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The endometrial glands of the pregnant sheep: an ultrastructural study

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INTRODUCTION

During pregnancy, the ruminant endometrium secretes an albuminous fluid, known as the uterine milk, into the space between the uterine epithelium and the chorion. This is absorbed and processed by the fetal trophoblast and, being rich in protein, is probably of importance for the nutrition of the fetus. Although it was initially considered to be produced in the cotyledons, it is now generally accepted that the uterine milk is mainly formed from the intercotyledonary endometrium. In these regions, endometrial glands are numerous and, in the mare, Kolster (1902) found evidence of the release of areas of the glandular epithelium into the lumen and their incorporation into the milk. The nature and development of the somewhat similar process which occurs in the endometrial glands of the sheep has now been studied at the ultrastructural level in specimens of intercotyledonary endometrium obtained at about mid-pregnancy and towards the end of gestation.

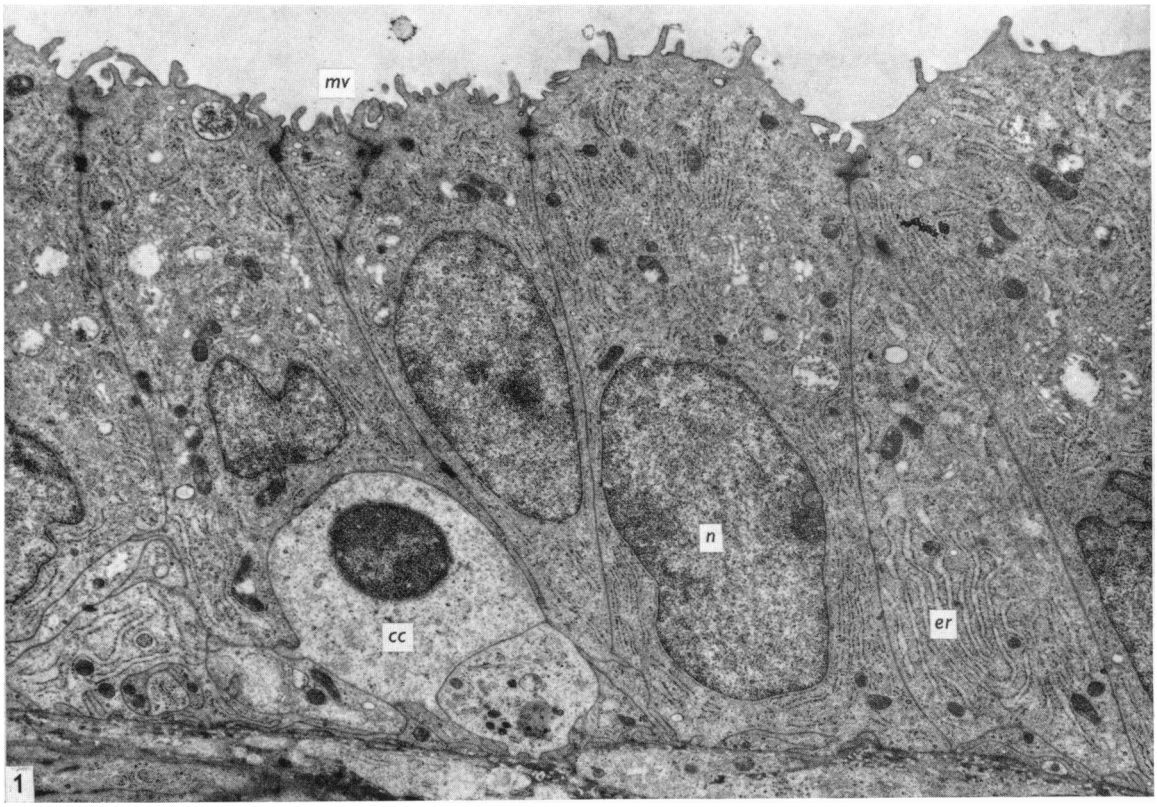
MATERIALS AND METHODS

Specimens of intercotyledonary endometrium were obtained from sheep calculated to be approximately 63 and 67 days pregnant and from seven others with pregnancies of between 116 and 142 days. After fixation for one hour in 2% glutaraldehyde in cacodylate buffer (Gordon, Miller & Bensch, 1963), the specimens were post-fixed in buffered osmium tetroxide (Palade, 1952). They were then dehydrated in ethyl alcohol and embedded in TAAB resin. Thick sections cut on a Porter–Blum ultramicrotome were mounted on glass slides and stained with toluidine blue for light microscopy. Ultrathin sections were mounted on uncoated grids and stained with lead salts (Karnovsky, 1961; Reynolds, 1963) before being examined and photographed in a Siemens Elmiskop 1 or Philips EM 300 electron microscope.

RESULTS

Light microscopy

In all the specimens, the glands communicated with the uterine cavity through a short duct lined by a columnar epithelium similar to that present on the surface of the endometrium. Branching normally occurred near the base of the duct and the rest of the glands were generally simple in form. In material obtained at about mid-pregnancy



