Virchows Arch. A. Path. Anat. and Histol. 372, 205–226 (1976)

Virchows Archiv A

The Fine Structure of the Normal, Resting Terminal Ductal-Lobular Unit of the Female Breast

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Summary. The fine structure of the terminal ductal-lobular unit (TDLU) of the normal, resting female breast is described. Tissue used conformed to strict criteria for normal. 4 distinct cell types, were observed, epithelium, myoepithelium, macrophages and lymphocytes. A gradation in structure, especially of myoepithelium, was found between the ductule tip and intralobular duct, apparently reflecting the age and function of each region. Cilia originating from the myoepithelial cells are possibly sensory in function. Epithelial vesicular inclusions may represent minimal secretory activity, transport of material across the cell, or be lysosomal in nature. The capillaries in close contact with the delimiting fibroblasts of the epithelial-stromal junction (ESJ) are described and the zone designated the capillary-fibroblast junction (CFJ). The CFJ is conceived as a regulatory unit similar to the ESJ, and may contribute to dysplasia due to biochemical or functional alterations, or to disruption of the microvascular geometry.

Key words: Breast – Ultrastructure.

Introduction

There have been a number of publications on various aspects of the ultrastructure of the normal human mammary gland. Ozzello (1970, 1971, 1974) remains the only author to have described strictly defined normal tissue, obtained from biopsy of a 21 year old Cancerphobia patient. A review of the literature indicates the wide range of tissue used in previous studies. These include: unqualified "normal" or "essentially normal" (Carter et al., 1969; Murad and Von Haam, 1968; Tannenbaum et al., 1969; Salazar and Tobon, 1974; Kern and Dermer, 1972; Sykes et al., 1968), the fourth month of pregnancy (Toker, 1967), adjacent to benign or malignant disease (Barton, 1964; Waugh and Van der Hoeven, 1962; Haguenau, 1959; Haguenau and Arnoult, 1959; Fanger and Ree, 1974; Ahmed, 1971; Takahashi, 1958), and mixtures of normal and post-menopausal tissue (Murad and Scarpelli, 1967; Murad et al., 1967). Some reports are brief and

appended to longer articles on diseased material (Bassler, 1968; 1970) or describe specific aspects such as the myoepithelium (Murad and Von Haam, 1968; Takahashi, 1958; Haguenau, 1959) or variation with the menstrual cycle (Fanger and Ree, 1974). Others have described developmental aspects of the mammary gland (Salazar and Tobon, 1974; Tobon and Salazar, 1974; 1975).

Despite the broad spectrum of literature, the overall structure of the terminal ductal-lobular unit (TDLU) (Wellings et al., 1975) has not been fully described, and the number of cell types remains obscure. Nomenclature for the various components of the gland tree is also confused. This study presents a detailed description of the TDLU using strictly defined material similar to Ozzello (1970, 1971, 1974) as a basis for further investigations on both normal and diseased tissue. A description of the major ducts of the mammary gland is the subject of a further report.

Materials and Methods

Tissue was routinely collected at operation from young females undergoing reduction mammaplasty (breast reduction) for cosmetic purposes. The portion of tissue excised from the lower quadrants of the breast was received immediately upon removal and screened for gross abnormalities.

For electron microscopy the white stromal tissue bearing the glandular elements was dissected out and placed in 3% glutaraldehyde in either 0.16 M phosphate buffer (330 mOsm) or 0.1 M phosphate buffer (220 mOsm). After dicing, 1–2 mm pieces of the tissue were fixed for 3 h in the 3% glutaraldehyde. Subsequent to this was a 0.1 M phosphate buffer wash, postfixation in 1% Millonig's osmium tetroxide (330 mOsm), a further 0.1 M phosphate buffer wash and dehydration in ethanol. Finally the specimens were flat embedded in foil trays with Araldite.

After polymerization suitable epithelial areas were selected with a high power binocular microscope and sectioned using either a Huxley Mk. II or L.K.B. III ultra-microtome. Thick sections for orientation purposes were stained with 0.25% toluidine blue and borax. Thin sections were collected on bare copper grids, stained with uranyl acetate and lead citrate and examined using an A.E.I. EM6B electron microscope.

Material adjacent to that taken for electron microscopy, and also random samples, were processed for light microscopy and screened for histologic evidence of disease.

Tissue described in this report was from two donors and conformed to the following criteria: a) no clinical or gross evidence of disease in either breast; b) no histological evidence of disease in the breast sampled; c) no history of hormonal disfunction or administration of hormones directly or indirectly affecting the mammary gland, including oral contraceptives; d) nopregnancies; e) regular menstrual cycles; f) no previous breast disease or biopsy; g) no known familial history of breast cancer; h) no history of major surgery or disease.

One donor was 23 years of age, point in menstrual cycle not known, with some previous use of a tranquilizer "Tranxene" (Boehringer, Ingelheim) taken for approximately 2 weeks, 12 months previously. The second donor was aged 20, at day 28 in her menstrual cycle at operation and with no known use of tranquilizers.

Results

The nomenclature of Wellings et al. (1975) was used throughout (Fig. 1).

A) TDLU-Subgross Structure

Lobules arose from small and large ducts, and consisted of varying numbers of ductules (Figs. 2, 3). Ductules ranged from long, well differentiated types

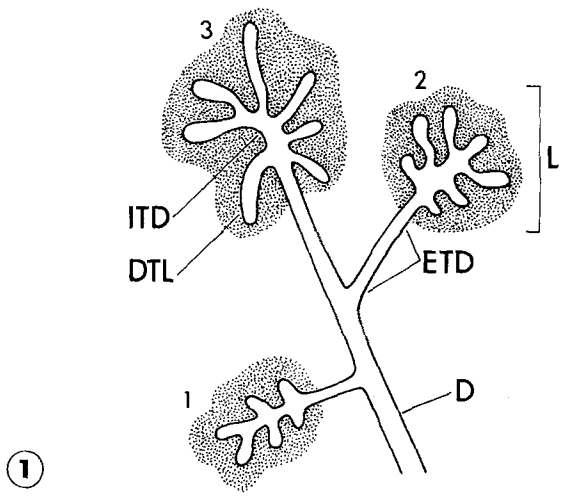


Fig. 1. Diagrammatic representation of the terminal ductal lobular unit (TDLU), modified from Wellings et al. (1975). *DTL* ductule (synonymous with acinus, terminal ductule, alveolus); *ITD* intralobular terminal duct, drains DTL's; *L* lobule, DTL's and ITD combined; *ETD* extra lobular terminal duct, drains lobule; *D* duct, drains a collection of lobules. TDLU comparises DTL, ITD and ETD. DTL's may vary in length from small and short (*1*), to medium (*2*) and long (*3*). The number of DTL's in a lobule may also vary

to small, poorly developed ductules with little or no lumen (Fig. 1). No true acini were found. The outline of small ductules and the tips of well differentiated types was smooth. Where small ductules joined the intralobular duct myoepithelial extensions were sometimes seen to project into the stroma. Epithelial structures were surrounded by a fibroblast layer delimiting the epithelial-stromal junction (ESJ). Exterior to this was the capillary supply in close contact with the ESJ fibroblasts (Fig. 22). Epithelial structures lay in a collagenous stroma with capillaries, scattered fibroblasts, mast cells, and occasional lymphocytes.

B) Ductules

The major cellular components comprised an inner, single layer of epithelial cells abutting onto the lumen and a discontinuous outer layer of myoepithelial cells. Interspersed between these major cell types was a heterogenous group of cells termed basal clear cells (BCC) (Figs. 4, 20, 21). The lumen sometimes contained amorphous or granular debris (Fig. 9).

i) Epithelial cells

The majority of epithelial cells were cuboidal or columnar, with a pale granular cytoplasm, termed "B" cells, with occasional dark cells ("A" cells) (Haguenau and Arnoult, 1959). Their long axis was radial to the lumen. The luminal surfaces had variable numbers of single or branched microvilli with longitudinal core

filaments and often with *antennulae microvillares*. Micropinocytosis was scarce, but occurred on all cell surfaces. Some vesicles were the coated type. At their apical tips adjacent epithelial cells were attached by typical terminal bars (Fig. 9). Elsewhere contiguous epithelial and myoepithelial cells had desmosomal contacts and interdigitating microvilli, the latter being more common in the basal region. Fibrils extending from desmosomes and terminal bars were not extensively developed (Fig. 9).

The supranuclear cytoplasm often contained a typical diplosomal centriole, and a golgi apparatus, poorly developed, with empty saccules. Rough endoplasmic reticulum was not extensive, and occasionally closely associated with mitochondria (Fig. 9). Mitochondria were large, round or ovoid, sometimes exhibiting granules in the intercristal matrix. Free ribosomes, glycogen granules, mitochondria, endoplasmic reticulum and ribosomes were dispersed throughout the cytoplasm. Fine filaments were frequent but not dense, and showed no orientation except occasionally around the nucleus (Fig. 5), nor did they show dense zones as in myoepithelial cells. Microtubules were rare.

