J. Anat. (1984), **138**, 2, pp. 287–296
With 12 *figures*
Printed in Great Britain

An ultrastructural study of the sinus epithelium in the mammary gland of the lactating ewe

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(*Accepted* 6 *July* 1983)

INTRODUCTION

In the mammary gland of ruminants, the integrity of the epithelium of the sinuses and large ducts maintains a so-called 'blood–milk barrier' by which lactose and other milk components are excluded from the blood, even when milk is stored in the mammary gland for long periods between sucklings or milkings (Linzell & Peaker, 1971). Moreover, the anatomy of the gland is such that, when pathogenic bacteria penetrate the teat canal, the sinus epithelium is the first part of the organ exposed to the risk of toxin-mediated tissue damage and the accompanying changes in epithelial permeability that this implies. It follows that for studies in which the objective is to examine the earliest cellular changes in the mammary gland following infection with pathogens, a knowledge of the normal microscopical structure of the sinuses is necessary. The present investigation of the teat and lactiferous sinus epithelium of the ewe is preliminary to a study of the pathological changes in the sinuses and large ducts associated with experimental ovine mastitis. Although Turner (1952) has given some information on the structure of the sinus epithelia in the ewe, it is not detailed and recent microscopical studies of the ovine mammary gland have been confined to the alveolar secretory tissue (Lee & Lascelles, 1969; Kovachev, 1979*a*, *b*).

MATERIALS AND METHODS

Animals

A total of 23 primiparous ewes in mid-lactation were examined of which 20 were Merino ewes raised at C.S.I.R.O. Armidale, New South Wales and 3 were Southdown ewes from N.I.R.D., Shinfield. Milk from each of the glands used was free from pathogenic bacteria as judged by a bacteriological examination at the beginning of the study. After slaughter, the udder was removed and each gland was gently infused through the teat canal with 3 % glutaraldehyde in 0·1 M cacodylate-HCl buffer (pH 7·2) by means of a syringe fitted with a cannula. After 1 hour, each half of the udder was dissected and epithelium from the teat and lactiferous sinuses was removed for microscopic examination.

Light microscopy

Tissue taken from glands which had been treated with glutaraldehyde was fixed further by treatment with 10 % neutral buffered formalin for 24 hours. It was then embedded in paraffin wax, sectioned at a thickness of 5 μm and stained with haematoxylin and eosin.

Transmission electron microscopy

Samples of fixed tissue were removed from each gland and washed for 2 hours in 0·2 M cacodylate-HCl buffer (pH 7·2). Thin strips (1 mm wide and 1 mm deep) of sinus epithelium were separated from the underlying connective tissue and fixed for 1 hour in 1 % osmium tetroxide in 0·1 M cacodylate buffer at pH 7·2. They were then washed in distilled water, stained *en bloc* for ½ hour in 1 % aqueous uranyl acetate and dehydrated in a graded series of acetone–water mixtures and in 100 % acetone. After embedding in Araldite, thin sections were cut on a Reichert OmU3 ultramicrotome and stained with lead citrate before examination in a Hitachi 600 electron microscope. The proportion of secretory cells in the lactiferous sinus epithelium of each gland was estimated by examining 100 epithelial cells in each of 10 areas selected at random from four blocks of tissue.

Scanning electron microscopy

Pieces of epithelium approximately 10 mm × 10 mm were carefully removed from larger specimens of excised sinus. They were washed for 2–3 hours in 0·2 M cacodylate-HCl buffer (pH 7·2) and then post-fixed in 1 % osmium tetroxide in 0·1 M cacodylate buffer for 1 hour. After washing in distilled water for ½ hour to remove excess osmium tetroxide, samples were dehydrated in acetone as above and dried in a Polaron E3000 critical point drying apparatus using liquid carbon dioxide. They were then attached to aluminium stubs with Araldite epoxy resin, coated with gold in an Edwards 150 sputter coater and examined either in an ISI IIIA or Hitachi 520 scanning electron microscope.