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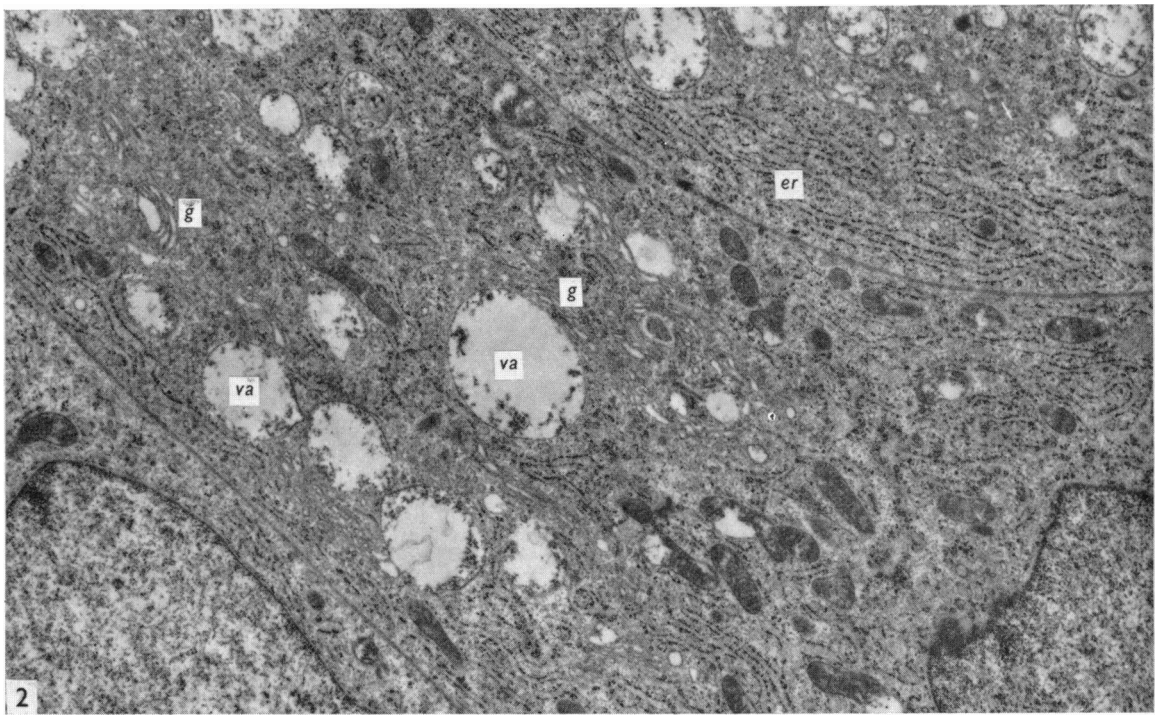

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the basal parts of the glands were often coiled around a central core of vascular fibrous tissue and, in these areas, the epithelium was usually columnar in form and there was little cellular debris in the lumen. Near the surface the glandular profiles were often irregularly shaped and the lumen was filled with degenerating cells; the epithelium also sometimes appeared to be stratified, and there was some increase in the degree of vacuolation of the epithelial cell cytoplasm.

In the older specimens the coiling of the basal parts of the glands was less pronounced, and foci of epithelial stratification were often present. Masses of degenerating cells could often be seen in the lumen and, both in these regions and near the surface, there was marked vacuolation of the epithelial cell cytoplasm.

At all stages of development, small cells with deeply staining nuclei and pale cytoplasm were apparent in the epithelium, and similar cells were often seen in the epithelium lining the ducts.

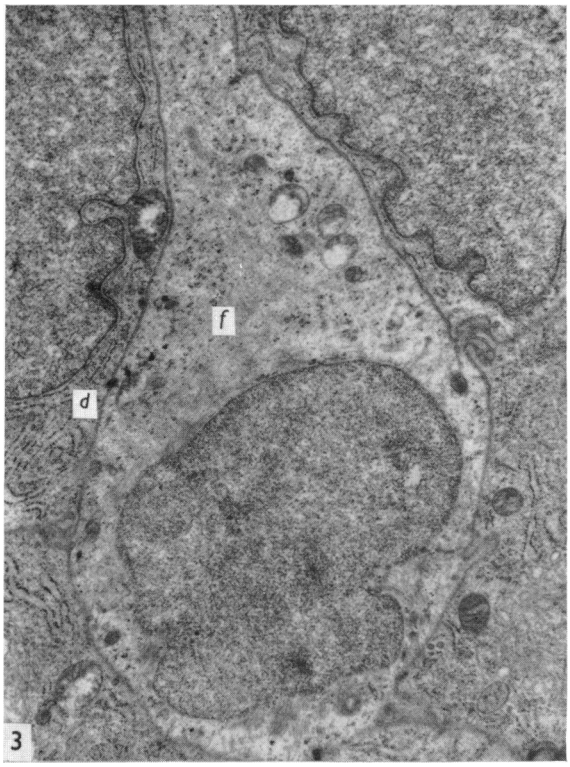
Electron microscopy

The luminal surface of the columnar epithelium lining the basal parts of the glands in the younger specimens was covered by irregular microvilli of variable length (Fig. 1). The lateral cell membranes were smooth and closely apposed and were united both by occasional desmosomes and, near the surface, by junctional complexes, which were composed of a superficial zonula occludens, a zonula adhaerens, and associated maculae adhaerentes. At the base of the cells interdigitating cytoplasmic processes were present, and the epithelium was separated from the underlying connective tissue and the network of small vessels which surrounded the glands by a thin and normally regular basement membrane. The cell nuclei were usually basal in position and only slightly indented (Fig. 1), and were characterized by an evenly distributed chromatin and a highly differentiated nucleolus. The cytoplasm contained numerous polyribosomes and large amounts of granular endoplasmic reticulum, the sacs of which were often arranged in parallel array (Fig. 1) and were almost entirely invested by a single layer of ribosomes. The electron-dense mitochondria were disposed either among the ribosomes or between the sacs of endoplasmic reticulum, and were only occasionally present in the region of the Golgi apparatus. This was typically supranuclear in position and, in section, consisted of one or more groups of cisternae. The small vesicles associated with the apparatus were numerous and were both coated and uncoated in type; similar structures were often related to the surface membrane and, in the case of the coated vesicles, also to the lateral and basal parts of the membrane. Large vacuoles containing a reticulum of material, or peripheral deposits of a more electron-dense but otherwise similar substance, were also present in the supranuclear cytoplasm, and were often related both to the Golgi apparatus and to the surface membrane.

