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A morphological study of the changes which occur during pregnancy in the human breast

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Summary. In this study the structural changes which occur during human pregnancy were examined by light and electron microscopy. Pregnancy was associated with proliferation and differentiation of the epithelial cells within the lobules. Proliferation was continuous throughout pregnancy with a progressive increase in the size of the lobules. The highest level of mitosis was observed in the first trimester with lower levels in the second and third trimesters. Unexpectedly a number of apoptotic cells were observed during pregnancy. Differentiation was initiated in the second trimester with an increase in the amount of rough endoplasmic reticulum and the appearance of a hypertrophic Golgi body and lipid droplets within a number of epithelial cells. A number of small vacuoles were present close to the apical plasmalemma of a few epithelial cells. As the pregnancy proceeded there was an increase in the number of cells exhibiting these features. There was also an increase in the size of the lipid droplets and the number of apical vacuoles. The apical vacuoles which have not been described previously range in size from 150–600 nm with the contents of the larger vacuoles having a whorled or labyrinth-like appearance.

Key words: Breast – Human – Pregnancy – Ultrastructure – Morphology

The human breast undergoes marked changes during pregnancy with the controlled proliferation and enlargement of the lobular units in preparation for the synthetic and secretory activity which occurs during lactation. There have been few reports in which the fine structure of the pregnant breast has been examined (Waugh and Van der Hoven 1962; Toker 1967; Salazar et al. 1975). However, these studies do not examine in detail the progressive

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changes which occur during pregnancy. Information of this kind may assist in our understanding of the physiological development prior to lactation and also the normal reaction of the target tissue to given hormonal changes.

In this paper we describe the ultrastructural changes which occur during pregnancy as observed using both scanning and transmission electron microscopy and correlate these with the light microscopic appearances.

Materials and methods

In this study samples of normal appearing breast tissue were obtained at biopsy from eight pregnant women. The biopsies were performed for clinical reasons, the histopathology being reported as fibroadenoma in 4 cases, fibrosis in two and no significant features in two. Details of their age, parity, and stage of pregnancy are given in Table 1.

Light microscopy. The tissue was fixed in Carson's fluid (Carson et al. 1973), dehydrated in ethanol and embedded in glycol methacrylate (GMA). Sections were cut at 1 μm and stained with either haematoxylin and eosin or Feulgen counter stained with fast green (Ferguson and Anderson 1981a).

Transmission electron microscopy. The tissue was processed as described previously (Ferguson and Anderson 1981b and c) but can be summarised as follows: the tissue was fixed in glutaraldehyde, post fixed in osmium tetroxide and embedded in Araldite. The thin sections were stained with uranyl acetate and lead citrate prior to examination with either an AEI Corinth 275 or a Philips 400 electron microscope.

Scanning electron microscopy. Samples of tissue approximately 10 mm × 10 mm × 4 mm were excised and fixed in either Carson's fluid (Carson et al. 1973) or 3% glutaraldehyde in cacodylate buffer pH 7.2. After a primary fixation of at least 4 h the tissue blocks were either sliced to produce a new face or chopped at 200 μm using a Sorval TC-2 Tissue Sectioner. In both cases, the tissue was post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 h and dehydrated through a graded acetone series. At this stage the tissue blocks and slices were examined using a dissecting microscope. It was found that parenchymal structures were stained black with the osmium while the connective tissue was grey/white in appearance. Blocks or slices in which the cut surface passed through lobules or ducts were selected. The samples were critical point dried, mounted on stubs, and sputter coated with gold/paladium. The blocks and slices were then examined using the Cambridge Stereoscan S180 scanning electron microscope.

