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Involution of the sheep mammary gland

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ABSTRACT

Changes in the ovine mammary gland epithelium during initiated involution were studied by light and electron microscopy. Apoptosis of the duct and alveolar epithelial cells was first identified at 2 d after weaning, reached a peak at 4 d and then progressed gradually thereafter. Apoptotic cells were phagocytosed by intraepithelial macrophages and alveolar epithelial cells. Occasional apoptotic epithelial cells were observed in the alveolar and duct lumina. The highly vacuolated cells in the alveolar and duct lumina were confirmed to be macrophages as they were $CD45^+$, MHC class II^+. Changes in myoepithelial cells involved shrinkage and extension of cytoplasmic processes into the underlying stroma and no apoptosis was observed. Regression of the blood capillaries was also by apoptosis. The resulting apoptotic bodies were either taken up by adjacent endothelial cells or were shed into the capillary lumen to be phagocytosed later by mural endothelial cells or blood monocytes. The mammary glands were completely involuted by 30 d after weaning. It was concluded that the mammary gland involutes by apoptosis, a process which allows deletion of cells without the loss of the basic architecture and the integrity of the epithelial lining of the gland.

Key words: Ewe; mammary gland regression; apoptosis.

INTRODUCTION

The nature and the extent of glandular epithelial cell destruction during mammary involution is controversial. According to Helminen & Ericsson (1968), in rats, after cessation of suckling at weaning, the increase in intramammary pressure due to accumulation of milk in the lumina of alveoli and ducts causes degeneration of secretory cells and subsequent disruption of alveolar and lobular structures. Other workers (Walker et al. 1989), in their studies on involution of lactating mammary glands in mice and rats, attributed the involution process to cell death by 'apoptosis' (physiological or programmed cell death involving nuclear DNA fragmentation, in contrast to necrosis which is associated with cell injury). Studies on ruminant mammary glands, however (Holst et al. 1987; Sordillo et al. 1988), have shown that the mammary epithelial cells do not regress to the same extent as observed in rat mammary glands and they appear to maintain some synthetic and secretory activity throughout the nonlactating period. Moreover, sloughing of mammary epithelial cells into alveolar lumina was not observed in goat (Sordillo et al. 1984), sheep (Lascelles & Lee, 1978) or cow mammary gland (Holst et al. 1987; Sordillo & Nickerson, 1988) during involution.

It has been reported that the early period of mammary gland involution coincides with a period of acutely increased susceptibility to intramammary infection (Nickerson, 1989; Oliver & Sordillo, 1989) and one of the reasons given was that this could be due to the loss of the physical barrier by sloughing of the alveolar epithelium. In view of this hypothesis it is important to examine whether regression of the mammary gland is brought about by sloughing of the alveolar and ductal epithelium or by apoptosis, a process which would allow the deletion of alveolar epithelial cells without the loss of the integrity of the epithelial lining of the gland. This study was therefore designed to investigate the mechanism of mammary gland regression in the sheep. Attention was also

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focused on whether changes occur in the blood capillaries and myoepithelial cells as the gland regressed.

MATERIALS AND METHODS

Tissues

All sheep used in this study were healthy and their mammary glands were palpated and found to be free of abnormality. Mammary gland tissues were obtained for light and electron microscopy and immunocytochemistry from sheep at 5 d after parturition (d 0) (n = 2) and at 2 (n = 3), 4 (n = 3), 7 (n = 3), 15 (n = 3), 30 (n = 3) and 60 (n = 3) d after lambs were weaned at 5 d after birth. All animals were killed by an overdose of pentobarbitone sodium (Nembutal, Ceva Chemicals, Victoria, Australia) prior to tissue collection.

Light microscopy

Specimens for routine light microscopy were obtained from the lower, middle and upper regions of the left gland. At each level, 3 samples were taken, fixed in Bouin's fixative for 24 h, processed and embedded by conventional methods. Paraffin sections (4–5 μm) were prepared and stained routinely with haematoxylin and eosin.

Electron microscopy

The right mammary glands were fixed by perfusion via the external pudendal artery with a fixative containing 2.8% paraformaldehyde, 5% glutaraldehyde and 0.6% picric acid in 0.15 M cacodylate buffer, pH 7.2. After initial fixation of the whole gland, small samples of mammary tissue were taken from the lower, middle and upper regions of the gland. These samples of tissue were then cut into smaller pieces of approximately 1 mm^3 while being immersed in the fixative. The tissues were placed in fresh fixative for a minimum of 12 h at 4 °C before being processed. After washing 4 times in cacodylate buffer, the samples were postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature on a vertical rotator. The samples were then washed in distilled water twice and dehydrated in a series of graded acetone solutions before being embedded in Araldite (Biorad, USA). The blocks were polymerised at 60 °C for 48 h. Some specimens were stained en bloc during dehydration using a solution containing 2% uranyl acetate in 70% acetone.

Semithin sections (1 μm) were mounted on glass microscope slides and stained with a solution of 1% methylene blue and 1% sodium tetraborate. Ultrathin sections were collected on acetone-cleaned uncoated 200 mesh copper grids. These sections were stained with a 5% aqueous solution of uranyl acetate for 10 min and then with Reynold's lead citrate for 10 min.

