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Ultrastructural characterisation of the proliferative (stem?) cells within the parenchyma of the normal "resting" breast

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Summary. In this study the proliferative (stem?) cells within the parenchyma of the normal "resting" breast were characterised by the ultrastructural examination of 60 mitotic cells. The parenchyma consists of epithelial and myoepithelial cells plus a few intraepithelial lymphocytes and macrophages. The majority of mitotic cells were randomly distributed throughout the lobules with a few present in ducts. In all cases the cells were identified as luminally positioned polarised epithelial cells. The proliferating cells had similar cytoplasmic features and were indistinguishable from adjacent interphase epithelial cells. No evidence was found for the division of subluminal epithelial or myoepithelial cells. These observations would be consistant with a single cell type giving rise to both epithelial and myoepithelial cells.

Key words: Normal Human Breast – Mitosis – Ultrastructure

Introduction

In the human breast little is known of the identity of the proliferating (stem) cells or the mechanism by which the normal architecture of the parenchyma is maintained. It has been shown by in vitro culture of cloned cells from a rat mammary tumour that a single (stem) cell can produce both epithelial and myoepithelial-like cells (Rudland et al. 1980; Bennett 1980). In the rat mammary gland the epithelial cells have been divided into three subgroups on the basis of their cytoplasmic density and it has been proposed that cells with intermediate features represent the stem cell population (Russo et al. 1976a and b). However, due to differences in the state of development of the "resting" mammary gland in the rat and the human (Short and Drife 1977) it is difficult to extrapolate from the rat to the human.

Although it has been proposed that stem cells exist in the human breast there is little evidence of a morphologically identifiable stem cell population

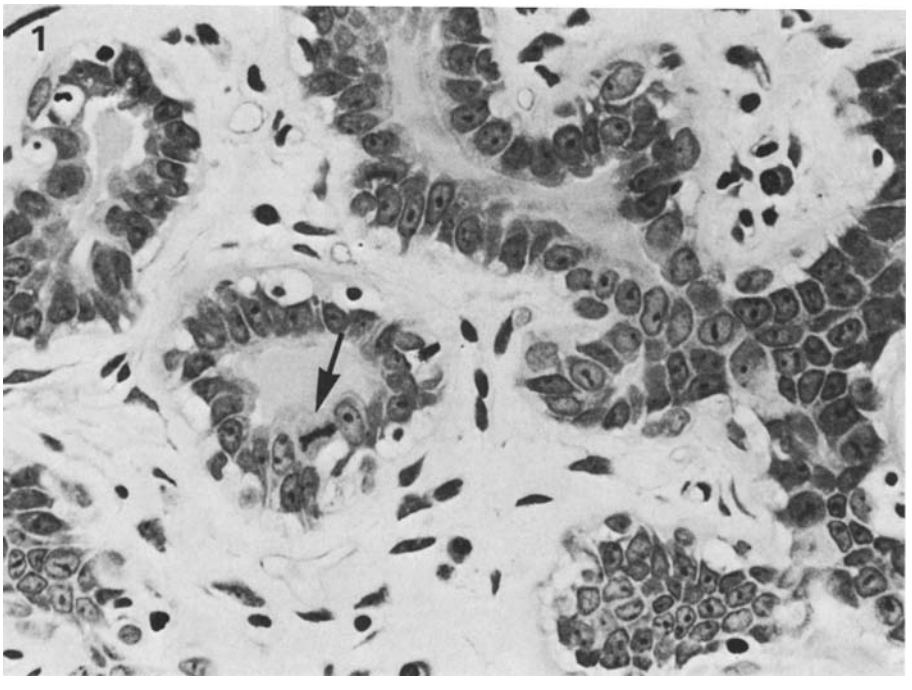

Fig. 1. Light micrograph of part of a lobule. Note the mitotic cell (*arrow*) present in one of the ductules. Haematoxylin and eosin stained/Glycol metharcylate embedded. × 800

(Ozzello 1971; Stirling and Chandler 1976; Salazar and Tobon 1974; Toker 1967). However, it should be possible to gain information on the identity of the proliferative (stem) cells by examining the mitotic cells. In this study the identity and ultrastructural characteristics of the proliferating cells within the breast parenchyma are described.

