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An ultrastructural study of mitosis and cytokinesis in normal 'resting' human breast

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Summary. The parenchyma of the normal "resting" human breast was examined by electron microscopy to characterize the cells undergoing mitosis and the mechanism by which the normal tissue architecture is maintained during this process. In this study of 112 mitotic cells, it was found that the mitotic cells were luminally positioned, polarised epithelial cells with no evidence of myoepithelial cell division. Ultrastructurally, the nuclear and cytoplasmic changes were consistent with previous reports of mitosis in other tissues. However, unlike all previous reports, two specific orientations of the nuclear spindle and thus the planes of cytokinesis were observed. In a few cases the spindle formed parallel to the lumen and division resulted in two luminally positioned daughter cells. However, in the majority of mitotic cells the spindle was approximately at right angles to the lumen and this orientation resulted in a luminally and a basally positioned daughter cell. It is proposed that the abnormally positioned basal daughter cell could develop into a myoepithelial cell or undergo deletion (apoptosis). Thus the two orientations of mitosis may explain the mechanism by which the epithelial and myoepithelial cell populations were maintained by a single progenitor cell without disrupting the integrity of the tissue architecture.

Key words: Normal resting breast – Ultrastructure – Mitosis – Cytokinesis – Human

The parenchyma of the mammary gland consists of branching duct systems which radiate from the nipple and end in the specialised milk producing units termed lobules. However, the stage at which the various parenchymal components develop differs between species with respect to sexual maturity and pregnancy (Short and Drife 1977). In its developmental sequence the human differs from all other species in that the breast undergoes full structural differentiation with the formation of ducts and lobules at puberty (Short and Drife 1977). This gives rise to a highly organised structure which is maintained throughout reproductive life irrespective of pregnancy and lactation.

In addition, in the westernized world, sociological changes have resulted in physiological changes which affect the human breast (Short 1976). With the artificial suppression of fertility, the breast spends extended periods in a "resting" i.e. non-lactating condition, and is repeatedly exposed to the hormonal fluctuations associated with the menstrual cycle. These sociological changes have been coincident with an increased incidence of breast disease (Short and Drife 1977). In the "resting" breast it has been shown that the cyclical hormonal fluctuations result in changes in the incidence of mitosis with elevated levels during the luteal phase (Meyer 1977; Ferguson and Anderson 1981a; Anderson et al. 1982). The effect of this repeated low level stimulation on the normal morphology of the breast parenchyma is unknown.

Morphological studies of the parenchyma of the breast have shown it to consist of two types of epithelial cells (epithelial and myoepithelial) which have an organised and specific spatial relationship (Ozzello 1971; Stirling and Chandler 1976). In a highly differentiated gland such as the breast, the orientation of mitosis must be controlled in such a manner as to maintain the normal tissue architecture. However, there have been no detailed morphological studies of mitosis in the normal "resting" breast. The present paper describes the results from an ultrastructural analysis of mitosis which was undertaken to characterize the cells undergoing mitosis and to examine the process in relation to the maintenance of the normal structural architecture of the breast parenchyma.

Materials and methods

Samples of normal breast tissue were obtained from biopsies of 50 women of reproductive age (17–40 years). The criteria used to select tissue for inclusion in the study were as described previously (Ferguson and Anderson 1981a). The patients position in the menstrual cycle was calculated from the dates of the menses prior and subsequent to the biopsy.

The tissue was obtained directly from the operating theatre and portions were processed for subgross examination, histology and electron microscopy. The samples for subgross and histological examination were processed as described previously (Manton et al. 1981; Ferguson and Anderson 1981a).

