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The Fine Structure of Ducts and Subareolar Ducts in the Resting Gland of the Female Breast

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Summary. The ultrastructure of the ducts and subareolar ducts of the resting female breast have been described. In transverse section ducts have longitudinal folds, some of which are solid ridges of cells. Four distinctive cell types were distinguished; epithelial cells, lymphocyte type cells, basal clear cells and myoepithelial cells. The epithelium is single layered, multiple at folds, and similar in general morphology to the terminal ductal-lobular unit. Well developed terminal bars may prevent cellular disruption during contraction, and apical cytoplasmic vesicles represent possible secretory material. Basal cytoplasmic bodies may represent transport of secretory products into or from the periductal stroma. Intranuclear vesicles may also be linked to secretory activity. The myoepithelium is well differentiated with numerous cytoplasmic filaments and 9+0 cilia, forming a discontinuous layer around the epithelium. The basal lamina is generally multilaminate. Capillaries are 1–5 μm in diameter and exterior to the delimiting fibroblasts of the epithelial-stromal junction.

Key words: Breast – Ultrastructure.

Introduction

Other reports on mammary gland ultrastructure (Stirling and Chandler, 1976b) have dealt solely with aspects of the terminal ductal-lobular unit (TDLU) (Wellings et al., 1975). Although the latter is the major site of mammary dysplasia the ducts are also involved in many disease conditions. The subareolar ducts also provide an ideal source of mammary epithelium for tissue culture studies (Flaxman, 1974). This description provides a comparison for further studies and evaluation of both normal and diseased tissue, and attempts to further the understanding of cell types and ultrastructural morphology throughout the whole gland tree.

Materials and Methods

Tissue was routinely collected at operation from young females undergoing reduction mammaplasty for cosmetic purposes. Tissue excised from the lower quadrants of the breast, and from the subareolar zone, was received immediately on removal and screened for gross abnormalities. After dissection, material was processed for electron and light microscopy as described elsewhere (Stirling and Chandler, 1976b). Tissue was examined for histological evidence of disease.

For this study, tissue was used from two donors described previously (Stirling and Chandler, 1976b) who conformed to the strictly defined criteria for normal resting breast. Subareolar ducts were obtained from two further donors. The first patient was age 21, gravida 1, para 1 (at 19), with no lactation. Menstrual cycles were regular, with some use of oral contraceptives for 1 year (Minovlar, Schering). There was no nipple discharge, no gross or histological evidence of disease in the breast sampled, no history of breast disease or hormonal disfunction and no known familial history of breast cancer. The second patient was age 27, had no nipple discharge, and conformed to normal criteria except for some localized areas of epithelial apocrine metaplasia visible in histologic sections which were avoided for electron microscopy. Neither donor had any known recent use of tranquilizers, and both were at day 14 in the menstrual cycle.

Results

The nomenclature of Wellings et al. (1975) is used throughout.

a) Subgross Structure of Ducts and Subareolar Ducts

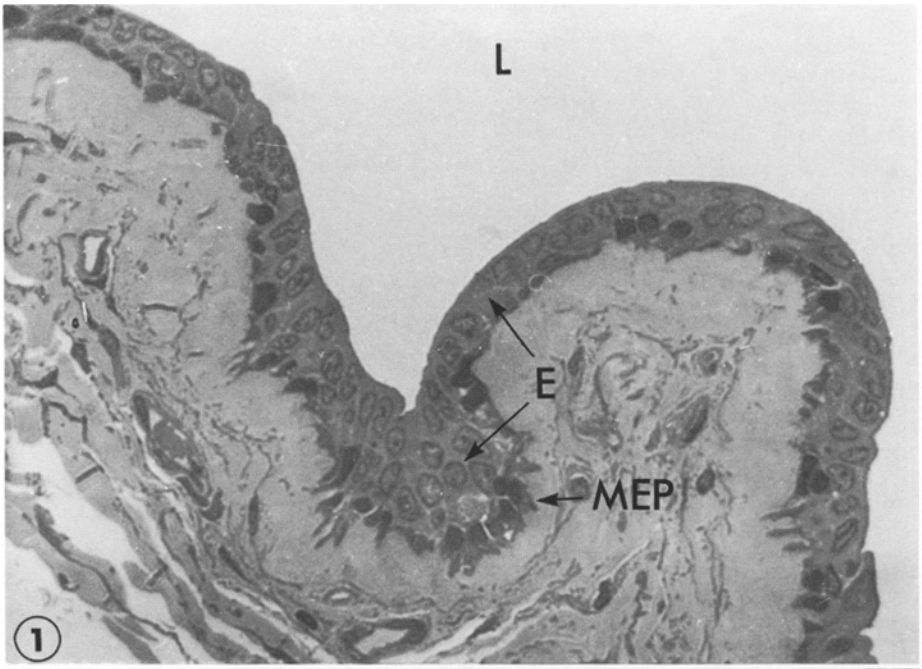
In the whole specimen, subareolar ducts were large and approximately 2–3 mm in diameter. Smaller ducts were not easily discerned from background stroma. In transverse section all ducts had a corrugated appearance due to longitudinal folds (Fig. 1), some of which were large and comprised of a solid column of epithelial cells with an outer layer of myoepithelium. Some smaller ducts were circular in cross section. The luminal epithelium of subareolar ducts was single or multiple at longitudinal folds. The outer myoepithelium was single and often closely packed at longitudinal grooves, intermittent elsewhere. Smaller ducts were similar, but the myoepithelium was generally continuous. In all ducts the myoepithelium showed prominent projections into the surrounding stroma. Secretory material was often seen in duct lumina.

b) Ultrastructure: Subareolar Ducts

i) Epithelium. Epithelial cell cytoplasm varied in density from pale to dark (Fig. 9), the bulk of the epithelium having an intermediate density corresponding to the 'B' cells of the TDLU described elsewhere (Stirling and Chandler, 1976b). Overall cell shape was cuboidal or columnar with the long axis orientated at right angles to the lumen. Luminal microvilli varied in density, length, and morphology, being single, branched, or swollen ended (Fig. 2). The surface of the duct undulated gently due to irregularities in cell height, there were also occasional deep pits containing long microvilli (Fig. 3).