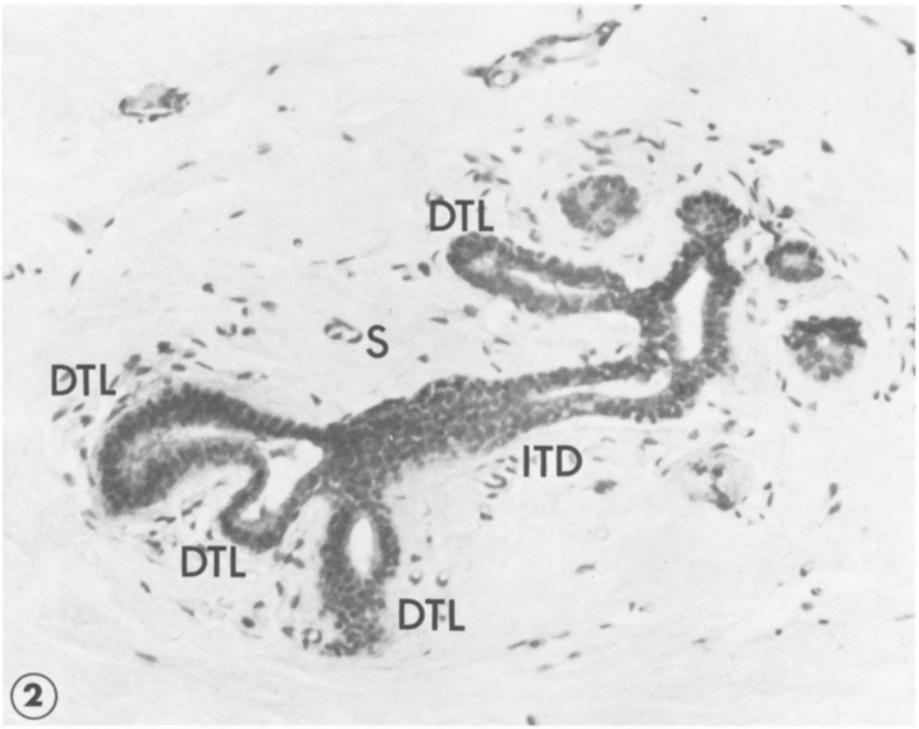
Several types of cytoplasmic vesicle were found. At the luminal surface small membrane bound vesicles containing pale granular material were noted (Fig. 6), together with elongate vesicles, similar to smooth endoplasmic reticulum with dark granular contents (Fig. 7). Also in the supranuclear cytoplasm were 2 types of larger membrane bound vesicle, one type being pale and apparently containing lipid, and another of similar size but dark and granular (Figs. 8, 9) with occasional internal vesicles and inclusions (Fig. 10). Slightly smaller dark vesicles were often closely associated with the nucleus (Fig. 11), sometimes being sited in a groove or indentation in the nuclear surface. Collections of dense vesicles were also found in the basal region of epithelial cells which extended to the basal lamina. These were round or elongate, and often occurred in small groups (Fig. 12).

Nuclei were round or slightly elongate with a smooth or undulating perimeter, deep indentations being scarce. The nuclear envelope had surface ribosomes, and an internal fibrous lamina. Nuclear pores were common. Heterochromatin was limited to a thin irregular peripheral zone giving the nuclei a pale appearance. Nucleoli were common. Two types of nuclear inclusion were found, perichromatin granules and nuclear pale bodies or "Sykes bodies" (Sykes et al., 1968), of which there were sometimes several per nucleus (Fig. 26).

Epithelial cells extending to the basal lamina showed no exceptional modifications except that some had infrequent coated micropinocytotic vesicles at the basal surface (Fig. 13). Focal densities on the basal membrane were possibly points of attachment (Fig. 14). There were no well developed hemidesmosomes.

Fig. 2. Light micrograph of a lobule showing ductules (*DTL*) and intralobular terminal duct (*ITD*). The lobule is surrounded by the loose vascular collagenous intralobular stroma (*S*). × 229

Fig. 3. Light micrograph of a group of ductules (*DTL*). Epithelium (*E*). Myoepithelium (*M*). Ductule lumen (*L*). Delimiting fibroblasts of the epithelial stromal junction (*F*). Capillary (*C*). Collagenous intralobular stroma (*S*). × 570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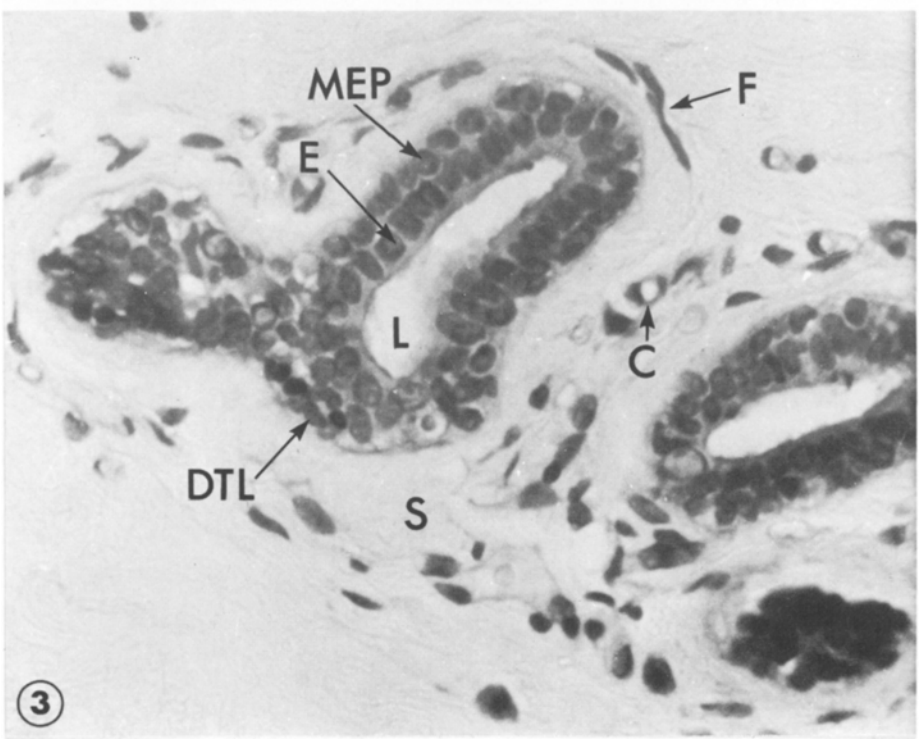
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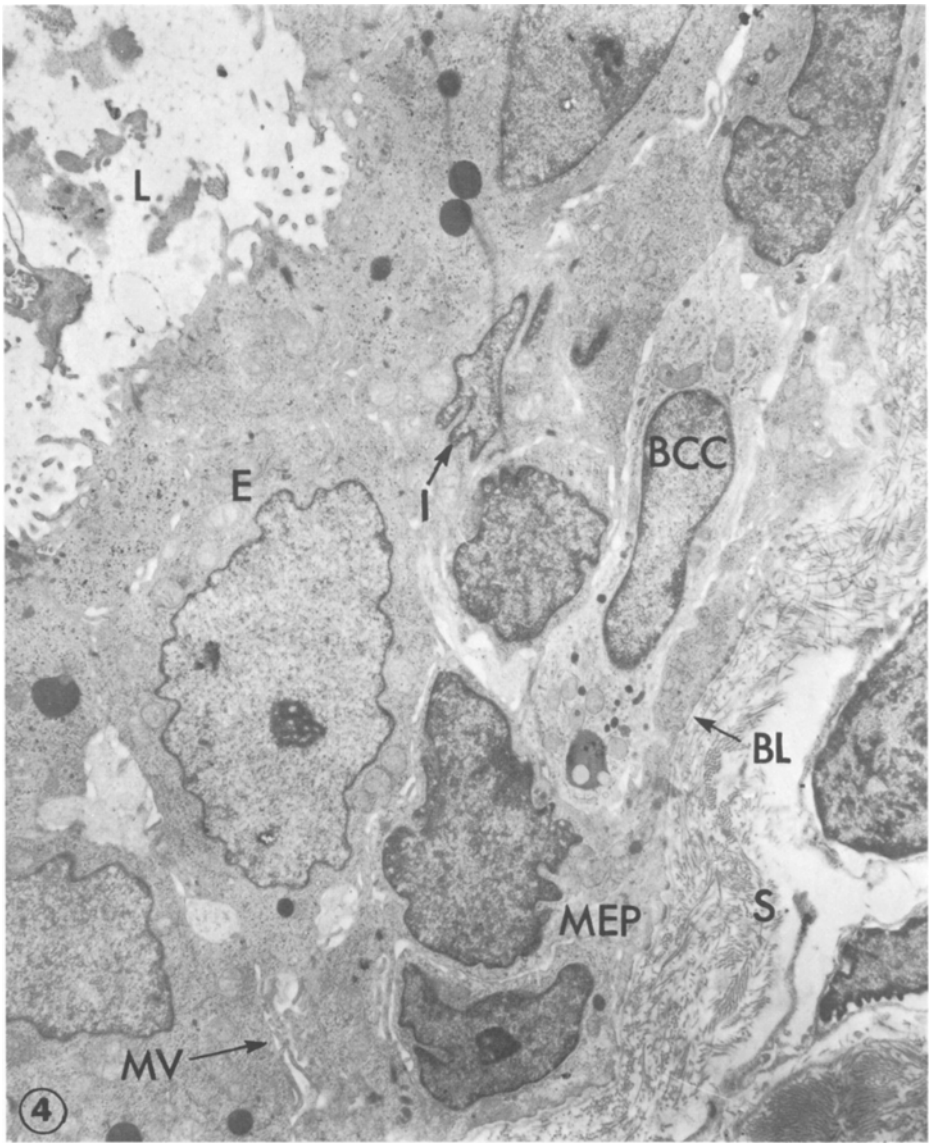


