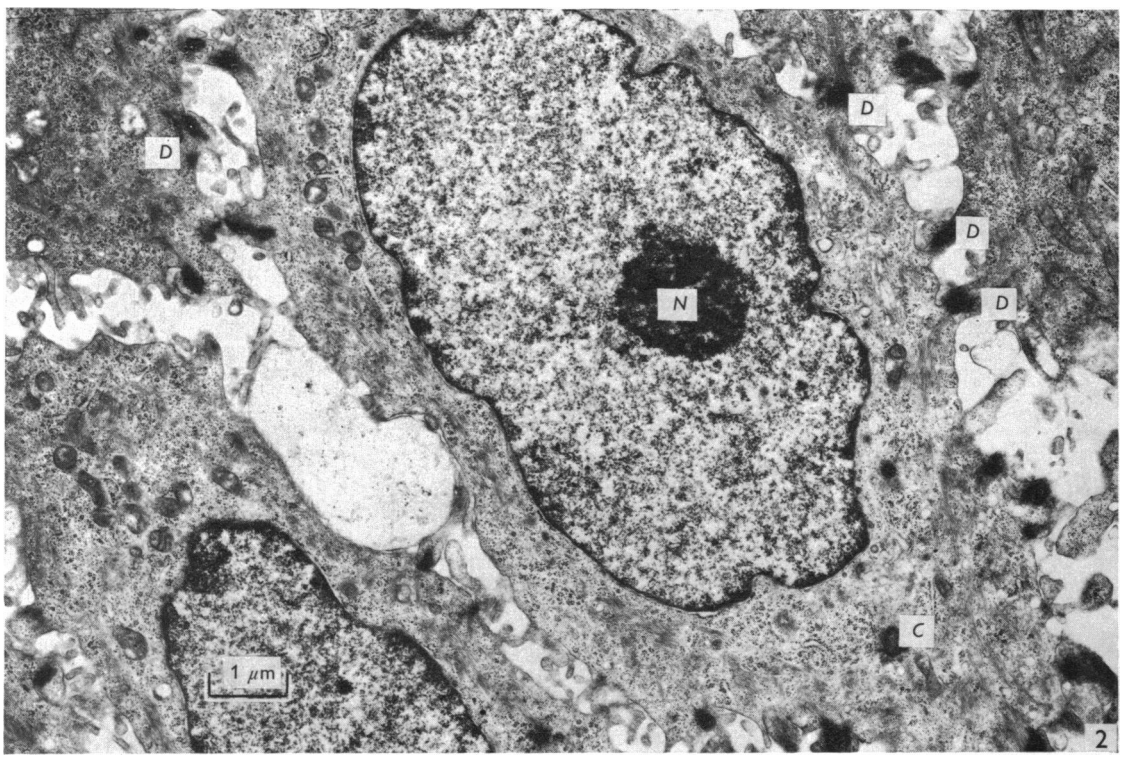
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The base, resting on the basal lamina, shows numerous hemidesmosomes, each consisting of a single dark plate with inserted tonofilaments, along with the extracellular lamina which is typical of this structure. The basal lamina itself, 40–55 nm in thickness, is separated from the base of the epithelium by a pale interspace of about 50–65 nm. (Fig. 1).

In the intermediate zone of the epithelium the cells are larger and flatter than basal cells (Fig. 2). They may have coarser bundles of tonofilaments, but they are similar to basal cells with respect to their mitochondria, endoplasmic reticulum, ribosome distribution and Golgi apparatus.

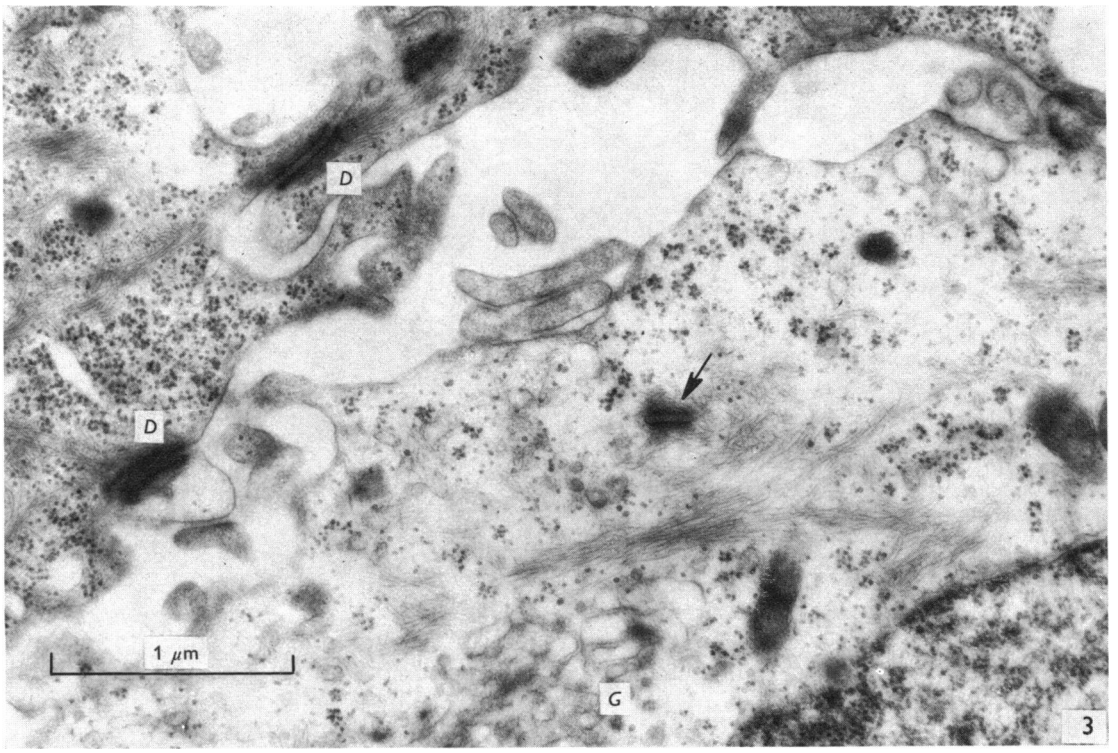
The desmosomes appear more prominent in the superficial part of the intermediate layer. However, at some sites, often where the intercellular space is widened and conventional desmosomes are absent, occasional intracytoplasmic desmosomes are encountered (Fig. 3). These paradoxical structures have the morphology of typical desmosomes, including a laminated structure and inserted tonofilaments, but they lie embedded within the cytoplasm instead of forming an attachment with another cell. These intracytoplasmic desmosomes have no demonstrable link with the cell surface or with any intracytoplasmic membrane system. A further feature of the intermediate zone is the occurrence of 'membrane-coating granules' in the more superficial cells. These are described more fully below. Occasional single cilia project into the intercellular space (Figs. 4, 5).

The cells of the superficial zone of the mucosa are flattened, lying parallel to the surface. They retain their nuclei and have diffuse tonofilaments in a rather pale cytoplasmic matrix. Cytoplasmic vacuolation is common in the deeper cells of this zone. This is associated with the presence of glycogen. There are fewer desmosomes at this level and the cytoplasmic projections at the cell surface only rarely appear to interdigitate. At the luminal free surface there are many finger-like projections, probably representing sectioned folds or flaps of cytoplasm (Fig. 6). The cell membrane is particularly prominent here, due to a significant increase in the thickness and staining reaction of its inner cytoplasmic leaflet (Fig. 7), while the outer leaflet and the pale interspace remain unaltered. The presence of a delicate carbohydrate-rich external cell coat is demonstrated by the periodic acid technique (Fig. 8). 'Membrane-coating' granules are seen in the cells of the superficial layer.