Fig. 1. Subareolar duct, transverse section showing a fold in the duct wall. Epithelium (*E*), myoepithelium (*MEP*), duct lumen (*L*). Araldite section, 0.25% Toluidine blue and borax stain. ×640

Fig. 2. Luminal surface of an epithelial cell (*E*) in a subareolar duct. Normal microvilli (*MV*), swollen microvilli (*MVs*) and branched microvilli (*MVb*) are present. Duct lumen (*L*). ×32,550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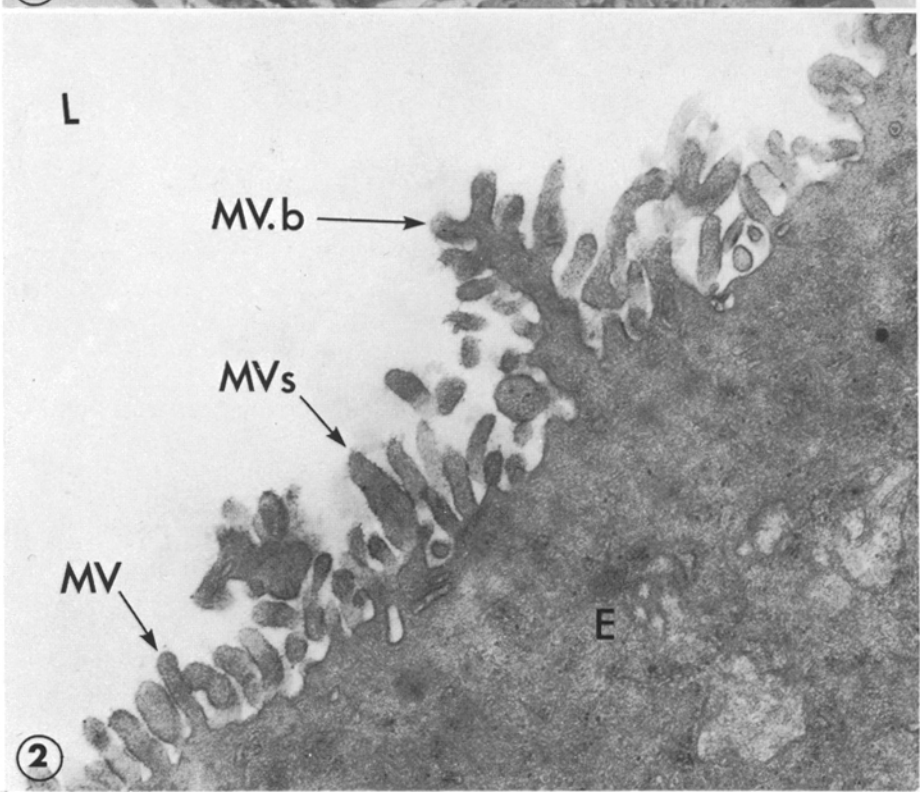
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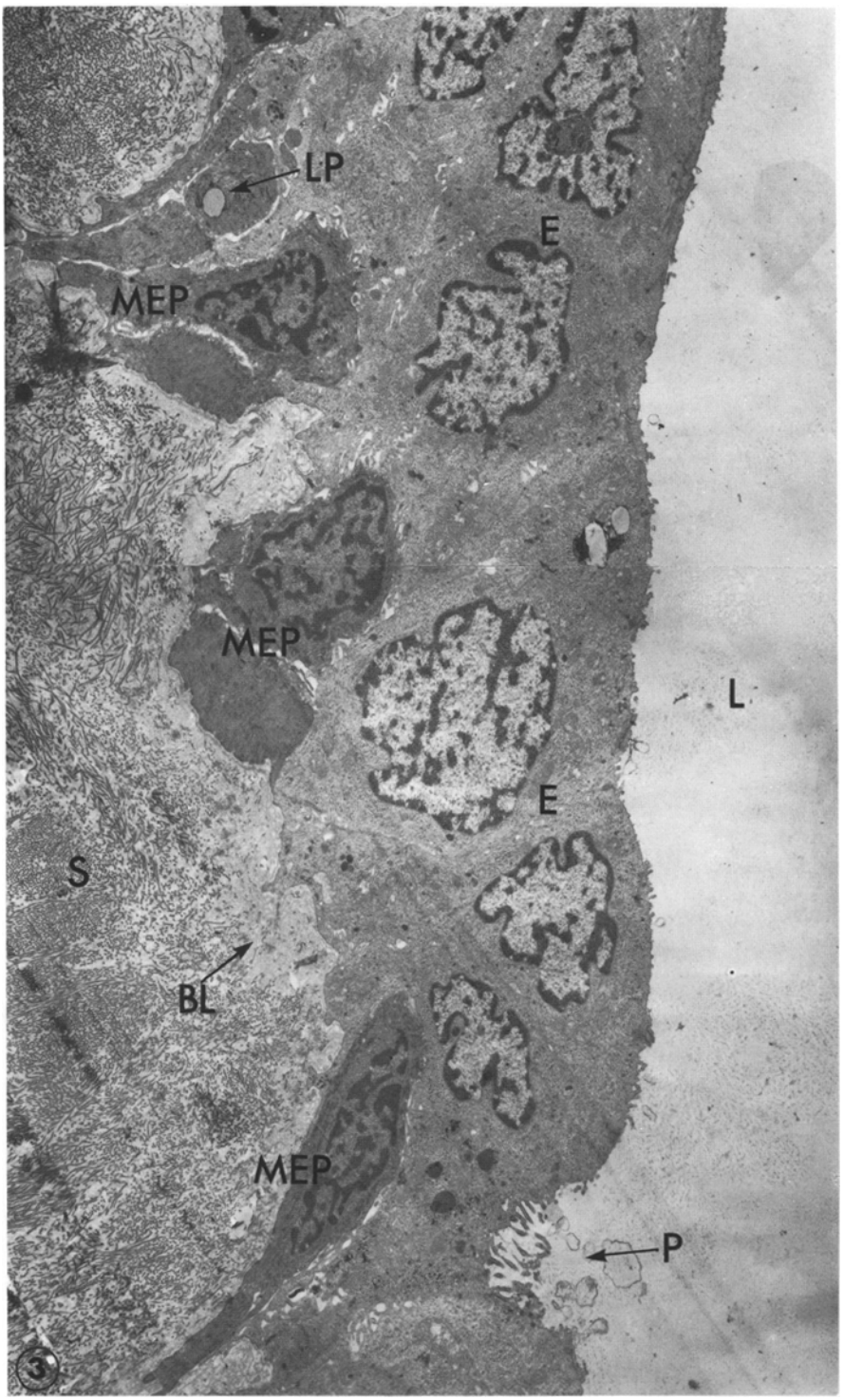
LP
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3