Fig. 4. Ductule tip with single basal lamina (*BL*). A discontinuous myoepithelial layer (*MEP*) lies exterior to the epithelium (*E*) with a basal clear cell (*BCC*) between. The latter has no secondary lysosomes and is possibly a resting, inactive macrophage. Interdigitating microvilli (*MV*) between cells are common and myoepithelial nuclei are indented (*I*). Lumen (*L*). Stroma (*S*). ×4,000

Fig. 5. Filaments (*F*) in the epithelial cytoplasm (*E*) may be orientated around the nucleus (*N*). ×69,900

Fig. 6. Vesicles (*V*) surrounded by a unit membrane and containing pale granular material in the tip of an epithelial cell (*E*). Terminal bar (*T*). Microvilli (*MV*). Lumen (*L*). ×93,200

Fig. 7. Elongate vesicles (*V*) containing dark amorphous material in the tip of an epithelial cell (*E*). Ductule lumen (*L*) contains similar dark material. ×23,400

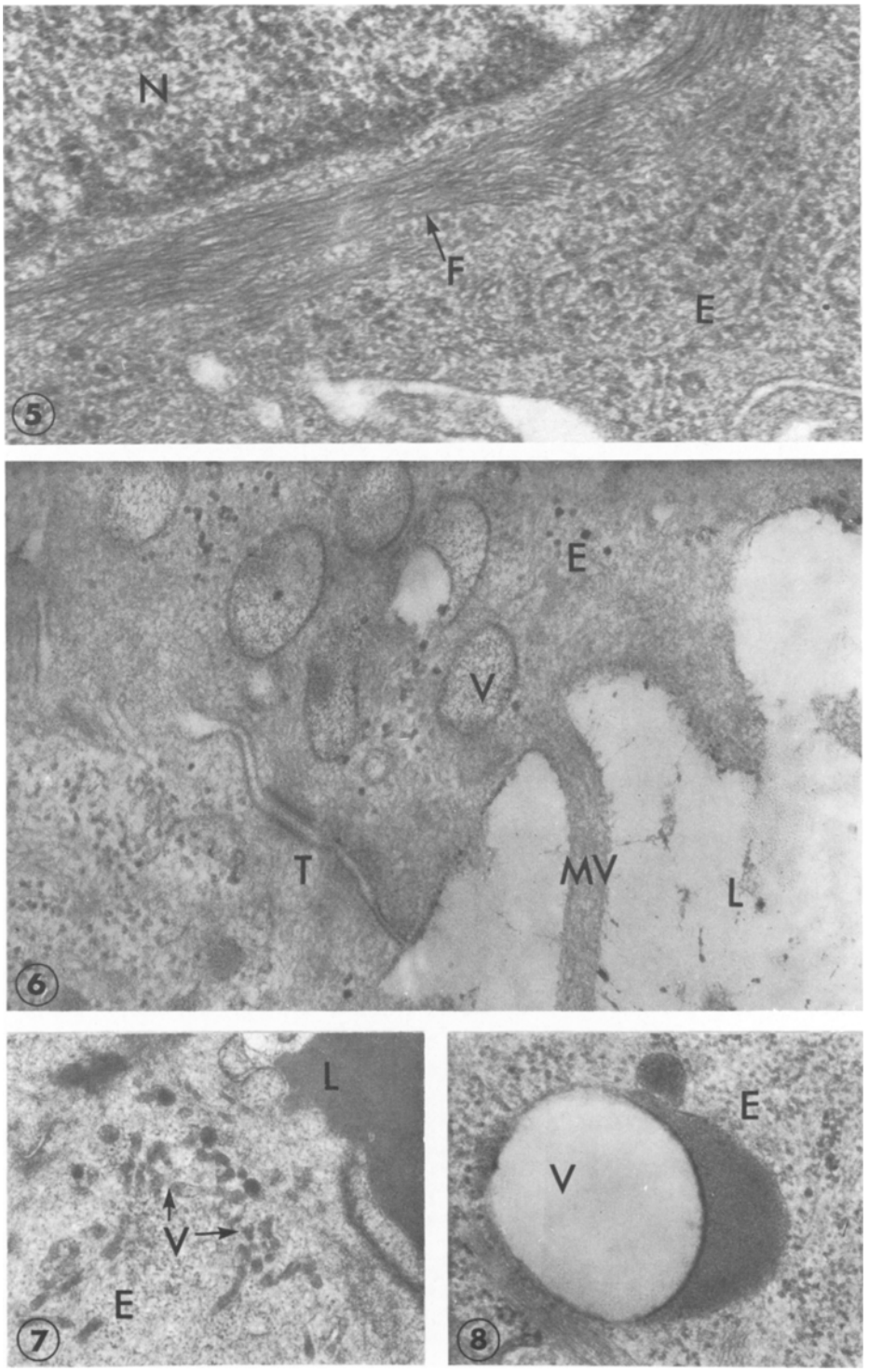


Fig. 8. A possible lipid filled vesicle (*V*) with associated peripheral dark zone. Epithelial cytoplasm (*E*). ×54,900

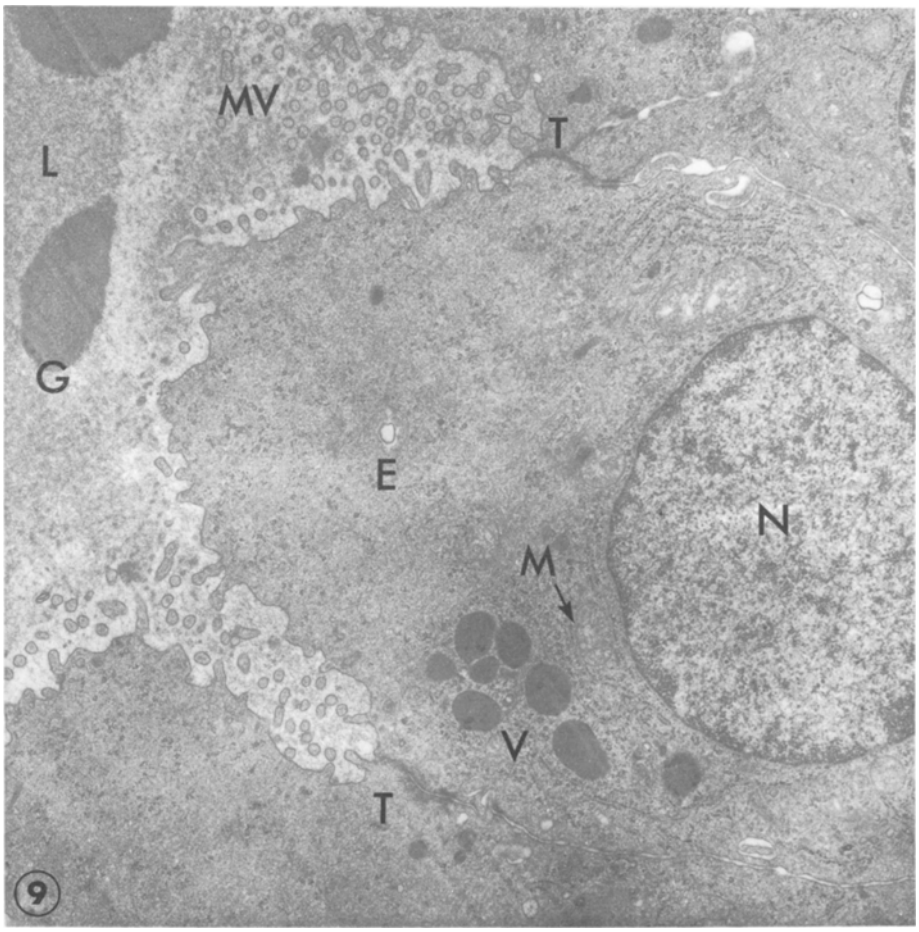


Fig. 9. Tip of a ductule epithelial cell (*E*) with numerous apical microvilli (*MV*) extending into the lumen (*L*) which also contains granular material (*G*) of various densities. Terminal bars (*T*) between adjacent cells have few fibrils extending into the cytoplasm. A mitochondrion (*M*) has rough endoplasmic reticulum in close association and dark vesicles (*V*) are adjacent. Nucleus (*N*). ×12,530

