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Histological Changes in the Pregnant, Lactating and Involuting Mammary Gland of the Guinea Pig

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ABSTRAK

Perubahan histologi kelenjar susu tikus belanda pada pertengahan dan akhir kebuntingan, 1 hari selepas beranak, kemuncak laktasi dan 2, 3, 4, 8 dan 12 hari selepas cerai susu telah dikaji. Tumbesar tisu kelenjar berlaku dengan cepat pada akhir kebuntingan hingga ke kemuncak laktasi. Sel-sel plasma didapati banyak di ruang antara alveolus dari pertengahan kebuntingan hingga laktasi. Involusi kelenjar susu ialah proses otolitik yang dibantu oleh sel-sel makrofaj, diiringi dengan alveolus-alveolus yang kecut dan bertambahnya tisu perantaraan antara alveolus. Ciri ini jelas pada hari ke 3 selepas cerai susu dan ia bermula dari periferi kelenjar susu. Sel-sel makrofage terdapat dengan banyaknya dalam tisu perantaraan dan dalam saluran alveolus dan duktus alveolus. Pada hari ke 8 alveolus-alveolus tidak dapat lagi dikenali dan pada hari ke 12 selepas cerai susu hanya sisa-sisa saluran dan alveolus sahaja yang kelihatan.

ABSTRACT

The histological changes of mammary glands of guinea pigs during mid and late pregnancy, 1 day after parturition, peak lactation and 2, 3, 4, 8 and 12 days after weaning were studied. Rapid growth of the glandular tissue occurred towards late pregnancy and was completed by peak lactation. Plasma cells occurred in large numbers in the interalveolar connective tissue during the period between mid-pregnancy and lactation. Mammary involution was an autolytic process aided by the action of macrophages accompanied by shrunken alveoli and increased interalveolar connective tissue. This feature was evident at 3 days after weaning which began from the periphery of the glands. The number of macrophages were abundant in the connective tissue and alveolar and ductal lumina. By day 8, the alveoli were no longer recognisable and at 12 days after weaning only remnants of ductules and alveoli were present.

INTRODUCTION

The mammary gland is a modified cutaneous organ (Sisson 1975) with a wide diversity of physiological and biochemical functions. The histological structure of this organ varies throughout the productive life of an animal. The histology of the mammary gland during the productive life of the cow (Feldman, 1961), sheep (Lee and Lascelles, 1970) and rat (Jeffers, 1935a; 1935b Tateyama *et al.*, 1981) have been studied. However relatively little work has been done on the histology of guinea pig's mammary gland during its productive life. The aim of the present investigation is to give a detailed description of the histological changes of

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mammary gland of the guinea pig during well defined stages or pregnancy, lactation and involution with emphasis made on cell population at each stage.

MATERIALS AND METHODS

Animals

A total of 18 guinea pigs of Dunkin-Hartley strain were used in these experiments, 6 of which were pregnant and the remaining 12 were lactating. Two each of the pregnant guinea pigs were sacrificed at 30 – 32 days of pregnancy (mid-pregnancy) two at 55 – 60 days of pregnancy (late pregnancy) and at 1 day after parturition.

Of the 12 lactating guinea pigs, 2 were killed at the peak of lactation that is 6 – 8 days after parturition. The remaining 10 guinea pigs were separated from their pups at the peak of lactation to initiate involution of the mammary glands and two in each group were killed at day 2, 3, 4, 8 and day-12 after weaning.

All the guinea pigs were killed by intraperitoneal administration of an overdose of 23% ethyl carbonate (BDH, United Kingdom). The mammary glands were fixed by perfusion through the external pudendal arteries with 4% glutaraldehyde.

Sampling of Tissue

Each mammary gland was divided into 2 equal halves. One half was used for frozen and araldite sections. The other half was further divided into 3 equal blocks, from medial laterally, and used for paraffin sections.

