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On the ultrastructure of the canine mammary gland during pregnancy and lactation

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INTRODUCTION

The Beagle plays an important role as a standard experimental animal in toxicological and cancer research. In the last few years several synthetic gestagens had to be withdrawn from the market as long term administration of certain progestagen and progestagen–estrogen combinations had caused an increased rate of mammary tumours in the Beagle (Frank *et al.* 1979). The relevance of these observations for the human situation is difficult to assess. Contrary to the numerous publications dealing with various aspects of the ultrastructure of normal and diseased human mammary gland (Langer & Huhn, 1957; Takahashi, 1958; Waugh & van der Hoeven, 1962; Toker, 1964; Murad & v. Haam, 1968; Carter, Yardley & Shelley, 1969; Tannenbaum, Weiss & Marx, 1969; Gros & Girardie, 1970; Hagenau & Arnoult, 1959; Stirling & Chandler, 1976) there is comparatively little information on the fine structural changes of the canine mammary gland during gestation and lactation. Ultrastructural studies have been made by Sekhri & Faulkin (1970), Pulley (1973) and Bomhard & Kappes (1975). Sekhri & Faulkin (1970) concentrated on the changes in the mammary gland epithelium and did not pay much attention to the myoepithelial cells. On the other hand Pulley (1973) dealt mainly with the myoepithelial cells which play an important role in the development of mixed mammary tumours in the dog. In these older studies small numbers of animals of unknown age were generally used.

The aim of the present investigation is to give a more detailed description of the ultrastructural changes of the Beagle mammary gland during well defined stages of gestation and lactation.

MATERIAL AND METHODS

Mammary glands of 30 Beagle bitches (between 1 and 3 years old) of the stock of Schering AG were used for this investigation. The animals were randomly placed in 6 groups: primiparous Beagles on days 20, 30, 40, 50 and 60 of pregnancy; and on the third to sixth day of lactation.

The animals were killed by exsanguination following hexobarbital anaesthesia.

Tissue samples, approximately 1 mm thick, were rapidly removed from the mammary glands and immersed in 2 % buffered glutaraldehyde (0·1 M cacodylate buffer, pH 7·2). After one hour the specimens were transferred to 0·1 M cacodylate

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buffer with 7 % sucrose and washed for two hours. After post-fixation in 1 % cacodylate-buffered osmium (pH 7·2) the samples were dehydrated in a graded series of ethanol, immersed in acetone and embedded in ERL.

Semithin sections for light microscopy were stained with 1 % Azur II–methylene blue. Ultrathin sections were cut on a Reichert ultramicrotome (OM U3), routinely stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10A electron microscope.

RESULTS

Day 20

On day 20 of pregnancy (Fig. 1) the parenchyma of the mammary gland consisted of cords of epithelial cells originating from short canalized ducts. Neighbouring epithelial cells were in contact by thin, finger-like cytoplasmic projections which bridged large intercellular spaces. The outstanding features of this stage were the great variation in cellular shape and the large (usually indentated) nuclei which occupied most of the cytoplasm. Many nuclei possessed pronounced nucleoli. The scarce, generally electron-dense cytoplasm contained numerous free ribosomes and a few mitochondria with transverse cristae. The glandular ducts were lined by a stratified epithelium consisting of two or three cell layers (Fig. 2). These cells were of similar appearance to the epithelial cells of the solid cords and exhibited a low degree of differentiation. In this early stage of gestation, a distinction between prospective secretory cells and prospective myoepithelial cells was difficult to establish by their ultrastructural features. Generally, prospective myoepithelial cells were located at some distance from the prospective secretory cells and showed a more electron-dense cytoplasm and a more elongated nucleus.

Day 30

On day 30 (Fig. 3) the proliferation of the glandular parenchyma into the surrounding connective tissue had proceeded further. Dominant features of the epithelial cells were still the variation of their shape, the largeness and irregular outlines of their nuclei, their comparatively electron-dense cytoplasm and their paucity of cellular organelles. Prospective myoepithelial cells (Fig. 4) appeared to make a closer contact with prospective secretory cells.

Day 40

At this stage of gestation the secretory elements had considerably enlarged (Fig. 6) and from this stage onward the glandular parenchyma showed the alveolar arrangement which is regarded as typical for this organ. The glandular epithelium consisted of a luminal layer of predominantly isoprismatic secretory cells and a surrounding layer of flat myoepithelial cells. The myoepithelial cells formed a continuous stratum around the lining cells of the ducts but were discontinuous around the alveoli. The luminal face of the secretory cells bore a few, short, microvilli (Fig. 7). Lateral cell membranes of neighbouring secretory cells were comparatively straight and did not show much interdigitation. Close to the lumen, typical junctional complexes were found (Fig. 7). The nuclei were large and showed a high degree of indentation. Bilobated nuclei were frequently observed.