Fig. 10. A group of vesicles of several types in an epithelial cell (*E*). A small vesicle (*V.i*) contains smaller pale vesicles and is similar to a multivesicular body, the second type (*V.l*) is associated with a possible lipid vesicle and contains dark granules, the third type (*V.d*) also contains dark granules and is similar to the vesicles of Figure 9. ×60,000

Fig. 11. A dark vesicle (*V*) in an epithelial cell (*E*) in close association with the nucleus (*N*). ×80,000

Fig. 12. Groups of vesicles often occur in the basal area of epithelial cells (*E*) extending to the basal lamina (*BL*). There may be several types including dark vesicles associated with possible lipid zones (*V.l*), vesicles containing dark granules (*V.d*) and smaller elongate vesicles (*V.e*) which also contain amorphous granular material. ×30,000

Fig. 13. Base of an epithelial cell (*E*) extending to the basal lamina (*BL*) which is single. There is a coated micropinocytotic vesicle (*MP*) and a microtubule (*MT*) within the cytoplasm. ×53,200

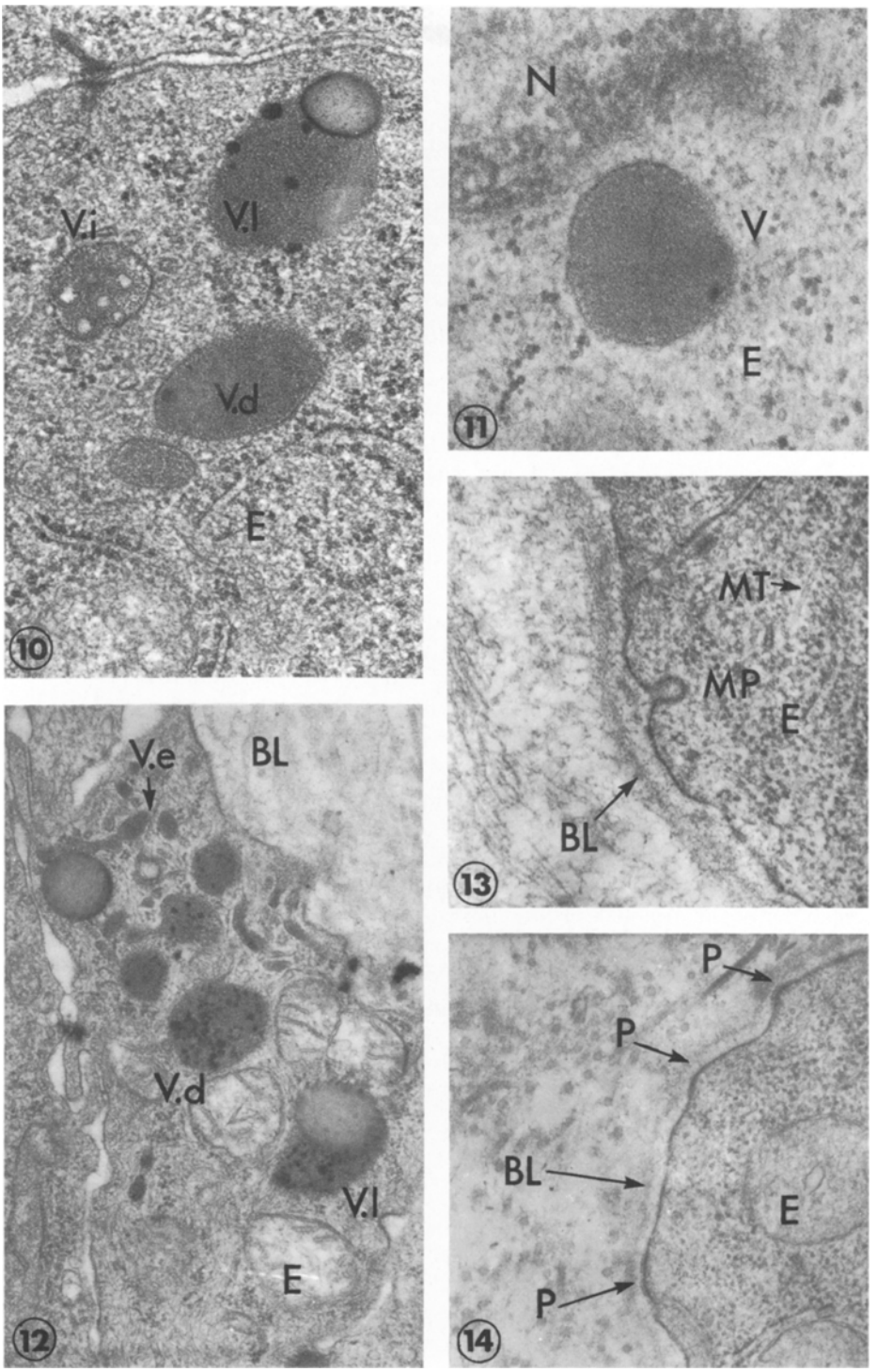


Fig. 14. Base of an epithelial cell (*E*) extending to a single basal lamina (*BL*). Dense plaques (*P*) on the cell membrane are visible but there are no fully developed hemidesmosomes. ×39,900

ii) Myoepithelium

The myoepithelium formed a discontinuous outer layer surrounding the epithelium (Fig. 4). Myoepithelial cells were irregular in shape, but generally cuboidal or triangular with cytoplasmic extensions reaching across the base of the epithelium. They did not extend to the lumen. Three dimensional structure appeared to be conical or stellate. The cytoplasm was granular, being similar in density, or slightly darker than the epithelium. Microvilli were common on all surfaces except that in contact with the basal lamina, and interdigitated with those of contiguous cells (Figs. 4, 15). Micropinocytosis was common, especially at the base (Fig. 16).

Adjacent myoepithelial cells were attached by desmosomes and to the basal lamina by well developed laminate hemidesmosomes. Hemidesmosomes were often sited on small cellular extensions (Figs. 16, 19).

Centrioles were diplosomal, and sited in the supranuclear cytoplasm. The distal centriole formed the basal body of a short 9+0 cilium (Fig. 17) which projected into the epithelial-myoepithelial intercellular space. A well developed golgi apparatus was found in close proximity to the diplosome.

Rough endoplasmic reticulum was poorly developed and sometimes closely associated with mitochondria. Mitochondria were smaller than those of the epithelial cells (Fig. 15), also having intercristal granules. Free ribosomes were scattered throughout the cytoplasm, microtubules were scarce.

