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The Ultrastructure of the Sheep Parotid Gland

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Summary. The sheep parotid is a compound tubular gland; its ultrastructure reflects the function of this gland to secrete large amounts of fluid with very little protein. The cells of the secretory tubules possess extensively folded lateral plasma membranes and a fairly large number of mitochondria. Rapid equilibration of water across the epithelium is assured by the close proximity over large areas of intercellular spaces and the wide secretion canaliculi. Numerous long microvilli extend into the latter. Although secretion granules may be quite numerous, there is evidence that many of these granules are not discharged but undergo degradation by lysosomal enzymes. The intercalated ducts are often dilated but excessive distension is probably prevented by bundles of microfilaments in their epithelial cells.

Key words: Parotid – Sheep – Ultrastructure – Lysosomes – Enzyme cytochemistry.

Introduction

The main secretion products of salivary glands are water, electrolytes and proteins. The process of protein secretion has been studied extensively both in its physiological and its morphological aspects in salivary glands of a relatively small number of mammals. Physiological studies on fluid and electrolyte secretion have generally been performed on a wider range of animals and glands (cf. Young and van Lennep, 1977a). Considerations of the morphological aspects of fluid secretion have been largely based however on ultrastructural studies of typically serous, protein secreting glands such as the parotid and mandibular glands of the rat. It would seem to be more logical to base such studies on the parotid gland of the sheep, an animal that is known to secrete large amounts of fluid with a very low protein content.

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Although the fine structure has been described of the parotid gland of the ox (Shackleford and Wilborn, 1969) and of a number of African wild ruminants (Kayanja and Scholz, 1974), only a few brief notes have so far been published on the fine structure of the sheep parotid (Blair-West et al., 1969; Patterson et al., 1973, 1976) and on the enzyme histochemistry of this gland (Chauncey and Quintarelli, 1961; Blair-West et al., 1969).

The present study was undertaken to form a basis for a correlative investigation of morphological changes associated with varying physiological conditions. Although the animals used in this study varied in age as well as sex, and most animals were in fact submitted to such experiments as sodium depletion by cannulation of the parotid duct (Denton, 1957), and stimulation by infusion of acetylcholine, the present report will deal only with the general ultrastructural features and enzyme cytochemistry of the sheep parotid. The paper by Blair-West et al. (1969) reports some difference in cytology of the parotid glands of sodium depleted animals as compared with sheep of normal sodium status. Our preliminary studies have shown that no conclusions in this respect are valid unless experimental as well as preparative procedures for electron microscopy are standardized as much as possible.

Materials and Methods

Parotid glands from seven animals were examined. Four of these were approximately four months old wethers, the other three were adult ewes. Pieces of gland were removed under general anesthesia both from otherwise untreated animals and from animals which had undergone sodium depletion by cannulation of the parotid duct, from unstimulated glands and from glands which had been stimulated for various periods by close arterial infusion of acetylcholine. In one instance 50 mg horseradish peroxidase (Sigma grade II) was infused simultaneously with acetylcholine through an artery supplying the gland and the tissue fixed six minutes after start of the infusion.

All tissues intended for electron microscopy were fixed in either full strength or half strength Karnovsky's formaldehyde-glutaraldehyde fixative (Karnovsky, 1965), and after rinsing in buffer, postfixed in one percent buffered osmium tetroxide, dehydrated and embedded in Spurr's low viscosity embedding medium. For enzyme cytochemistry, tissues were fixed for one to two hours in diluted (50%) Karnovsky's fixative and rinsed in buffer; 40 µm thick cryostat sections were incubated in the appropriate medium, rinsed in buffer, followed by post-osmification, dehydration and embedding. Acid phosphatase was demonstrated by incubation for 30–60 min at 37° C in a medium containing 50 mM acetate buffer at pH 5.5, 6.5 mM sodium glycerophosphate, 3.5 mM lead nitrate and 0.2 M sucrose. As controls, some sections were incubated in the above medium to which had been added 10 mM sodium fluoride.

For the demonstration of peroxidases, cryostat sections were incubated for one hour at room temperature in 50 mM Tris-HCl buffer pH 7.6, containing 1.4 mM diaminobenzidine, 5 mM manganese sulfate and 0.001% hydrogen peroxide.

For light microscopy both 1 µm epoxy sections, stained with toluidine blue, and paraffin sections were used. The latter were from material fixed in either 4% formaldehyde (prepared from paraformaldehyde) or ice-cold acetone and dehydrated and embedded rapidly to preserve some of the enzyme activities. Alkaline phosphatase was only examined on paraffin sections, incubated for 20–30 min in a standard medium containing 20 mM sodium diethylbarbiturate, 20 mM sodium glycerophosphate, 78 mM calcium chloride and 9 mM magnesium sulfate. For light microscopical demonstration of acid phosphatase both paraffin and cryostat sections were incubated in the same media as used for electron microscopic demonstration of this enzyme. Other paraffin sections were stained according to the periodic acid-Schiff (PAS) method, with alcian blue at pH 1.0 and at pH 2.5, as well as with aldehyde fuchsin.

Results

Histologically, the sheep parotid is a compound tubular gland. Its secretory endpieces are long and branched tubules with a wide, irregularly shaped lumen and an epithelium of variable height (Fig. 1). The tubules are drained by intercalated ducts which again may vary from narrow ducts with a cuboidal epithelium to highly distended ducts lined with an almost squamous epithelium (Figs. 1, 2). The intercalated ducts open into striated ducts with an abrupt change in epithelium. These striated ducts do not lie randomly scattered in the lobule as they do in most eutherian mammals, but run mostly in small bundles in the center of the lobule, although this concentration of striated ducts in the lobule center is less conspicuous than in the mandibular glands of some marsupials and the monotreme *Ornithorhynchus anatinus* (Young and van Lennep, 1977b; Blood et al., 1977).