Results

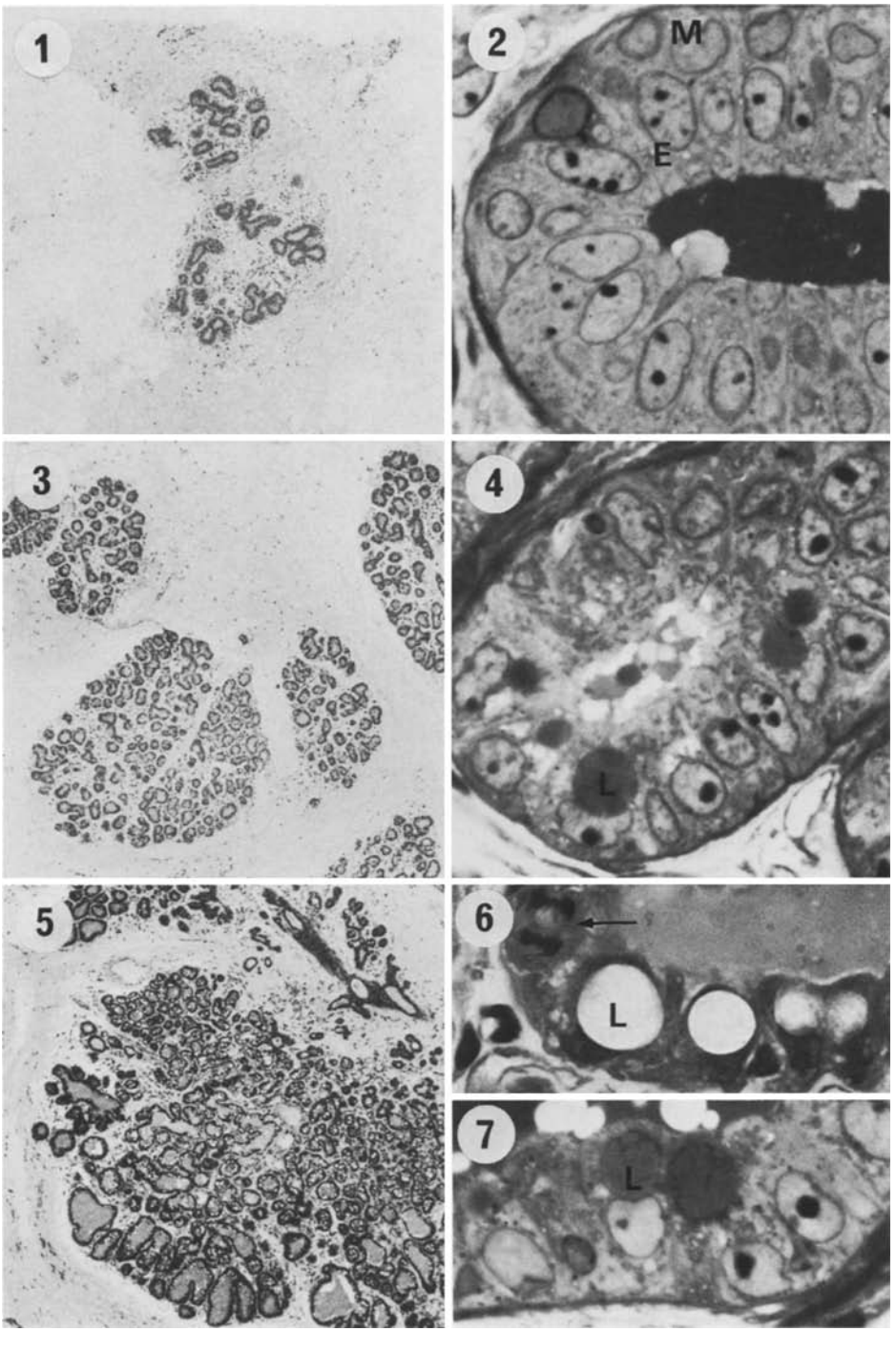
Light microscopy. The breast tissue from patients in the first trimester of pregnancy was found to contain lobules which were similar to those observed in the 'resting' breast (Ferguson and Anderson 1981a). The average number of ductules per lobule was within the range observed in the 'resting' breast tissue (Anderson et al. 1982) with two of the pregnant patients having relatively small lobules (Table 1). The constituent ductules were loosely packed (Fig. 1). The epithelial cells appear as a single layer with a distinct layer of underlying myoepithelial cells and a few clear cells (Fig. 2).

During the second and third trimesters the lobules increase in size (cf Figs. 3 and 5) and are comprised of a higher number of tightly packed ductules[1] (Table 1). The most obvious histological change was the presence

[1] At this stage the lobular subunits could be termed acini but to prevent confusion the term ductule is retained throughout the paper

Table 1. Details of the pregnant women

No.	Age	Preg/Weeks	Parity	Ductules/Lob	MIT/LOB	APT/LOB	MIT/Duct	APT/Duct
1	26	7	0+0	8.8	1.6	0.0	0.18	0.0
2	26	8	1+0	8.3	1.02	0.8	0.123	0.097
3	32	8	0+0	20.5	1.0	0.3	0.049	0.015
4	31	21	3+0	31.2	0.35	0.35	0.012	0.011
5	21	22	0+0	39.0	0.41	0.23	0.01	0.006
6	22	24	0+0	45.8	0.6	0.6	0.013	0.013
7	22	28	0+0	41.2	0.56	0.17	0.014	0.004
8	32	34	2+0	45.4	1.13	0.44	0.025	0.01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of large translucent vacuoles in the anterior cytoplasm of a number of epithelial cells. In toluidine blue stained araldite sections, these could be identified as lipid droplets (Fig. 4). The development of the lipid droplets was not synchronised and varied between and within lobules. It was also noted that there was a difference in the degree of differentiation shown in the breast tissue between patients in the second trimester. By the third trimester the majority of the epithelial cells of the ductules possessed large lipid droplets (Figs. 6 and 7) while the epithelial cells of the ducts and extralobular terminal ducts were negative for lipid globules.