In the areas of epithelial stratification which were present in the upper parts of the glands occasional superficial cells were globular in form and remained connected to the underlying cells only over a small area of their surface. Although most of the

Fig. 1. Columnar epithelium at the base of a gland in a 67-day specimen. *mv*, surface microvilli; *n*, nucleus; *er*, granular endoplasmic reticulum; *cc*, clear cell. × 8000.

Fig. 2. Cells in an area of epithelial stratification near the duct of a gland in a 67-day specimen. *er*, granular endoplasmic reticulum; *va*, cytoplasmic vacuoles; *g*, Golgi apparatus. × 16500.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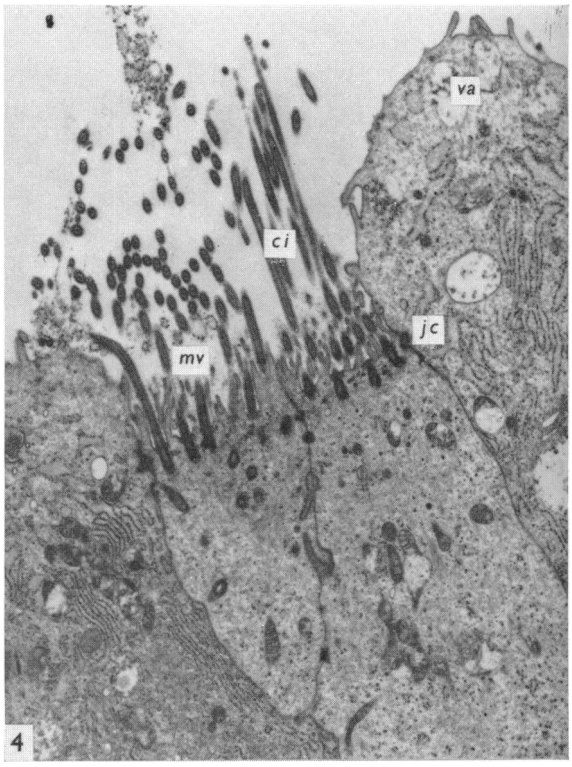
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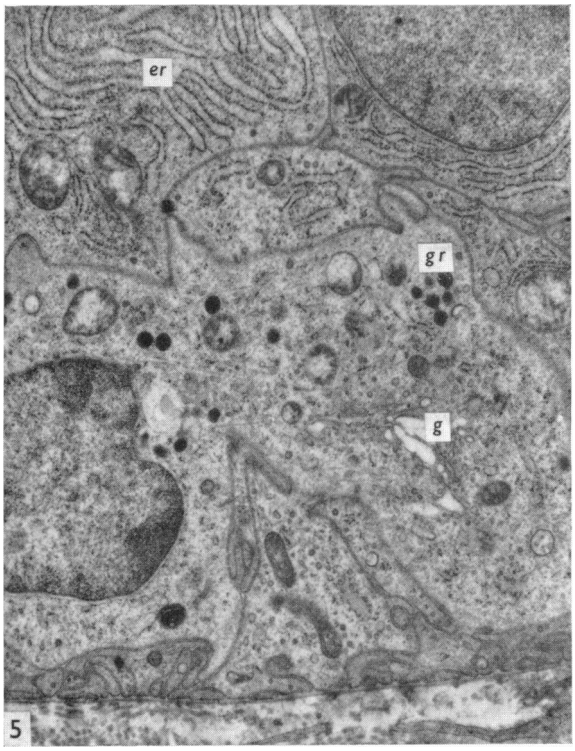
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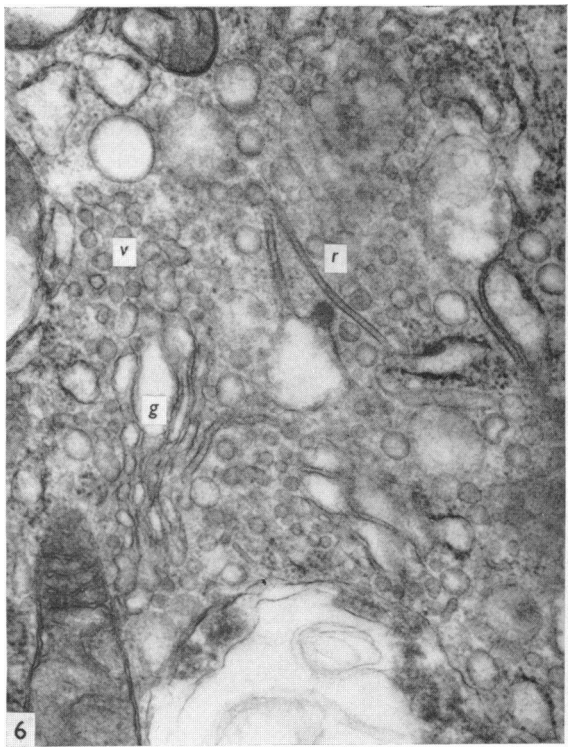
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epithelial cells also contained large amounts of endoplasmic reticulum (Figs. 2, 5), polyribosomes were less numerous than in the basal parts of the glands and the close association between the mitochondria and the endoplasmic reticulum was less apparent. The mitochondria were often poorly preserved, and some appeared to be in the process of transformation into structures resembling the supranuclear vacuoles. The vacuoles varied considerably in size, but were still frequently seen close to the highly developed Golgi apparatus (Fig. 2) and to the surface membrane (Fig. 4).

