Virchows Arch [Pathol Anat] (1984) 402:319–329

Virchows Archiv A

Basal clear cells of the normal human breast

Caroline A. Smith, Paul Monaghan, and A. Munro Neville
Ludwig Institute for Cancer Research, Clifton Avenue, Sutton,
Surrey, SM2 5PX, Great Britain

Summary. The ductal system of the human breast consists of two major cell types: epithelial and myoepithelial. In some reports a third cell type, given various names is mentioned. In this study it is called a basal clear cell. The role of this cell, unlike that of the epithelial and myoepithelial cells, remains unclear, although it has been suggested that it may have a stem cell function. We illustrate here that there is an ultrastructural transition between the basal clear and myoepithelial cell; suggesting that it acts as a precursor of the myoepithelial cell, and may not be a stem cell for both epithelial and myoepithelial cells.

Key words: Normal Human Breast – Ultrastructure Basal Clear Cells

A series of in vitro studies of chemically induced rat breast tumour cell lines (Rudland et al. 1980; Bennett 1980) has provided considerable evidence for the presence of a population of stem cells in rat breast. Such cells are postulated to be capable of giving rise to both epithelial and myoepithelial cells (Rudland et al. 1980). Ultrastructural studies of rat breast have identified 3 major cell types within mature breast epithelium. These are epithelial cells, whose major function during pregnancy is secretion of milk products, myoepithelial cells, with a contractile function, and basal clear cells, which have been proposed to constitute a stem cell population (Radnor 1972a and b; Rudland et al. 1980; Bennett 1980). – The situation for human breast epithelium is less clear, particularly as no comparable in vitro models exist to study the relationships between the various cell types in human breast.

Ultrastructural studies of normal human breast have provided inconsistent results. With variations in both the source of material, and different fixation protocols (Ozzello 1971; Stirling and Chandler 1976; 1977; Waugh et al. 1962; Fanger et al. 1974; Ahmed 1980; Carter et al. 1969; Salazar

Offprint requests to: C.A. Smith at the above address

and Tobon 1974; Tannenbaum et al. 1969; Sykes et al. 1968) several different estimates for the number of cell types in human breast have been reported. Essentially, however, authors are in agreement that they consist of epithelial and myoepithelial cells (Carter et al. 1969; Murad et al. 1967, Murad and von Haam 1968; Sykes et al. 1968; Tannenbaum et al. 1969) with other authors additionally reporting intermediate cells (Ozzello 1971; Fisher 1976), indeterminate cells (Ozzello 1971), clear cells (Bässler 1958; Doerr et al. 1978), and basal clear cells (Stirling and Chandler 1976, Toker 1967).

Whilst the existence of a stem cell population has been postulated in human breast (Ozzello 1971; Hamperl 1970; Stirling and Chandler 1976a; Salazar and Tobon 1974; Tobon and Salazar 1974; Toker 1967) no clear supporting evidence has emerged.

We have undertaken an ultrastructural study of strictly defined (Ozzello 1971) Normal human breast, with particular reference to the structure of the basal clear cell, in order to determine whether, such an ultrastructural approach is capable of indicating the relationship between the major cells types in the human breast.

Materials and methods

Normal human breast tissue was obtained at surgery from reduction mammoplasties carried out for cosmetic purposes. Thirty two such samples have been examined from patients ranging from 19–40 years of age all nulliparous and with no known hormonal dysfunction or previous endocrine therapy including oral contraceptives (Ozzello 1971). Light microscopic examination of the breast tissue in each instance showed no evidence of disease in either breast.

Reduction mammoplasty tissue consists largely of fat, but when viewed with a dissecting microscope the ductal tissue can be distinguished by its thread-like appearance, whilst lobulo-alveoli appear as minute nodules. These separate areas were dissected carefully from the fatty stroma and cut into cubes of 1–2 mm.

Samples were fixed for a minimum of one hour in 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.3; 330 mOsM, by addition of sucrose) at 4° C, and post-fixed in 1% osmic acid in phosphate buffer (pH 7.3) for a minimum of one hour at 4° C.

Tissue was dehydrated in ethanol, and embedded via propylene oxide, in Epon Araldite (Mollenhauer 1964).

Semithin sections (1.5–2.0 μm) were stained with 1% toluidine blue for light microscopy, and suitable areas were selected for electron microscopy.

Ultrathin sections (0.05–0.07 μm) were cut on a Reichert OMU4 ultramicrotome using a diamond knife. The sections were double stained (Stempak and Ward 1964) with 15% uranyl acetate in methanol and Reynold's lead citrate pH 12 (Reynold 1963) and viewed in a Philips E.M. 400 electron microscope.