Araldite Sections

Tissues were post-fixed in 4% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 with 2% sucrose. The tissues were further processed at room temperature through 1% osmium tetroxide in 0.1 M-veronal acetate buffer at pH 7.2 for 1 hr. Tissues were then stained *en bloc* in 2% uranyl acetate in water for 30 min., dehydrated in ethanol and embedded in Araldite. Semi-thin sections were stained with Jeon's stain (Jeon, 1965) for light microsocopy.

Paraffin and Frozen Sections

Paraffin sections 7 um thick were stained with Erlich's haemataxylin and eosin, and Feulgen stain (Pearse, 1968). Plasma cells and mast cells were differentiated by staining sections with Alcian blue and methyl green-pyronin Y (Boxer & Chadwin, 1966). Frozen sections of 10 – 15 um thick were stained with Oil red O (Culling, 1957).

RESULTS

During mid-pregnancy the mammary glands were still small. Histologically, lobules closer to the papillary duct (a single large duct which received smaller ducts draining the lobules) were more developed than lobules closer to the periphery of the glands. These lobules around the papillary duct consisted of alveoli which were slightly distended and their lumina filled with homogenous secretions (*Fig. 1*).

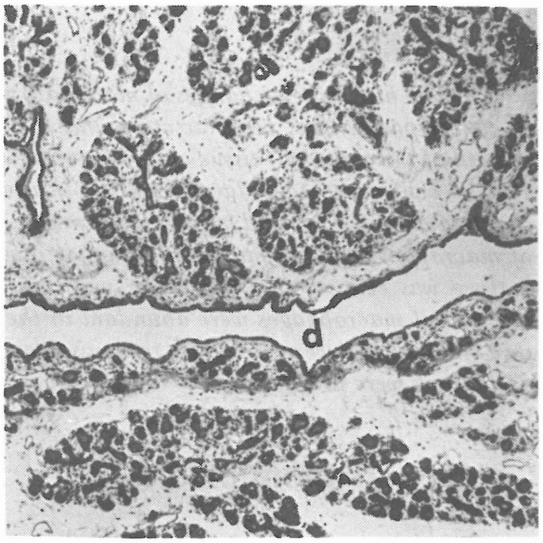

Fig. 1. Mammary gland of guinea pig in mid-pregnancy. Note lobules at the vicinity of the papillary duct (d). Each lobule is separated from one another by loose connective tissue. Note the presence of homogenous secretions within the alveolar lumina. H and E, × 77.

Alveolar epithelial cells were cuboidal with large spherical nuclei and scanty pyroninophilic cytoplasm. At this time pyroninophilic cells with clock-face nuclei (identified as plasma cells) were plentiful in the interalveolar connective tissue (*Fig. 2*).

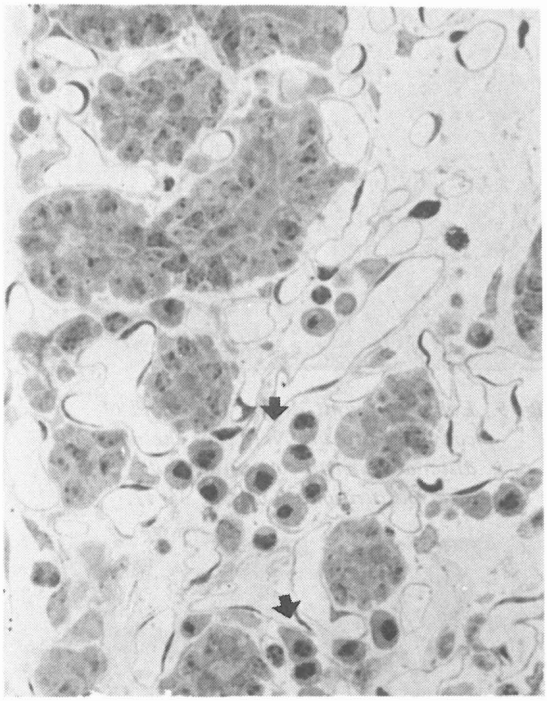

Fig. 2. A lobule with ducts and rudimentary alveoli in mid-pregnant mammary gland of guinea pig. Note the clusters of plasma cells (arrows) in the inter alveolar connective tissue. Araldite section, Azure II, × 194.