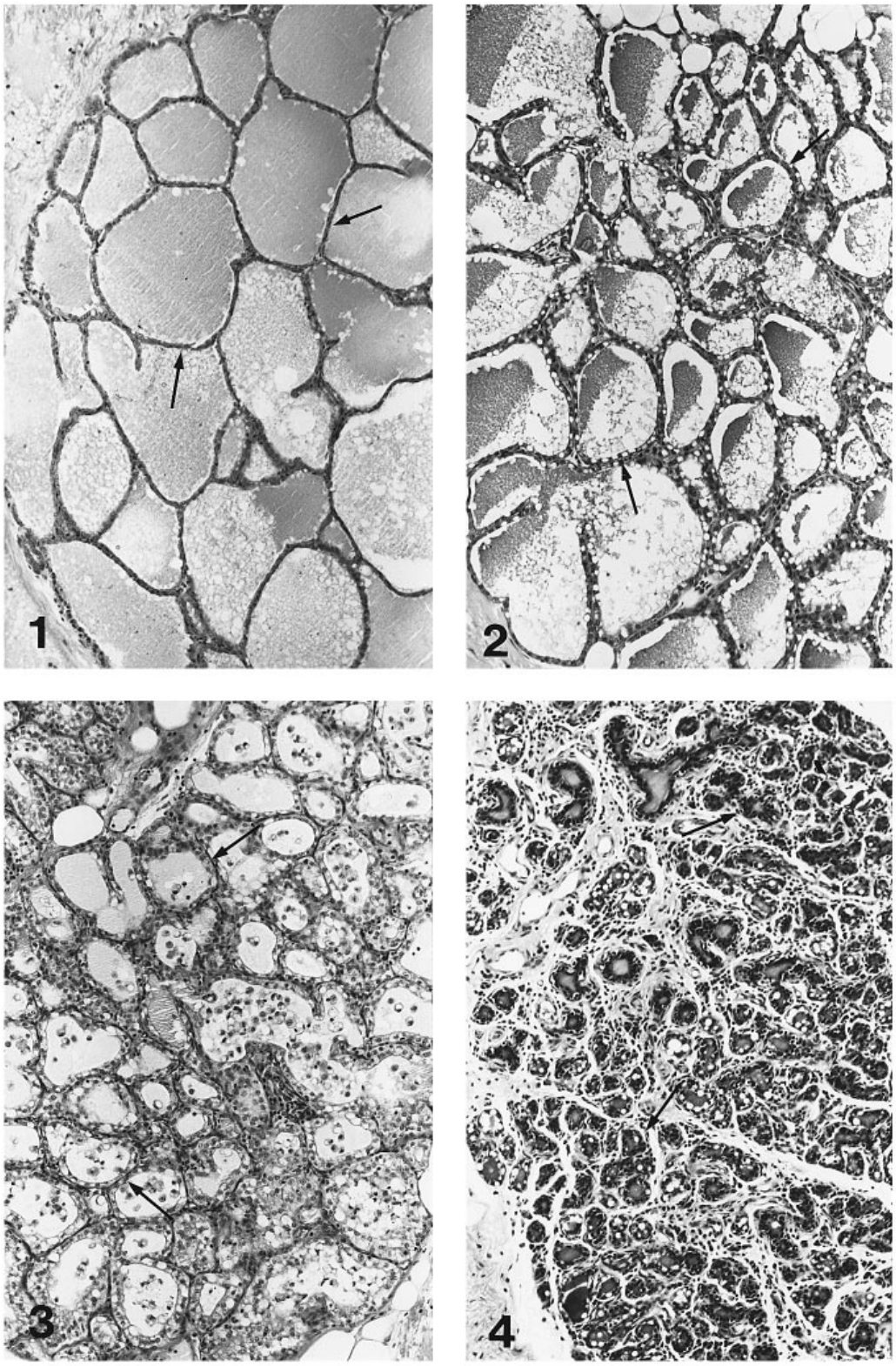
Quantitation of cells undergoing apoptosis

Araldite sections (1 μm) stained with 1% methylene blue and 1% sodium tetraborate were used for quantitation of apoptotic cells in the alveolar epithelium. Only cells with a prominent nucleus were counted. Cells counted were epithelial cells, apoptotic cells (epithelial cells or macrophages containing apoptotic bodies also counted as apoptotic cells), lymphocytes and macrophages. A total of 60 microscopic fields were counted for the 3 samples collected from each gland with a ×100 oil immersion objective.

Statistical analysis was carried out with Student's t test to assess if there was any significant difference ($P < 0.01$) between the means of the sample groups. All values are reported as mean ± standard error of the mean (S.E.M.).

Immunocytochemistry

For this study it is important to identify whether the highly vacuolated mononucleated cells in the lumina of alveoli and ducts are desquamated alveolar epithelial cells or macrophages containing ingested lipid droplets. Consequently immunocytochemical studies were carried out to differentiate these 2 cell types. All tissues used for this study were embedded in OCT compound (Miles Scientific, Naperville, Il), snap-frozen using liquid nitrogen and then stored at −70 °C. For immunocytochemical staining, 6 μm frozen sections were cut, fixed for 10 min in cold ethanol, air dried and then stained, using the indirect immunoperoxidase technique as previously described (Lee et al. 1985). Briefly, tissue sections were incubated with the first antibody for 45 min at room temperature. Slides were washed with phosphate-buffered saline (PBS) and then incubated with peroxidase-conjugated rabbit antimouse Ig (DAKO-immunoglobulins, Glostrup, Denmark) diluted 1:80 in PBS containing 5% normal sheep serum. After thorough washing in PBS, sections were incubated in 0.06% (w/v) diaminobenzidine tetrahydrochloride (Sigma Chemicals, St Louis, MO) in PBS containing 0.05% (v/v) hydrogen peroxide for 10 min at room tem-



Figs 1–4. Light micrographs of paraffin sections of uniform magnification showing that the alveoli (arrows) reduce in diameter with progression of involution. Fig. 1, 2 d, Fig. 2, 4 d, Fig, 3, 7 d, Fig. 4, 30 d after weaning. $\times 120$.

perature. For control either PBS or 1 % normal sheep serum in PBS was substituted for the first antibody.

Monoclonal antibodies (*mAb*)

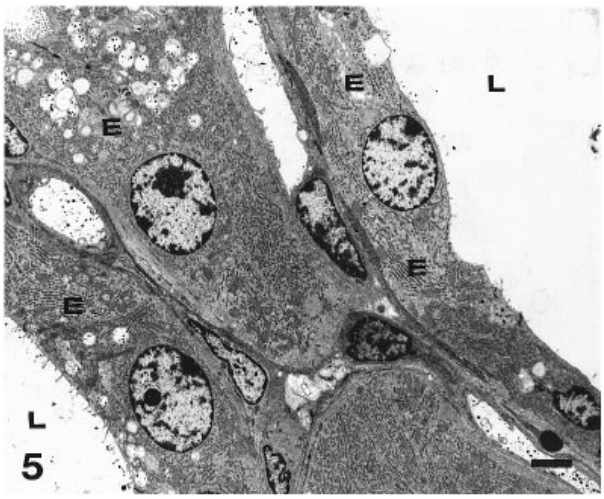
A panel of monoclonal antibodies (mAb) (kindly provided by Dr M. R. Brandon, Centre for Animal Biotechnology, University of Melbourne), raised against ovine leucocyte surface antigens, was used for immunoperoxidase staining. These included anti-CD45R (Mackay et al. 1987), anti-CD8 (Maddox et al. 1985*b*); anti-CD5 (Mackay et al. 1985); anti-CD4 (Maddox et al. 1985*b*); anti-CD45 (Maddox et al. 1985*a*) and anti-MHC class II (Puri et al. 1987).