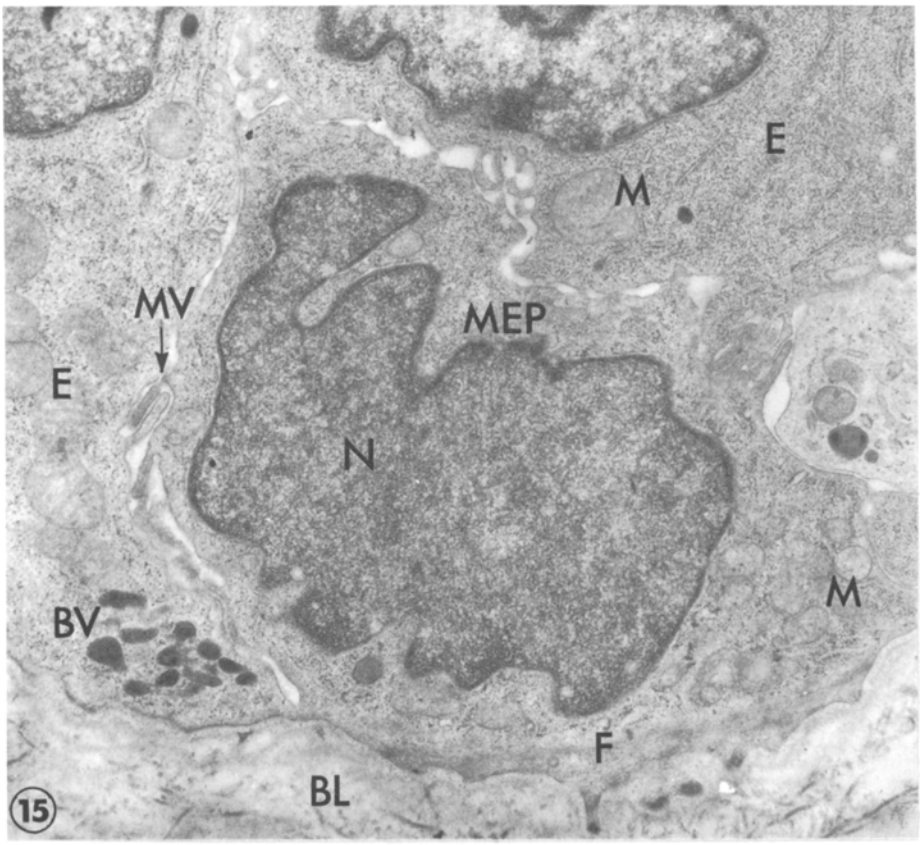
Differentiation of the myoepithelium depended upon its position in the ductule in relation to the intralobular duct. Cells at the ductule tip or in small ductules were poorly differentiated (Fig. 15) and difficult to distinguish from epithelial cells. Progressing toward the intralobular duct, myofilament development increased, initial filaments being orientated parallel to the basal lamina. In the well differentiated cells in close proximity to the intralobular duct the basal region of the cell was packed with myofilaments showing dense zones, the remainder of the organelles being limited to the apex of the cell (Fig. 18).

Cytoplasmic inclusions included lipid bodies and dark vesicles similar to those of the epithelium, but few in number. Some myoepithelial cells contained groups of small vesicles in the basal region, similar to those of the epithelium (Figs. 16, 19), but it was often difficult to distinguish between detached regions of epithelial and myoepithelial cytoplasm.

Fig. 15. A poorly differentiated myoepithelial cell (*MEP*) in a ductule tip. The nucleus (*N*) is dark and invaginated. Contractile filaments (*F*) are poorly developed and indistinct. Mitochondria (*M*) are much smaller than those of adjacent epithelial cells (*E*). Interdigitating microvilli (*MV*) are seen and an epithelial cell extending to the basal lamina (*BL*) has a group of basal vesicles (*BV*). ×16,700

Fig. 16. Micropinocytosis (*MP*) is common at the base of myoepithelial cells (*MEP*). Dark vesicles (*V*) are also seen. Hemidesmosomes (*HD*). Basal lamina (*BL*). ×60,000

Fig. 17. 9+0 cilia (*C*) project from the myoepithelial cell (*MEP*) into the intercellular space between myoepithelium and epithelium (*E*). There is usually an associated golgi apparatus (*G*). ×54,900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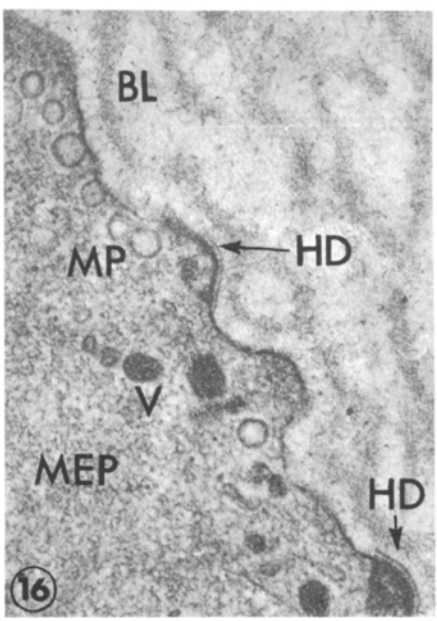
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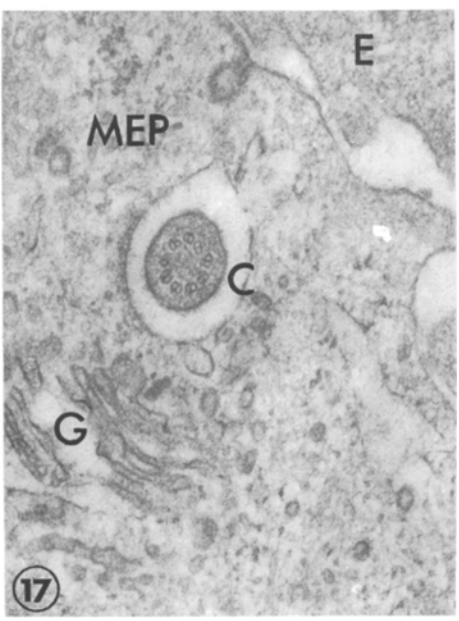
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Nuclei showed greater diversity than the epithelium, being round, or elongate with smooth to deeply indented margins (Figs. 4, 18). Nuclei were perpendicularly orientated in relation to epithelial nuclei. The nuclear envelope was similar to the epithelium. Heterochromatin was extensive with little euchromatin, giving the nucleus a dark granular appearance. Nucleoli, perichromatin granules and Sykes bodies were observed but obscured by heterochromatin. Pseudoinclusions formed by deep indentations were common (Fig. 18).

iii) Basal Clear Cells

Macrophage Type. Two types of macrophage-like cell were observed: 1) with secondary lysosomes (Fig. 20); 2) with no secondary lysosomes (Fig. 4). The cells were similar in other respects. Size was variable, the cells were irregular in shape, with pale cytoplasm and numerous cytoplasmic extensions between adjacent epithelial and myoepithelial cells. There were no microvilli. Micropinocytosis was infrequent, but occasionally appeared to be pinching off small portions of cytoplasm from adjacent cells (Fig. 20). There were no desmosomal attachments to contiguous cells. Cytoplasmic organelles were randomly dispersed (Fig. 20). The golgi was poorly developed with empty saccules. Rough endoplasmic reticulum was moderately developed and clearly visible against the pale cytoplasm, often containing pale granular material. Mitochondria were similar to the epithelium. Free ribosomes, and polysomes were present together with occasional cytoplasmic filaments and microtubules.

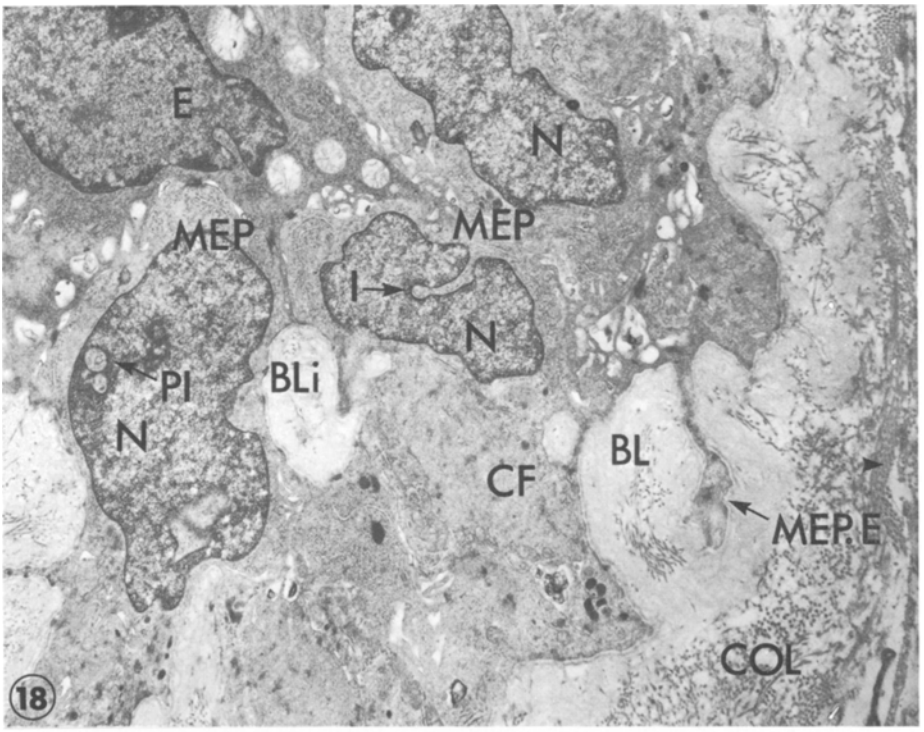
Numbers of primary and secondary lysosomes, and vesicles containing phagocytised material varied. Vesicles of the latter type ranged in size from mitochondrial to nuclear size (Fig. 20).