Results

Epithelial cells form a continuous layer, lining the lumen of both ducts and lobulo-alveoli. Such cells have microvilli on their luminal surfaces; junctional complexes link adjacent epithelial cells at their apical margins (Fig. 1). No major differences were observed between epithelial cells of duct or lobulo-alveolus, although, in the lobulo-alveolus, their columnar shape tends to be tapered towards the lumen in order to form the spherical contours of the lobulo-alveolus. Epithelial cells occasionally extend to the basal lamina, but there these cells neither form hemidesmosomes nor develop micropinocytotic vesicles, as found in myoepithelial cells.

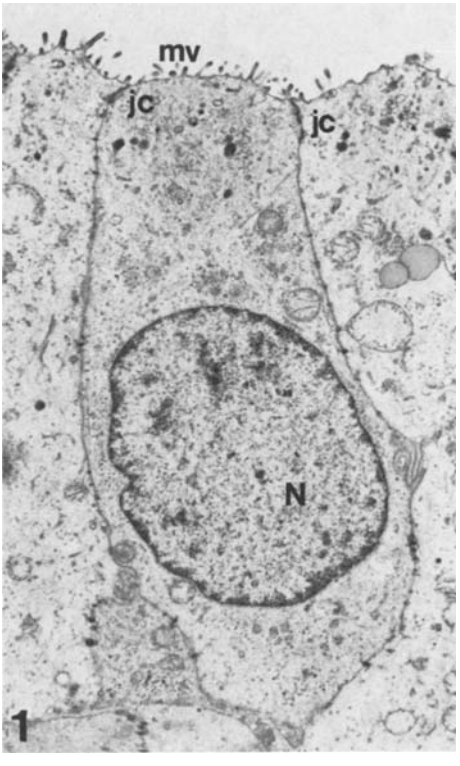


Fig. 1. An electron micrograph of an epithelial cell; showing pale staining cytoplasm, columnar shape, luminal microvilli. (*mv*), junctional complexes (*jc*) in the apical region and a round, basally situated euchromatic nucleus (*N*). Mag. ×6,012

Myoepithelial cells, characterised by the presence of myofilaments, with focal densities, micropinocytotic vesicles and hemidesmosomes, rest on the basal lamina. They show changes in their morphology dependent upon their position within the breast. In the ducts, these cells form a single continuous layer of electron-dense cells between the epithelial cells and the stroma. They have an irregular outline, with prominent peg-like projections into the stroma, which can be observed both at the light and electron microscopic level (Figs. 2a and b).

In the lobulo-alveolus, the myoepithelial cells are less electron-dense than in the ducts, form a discontinuous layer between epithelial cells and the stroma, and lack the prominent projections into the stroma again evident at both light and electron microscopic level, (Figs. 3a and b). In both regions the irregular nucleus is characterised by clumped heterochromatin.

The basal clear cell has an irregular shape, is larger than both epithelial and myoepithelial cells and has a very pale staining cytoplasm. The basal clear cells are more numerous in the lobulo-alveolus than main duct, and are abundant especially at its extreme tip. The basal clear cell is found between epithelial and myoepithelial cells. It has no microvilli or membrane interdigitations with neighbouring cells, but does have occasional small desmosomes between it and adjacent epithelial and myoepithelial cells. Cytoplasmic organelles are sparse, with a few round or elongated mitochondria, occasional single long strands of RER; free ribosomes; no Golgi apparatus;