Throughout this development it was possible to identify both mitotic (Fig. 6) and apoptotic cells within the lobules. These events were quantified by comparing the average number of events per lobule and per ductule (Table 1). The latter ratio took into account variation in lobular size with stage of pregnancy and was used in comparisons between trimesters. In the first trimester there were elevated levels of mitosis with lower levels of apoptosis. In all 3 patients the level of mitosis was equivalent to the highest values observed at the mitotic peak in the 'resting' breast (cf Table 1 and Fig. 1, Ferguson and Anderson 1981a). In the second trimester there was a diminished level of mitosis with an equivalent level of apoptosis and a similar pattern was maintained in the third trimester (Table 1).

Electron microscopy. Ultrastructurally the ducts and ductules of the first trimester were similar in appearance to those reported for the 'resting' breast (Stirling and Chandler 1976 and 1977). The epithelial cells varied between cuboid and columnar and contained a nucleus, a number of mito-

Figs. 1–24. The morphological features of human pregnancy are illustrated by light microscopy (Figs. 1–7), SEM (Figs. 8–10) and TEM (Figs. 11–24). The following abbreviations are used: *A* apical vacuoles; *Ca*, capillary; *D*, desmosome; *E*, epithelial cell; *ER*, rough endoplasmic reticulum; *G*, Golgi apparatus; *I*, intermediate junction; *L*, lipid droplet; *Lu*, lumen; *M*, myoepithelial cell; *Mi*, mitochondrion; *N*, nucleus; *T*, tight junction

Fig. 1. Lobules from the 1st trimester of pregnancy. H & E stained/GMA embedded ×65

Fig. 2. Detail of a ductule from the 1st trimester showing the distinct epithelial and myoepithelial cell layers. Toluidine blue stained/Araldite embedded ×1,600

Fig. 3. Lobules from the 2nd trimester. H & E stained/GMA embedded ×65

Fig. 4. Detail of a ductule from the 2nd trimester showing a few of the epithelial cells containing lipid droplets. Toluidine blue stained/Araldite embedded ×1,600

Fig. 5. Lobules from the 3rd trimester. H & E stained/ GMA embedded ×65

Fig. 6. Detail of part of a ductule from the 3rd trimester showing the epithelial cells with large clear vacuoles. An epithelial cell can also be seen undergoing mitosis. H & E stained/GMA embedded ×1,600

Fig. 7. Part of a ductule showing that the large vacuoles within the epithelial cells are lipid droplets. Toluidine blue stained/Araldite embedded ×1,600

chondria, a small Golgi body, a few strands of rough endoplasmic reticulum (rER) (Fig. 11) with a few of the cells also containing small lipid droplets. At this stage there was no polarisation of the organelles (Fig. 11). Between the epithelial and myoepithelial cells a few wandering cells (monocytes, macrophages and lymphocytes) were observed. These cells are probably equivalent to the clear cells seen with the light microscope. The myoepithelial cells were conical in appearance with a flattening along the basal lamina (Fig. 11). These cells formed an outer layer with few of the epithelial cells in direct contact with the basal lamina. The SEM provided little information on the lobules at this stage due to the very narrow lumen of the ductule. However, in the larger ducts the luminal surface of the epithelial cells was shown to possess short microvilli (Fig. 8). The microvilli were particularly concentrated at the cell junctions thus outlining the hexagonal packing of the cells (Fig. 8). The intralobular stroma comprised collagen fibres, fibroblasts, plasma cells, monocytes and capillaries. At this stage the epithelial stromal junction (ESJ) (Ozzello 1970) was intact.

During the second trimester the epithelial cells showed a slight increase in the number of mitochondria and a marked increase in the amount of rER which in certain cases formed parallel arrays or whorls (Figs. 13 and 15). The Golgi body was much more extensive and appears active with the formation of large and small vesicles (Fig. 14). In addition a large number of the cells contained lipid droplets of various sizes (Fig. 13). The synthesis of these bodies did not appear to be associated with any particular organelle (Fig. 15). At this stage there was a polarisation of certain of these structures with the larger lipid droplets situated in the luminal cytoplasm with the parallel arrays of rER present in the basal cytoplasm (Fig. 13). In addition a number of the cells contained either single or groups of randomly distributed large vacuoles containing varying amounts of osmiophilic material (Fig. 16). In the luminal cytoplasm of a few of the epithelial cells were small vacuoles. These apical vacuoles were of variable size with the smaller ones (150–400 nm) containing homogenous dense material (Fig. 21) while the material within the larger vacuoles (400–600 nm) had a characteristic whorled or labyrinth-like appearance (Figs. 20, 22, 23 and 24).