The overall structure of the epithelium was found to be similar to that of the terminal ductal-lobular unit (TDLU) (Stirling and Chandler, 1976b). Contiguous epithelial cells were attached at their apices by terminal bars, often with well developed filament systems extending across the cell tip (Figs. 4, 11).

A wide variety of membrane bound vesicles were observed in the cell apex. These included pale 'lipid bodies', dark amorphous bodies (Fig. 5), and large vesicles containing small inclusions of various densities (Fig. 6). Similar amorphous bodies were commonly observed in close proximity to the basement membrane (Figs. 7, 10, 14) and occasionally in association with the nucleus. Accumulations of small dark granules within the cytoplasm may have been glycogen, but were not common.

Nuclei were variable from large and pale, to indented and dark. Heterochromatin was peripheral with sporadic central deposits. Some nuclei contained pale membrane bound vesicles (Fig. 8) as well as "Sykes bodies" (Sykes et al.,

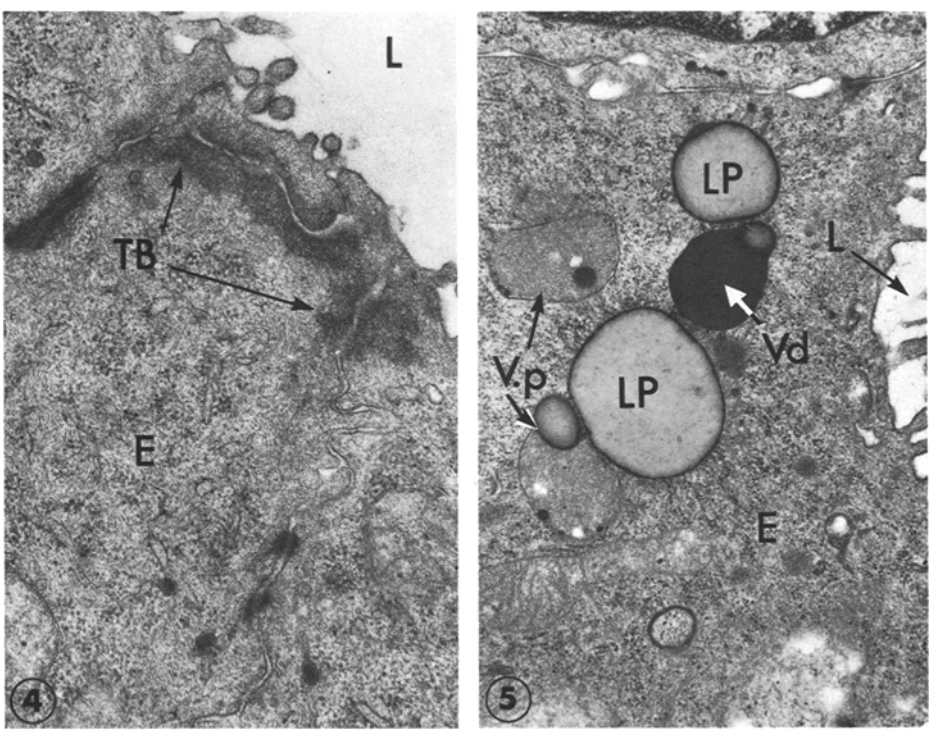


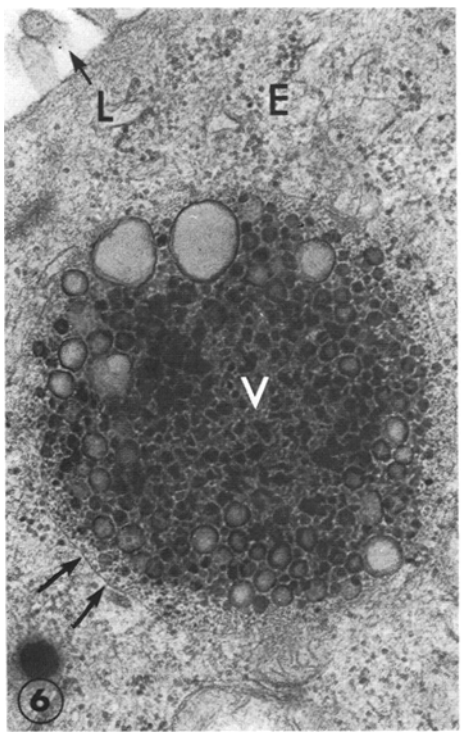
Fig. 3. Subareolar duct (transverse section) with continuous epithelium (*E*) and a discontinuous myoepithelial (*MEP*) layer. Myoepithelial cells show occasional lipid bodies (*LP*), the epithelial layer sometimes has deep pits containing long microvilli (*P*). Periductal stromal (*S*), basal lamina (*BL*), duct lumen (*L*). × 4,800