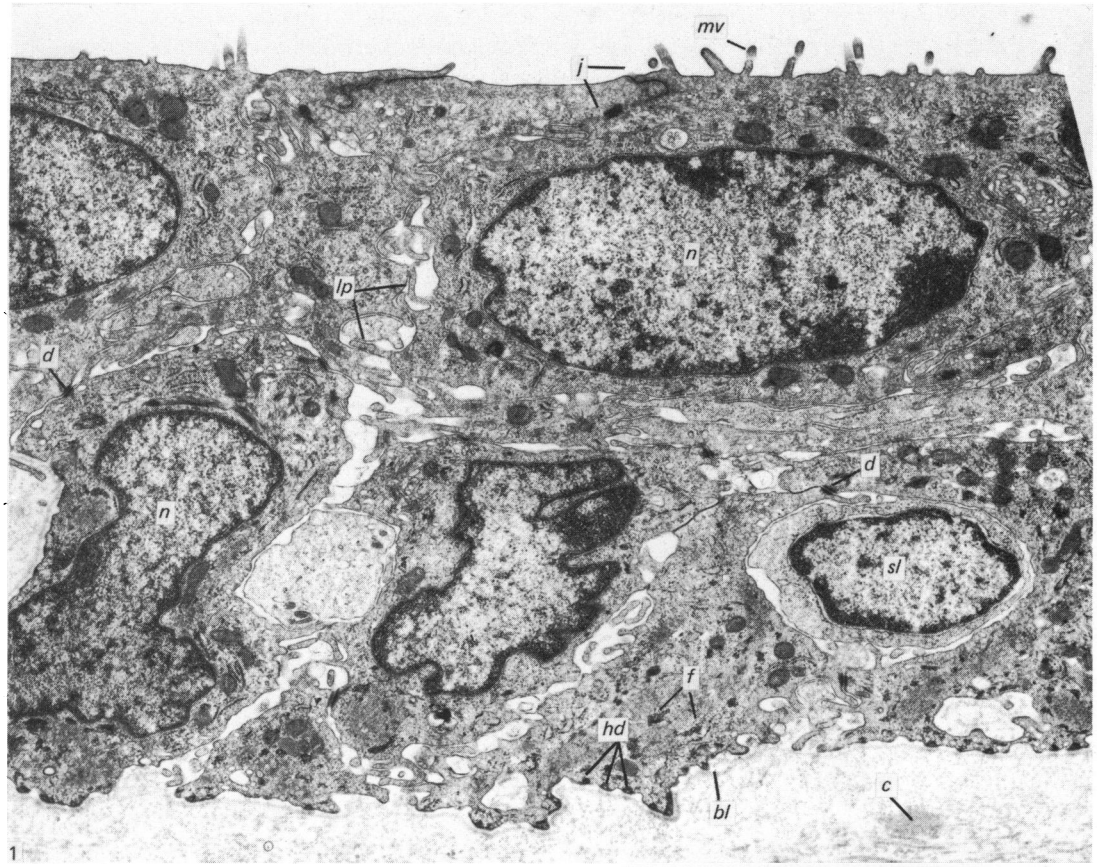
RESULTS

In the teat and lactiferous sinuses of the ovine mammary gland most of the epithelial lining was composed of a double layer of non-secretory cells (Fig. 1). The basal layer consisted of flattened cells with dendritic basal processes whose cytoplasm contained numerous microfilaments and coarser, electron-dense filaments which were usually arranged into large bundles (Figs. 1, 2). Collectively, these filaments represented the major component of the cytoplasm and they were aligned in one direction parallel to the basal lamina (Fig. 2). The entire basal surface of these cells was in intimate contact with the basal lamina and was marked at frequent intervals by hemidesmosomes (Fig. 1). Basal cells were attached to their neighbours and to the cells of the overlying superficial layer by desmosomes (Fig. 1). The nucleus was very irregular in shape (Figs. 1, 2).