A narrow rim of heterochromatin was seen lining the inner nuclear membrane and many of the nuclei possessed distinct nucleoli. Rod-shaped or elongated mitochondria (Fig. 7) were evenly dispersed in the cytoplasm and the Golgi apparatus,

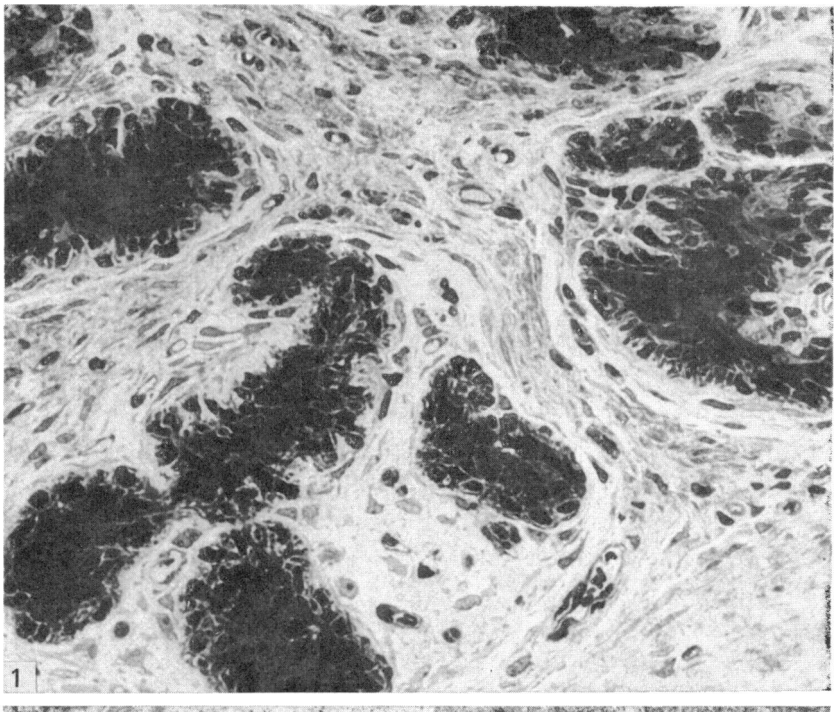
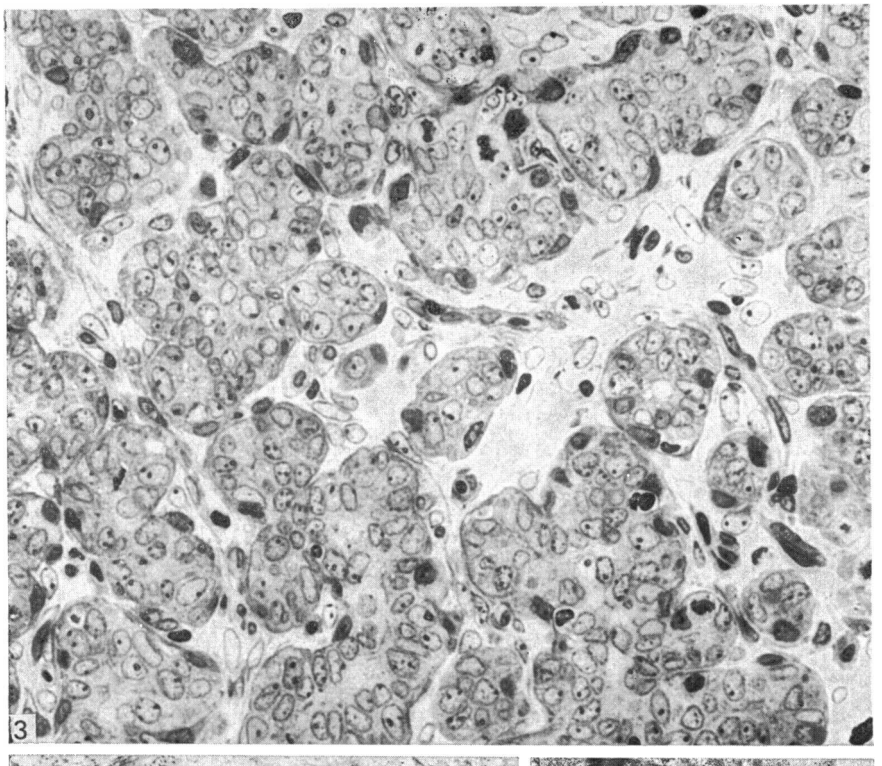
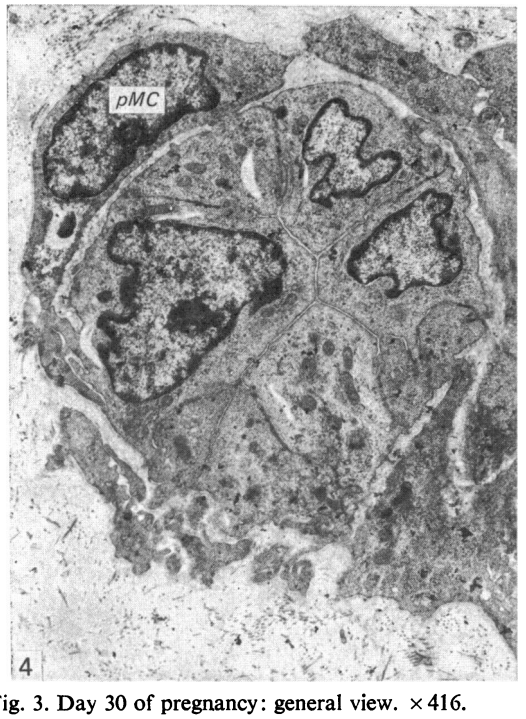