The samples for electron microscopy were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated in ethanol, and treated with propylene oxide prior to embedding in Araldite or Emix. Thin sections were stained

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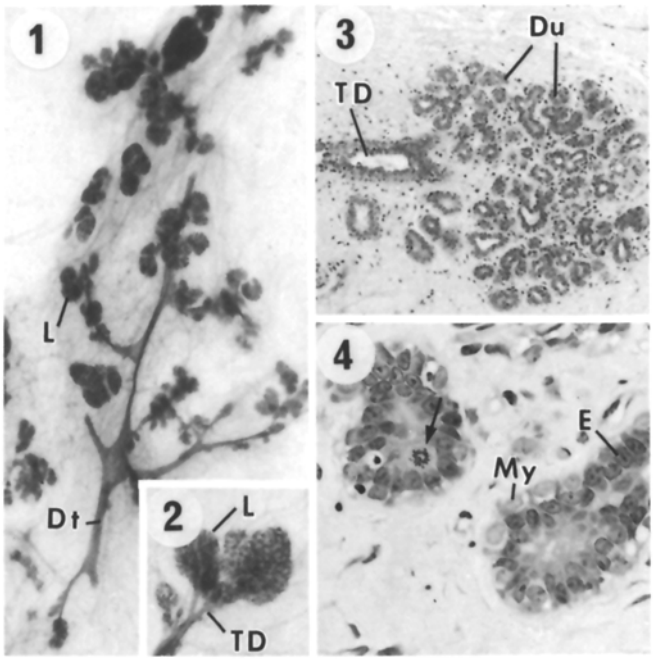


Abbreviations: *C* centriole; *cER* confronting strands of rough endoplasmic reticulum; *D* daughter cell; *De* desmosome; *Dt* duct; *Du* ductule; *E* epithelial cell; *G* Golgi apparatus; *H* hemidesmosome; *I* intermediate junction; *IL* intraepithelial lymphocyte; *K* kinetochore; *L* lobule; *Lu* lumen; *Ly* lysosome; *Mt* microtubule; *My* myoepithelial cell; *NM* nuclear membrane; *Pr* prophase cell; *T* tight junction; *TD* terminal duct; *V* vesicles

Fig. 1. Subgross appearance of part of the branching duct systems. ×10

Fig. 2. Detail showing the terminal ducts connected to the lobules. ×20

Fig. 3. Light micrograph showing the cross sectioned ductules which make up the lobule. GMA embedded/haemotoxylin and eosin stained. ×100

Fig. 4. Part of the lobule showing the ductules to consist of a single layer of epithelial cells with underlying myoepithelial cells. Note the cell in mitosis (*arrow*). GMA embedded/H and E stained. ×400

with uranyl acetate and lead citrate and examined in a Philips 400 or Jeol 100 CX electron microscope.

Mitosis. The histological examination confirmed the normality of the parenchymal structures and was also used to identify patients with elevated levels of mitosis (Ferguson and Anderson 1981a). The mitotic cells were initially identified by light microscopy of 1 μm-thick Azure A-stained sections. Thin sections of suitable areas were cut and examined by electron microscopy. The observations presented in this paper were based on the ultrastructural examination of 112 mitotic cells with semi-serial sections being studied in the majority of cases. The number of cells in each phase of mitosis was as follows: 13 prophase, 12 prometaphase, 36 metaphase, 10 anaphase and 41 telophase.

Results

The human breast consists of 12–20 branching duct systems which radiate from the nipple and end in specialised lobular units (Figs. 1, 2). The parenchyma is embedded in variable amounts of fatty connective tissue. The lobules are composed of a number of blind ending ductules which connect with the intralobular portion of the terminal duct (Fig. 3). The ducts and lobules consist of a continuous single layer of epithelial cells with underlying myoepithelial cells (Fig. 4).

Ultrastructure

The epithelial cells lining the lumen of the ducts and ductules varied between cuboid and columnar in shape with the nucleus located in the mid to basal cytoplasm (Fig. 5). The interphase nucleus had homogeneous nucleoplasm and one or two nucleoli. The cytoplasm contained a number of mitochondria, a small Golgi body situated close to the nucleus, a few strands of rough endoplasmic reticulum (rER) and two centrioles located close to the luminal surface. The epithelial cells were joined at the luminal extremities of the lateral borders by junctional complexes consisting of tight and intermediate junctions and desmosomes (Fig. 6).