In the third trimester the number of epithelial cells possessing lipid droplets increased (Fig. 19). The average size of the lipid droplets also increased to occupy most of the anterior cytoplasm (Figs. 10 and 19). In addition the apical vacuoles were more numerous (Fig. 19) and in certain cases these vacuoles were observed fusing with the plasmalemma and releasing their contents into the lumen. With the SEM it was seen that the majority of epithelial cells had a flat luminal surface with a few short microvilli and small blebs (Figs. 9 and 10). Throughout pregnancy the apical junctional complex remained intact (Fig. 12).

During this development there was a progressive flattening of the myoepithelial cell with the development of attenuated cytoplasmic processes forming an open meshwork around the epithelial cells. Within the cytoplasm there was a marked increase in the number of myofilaments with associated dense bodies (Fig. 18). During gestation the myoepithelial cells proliferate

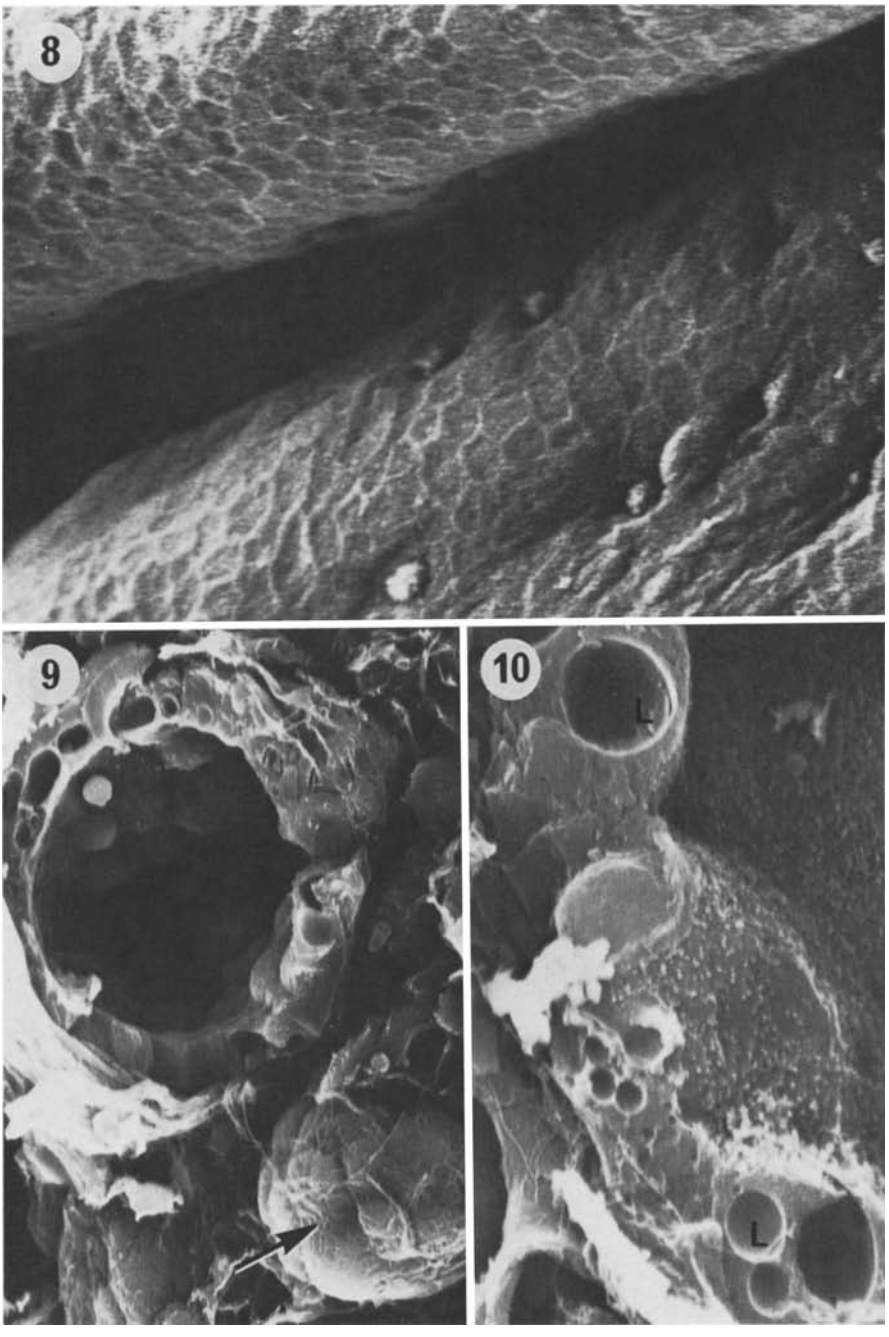

Fig. 8. Part of a large duct showing the surface appearances of the epithelial cells. × 3,000