Closer to the periphery of the glands, the lobules were smaller and were separated by wide bands of connective tissue. These lobules consisted mainly of ductules terminating into a few alveoli (*Fig. 3*).

By late pregnancy, most of the lobules in the mammary glands were well developed. Fat droplets were observed in the lumina of alveoli and the ducts. Predominantly, neutrophils together with foamy macrophages (Nordin and Lee, 1982) were found in the lumina. In Oil red O stained sections, the alveolar epithelial cells

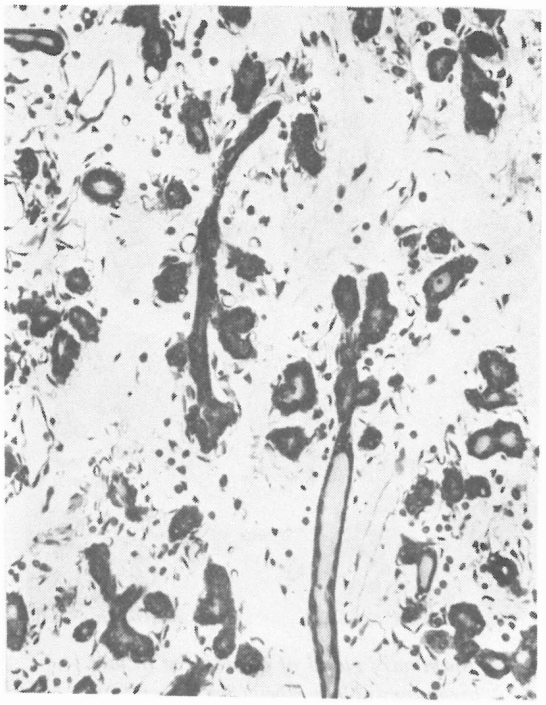

Fig. 3. Lobules at the periphery of the gland are made up of rudimentary alveoli and ductules in the mid-pregnant guinea-pig mammary gland. H and E, × 188.

were seen to contain fat droplets in the apical region. Visible cytoplasm (not occupied by fat droplets) was very pyroniniphilic. The most striking feature of the epithelial cells was the presence of numerous mitotic figures at different stages of division (*Fig. 4*). Some epithelial cells contained 2 nuclei, often referred to as binucleated cells. The location and incidence of plasma cells were similar to those observed during mid-pregnancy.

Twenty four hours after parturition the mammary glands produced copious quantities of milk and became grossly distended. Histologically, the alveoli were markedly distended and filled with secretions (*Fig. 5*) and alveolar epithelial cells appeared stretched and flattened (*Fig. 6*). Plasma cells were still predominant and occurred either singly or in clusters of 2 to 3 cells in close proximity to the basal region of the epithelial cells (*Fig. 6*). Isolated mitotic figures were commonly found.

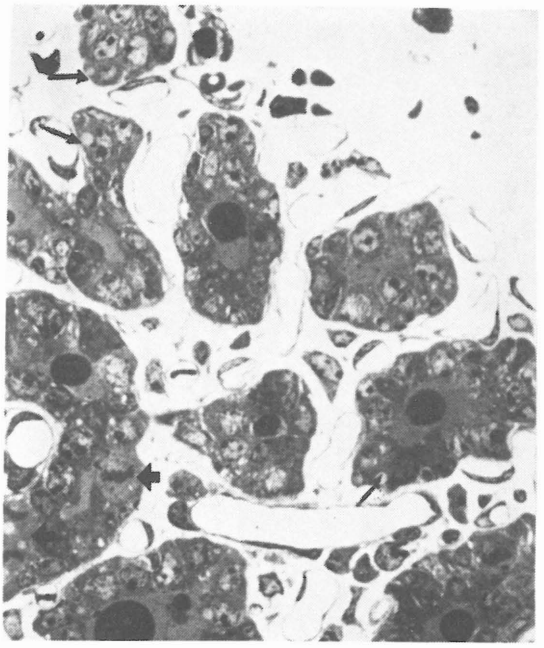

Fig. 4. Mammary gland of guinea pig in late pregnancy. Note alveoli with secretions containing dark staining material. Note the presence of numerous mitotic figures (large arrows) in the epithelial cells. Some of them contain fat droplets (small arrows). Araldite section, Azure II, × 471.