Fig. 5. Electron micrograph of a ductule lined by epithelial cells with underlying myoepithelial cells plus an intraepithelial lymphocyte. A cell in prophase and the two daughter cells (*D1* and *D2*) resulting from mitosis are illustrated. ×4500

Fig. 6. Junctional complex adjacent to the lumen. ×25000

Fig. 7. Part of a myoepithelial cell showing myofilaments with dense bodies (*arrows*). Note the formation of hemidesmosomes and pinocytotic vesicles (*arrow head*) along the plasmalemma adjacent to the basal lamina. ×40000

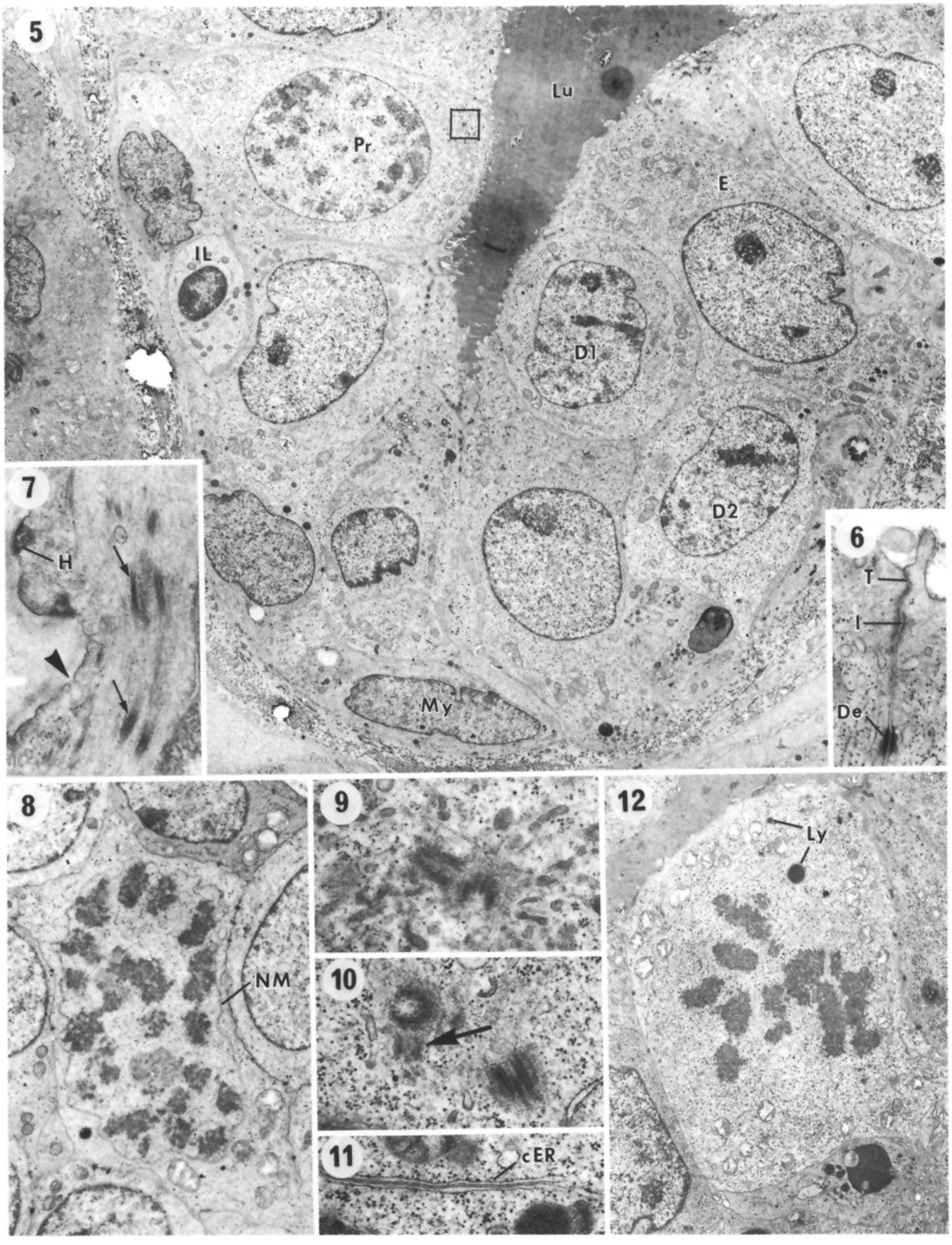
Fig. 8. Very early prometaphase. The condensed chromosomes are present within an irregular nuclear membrane. ×5500