In addition to occasional cells with a shrunken nucleus and an electron dense cytoplasm, ciliated cells could often be seen in the upper parts of the glands at this stage of development. These cells were irregularly shaped and sometimes occurred in groups of two or more. Although their epithelial origin was indicated by their attachment to the other cells by desmosomes (Fig. 3) and junctional complexes (Fig. 4), they contained little endoplasmic reticulum and only a small Golgi apparatus. Fibrillar material was abundant in the cytoplasm (Fig. 3) and tended to be concentrated around the basal bodies of the elongated cilia, which were associated at the surface of the cells with numerous finger-like microvilli (Fig. 4).

In the older specimens the fine structure of the epithelial cells was much more variable than at the earlier stage of development. In the areas in which the epithelium remained unilaminar the surface of the cells was often dome-shaped and the junctional complexes uniting the cells were situated low down on the lateral membranes. The interdigitating folds present at the base of the cells were generally more complex and the number of small vessels lying close to the under surface of the basement membrane appeared to be reduced. The cell nuclei were usually irregular (Fig. 7) and not only was there frequent marginal clumping of the chromatin but the nucleolus was also often homogeneous in structure. Most cells still contained much endoplasmic reticulum, but the individual sacs were commonly dilated and irregular in form (Fig. 7) and in some cells they were fragmented and only incompletely covered with ribosomes. Free ribosomes were few and the mitochondria were scattered throughout the cytoplasm. The Golgi apparatus was still usually prominent, however, and rod-like structures could often be seen among the numerous small vesicles associated with the apparatus (Fig. 6). A considerable variety of vacuoles, only a proportion of which retained the features of those present in the younger specimens, was also present in the cytoplasm (Fig. 7).

Although the development of areas of epithelial stratification in the basal parts of the glands was accompanied by the appearance of occasional ciliated cells, these were never as numerous as in the superficial parts of the glands in the younger specimens. In these areas the basal plasma membrane was sometimes folded, but there

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Fig. 3. Base of ciliated cell in the epithelium of the upper part of a gland at 67 days. *d*, desmosome; *f*, fibrillar material. × 9000.

Fig. 4. Ciliated and adjacent cells in an area of epithelial stratification at 67 days. *va*, cytoplasmic vacuoles; *jc*, junctional complex; *ci*, cilia; *mv*, microvilli. × 6000.

Fig. 5. Clear cell between the basal epithelial cells in the upper part of a gland at 67 days. *er*, granular endoplasmic reticulum in epithelial cell; *g*, Golgi apparatus; *gr*, dense granules in clear cell cytoplasm. × 8000.

Fig. 6. Region of the Golgi apparatus (*g*) in an epithelial cell at 114 days. *v*, cytoplasmic vesicles; *r*, rod-like structures. × 31000.

was little evidence of the development of major infoldings of the basement membrane, and changes similar to those seen in the cells in the unilaminar areas often occurred in an orderly fashion from the base of the epithelium to the surface. While, therefore, the basal cells still contained large amounts of endoplasmic reticulum, marked dilatation of the sacs was normally apparent only in the more superficially placed cells. Near the surface, fragmentation of the sacs was usual (Figs. 8, 9), and this was