The membrane-coating granules of the human oesophageal mucosa are similar to those seen in other non-keratinized epithelia. They are small heterogeneous oval or round granules, from 60 to 350 nm in diameter, limited by a trilaminar membrane (Fig. 9). The location and size of the dense element within its pale halo is variable: this is at least partly a plane-of-section effect. A carbohydrate-rich component of the vacuole is stained by the periodic acid technique (Fig. 10). Some granules show a faint internal lamination (Fig. 11), but this is not as pronounced as in keratinized epithelium, where the distinctive periodicity of such granules is their most striking property.

Fig. 1. Base of squamous epithelium, showing basal lamina (*BL*) and hemidesmosomes (*H*). Numerous free ribosomes are seen in the cytoplasm.

Fig. 2. Intermediate zone of squamous epithelium, showing the typical appearance of the cells and their interrelationships. Prominent desmosomes are seen (*D*). Note nucleolus (*N*) and a centriole (*C*).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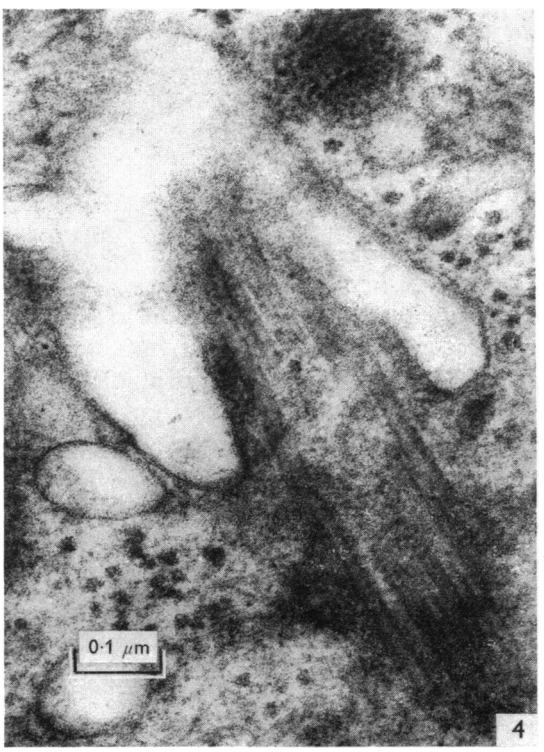
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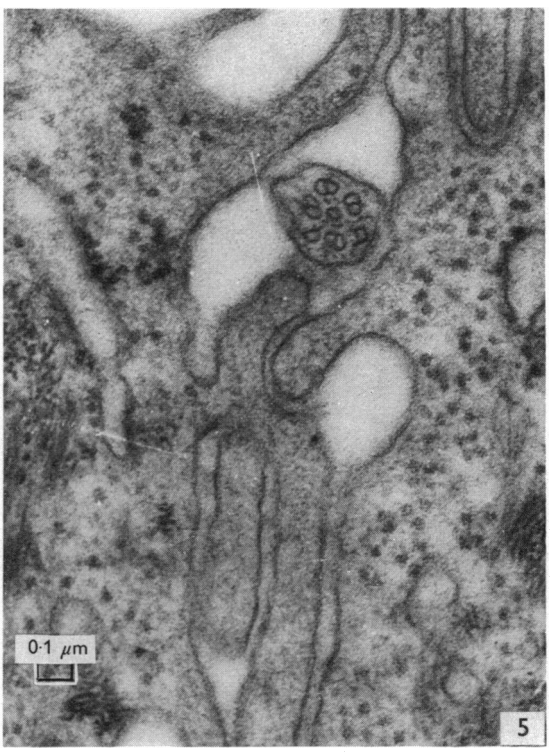
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Membrane-coating granules occur in the distal cells of the intermediate zone of the human oesophagus, and in the superficial zone. The granules gather at the margin of the cell, and dense material resembling their contents is often seen in the intercellular space (Fig. 12).

There are two other features of note in the oesophageal epithelium. The presence of Langerhans cells is reported in detail elsewhere (Al Yassin & Toner, 1977). The difficulties of distinguishing in some instances between Langerhans cells and lymphocytes raised the question of a relationship between these two cell types. In general, however, the balance of evidence favours a histiocytic origin for the Langerhans cell. Nuclear bodies are occasionally encountered at all levels in the oesophageal epithelium. They consist of aggregates of microfibrillar material, up to 600 nm in diameter, and not demarcated positively from the nuclear substance. The nature of these structures remains obscure.

Submucosal glands

The oesophageal glands are situated in the submucous connective tissue, each connected to the surface by a straight duct. They are composed of lobules, each consisting of acinar and ductal structures. The acini are lined by predominantly mucus-secreting columnar epithelium, the ducts by cuboidal or stratified epithelium.

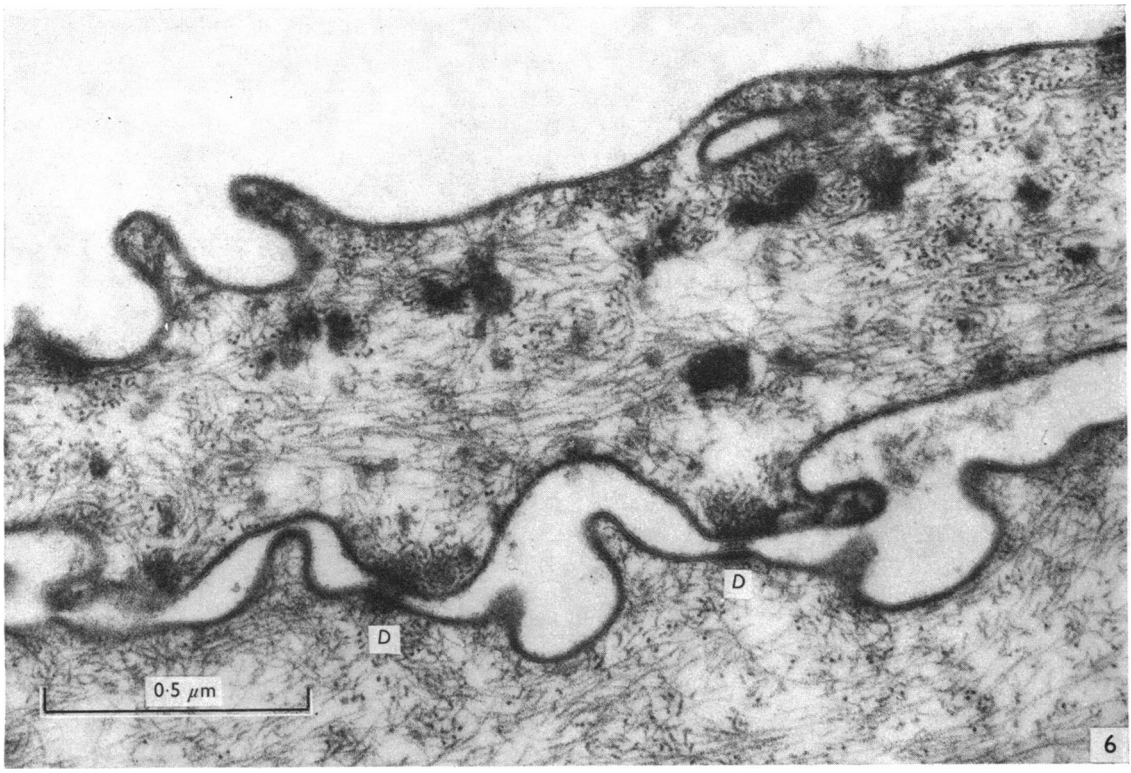
Four acinar cell types can be identified in human oesophageal submucosal glands. The predominant mucous cells are recognized by their numerous large pale secretion granules. Occasional subsidiary secretory cells are seen, with similar cytoplasmic characteristics, but different granule patterns. Myoepithelial cells are readily identified by their characteristic location and cytoplasmic filaments. Finally, typical oncocytes are sometimes encountered close to the origin of the duct system.