Nuclei were ovoid or elongate, smooth in outline, rarely indented, and pale, the heterochromatin being peripheral and scant (Fig. 20).

Lymphocyte Type. An uncommon but distinctive cell type (Fig. 21) was observed, characterised by a pale granular cytoplasm with an almost total lack of organelles, having only sparse profiles of rough endoplasmic reticulum, free ribosomes and polysomes. The cell surface was ruffled, detached from surrounding cells, and with no apparent membrane attachments. The nucleus was smooth and ovoid with well developed peripheral heterochromatin, the nucleus appearing dark.

Fig. 18. Myoepithelial cells (*MEP*) in the intralobular duct are well differentiated with large areas of contractile filaments (*CF*), other organelles restricted to the cell apex. Indentations (*I*) in myoepithelial cell nuclei (*N*) may appear as pseudoinclusions (*PI*). The myoepithelium is irregular in outline with prominent extensions (*MEP.E*) which often isolate small islands (*BL i*) of the multilaminate basal lamina (*BL*). Collagen fibres (*C*) are adjacent to the basal lamina and also embedded in it. ×7,000

Fig. 19. The myoepithelial cells (*MEP*) often show well developed hemidesmosomes (*HD*) on small protrusions. Basal lamina (*BL*). Collagen (*C*). Dark vesicles (*V*) can be seen adjacent to a long tubular mitochondrion (*M*). ×54,900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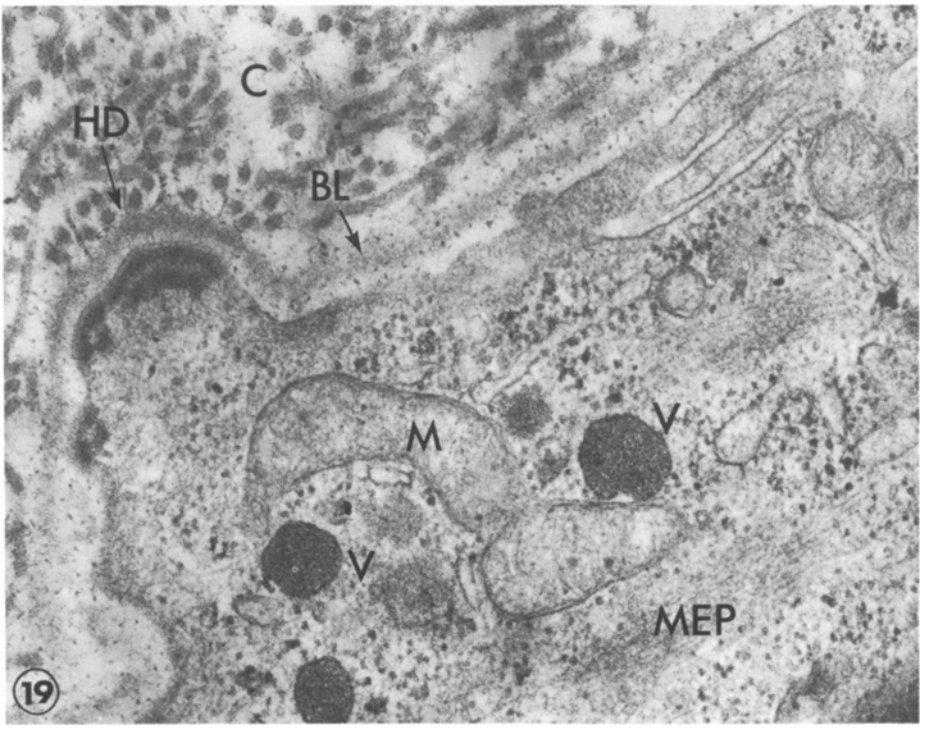
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C) Intralobular Duct

Intralobular ducts were similar in cellular component to well differentiated ductules. Epithelial cells were columnar, myoepithelial cells well differentiated with numerous myofilaments, basal clear cells were similar to those of the ductules. Myoepithelial cells often projected into the surrounding stroma giving the duct a ragged appearance. Few epithelial cells extend to the basal lamina. The basal lamina was usually well developed and multilaminate (Fig. 18).

D) Stromal Elements of the ESJ

A continuous basal lamina surrounded the ductular system. At the ductule tip the basal lamina was single (Fig. 4), becoming thicker and multilaminate towards the intralobular duct (Fig. 18). Between the basal lamina and the delimiting fibroblasts of the ESJ was a zone of collagen fibres (Fig. 22) with small interspersed elastin deposits. Collagen fibres were often embedded in the basal lamina where the latter was multilaminate (Fig. 18).

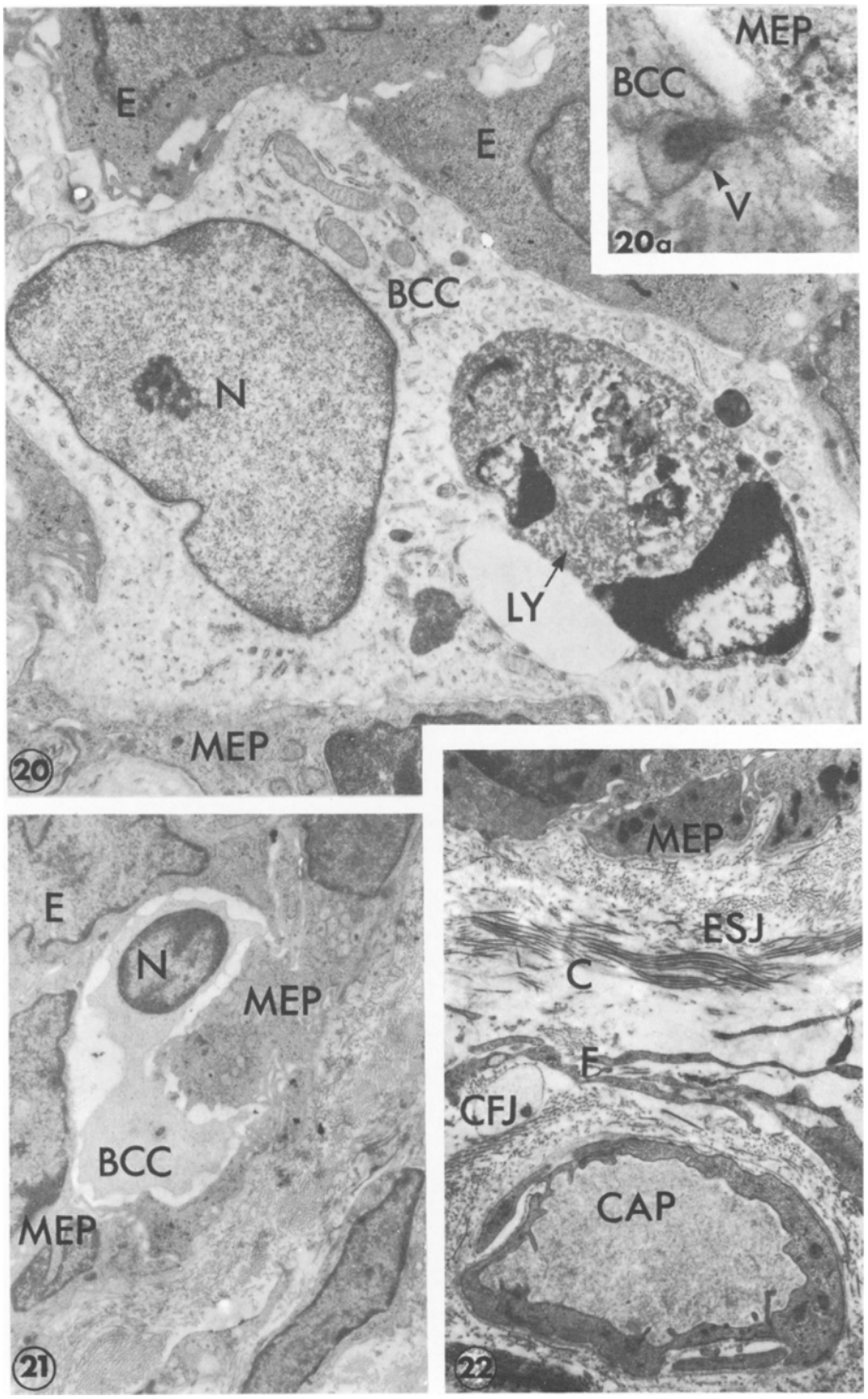
E) Ductule Capillaries

There was a discrete capillary supply in close association with the delimiting fibroblasts of the ESJ of ductules and intralobular ducts (Fig. 22). Capillaries were similar in structure to skeletal types and conformed to the A-1α type in the classification of Bennet et al. (1959). In cross section the wall was usually continuous with rare fenestrations (30–55 nm) (Fig. 24) and comprised 2–3 endothelial cells. Total diameter varied between 1–5 μm. The outer basal lamina was continuous and often multiple. Capillaries were always exterior to the delimiting fibroblasts of the ESJ (Fig. 22). Specializations of the endothelial luminal membrane included marginal folds at interdigitated junctions, occasional protrusions similar to microvilli and numerous micropinocytotic vesicles. The outer surface of the endothelium was smooth or undulating and distinct projections such as microvilli were absent. Micropinocytotic activity was more pronounced than on the luminal wall. Rarely, surface plaques similar to the hemidesmosome-like