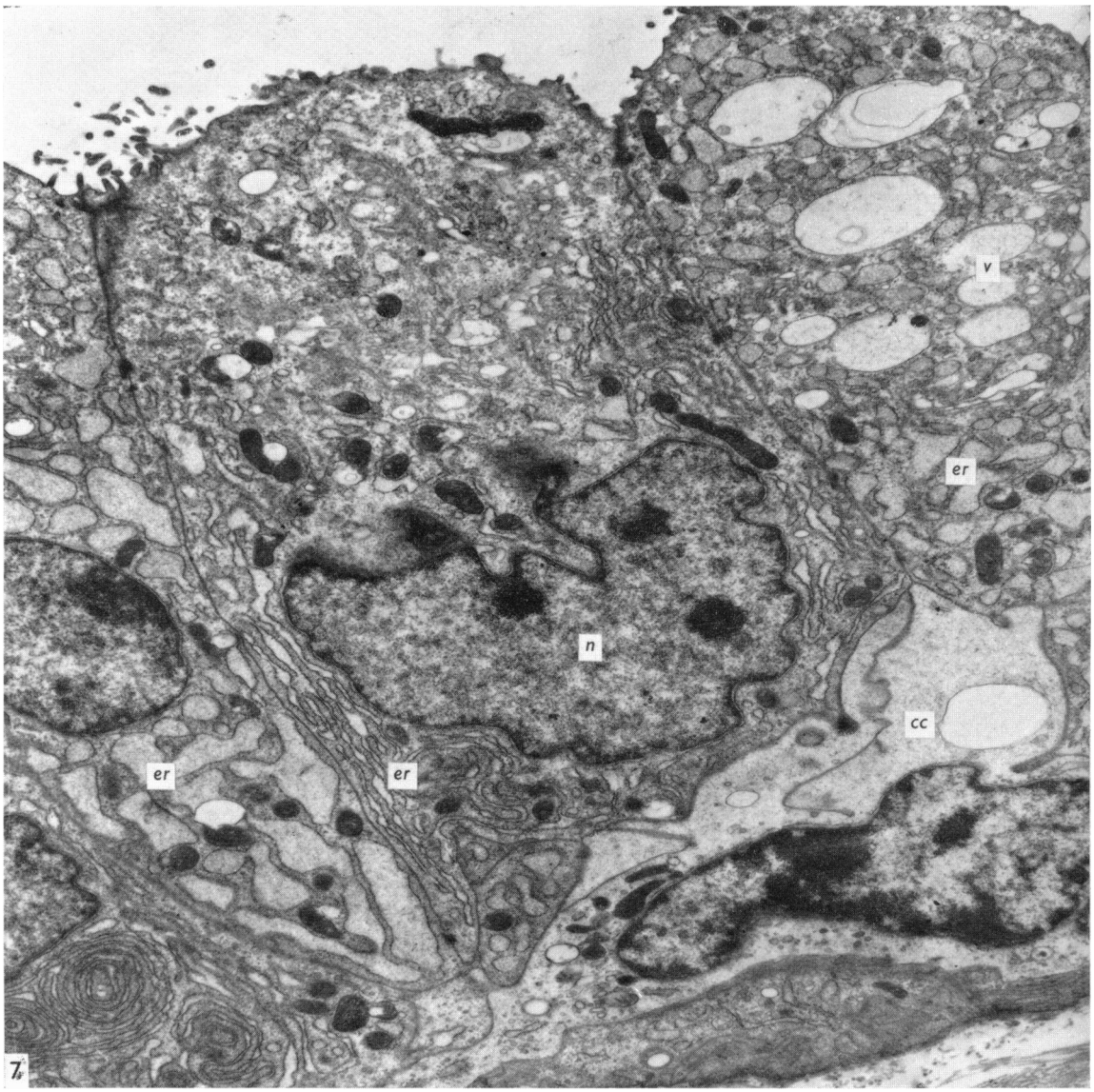


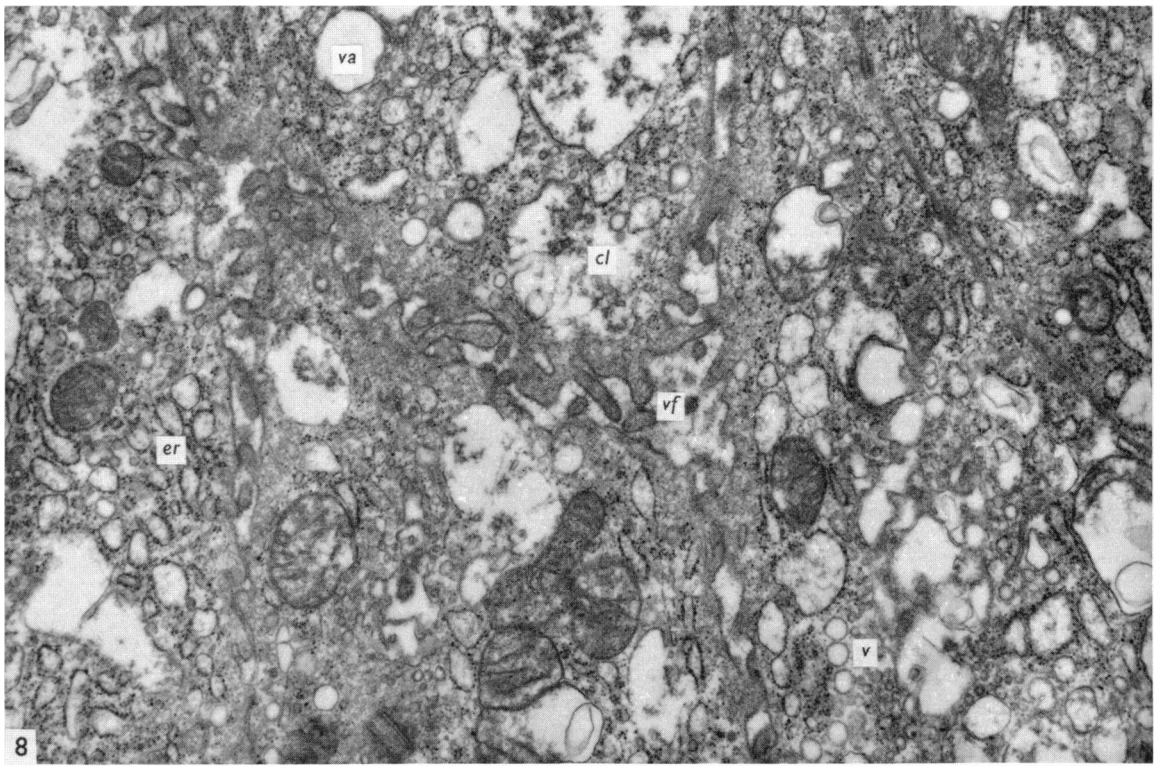
Fig. 7. Unilaminar epithelium in a gland at 142 days. In the central cell the granular endoplasmic reticulum (*er*) resembles that seen in the younger specimens, but in the other cells the sacs are irregularly dilated. *n*, nucleus; *v*, cytoplasmic vacuoles; *cc*, clear cell. × 9000.

associated with gross irregularity of the nuclei and condensation of the chromatin (Fig. 9). In such cells the Golgi apparatus was dispersed and the individual cisternae were commonly dilated (Fig. 9); cytoplasmic vesicles and vacuoles were also numerous (Fig. 8) and clear areas which appeared to be produced by disruption of the vacuolar membrane were often visible in the cytoplasm (Fig. 8). At these levels desmosomes were rarely apparent and villous processes were often present in narrow intercellular spaces between the cells. In some instances these were covered by fine fibrillar material and were at least as electron-dense as the membranes of the basal cells (Fig. 8), but in other cases the membranes were difficult to demonstrate (Fig. 9) and the cells were indistinguishable from the masses of degenerate material present in the lumen.

Although the apparently syncytial masses of degenerating cells present in the lumina of the glands normally contained nuclei and numerous cell organelles, a similar degree of organization was not usually apparent in the material present in the lumina of the ducts, and it was often formed only by irregular masses of a substance which was both electron-dense and more or less homogeneous in appearance (Fig. 10). The duct lumen was also often narrow and in some instances the microvilli on the surface of the epithelial cells formed interdigitating folds with those of the cells on the opposite wall (Fig. 10). Interlocking processes were also present between the closely apposed lateral membranes of the epithelial cells (Fig. 13) and the membranes were united at the surface by junctional complexes and elsewhere by occasional desmosomes. Hemidesmosome-like plaques were numerous on the basal plasma membrane and were often associated with club-like evaginations of the membrane (Fig. 13). The basement membrane was thick and homogeneous (Fig. 13) and capillary vessels were common in the connective tissue immediately beneath the membrane.