Fig. 4. The terminal bars (*TB*) at the tips of ductal epithelial cells (*E*) are well developed. Duct lumen (*L*). × 32,000

Fig. 5. Tip of an epithelial cell (*E*) with a variety of vesicular inclusions. Pale vesicle (*Vp*), dark vesicle (*Vd*), the third (*LP*) possibly contains lipid. Lumen (*L*). × 19,500

1968) and perichromatin granules. Nucleoli were prominent. Cells extending to the basal lamina often had finger-like extensions protruding into the basal lamina (Figs. 9, 10) similar to those of the myoepithelium.

ii) Myoepithelium. Spindle shaped myoepithelial cells were irregularly spaced along the base of the epithelium (Fig. 3). At indentations in the duct the cells were closely packed, elsewhere they were intermittent with epithelial cells extending to the basal lamina. The general structure of the myoepithelium was similar to that of the TDLU (Stirling and Chandler, 1976b), being the well differentiated type with numerous contractile filaments bearing focal densities, 9+0 cilia,



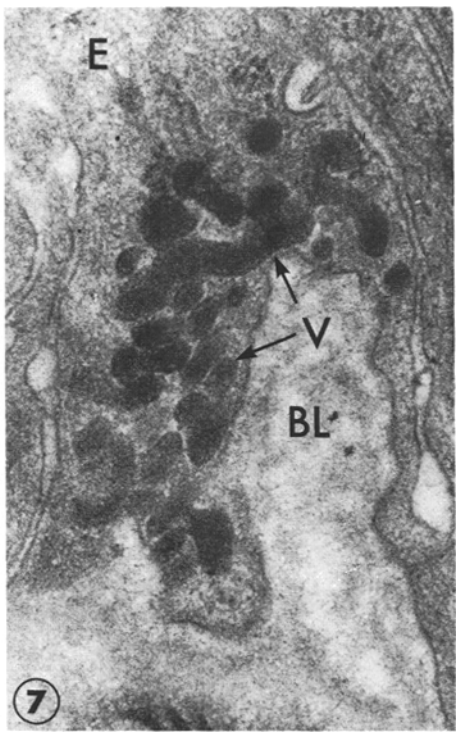


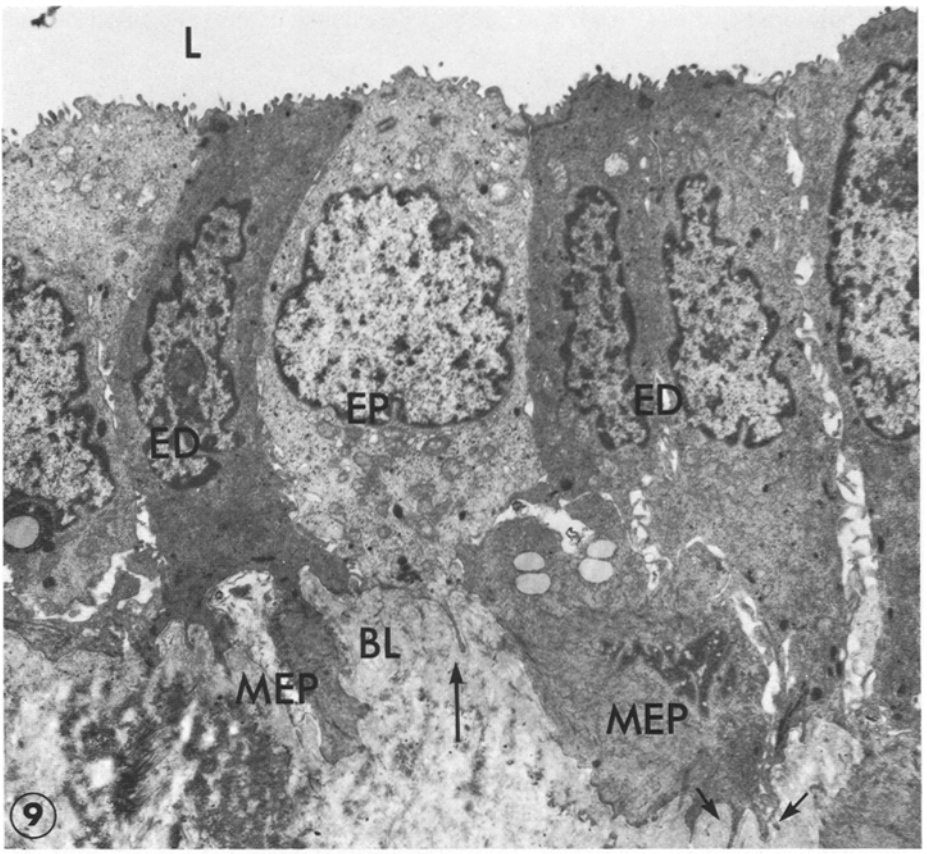
Fig. 6. Large membrane bound (small arrows) vesicle (*V*) containing a variety of both light (possibly lipid) and dark granules. Tip of epithelial cell (*E*), lumen (*L*). ×45,000

Fig. 7. Vesicular bodies (*V*) in the base of an epithelial cell (*E*) which extends to the multiple basal lamina (*BL*). ×54,900

Fig. 8. Epithelial nucleus (*EN*) with a membrane bound (arrow) vesicular inclusion (*V*). ×73,200

Fig. 9. Subareolar duct, transverse section, showing differing densities of the epithelial cells, dark type (*ED*), pale type (*EP*). Epithelial cells extending to the basal lamina (*BL*) show similar extensions (long arrow) to the myoepithelial (*MEP*) extensions (short arrows). Duct lumen (*L*). ×6,000