Fig. 9. Part of a lobule in which cut and uncut (*arrow*) ductules can be seen. × 2,100

Fig. 10. High magnification showing the luminal surface of the ductule with short microvilli and the cut portion of the epithelial cells exhibiting lipid droplets. × 9,000

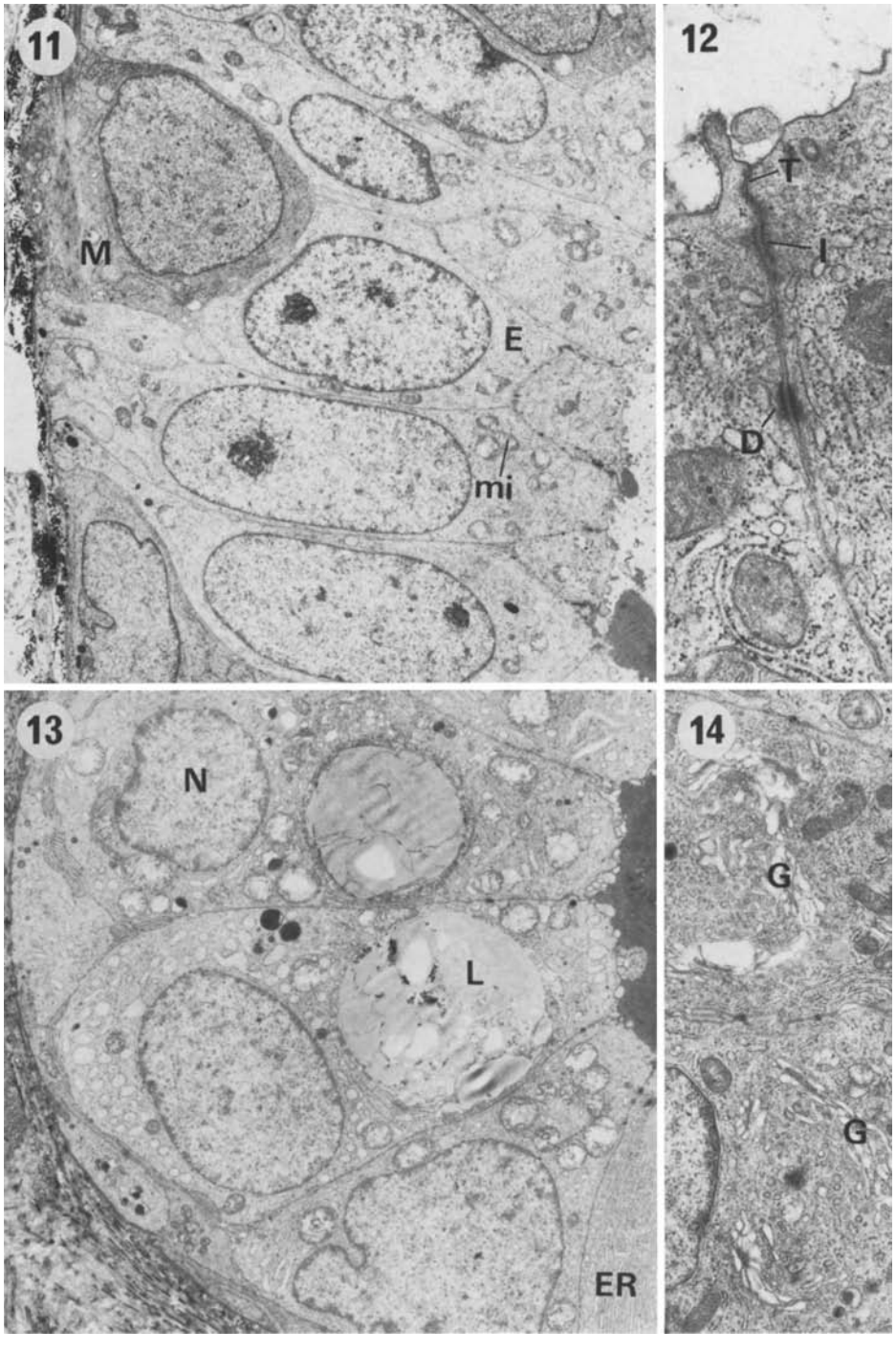

Fig. 11. A section through part of a ductule from a patient in the 1st trimester showing the epithelial and myoepithelial cells. × 4,000

