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Myoepithelium in involuting mammary glands of the rat

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INTRODUCTION

Ultrastructural aspects of the process of involution in the mammary gland have been described by Wellings & De Ome (1963) and Sekhri, Pitelka & De Ome (1967) in the mouse, Verley & Hollman (1967) and Hollman & Verley (1967) in mice, rats and rabbits, and Helminen & Ericsson (1968*a*, *b*), Helminen, Ericsson & Orrenius (1968) and Brandes, Anton & Barnard (1969) in the rat. Of these, only Verley & Hollman (1967) and Hollman & Verley (1967) have attributed a functional role to the myoepithelial cells, showing that they are resistant to glandular involution and act as bridges between acellular portions of alveoli, holding surviving cells together and preventing disruption of the glandular tree. Their ultrastructural studies, however, gave no details of how the process of regression affected the cytology of the myoepithelial cells.

Material taken from the mammary glands of rats at various times after weaning has therefore been examined, with special reference to ultrastructural changes occurring in the myoepithelium.

MATERIALS AND METHODS

The animals used for this study were female Sprague–Dawley rats 44 hours, 4 and 13 days after weaning. The procedure adopted has already been detailed (Radnor, 1972).

OBSERVATIONS

Forty-four hours after weaning, the alveoli were distended with accumulated milk products and the myoepithelium had a well-developed myofilament system, with associated dense bodies and dark filament attachment areas along the plasma membrane (Fig. 1).

Nuclei were osmiophilic and usually irregular in shape, the chromatin being evenly dispersed, apart from slight aggregations at the periphery of the nucleus. Clusters of ribosomes were numerous in the perinuclear area, and mitochondria were generally small. Although vesicles were sometimes seen in the apical part of the cells, well-developed Golgi dictyosomes were not found. Multivesicular bodies and occasional fat droplets were seen.

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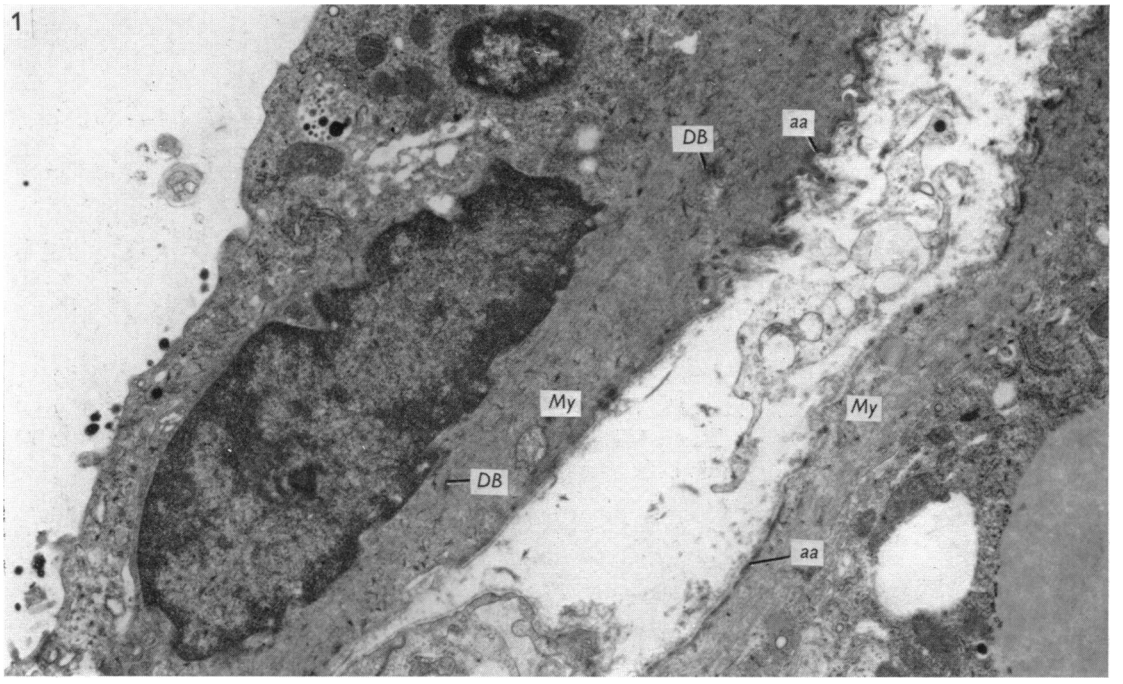

Fig. 1. Forty-four hours involution. Myoepithelial cells (*My*) showing irregular contours, attachment areas (*aa*) and dense bodies (*DB*). × 10000.