Fig. 1. Section through the lactiferous sinus epithelium showing the two cell layers. Cells of the superficial layer are joined by a full junctional complex (*j*), bear microvilli (*mv*) and have lateral cell processes (*lp*). Desmosomes (*d*) are visible where cells of both layers make contact and hemidesmosomes (*hd*) mark the contact of basal cells with the basal lamina (*bl*). Cytoplasmic filaments (*f*) in the basal cells are arranged at right angles to the plane of this micrograph. A small lymphocyte (*sl*) lies between two basal cells. *n*, nucleus; *c*, collagen. Transmission electron micrograph. × 11 500.

Fig. 2. A basal cell sectioned parallel to the plane of the epithelium showing the irregularly shaped nucleus (*n*) and the unidirectional orientation of both thick and thin cytoplasmic filaments. *bl*, basal lamina; *c*, collagen; *f*, cytoplasmic filaments. Transmission electron micrograph. × 9000.

Fig. 3. A mitotic figure in the superficial layer of the lactiferous sinus epithelium. Transmission electron micrograph. × 5000.



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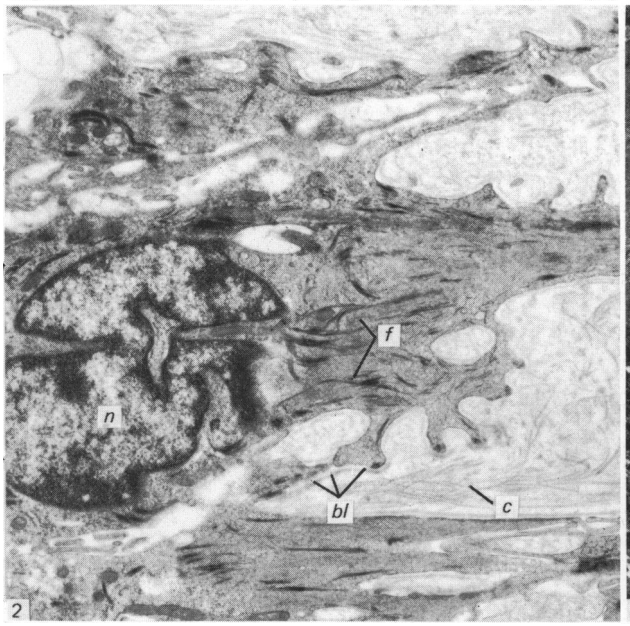

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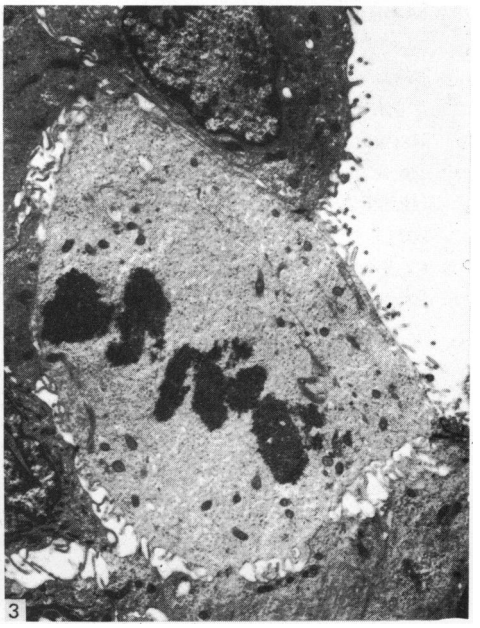

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In the superficial layer, the lateral membranes of neighbouring cells were joined by the interdigitation of cell processes and, on their luminal border, by a full junctional complex (Fig. 1) of the type described by Farquhar & Palade (1963). The cells were cuboidal or columnar in shape, depending on the state of distension of the sinus, and sometimes extended one or more narrow processes between the cells of the basal layer to make contact with the basal lamina. The nucleus was irregular in shape with one or more prominent indentations; mitotic figures and other signs of cell division were not common, but did occur (Fig. 3). Macrophages together with large and small lymphocytes were commonly found sandwiched between cells in both layers of the epithelium (Fig. 1). They could be recognised not only from their specific fine structural features but also because, unlike the epithelial cells, they did not produce desmosomes or any other sign of permanent contact where their cell membrane was closely apposed to that of another cell.