Fig. 1. Day 20 of pregnancy: general view. ×416.

Fig. 2. Day 20 of pregnancy: glandular ducts are lined by two or three layers of epithelial cells. Intercellular channels are present. *L*, lumen. ×7888.





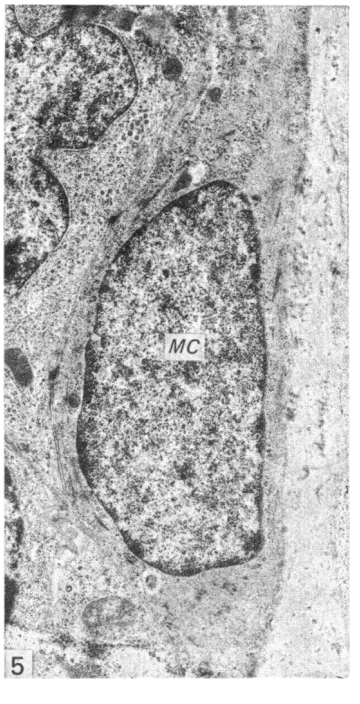
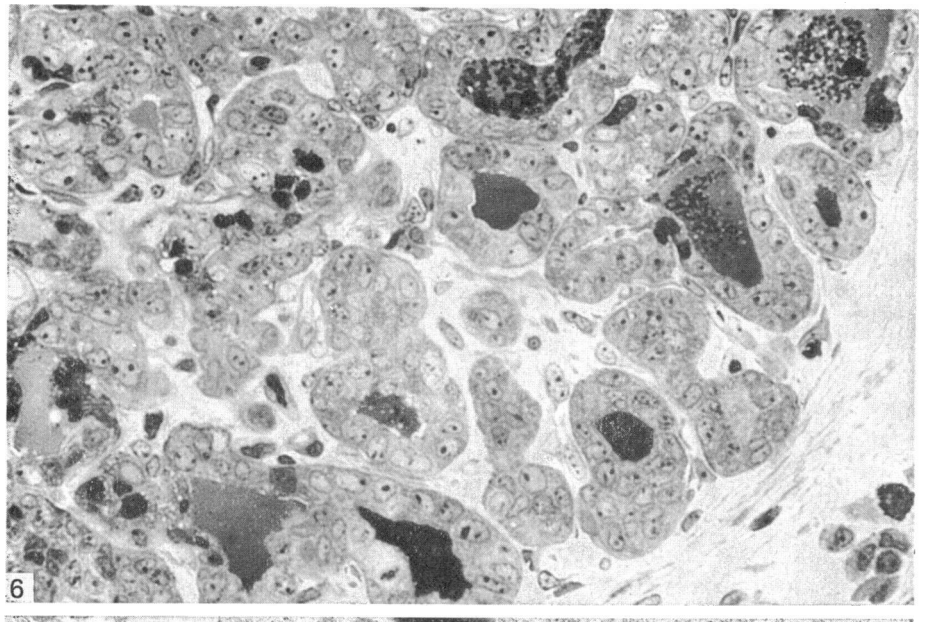


Fig. 3. Day 30 of pregnancy: general view. ×416.

Fig. 4. Day 30 of pregnancy: secretory elements show slit-like lumina; *pMC*, prospective myoepithelial cell. ×6448.

Fig. 5. Day 40 of pregnancy: myoepithelial cells (*MC*). ×7888.