RESULTS

Alveolar epithelial cells

The present studies revealed that at 2 d after weaning, the alveoli and ductules were generally markedly distended, but from 4 d thereafter, there was a progressive reduction in alveolar diameter (Figs 1–4). There were only slight variations in the progression of involution in different animals of the same group. Closer examination revealed that at 2 d after weaning most of the alveolar epithelial cells were flattened but they remained intact (Fig. 5) and some of them were seen with stasis vacuoles in their cytoplasm (Fig. 6). However, the occasional alveolar epithelial cell undergoing early apoptosis was observed interspersed between unaltered epithelial cells. These apoptotic cells were identified by the condensation of chromatin into patches or crescents lying against the nuclear envelope (Fig. 7). The cytoplasm of these cells contained well preserved rough endoplasmic reticulum, mitochondria and some secretory vesicles. In alveolar epithelial cells at a more advanced stage of apoptosis, their cytoplasm was seen with numerous vacuoles, some of which contained clusters of dense material and their nuclei showed mild convolution or fragmentation (Fig. 8). Sometimes portions of apoptotic alveolar epithelial cells were seen within the cytoplasm of presumed macrophages. These macrophages had extensive pale cytoplasm and were usually located between the alveolar epithelial cells and the basement membrane (Fig. 9). Vacuoles containing apoptotic bodies were sometimes seen in the cytoplasm of alveolar epithelial cells (Fig. 10). The lumina of the alveoli were filled with secretion containing numerous casein micelles and lipid droplets. At this stage very few free cells were observed in the lumina.

By 4 d after weaning, the alveoli were less distended than previously and most of the alveolar epithelial



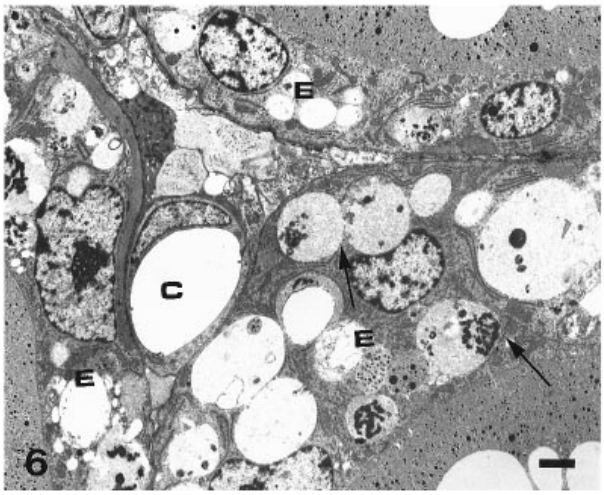


Fig. 5. Electron micrograph showing flattened alveolar epithelial cells (E); all organelles within them appear normal; 2 d after weaning. L, lumen. Bar, 2 μm.

Fig. 6. Electron micrograph showing flattened alveolar epithelial cells (E) containing stasis vacuoles (arrows); 2 d after weaning. C, capillary. Bar, 1 μm.

cells were seen with large empty vacuoles (Fig. 11) and autophagosomes were present (Fig. 12) in their cytoplasm. However, the most striking feature was the frequent appearance of cells undergoing apoptosis (Fig. 11). Some apoptotic bodies were seen within lining epithelial cells and some were engulfed by macrophages. That most of the cells undergoing apoptosis were alveolar epithelial cells was evidenced by their characteristic ultrastructural features. Apoptotic bodies were sometimes observed in the lumina of alveoli (Fig. 13). Macrophages, neutrophils and lymphocytes were found in the interalveolar areas as well as in the lumina of alveoli and ducts.

By 7 d, the alveoli were still well defined, but were much smaller than at 4 d and they contained vacuolated cells and much less secretion in their lumina (Fig. 3). The epithelial cells were highly