Myoepithelial cells which may be demonstrated by the histochemical method for alkaline phosphatase (Silver, 1954) surround the secretory tubules and parts of the intercalated ducts (Fig. 10). A few myoepithelial cells may be found associated with the striated ducts. The intralobular stroma is scanty and contains a few fibrobasts and plasma cells, capillaries and sinusoidal vessels (Blair-West et al., 1969).

The Secretory Tubules

The epithelial cells can best be described as consisting of a cell body containing the nucleus, rough endoplasmic reticulum, Golgi complexes, secretion granules and mitochondria, and the basolateral expansions of folds which occupy approximately one third of the epithelial volume (Figs. 4, 5). The apical part of the cell body usually bulges out into the lumen of the tubule (Fig. 5), bearing a variable number of microvilli. The lumen forms diverticula between the cell bodies, probably representing the secretion canaliculi seen in other serous exocrine glands. The plasma membranes lining the diverticula possess brush borders of long, sometimes branched or forked microvilli (Figs. 4, 6, 9). Almost the entire baso-lateral plasma membrane of the epithelial cell is thrown into broad folds which, at the lateral aspects of the cell, interdigitate with folds from neighbouring cells, except where a myoepithelial cell intervenes. Consequently, the cell body rests only with a relatively narrow base with a smooth basal membrane on the basement lamina.

The lateral intercellular spaces are often separated from the lumen of the secretion canaliculus only by a very narrow strip of cytoplasm containing the junctional complex. This strip is frequently no thicker than a lateral fold but contains 6 nm filaments, inserted in the zonula adhaerens, and some microtubules. Such filaments and tubules are lacking in the lateral folds although they do appear to contain an indistinct central layer of fine filamentous material. The lateral intercellular spaces are usually fairly narrow in unstimulated glands and frequently contain an electron-dense material (Fig. 9).

The basal region of the cell body usually contains a small number of stacked cisternae of rough endoplasmic reticulum (RER) but the amount of RER varies

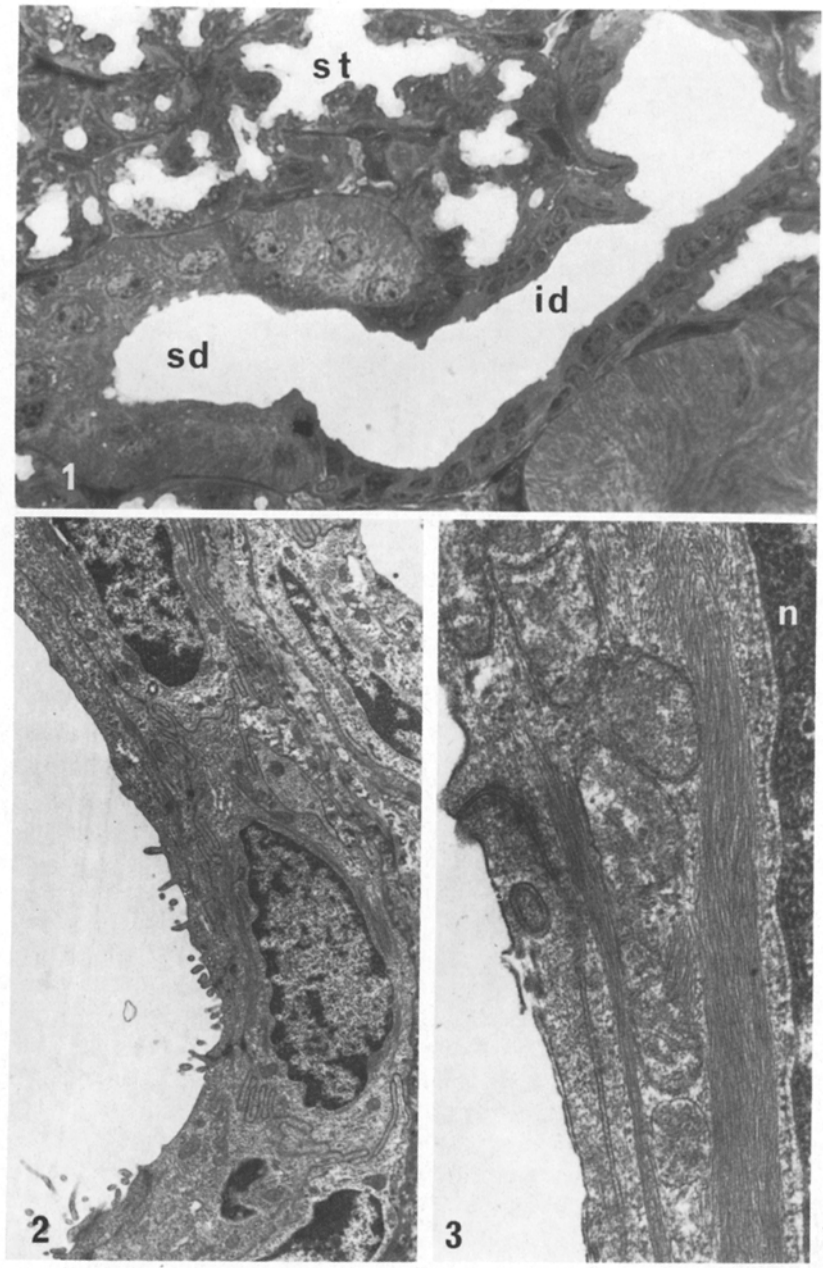


Fig. 1. Light micrograph of epon section showing secretory tubules (*st*), intercalated duct (*id*) and striated duct (*sd*). Note the wide lumina of both secretory endpieces and ducts. × 810

Fig. 2. Low power electron micrograph of the epithelium of a distended intercalated duct. × 7290

Fig. 3. Apical region of intercalated duct cell with bundles of 6 nm filaments. *n* nucleus. × 38,700