Fig. 9. Prophase. Detail of centrioles from a serial section of the enclosed area in Fig. 5. ×35000

Fig. 10. Centrioles from late prophase illustrating the presence of a daughter centriole (*arrow*). ×35000

Fig. 11. Part of a cell in prometaphase illustrating the confronting strands of rough endoplasmic reticulum plus a few lysosomes. ×30000

Fig. 12. Prometaphase. The randomly oriented chromosomes are centrally located. ×5600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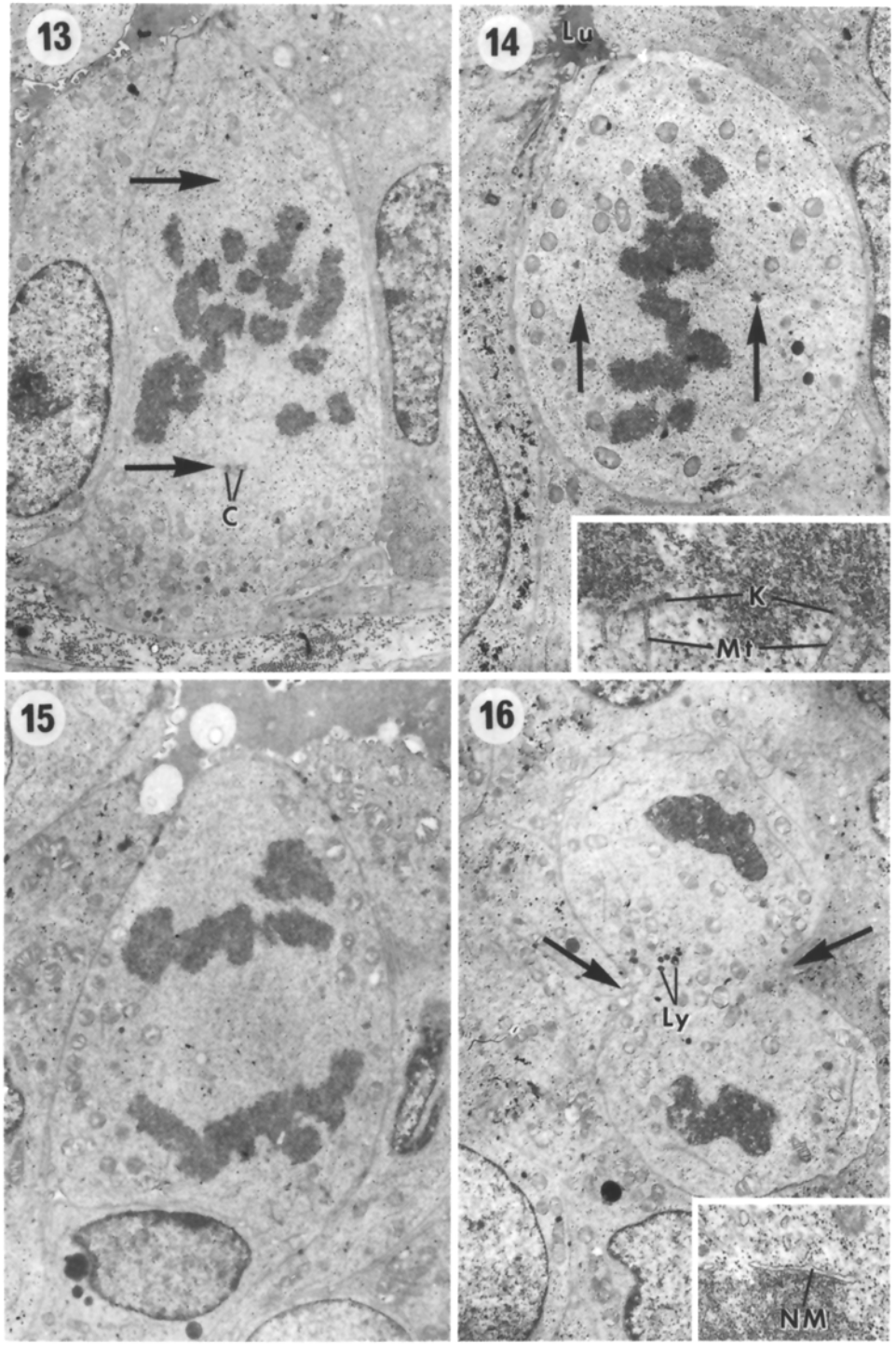