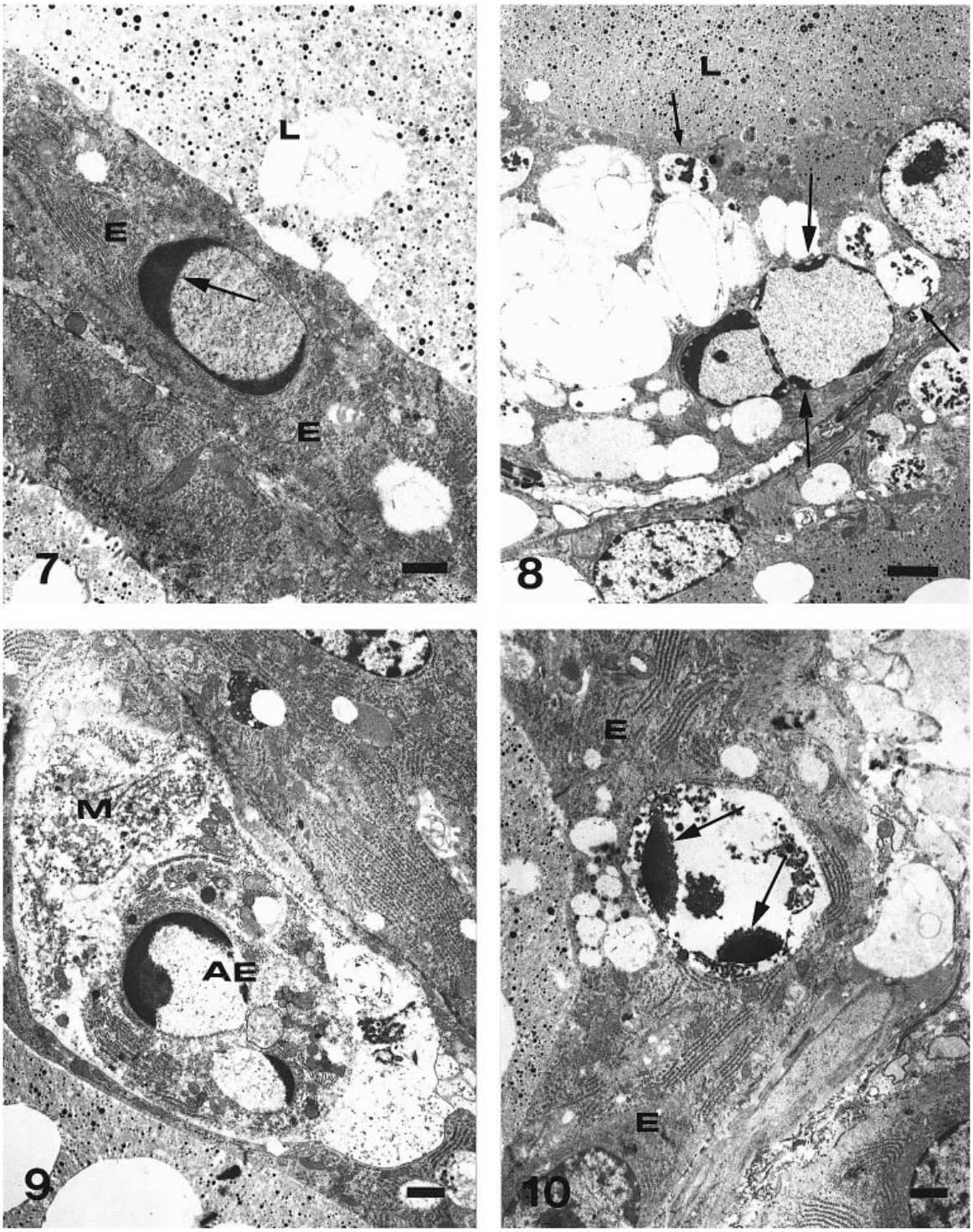


Fig. 7. Electron micrograph showing an alveolar epithelial cell (E) undergoing early apoptosis. The chromatin has condensed into crescents (arrow). L, lumen. Bar, 1 μm.

Fig. 8. Electron micrograph showing nuclear fragmentation (large arrows) in an alveolar epithelial cell. Stasis vacuoles (small arrows) are present within the cytoplasm; 2 d after weaning. L, lumen. Bar, 2 μm.

Fig. 9. Electron micrograph showing an apoptotic alveolar epithelial cell (AE) phagocytosed by an intraepithelial macrophage (M) which is probably undergoing degeneration; 2 d after weaning. Bar, 1 μm.

Fig. 10. Electron micrograph showing degraded apoptotic bodies (arrows) within the cytoplasm of an apparently normal alveolar epithelial cell (E). Bar, 1 μm.

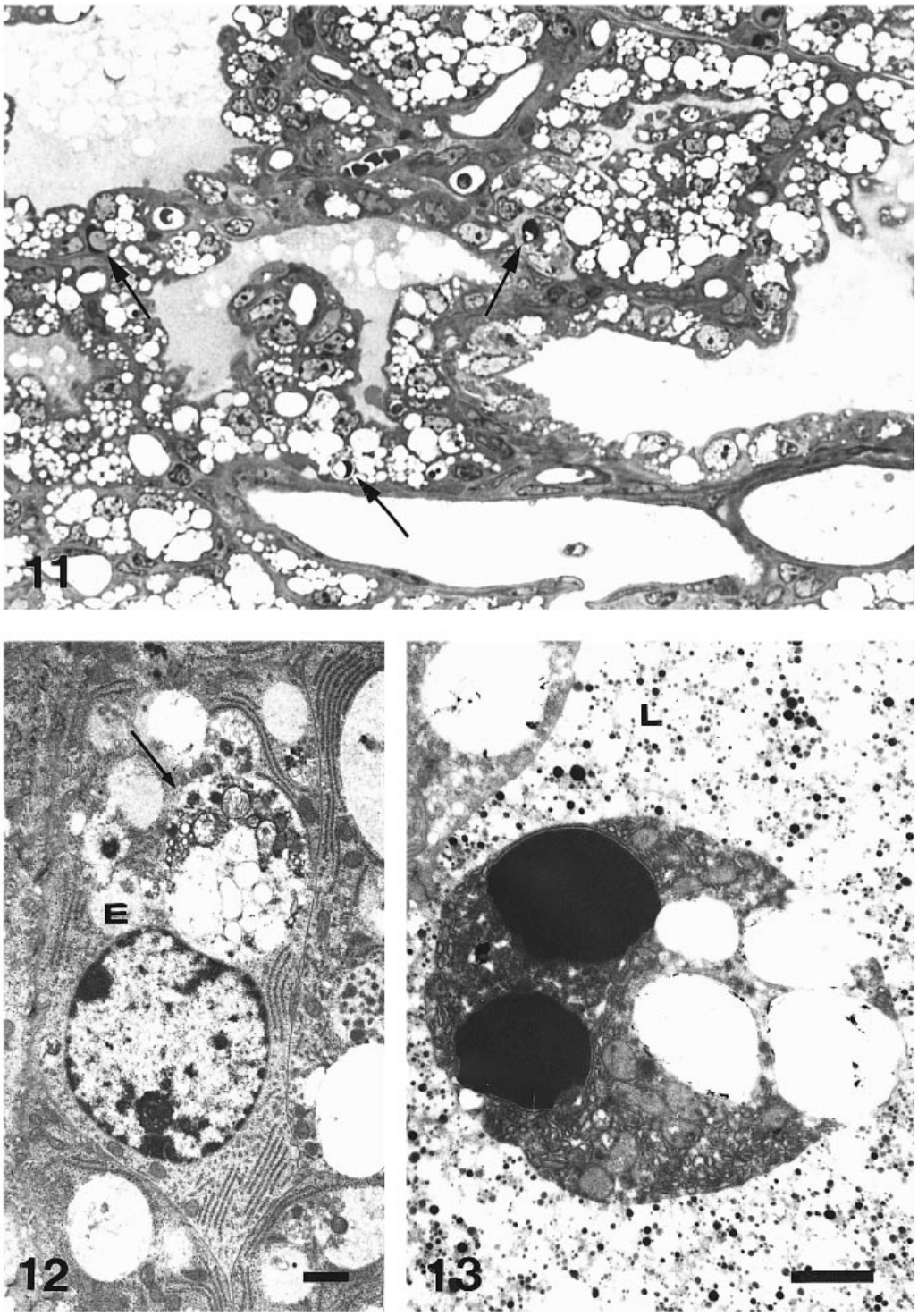


Fig. 11. Light micrograph of a semithin section from a mammary gland 4 d after weaning showing that most of the alveolar epithelial cells are highly vacuolated. Several apoptotic bodies (arrows) are seen within the alveolar epithelium. × 640.