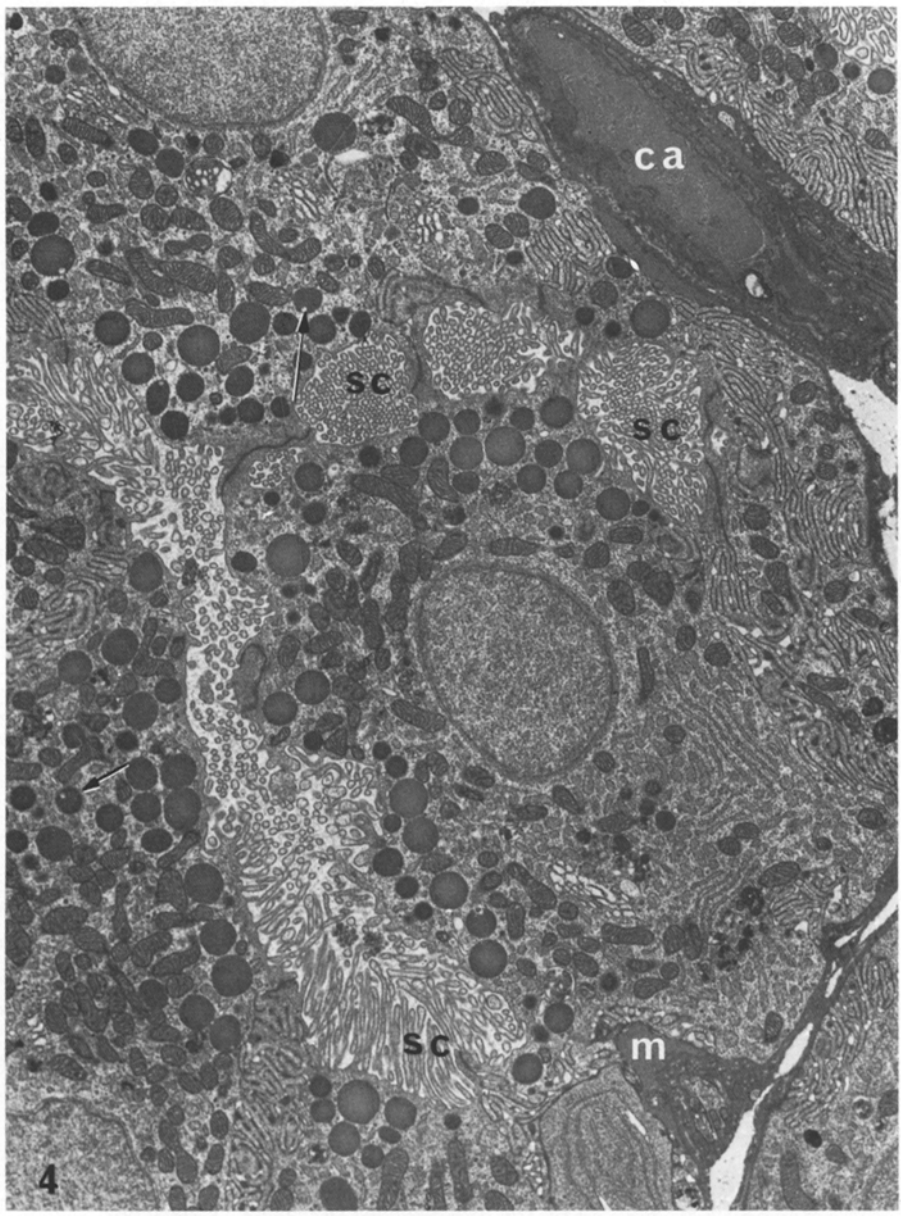


Fig. 4. Epithelium of a secretory tubule showing the central lumen and the secretion canaliculi (*sc*) with their brush borders of microvilli. Note the abundance of mitochondria and two types of secretion granules. Some of the electron dense granules of type I show a peripheral rarefaction (arrowed). *m* myoepithelial cells; *ca* capillary. × 8000

