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Ultrastructural changes in porcine mammary tissue during lactogenesis*

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INTRODUCTION

Ultrastructural changes occurring in mammary tissue at lactogenesis have been studied in several species including the rat (Chatterton, Harris & Wynn, 1975), rabbit (Bosquet, Glechon & Denamur, 1969), dog (Sinowatz, Wrovel, Etreby & Sinowatz, 1980) and cow (Feldman, 1961; Saake & Heald, 1974). Histological and metabolic changes occurring in porcine mammary tissue during pregnancy and lactation are reported by Kensinger *et al.* (1982). Wooding (1977) has briefly described the relative amounts of organelles present in mammary tissue of the pig on Days 30 and 111 of pregnancy and during lactation.

Lactation failure is a major economic problem in the swine industry and contributes to neonatal mortality in this species (Cross, Goodwin & Silver, 1958; Penny, 1970; Martin, Hartmann & Gooneratne, 1978). However, little is known concerning the structural changes occurring in mammary tissue before or after parturition in swine. The current study was conducted to describe in greater detail the cytological as well as the ultrastructural changes which occur in porcine mammary tissue during the periparturient period.

MATERIALS AND METHODS

Tissue for ultrastructural analysis was obtained by biopsy as previously reported (Kensinger *et al.* 1982). At biopsy, gilts were anaesthetised with sodium thiamylal and anaesthesia was maintained with metofane. In all 27 gilts, a single mammary tissue biopsy of approximately 6 g was taken from the penultimate inguinal gland on the right side. Mammary biopsies on the day of parturition were performed 4 to 12 hours after the last pig was born. Tissue was obtained from three animals each on Days 90, 105, 112 of pregnancy, day of parturition (Day 115) and Day 4 of lactation. These days were chosen because Kensinger *et al.* (1982) determined that, by Day 90 of pregnancy, near maximum concentrations of DNA had been attained in the porcine mammary gland. Examination of RNA concentrations and RNA:DNA ratios indicated that after Day 90 of pregnancy the mammary epithelial cells underwent differentiation as they acquired the capacity to synthesise and secrete milk.

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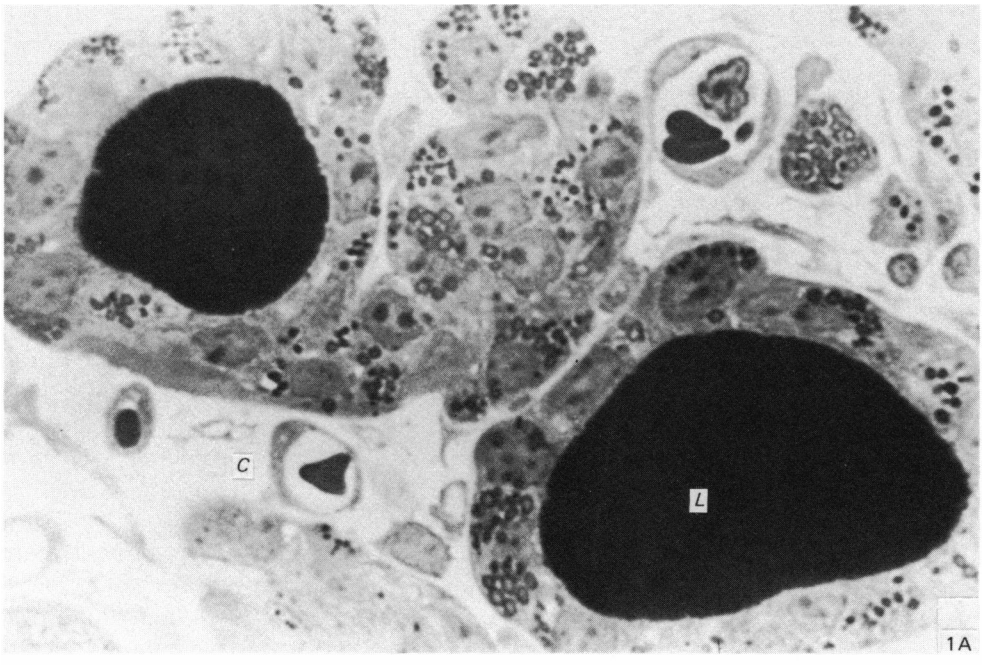
Therefore, tissues from Days 90, 105, and 112 of pregnancy, the day of parturition, and Day 4 of lactation were used for ultrastructural examination.