Fig. 13. Metaphase. The nuclear spindle is orientated at right angles to the lumen (*position of the poles arrowed*) with the chromosomes aligned in the central region. ×4600

Fig. 14. Metaphase. The chromosomes are aligned in the central region of a nuclear spindle orientated parallel to the lumen (*nuclear poles arrowed*). ×5000. *Insert:* Junction between the microtubules and the kinetochores. ×44000

Fig. 15. Late anaphase. The intertwined chromosomes are located at the nuclear poles of a spindle orientated at right angles to the lumen. ×5000

Fig. 16. Telophase. A waist-like constriction is present midway between the daughter nuclei (*arrows*). ×5500. *Insert:* Detail of the periphery of a nucleus illustrating the partially reformed nuclear membrane. ×20000

The myoepithelial cells form a continuous layer beneath the epithelial cells of the ducts and a discontinuous layer around the ductules (Fig. 5). They contain organelles similar to those present in epithelial cells, but were characterised by the presence of parallel arrays of myofilaments (10 nm thick) with dense bodies and the formation of hemidesmosomes and pinocytotic vesicles at the plasmalemma adjacent to the basal lamina (Fig. 7). In addition, variable numbers of intraepithelial lymphocytes and macrophages were observed situated between the epithelial and myoepithelial cells (Fig. 5).

Mitosis

The majority of mitotic cells were observed randomly distributed within the ductules of the lobules (Fig. 4) although a few cells were also located within ducts. Where orientation was possible (depending on the plane of section), the dividing cells were identified as luminally positioned epithelial cells (Figs. 5, 12). Of the 112 mitotic cells examined there was no evidence of the dividing cells being either myoepithelial cells or subluminally positioned epithelial cells.