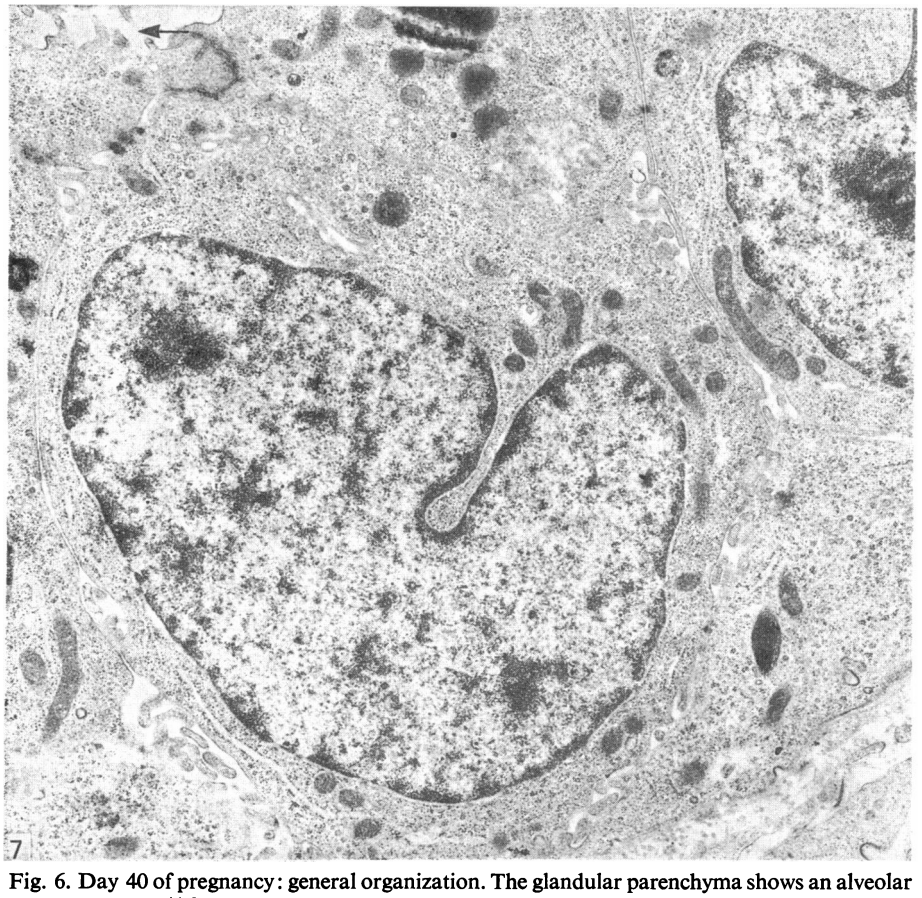


Fig. 6. Day 40 of pregnancy: general organization. The glandular parenchyma shows an alveolar arrangement. ×416.

Fig. 7. Day 40 of pregnancy: the luminal surface of the secretory cells bears a few, short microvilli (arrow). Rod-shaped mitochondria are dispersed in the cytoplasm, which is further characterized by numerous free ribosomes. ×40000.

situated supranuclearly, comprised a few dilated cisternae and vacuoles. The rough endoplasmic reticulum was well developed and numerous free ribosomes occurred in the cytoplasm. In many of the secretory cells small lipid droplets with an average diameter of 1 μm were observed and myelin figures were occasionally present. On day 40, myoepithelial cells were clearly discernible and displayed features of typical contractile 'basket cells'.

Some desmosomes attached the myoepithelial cells to the basal plasma membrane of neighbouring secretory cells (Fig. 5). The nuclei of the myoepithelial cells were oval or elongated and usually smaller than those of the secretory cells. The outstanding feature of the myoepithelial cells from day 40 onward was the abundance of myofilaments which occupied most of the cytoplasm. They were orientated parallel to the long axis of the cells and only the nuclear poles were devoid of them. Here a few mitochondria, a poorly developed Golgi apparatus, some cisternae of rough endoplasmic reticulum and a moderate amount of glycogen granules were observed.

Day 50

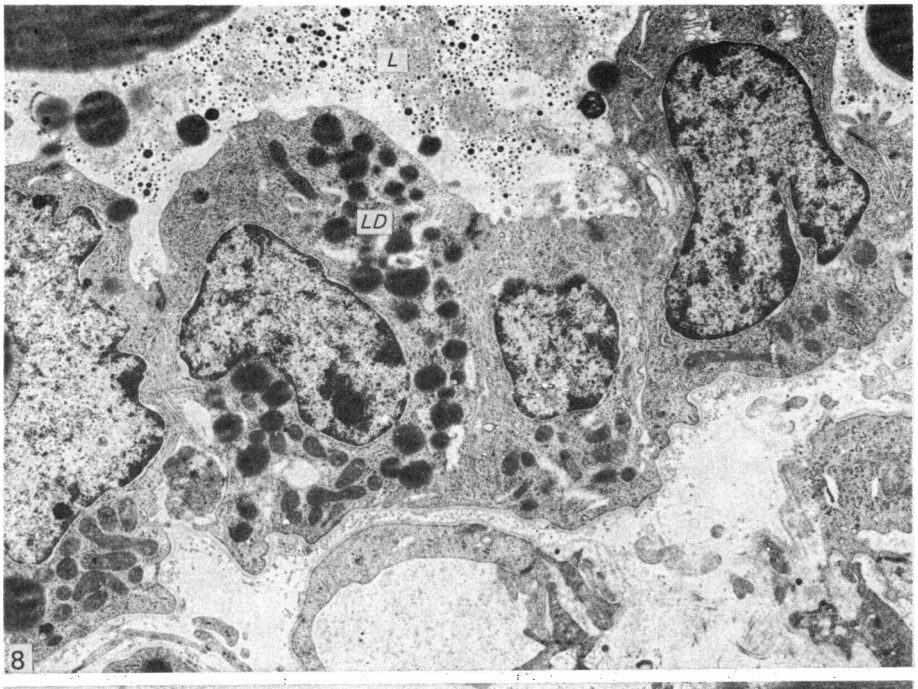
The alveoli were dilated by a large amount of the secretory material which distended their lumina (Fig. 8). The height of the glandular epithelium was reduced (approximately 7·5 μm) as compared to day 40; the nuclei appeared irregularly shaped and more heterochromatic than in earlier stages of gestation. The apical parts of the secretory cells were dome-shaped and protruded into the alveolar lumen. A few short microvilli were occasionally seen at the apical plasma membrane. Many lipid droplets of varying size (0·3–8·0 μm) occupied a large part of the cytoplasm and rough endoplasmic reticulum and Golgi apparatus were well developed. Mitochondria were now mainly concentrated beneath the nuclei; they were oval and approximately 0·5 μm long. Numerous myelin figures, free ribosomes and glycogen granules occurred dispersed in the cytoplasm. A few cells, widely dispersed in the glandular epithelium, displayed a different appearance; they possessed large nuclei, long (1 μm) microvilli and a poorly developed cytoplasm. Myoepithelial cells, similar to those present on day 40, surrounded the secretory cells.