Fig. 12. Electron micrograph showing an alveolar epithelial cell (E) containing autolysosomes (arrow) within the cytoplam; 4 d after weaning. Bar, 1 μm.

Fig. 13. Electron micrograph showing an apoptotic body of epithelial origin in the lumen (L) of an alveolus; 4 d after weaning. Bar, 1 μm.

vacuolated and the remaining cytoplasm contained small dense mitochondria, some rough endoplasmic reticulum and numerous free ribosomes. Macrophages were commonly found in the alveolar epithelium and many were seen with numerous residual bodies, presumably end products of phagocytosed

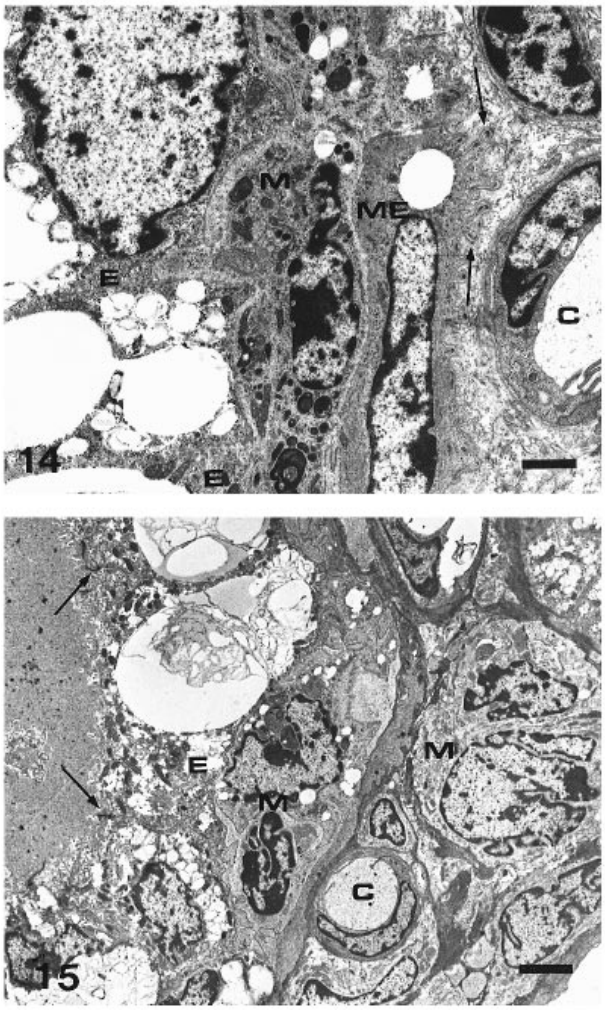


Fig. 14. Electron micrograph showing an intraepithelial macrophage (M) containing numerous heterolysosomes and residual bodies in its cytoplasm; 7 d after weaning. An alveolar epithelial cell (E) is highly vacuolated and a myoepithelial cell (ME) possesses numerous cytoplasmic processes (arrows) extending into the underlying connective tissues. C, capillary. Bar, 1 μm.

Fig. 15. Electron micrograph of a section from a mammary gland 15 d after weaning showing the presence of vacuoles of different sizes in the alveolar epithelial cells (E) and intercellular junctions (arrows) at the apical lateral borders of the cells. Macrophages (M) are present at the basal region of the alveolar epithelium and in the interalveolar area. C, capillary. Bar, 2 μm.

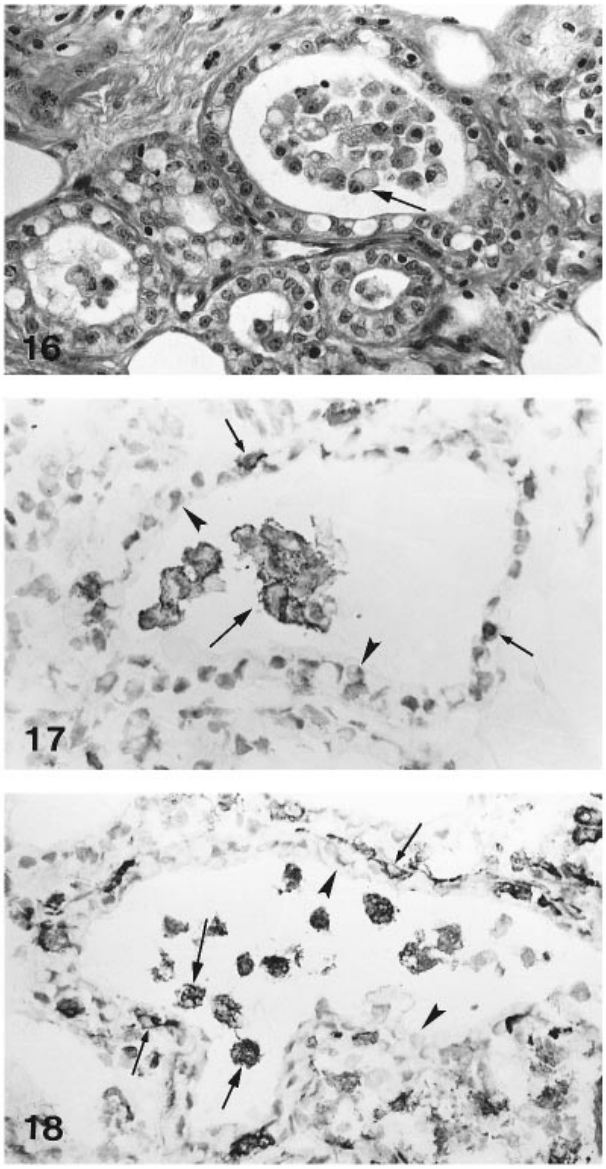


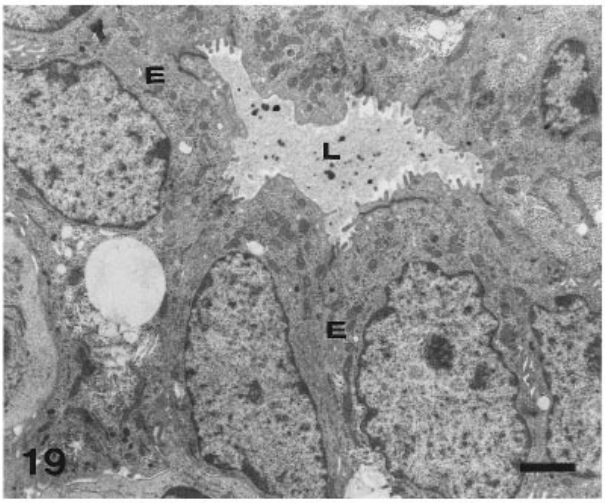
Fig. 16. Light micrograph of a paraffin section showing numerous highly vacuolated macrophages (arrow) within the lumen of a ductule; 15 d after weaning. ×400.