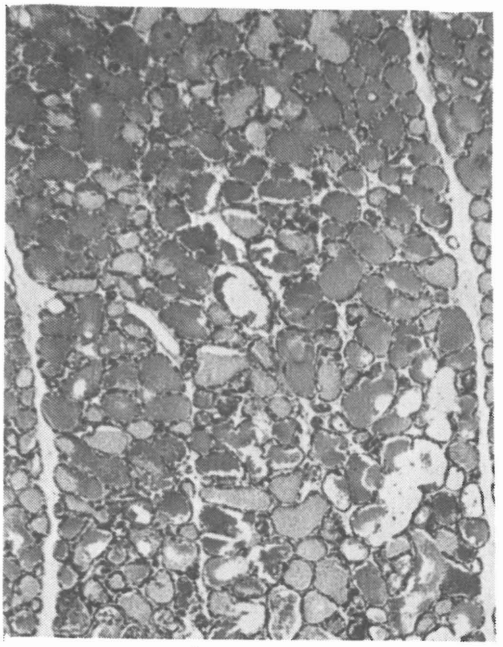

Fig. 5. Lobules separated by a thin band of connective tissue. Note that the markedly distended alveoli containing milk. 24 hrs after parturition, H and E, × 47.

At peak lactation, the mammary glandular structure was basically similar to that observed at 24 hours after parturition. Two days after weaning, the alveoli were still well defined and their lumina were filled with secretion of frothy appearance. The incidence of neutrophils was somewhat increased and occasional lymphocytes and foamy macrophages were also observed. Epithelial cells were cuboidal and filled with fat droplets and eosinophilic granules. Plasma cell was the main cell type seen in the connective tissues between the alveoli.

The size of the glands had involuted considerably at 3 days after weaning. At the periphery of the glands, the alveoli were shrunken and their lumina filled with distinct fatty secretions. Degenerating epithelial cells were a predominant feature of the histological picture and in their lumina, foamy macrophages were more

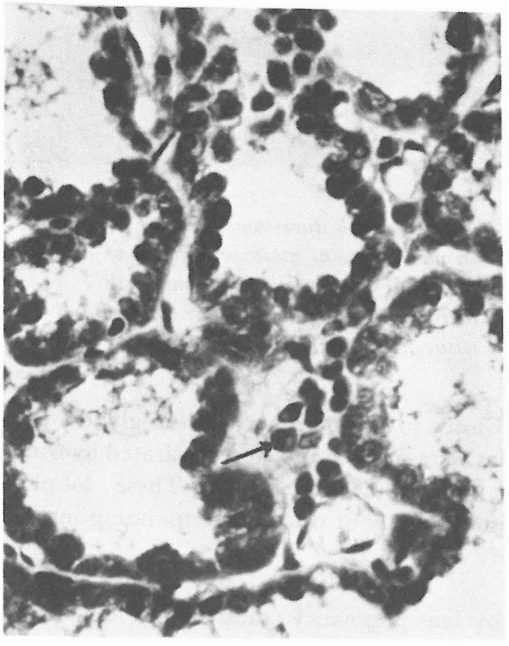

Fig. 6. The alveolar epithelium is flattened. Note that some plasma cells are located at close proximity to the basal region of the epithelium (arrow). 24 hrs after parturition, H and E, × 471.