Immediately after biopsy, tissue was cut into pieces approximately 1 mm^2, and fixed for 2 hours in 2 % glutaraldehyde buffered at pH 7·4 with 0·2 M Sym-Collidine (2,4,6-trimethylpyridine; Polysciences, Inc., Warrington, PA). After a 1 hour rinse in Sym-Collidine buffer, tissues were postfixed in Sym-Collidine buffered osmium tetroxide (2 %), pH 7·4, for an additional one hour. Tissues were then dehydrated in a graded series of ethanol solutions, equilibrated in acetone, and embedded in Spurr's low viscosity embedding medium (Polysciences, Inc.). Subsequently, 1 μm thick sections of tissues were made with an LKB Ultratome III ultramicrotome, stained with toluidine blue, and observed with a Leitz light microscope. Ultrathin sections (60–100 nm) were then made, post-stained in uranyl acetate and lead citrate, and examined with an Hitachi HU11E electron microscope.

RESULTS

Mammary tissue from a Day 90 pregnant pig is shown in Figure 1A. Two alveoli with a single layer of epithelial cells surrounding the lumina were visible. Also present were developing alveolar structures composed of solid balls of cells which had not developed distinct lumina. There were also two blood vessels containing red blood cells and a lymphocyte (Fig. 1A). Preservation of the normal biconcave structure of erythrocytes indicated that no appreciable osmotic shock had occurred during the fixation process. Secretions present in these tissues stained densely due to the high protein concentration. In the basal portion of several cells, areas of the cytoplasm were occupied by small lipid droplets (Fig. 1A, B). A typical mammary epithelial cell on Day 90 of pregnancy (Fig. 1B) had a large, irregularly shaped nucleus with one or two prominent nucleoli. A few lipid droplets were present in the basal portion of some cells (Fig. 1B), and numerous oval and rod shaped mitochondria with visible cristae were scattered throughout the cytoplasm (Fig. 1B). Some agranular endoplasmic reticulum was present in the basal cytoplasm of the cell, but there was very little granular endoplasmic reticulum and numerous free ribosomes were observed. The Golgi apparatus was a series of stacked plates and was located in a paranuclear region of the cell. No Golgi vacuoles or secretory vesicles were apparent and the apical cell membrane contained a few, short, club shaped microvilli.

Thick sections of mammary tissue at Day 105 of pregnancy (Fig. 2A) showed an increased area occupied by alveolar lumina. At this time, all alveoli in the field contained densely staining secretion. Other features were an increase in size of the cytoplasmic lipid droplets and an increased number of lipid droplets within the alveolar lumina (Fig. 2A). Electron microscopy on Day 105 (Fig. 2B) suggested that the process of differentiation or lactogenesis had begun. Secretory cells assumed a more cuboidal appearance as secretions began to accumulate within the lumina. Nuclei were more regular in shape (Fig. 2B), and although the size of the lipid droplets had increased, they still occupied either a basal or paranuclear position. The Golgi apparatus assumed a more supranuclear position, but no secretory vesicles were seen. The cytoplasm was more electron-dense than on Day 90; higher magnifications (not shown) due to an increased number of polysomes, but very little agranular endoplasmic reticulum was present. The apical cell membrane still contained only a few short microvilli (Fig. 2B).