In other respects, the cells of the duct epithelium could be classified into two distinct types. One was more numerous in the younger specimens and contained a pyknotic nucleus and what appeared to be dilated sacs of endoplasmic reticulum in an extremely dense and finely granular cytoplasm. The second was more common, and was characterized by a much paler cytoplasm and a regular nucleus which showed little evidence of aggregation of the chromatin. In these cells, the cytoplasm contained scattered microtubules and bundles of fine filaments. Granular endoplasmic reticulum and ribosomes were not abundant, but the mitochondria were numerous and were often vacuolated (Figs. 11, 13). The supranuclear Golgi apparatus was well developed (Fig. 11) and uncoated vesicles similar to those associated with the apparatus were often present in the region of the thick terminal web at the surface of the cells (Fig. 10), and sometimes communicated with the plasma membrane at the base of the microvilli.

Although the clear cells seen in the epithelium with the light microscope were usually situated near the basement membrane, they were also occasionally present near the surface. They were not seen to be connected to the epithelial cells by desmosomes, and often appeared to be dendritic in nature (Figs. 5, 7, 12). Infolding of the plasma membrane into the cells was also not uncommon (Figs. 5, 7) and, although always apparently mononuclear, the cells not infrequently occurred in pairs. Their cytoplasm rarely contained more than a few short sacs of endoplasmic reticulum (Fig. 12) and scattered polyribosomes. The Golgi apparatus (Fig. 5) was generally



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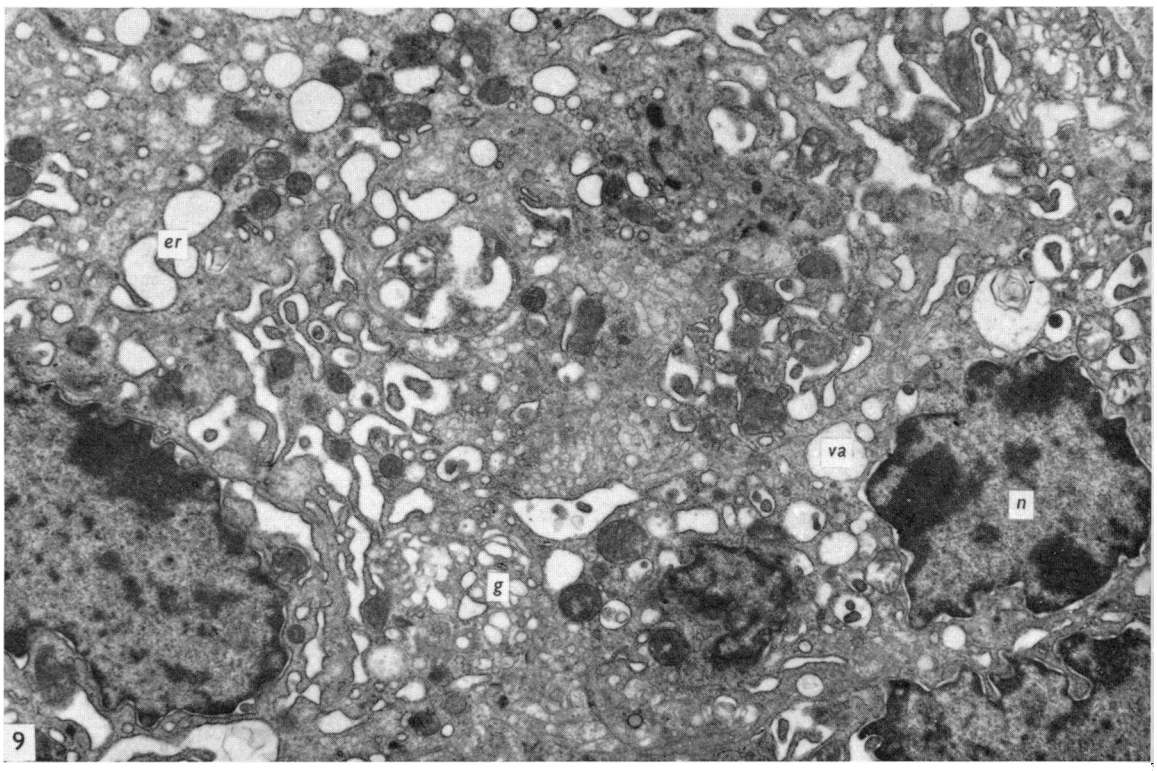

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small and mitochondria were few (Fig. 12), but a considerable variety of vesicular and other bodies was present in the cytoplasm. Some of these were devoid of content but others contained granular material, and in some cells the granules were filled with a homogeneous material of high electron density (Fig. 5).