Fig. 12. Detail showing the anterior junctional complex between epithelial cells. × 26,000

Fig. 13. Part of a ductule from the 2nd trimester showing epithelial cells containing large lipid droplets and parallel arrays of rER. × 4,000

Fig. 14. Portion of two epithelial cells showing extensive Golgi bodies (2nd trimester). × 10,000

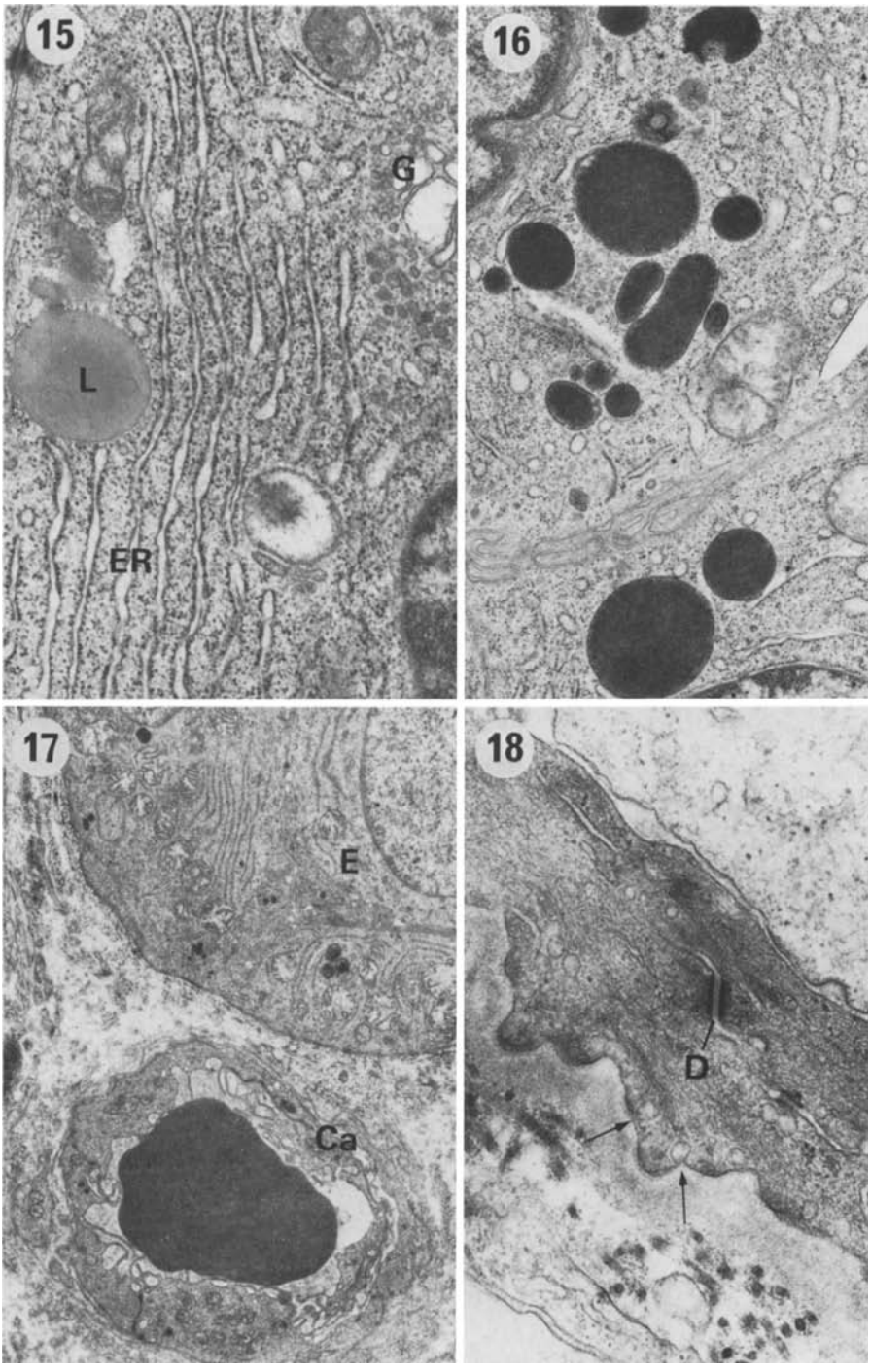

Fig. 15. A detail of part of an epithelial cell showing the rER, Golgi body and a small lipid droplet (2nd trimester). × 27,000