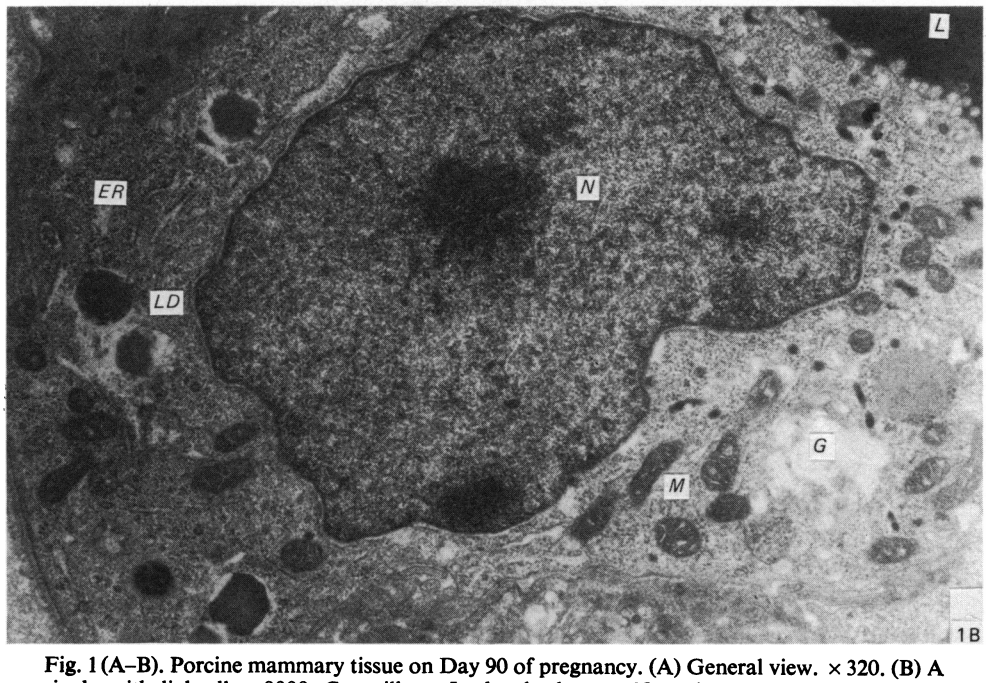


Fig. 1 (A–B). Porcine mammary tissue on Day 90 of pregnancy. (A) General view. × 320. (B) A single epithelial cell. × 9000. *C*, capillary; *L*, alveolar lumen; *N*, nucleus; *ER*, agranular endoplasmic reticulum; *LD*, lipid droplet, *G*, Golgi membranes; *M*, mitochondrion.