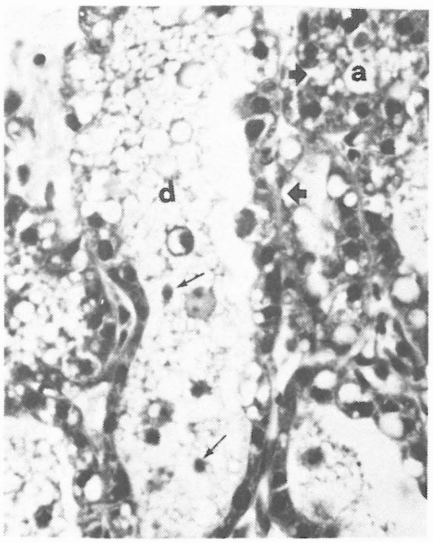

Fig. 7. Alveoli (a) and a ductule (d) at the periphery of the gland. Note that the cellular outline of some epithelial cells are not discernible (large arrows). Within the lumen of the ductule are foamy macrophages and cellular debris (small arrows). 3 days after weaning, H and E × 471.

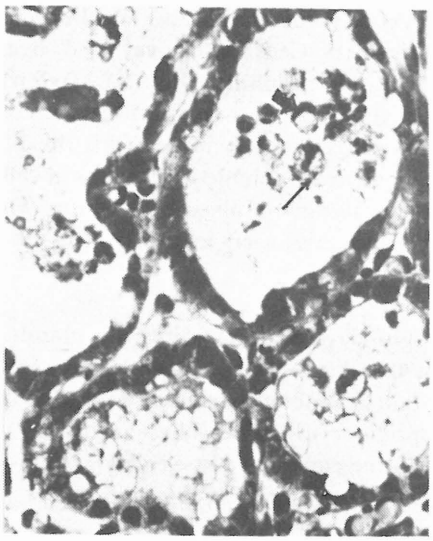

Fig. 8. Alveoli located adjacent to the papillary duct. Note that they are still well defined. Their lumina contain many foamy macrophages (small arrow) and 2 desquamated epithelial cell (large arrow). 3 days after weaning, H and E, × 471.

commonly seen *(Fig. 7)*. Sections stained with Feulgen stain (Pearse, 1968) revealed that the cells in the lumina were karyorrhectic and pyknotic cells.

Closer to the papillary duct, the alveoli were still well defined but in most cases they were not filled with secretion; this apparently had been removed during tissue processing *(Fig. 8)*. Large fat droplets were sometimes seen at the apical region of the epithelial cells and their nuclei were larger and stained lightly. In the lumina, foamy macrophages were abundant *(Fig. 8)*. Desquamated epithelial cells and neutrophils were occasionally observed *(Fig. 8)*.

At 4 days after weaning most of the secretions in the mammary glands had been resorbed as judged by the size of the glands. Alveoli were now difficult to recognise as distinct structures. Epithelial cells contained single fat-filled vacuoles and in the majority of the cells the nuclei were no longer visible *(Fig. 9)*. Connective

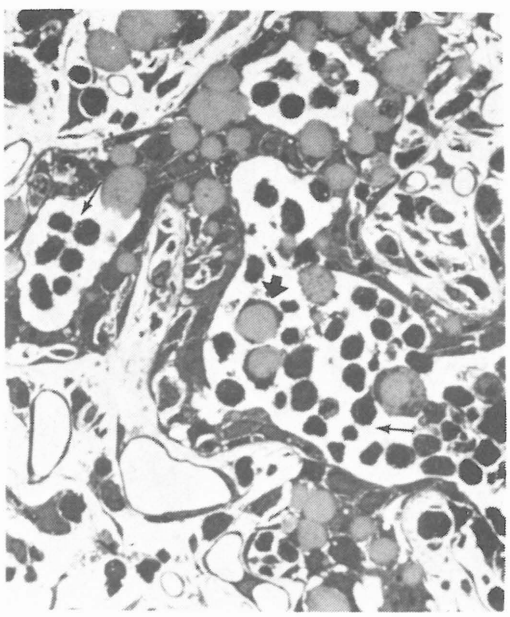

Fig. 9. Alveoli with epithelial cells containing large fat droplets. In the lumina are present numerous macrophages (small arrows) and 2 – 3 desquamated epithelial cells. (large arrow) 4 days after weaning, Araldite section, Azure II, × 486.