Day 60

This late stage of gestation was characterised by the numerous lipid droplets of varying size (0·5 to 10 μm) which occupied a large area of the apical cytoplasm of the secretory cells (Fig. 9). Many lipid droplets, usually surrounded by some cytoplasmic material, were also found in the lumina of the alveoli. The amount of secretion in the alveoli had obviously further increased as compared with day 50 and had caused an additional distension of the alveoli, accompanied by a reduction of the epithelial height. The nuclei of the secretory cells exhibited irregular outlines and some possessed distinct nucleoli. The hypertrophic Golgi apparatus consisted of a complex system of dilated cisternae and numerous vacuoles and vesicles. The

Fig. 8. Day 50 of pregnancy: alveoli are dilated by a large amount of secretory material within the lumen (*L*). The apical parts of the secretory cells are often dome-shaped and protrude into the lumen. Many lipid droplets (*LD*) occupy large portions of the cytoplasm. × 6448.

Fig. 9. Day 60 of pregnancy: a characteristic feature of the secretory cells are numerous lipid droplets (*LD*) of varying size. The hypertrophic Golgi apparatus (*GA*) comprises dilated cisternae and numerous small vesicles. *MC*, myoepithelial cell. × 6448.



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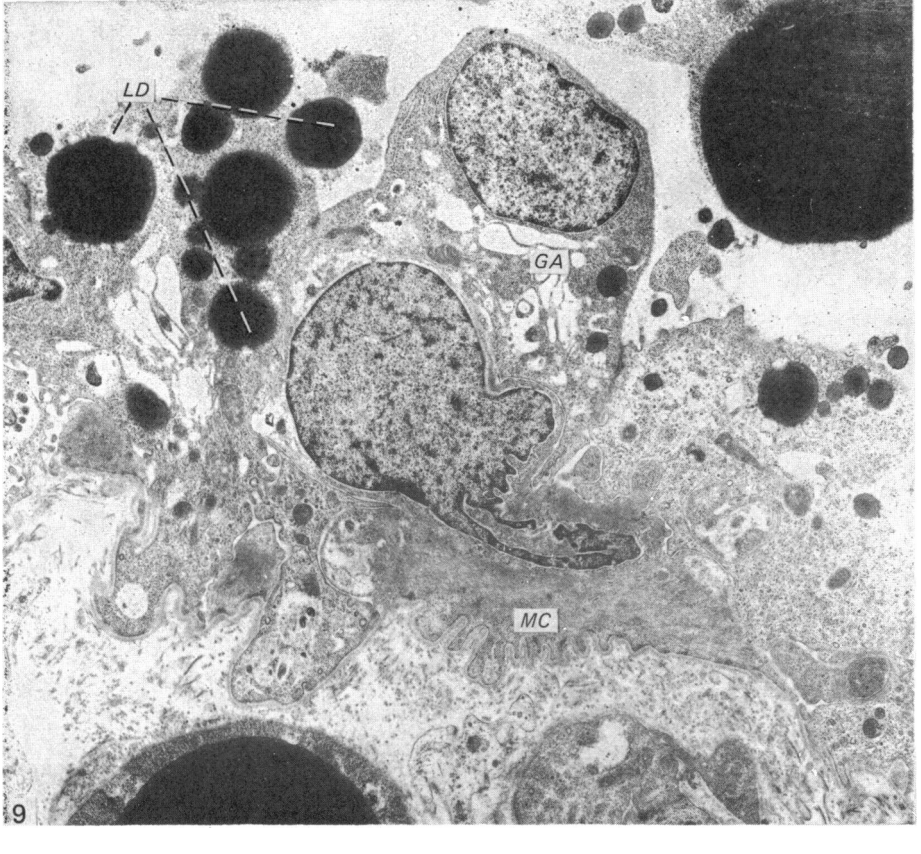