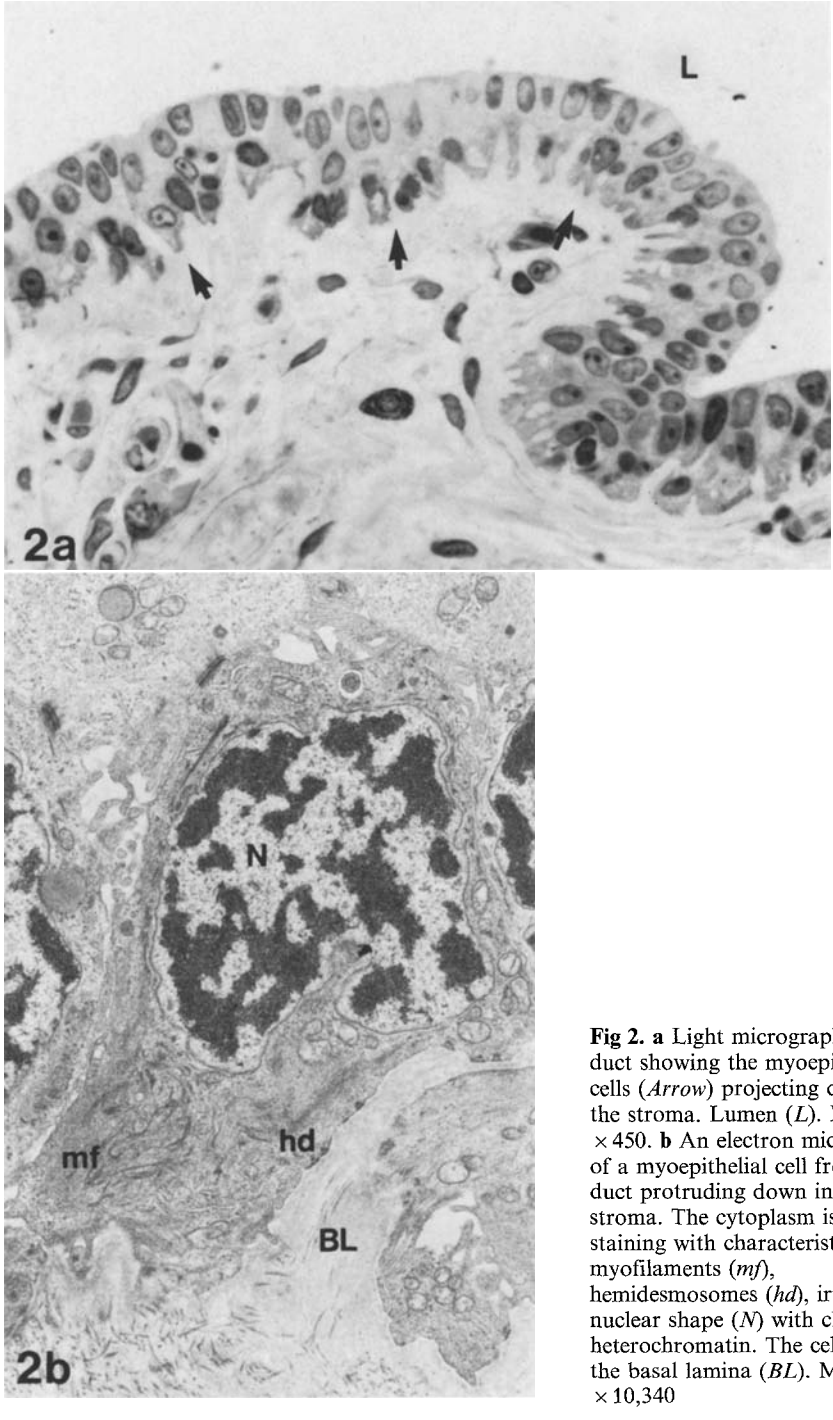


Fig 2. a Light micrograph of main duct showing the myoepithelial cells (*Arrow*) projecting down into the stroma. Lumen (*L*). Mag. ×450. **b** An electron micrograph of a myoepithelial cell from main duct protruding down into the stroma. The cytoplasm is dark staining with characteristic myofilaments (*mf*), hemidesmosomes (*hd*), irregular nuclear shape (*N*) with clumped heterochromatin. The cell rests on the basal lamina (*BL*). Mag. ×10,340