Fig. 10. Basal vesicles (*V*) in the base of an epithelial cell (*E*) extending to the basal lamina (*BL*). Several prominent extensions (*EXT*) protrude from the epithelial cell into the periductal stroma, and a micropinocytotic vesicle (*MP*) can also been. ×30,000


L
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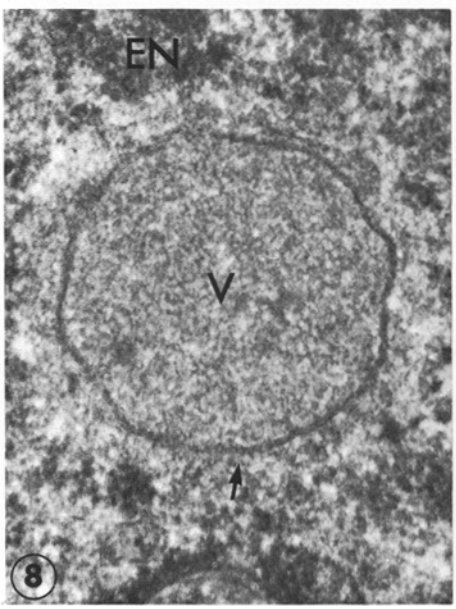
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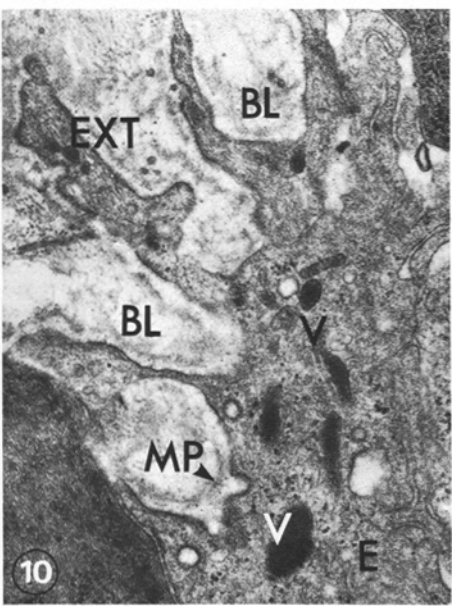
EXT
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large pale lipid bodies (Fig. 3) and prominent extensions into the basal lamina (Figs. 3, 9).

c) Ultrastructure of Ducts

i) Epithelium. In general the epithelium showed no major differences to those of the subareolar ducts (Fig. 11). Both apical (Figs. 5, 12, 13) and basal cytoplasmic inclusions (Fig. 14) of various types were found, as were membrane bound intranuclear vesicles (Fig. 8).

ii) Myoepithelium. Myoepithelial cells were spindle shaped (Fig. 15) and well differentiated with numerous filaments bearing focal densities (Fig. 15). Cells often extended well into the stroma giving the duct a ragged appearance (Figs. 11, 15). The myoepithelial layer was almost complete, but some epithelial cells did extend through to the basal lamina. Groups of myoepithelial cells in close contact often surrounded small islands of basal lamina (Fig. 15). Cells showed typical 9+0 cilia and lipid bodies.