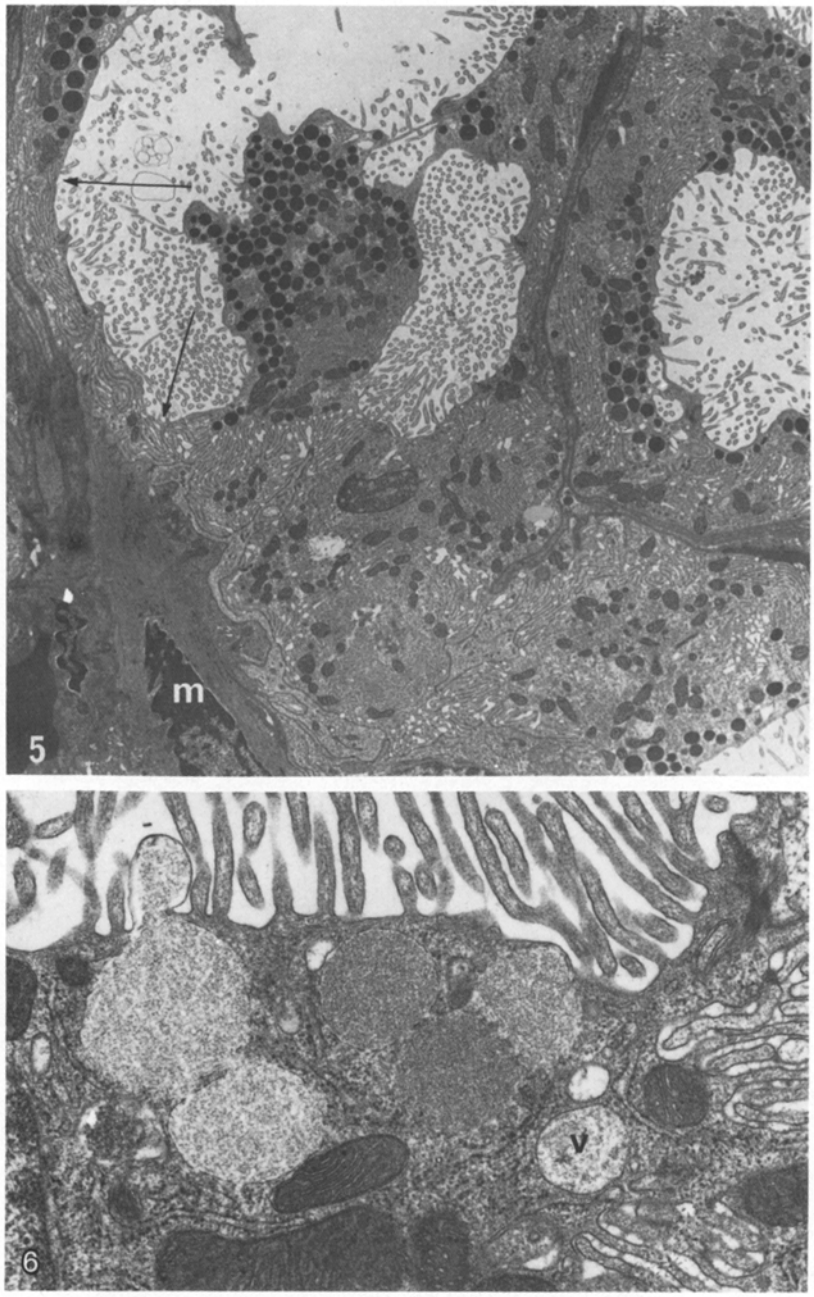


Fig. 5. Dilated secretory tubules with mainly type I granules. The cell body of one epithelial cell bulges out into the lumen, leaving most of the wall of the tubule to be formed by the expanded lateral folds (arrowed). *m* myoepithelial cell. × 5400

Fig. 6. Apical region of epithelial cell with type II granules that are partly fused. One granule appears to undergo exocytosis. The large vesicle (*v*) is probably the remainder of a degenerated granule. Note the forked microvilli. × 26,100

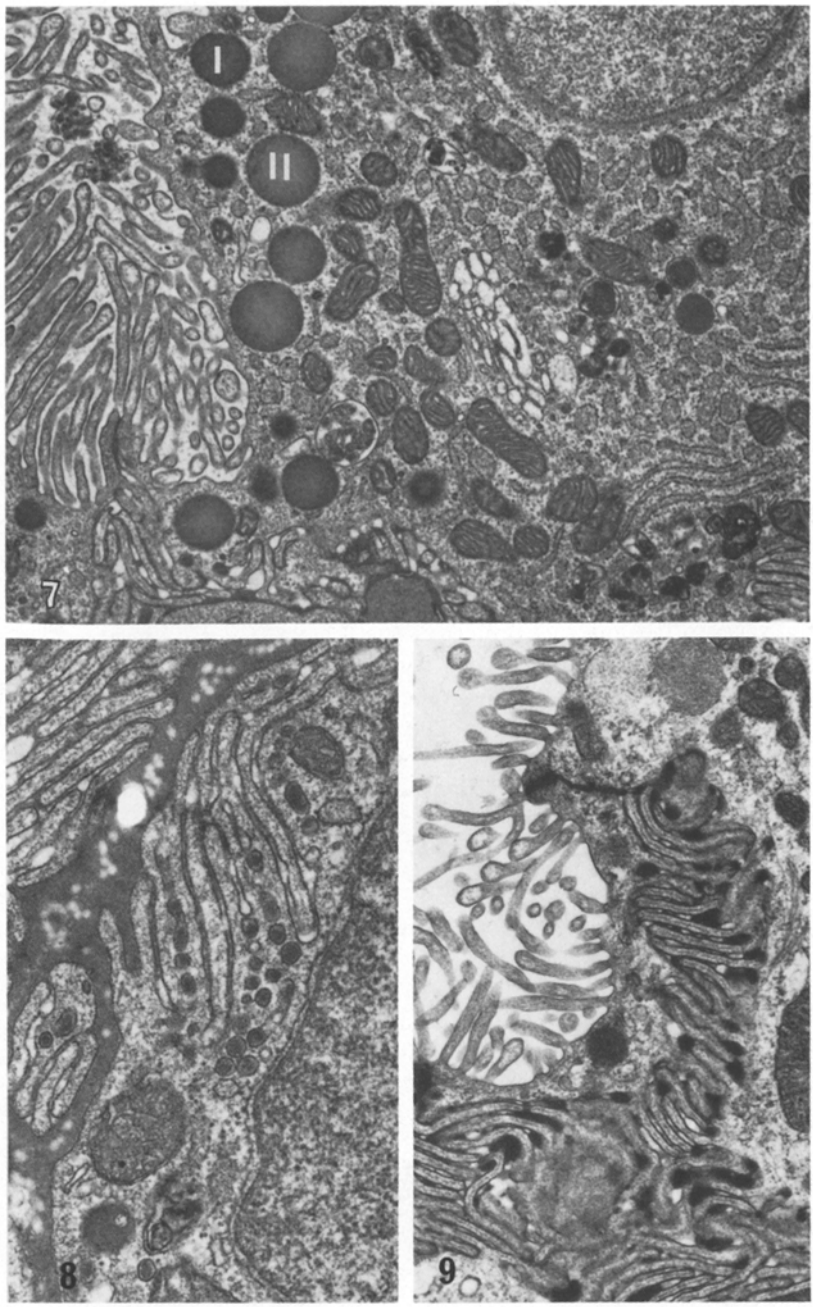


Fig. 7. Section of an epithelial cell shown in Fig. 4. Besides secretion granules of both types (I, II) the cytoplasm contains a large number of secondary lysosomes that are presumably secretion granules undergoing degradation. The lumen of the secretion canaliculus contains small aggregates of dense material. × 16,200

Fig. 8. Small vesicles containing an electron dense substance are lined up in the direction of the lateral folds of an epithelial cell. × 32,400

Fig. 9. The lateral intercellular spaces often contain an extremely dense material. × 16,200

a great deal from cell to cell. The lateral and apical regions of the cell body contain the secretion granules, Golgi complexes, assorted vesicles, including secondary lysosomes, as well as scattered profiles of RER and a fair number of microtubules. Mitochondria are particularly abundant in the secretory cell of the sheep parotid. They are presumably mostly elongated but because of their random orientation appear in a variety of shapes in the micrographs.