The cell in prophase was characterised by a more cen-

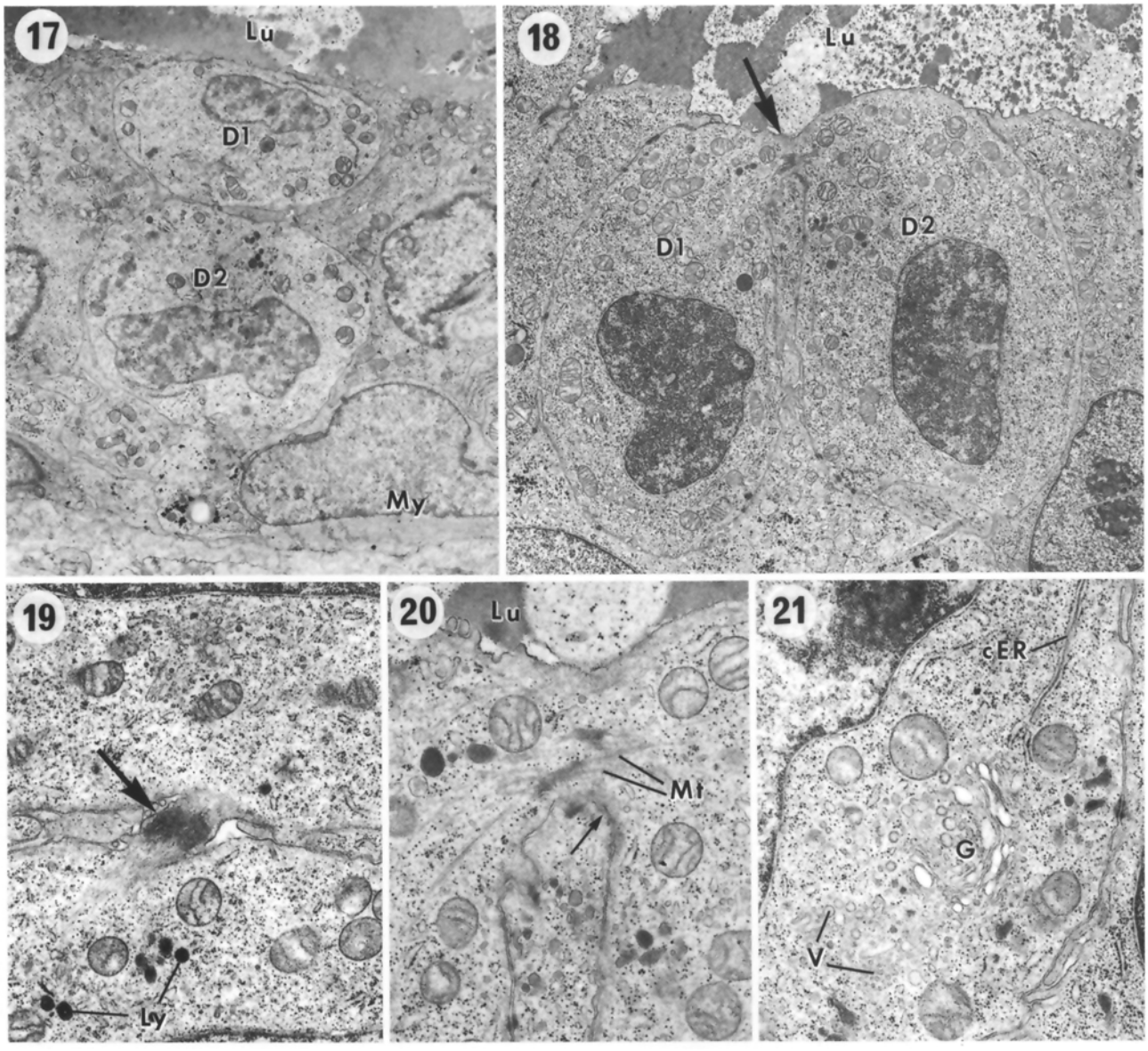

Fig. 17. Late telophase. Cytokinesis has occurred parallel to the lumen resulting in a luminally (*D1*) and a basally (*D2*) positioned daughter cell. Dense heterochromatin is distributed throughout the irregularly shaped nuclei. ×4500

Fig. 18. Late telophase. Cytokinesis has occurred at right angles to the lumen resulting in two luminally positioned daughter cells. The cytoplasmic bridge connecting the daughter cells is eccentrically located (*arrow*). ×6000

Fig. 19. Detail from a serial section of the cell in Fig. 17 showing the daughter cells still joined by a centrally located midbody (*arrow*). ×11500

Fig. 20. Enlargement of the cytoplasmic bridge in Fig. 18 showing a few microtubules embedded in a dense matrix. Note the junctions formed between the cells beneath the cytoplasmic bridge (*arrow*). ×17000

Fig. 21. Cytoplasm of a daughter cell at similar stage to Fig. 17 showing the extensive Golgi apparatus with groups of small vesicles, lysosomes, mitochondria and confronting rER. ×14500

trally located nucleus within which patches of condensed heterochromatin were observed (Fig. 5). The organelles were similar to those of interphase cells. However, the centrioles were observed closer to the nucleus (Fig. 9) and was followed by the appearance of daughter centrioles (Fig. 10).

During prometaphase the chromatin condensed to form individual chromosomes enclosed by an irregular nuclear membrane (Fig. 8) which broke down completely in the latter stages (Fig. 12). Within the cytoplasm the Golgi apparatus had disappeared but groups of small coated and uncoated vesicles had appeared. In addition, a number of examples of confronting cisternae of rough endoplasmic reticulum (rER) had appeared (Fig. 11).

Metaphase was associated with the formation of the nuclear spindle with the chromosomes lining up along the mid portion of the spindle (Figs. 13, 14). Certain of the