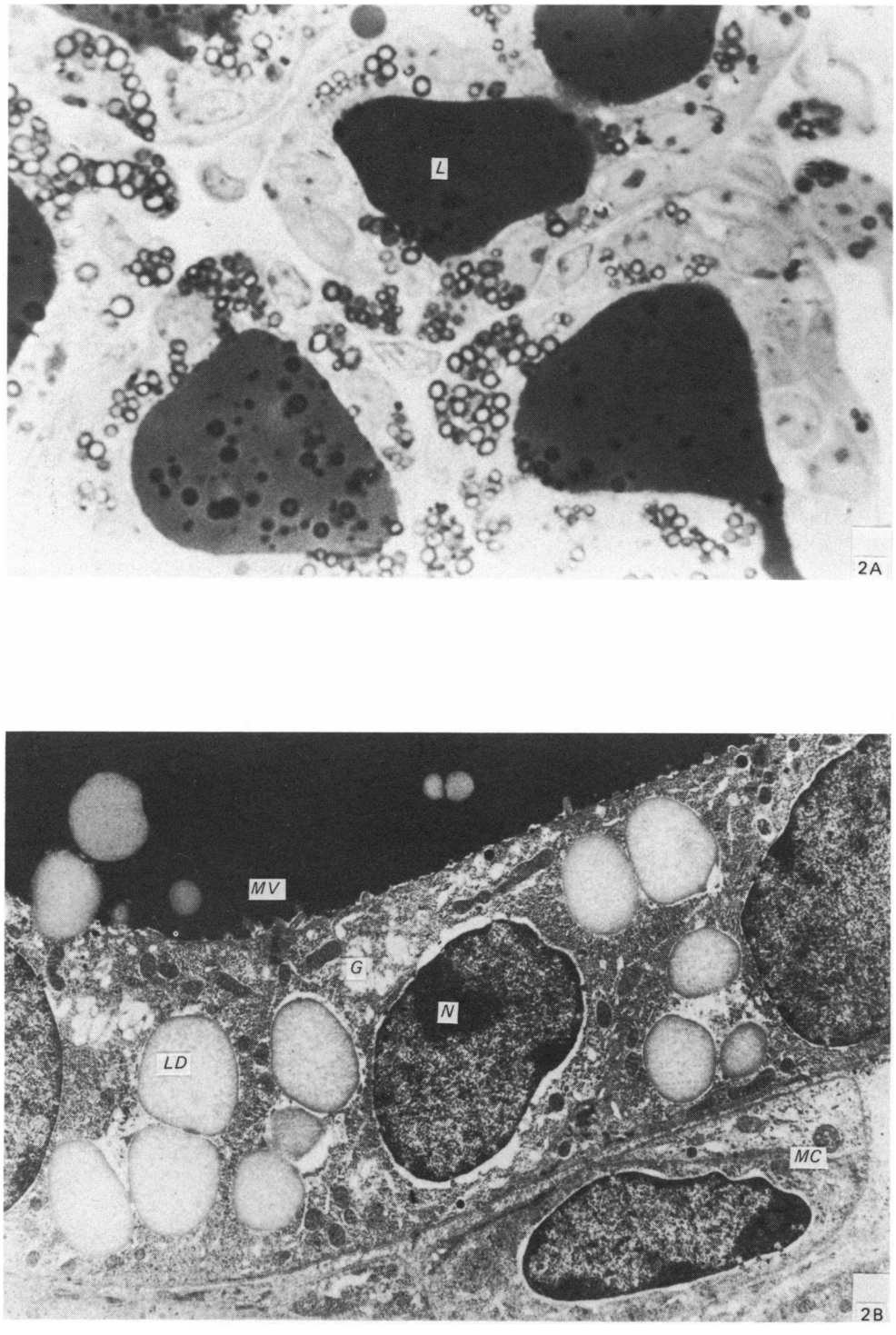


Fig. 2(A–B). Porcine mammary tissue on Day 105 of pregnancy. (A) Several alveoli. × 320. (B) An epithelial and a myoepithelial cell. × 4600. *L*, alveolar lumen; *MV*, microvilli; *G*, Golgi membranes, *LD*, lipid droplet; *N*, nucleus; *MC*, myoepithelial cell.

By Day 112 of pregnancy (Fig. 3A), the cytoplasm was dominated by lipid droplets and there was a slight increase in the number of lipid droplets present in the secretion. However, the processes of synthesis and secretion were not yet fully coupled as the cells retained the majority of the lipid synthesised (Fig. 3A). On Day 112, the mammary epithelial cell nuclei, although still somewhat irregular in form, appeared to occupy a smaller portion of the cytoplasm than previously (Fig. 3B). Numerous mitochondria were observed in all parts of the cytoplasm. While there were numerous polysomes throughout the cytoplasm, granular endoplasmic reticulum was observed in the basal aspects of some cells (Fig. 3B). A large portion of the cytoplasm was occupied by lipid droplets and some were in the process of being extruded from the cell. Although it was difficult to detect discrete areas of Golgi apparatus, the appearance of densely staining protein granules within the secretion suggested that the Golgi was functional to some degree (Fig. 3B). Microvilli on the apical cell membrane had elongated by Day 112 compared to those in tissue from Day 90 and Day 105.

Mammary biopsies on the day of parturition were performed approximately 6 hours after the last piglet was born and after the litter had nursed the dam. Consequently, several micrographs of tissue taken at the day of parturition (Fig. 4A) showed alveoli with relatively small lumina. Secretions within the majority of these lumina did not stain densely, but had the appearance of normal milk, suggesting that the colostrum had been removed. Nuclei of the epithelial cells were rounded and lipid droplets were evident in the apex of most cells (Fig. 4A). With the electron microscope (Fig. 4B), milk of normal appearance could be observed within the lumina which contained numerous lipid droplets and electron-dense protein granules. Nuclei were rounded and the cytoplasm was rich in organelles. Cells had considerable amounts of granular endoplasmic reticulum in the basal cytoplasm and adjacent to the nucleus. There were well defined areas of expanded Golgi apparatus lateral and apical to the nucleus and lipid droplets accumulated in the apical ends of the cells (Fig. 4B). Microvilli on the apical cell membrane had elongated compared to three days earlier, coincident with the process of active milk secretion.

Mammary tissue on Day 4 of lactation was characterised by alveoli with very large lumina (Fig. 5A), suggesting that the tissue was very active in milk synthesis and secretion. Indeed cells lining some alveoli were almost squamous in appearance. Most nuclei were rounded and had prominent nucleoli and the apex of most cells contained numerous lipid droplets (Fig. 5A). Secretions within the lumina contained numerous densely staining granules and lipid droplets of various sizes. Ultrastructural examination of mammary secretory cells on Day 4 of lactation showed that the cytoplasm was rich in organelles (Fig. 5B). Dilated cisternae of the granular endoplasmic reticulum were present throughout the cell but were particularly concentrated in the basal cytoplasm. The Golgi apparatus was well developed with numerous dilated vacuoles and secretory vesicles present in the supranuclear region (Fig. 5B). In addition, numerous microvesicles were observed just inside the apical membrane of the cell. Secretory vesicles containing numerous condensed protein granules were being transported toward the lumina and elongated microvilli were present on the apices of all secretory cells. At this stage, many cells had lipid droplets which were in the process of being extruded into the lumen suggesting that synthesis and secretion were fully coupled in cells, a feature typical of early lactation.