The secretion granules show a certain amount of variability in size and electron density. Many of the granules are relatively small and quite electron dense (type I granules). Such type I granules frequently show a peripheral electron lucent spot or rarefaction (Fig. 4). Type II granules are generally larger and have a less electron dense matrix. The low electron density of some of the type II granules and their tendency to fuse with one another (Fig. 6) are features typical for granules of mucous secreting salivary gland cells. In the granules of the sheep parotid however carbohydrate content must be very low since these granules did not stain with either PAS or alcian blue.

Discharge by exocytosis was never observed from type I granules, but type II granules could occasionally be seen to bulge out into the lumen, suggesting a form of discharge (Fig. 6). There seems little doubt that the two types described form a single class of granule since intermediate forms are very common.

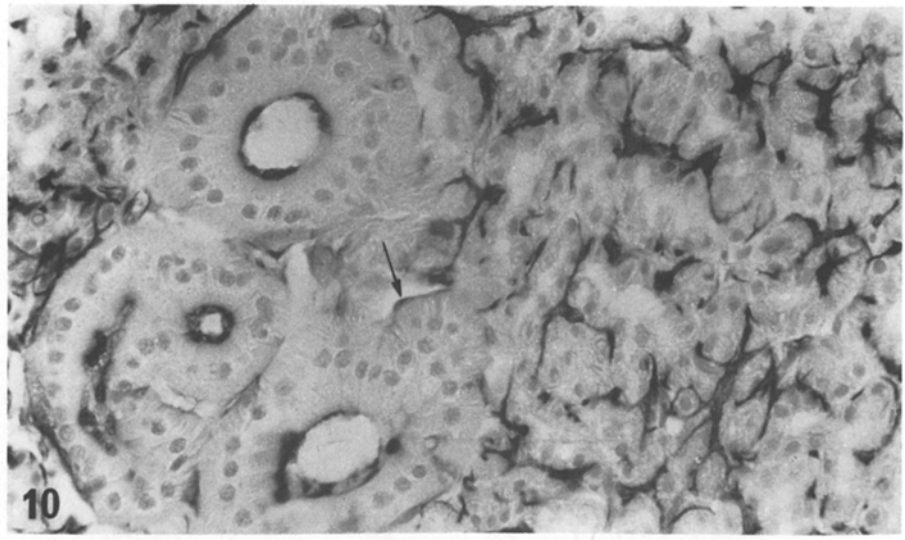
The miscellaneous vesicles referred to earlier comprise organelles obviously belonging to the class of secondary lysosomes (Fig. 7), as well as large electron lucent vesicles with some small denser inclusions (Fig. 6) which may or may not belong to the same class, and smaller smooth membrane vesicles associated with the Golgi apparatus and the GERL, or with the basolateral folds. The latter vesicles often contain material of similar density as that found in the intercellular spaces (Fig. 8).

Intercalated Ducts

The epithelial cells of the intercalated ducts have relatively smooth plasma membranes with little folding (Fig. 2). Mitochondria are less numerous than in either secretory tubule cells or striated duct cells. The apical cytoplasm contains a few organelles such as a centriole with striated rootlets, some vesicles and occasionally a dense granule, but the most conspicuous feature of the cytoplasm of intercalated duct cells is the large number of bundles of cytoplasmic filaments of 6 nm (Fig. 3) and 9–10 nm diameter.

Striated Ducts

The epithelium of these ducts has the appearance typical for striated ducts, with deep basal infoldings and associated mitochondria. The supranuclear region contains a fairly large number of smaller mitochondria. The apical surface is either smooth or has some short microvillous processes. Apical blebbing was observed in some striated ducts.



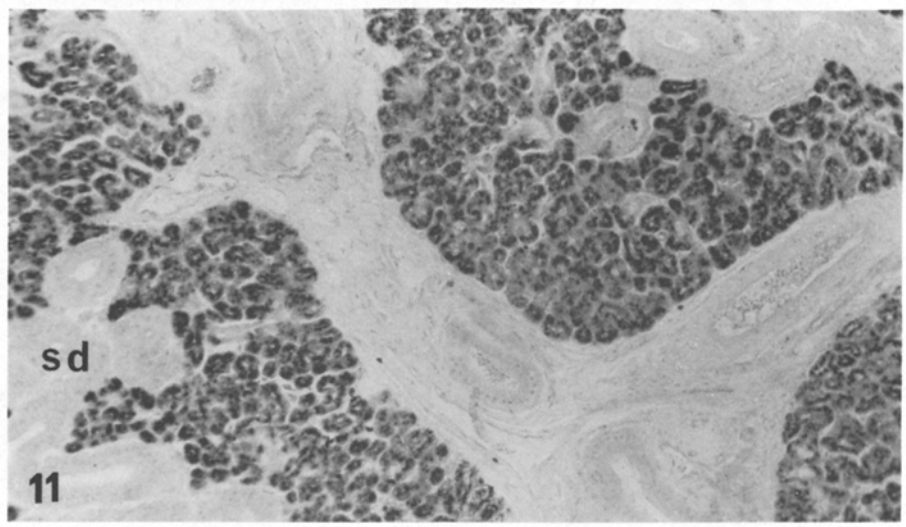


Fig. 10. Non-specific alkaline phosphatase. Numerous myoepithelial cells are associated with the secretory tubules. A few myoepithelial cells lie against striated duct epithelium (arrowed). Note the reaction product on the luminal surface of the striated ducts. ×380

Fig. 11. Non-specific acid phosphatase reaction in the parotid gland of a ewe fed ad lib. *sd* striated ducts. ×100

Cells with conspicuous cytoplasmic granules are fairly commonly found in the basal region of the epithelium. Two types of such granular cells may be distinguished. The most common type is probably an intraepithelial mast cell; the same cell may also be found in the stroma surrounding the striated duct epithelium. The other type is characterized by the possession of very large acidophilic granules which appear homogeneous in the electron microscope. Superficially, in the light microscope, the cell resembles an eosinophile leukocyte, but ultrastructurally it appears more like a "globular leukocyte" (German: Schollenleukocyt).