When examined by scanning electron microscopy, the areas of non-secretory epithelium superficially resembled a flat patchwork quilt, each cell possessing numerous microvilli (Fig. 4). Prominent pseudopodia similar to those described from sinus cells of the cow (Brooker, 1983) were observed, but were uncommon. A marked feature of some non-secretory cells in the lactiferous sinus and large ducts was a single cilium up to 4 μm long (Fig. 4). Transverse sections of cilia showed that they contained an axoneme whose tubules were arranged in a 9+0 pattern, although towards the tip some of the outer tubules became translocated to the centre of the axoneme (Fig. 5). The cilia appeared to be flexible at their base for, within the same microscope field, some projected at right angles to the epithelial surface whilst others lay parallel to the cell apex or were in contact with microvilli (Fig. 6). Several inconspicuous striated rootlets ran from the basal body of each cilium into the cytoplasm (Fig. 7). Although many ciliated cells were examined by transmission electron microscopy to determine whether they were the modified ends of sensory axons or whether they were innervated by axons, no anatomical evidence was found to suggest that their function was sensory. Ciliated cells were also found in the teat sinus but in small numbers.

In addition to non-secretory cells, the epithelial lining of the lactiferous sinus and large ducts contained large numbers of cells whose ultrastructural features were identical to those of nearby secretory cells which formed the alveoli of the mammary gland proper. Because they were larger than the adjacent non-secretory cells, they projected above the general level of the sinus epithelium and were therefore easy to identify, especially when examined by scanning electron microscopy (Fig. 8). These sinus secretory cells were either cuboidal, like alveolar cells, or, where packed tightly together, they were columnar (Fig. 9). In some animals the secretory cells extended for a considerable distance into the large ducts. Each cell contained abundant

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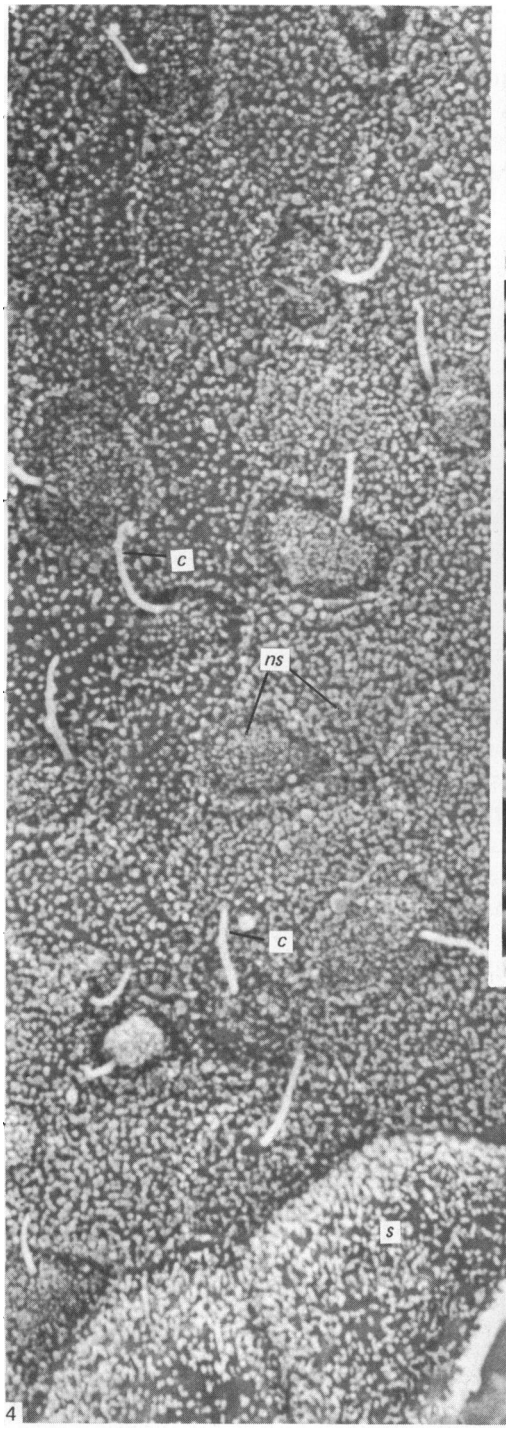
Fig. 4. Surface of the lactiferous sinus epithelium. Most of the cells in this field are non-secretory (*ns*). Cells bear numerous microvilli and sometimes a cilium (*c*). Note the difference in size between secretory (*s*) and non-secretory cells. Scanning electron micrograph. × 4000.