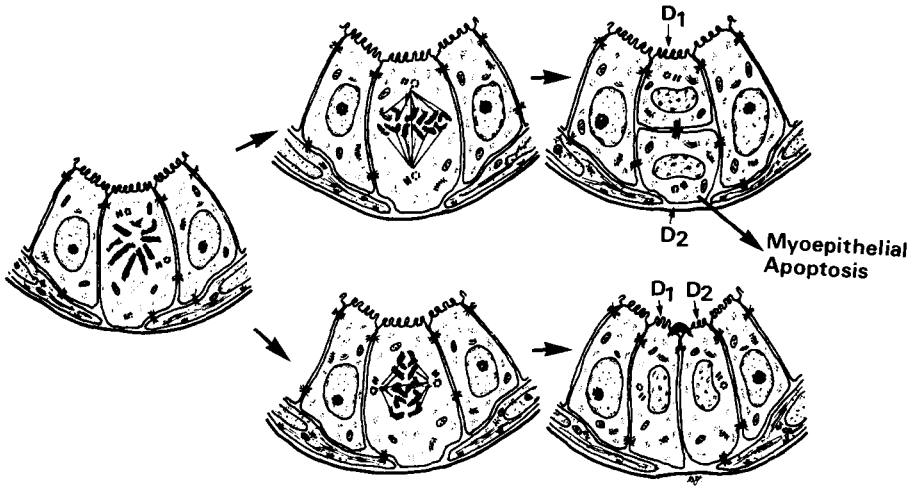

Fig. 22. Diagram illustrating the two orientations of the nuclear spindle and planes of cytokinesis observed in the parenchyma of the "resting" breast with the proposed fate of the basal daughter cell

microtubules were attached to the kinetochore plates (Fig. 14, insert) of the chromosomes, while others passed between the poles.

From the examination of 40 suitably sectioned cells (where the plane of sectioning allowed orientation) there appeared to be two distinct orientations of the nuclear spindle with respect to the lumen of the ductule. In the majority of cells the spindle was formed perpendicular to the lumen with one nuclear pole close to the lumen and the other in the basal region of the cell (Fig. 13). In other cells the spindle was orientated parallel to the lumen (Fig. 14).

During anaphase daughter chromosomes separated and moved towards the opposite nuclear poles. In cells with the nuclear spindle at right angles to the lumen this results in one set of chromosomes close to the luminal surface with the other in the basal cytoplasm (Fig. 15). By contrast, in cells with the spindle parallel to the lumen the two sets of chromosomes were positioned close to the lateral surfaces of the cells (Fig. 4; Ferguson 1985).

Early telophase was associated with continued condensation of the chromatin (Fig. 16) and reformation of the nuclear membrane (Fig. 16, insert). In the latter stages, the nuclei became more spherical with dispersed chromatin similar to that of interphase cells (Fig. 5). Within the cytoplasm the most obvious change was the reformation of the Golgi apparatus into an active appearing organelle within both developing daughter cells (Fig. 21). The confronting cisternae of rER were retained and only disappear after separation of the daughter cells.

Cytokinesis. Cytokinesis was initiated in early telophase by an invagination of the plasmalemma to form a waist-like constriction; the cleavage furrow (Fig. 16). This invagination was located at right angles to the orientation of the nuclear spindle and mid-way between the daughter nuclei. At this stage, groups of microtubules and lysosomes were observed in the central region of the cells. The invagination continued until the cells were connected by a narrow cytoplasmic bridge, the midbody. This consisted of a large number of microtubules embedded in an osmiophilic matrix (Fig. 19).

From the examination of over 40 suitably sectioned cells, it was concluded that the orientations of the nuclear spindle resulted in two distinct planes of cytokinesis. An extensive search of individual and serial sections failed to reveal any intermediate orientations. In those cases with the spindle at approximately right angles to the lumen the cleavage furrow forms parallel to the lumen, resulting in luminally- and basally-positioned daughter cells (Figs. 5, 17). In this case there was a concentric invagination resulting in a centrally positioned midbody (Fig. 19). The final separation and fate of the midbody was not observed. In the cells with the spindle parallel to the lumen, the cleavage furrow occurred at right angles to the lumen resulting in two luminally positioned daughter cells (Fig. 18). In the later stages of the process the cytoplasmic bridge of the midbody was eccentrically located protruding into the lumen (Fig. 20). The two orientations of mitosis and cytokinesis are diagrammatically represented in Fig. 22.

Irrespective of the orientation of mitosis, the luminal seal appeared to be maintained throughout the process with the retention of the apical junctional complex. Where lateral division occurred, both daughter cells retained the apical junctional complexes, and in the later stages of cytokinesis new junctions were formed around the protruding midbody (Fig. 20).