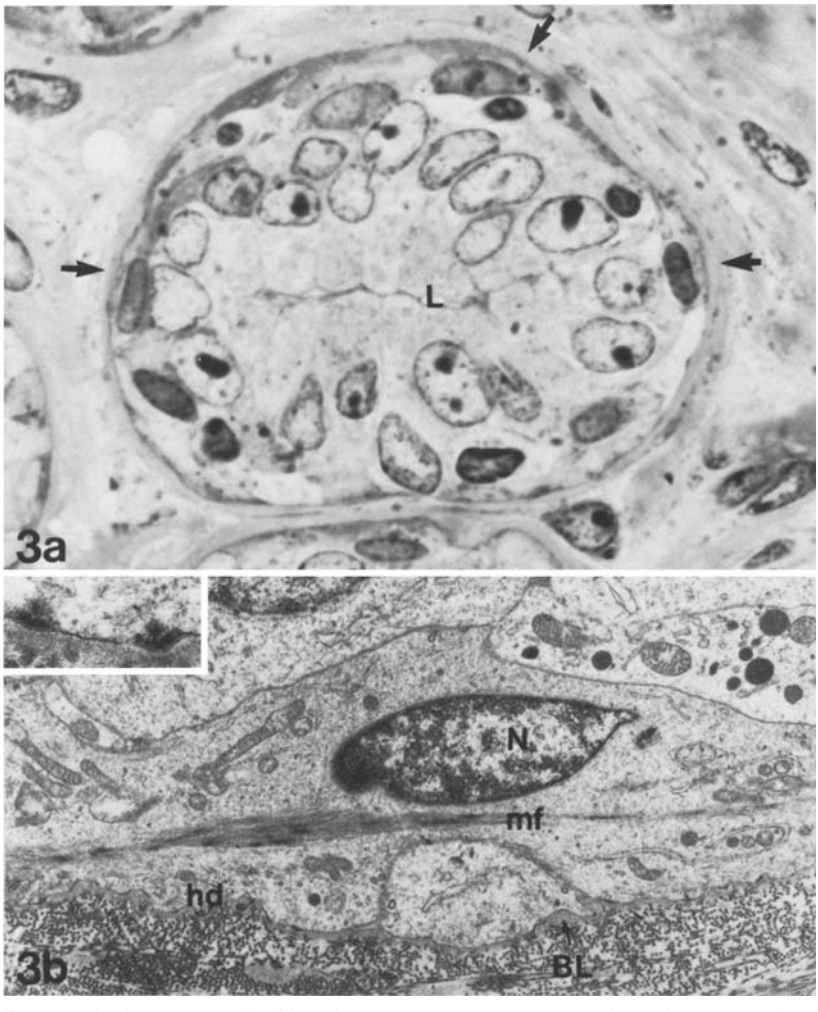


Fig. 3a. Light micrograph of lobulo-alveolus where myoepithelial cells (*Arrow*) do not protrude into the stroma. Lumen (*L*). Mag. ×985. **b** An electron micrograph of a myoepithelial cell from lobulo-alveolus in contrast to that found in the main duct has pale staining cytoplasm and an attenuated/elongated shape. The cytoplasmic features, such as myofilaments (*mf*), hemidesmosomes (*hd*) and irregular nucleus (*N*) with clumped heterochromatin, are similar to those of a myoepithelial cell found in main duct. The cell rests on the basal lamina (*BL*). Mag. ×10,500. A high power electron micrograph of hemidesmosomes from the basal membrane of a myoepithelial cell. (*Insert*) Mag. ×38,500

no filaments; occasional dark or pale lipid droplets (Fig. 4). The nucleus is round with uniform or clumped heterochromatin. The outer nuclear envelope has associated ribosomes and nuclear pores.

Occassionally, basal clear cells are found resting on the basal lamina in the lobulo-alveolus. In such cases, these cells, although having some features of basal clear cells, also have some or, all of the features characteris-