The mucous cells are closely packed, pyramidal in shape, and connected by conventional junctional complexes. The bulging luminal surface (Fig. 13) bears short sparse microvilli. The nucleus is pushed to the periphery of the cell by the accumulated mass of secretion. These mucus granules are pale, and sometimes foamy in texture. With the fixation procedures used in this study, individual limiting membranes are not observed, and granules frequently coalesce. There is a rim of well organized granular endoplasmic reticulum at the basal and perinuclear areas, and an elaborate Golgi system is seen in favourable sections (Fig. 17). The mitochondria are scattered towards the cell base. They have no distinctive features. There are occasional microtubular inclusions within mitochondria. These tubules, 15–25 nm in diameter, are orderly in their arrangement, are usually straight or slightly curved, and in longitudinal section they display a fine transverse periodicity. Finally, there are intracytoplasmic membrane-limited micro-fibrillar bundles associated with pale vacuoles (Fig. 14). These are distributed throughout the cytoplasm, often lying between secretion granules.

Fig. 3. Several typical desmosomes (*D*) with associated tonofilaments. An intracytoplasmic desmosome (arrow) lies in association with other tonofilament aggregates. Note the Golgi system (*G*).

Fig. 4. Rudimentary cilium originating in a basal body.

Fig. 5. Transverse section of a rudimentary cilium in the intercellular space, between squamous cells.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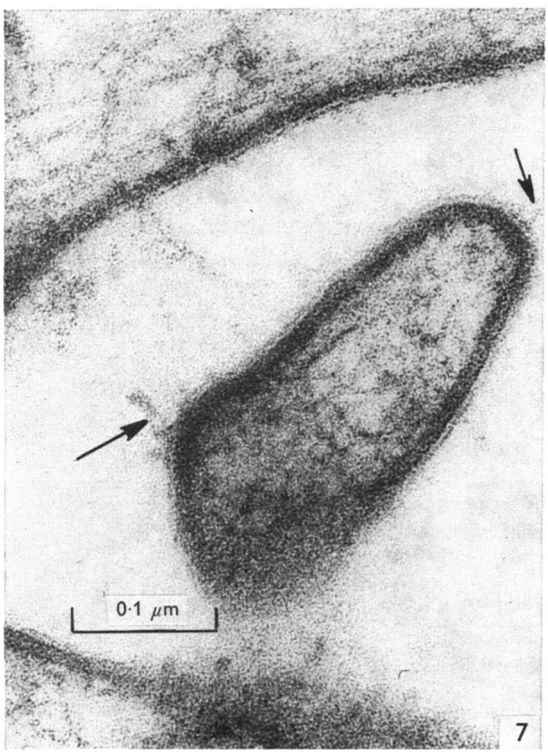
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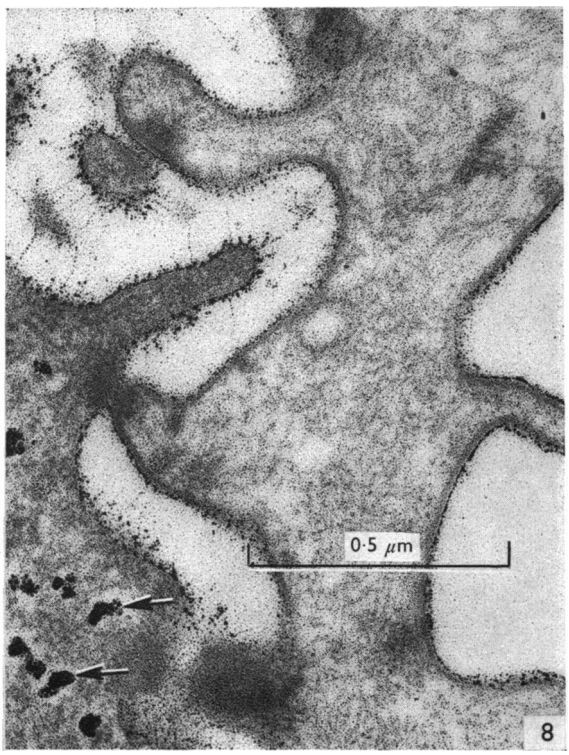
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The less common subsidiary secretory cells of the acinus (Fig. 15) are interposed between mucous cells, or lie at the junction of acinus and duct. The secretory granules are distinct from those of the mucous cells, being membrane-limited, smaller, rather more dense, and homogeneous or stippled in texture. In other respects these subsidiary cells are not dissimilar to the mucous cells. A further group of cells distinguished by the presence of numerous cytoplasmic filaments has smaller granules, often with a denser core and pale halo (Fig. 16). Occasional similar cells (Fig. 13) have few of these distinctive granules, or even none at all. Such cells have a less elaborate endoplasmic reticulum than their granulated counterparts.

The myoepithelial cells (Fig. 17) are flattened spindle-shaped cells, connected by desmosomes to the overlying epithelial cells. They rest upon the continuous acinar basement membrane. Their cytoplasm contains sparse mitochondria and few membrane systems, but is rich in myofilaments similar to those of smooth muscle, with typical attachment zones at the cell base.

Oncocytes are found in small numbers in the submucosal glands, occasionally in small clusters between duct and acinus (Fig. 18). Their characteristic numerous closely packed mitochondria may appear round, oval or elongated (Fig. 19). They have numerous cristae and occasional dense intramitochondrial granules. There is, in addition, a small Golgi system, a moderate complement of free ribosomes, and a scattering of membrane-limited dense bodies. The centrally placed nucleus has a slightly irregular outline. The oncocytes have no unusual surface features. They are connected to adjacent cells by junctional complexes and desmosomes, they rest on the basement membrane without basal specializations, and their free surfaces have few or no microvilli.

Various other features which have been described in previous studies of salivary tissue were not encountered in the present work. These include secretory canaliculi, intraepithelial nerve terminals and intranuclear inclusions. Unmyelinated nerves are, however, frequently found in the periacinar connective tissue, often close to the basal lamina. As well as mitochondria, these axons contain small agranular and large dense-cored vesicles.