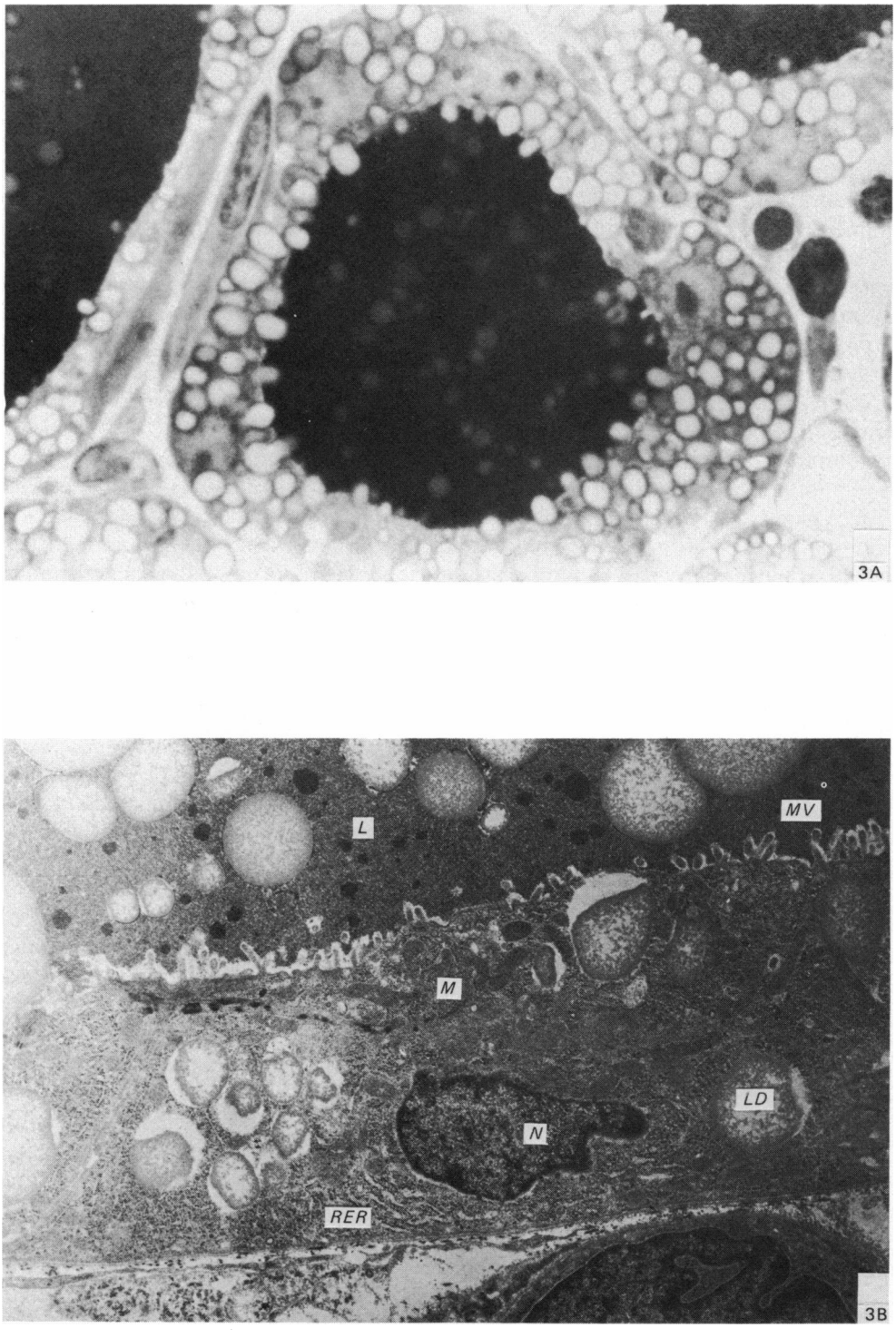
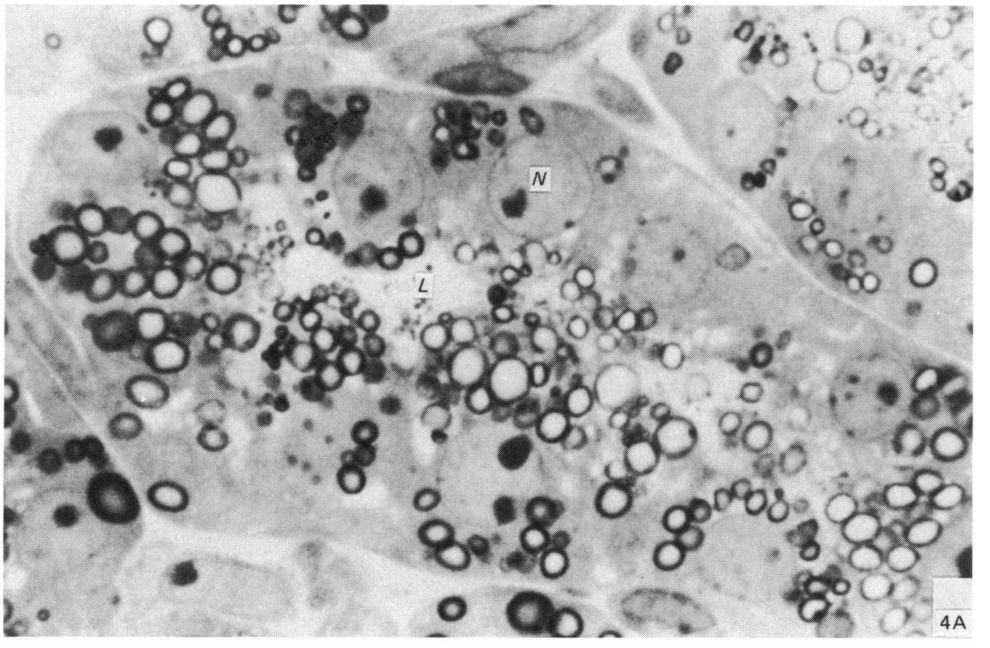