Fig. 16. Vacuoles of various size with osmiophilic contents present within the cytoplasm of epithelial cells. × 17,000

Fig. 17. In this section a capillary can be seen lying adjacent to a ductule with no intervening fibroblast. × 5,000

Fig. 18. Parts of two myoepithelial cells joined by a desmosome are illustrated. The cytoplasm is packed with myofilaments and pinocytotic vesicles are forming along the plasmalemma (*arrows*). × 40,000

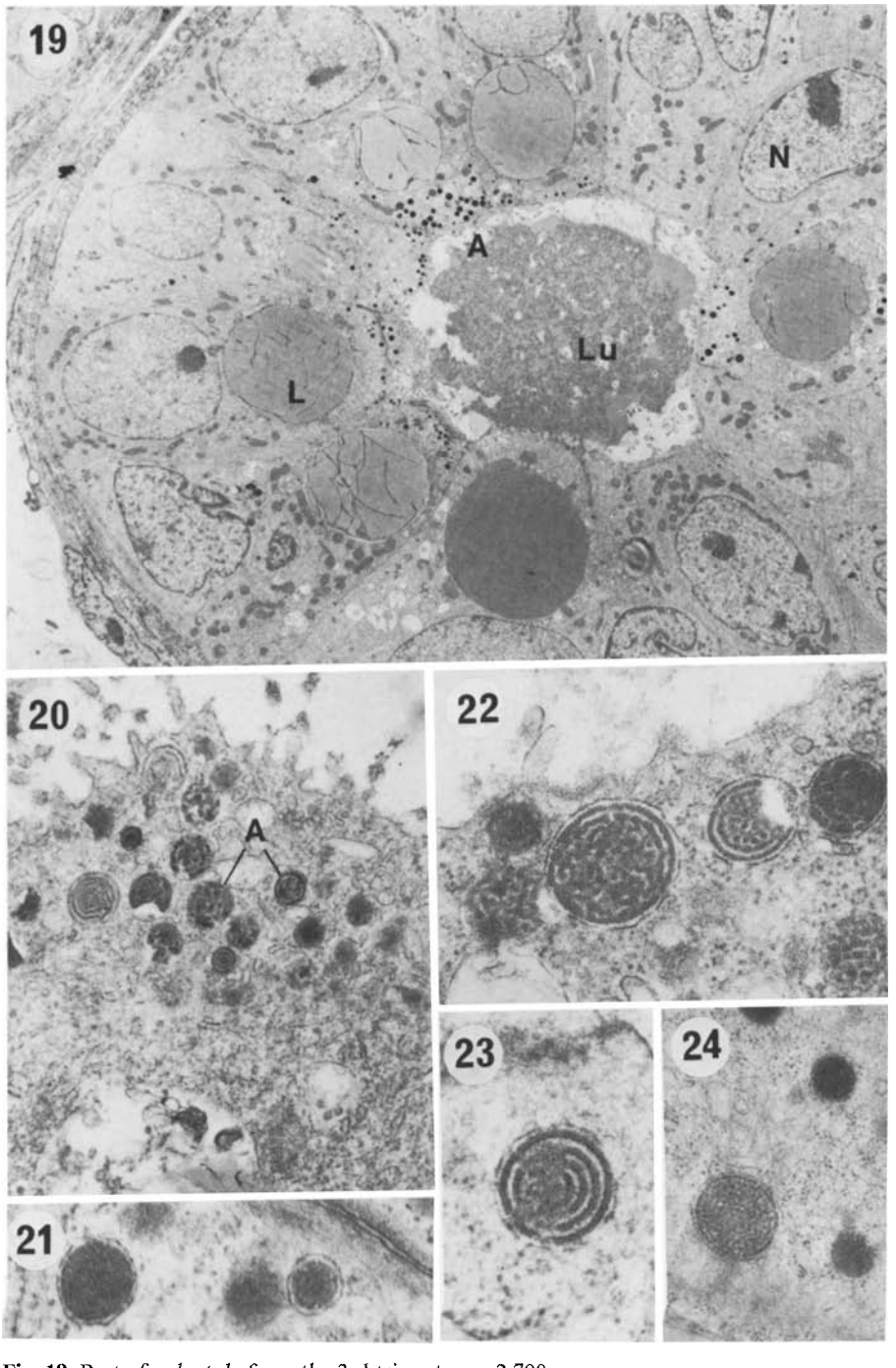

Fig. 19. Part of a ductule from the 3rd trimester. ×2,700

Fig. 20. In this section the variable appearance of the contents of the apical vacuoles is illustrated. ×18,000

Fig. 21. Detail of the smaller apical vacuoles. ×40,000

Figs. 22–24. Details of the larger apical vacuoles. ×45,000; ×58,000; ×24,000

less than the epithelial cells as evidenced by the increased ratio of epithelial to myoepithelial cells.