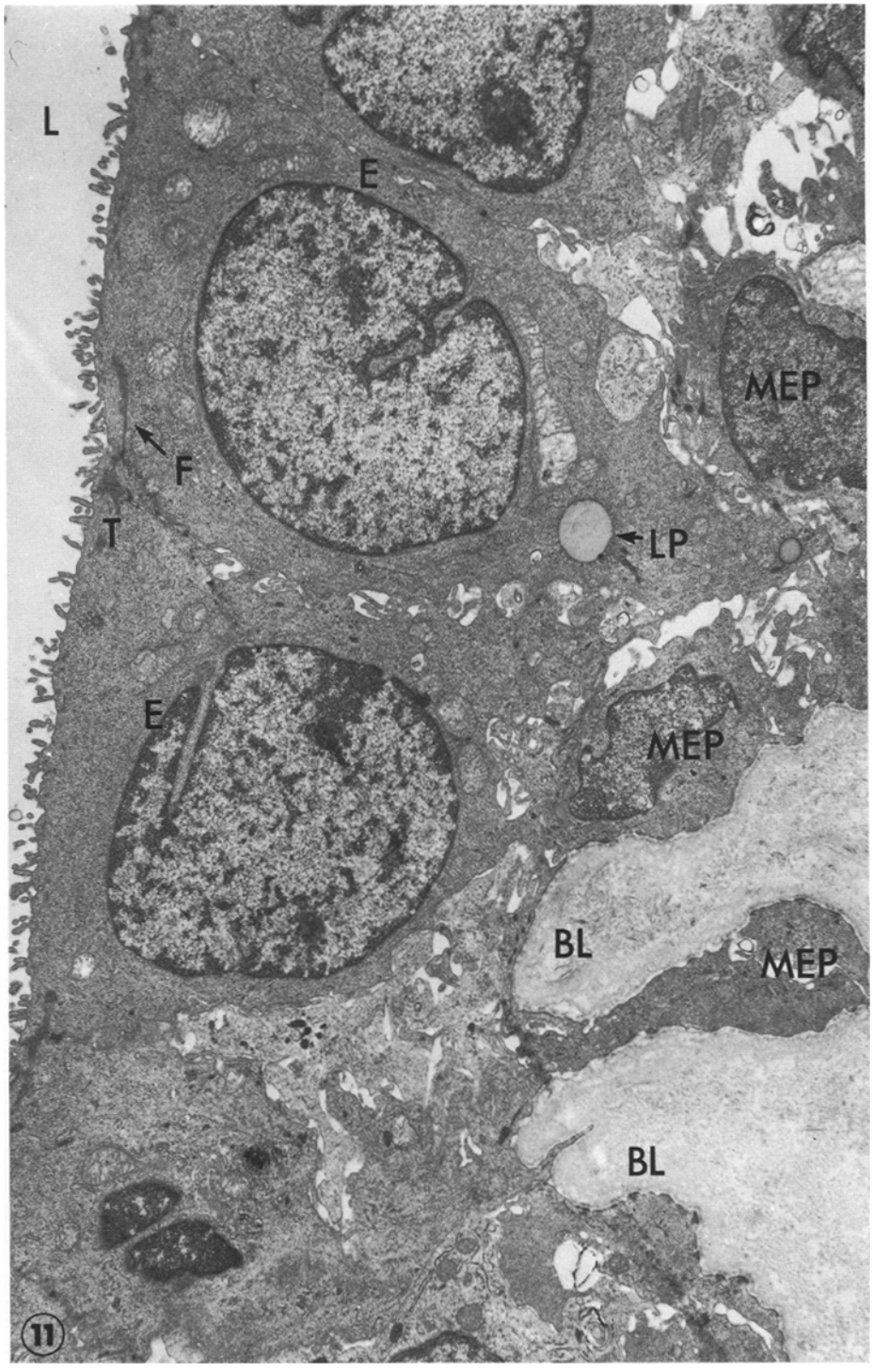
d) Basal Clear Cells

Two basal clear cell types were seen between epithelium and myoepithelium in both ducts and subareolar ducts. The lymphocyte type was small with pale cytoplasm containing few organelles (Fig. 16). The second type was a group with heterogenous morphology, similar in general structure to the epithelium but with paler cytoplasm. Organelle content was normal but desmosomal contacts with contiguous cells were poorly developed (Fig. 17). There were no distinctive macrophages with secondary lysosomes.

e) Capillaries and Epithelial-Stromal Junction (ESJ)

In all ducts the basal lamina was usually multiple and abundant amorphous background material was common (Figs. 3, 11, 15). Elastin and dense bands of collagen extended from the basal lamina to the delimiting fibroblasts of the ESJ (Fig. 15). The periductal stroma and capillary-fibroblast junction was also comprised of collagen bands and elastin. The capillaries were always situated to the exterior of the ESJ fibroblasts, in cross section comprising 2–3 endothelial cells and approximately 1–5 μm in diameter (Fig. 18). Microvilli were seen on the endothelial luminal surface, but rarely on the outer wall. The capillaries had associated perivascular cells and the basal lamina was single or multiple.

Fig. 11. Small duct, transverse section. Small ducts are similar in general morphology to subareolar ducts. Terminal bars (*T*) have well developed fibrils (*F*) extending into the epithelial cell (*E*) cytoplasm. An epithelial cell shows a prominent lipid vesicle (*LP*). The discontinuous myoepithelium (*MEP*) gives the duct a ragged outline. The basal lamina (*BL*) is multiple with amorphous background deposits. Duct lumen (*L*). ×7,500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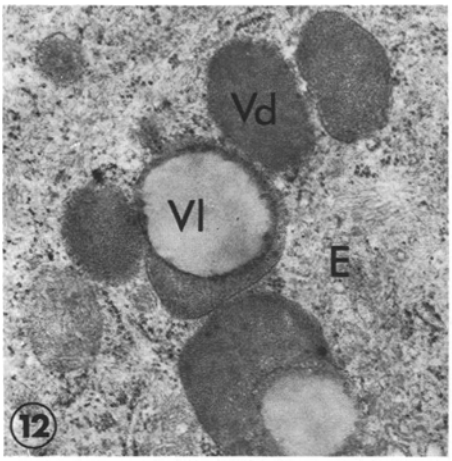
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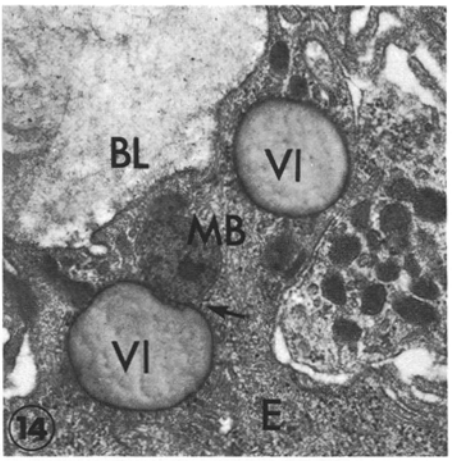
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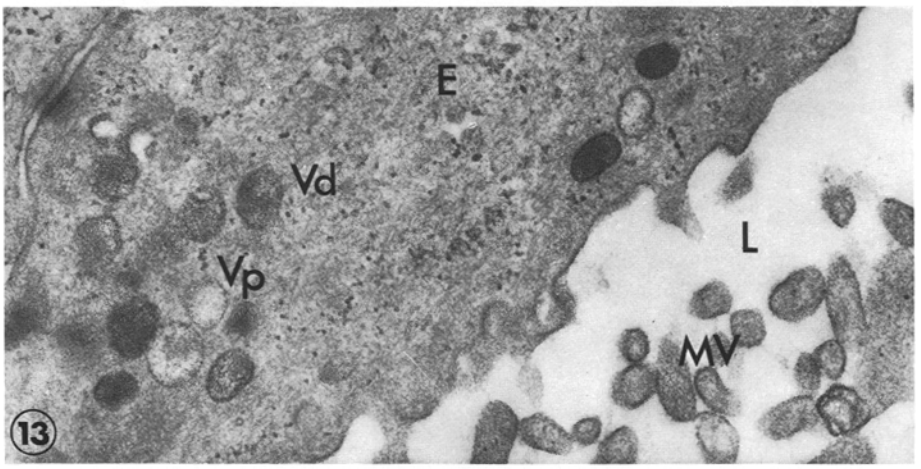
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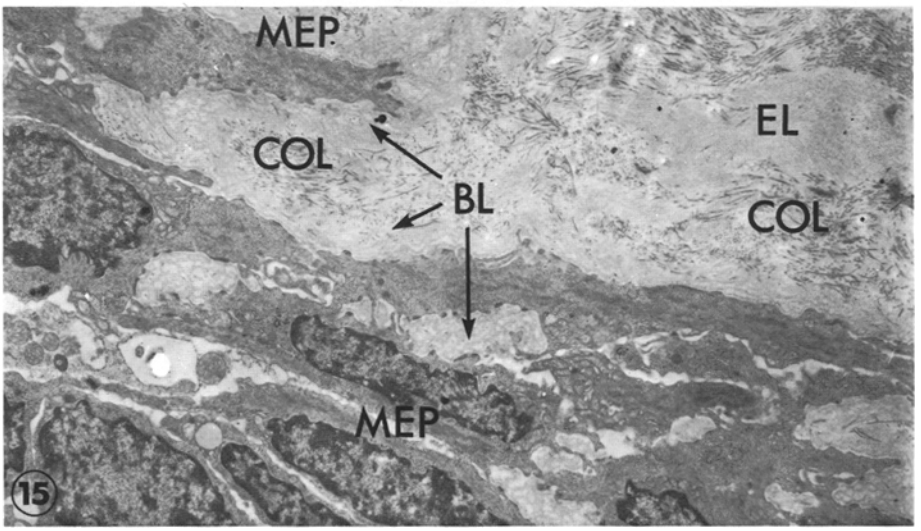
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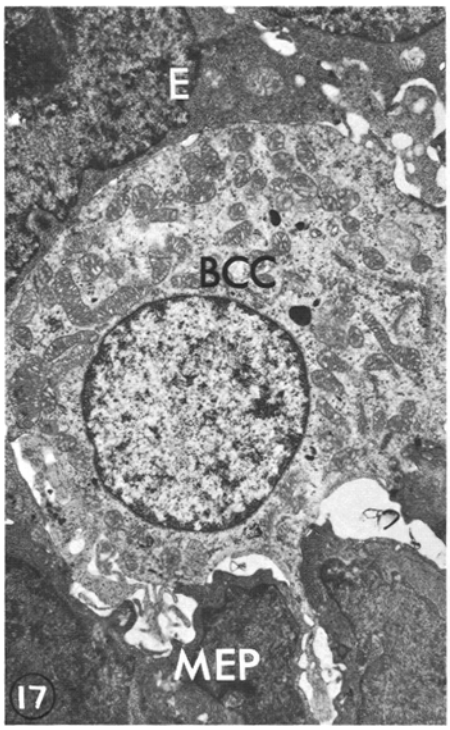