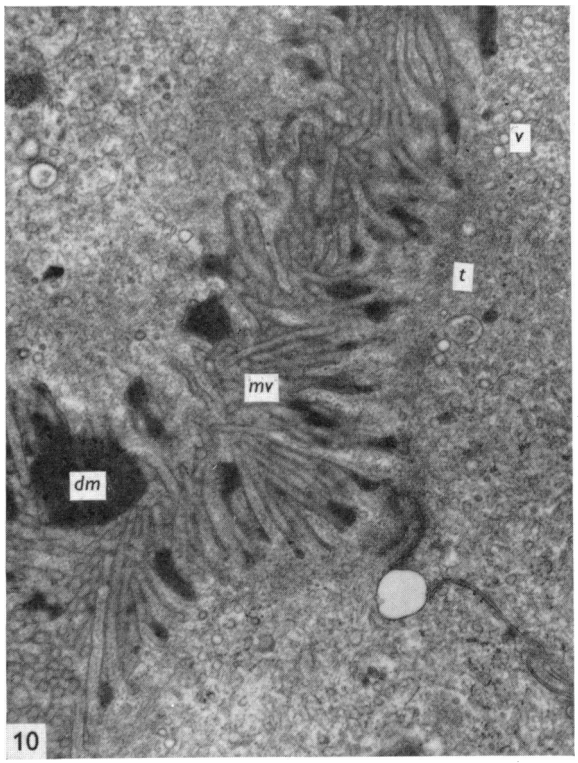
DISCUSSION

Although, in the sheep, the intercotyledonary endometrium remains thin, and the growth of the glands during pregnancy is probably limited compared to that seen in animals such as the horse, the early development of epithelial stratification and its subsequent extension into the basal coils of the glands indicates that, even in this species, the glandular epithelium continues to proliferate throughout pregnancy. In specimens obtained at about the 60th day most of the cells in the stratified areas were morphologically similar to those of the predominantly columnar epithelium present in the basal coils of the glands, and contained nuclei in which the chromatin was evenly distributed and the nucleolus well developed, as well as large amounts of granular endoplasmic reticulum. Such features are indicative of a high rate of protein synthesis and may be related either to the production of the materials required for cell proliferation or to a role of the cells in the secretion of the uterine milk. Their association in the basal coils with numerous free ribosomes favours the former view, but the similarity of the reticulum to that of many glandular cells suggests that it contributes to the production of materials for secretion. The high degree of development of the Golgi apparatus also suggests secretory activity, and the close proximity of the cytoplasmic vacuoles to the apparatus and to the surface membrane suggests involvement in the transmission of materials produced in the apparatus to the lumen. There are, however, a number of other ways in which such vacuoles may be formed and, although the mitochondria were often poorly preserved and the presence of transitional forms between these structures and the vacuoles may have been due to inadequate fixation, it is possibly significant that forms similar to the vacuoles have often been demonstrated among mitochondria isolated in media which produce a significant degree of swelling (Malamed, 1965; Caplan & Greenawalt, 1966; Wlodawer, Parsons, Williams & Wojtczak, 1966; Myron & Connelly, 1971).

In specimens obtained at later stages of gestation, cells with features similar to those present at earlier stages of development were concentrated in the basal layers of the stratified areas, and therefore could no longer be involved in secretory activity. In most of the cells there was now evidence of the onset of changes in the structure of the nuclei which were probably related to a progressive decline in the synthetic activity of the epithelium. The development of these changes could also be correlated with alterations in the morphology of the sacs of endoplasmic reticulum and in the number of cytoplasmic vesicles, which were similar to those seen in the cells of the amniotic and chorionic mesenchyme during their development into Hofbauer cells (Hoyes,

Fig. 8. Cells near the surface of a stratified area at 142 days. *er*, granular endoplasmic reticulum; *va*, vacuole; *v*, vesicles; *cl*, clear areas in cytoplasm; *vf*, villous folds in an intercellular space. × 20000.

Fig. 9. Upper layers of a stratified area at 142 days. The plasma membranes are indistinct. *er*, granular endoplasmic reticulum; *n*, nucleus; *g*, Golgi apparatus; *va*, cytoplasmic vacuole. × 8500.



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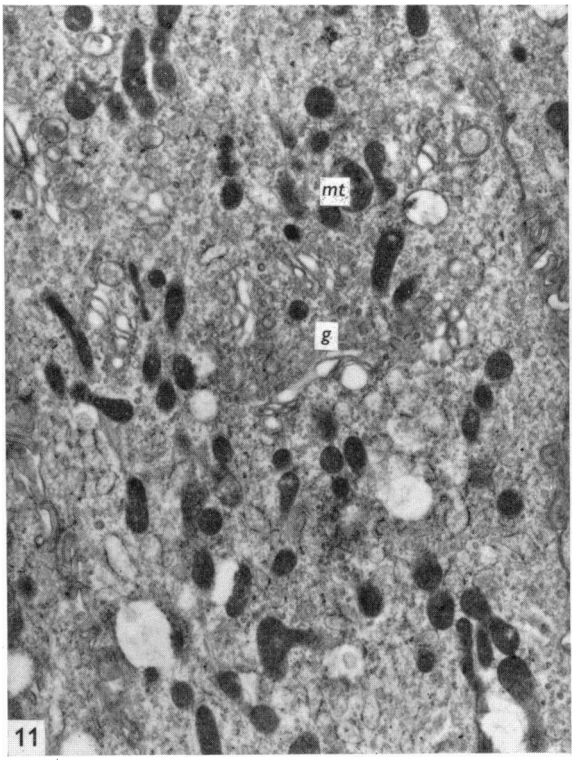

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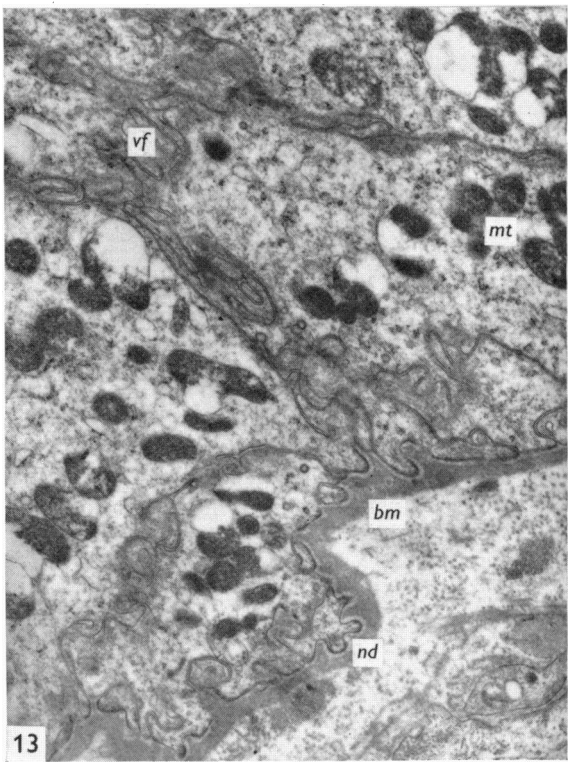