tissues between the alveoli and lobules were now more distinct. Cells with oval and irregular shape with alcianophilic cytoplasm (referred as mast cells) were commonly found in the connective tissue in close association with the plasma cells. Foamy macrophage was the main cell type seen in the lumina of alveoli and ducts *(Fig. 9)*. This cell was also seen in the lymphatics *(Fig. 10)*.

Eight days after weaning the glands were almost involuted. Alveolar remnants were seen to contain degenerating cells and large cells with eosinophilic cytoplasm. These cells were also found in the connective tissue *(Fig. 11)*. By now, plasma cells were very few in number.

Twelve days after weaning, the degenerating cells had virtually disappeared and the alveolar and ductular remnants were lined with 1 or 2 layers of closely packed cuboidal epithelial cells *(Fig. 12)*. Foamy macrophages were seldom seen at this time. However, large cells with eosinophilic cytoplasm were commonly seen.

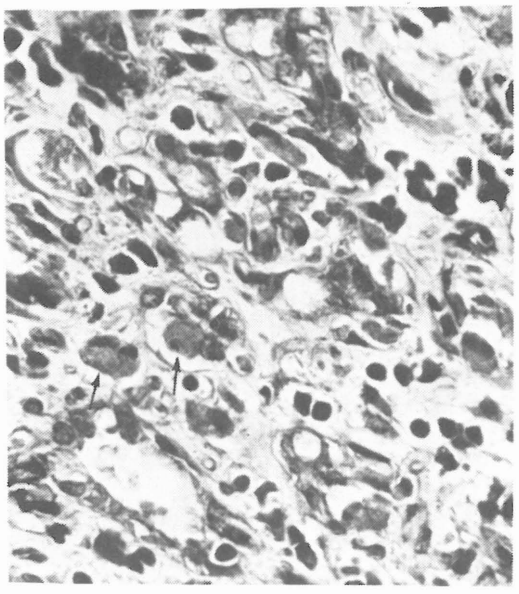

Fig. 11. A lobule with alveoli difficult to recognize as distinct structures. Within the remnants of the alveoli and in the connective tissue are present large eosinophilic cells (arrows). 8 days after weaning, H and E, × 486.

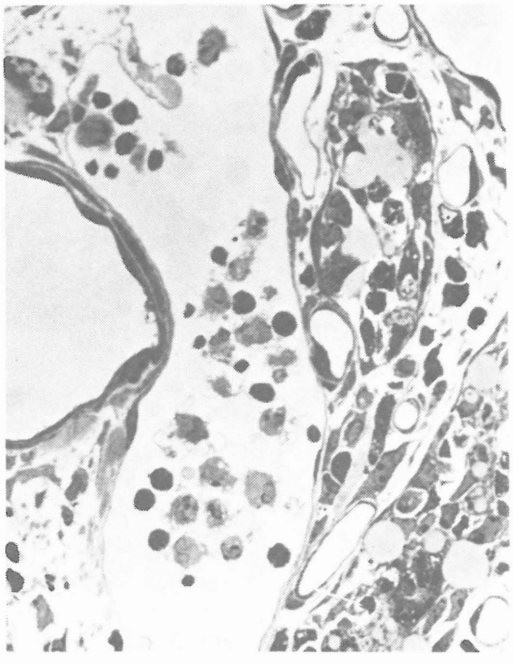

Fig. 10. A large lymphatic vessel containing macrophages and lymphocytes in its lumen. 4 days after weaning. Araldite section, Azure II, × 486.