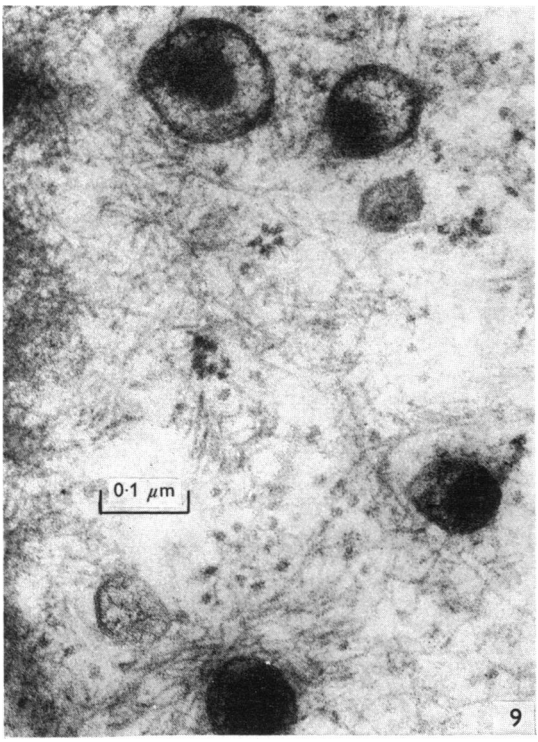
Ducts of submucosal glands

The lining of the ducts ranges from flattened cuboidal epithelium (Fig. 20) at the junction of acinus and duct, through two layers (Fig. 21), to a stratified pattern. The luminal surface is covered by short irregular microvilli; the contact surfaces have junctional complexes, desmosomes, and some interdigitations. Evenly distributed microfilaments are more numerous in the cuboidal than in the columnar cells. Granular endoplasmic reticulum is very sparse, but free ribosomes are present in

Fig. 6. Surface of squamous epithelium, showing flattened cells with diffuse filamentous contents and residual desmosomes (*D*). Note the prominence of the cell membrane.

Fig. 7. High magnification of the cell membrane of cells in the superficial zone of the epithelium. The inner lamina shows a pronounced linear thickening. Traces of cell coat material can be made out (arrows).

Fig. 8. Carbohydrate stain, to demonstrate the cell coat. Note the presence of glycogen granules (arrows).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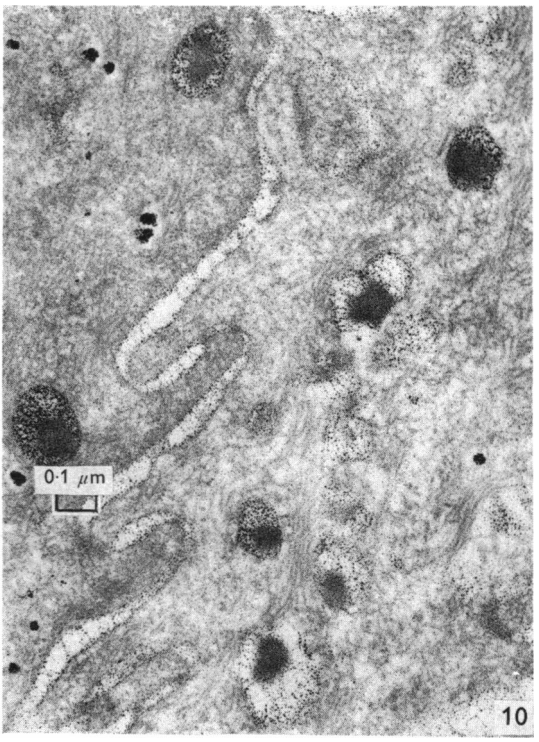
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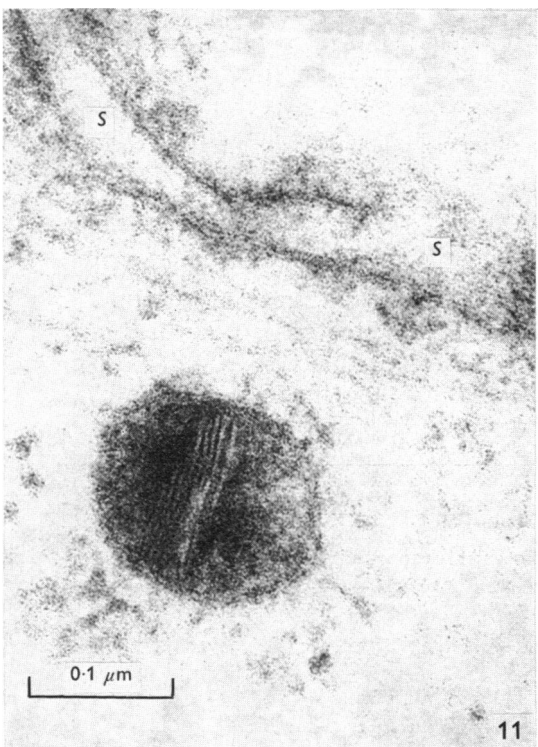
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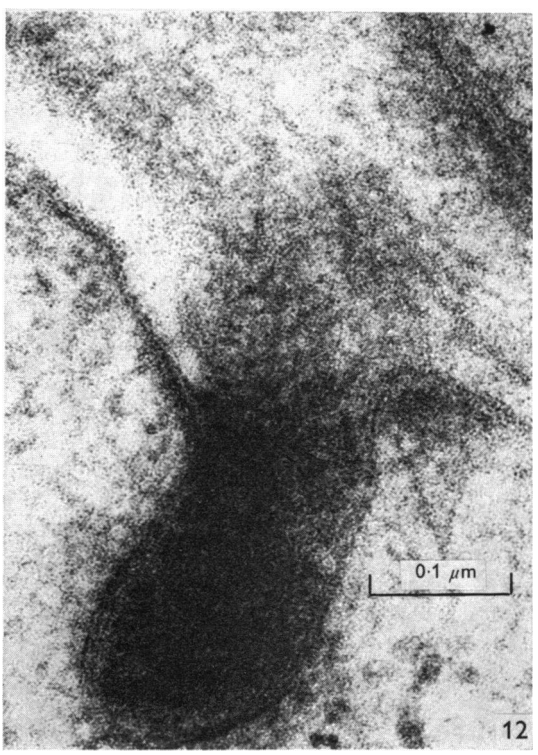
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moderate numbers. In the apical cytoplasm there are some empty smooth-walled vesicles from 70 to 140 nm in diameter, along with membrane-limited multilocular bodies and pleomorphic dense granules. Myoepithelial cells, similar to those of the acinus, are present in those ducts which have a simple epithelial lining, but are absent from ducts lined by stratified epithelium.

DISCUSSION

The squamous epithelium of the human oesophagus closely corresponds to the non-keratinized epithelium of the buccal mucosa (Zelickson & Hartmann, 1962; Hashimoto, Dibella & Shklar, 1966) and cervical mucosa (Hackemann, Grubb & Hill, 1968). This applies in particular to the membrane-coating granules (Grubb, Hackemann & Hill, 1968; Hayward & Hackemann, 1973). These granules lack the distinctive lamination seen in keratinizing epithelium (Matoltsy & Parakkal, 1965; Rowden, 1966), although traces of this differentiation were occasionally recognized. Absent also was any evidence of keratohyalin granules in the oesophagus, despite their identification in non-keratinized cervical squamous epithelium (Hackemann *et al.* 1968).

There are two features of the oesophageal epithelium which have not been reported at equivalent sites. These are the intracytoplasmic desmosomes and the nuclear bodies. Intracytoplasmic desmosomes are of rare occurrence, tending to be seen particularly at points where adjacent cells seem to have pulled apart and where they lack conventional desmosomal adhesion. This might be taken to support the view that their occurrence is related to defective intercellular adhesion (Mishima & Pinkus, 1968; Seiji & Mizuno, 1969; Takaki, Masutani & Kawada, 1971). Perhaps the residual hemidesmosome is retracted into the cell and pinched off, forming a twin for itself from the resultant small intracellular vacuole. The nuclear bodies encountered in the present study are equivalent to similar structures described as a normal cellular component at other sites (Buttner & Horstmann, 1967; Dupuy-Coin, Lazar, Kalifat & Bouteille, 1969). While their normal occurrence in human oesophagus can be affirmed, their functional significance remains obscure.