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1970, 1971). In the stratified areas, they could also be equated with the transformation of the cells into the syncytial masses of degenerate material present in the lumen. The formation of dome-shaped elevations from the surface and the recession of the junctional complexes towards the base of the cells, which were often evident in the unilaminar areas, may also be representative of an early stage in the development of processes for the detachment of cells from the epithelium. Such detachment was clearly evident only in the stratified areas in the younger specimens, but the appearance of intercellular spaces and associated villous folds in the upper layers of the corresponding areas in the older specimens may also be related to processes of this kind and suggests that, even at this stage of development, the transformation of the cells into a syncytium is often preceded by a degree of intercellular dissociation.

The glands of the bovine endometrium contain larger amounts of endoplasmic reticulum at oestrus than they do in the luteal phase of the cycle (Kojima & Selander, 1970), and this suggests that the development of synthetic activity in the glands of the pregnant sheep is controlled by oestrogen. Ciliated cells similar to those seen in the glands in the present study also appear to be more numerous in the bovine endometrium at oestrus (Kojima & Selander, 1970) and their frequent occurrence in the glands of the human endometrium in endometrial hyperplasia (Fruin & Tighe, 1967), a condition which is often attributed to a failure of ovulation and the continued exposure of the endometrium to the unopposed action of oestrogen, represents further evidence that their differentiation is influenced by this hormone. The appearance of the hyperplastic endometrium may also be related to that of the pregnant mare (Kolster, 1902), and the stratification and occasional development of a syncytium in the glands in the later and probably precarcinomatous phases of this condition may be related to their morphology in the later stages of pregnancy in the sheep. It is possible, however, that the atypical hyperplasia which precedes the development of carcinoma in the human endometrium is due to factors other than oestrogen. Although the levels of this hormone in the blood of the sheep are higher during pregnancy than at oestrus, a significant increase in the amount of unconjugated oestrogen in the circulating blood is normally apparent only in the days preceding parturition (Challis, 1971).

In addition to the material which is produced by the breakdown of cells of the glandular epithelium, the uterine milk contains numerous leucocytes. It is generally accepted that these are largely maternal in origin and the frequent occurrence of lymphocyte-like clear cells between the epithelial cells supports the view that at least a proportion reach the milk by migration through the glandular epithelium. Although some of these cells contained dense granules they were never seen to be multi-

Fig. 10. Dense material (*dm*) in the narrowed lumen of a duct at 127 days. *mv*, interdigitating microvilli of apposing cells; *v*, cytoplasmic vesicles; *t*, terminal web. × 14500.

Fig. 11. Supranuclear cytoplasm of duct epithelial cell. *mt*, mitochondria; *g*, Golgi apparatus. 127-day specimen. × 10000.

Fig. 12. Clear cell between duct epithelial cells. *er*, granular endoplasmic reticulum; *mt*, mitochondrion. 127-day specimen. × 6000.

Fig. 13. Basal region of duct epithelial cells. *vf*, interlocking folds of adjacent cell membranes; *nd*, hemidesmosome-like plaques in basal membrane; *bm*, basement membrane; *mt*, mitochondria. 127-day specimen. × 11000.

nucleated and the differences in their morphology were not sufficiently pronounced to enable them to be classified into more than a single population.

The presence of numerous mitochondria in the more common of the two types of cell lining the ducts of the glands is consistent with a high rate of metabolic activity, and the occurrence of elongated rod-like microvilli on the surface and of numerous villous folds on the lateral membranes is suggestive of their involvement in processes of ionic exchange. The presence of a well-developed Golgi apparatus and of numerous small vesicles in the cytoplasm is also consistent with such processes, but the relationship of the vesicles to the surface membrane was such that they could not be definitely regarded as being either secretory or absorptive in nature. Since, however, the material in the duct lumen was often electron-dense, it is possible that the cells are capable of extracting water from the glandular secretions and assist in its transformation into the viscid fluid present in the uterine lumen.

SUMMARY

In specimens of intercotyledonary endometrium obtained from sheep at about mid-pregnancy the morphology of the glandular epithelium was consistent with a high rate of protein synthesis. In older specimens the epithelial stratification which was initially apparent in the upper parts of the glands had extended into the basal coils and the morphology of the cells had undergone a number of changes which could be correlated with their gradual transformation into the syncytial masses of degenerate material present in the glandular lumen. The role of oestrogen in the production of the changes in the structure of the epithelium and their relationship to those seen in endometrial hyperplasia in the human was discussed. The lymphocyte-like cells which were common in the epithelium were equated with the leucocytes present in the uterine milk, and the structure of the cells lining the ducts was correlated with the involvement of the epithelium in processes of ionic exchange.

I am indebted to Dr D. A. Nixon and Miss Pauline Alexander of the Department of Physiology, St Mary's Hospital Medical School, for making available the material used in this study and to Professor K. A. Porter of the Department of Histopathology and Experimental Pathology, St Mary's Hospital Medical School, for permission to use the Philips electron microscope provided by the Wates Foundation.

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