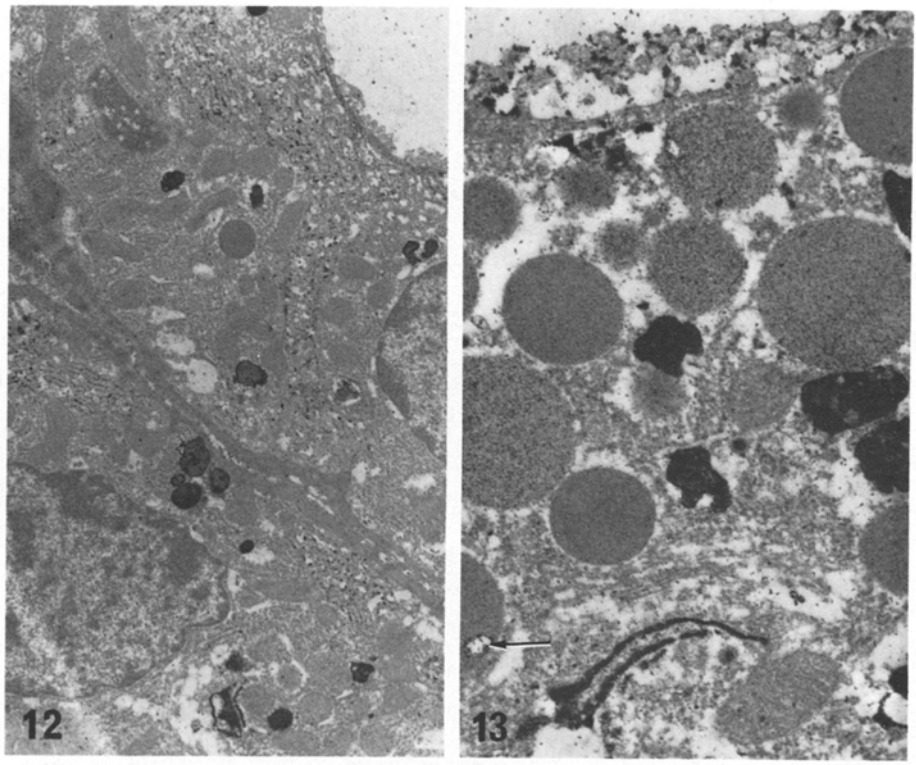


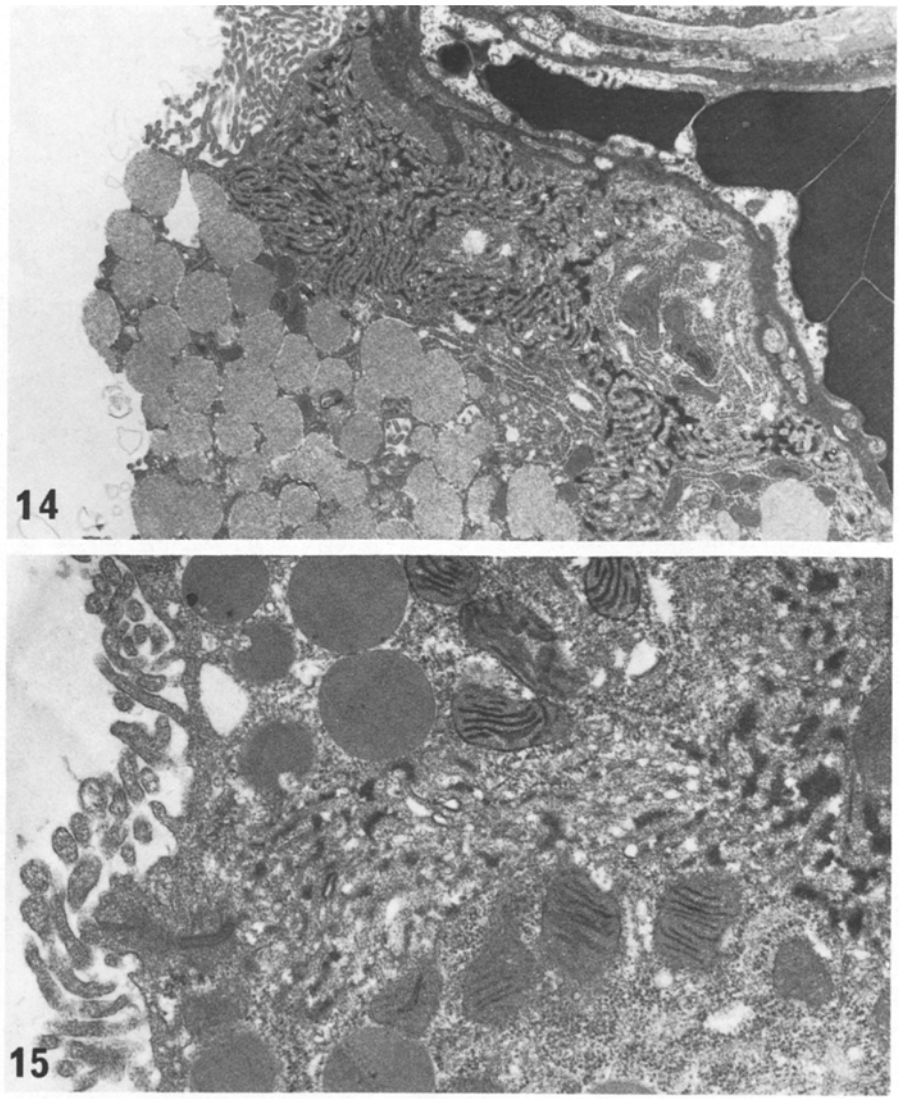
Fig. 12. Non specific acid phosphatase. Reaction product is present in secondary lysosomes and in the lateral intercellular spaces. ×8100

Fig. 13. Acid phosphatase reaction product is found in secondary lysosomes, in the apical brush border and in some cisternae, probably belonging to the GERL system. A fine diffuse precipitate of lead phosphate is present in some of the secretion granules. Reaction product is also seen in a rarefaction (arrow). ×27,000

Myoepithelial Cells and Innervation

Many stellate myoepithelial cells surround the secretory endpieces and intercalated ducts (Fig. 10). Their cell processes occur mostly at the intercellular regions, i.e. beneath the lateral folds of the epithelial cells. Ultrastructurally, the myoepithelial cells exhibit the common features of such cells: abundance of filaments, caveolar invaginations of the plasma membrane, and hemidesmosomes or dense plaques at the side facing the basal lamina. the same features were also observed in a few myoepithelial cell processes at the base of the striated duct epithelium, although the number of cytoplasmic filaments in such cases were smaller.