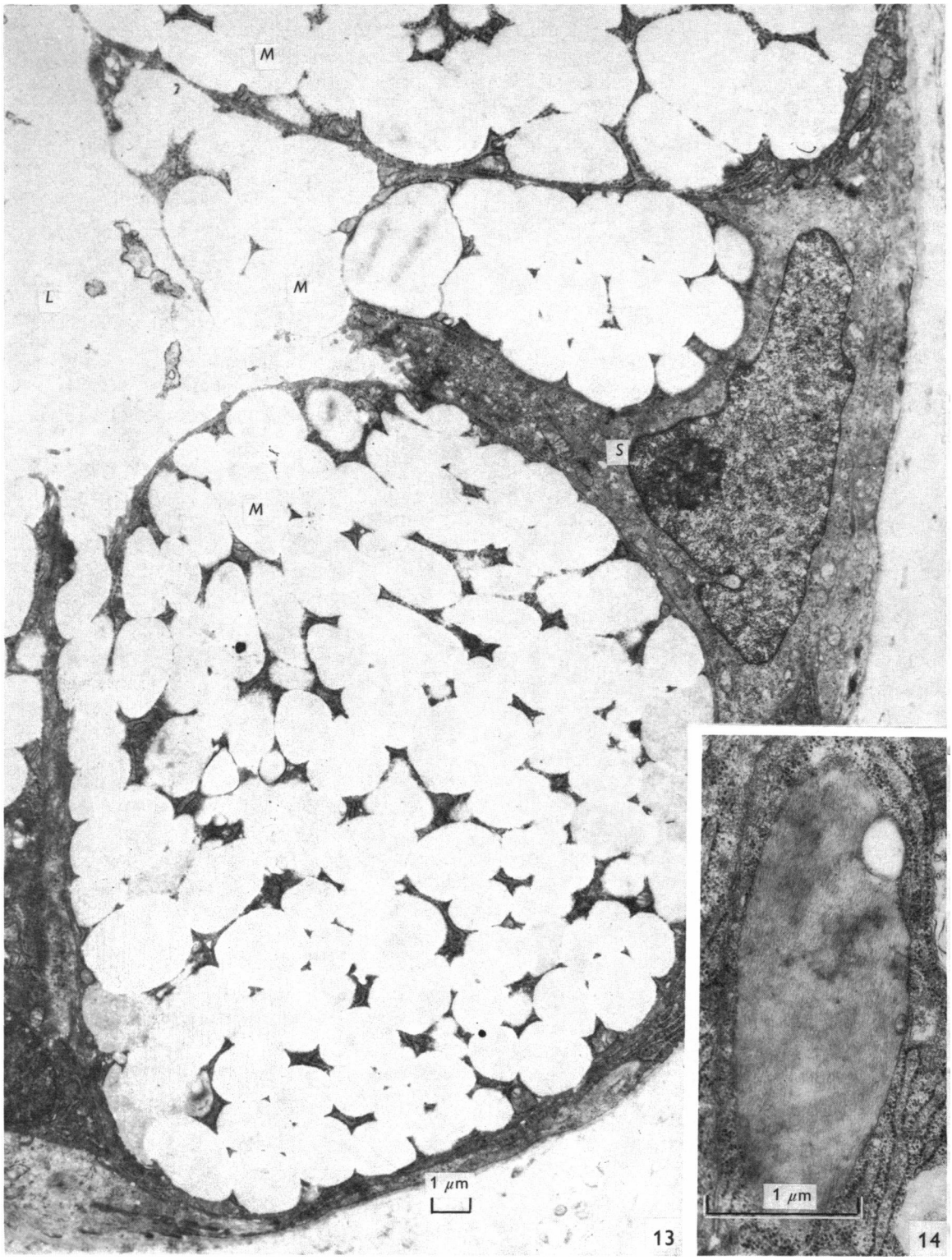
The submucosal glands of the human oesophagus can best be compared with the human labial salivary glands (Tandler, Denning, Mandel & Kutscher, 1969). Their principal mucous cells are virtually identical in many respects, such as general granule morphology, the occurrence of 'duplex' inclusions, and the vacuolar and fibrillar aggregates described above. Tandler *et al.* (1969) believe that these inclusions are discharged along with the mucus, but we have no direct evidence to support this view. Somewhat similar filamentous bundles have been described in adipose cells in

Fig. 9. 'Membrane-coating granules' of upper intermediate zone of the epithelium. Notice the limiting membrane, pale halo and dense core. No periodicity is seen.

Fig. 10. Carbohydrate stain, indicating the presence of a positive staining component in the halo of the membrane-coating granule. Note the presence of glycogen granules.

Fig. 11. A faint lamellation is evident in this granule, which is probably a 'membrane-coating granule'. Note the nearby cell surface and intercellular space (*S*).

Fig. 12. 'Membrane-coating granule' discharging into the intercellular space.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relation to lipid droplets, but again their function is unknown (Wood, 1967). The occasional arrays of microtubules observed in the present study within mitochondria have not been reported in previous studies of salivary tissue.

The subsidiary acinar secretory cells described above have granules of greater density than those of the more numerous mucous cells. They resemble to some extent the mucous granules of gastric mucous cells (Lillibridge, 1964; Rubin, Ross, Sleisenger & Jeffries, 1968). The labial salivary glands (Tandler *et al.* 1969) have been reported as having a similar degree of variation in granule morphology. The cells containing smaller granules of heterogeneous pattern, or, on occasion, few if any granules, may correspond to the 'inactive mucous cells' of Goetsch (1910). Perhaps they are immature forms. More likely they are transitional between acinar and duct cells, in a situation analogous to that of the centro-acinar cells of the pancreas.

The myoepithelial cells of oesophageal glands are confined to the acini and adjacent ducts. They display no unusual features and can be compared directly to those of salivary tissue (Tandler, Denning, Mandel & Kutscher, 1970). It is curious that Goetsch (1910) in an otherwise meticulous histological study made no mention of these cells.