Fig. 5. Transverse section near the tip of a cilium of an epithelial cell. A pair of peripheral tubules occupies the centre of the axoneme at this level. Transmission electron micrograph. × 140000.

Fig. 6. Scanning electron micrograph of a recurrent cilium touching the microvilli of an adjacent epithelial cell (arrow). × 22000.

Fig. 7. Longitudinal section of the cilium of an epithelial cell showing the basal body (*bb*), the axoneme (*ax*) and one striated rootlet (arrow). Transmission electron micrograph. × 34000.



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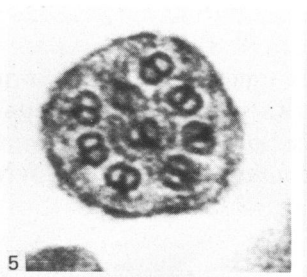

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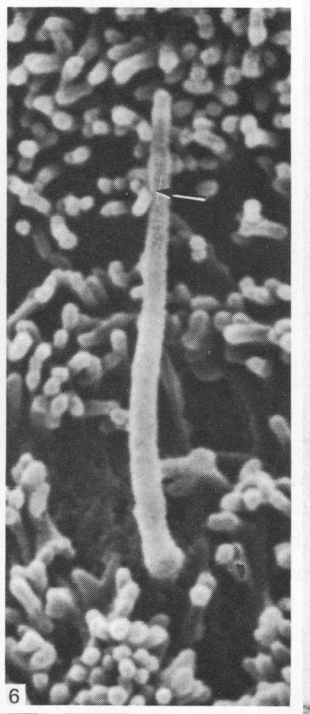

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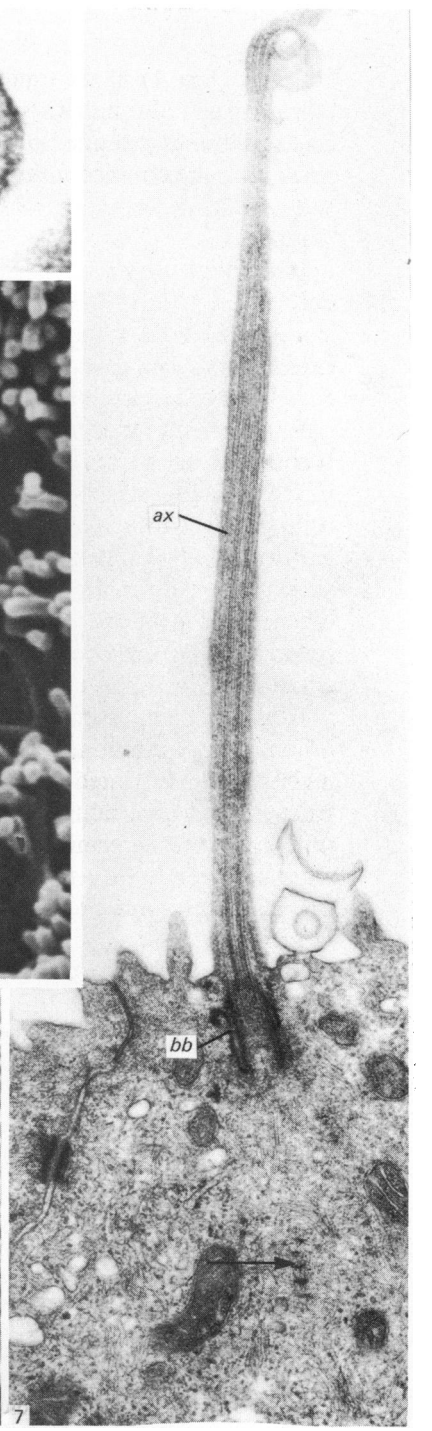