Fig. 17. Light micrograph of a frozen section stained by the indirect immunoperoxidase technique with anti-CD45 mAb to localise leucocyte common antigen on the surface of the vacuolated macrophages; 15 d after weaning. The entire surface of virtually all the vacuolated macrophages within the lumen of a ductule (large arrow) and the surface of a few intraepithelial leucocytes (small arrows) within the duct epithelium (arrowheads) are stained. The duct and alveolar epithelial cells are not stained. ×560.

Fig. 18. Light micrograph of a frozen section stained by the indirect immunoperoxidase technique with an anti-MHC class II mAb. The cytoplasm of the vacuolated macrophages (large arrows) within the duct lumen are intensely stained. Some leucocytes located at the basal region of the duct epithelium are also stained (small arrows) but the duct epithelial cells (arrowheads) are not. ×400.

apoptotic bodies, in their cytoplasm (Fig. 14). The interalveolar connective tissue appeared thicker than at 4 d and lymphocytes and macrophages were commonly found.

At 15 d after weaning, the alveolar epithelial cells appeared similar to those seen at 7 d, although the cytoplasmic vacuoles were now more pronounced and tight junctions were invariably observed between neighbouring epithelial cells (Fig. 15). Intraepithelial macrophages with engulfed material at different stages of degradation within their cytoplasm was a common feature. A very striking feature was the presence of numerous highly vacuolated cells in the lumina of alveoli and ducts (Fig. 16). Immunocytochemical staining with a panel of monoclonal antibodies



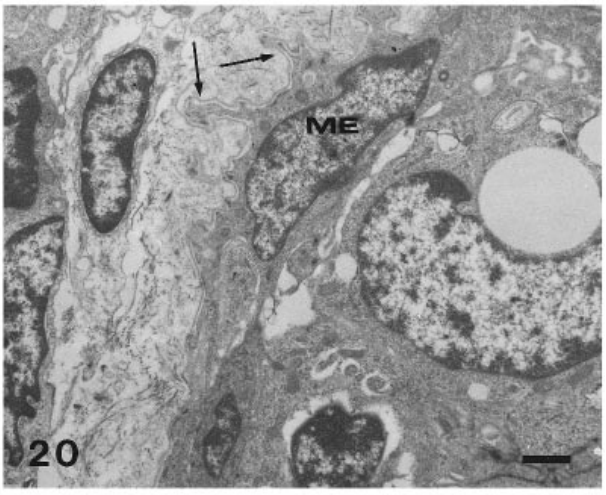


Fig. 19. Electron micrograph showing that the alveolar epithelial cells (E) lining the markedly reduced alveolar lumen (L) present no evidence of secretory activity although some contain vacuoles in their cytoplasm; 30 d after weaning. Bar, 2 μm.

Fig. 20. Electron micrograph showing a shrunken myoepithelial cell (ME) with its long cytoplasmic processes surrounded by basement membrane (arrows); 60 d after weaning. Bar, 1 μm.

revealed that these cells were $CD45^+$, MHC class II^+ (Figs 17, 18), but $CD4^-$, $CD8^-$ and $CD45R^-$ (not shown), thus indicating that they were macrophages and not epithelial cells. The alveolar and ductal epithelial cells were $CD45^-$, MHC class II^-.

At 30 and 60 d after weaning the alveoli presented the picture of a resting gland (Fig. 4). The alveoli had reduced markedly in size and they appeared as collapsed masses of cells with little or no lumen. The closely packed, cuboidal epithelial cells contained numerous ribosomes, mitochondria, a small Golgi apparatus as well as a small amount of rough endoplasmic reticulum (Fig. 19). There was no evidence of extensive sloughing of alveolar epithelial cells at any of the stages studied.

Table. *Percentages of apoptotic bodies in the mammary gland epithelium**

Days after weaning	Number of animals	Percentages of cells in apoptosis
0	2	0.0 (0.0)[a]
2	2	2.9 (1.2)[b]
4	2	3.6 (0.2)[b]
7	2	1.0 (0.1)[a]
15	2	0.8 (0.3)[a]

* Figures in brackets are standard errors. Different superscripts indicate significant differences ($P < 0.01$).

Quantitation of apoptotic bodies

The above studies indicated a dramatic increase in the appearance of apoptotic bodies in the alveolar epithelium 2–4 d after weaning, followed by a gradual decline thereafter. Consequently, a quantitative study based on 60 microscopic fields on Araldite sections with a $\times 100$ oil immersion objective was carried out to substantiate this observation. As shown in the Table, no apoptotic bodies were observed in the specimens counted for d 0 after weaning. However, as involution advanced there was a significant occurrence of apoptosis ($P < 0.01$) at d 2 after weaning. The percentages of apoptosis reached a peak (3.6 ± 0.3) by d 4 after weaning and then declined dramatically ($P < 0.01$) by d 7 and 15.

Myoepithelial cells

The myoepithelial cells at 2 d after weaning were basically similar to those at d 0. They were located between the alveolar epithelial cells and the basement membrane. They had abundant myofilaments and organelles such as mitochondria and rough endoplasmic reticulum which were located close to the nucleus, whereas numerous pinocytotic vesicles were situated mainly at the periphery of the cells.