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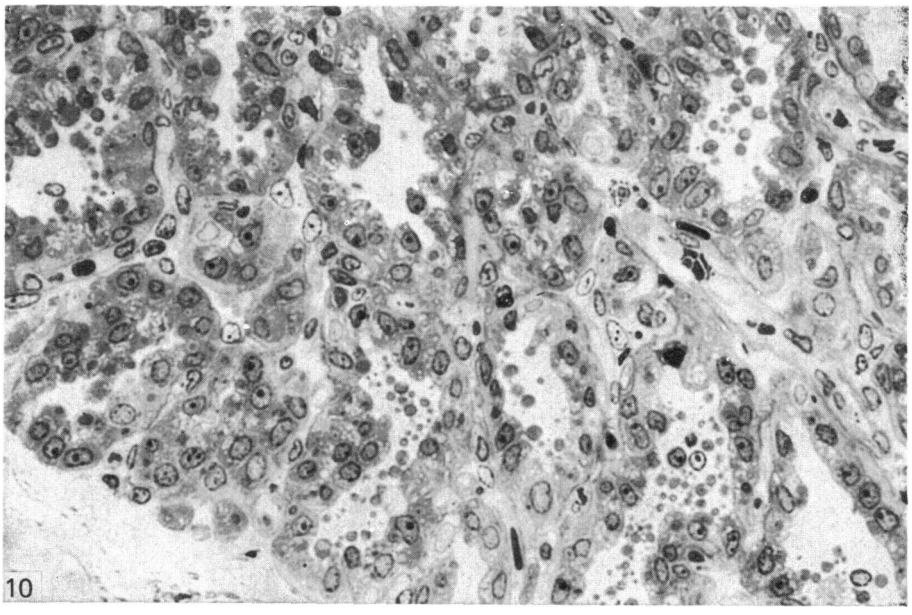