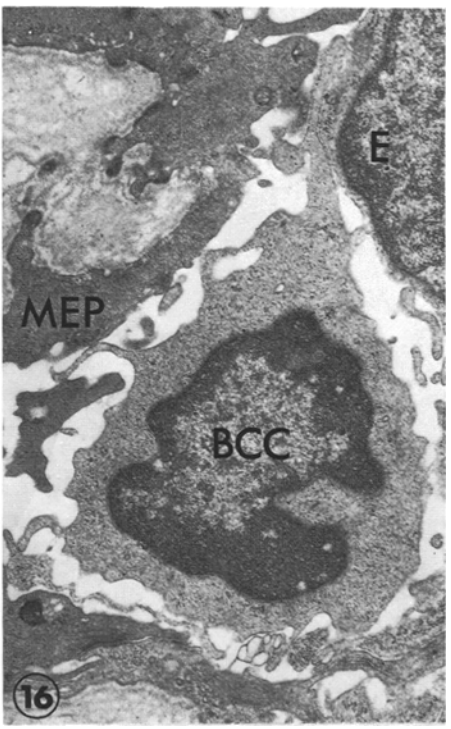

Fig. 12. Vesicles in the tip of an epithelial cell (*E*). Dark vesicles (*Vd*) often have associated light areas (*Vl*) presumably containing lipid. ×32,100

Fig. 13. Small epithelial (*E*) apical vesicles containing both pale (*Vp*) and dark (*Vd*) amorphous granular material. Duct lumen (*L*), microvilli (*MV*). ×42,000

Fig. 14. Basal vesicles in an epithelial cell (*E*) extending to the basal lamina (*BL*). Lipid vesicles (*Vl*) and a type of multivesicular body (*MB*) are both present. Possible interchange of material is occurring between a lipid vesicle and the multivesicular body (arrow). ×24,600

Fig. 15. Edge of a duct showing elongated shape of myoepithelial cells (*MEP*). The basal lamina (*BL*) is multiple with amorphous background deposits. Small islands of basal lamina occur due to the irregular nature of the myoepithelial layer. Collagen fibres (*COL*) are both external to, and embedded in the basal lamina. Elastin (*EL*). ×4,800

Fig. 16. Basal clear cell (*BCC*), lymphocyte type with no obvious organelles, dark nucleus and no attachments to contiguous cells. Epithelium (*E*) myoepithelium (*MEP*). ×13,000

Fig. 17. Basal clear cell (*BCC*), indeterminate type, possibly an inactive macrophage or undifferentiated cell. Epithelium (*E*), myoepithelium (*MEP*). ×6,500