Discussion

The majority of our knowledge on the ultrastructural changes associated with mitosis has been gained from in vitro studies (Erlandsen and Harven 1971; Robbins and Gonatas 1964). There have been a number of investigations of in vivo cell division (Zeligs and Wollman 1979a) but these have been rather fragmentary except for a detailed study of mitosis in the stimulated thyroid of the rat (Zeligs and Wollman 1979a–d). The reason for this is that in normal tissue there are few mitotic cells; this makes their study tedious and time consuming.

The present study confirmed the preliminary observations (Ferguson 1985) that the proliferative cells within the breast parenchyma are luminally positioned polarised epithelial cells which are indistinguishable morphologically from the interphase epithelial cells.

The changes in the nuclear morphology associated with mitosis are similar to those described previously and are used to identify the various stages of cell division. The cytoplasmic changes are also basically similar to those described previously (Erlandson and Harven 1971; Zeligs and Wollman 1979a, b). In the breast, as in the thyroid, the most obvious changes were associated with the breakdown and reformation of the Golgi body and the appearance and disappearance of the confronting strands of rER. The functional significance of this is unknown although it may reflect a generalised effect on cytoplasmic membranes since it coincides with the breakdown and reformation of the nuclear membranes.

It was found that the junctional complexes adjacent to the lumen and the desmosomes were retained throughout the entire mitotic process irrespective of the orientation of cell division. This maintenance of the luminal seal during mitosis is similar to that observed in the thyroid (Zeligs and Wollman 1979c), pancreas (Lütcke et al. 1987) and small intestine (Jinguuji and Ishikawa 1986).

Orientation of mitosis. In the unorganised proliferation associated with in vitro cell culture it is likely that mitosis is randomly orientated. However, within the confines of a differentiated tissue, it is probable that cell division will be orientated in such a manner as to maintain the structural integrity of the tissue (Potten 1981). This would be particularly true of glands lined by polarised epithelial cells. In studies of the pancreas (Pictet et al. 1972; Lutcke et al. 1987), and thyroid (Zeligs and Wollman 1979c), it has been reported that there is a fixed orientation of the mitotic spindle and cytokinesis which resulted in two luminally-positioned daughter cells. However, in the breast, two specific orientations have been observed. One orientation of mitosis is similar to that described for the pancreas and thyroid and results in two luminally-positioned daughter cells. This mechanism increases the number of luminal epithelial cells by one while maintaining the normal architecture.

The second orientation, with the spindle at right angles to the lumen and which results in a luminally- and a basally-positioned daughter cell, has not been described previously. However, the basally-positioned daughter cell is in an unexpected position since the normal breast parenchyma is lined by a single layer of epithelial cells. Although the fate of the basal daughter cannot be followed morphologically, two possible outcomes are proposed. The first is that it may differentiate into a myoepithelial cell. This is consistent with the observation that, in rats, myoepithelial cells appear to differentiate from basally-positioned undifferentiated epithelial cells (Radnor 1972; Ormerod and Rudland 1984). It is also supported by the observations in an elegant in vitro study of human breast tissue pulse labelled with H^3-thymidine, in which myoepithelial cells appeared to develop from a progenitor epithelial cell (Joshi et al. 1986). However, although 'resting' breast is repeatedly exposed to proliferative stimulation with each menstrual cycle, there is little evidence of a continuous growth of the lobular units (Anderson et al. 1982) and therefore homeostasis of cell number. A second alternative is, therefore, that a number of the basal daughter cells are deleted by apoptosis. This alternative is supported by the observations that the peak of apoptosis occurs 3 days after the peak of mitosis (Ferguson and Anderson 1981a; Anderson et al. 1982), and that the early apoptotic cells are located in a similar position to the basally positioned daughter cell (Ferguson and Anderson 1981b).

Thus, the two orientations of mitosis would allow a single luminally positioned proliferative cell to give rise to both epithelial and myoepithelial cells. It could also represent an adaptation to prevent cellular proliferation in the 'resting' breast. It is possible that breakdown in the control of this process could lead to abnormal epithelial proliferations associated with pathological lesions of the breast.

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