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granular endoplasmic reticulum and mitochondria, lipid droplets, stacks of Golgi saccules and Golgi vesicles containing casein micelles or casein subunits in various stages of aggregation (Fig. 10). The nucleus was quite unlike that of the non-secretory cells for it had no indentations, usually had a round profile and contained an eccentrically placed nucleolus. The secretory cells occurred in numerous small groups or as extensive tracts of continuous secretory epithelium. For the most part, they rested directly on the basal lamina, thus forming an epithelium only one cell deep. However, long strap-like cells did occur which closely resembled the myo-epithelial cells found in secretory alveoli, both in appearance and position. It is worth noting that, as in the secretory alveoli (Kovachev, 1979*a*), when a secretory cell was in direct contact with the basal lamina, its basal surface was thrown into deep folds but that where a myoepithelial cell was interposed, the cell membrane was smooth. Secretory cells were never observed to possess a cilium.

In this study, the proportion of secretory cells in the lactiferous sinus epithelium was found to vary from $< 5\%$ to $> 50\%$ (mean $\pm$ standard deviation: $16{\cdot}5 \pm 15{\cdot}9$; $n = 23$) of the total. In some cases, secretory cells were found only with difficulty but in others, most of the lactiferous sinus was lined with these cells. When prepared for scanning electron microscopy, some secretory cells possessed a conspicuous artefact in the form of an apical concavity. This marked the site of a cytoplasmic fat droplet which had been extracted during tissue processing, leading to local collapse of the cell membrane. No equivalent feature was found in cells examined by transmission electron microscopy.

In the teat sinus, secretory cells were rarely incorporated into the lining epithelium but, in some animals, many secretory alveoli producing casein micelles and milk fat globules opened directly into the sinus (Fig. 11). When these openings were numerous, the teat sinus epithelium was thrown into complex folds (Fig. 12). In the wall of the lactiferous sinus, there were secretory alveoli and lobules of secretory cells which drained directly into the lumen of the gland. The ultrastructural criteria by which secretory cells were identified were identical to those given above.

DISCUSSION

In its basic architecture of two cell layers, the sinus epithelium of the ewe is very similar to that of other ruminants (Venzke, 1940; Linzell & Peaker, 1971). However, the presence of such large numbers of ciliated cells is peculiarly ovine. Since the

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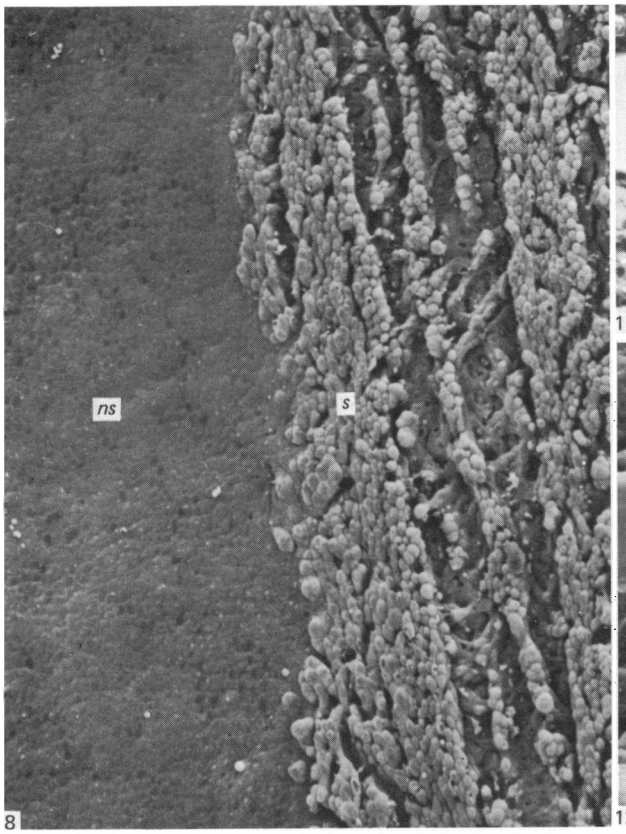
Fig. 8. Surface of the lactiferous sinus showing the different appearance of adjacent areas of secretory (*s*) and non-secretory (*ns*) epithelium. The larger secretory cells are less organised than in the secretory tissue proper. Scanning electron micrograph. × 250.