Materials and methods

Samples of normal "resting" breast tissue were obtained from biopsies of 30 women in the age range 16–40 years. The criteria of normality were as described previously (Ferguson and Anderson 1981a). Samples were processed for histology and electron microscopy by techniques previously described (Ferguson and Anderson 1981a, b and c). The ultrastructural technique can be summarised as follows: tissue was fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in Emix. The thin sections were stained with uranyl acetate and lead citrate prior to examination with either a Philips 400 or Jeol 100 CX electron microscope.

The histological examination identified those samples with peak levels of mitosis (Ferguson and Anderson 1981a) and the ultrastructural study was then concentrated on these cases. Thin sections were cut of mitotic cells initially identified in 1 μm Azur A stain sections. The observations presented in this study were based on the examination of 60 dividing cells at various stages of mitosis.

Figs. 2–8. Represent electron micrographs of mitotic cells present in the parenchyma of the normal "resting" breast. The following abbreviations are used throughout: *A*, apical vesicles; *C*, chromosome; *D*, desmosome; *E*, epithelial cell; *I*, intermediate junction; *IL*, intraepithelial lymphocyte; *L*, lumen; *My*, myoepithelial cell; *T*, tight junction

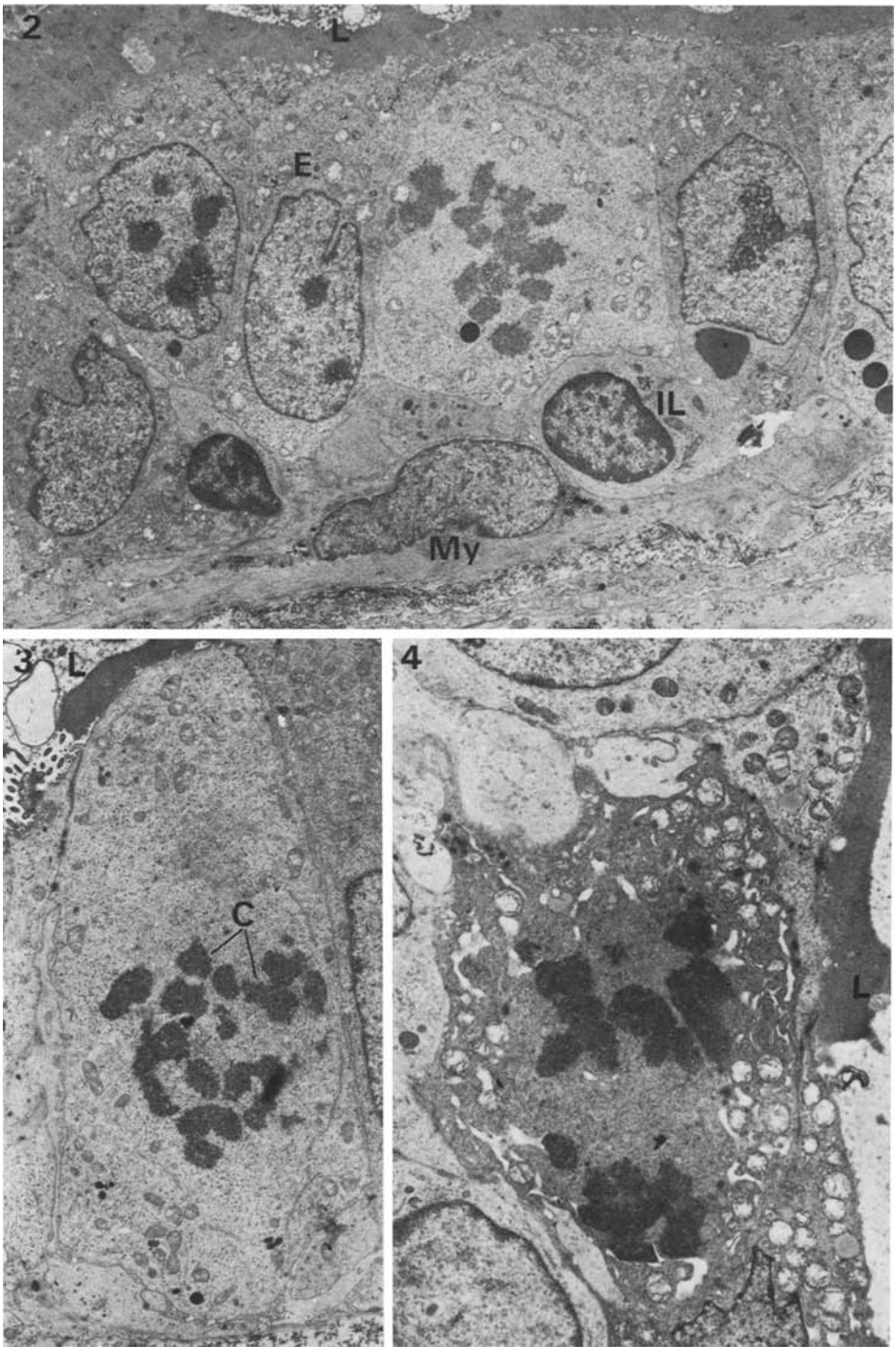

Fig. 2. Section through part of a ductule illustrating the luminal positioned mitotic cell, adjacent interphase epithelial cells, underlying myoepithelial cell and intraepithelial lymphocytes. × 4,000