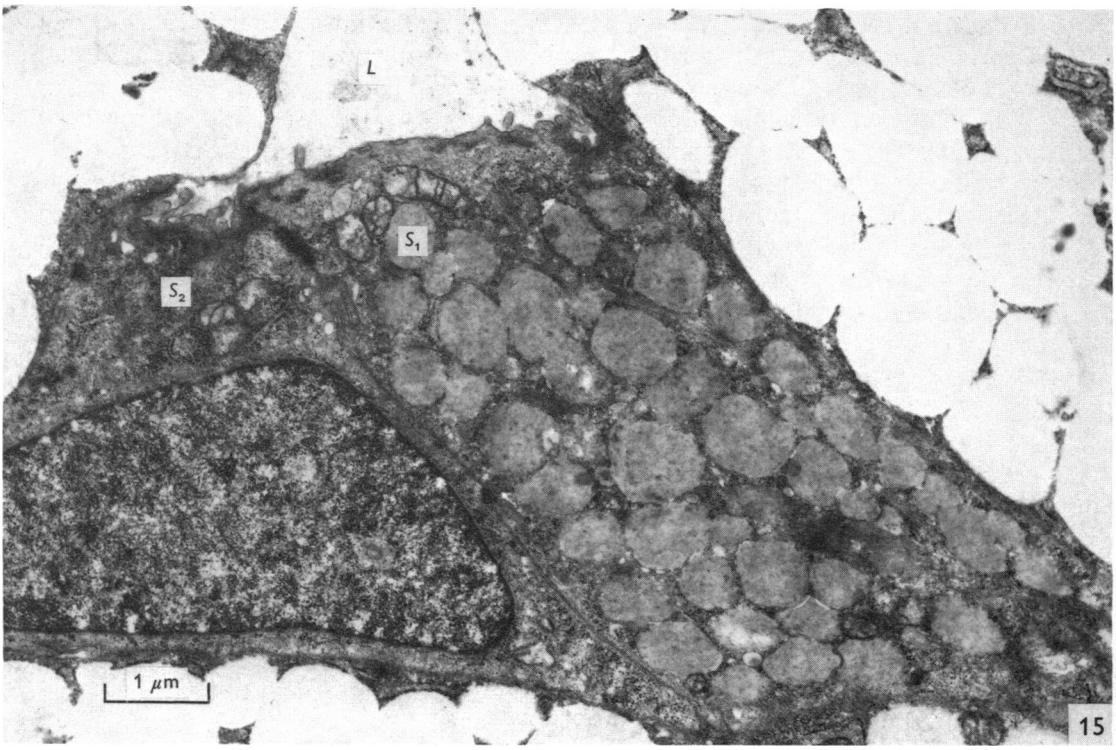
The oncocytes of the present study correspond to those previously reported in normal salivary glands (Tandler, 1966*b*; Bogart, 1970) and elsewhere (Roth, Olen & Hansen, 1962; Tandler & Shipkey, 1964; Balough & Roth, 1965; McGavran, 1965; Tandler, 1966*a*). The ultrastructure of these cells, and their occurrence at the junction of duct and acinus, give no clue to their origins, thought variously to be from duct cells (Chauncey, Shklar & Brooks, 1962; Shklar & Chauncey, 1965), from myoepithelial cells (Hubner, Paulussen & Kleinsasser, 1967) or from acinar secretory cells (Voth, 1962). The distinctive oncocytoma (Tandler, 1966*a*), which shares similar ultrastructural features, is described in various situations, but, surprisingly, apparently not in the oesophagus.

The main duct cells described above do not appear to contribute mucus to the secretion of the gland, nor do they appear to have the large-scale fluid and electrolyte transfer functions which are inferred from the ultrastructural features of striated duct cells in salivary tissue. It is uncertain whether the small apical vesicles reflect a secretory or an absorptive function.

Finally, the innervation of the oesophageal submucosal glands appears to be by indirect contact, since neural elements have been found only in periacinar connective tissues, and do not enter the epithelium, as has been reported in salivary tissue (Tandler & Ross, 1969; Hand, 1970). This difference may well be related to different functions, the more sophisticated direct control of the salivary tissue perhaps reflecting the immediate responsiveness of salivary secretion to stimulation from higher centres.

Fig. 13. Submucosal gland cells, mainly of the principal mucous-secreting type (*M*), with empty granules awaiting release into the lumen (*L*). A subsidiary cell without granules is present (*S*).

Fig. 14. A 'duplex' inclusion in a principal cell, consisting of filamentous material with a single vacuole; note the surrounding granular endoplasmic reticulum.



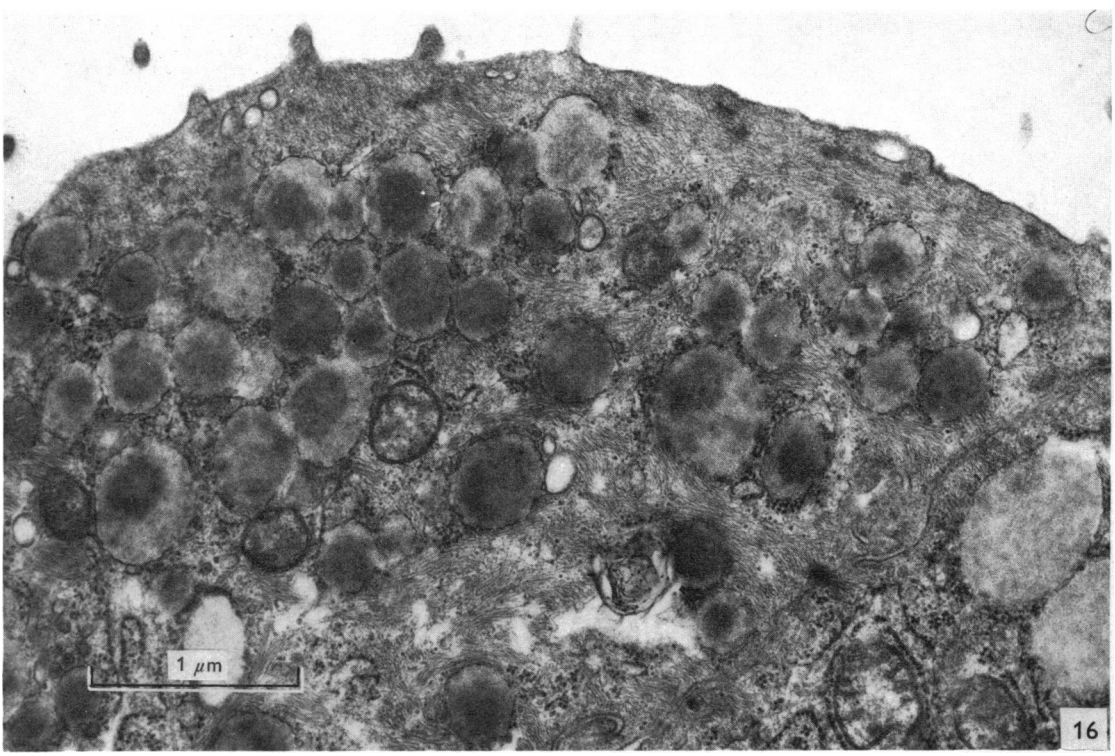


Fig. 15. Principal cells are identified by their empty granules. Two subsidiary cells are seen, one with homogeneous granules (S_1) and one with no granules (S_2). The lumen is shown (L).

Fig. 16. A further subsidiary cell type in the submucosal gland is identified by its heterogeneous granules and its numerous cytoplasmic filaments.