Fig. 9. Light micrograph of the lactiferous sinus epithelium showing groups of columnar secretory cells. Adjacent alveolar secretory cells are cuboidal (arrows). Haematoxylin and eosin. × 190.

Fig. 10. Lactiferous sinus epithelium showing the ultrastructural differences between adjacent areas of secretory and non-secretory (*ns*) cells. Secretory cells contain fat droplets (*f*), abundant granular endoplasmic reticulum (*rer*) and Golgi vesicles (*gv*) containing casein micelles. *cp*, capillary; *n*, nucleus. Transmission electron micrograph. × 5000.

Fig. 11. Light micrograph of the teat sinus epithelium showing the opening of sinus secretory alveoli (*ss*) to the lumen. Haematoxylin and eosin. × 190.

Fig. 12. Surface view of the teat sinus epithelium showing large folds in the wall (arrows); the irregular topography in the areas between the major folds is caused by the opening of many sinus secretory alveoli to the surface. Scanning electron micrograph. × 170.



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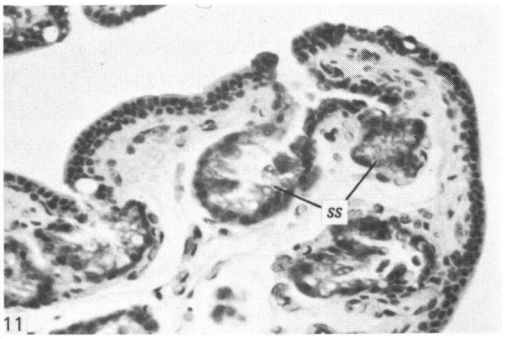

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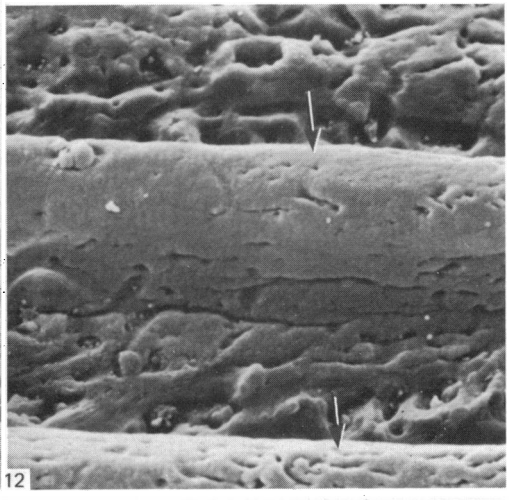
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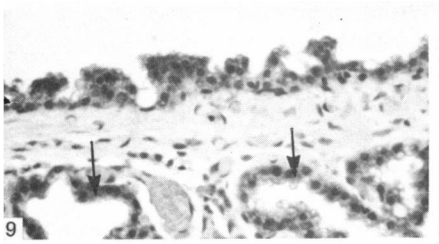
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axoneme of each cilium lacks the two central tubules which, with the exception of some spermatozoa (Phillips, 1974), are usually necessary for motility, it may be inferred that they are not actively motile. Moreover, although the 9+0 arrangement of tubules is often associated with cilia that are sensory in nature, it is unlikely that the ciliated cells described in the present study are receptors since they do not appear to be the modified ends of axons nor are they innervated by axons. Their functional significance is therefore unclear.