Fig. 20. A large basal clear cell (*BCC*), active macrophage type. There is a large nuclear sized secondary lysosome (*LY*). The nucleus (*N*) is pale and smooth in outline. Epithelium (*E*). Myopeithelium (*MEP*). ×9,150. Insert 20a. Detail of Figure 20. A small vesicle (*V*) at the basal clear cell (*BCC*) surface may be pinching off a small portion of the adjacent myoepithelial cell. ×106,800

Fig. 21. A small basal clear cell (*BCC*), lymphocyte type. The nucleus (*N*) is ovoid and dark. Epithelium (*E*), Myoepithelium (*MEP*). ×5,500

Fig. 22. The epithelial stromal junction (*ESJ*) extends from the myoepithelium (*MEP*) to the delimiting fibroblast zone (*F*), and includes a layer of collagen fibres (*C*). The capillary fibroblast junction extends from the fibroblast zone (*F*) to the capillary lumen (*CAP*). In normal tissue capillaries are always external to this fibroblast layer. ×7,500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specializations of the perivascular cells were seen (Fig. 23). Nuclei ranged from smooth to indented and contained pale bodies (Sykes bodies) similar to those found in ductular epithelium (Fig. 26). Fine filaments were present throughout the cytoplasm, sometimes being well developed in the perinuclear cytoplasm (Fig. 26). Cytoplasmic inclusions included possible lysosomes, dark and light variants of the Weibel-Palade body (Weibel, Palade, 1964) (Fig. 25) and an occasional lipid body, similar to those observed in the ductule epithelium. Pinocytosis vesicles were not common and often had associated micropinocytosis at their surface. Micropinocytosis also occurred at endothelial interdigitated junctions, and developed as "micropinocytosis channels" at the endothelial luminal surface. Vesicles also coalesced in the cytoplasm.

The perivascular cells (Fig. 23) formed a discontinuous layer, their cytoplasmic extensions often sited in shallow indentations in the endothelium. Contiguous perivascular cells did not come into contact. Perivascular cells were sometimes in direct contact with the endothelium, but more often separated by a variable thickness of basal lamina contributed by both cells, which was often multiple or had fused to become an amorphous layer (Fig. 23). Collagen fibres appeared to penetrate the basal lamina complex between the two cell layers.

The surface of perivascular cells was flattened or undulating with micropinocytotic vesicles which were more numerous on the side furthest from the endothelium (Fig. 23). Dense surface plaques on the cell membrane were also more common on the latter surface. Fine cytoplasmic filaments appeared to be less common than in endothelium, and no Weibel-Palade bodies or Sykes bodies were observed. Perivascular cells were similar in general appearance to both fibroblasts and endothelium. They were distinguished from fibroblasts by the presence of a basal lamina, but were often difficult to distinguish from endothelium except by their spatial relationship to the capillary wall.

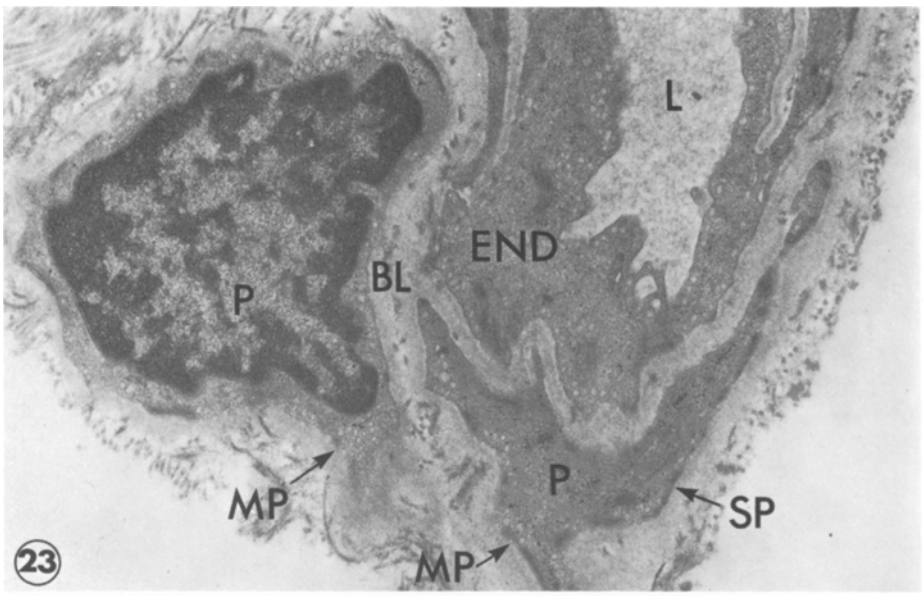
Intercellular elements consisted of the basal laminae of endothelial and perivascular cells, collagen, and the background matrix of amorphous deposits, all of which were essentially part of the intralobular stroma. Variation in the amount of these components was mainly determined by the position of the capillary in relation to the delimiting fibroblasts of the ESJ.

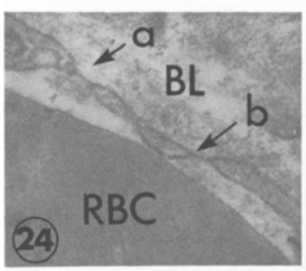
Fig. 23. Perivascular cells (*P*) are in close association with the capillary endothelium (*END*) and the basal lamina of the two cells may fuse to become an amorphous layer (*BL*). Dense surface plaques (*SP*) are common on the outer surface of perivascular cells, but rare on endothelial cells. Micropinocytotic vesicles (*MP*). Capillary lumen (*L*). ×11,500

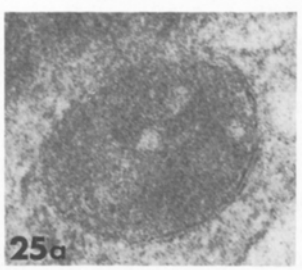
Fig. 24. Fenestrations (arrows) occasionally penetrate the capillary endothelium: *a* 50 nm; *b* 40 nm. Red blood cell (*RBC*), basal lamina (*BL*). ×60,000

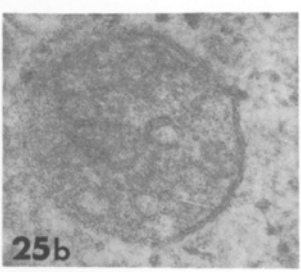
Fig. 25a Weibel-Palade body, dark type, capillary endothelium. ×133,600. **b** Weibel-Palade body, pale type, capillary endothelium. ×100,200

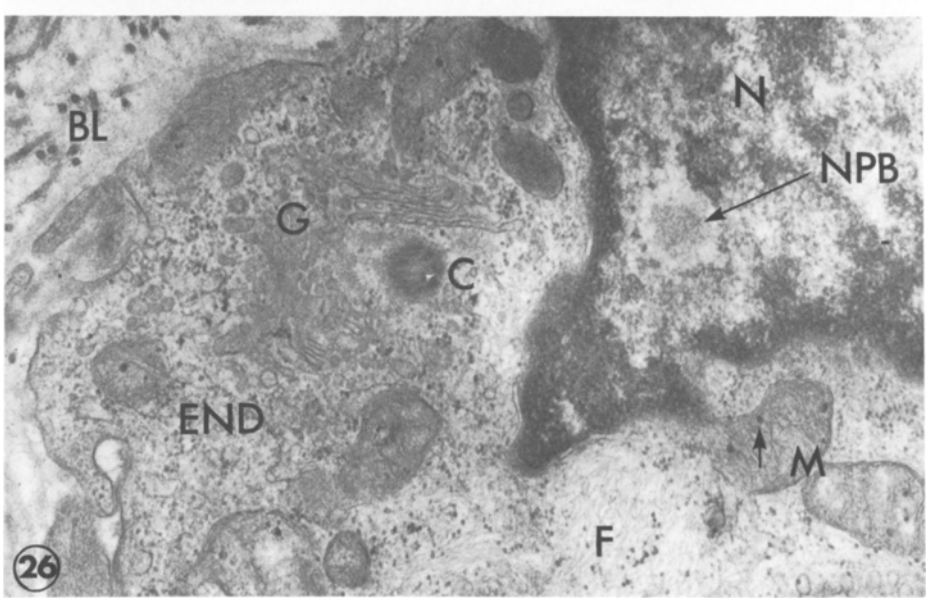
Fig. 26. Endothelial cell (*END*) containing a centriole (*C*), adjacent golgi (*G*) and mitochondria (*M*) with intercristal granules (arrow). The nucleus (*N*) contains a nuclear pale body (*NPB*) or "Sykes body" similar to those of the epithelium and myoepithelium. Cytoplasmic filaments (*F*) are seen in close proximity to the nucleus. Basal lamina (*BL*). ×27,450