Fig. 3. A light staining mitotic cell in prometaphase. × 4,700

Fig. 4. A dense staining mitotic cell in anophase. × 4,500

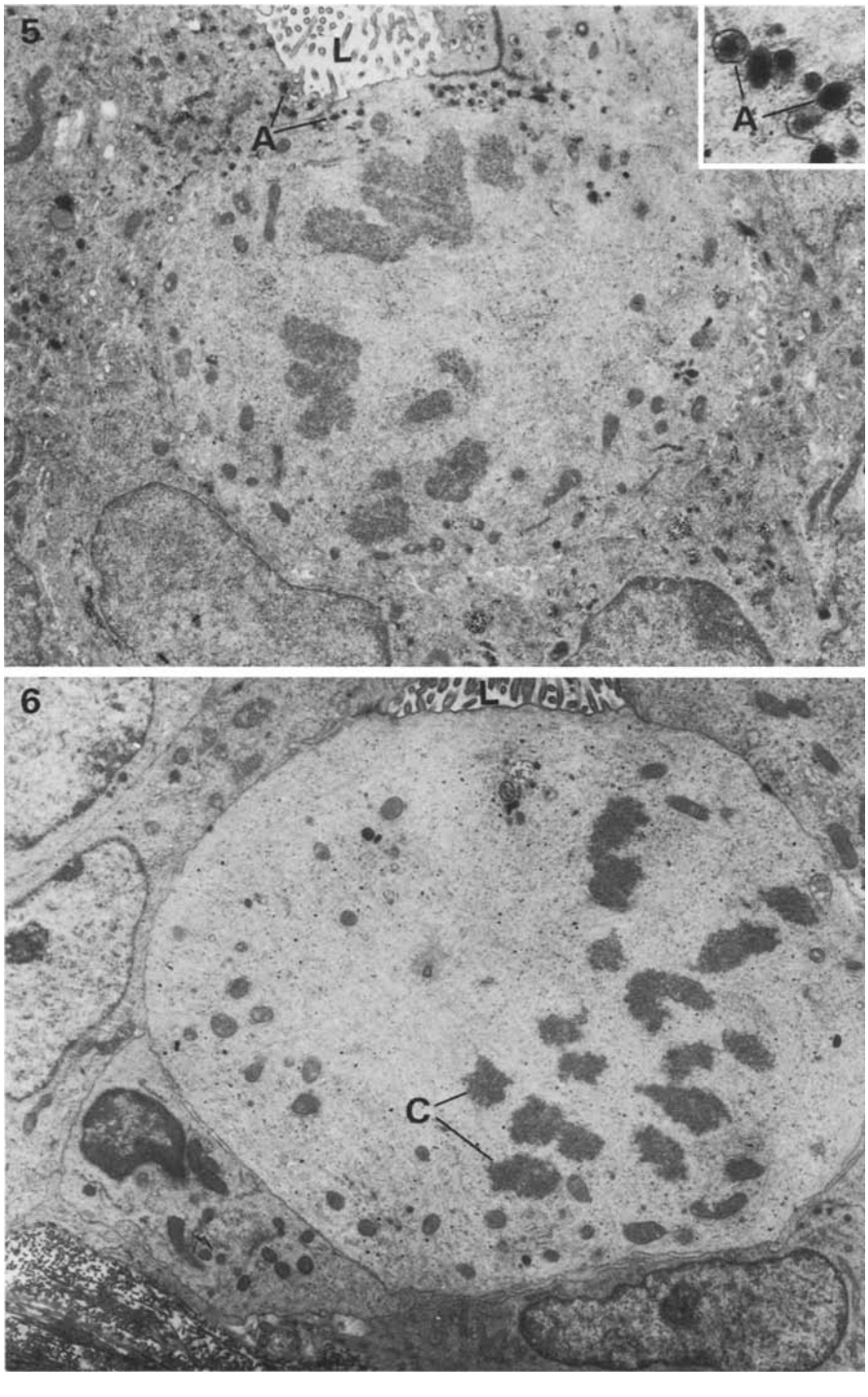

Fig. 5. A mitotic cell in prometaphase which possesses dense core apical vesicles. Note similar granules present in the adjacent interphase cells. × 6,000
Insert. Detail of the apical vesicles. × 24,000

Fig. 6. A mitotic cell at a similar stage to that in Fig. 5. Note the absence of the apical vesicles in both the mitotic and interphase cells. × 5,500

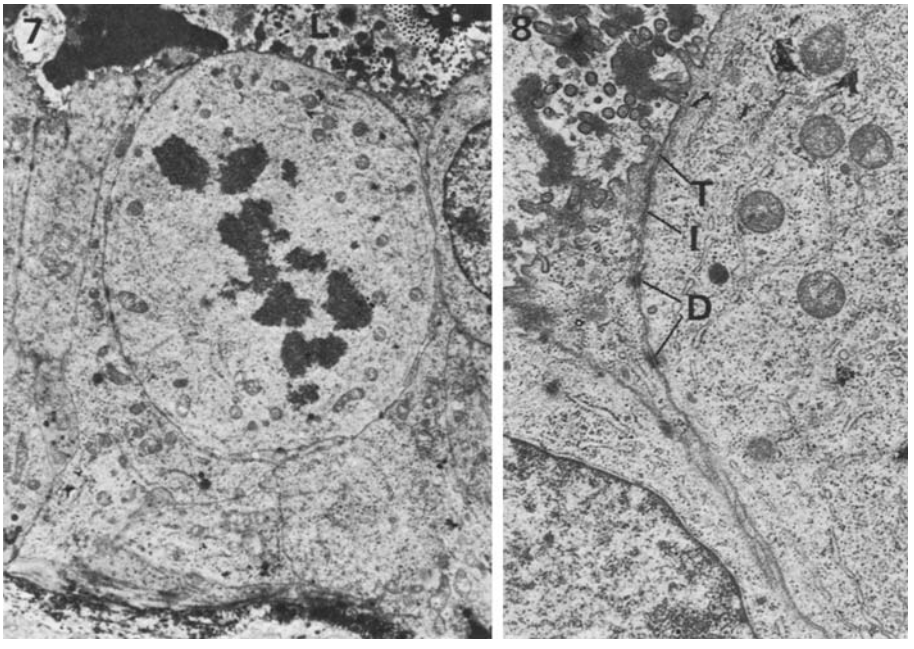

Fig. 7. Low power of a luminally positioned mitotic cell in metaphase. × 4,300

Fig. 8. Detail of the apical junction complex between the mitotic and interphase epithelial cells shown in Fig. 7. × 14,500

Results

The parenchyma of the breast consists of a number of branching duct systems ending in specialised lobules comprising a number of ductules. These structures were lined by a layer of columnar or cuboidal epithelial with underlying myoepithelial cells and a few intraepithelial lymphocytes and macrophages (Figs. 1 and 2). The ultrastructural features of these cells were as described previously (Ozzello 1971; Stirling and Chandler 1976; Ferguson 1985).