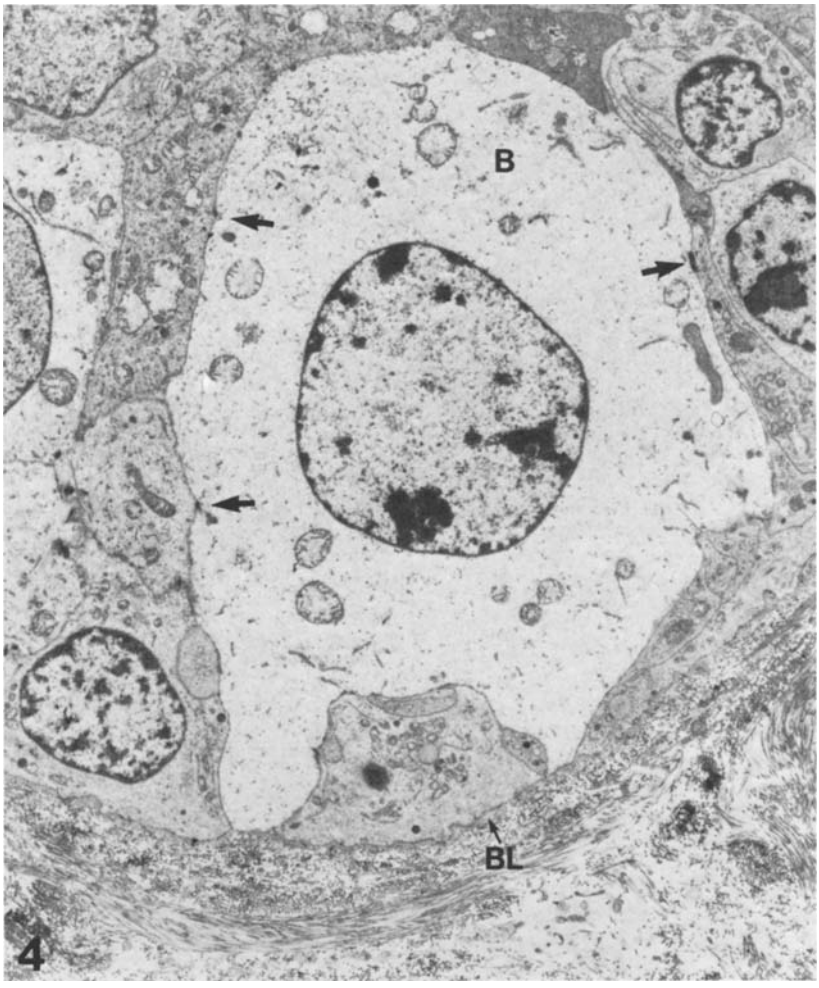


Fig. 4. An electron micrograph of a basal clear cell (*B*) in lobuloalvèolus. This cell is a much larger cell than the epithelial or myoepithelial cells, and has electron-lucent cytoplasm. Cytoplasmic organelles are scant; the nucleus is round with no heterochromatin. This cell is touching the basal lamina (*BL*) in places, but is still largely residing between the epithelial and myoepithelial cells, and has desmosomes (*Arrow*) attaching it to these cells. Mag. × 5,942

tic of myoepithelial cells. Some cells rest on the basal lamina with hemidesmosomes, structures not found in epithelial cells (Ozzello 1971; Stirling and Chandler 1976, 1977), which attach them to the basal lamina, but lack cytoplasmic filaments (Fig. 5a). Other cells are seen to have a few cytoplasmic filaments with focal densities in the basal cytoplasm (Fig. 5b). These basal clear-like cells, with hemidesmosomes and myofilaments, have also been observed with an irregular shaped nucleus and increasing heterochromatin content (Fig. 5c). These cells display most of the differentiated features of myoepithelial cells, but they are rounded in shape and pale

staining. With increasing distance from the tip of the lobulo-alveoli, the pale myoepithelial cell (Fig. 5d) becomes more electron dense, and shows projections into the stroma described in myoepithelial cells in major duct.

Thus, there appears to exist, a transition between basal clear cells positioned between the epithelial and myoepithelial cells, through a cell on the basal lamina showing characteristics of both basal clear cells and myoepithelial cells, to fully differentiated myoepithelial cells.

A similar relationship with cells exhibiting features intermediate between basal clear and epithelial cells was not observed.

Discussion

The precise inter-relationships between breast cell types in general, and the question of whether there is a stem cell population in adult human breast epithelium in particular are important issues in an understanding of both normal and neoplastic processes in human breast.

Clearly, ultrastructural studies are not an ideal means to approach the dynamic processes involved in the relationships between the various cell types in the human breast. At present, however, it remains one of the few methods available for such investigations.

There is no clear concensus in published reports of the ultrastructure of the human breast as to whether the basal clear cell is an epithelial or a macrophage type cell (Ozzello 1971; Stirling and Chandler 1976 and 1977). It is important to confirm the epithelial nature of the basal clear cells. The presence of desmosomes observed between basal clear cells and epithelial and myoepithelial cells indicates that the basal clear cell is indeed an epithelial cell. Basal clear cells are rarely observed in major ducts, and are most commonly observed in lobulo-alveolar regions where they are situated between epithelial and myoepithelial cells. Occasionally they rest on the basement membrane, and cells in this position exhibit one or more features of myoepithelial cells (hemidesmosomes, myofilaments and micropinocytotic vesicles).

Cells showing these myoepithelial characteristics should perhaps be designated pale myoepithelial cells, rather than any form of basal clear cell. The further these cells are positioned away from the lobule tip, the more they resemble 'classical' ductal myoepithelial cells.

Despite the ultrastructural spectrum observed between basal clear cells and myoepithelial cells, the lack of a similar association between basal clear cells and epithelial cells argues against the basal clear cell being a stem cell in the sense of giving rise to both epithelial and myoepithelial cells in the resting adult human breast.