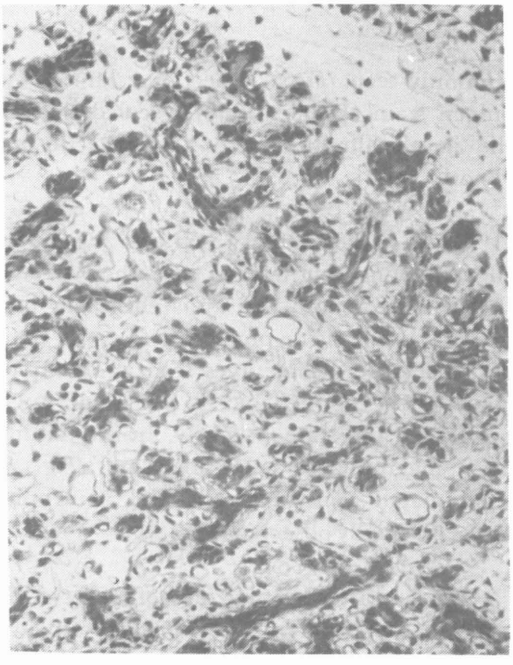

Fig. 12. Showing markedly shrunken lobules containing mainly ductular and alveolar remnants. 12 days after weaning, H and E, × 192.

DISCUSSION

At mid-pregnancy the lobular development was only concentrated around the papillary duct. During this stage the alveolar epithelial cell cytoplasm had already started synthesising protein as evident from its pyronin stain affinity. Secretory activity of these alveoli had also started at this time as their lumina were filled with homogenous secretions.

As parturition approached there was a rapid development of the alveoli as apparent from the numerous mitotic figures present in the alveolar epithelium. Most of the lobules were well developed at this stage and secretory activity of these alveoli had been very vigorous. The alveolar epithelial cell cytoplasm had started to synthesise fat and the alveoli showed a striking enlargement due to the accumulation of secretions containing numerous fat droplets. This is similar to the mouse, where the synthesis of fat also occurred at the end of pregnancy (Tateyama *et al.*, 1981).

After parturition very few isolated mitotic figures were present in the alveolar epithelium which suggested that alveolar development at this time was minimal. This pattern of lobular development after parturition is similar to that described by Nelson *et al.* (1962), in rat (Munford, 1964), in sheep (Anderson, 1979) and in goat (Anderson *et al.*, 1981).

The fatty vacuolation and other degenerative changes in the alveolar epithelial cells observed as involution progressed was essentially similar to those described by other workers who studied involution in rats (Jeffers, 1935a, 1935b), guinea-pigs (Hesselberg and Loeb, 1937) and sheep (Lee *et al.*, 1969; Lee & Lascelles, 1970). On the second day abundant eosinophilic granules accumulated within the cytoplasm of the alveolar epithelium. This was probably protein materials which were not secreted by the cells due to impedance of the secretory mechanisms of the cells as a result of the increased intramammary pressure. Between 3 and 8 days after weaning, the epithelial cells had lost their internal structure; the nucleus became pyknotic and eventually disappeared while the cytoplasm was ultimately replaced by fat. By the twelfth day, the glandular tissue had completely involuted leaving only alveolar and ductular remnants.

Large number of plasma cells were observed in the inter alveolar connective tissue during mid and late pregnancy, lactation and early involution. These cells could be active producers of IgA as this class of immunoglobulin has been reported to be the major class in mammary secretions of this species (McDowell *et al.*, 1971). However, in contrast to the sheep, large numbers of lymphocytes in the interlobular connective tissue during late pregnancy, lactation and involution were observed (Lee *et al.*, 1969; Lee and Lascelles, 1970).

The foamy macrophages observed within the alveoli and ducts during pregnancy could have migrated from the surrounding connective tissue in response to the large amount of fat in the colostrum. During the course of involution these cells persisted in the alveolar and ductal lumina. Lee & Lascelles (1970) made a similar observation in the mammary glands of sheep. It is interesting to note that these cells were seldom seen in the glands at the advance stage of involution where alveoli were mostly dry. This further suggests that the foamy macrophages may have migrated into the lumina only in response to the fat and cellular debris.

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