Fig. 3 (A–B). Porcine mammary tissue on Day 112 of pregnancy. (A) A single alveolus. × 400. (B) An epithelial cell. × 4600. *L*, alveolar lumen; *MV*, microvilli; *M*, mitochondria; *N*, nucleus; *LD*, lipid droplet; *RER*, granular endoplasmic reticulum.



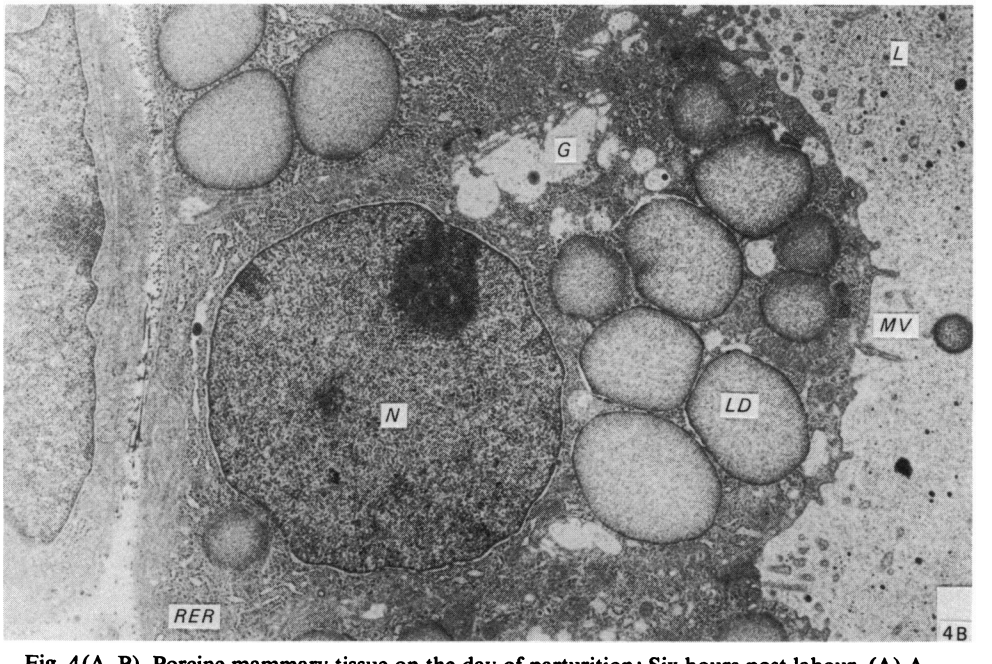
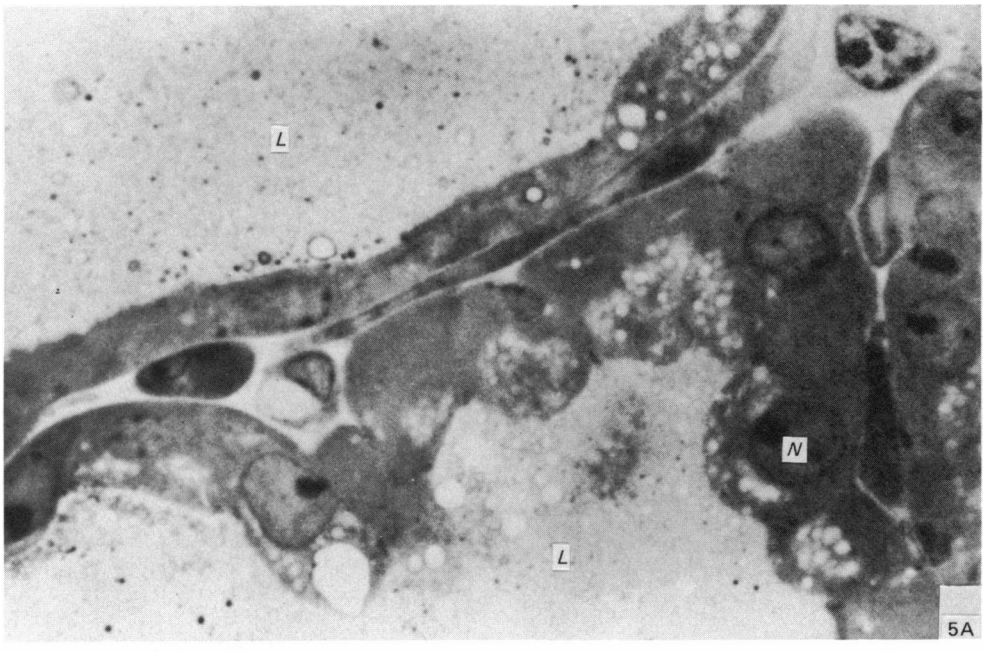