In vitro studies of normal human breast might be expected to assist in resolving the relationship between the cell types of human breast. However, during studies on a large number of samples of normal human breast, digested by collagenase, and cultured in collagen gels (Foster et al. 1983) no basal clear cells were observed. Whether the basal clear cells are more susceptible to mechanical damage during the digestive preparation of breast

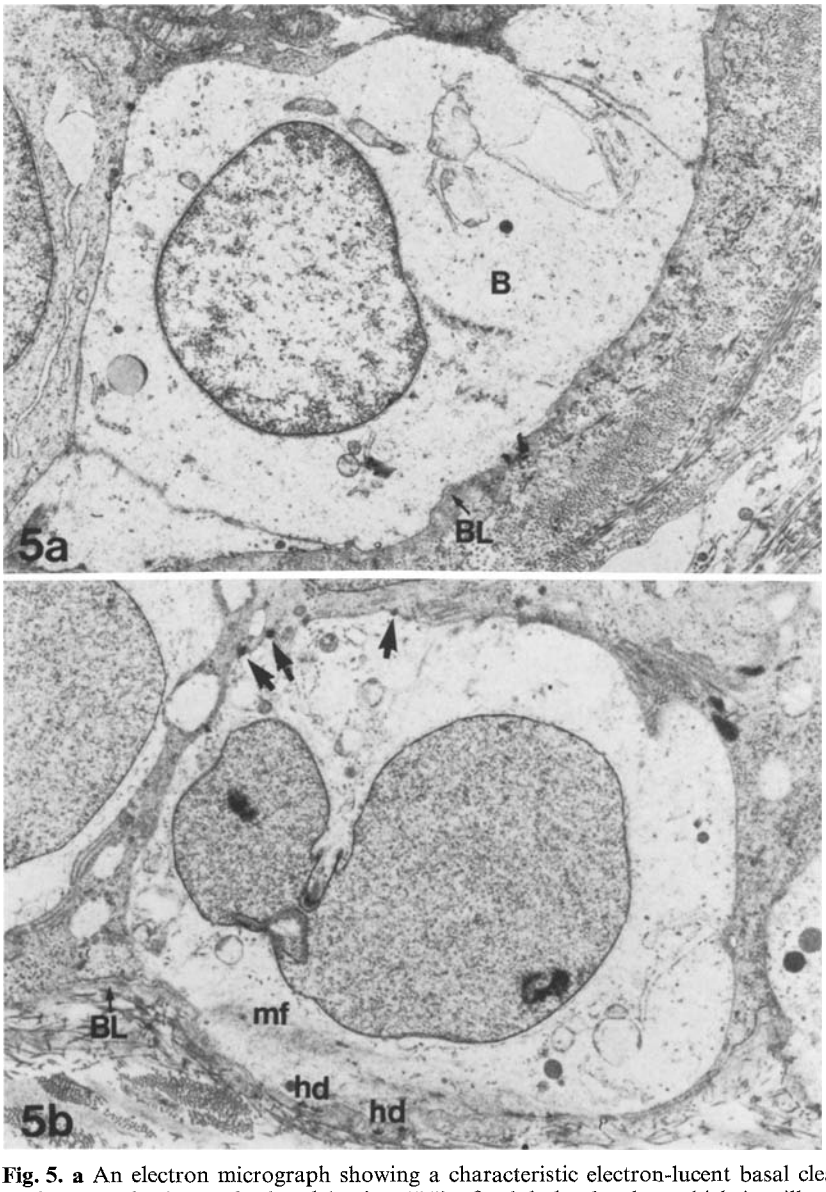
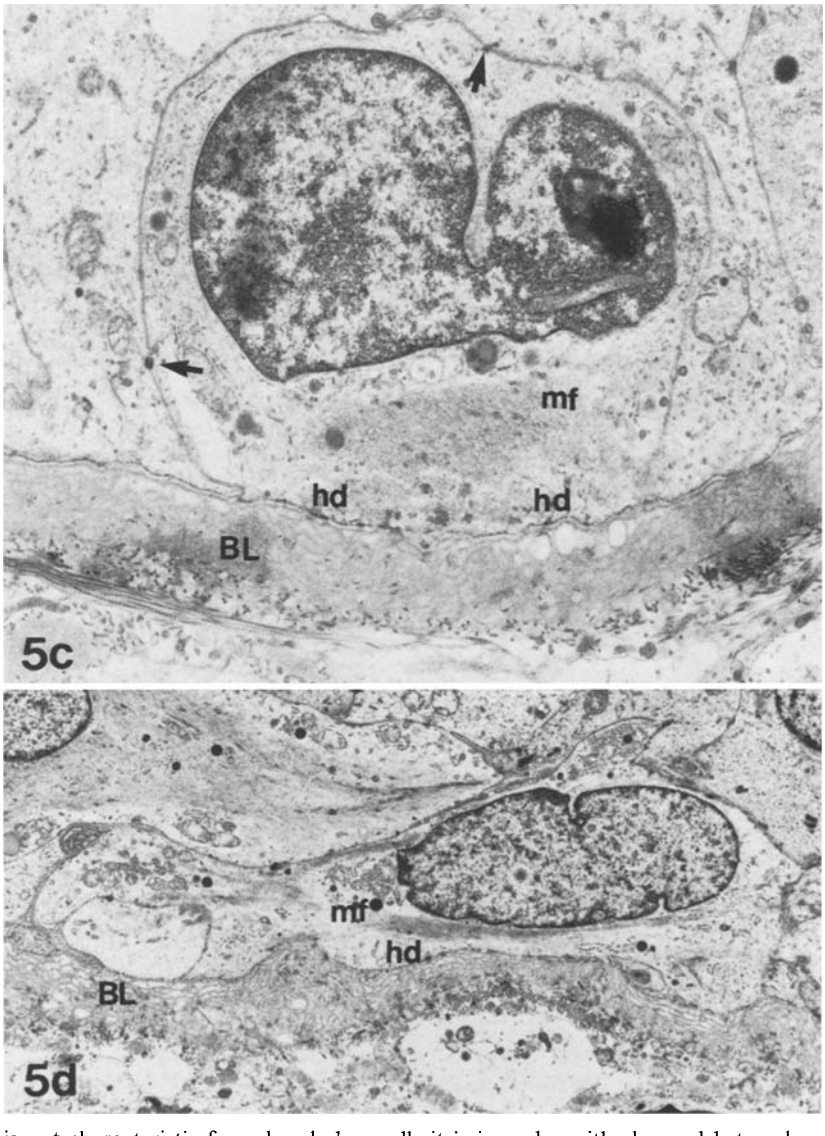