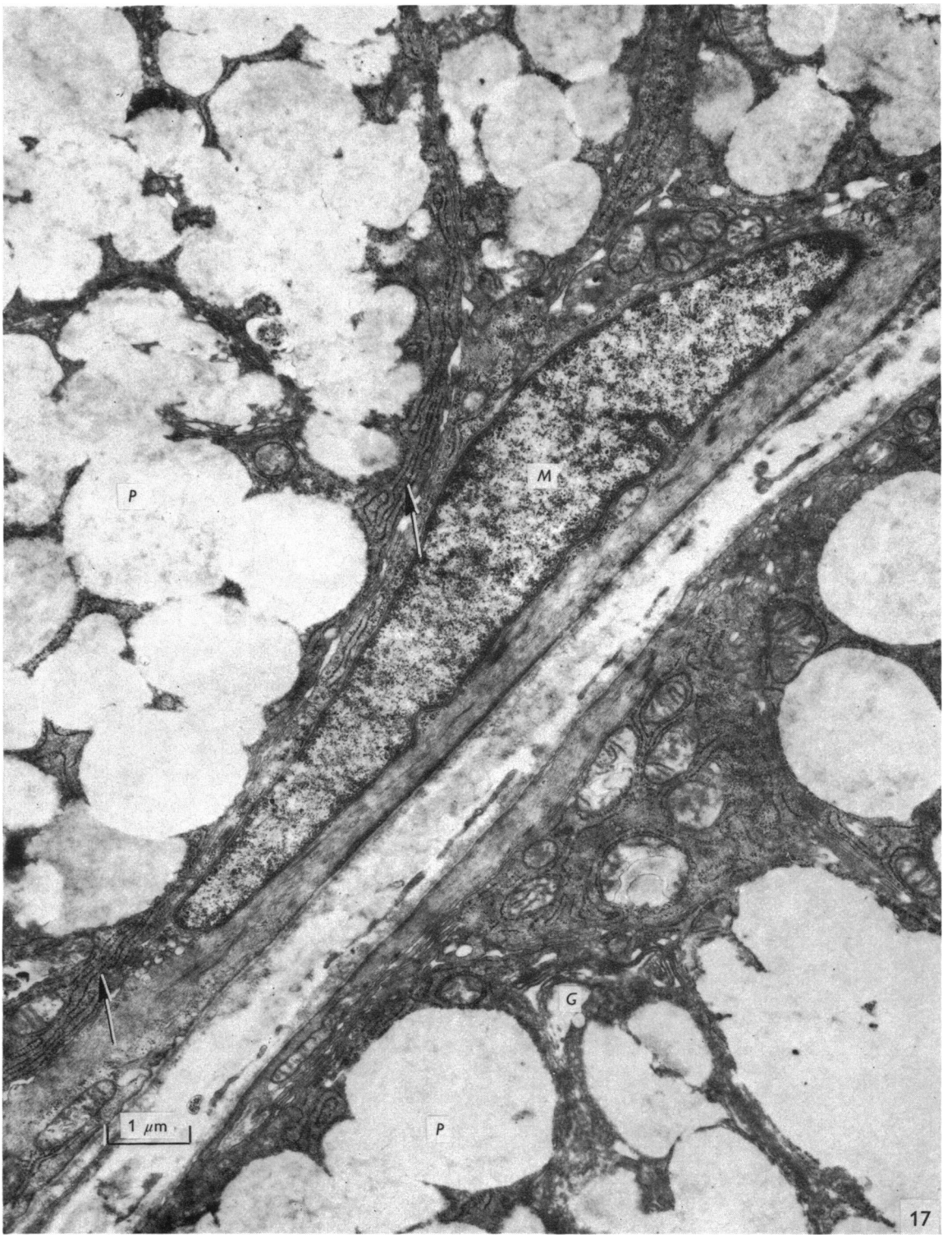
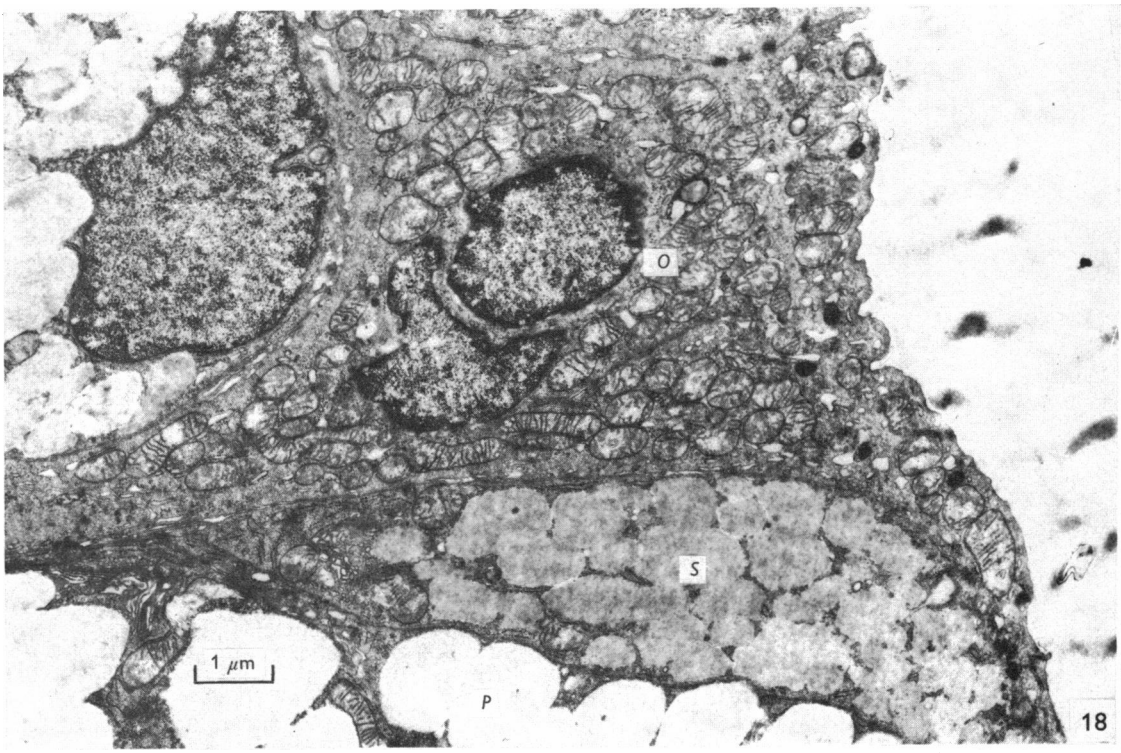


Fig. 17. The bases of two gland acini are shown, with intervening connective tissue. Note the typical principal cells (*P*) with empty granules, elaborate granular endoplasmic reticulum (arrows) and Golgi system (*G*). A myoepithelial cell (*M*) is seen at the acinar base.