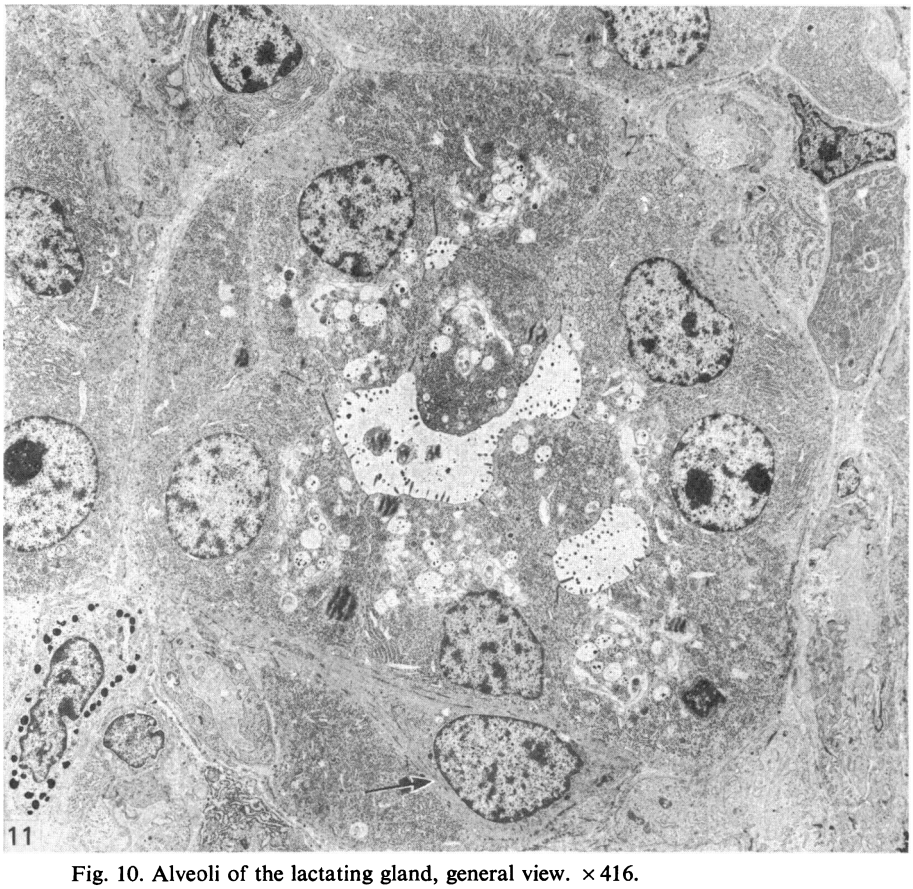
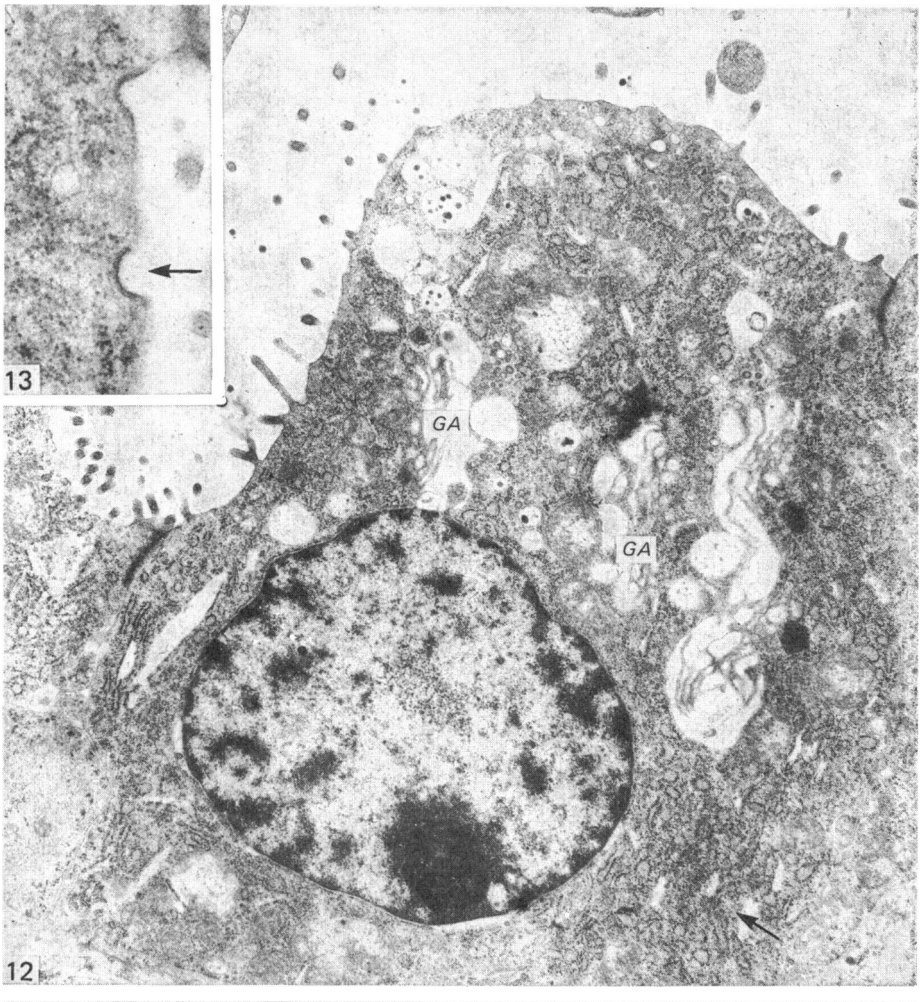


Fig. 10. Alveoli of the lactating gland, general view. ×416.

Fig. 11. Lactating gland; a myoepithelial cell is indicated by the arrow. ×2624.



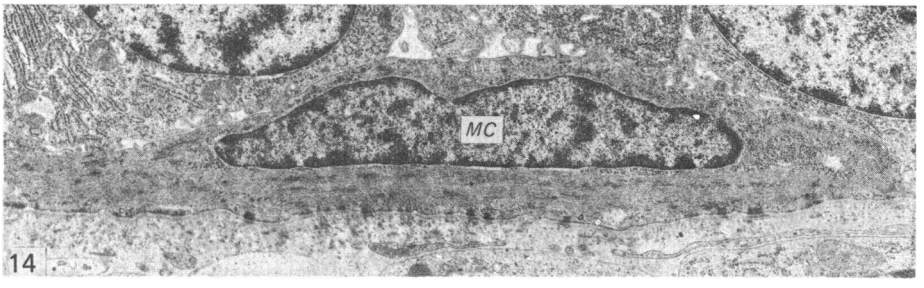


Fig. 12. Lactating gland: during the stage of synthesis of secretory material rough endoplasmic reticulum is abundant (arrow). The Golgi apparatus (*GA*) is also extensively developed. × 10032.

Fig. 13. Lactating gland: secretion of protein granules occurs normally by reversed pinocytosis (arrow). × 32000.

Fig. 14. Lactating gland: myoepithelial cell. × 10032.

cisternae of the rough endoplasmic reticulum were well developed and showed an increasing number of attached ribosomes. Additionally, many free ribosomes were dispersed throughout the cytoplasm.

The myoepithelial cells were basically similar to those seen in earlier stages of gestation but now, due to the general dilation of the alveoli, their shape appeared even more flat and attenuated.