Bundles of non-myelinated nerve fibres are common in the interstitium of the lobule. Hypolemmal (intraepithelial) nerve terminals have never been observed in the secretory endpieces, although they have been found in intercalated duct epithelium.



Figs. 14 and 15. Secretory tubule epithelium of a sheep parotid gland infused with horseradish peroxidase simultaneously with acetylcholine. The peroxidase has penetrated into the lateral intercellular spaces but not into the junctional complex. The mitochondrial cristae show reaction for cytochrome oxidase. Figure 14: ×8800, Figure 15: ×25,000

Enzyme Histochemistry

Alkaline Phosphatase. As had already been pointed out by Silver (1954) and Blair-West et al. (1969), the myoepithelial cells of the sheep parotid show up well with the Gomori method for the demonstration of alkaline phosphatase. Capillary endothelium shows only a weak raction or no reaction at all. When the latter is the case, it is possible to establish unequivocally the presence of myoepithelial cell processes along many of the striated ducts (Fig. 10). It

is likely that most of such processes are extensions of cells otherwise mainly associated with secretory endpieces. A curious feature is the frequent presence of alkaline phosphatase activity on the luminal side of the striated duct epithelium.

Acid Phosphatase. Light microscopically, some glands showed an abundance of acid phosphatase positive granules (Fig. 11). The reaction was completely inhibited by 10 mM fluoride. Ultrastructurally the enzyme appeared to be present in various spheroidal bodies that may be described as primary and secondary lysosomes as well as in some cisternae near the Golgi apparatus and presumably belonging to the GERL system (Figs. 12, 13). Some diffusely scattered reaction product is found in many of the type II granules, whereas type I granules are generally devoid of acid phosphatase activity except sometimes in the peripheral rarefaction, which may show a considerable amount of reaction product. Lead phosphate is furthermore found in the lateral intercellular spaces and on the microvilli on the apical surface of the cells.

Peroxidase. In the gland perfused with horseradish peroxidase the enzyme was found to occupy the lateral intercellular spaces (Figs. 14, 15) but it did not appear to penetrate the tight junctions. A few secretory cells showed presence of endogenous peroxidase in the cisternae of the RER. A positive reaction was also found in many mitochondria; this reaction must be ascribed to cytochrome oxidase.

Discussion

Although the parotid gland is a compound acinar gland in the great majority of mammals (reviewed by Young and van Lennep, 1977b), compound tubular glands have been described for a number of artiodactyla (van Lennep, 1957; Shackleford and Klapper, 1962; Shackleford and Wilborn, 1968; Kayanja and Scholz, 1974) and possibly in seals and sea lions (Kubota and Horiuchi, 1963; Fava-de-Moraes et al., 1966; Messelt and Blix, 1973). Tubular secretory endpieces are also found in the parotid of the monotreme *Ornithorhynchus anatinus* (Young and van Lennep, 1977b). Unfortunately, some authors persist in using the term "acinus" when referring to any secretory endpiece irrespective of its shape, thereby ignoring the possible functional implications of the differences in geometry of the secretory endpieces.

The parotid glands of the sheep and presumably all ruminants, including the camel, secrete large amounts of fluid and very little protein. Since water transport is the result of electrolyte transport such parotid glands must be considered primarily as electrolyte secreting glands. It is probably no coincidence that vertebrate salt glands, which must be considered the most specialized electrolyte secreting glands, all have tubular secretory endpieces, irrespective of the anatomical origin of the gland (Young and van Lennep, 1977a). It should be remembered that for the secretion of proteins which can be compacted and stored in serous secretion granules, a large cytoplasmic volume is required to contain the rough endoplasmic reticulum needed for the synthises of these proteins, whereas the requirements for electrolyte and water transport are extensive intercellular channels and, in the case of isotonic secretion, a large apical

surface area for water equilibration. The different requirements for volume to surface area ratios in these two cases may account for the spheroidal shape of the secretory endpiece in glands whose prime function is the secretion of proteins (other than acid glycoproteins) and the tubular shape where water and electrolyte secretion is mainly required.

The morphology of the secretory epithelium of the sheep parotid reflects in many aspects the classical view of electrolyte transporting epithelia, such as the presence of strongly folded baso-lateral plasma membranes and/or apical plasma membranes. The sheep parotid epithelium in fact bears a striking resemblance to other epithelia with a similar function, e.g. the epithelium of the salivary glands in the blowfly *Calliphora* (Oschman and Berridge, 1971). Where the primary fluid is isosmotic with plasma there must be ample opportunity for water to flow through the epithelium for osmotic equilibration. In acinar secretory endpieces and in serous demilunes this is achieved by long intercellular secretion canaliculi. In the sheep parotid the secretion canaliculi are wide and their limiting plasma membranes are expanded by the presence of many microvilli in a similar way as the intracellular secretion canaliculi of *Calliphora* and the intercellular spaces are in close proximity to the lumen over a considerable area; both factors would allow complete equilibration, assuming a reasonable permeability of the plasma membranes for water.

Direct filtration as a means of fluid secretion can be excluded since first, the sheep parotid is known to continue to secrete up till 15 minutes after death, second, the tight junctions apparently do not permit passage of extracellular tracers such as horseradish peroxidase, that have no difficulty in passing through the glomerular filter of the kidney and third, because the primary saliva, although isosmotic with plasma, has sodium and potassium concentrations that are significantly different from those of plasma (Compton and Young, 1976).