From 4 to 7 d after weaning the outline of the myoepithelial cells had become irregular and on the basal side of the cells distinct cytoplasmic processes enveloped by the basement membrane were seen extending into the underlying connective tissue (Fig. 14). At this stage, apoptosis was commonly seen in the alveolar epithelial cells but never in the myoepithelial cells, although these latter had become somewhat shrunken. By 30 d, the outline of the myoepithelial cells became considerably more irregular in form and their cytoplasmic processes protruded deeper into the underlying stroma. There were fewer myofilaments,

but dark filament-attachment plaques and pinocytotic vesicles were present.

By 60 d the myoepithelial cells had decreased markedly in size and their cytoplasm had condensed around the nucleus but a few cytoplasmic processes were still present (Fig. 20). There were fewer myofilaments than previously and there were only occasional mitochondria and some pinocytotic vesicles. Apoptosis of myoepithelial cells was never observed in any of the stages studied.

Blood capillaries

Numerous blood capillaries of the continuous type were found in the interalveolar areas. The cytoplasm of the endothelial cells were seen to contain sparse organelles including a few mitochondria, rough endoplasmic reticulum and occasional dense granules, presumably lysosomal granules. Numerous pinocytotic vesicles were seen scattered in the cytoplasm. The luminal surfaces of the endothelial cells were often irregular, with many villous projections extending into the lumen (Fig. 21). The basal surfaces were more regular and they were covered by a continuous layer of basal lamina. However, regression of the blood vessels of the mammary gland somewhat paralleled that of the parenchyma. Abnormalities of some capillaries and vessels, presumably small venules, were recognised 4–15 d after weaning. These abnormalities were observed in the contents of the capillary lumen, the wall of the capillary or both and they varied in their nature and degree between different individual capillaries within each gland. In some capillaries the endothelial cells were seen to contain apoptotic bodies within their cytoplasm (Fig. 22), whereas in others, their lumina were filled with apoptotic endothelial cells which were still attached to the mural endothelial cells (Fig. 23). In some capillaries apoptotic bodies enclosed within the cytoplasm of apparently normal endothelial cells protruded into the lumen (Fig. 24). This appearance was suggestive of phagocytosis of degenerating material from the lumen by normal endothelial cells.

DISCUSSION

The results of this study clearly showed that the mammary gland of the ewe regressed by apoptosis, reaching a peak 4 d after initiated weaning and continued gradually thereafter. The gland became completely regressed by 30–60 d after weaning. This mechanism of cellular deletion in mammary gland involution is consistent with reports on studies in mammary gland involution in mice and rats (Walker et al. 1989; Strange et al. 1992; Guenette et al. 1994). Similar to these species, the apoptotic cells were subsequently phagocytosed by alveolar epithelial cells and intraepithelial macrophages. However in mice, numerous apoptotic bodies were shed into alveolar lumina. This phenomenon was reported to be much less obvious in rats, an observation similar to that made in our study in the sheep. Similar to mice and rats, some of the shed apoptotic cells showed excellent preservation of organelle morphology and plasma membranes but later they appeared to undergo swelling and lysis. It is conceivable that this method of cellular deletion allows the preservation of the physical epithelial barrier of the alveoli. However, it would be of interest to know what factor(s) govern which particular cells undergo such programmed cell death, and whether the proportion of epithelial cells lost over the period in question is commensurate with the amount of apoptosis actually seen.

It has been reported that the early period of mammary gland involution coincides with a period of acutely increased susceptibility to mammary infection (Nickerson, 1989; Oliver & Sordillo, 1989) and one of the reasons given is that this could be due to the loss of the physical barrier by sloughing of the alveolar epithelium. Our present study obviously excluded this possibility as it clearly showed that, apart from the occasional shed apoptotic epithelial cells, there was no evidence of extensive shedding of alveolar epithelial cells into the alveolar lumina. This is consistent with earlier work carried out in the sheep (Lascelles & Lee, 1978; Lee & Outteridge, 1981), goat (Sordillo et al. 1984) and cow (Holst et al. 1987; Sordillo & Nickerson, 1988).

Numerous highly vacuolated cells together with some lymphocytes were observed in the alveolar lumina 7–15 d after weaning. These highly vacuolated cells designated as 'cells of Donné' (Donné, 1844–45), created great controversy since their discovery. Many believed they were desquamated alveolar epithelial cells while others thought they were macrophages containing phagocytosed liquid droplets (review by Lascelles & Lee, 1978). However, our present studies further confirm that these vacuolated cells are macrophages and not desquamated epithelial cells as they are MHC class II^+ and $CD45^+$. The alveolar and duct epithelial cells showed no affinity to antibodies raised against these 2 antigens.

It is perplexing to find that though there is extensive leucocytic infiltration into the mammary gland during early involution, the gland at this stge is more prone to infection than in a fully involuted gland (Nickerson,