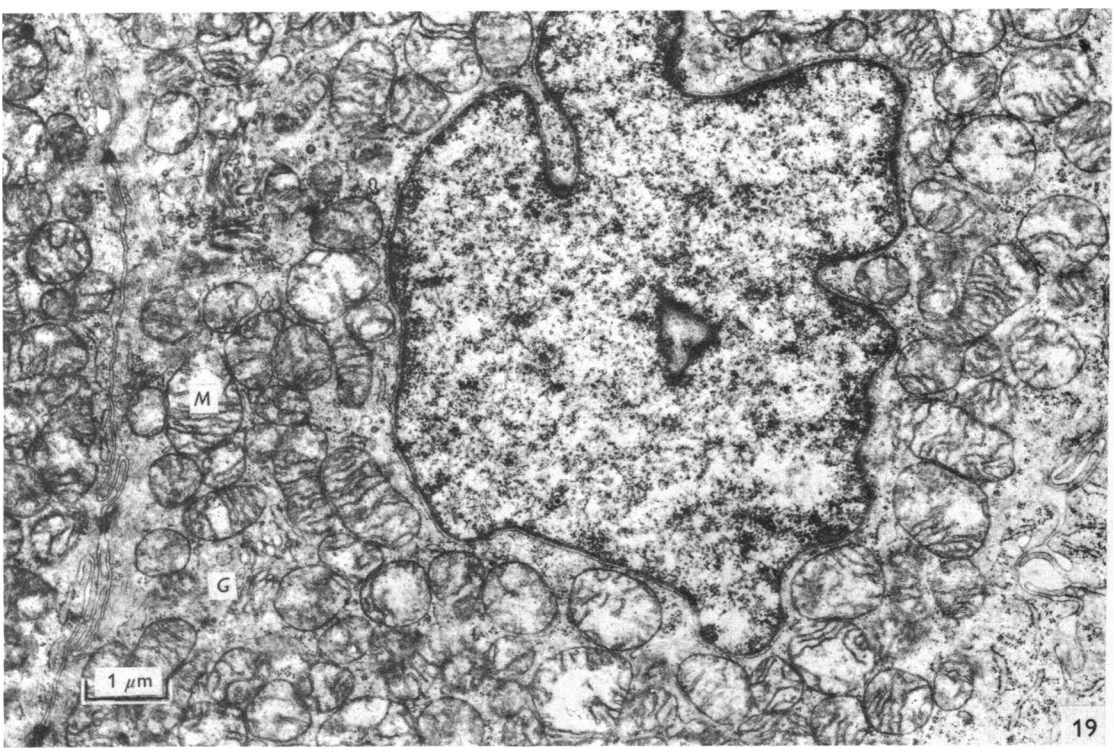
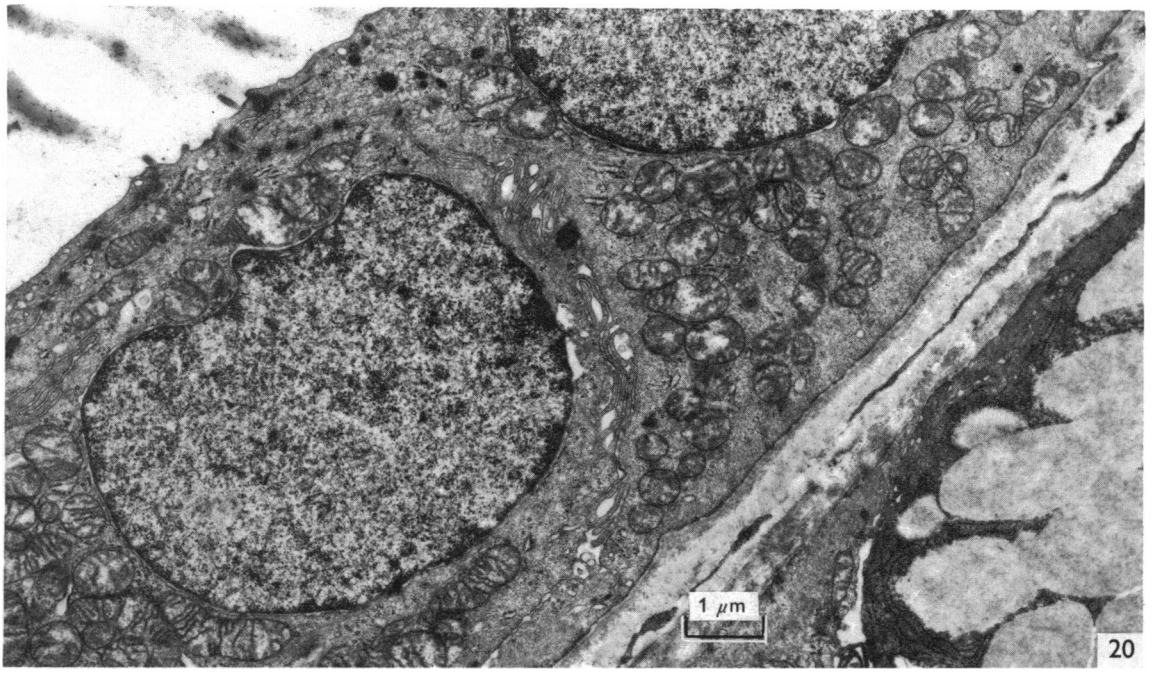


Fig. 18. Junction of acinus and duct. A principal cell (*P*), a subsidiary cell (*S*) and an oncocyte (*O*) can be identified by their distinctive features.

Fig. 19. Oncocyte, showing numerous mitochondria (*M*) and a small Golgi system (*G*).



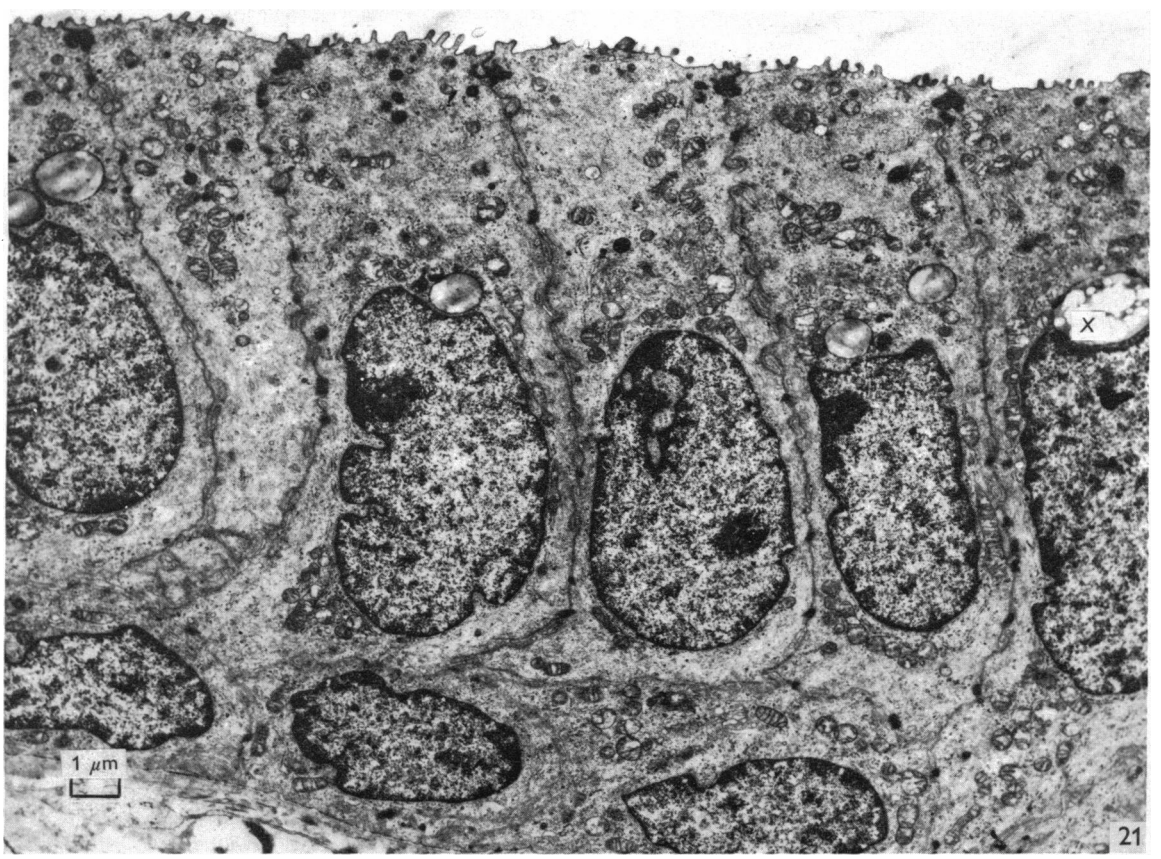


Fig. 20. Flattened epithelium of a small duct, adjacent to an acinus.

Fig. 21. Two-layered epithelium of a larger duct. Note the complex inclusions (*X*), probably lipid-rich.

SUMMARY

Normal squamous epithelium and submucosal glands of the human oesophagus have been studied in biopsy and resection specimens. The squamous epithelium has a structure similar to that seen in other histologically comparable sites, and contains Langerhans cells. Features of note within the squamous cells include microfibrillar intranuclear bodies and occasional intracytoplasmic desmosomes. The submucosal glands are comparable in many respects to the mucous-secreting minor salivary glands. Myoepithelial cells are observed within the acini and smaller ducts; occasional oncocytic cells are identified at the junction between duct and acinus. The innervation of these glands appears to be indirect.

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