Lactation

Depending on the stage of the secretory cycle the alveoli of the lactating gland showed variation of their form (Figs. 10, 11). Alveolar size, as well as height and ultrastructure of the alveolar epithelium, are markedly influenced by the amount of secretion into the lumina. During the stage of synthesis the rough endoplasmic reticulum was abundant, consisting of well developed cisternae filled with a moderately electron-dense material. Usually the cisternae of the endoplasmic reticulum were arranged in parallel arrays (Fig. 12) and many ribosomes were attached to the cisternae. The Golgi apparatus was well developed (Fig. 12) and varying numbers of mitochondria were evenly dispersed throughout the cytoplasm. The nuclei of the secretory cells were round to oval with a marginal zone of dense chromatin. Many of them showed pronounced nucleoli. In the apical cytoplasm, lipid droplets and (usually) a great number of electron-lucent granules were seen (Figs. 12, 13). The apical plasma membrane exhibited a few short microvilli (Fig. 12). During the stage of storage, epithelial height as well as the number of cellular organelles became reduced.

The myoepithelial cells (Fig. 14) appeared distended and flat; their morphology did not depend on the stage of secretory activity.

DISCUSSION

This investigation shows that the fine structure of the Beagle mammary gland is in many respects similar to that described for other mammals such as the rat (Bargmann & Knoop, 1959; Cowie & Tindal, 1971; Helminen & Ericsson, 1968; Radnor, 1972), mouse (Hollmann, 1959; Bargmann, Fleischhauer & Knoop, 1961), pig (Adamiker & Glawischnig, 1967*a*, *b*), cow (Feldmann, 1961) and man (Gros & Girardie, 1970; Stirling & Chandler, 1976; Langer & Huhn, 1957; Takahashi, 1958; Waugh & van der Hoeven, 1962; Toker, 1964; Murad & v. Haam, 1968; Hagenau & Arnoult, 1959). The glandular parenchyma consists of a luminal layer of secretory cells and the surrounding contractile myoepithelial cells. During pregnancy and lactation the secretory epithelial cells exhibit a marked change in cellular height, distinct variations of shape, size and location of their nuclei and a progressive development of the rough endoplasmic reticulum and Golgi apparatus. The secretory mechanism in the glandular epithelium of the Beagle mammary gland is basically the same as in other species. Lipid droplets are usually sequestered by apocrine extrusion. Occasionally, lipid droplets are pinched off together with some cytoplasmic material. Protein granules are extruded by reversed pinocytosis.

Our results clearly demonstrate that in the Beagle mammary gland active secretory processes take place 10 to 15 days before term. As the secretory material obviously does not leave the alveoli, the mammary gland of the Beagle during the late stages of gestation remains in a phase of physiological milk retention. In the last third of pregnancy and during lactation another special cell type can be found in the glandular

epithelium interspersed between ordinary secretory cells. They possess large nuclei, comparatively long microvilli and a poorly developed endoplasmic reticulum. In a similar location, Wrobel (unpublished observation) observed cells with high activity of non-specific esterases. The function of these interspersed cells is not clear at present but it is possible that they represent a pool of functionally inactive reserve cells.

Although the myoepithelial cells do not show such pronounced changes as the secretory cells, some distinct differences in their ultrastructure can be seen during pregnancy. In early pregnancy, prospective myoepithelial cells can be distinguished from adjacent secretory cells, at the ultrastructural level, only by their basal position immediately adjacent to the basal lamina. Usually their contact with the basal lamina is more extensive than that of the secretory cells. Broad intercellular channels frequently occur between neighbouring myoepithelial cells, but these differences are not very obvious. A clear discrimination of the two cell types can be made from day 40 onwards, where an abundance of myofibrils can be clearly demonstrated in the myoepithelial cytoplasm. The characteristic myofilaments consist of actin and myosin and intermediate filaments (Tateyama, Nosaka & Ashizawa, 1978). Langer & Huhn (1957) have demonstrated many morphological similarities between smooth muscle and myoepithelial cells. In contrast to ultrastructural examination, alkaline phosphatase histochemistry allows reliable identification of negative prospective secretory cells and positive prospective myoepithelial cells as early as day 20 of pregnancy (Wrobel, El Etreby & Günzel, 1974).

In the last third of pregnancy and during lactation, myoepithelial cells have adopted a characteristic arrangement. Whereas myoepithelial cells completely surround the epithelium of the excretory ducts, they form a discontinuous layer around the alveoli. The morphological changes observed in myoepithelial cells during pregnancy suggest that these cells are, like the secretory cells, subject to hormonal control.

SUMMARY

During pregnancy and lactation marked changes are observed in the fine structure of the secretory cells in the Beagle mammary gland: especially pronounced are differences in cellular height, shape and size of the nuclei and distribution of mitochondria. In later stages of pregnancy a proceeding development of those cellular organelles involved in synthesis and extrusion of secretory material (i.e. rough endoplasmic reticulum, Golgi apparatus) can be observed. Myoepithelial cells which can be first discerned from secretory cells by ultrastructural features from day 40 on show only minor variations of their ultrastructure during pregnancy and lactation.

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