The area of stroma was limited due to the tightly packed ductules but contained numerous capillaries, fibroblasts, plasma cells and monocytes. Although the ESJ was present in a number of cases there was an increased incidence of examples where capillaries (Fig. 17) and also plasma cells were situated adjacent to ductules without intervening fibroblasts.

Discussion

The structural changes observed during pregnancy can be related to two distinct processes involving proliferation and differentiation of the epithelium.

From our results (Table 1) it would appear that proliferation is continuous throughout gestation resulting in progressive increases in the size of the lobules (based on the number of constituent ductules). Although no definitive statement can be made on our quantitative results because of the small number of patients examined it is apparent that mitosis is highest on the first trimester with lower levels during the second and third trimesters. In these comparisons the ductule rather than the lobule was used as the common denominator because of the variation in lobule size with stage of pregnancy. The peak of mitosis in early pregnancy is similar to that observed for DNA synthesis in the pregnant mouse (Nagasawa and Vorherr 1977). The mechanisms regulating this proliferation are not known but are presumably hormonal. Elevated levels of oestrogen and progesterone occur during gestation (Tulchinsky et al. 1972) but studies of the 'resting' breast including samples from current users of oral contraceptives (Anderson et al. 1982) suggest that other factors are likely to be involved. Studies in vitro with mouse (Topper and Oka 1974) and human (Strum and Hillman 1981) mammary cells indicate that the regulation of proliferation is more complex than by the sex steroid hormones alone.

The presence of relatively frequent apoptotic cells during pregnancy was unexpected. In other organs withdrawal of trophic hormones has been considered to be a major factor in promoting apoptosis (Wyllie 1975; Hopwood and Levison 1976). However, in pregnancy the 'trophic' hormones for breast epithelium would be expected to show persistently increasing levels. Alternative mechanisms must therefore be sought to explain the persistence of apoptotic cells in breast tissue no longer subject to the hormonal fluctuations of menstrual cycles.

During the first trimester there were no histological or ultrastructural features which would differentiate the pregnant from the 'resting' breast. It was exclusively a phase of proliferation. However, with the initiation of cellular differentiation during the second trimester it was possible to identify tissue from pregnant women.

The process of differentiation initiated in the second trimester is completed during the third trimester. It is possible that differentiation is associated with the increased levels of prolactin which start to appear during

the second trimester (Tyson et al. 1972). However, from the results reported from in vitro studies (Topper and Oka 1974) it is possible that this is again an oversimplification. The cytoplasmic differentiation is not synchronised, with variations between and within lobules and also between patients at similar stages of pregnancy. This is similar to that reported for animals (Mills and Topper 1970). Histologically the distinguishing feature between pregnant and non-pregnant is the presence of large lipid droplets within the epithelial cells. However, ultrastructurally, a number of additional cytoplasmic changes were identified. These relate to a marked increase in the synthetic capabilities of the cells following increases in the amount of rER and hypertrophy of the Golgi body.

The groups of large vacuoles, some with osmiophilic contents, have previously been thought to be related to protein secretion (Salazar et al. 1975) but due to their lack of apical polarisation or fusion with the plasmalemma we feel they are more likely to represent components of the lysosomal system.

The small apical vacuoles with their characteristic contents appeared in the second and third trimesters, and have not previously been reported for animals or humans (Salazar et al. 1975; Wooding 1977). The vacuoles would appear to be of a secretory nature but their morphology differs from that of casein micelles observed during lactation. However, they closely resemble the membrane bound material identified as alpha lactalbumin in human breast tissue by ultrastructural immunochemical techniques (Clayton et al. 1982).

From our study it would appear that the myoepithelial cells do not undergo marked proliferation during gestation although they undergo cytoplasmic changes with the development of large numbers of myofilaments. Thus the ratio of myo- to epithelial cells drops as gestation progresses. This means that the myoepithelial cells become flattened with stellate attenuated processes. This is probably responsible for the apparent histological change from the two layer structure of the ductule in the first trimester where the lower layer consists of conical myoepithelial cells to the single cell layer seen in the third trimester where the myoepithelial cells are so attenuated that they are difficult to identify with the light microscope.

In conclusion, this study shows that the hormonal changes associated with pregnancy results in a continuous proliferation of the epithelial cells although a number of apoptotic cells are also present. The cytoplasmic changes are similar to those described for other lactating systems although this is the first study to describe the apical secretory vacuoles.

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