Fig. 5. a An electron micrograph showing a characteristic electron-lucent basal clear cell (*B*) resting completely on the basal lamina (*BL*) of a lobulo-alveolus, which is still attached to epithelial cells by desmosomes (*Arrow*). Mag. ×7,500. **b** An electron micrograph of a cell illustrating features characteristic of both basal clear cells and myoepithelial cells. The cytoplasm is electron-lucent and the nucleus round with no heterochromatin, whilst below the nucleus myofilaments (*mf*) and hemidesmosomes (*hd*) can be seen attaching the cell to the basal lamina (*BL*). Note the presence of desmosomes (*Arrow*). Mag. ×7,333. **c** An electron micrograph of a cell which has pale staining cytoplasm (it is neither electron-lucent as in the basal clear cell, nor electron-dense as for the myoepithelial cell). The nuclear morphology



is not characteristic for a basal clear cell; it is irregular with clumped heterochromatin, and myofilaments (*mf*) and hemidesmosomes (*hd*) are present joining the basal membrane and basal lamina (*BL*). The shape of the cell, however, is round and not elongated as a myoepithelial cell in lobulo-alveolus. Desmosomes are present between this cell and neighbouring epithelial cells (*Arrow*). Mag. ×10,966. **d** An electron micrograph of a cell displaying features similar to those of the cell in **c**, however, the shape of the cell is elongated characteristic of a myoepithelial cell in lobulo-alveolus: basal lamina (*BL*), myofilaments (*mf*), and hemidesmosomes (*hd*) are present. This cell appears to be a pale staining myoepithelial cell. Mag. ×4,983

tissue for culture is not known. Alternatively, it is known that the rest of the cells of the breast epithelium undergo an initial loss of many of their differentiated characteristic features, under the conditions used during tissue culture. This may render the basal clear cells indistinguishable from the epithelial and myoepithelial cells.

Our interpretation of the ultrastructural data on human breast is at variance with that reported for rat breast in that no evidence exists in the resting adult human breast for the basal clear cell being a stem cell. One possible explanation results from the fact that breast tumour cell lines have been induced by carcinogen treatment in virgin rats (Bennett et al. 1978), where the breast epithelium is composed of mainly ducts and terminal end buds (Russo and Russo 1978). The latter contain a large number of undifferentiated cells, which are not seen in the resting adult human breast. During pregnancy, in the rat mammary gland, the terminal end buds differentiate into lobulo-alveoli. In the adult human breast, terminal end buds have not been found or reported either before or during pregnancy (Ozzello 1971; Stirling and Chandler 1976 and 1977; Salazar and Tobon 1974). Thus, in the adult human breast we may be dealing with a different cell population from that found in the adult rat mammary gland.