L
END
BL
P
MP
P
SP
MP
23


a
BL
b
RBC
24


25a


25b


BL
N
NPB
G
C
END
M
F
26

Discussion

Ozzello (1970, 1971, 1974) first stressed the importance of specific criteria to which tissue used for a normal study should conform. Experience of tissue from a large number of reduction mammaplasty donors with a wide range of clinical backgrounds has shown how diverse the ultrastructure of the TDLU may be, when in histological material little or no significant differences can be detected between such specimens. The ultrastructural morphology of such glands presumably presents a confusing picture due to the effects of pregnancy, involution phenomena and the number of menstrual cycles experienced by the individual. The effects of drug administration especially are little known, but many in common use, such as tranquilizers, and other factors such as stress (Fluckiger, 1972), affect the plasma levels of hormones such as prolactin which may affect gland structure. Prolactin may also cause secondary effects by increasing plasma steroids such as progesterone (Lindner and Shelesnyak, 1967; McNatty et al., 1974) which could produce changes which confuse the interpretation of ultrastructural morphology. Considering these complicating factors the need for using strictly defined tissue must be stressed, and the use of material which may contain minimal dysplasias due to contiguous benign or malignant disease should be avoided.

Previous authors (Carter et al., 1969; Salazar and Tobon, 1974; Waugh and Van der Hoeven, 1962) found no difference in structure between the regions of the TDLU. In this study morphological differences were found along the ductal system, the most striking being the degree of myoepithelial differentiation and corresponding alterations in the development of the basal lamina. The poor differentiation of cells in ductule tips and smaller ductules suggests that these are young cells at growing points and are responsive to growth stimuli. The better differentiated regions of the TDLU will form the intralobular ducts and collecting ducts of the expanded gland after the formation of functional acini. Ellis (1965) suggested that myoepithelial function included production of basement membrane and intercellular substances, and Tannenbaum et al. (1969) suggested that the development of basal lamina corresponded to the density of packing of the myoepithelium, loosely packed cells having a multiple basal lamina, tightly packed cells a single lamina. The correlation here of single lamina with poorly differentiated myoepithelium (few myofilaments) and multiple basal lamina with areas of well differentiated myoepithelium (well developed myofilament content) suggests the development of basal lamina is related to time and ageing of the myoepithelium.

The $9+0$ myoepithelial cilia, fully described elsewhere (Stirling and Chandler, 1976) may have a sensory function. Ozzello (1970) discussed the importance of the myoepithelium in his concept of the epithelial stromal junction (ESJ). The cilium may represent an element in this system, acting as a sensor related to myoepithelial-epithelial activities, or it may play a part in the milk ejection complex acting as a mechano-receptor involved with myoepithelial contractility. Ellis (1965) also speculated that myoepithelium is involved in impulse transmission. Considering the role played by similar cilia in a transducing and conducting capacity in various receptor organs (Barber, 1974), the part of the myoepithelium in such processes may be worth further consideration.

The basic cell types encountered in this study are considered to be four types, epithelium, myoepithelium, macrophages and lymphocytes. The epithelium consisted of 2 cell types as described elsewhere (Bassler, 1968, 1970; Haguenau and Arnoult, 1959), namely light "B" cells with occasional dark "A" cells. The myoepithelium, as described above, showed a transition series from those cells at ductule tips with a poorly developed myofilament content, to those in well developed ductules and intralobular ducts with many myofilaments. This appears to reflect the ageing of the respective cells along the ductule.

The basal clear cells form a heterogeneous group, but are easily distinguished from both epithelium and myoepithelium. As they show no attachment to contiguous cells it is concluded that they are free to wander through the TDLU. Those cells with no lysosomes are regarded as being resting macrophages, those with secondary lysosomes as active. The role of macrophages within the TDLU is known to involve the uptake of secretory and cellular debris during regression after lactation (Richards and Benson, 1971), but their activities will presumably also include the phagocytosis of dead and effete cells, and the location of abnormal cells as part of the immune response. The number and activity of macrophages within the TDLU may vary with the menstrual cycle as oestrogen is known to increase macrophage activity (Vernon-Roberts, 1969) and further work on this topic may be rewarding.

It is possible to speculate that the "resting" type macrophage and the cell described as a lymphocyte may represent stem cells. Salazar and Tobon (1974) recently described a similar lymphoid type cell, suggesting it may be a stem cell. In this study, as none of these cell types showed cellular attachments and were present in only small numbers, they are not considered to be stem cells. No distinctive cell type that could have represented a stem cell was located, and the view of Slemmer (1974) that epithelial and myoepithelial cells represent separate populations, is supported.

The various vesicular inclusions found in the epithelium present a confusing problem. The small pale vesicles may represent the production of mucopolysaccharide which lines the ductular lumen. Other vesicles can be interpreted as storage secretion, a low level of active secretion, or resorption of secretory debris from the lumen. Alternatively, the dark bodies may be lysosomes. The occasional internal vesicles within dark bodies may be associated with lysosomal activities or may represent the transport of material in or out the vesicle. They could also be related to the formation of lipid bodies, especially where the latter are associated with a granular body. The dark vesicles found in close association with the nucleus are similar to those described by Szego (1974) as lysosomes involved in the transport and mediation of hormone action with the nucleus. Speculation that a similar phenomenon occurs in breast epithelium must await positive identification of their lysosomal nature.

The identification of the dark, basally located bodies in the epithelium is also difficult. Again they may represent uptake or secretion of material by the epithelium, or they may be lysosomal.

The implications and importance of the ESJ as a functional unit mediating exchange of materials between the circulation and mammary epithelium has been discussed by Ozzello (1970). For efficient exchange between blood and

cells the tissue must also maintain its microvascular geometry, the structure and function of the capillary endothelium and its associated structures. The ESJ may actively participate in the genesis of mammary dysplasia by loss of control of transport of materials to and from the epithelium. This same concept can be extended to the intralobular stroma situated between the delimiting fibroblasts of the ESJ and the capillaries in close contact with the ductal system.

Abnormal capillaries have been associated with several disease conditions. Ozzello (1971) described how disarray of the ESJ results in disruption of the microvascular geometry with capillaries approaching and coming into contact with epithelial structures. This is often found in areas of apocrine metaplasia (Stirling, unpublished data), and Ozzello (1971) also reported that in proximity to foci of apocrine metaplasia, vascular channels may be more numerous and of larger diameter than normal. (Gould and Snyder, 1974) described duplicated capillary basal laminae in subareolar carcinomas, as did Carstens (1974) in fibroadenomas, suggesting that the increased barrier between blood supply and epithelium may explain why fibroadenomas tend to undergo atrophy, hyalinization and calcification. Perivascular cells may also contribute to the cell population of fibroadenomas (Ahmed, 1971; Murad et al., 1967) and their possible synthetic capabilities (Rhodin, 1968) make them an important factor to consider in dysplasia.

The capillary bed is obviously a dynamic region which will undergo profound alteration in the various physiological states of the breast such as pregnancy, menstrual and hormonal status and in disease. It is the primary barrier to transport between blood and epithelium and it is probably influenced by many factors operating within and around tumours and by tumour products and stromal reactions. The capillary is also a site of activity in inflammatory reactions and endothelial surface components may be selective in the interaction between lymphocytes and their transcellular migration through the endothelium. The zone from the ESJ to the capillary lumen can be regarded as a functional unit, similar to the ESJ, and is designated the capillary fibroblast junction (CFJ). The CFJ may play an important role as both an initiator and contributor of various components to breast disease. The part played by the basal laminae of the capillary remains open to question. The finding of multilayered basal laminae around normal capillaries makes their significance in dysplasia obscure. It is likely that a certain amount of multilayering is normal, but extensive multilayering or biochemical alteration precedes, or is produced by certain pathological conditions.

Acknowledgements. We are indebted to the surgical staff of the Plastic Surgery Units at Chepstow (Gwent), Wordsley (West Midlands) and Frenchay (Avon) hospitals, for supplying tissue. Supported by M.R.C. Grant No. G-974-125C, and by the Tenovus Organisation. This paper forms part of a Ph.D thesis to be submitted by J.S. to the University of Wales, Cardiff.

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Received August 26, 1976