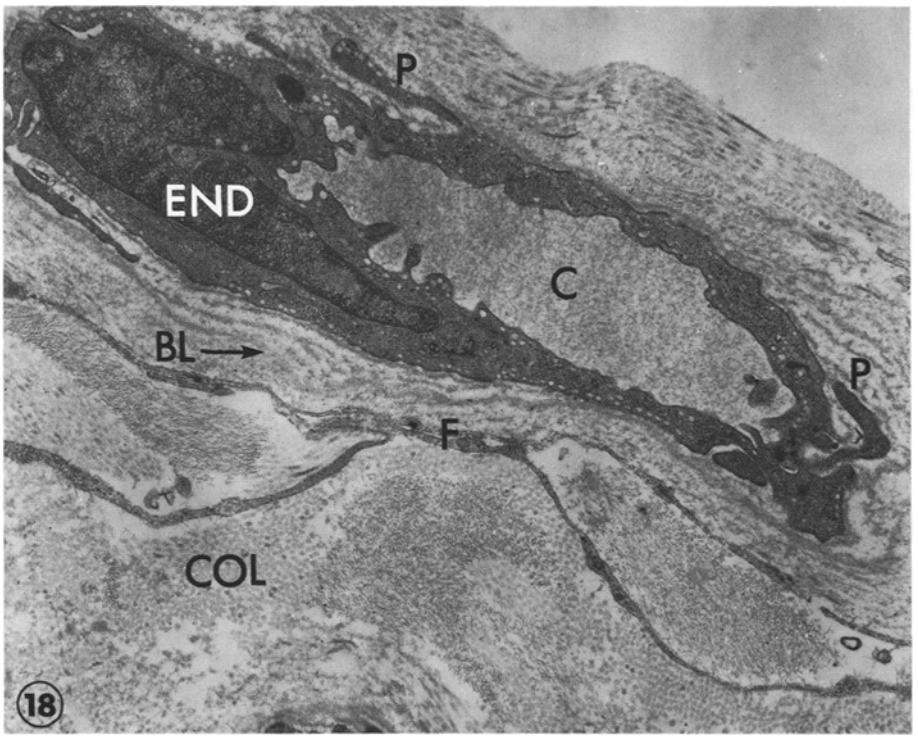

Fig. 18. Capillary (*C*) in close contact with the delimiting fibroblasts (*F*) of the epithelial-stromal junction. Periductal collagen (*COL*) is adjacent to the myoepithelium. The endothelial wall (*END*) of the capillary has intermittent perivascular cells (*P*) and a basal lamina which is sometimes multiple (*BL*). × 12,230

Fibroblasts could often only be distinguished from perivascular cells by their lack of basal lamina. Mast cells, macrophages and other cells of the reticulo-endothelial system were occasionally observed in the surrounding periductal stroma.

Discussion

The major ducts show distinctive features which separate them from the ductules of the TDLU (Stirling and Chandler, 1976b), notably in the development of the epithelial-stromal junction (ESJ), reflecting the differing functions of the two areas. The myoepithelium is well developed and responsible for ductal contraction, the thickened basal lamina presumably acting as attachment and providing mechanical support. The thickening of the basal lamina may also affect its role as a regulatory unit in the ESJ complex (Ozzello, 1970). The well developed terminal bars of the epithelium will provide protection against cellular disruption during contraction. The similarity in structure between ductu-

lar and ductal epithelium provides no clue for the apparent inability of ducts to form alveolar structures, suggesting that this may be a function of the ESJ, or of genetic expression. The function of myoepithelial cilia has been discussed at length elsewhere (Stirling and Chandler, 1976a).

Intranuclear bodies and vesicles similar to those described in the epithelium have been found in several other locations including human labial salivary glands (Tandler et al., 1969) and calf adrenal zona fasciculata (Weber et al., 1964). Weber et al. (1964) found that vesicular bodies appeared to develope from smaller nuclear bodies, these are similar to the "Sykes body" of mammary epithelium (Sykes et al., 1968). Transformation depended on ACTH stimulation and it was suggested that the bodies may represent the nuclear organizer or a trophic hormone receptor. The observations of Tandler et al. (1969) led them to conclude that the vesicles had no relation to possible viral infection, that some appeared to contain lipid deposits and there was a non-obligatory relationship between vesicular formation and epithelial protein secretion. The significance of intranuclear vesicles in breast epithelium is obscure, cells containing them showing no other distinctive morphological characteristics. Epithelial cells in the resting gland contained some secretory material, those containing nuclear vesicles generally did not. This may indicate that such vesicles are linked to secretory activity in a similar manner to the salivary gland (Tandler et al., 1969), contiguous cells having an asynchronous pattern of cyclic secretory activity similar to that proposed for lactating epithelium (Tobon and Salazar, 1975).

It is considered that the majority of the vesicular inclusions within the epithelial cytoplasm represent a low level of secretion. The morphology of such inclusions does not however resemble the secretory products of lactating epithelium (Salazar and Tobon, 1974; Tobon and Salazar, 1975). This suggests the small amounts of secretory material observed in the epithelium is not of the same type as that of the fully active cell, but forms through alternative processes due to the differences in physiological state and in hormonal milieu between resting and active glands (Tobon and Salazar, 1975). The location of vesicular inclusions at the base of the cell may represent the transport of material across the duct as suggested by Dossett (1958), a similar mechanism possibly existing in the ductules (Stirling and Chandler, 1976b).

The distribution of cell types within the ductal system is essentially similar to the ductules of the TDLU, except for the apparent lack of active macrophages. Macrophages are involved in the removal of dead and effete cells, in repair and involution, as well as in other functions (Carr, 1973). As the ductal system does not appear to be a site of extensive growth or acinar formation (Slemmer, 1974) the amount of cellular activity is presumably much lower than ductular areas, this resulting in low macrophage activity.

The lymphoid type cells are distinctive, but the remaining heterogenous group of basally located pale cells are problematical. Perhaps the most likely possibilities are that they are undifferentiated epithelial or myoepithelial cells. Despite the fact that some pale cells appear to have poorly developed desmosomal contacts with contiguous cells they may be related to cells described elsewhere as resting macrophages (Stirling and Chandler, 1976b). The inability

of ducts to form acini in vitro (Slemmer, 1974) mitigates against pale cells performing a stem cell function.

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