The occurrence of lymphocytes and other leucocytes in the secretory epithelium of pregnant and lactating mammals is well established (Helminen & Ericsson, 1968; Seelig & Beer, 1978, 1981) but there are few reports of their occurrence in the epithelium of large ducts and of the sinuses. Their true significance is not known but in the case of lymphocytes it has been suggested that they originate from the gut, enter the lumen of the gland (Seelig & Beer, 1978) and have a protective role to play when colostrum or milk is ingested by the immunologically immature suckling infant (Parmely & Beer, 1977).

The presence of alveolar secretory tissue in the wall of the sinuses which opens directly into the lumen of the gland is well established in the ewe (Turner, 1952). In other ruminants too these so-called 'accessory glands' are found in the teat and/or lactiferous sinuses although there is considerable variation in their numbers both between individuals and breeds (Linzell & Peaker, 1971). Venzke (1940) considers that the sinus secretory alveoli in the bovine mammary gland secrete normal milk because they contain fat globules and secretory granules. Pattison (1952) has come to the same conclusion about the glands in the teat sinus of the goats used in his study of experimental streptococcal mastitis. There seems little doubt from the present study that the sinus secretory alveoli of the ewe are identical in appearance to normal secretory tissue and that they produce the particulate components of normal milk.

Although secretory cells do not form part of the epithelial lining of the sinuses and large ducts in the bovine mammary gland, in the goat Linzell & Peaker (1971) have found cells in the intralobular and interlobular ducts which show signs of secretory activity. However, they possess fewer mitochondria and basal folds and less granular endoplasmic reticulum than alveolar secretory cells, and in only very few cases are secretory cells found in the lactiferous sinus. The present study has shown that in many ewes, secretory cells compose a large part of the lactiferous sinus epithelium and that they are morphologically identical to alveolar cells in higher regions of the mammary gland. These areas of secretory epithelium may be regarded as tracts of presumptive alveolar or 'accessory gland' epithelium which, for some reason, have failed to evaginate from the anlage of the sinus during the embryonic stages of mammary development.

The apical concavities observed in the secretory cells prepared for scanning electron microscopy were first described by Nemanic & Pitelka (1971) in mouse mammary gland. Using secretory alveolar tissue, they have shown that the artefact is produced by extraction of fat droplets during the dehydration stage in specimen preparation.

The coexistence of secretory and non-secretory cell populations in the sinus epithelium of the ewe suggests a use for this tissue in a variety of experimental studies of the mammary gland. It should, for example, be an ideal system with which to compare the response of both cell types to a variety of chemical stimuli. In addition to such comparative studies, there is considerable value in a population of secretory cells whose conformation favours experimental manipulation. In organ culture for

example, the use of a flat sheet of epithelium containing secretory cells rather than pieces of alveolar secretory tissue would provide ideal conditions for the uptake of nutrients and for gaseous exchange and would overcome the problems of congestion that may be caused by mammary duct closure (Forsyth & Jones, 1976).

SUMMARY

The teat and lactiferous sinus epithelium from the mammary glands of 23 lactating ewes was examined by light and electron microscopy. Most of the sinus epithelium consisted of two layers of non-secretory cells but, in the lactiferous sinus, cells with the same ultrastructural features as alveolar secretory cells were also found. Secretory cells sometimes occupied more than 50% of the total area of the sinus. Many non-secretory cells in the lactiferous sinus possessed a single cilium but they were less common in the teat sinus. 'Accessory glands', which opened directly into the lumen of the gland, were found beneath the epithelium in both the teat and the lactiferous sinuses. From their ultrastructure it was clear that these glands consisted of normal secretory alveoli and that they produced normal milk components.

It is suggested that the mixed population of secretory and non-secretory cells in the lactiferous sinus provides unique material for the experimental study of many aspects of mammary gland physiology.

The author is greatly indebted to Dr D. Watson and Dr I. Colditz of C.S.I.R.O. Armidale, New South Wales and to Dr A. J. Frost and Miss D. O. Boyle of the University of Queensland for providing tissues from Merino sheep. The assistance of Mrs K. Wells is also gratefully acknowledged.

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