The presence of large numbers of secretion granules in some sheep parotid glands may at first glance seem rather surprising since this gland is known to secrete only small amounts of protein. However, the amount of secretory material in the cell is very variable and the relatively poor development of the rough endoplasmic reticulum in many cells may point to a low turnover rate of protein as has been observed for instance in the granular ducts of rat mandibular glands (Matthews, 1974).

We have described two types of granules, differing in size and electron density. The presence of two types of granules was earlier reported by Patterson et al. (1976) who ascribed the differences in relative numbers of the two types to differences in age of the animals. We were unable to detect any correlation with age. The absence of any signs of exocytosis of one type of granule (type I) might be taken as indicative of a low rate of protein secretion, although admittedly, we did not examine sheep parotid glands after β-adrenergic stimulation, the most usual way of causing protein secretion in parotid glands.

Our observation of large numbers of secondary lysosomes and what are undoubtedly secretion granules undergoing lysis by lysosomal enzymes on the other hand suggests that a large proportion of the secretion granules are not normally discharged – at least in the absence of β-adrenergic stimulation – but are reabsorbed by the secretory cell. Such a lysosomal degradation of secretion

granules has been described earlier in the parotid gland of the rat after prolonged fasting (Hand, 1972) but we observed this phenomenon even in a ewe killed without prior fasting (see Fig. 11). This process of secretion granule degradation is not entirely clear. Type I granules did not show activity of acid phosphatase, except in the rarefaction where the latter was present. Many of the type II granules on the other hand show a weak diffuse activity of the enzyme. This fact suggests that the type II granule may in many cases represent an early stage in the degradation of a type I granule, resulting ultimately in a secondary lysosome. On the other hand, since exocytosis of type I granules was never observed, whereas discharge of type II granules was suggested in a number of our sections, it might be argued that where discharge of protein occurs this could be preceded by an uptake of water by the type I granules leading to the appearance of the more swollen and electron lucent granules, possibly followed by fusion of the latter. We have observed such a swelling and fusion of serous granules prior to their discharge in the parotid gland of the pouch young of the Australian possum *Trichosurus vulpecula* and in the deep mandibular gland of the echidna *Tachyglossus aculeatus* (unpublished observations). Swelling of the granules of the sheep pancreas seems to occur after stimulation with secretin but not after stimulation with pancreozymin (J. Blomfield, personal communication) and it is probably quite common during chain exocytosis (Young and van Lennep, 1977a).

The ultimate fate of the many secondary lysosomes is uncertain, but the presence in the tubular lumen of material resembling the dense material in these lysosomes suggests a possible luminal extrusion of some of their indigestible contents. It should be noted that Chauncey and Quintarelli (1961) failed to demonstrate any acid phosphatase activity in the sheep parotid. This may have been due to inappropriate fixation procedures, but some of our own glandular material also showed only a weak reaction. Such discrepancies may be due to a variability between animals or even between different parts of the same gland.

The presence of electron dense material in the intercellular spaces is a quite unusual feature. This material does not appear to stain with the PAS method and therefore cannot be compared with the glycoprotein coating often seen on the lateral membranes of other electrolyte transporting epithelia (Berridge and Oschman, 1972). The presence of vesicles, containing a substance of similar electron density, in the vicinity of the lateral folds (Fig. 8) suggests either a secretion of this material by the cell simultaneously with an addition to lateral plasma membrane, or an uptake of the dense material into the cell. We have at present no means of deciding which of the two alternatives is correct, but the finding of acid phosphatase activity in the lateral spaces favours the first alternative.

The Duct System

The intercalated ducts are peculiar in that they can become highly dilated. Although the maximal flow rate calculated per gram of gland may not be much higher in the sheep parotid than in many other salivary glands, it is conceivable that the relatively short intercalated ducts occasionally have to

handle large volumes of fluid coming from the long, branched and dilated tubular endpieces. In this regard it may be noted that Blair-West et al. (1969) estimated the ratio of endpiece fluid space to ductal fluid space in the sheep parotid to be approximately 66; 1. Although this figure was arrived at by retrograde injection and morphometric studies – methods which have severe limitations with regard to measurements of luminal spaces – we agree with these workers that the secretory endpiece luminal space greatly exceeds the spaces of striated and excretory duct lumina. The intercalated ducts are provided with some myoepithelial cells, but the bundles of tonofilaments and 6 nm actin filaments may well form the means of passive and active support preventing excessive distension and allowing the duct epithelium to resume its normal dimension.

The morphology of the striated ducts shows little differences from that of other mammals except for the tendency of the ducts to be bundled in the center of the lobules. The presence of myoepithelial cells in the striated duct epithelium is perhaps not as exceptional as a survey of the literature seems to suggest since it apparently does occur occasionally in other mammals (J.R. Garrett, personal communication). Because of their rarity, these myoepithelial cells are unlikely to be of much functional significance.

The function of the striated ducts in the sheep parotid presents some peculiar problems. The duct system in most mammals is thought to absorb sodium from the luminal fluid and secrete potassium into this fluid as is shown by the dependence of sodium and potassium concentration in the final saliva on the flow rate (Thaysen et al., 1954; Thaysen, 1960; Schneyer et al., 1972; Young, 1973). In the parotid of a sheep of normal sodium status however sodium and potassium concentrations appear to be largely independent of the flow rate (Coats and Wright, 1957; Compton, 1976). In animals depleted of sodium by cannulation of the parotid main duct, concentrations of sodium and potassium in the final saliva are however strongly dependent on the flow rate (Compton, 1976). This suggests a marked change in the electrolyte transporting function of the ducts in sodium depleted animals which might be reflected in a morphological change of the duct epithelium. Rabbits, kept for 100 days on a sodium deficient diet certainly show a hypertrophy of the striated ducts (Compton et al., 1975) but we have so far not found any marked differences between striated ducts in parotid glands of sodium replete and sodium deficient sheep.

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