Fig. 4(A–B). Porcine mammary tissue on the day of parturition: Six hours post labour. (A) A single alveolus. ×400. (B) A single secretory cell. ×6300. *N*, nucleus; *L*, alveolar lumen; *G*, Golgi membranes; *MV*, microvilli; *LD*, lipid droplet; *RER*, granular endoplasmic reticulum.



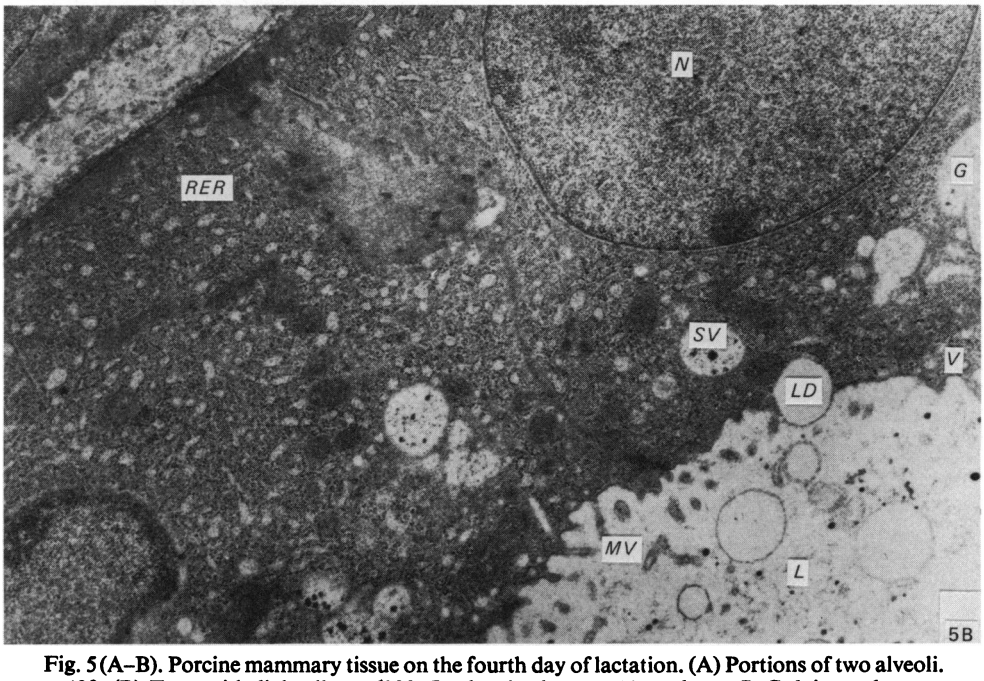


Fig. 5 (A–B). Porcine mammary tissue on the fourth day of lactation. (A) Portions of two alveoli. ×400. (B) Two epithelial cells. ×6300. *L*, alveolar lumen; *N*, nucleus; *G*, Golgi membranes; *RER*, granular endoplasmic reticulum; *SV*, secretory vesicle; *LD*, lipid droplet; *V*, microvesicle; *MV*, microvilli.