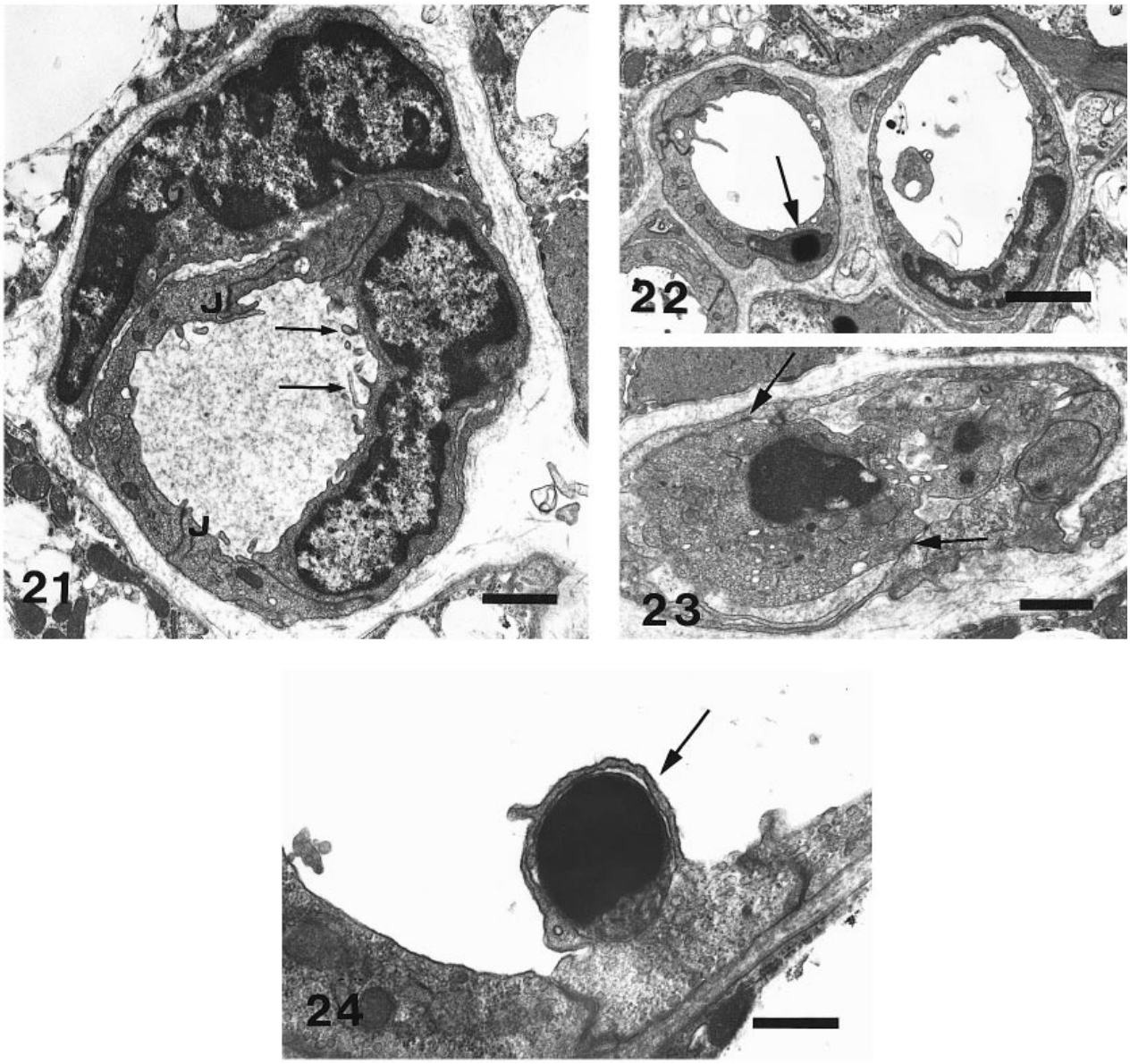


Fig. 21. Electron micrograph showing an apparently normal capillary with intercellular junctions (J) and numerous pinocytotic vesicles within the cytoplasm of the endothelial cells; 4 d after weaning. The luminal surfaces of the endothelial cells display many villous projections (arrows) extending into the lumen. Bar, 1 μm.

Fig. 22. Electron micrograph showing an apoptotic body (arrow) within the cytoplasm of an apparently normal endothelial cell; 4 d after weaning. Bar, 0.5 μm.

Fig. 23. Electron micrograph showing an apoptotic endothelial cell within the lumen of a capillary still attached (arrows) to a mural endothelial cell; 4 d after weaning. Bar, 1 μm.

Fig. 24. Electron micrograph showing an apoptotic body (arrow) enclosed within the cytoplasm of an apparently normal endothelial cell; 4 d after weaning. The apoptotic body protrudes into the lumen of the capillary. Bar, 0.5 μm.

1989). There are probably many explanations for this but 2 possible hypotheses are worth considering. (1) It is possible that the leucocyte types, especially the lymphocyte phenotypes, infiltrating into the gland at this stage are not sufficient (in terms of number of cells, types of cells or both), to afford the gland protection and (2) the phagocytotic leucocytes residing in the alveolar and ductal lumina are incapacitated since they are preoccupied with removing the lipid droplets and milk protein and are replete with engulfed material. Further experimental studies are needed to elucidate this point.

There appear to be species differences in relation to the fate of myoepithelial cells during mammary gland involution. In mice, deletion of myoepithelial cells was by apoptosis (Walker et al. 1989), while elimination of myoepithelial cells was not seen in rats (Radnor, 1972; Walker et al. 1989) nor in sheep in our present

study. The ultrastructural changes observed in these cells included infolding of the basal cell membrane and thickening and folding of the adjacent basal lamina. All these changes are similar to those described in rats and mice (Radnor, 1972; Martinez-Hernandez et al. 1976; Lascelles & Lee, 1978).

The preservation of myoepithelial cells during involution is probably vital as it has been suggested (Radnor, 1972; Lascelles & Lee, 1978) that they may provide a supportive framework for the alveoli during involution. In addition, recent studies (Dickson & Warburton, 1992; Talhouk et al. 1992; Anderson et al. 1994) have shown that during involution, myoepithelial cells synthesise matrix metalloproteinases, a family of enzymes capable of degrading the basal lamina. On the other hand myoepithelial cells have been shown to synthesise some basal lamina components (Warburton et al. 1992). Therefore, apart from providing support, myoepithelial cells also seem to play an important role in extracellular matrix remodelling during mammary gland involution. Further studies employing appropriate immunocytochemical techniques are necessary to elucidate this point.

Regression of blood capillary endothelial cells by apoptosis was detected at 4 d after weaning and it continued gradually. However this process was not as dramatic as that observed in mice and rats during the first 3–4 d after weaning (Walker et al. 1989). Such discrepancies are probably related to the size and the rate of regression of the glands in these species. The mammary gland of rats and mice are relatively much smaller and they regress at a much faster rate than in sheep. This is consistent with the regression of other small glands, for example the corpora lutea of sheep (O'Shea et al. 1977) in which extensive apoptosis of endothelial cells was observed during cyclical luteal regression.

It was concluded in this study that there was no evidence of extensive sloughing of alveolar epithelial cells into the lumina of alveoli and ducts as the mammary gland regressed. The main mechanism of mammary involution was by apoptosis and it was most active 2–4 d after weaning and then continued at a slower rate thereafter. This mode of cellular deletion is important as it allows the preservation of the physical barrier formed by the alveolar and ductal epithelium. This barrier has been claimed to be important in protecting the gland from bacterial infection.

It is generally accepted that it is necessary to have a dry period (resting period for the gland) between successive lactations to maximise milk production in the subsequent lactation. The present study clearly showed that in the sheep, the mammary gland became completely involuted by 30 d after weaning. Thus a resting period of 30–60 d prior to the next pregnancy may be recommended for this species.

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