It must be remembered, however, that these studies refer to the normal resting adult human breast and should not be expanded, without further verification to include foetal, pregnant, lactating or the involuting human breast.

References

Ahmed A (1980) Ultrastructural aspects of human breast lesions. Pathol Annu 15:411–443

Bässler R (1958) Beiträge zur Morphologie der kindlichen Brustdrüse. Frankfurt Z Pathol 69:37–52

Bennett DC (1980) Morphogenesis of branching tubules in cultures of cloned mammary epithelial cells. Nature 285:657–659

Bennett DC, Peachey LA, Durbin H, Rudland PS (1978) A possible mammary stem cell line. Cell 15:283–298

Carter D, Yardley JH, Shelley WM (1969) Lobular carcinoma of the breast. An ultrastructural comparison with certain duct carcinomas and benign lesions. John Hopk Med J 125:25–43

Doerr W, Seifert G, Uehlinger E (1978) Spezielle Pathologische Anatomie. Springer, Berlin Heidelberg New York Band 11 pp 28–88

Fanger H, Ree HJ (1974) Cyclic changes of human mammary gland epithelium in relation to the menstrual cycle – an ultrastructural study. Cancer 34:574–585

Fisher ER (1976) Ultrastructure of the human breast and its disorders. Am J Clin Pathol 66:291–375

Foster CS, Smith CA, Dinsdale EA, Monaghan P, Neville AM (1983) Human mammary gland morphogenesis in vitro: The growth and differentiation of normal breast epithelium in collagen gel cultures defined by electron microscopy, monoclonal antibodies, and autoradiography. Develop Biol 96:197–216

Hamperl H (1970) The myothelia (myoepithelial cells): Normal state, regressive changes, hyperplasia and tumours. Curr Topics Pathol 53:162–220

Mollenhauer HH (1964) Plastic embedding mixture for use in electron microscopy. Stain Technol 39:111–114

Murad TM, Grieder MH, Scarpelli DG (1967) The ultrastructure of human mammary fibroadenoma. Am J Pathol 51:663–679

Murad TM, Von Haam E (1968) The ultrastructure of fibrocystic disease of the breast. Cancer 22:587–600

Ozzello L (1971) Ultrastructure of the human mammary gland. Pathol Annu 6:1–59
Radnor CJP (1972a) Myoepithelium in the prelactating and lactating mammary glands of the rat. J Anat 112:337–353
Radnor CJP (1972b) Myoepithelial cell differentiation in rat mammary glands. J Anat 111:381–398
Reynold ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212
Rudland PS, Ormerod EJ, Paterson F (1980) Stem cells in rat mammary gland development and cancer, a review. J Roy Soc Med 73:437–442
Russo IH, Russo J (1978) Developmental stage of the rat mammary gland determinant of its susceptibility to 7,12-dimethylbenz(a)anthracene. J Natl Cancer Inst 61:1439–1449
Salazar H, Tobon H (1974) Morphologic changes of the mammary gland during development, pregnancy and lactation. In: Josimovich JB (ed) Lactogenic hormones, fetal nutrition and lactation. John Wiley and Sons, New York pp 221–227
Stempak JG, Ward RT (1964) An improved staining method for electron microscopy. J Cell Biol 22:697–701
Stirling JW, Chandler JA (1976) The fine structure of the normal resting terminal ductal-lobular unit of the female breast. Virchows Arch [Pathol Anat] 372:205–226
Stirling JW, Chandler JA (1977) The fine structure of ducts and subareolar ducts in the resting gland of the female breast. Virchows Arch [Pathol Anat] 373:119–132
Sykes JA, Recher L, Jernstrom PH, Whitescarver J (1968) Morphological investigation of human breast cancer. J Natl Cancer Inst 40:195–223
Tannenbaum M, Weiss M, Marx AJ (1969) Ultrastructure of the human mammary ductule. Cancer 23:958–978
Tobon H, Salazar H (1974) Ultrastructure of the human mammary gland I. Development of the fetal gland throughout gestation. J Clin Endocrinol Metab 39:443–456
Toker C (1967) Observations on the ultrastructure of a mammary ductule. J Ultrastruct Res 21:9–25
Waugh D, Van Der Hoeven E (1962) Fine structure of the human adult female breast. Lab Invest 11:220–228

Accepted October 13, 1983