DISCUSSION

Observations on mammary tissue from Day 90 of pregnancy are similar to the descriptions of midpregnancy mammary epithelium made by Hollmann (1974) and are consistent with those of Kensinger *et al.* (1982) which suggested that differentiation of epithelial cells of the mammary gland had not yet occurred. Changes in cellular organelles, compared to Day 90 mammary tissue, agreed with the increased RNA concentration observed in the previous study by Kensinger *et al.* (1982). Increased area occupied by alveolar lumina suggest that in the pig, as in the dog (Sinowatz *et al.* 1980), some secretion of colostrum takes place as early as 10 days prepartum.

The overall appearance of porcine mammary tissue on Day 112 apparently is similar to mammary tissue from several species in the 'immediate prepartum period'. Such tissue has been characterised as having little development of the granular endoplasmic reticulum, few secretory vesicles, and a large number of lipid droplets within the cell (Wooding, 1977). These ultrastructural observations are also consistent with changes in nucleic acids and metabolic activity. While increasing concentrations of RNA on Day 112 (Kensinger *et al.* 1982) suggest that cells are acquiring the cytoplasmic assemblies for protein synthesis necessary for lactation, estimates of lipid synthesis and oxygen consumption indicate that these processes are still much less active than is observed during lactation. Retention of the majority of *de novo* lipid by mammary tissue on Day 112 of pregnancy, three days prepartum, is consistent with events in the prepartum rat mammary gland (Chatterton *et al.* 1975), indicating that the processes of synthesis and secretion are not yet coupled.

Observations from tissue on the day of parturition indicate the appearance of milk within lumina along with lipid droplets and protein granules. Mammary tissue from the day of parturition, with the requisite arrangement of granular endoplasmic reticulum and Golgi complex, exhibits the distinct cellular polarity characteristic of lactating mammary epithelial cells (Saake & Heald, 1974).

The overall appearance of cells on Day 4 of lactation suggests that ultrastructural differentiation is complete. This is supported by the tremendous increase in rates of lipogenesis and substrate oxidation which have been reported previously for the same tissue samples by Kensinger *et al.* (1982). Results from the present study also agree with observations made by Wooding (1977) that there is a large increase in granular endoplasmic reticulum and Golgi vesicles and a pronounced decrease in lipid droplets in lactating porcine mammary tissue compared to tissue from Day 111 of pregnancy.

Martin and coworkers (1978) measured lactose in mammary secretions and progesterone and corticoids in plasma of sows. They concluded that "initiation of lactation occurred within 24 h of parturition". Results from the present study indicate that lactogenesis in the pig is a two stage process similar to that in other species (Strong & Dils, 1972; Mellenberger, Banman & Nelson, 1973; Mellenberger & Bauman, 1974*a*, *b*). Stage 1 of lactogenesis, or the initiation of structural and metabolic differentiation, begins between Days 90 and 105 of pregnancy. Stage 2 is the onset of copious milk secretion and occurs just prior to parturition, as concluded by Kensinger *et al.* (1982) and Martin and coworkers (1978). It is indicated by distinct structural changes in porcine mammary tissue as observed in the present study. Endocrine regulation of these processes in the pig has yet to be elucidated.

SUMMARY

Ultrastructural changes occurring in porcine mammary tissue were characterised between Day 90 of pregnancy and Day 4 of lactation. Porcine mammary tissue on Day 90 of pregnancy was composed of alveoli which contained negligible to moderate amounts of secretion. Epithelial cells of these alveoli were relatively undifferentiated.

The appearance and distribution of cellular organelles suggested that mammary epithelial differentiation had been initiated by Day 105 of pregnancy in the pig. A further increase in intracellular lipid droplets and granular endoplasmic reticulum suggested that differentiation had progressed by Day 112. On the day of parturition, secretions within the alveolar lumina assumed the appearance of normal milk (as opposed to colostrum) and the epithelia displayed a distinct cellular polarity characteristic of lactating mammary tissue. By Day 4 of lactation, differentiation of epithelial cells appeared to be complete, with dilated cisternae of the granular endoplasmic reticulum and with numerous secretory vesicles. Elongated microvilli were present and numerous cells contained lipid droplets which were being extruded into the lumina. Data from this and previous studies indicate that lactogenesis in the pig occurs in two stages. Stage 1 occurs between Days 90 and 105 of pregnancy, and Stage 2 between Days 112 of pregnancy and early lactation when the predominant feature is active milk secretion.

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