From the histological examination of the tissue the majority of mitotic cells were observed randomly distributed throughout the lobules (Fig. 1) with a few present within ducts. The ultrastructural examination identified the mitotic cells as luminally positioned polarised epithelial cells (Figs. 2–8). This was the case for all 60 mitotic cells examined. No evidence was found for basally positioned epithelial or myoepithelial cells undergoing mitosis.

The various stages of mitosis could be identified by the characteristic form and distribution of the chromosomes. The stages illustrated represent prometaphase (Figs. 2, 5 and 6), metaphase (Figs. 3, 7) and anophase (Fig. 4). In certain biopsies there was a marked variation in the cytoplasmic density of the epithelial cells but both light and dark cells were observed undergoing mitosis (Figs. 2–4). The mitotic cells retained their luminal posi-

tion and remained attached to the adjacent interphase cells by the anterior junctional complex and desmosomes (Fig. 8). The cytoplasmic features of the mitotic cells were similar to the adjacent interphase cells. Difference in the organelle compliment between mitotic cells reflected a similar difference in the interphase cells of the particular biopsies. For example apical secretory vesicles were present in a few mitotic cells but only in biopsies in which certain of the interphase cells also exhibited these vesicles (cf. Figs. 5 and 6). There were no morphological features, other than the nuclear changes, by which proliferating cells could be distinguished from nonproliferative epithelial cells.

Discussion

In the "resting" breast the number of dividing cells is normally extremely low making the study of mitosis difficult. However, it has recently been shown that the incidence of mitosis varies in phase with the menstrual cycle (Ferguson and Anderson 1981a; Anderson et al. 1982). By concentrating the ultrastructural study on tissue from patients with peak levels of mitosis it is possible to characterise the proliferative cells by the examination of a large number of dividing cells.

In ultrastructural studies of both human and animal mammary glands there have been various attempts to divide the epithelial cells into subpopulations based on their cytoplasmic density (Ormerod and Rudland 1984; Bassler 1970; Smith et al. 1984; Toker 1967). It has also been proposed that stem cells may be identified from morphological criteria (Russo et al. 1976a and b; Salazar and Tobon 1974). However, as has been discussed previously (Ferguson 1985), it is possible that certain of the morphological differences are related to changes which occur during fixation. In addition, the present study illustrates both light and dark cells undergoing mitosis which would be inconsistant with the proposal that they represent different physiological states. In the rat it has been suggested that cells with intermediate features represent the stem cell population (Russo et al. 1976a and b) but this must await confirmation. In the human, subluminal clear cells were identified as possible stem cells (Salazar and Tobon 1974), although these cells probably represented intraepithelial lymphocytes (Ferguson 1985). From the observations made in the present study it would appear that the proliferating cells possess no morphological feature to distinguish them from neighbouring interphase cells. This finding would be consistant with studies of stem cells from other tissues (skin, intestine) in which no distinguishing features are observed (Potten 1983).

In the human breast it is unknown whether both epithelial and myoepithelial cells divide or if one cell can give rise to both cell types. In the rat it has been proposed that a single stem cell can produce both cell types (Bennett 1980; Rudland et al. 1980). The finding that all 60 mitotic cells are similar in being luminally positioned epithelial cells with no evidence of myoepithelial cell division, would be consistent with the proposal that a single stem cell produces both cell types. Therefore, although it is imposs-

ible to identify stem cells morphologically in the human breast, new information on the proliferative cells has been obtained by the ultrastructural examination of mitotic cells.

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