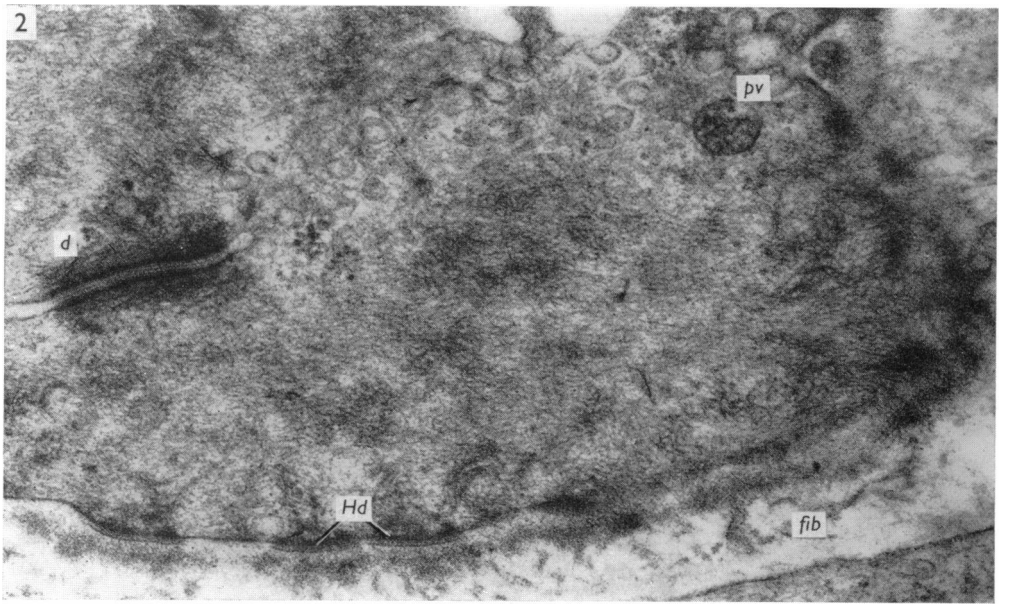

Fig. 2. Forty-four hours involution. Duct myoepithelium surrounded by banded fibrillar complexes (*fib*) and showing a large desmosome (*d*), hemi-desmosomes (*Hd*) and pinocytotic vesicles (*pv*). × 50000.

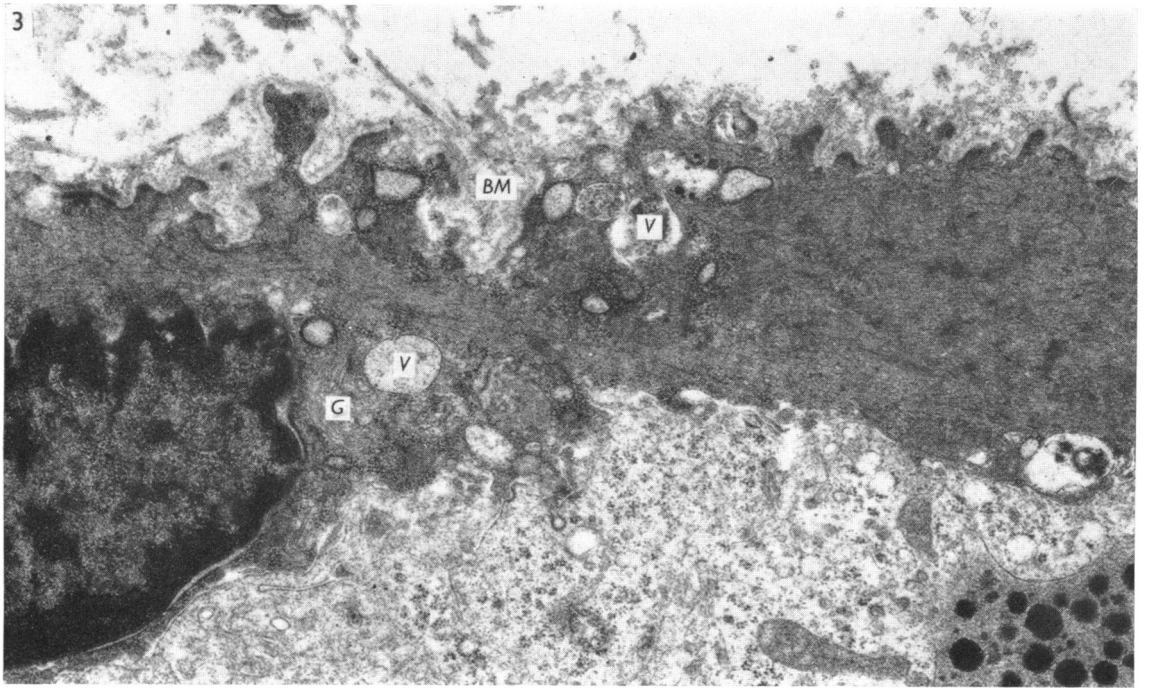

Fig. 3. Four days involution. A myoepithelial cell with 'feet' surrounded by basement membrane material (*BM*). A Golgi apparatus (*G*) and vesicles (*V*) can be seen. Note the dense filament attachment areas on the 'feet'. ×20000.

Despite the fact that the alveoli were distended with milk, the contours of myoepithelial cells were often irregular, and the myofilaments within them generally pursued a wavy course. The basal plasma membrane was frequently convoluted, the convolutions bearing no relationship to the distribution of dense attachment areas, which were numerous, and alternated with regions of pinocytotic activity. The thickness of the basement membrane was variable, but the lamina densa was well developed.

Around the ducts, myoepithelial cell processes protruded into the stroma, and were packed with myofilaments and associated dense bodies (Fig. 2). Hemi-desmosomes were plentiful, with one, or sometimes two, electron-opaque plaques in the lamina lucida immediately beneath the plasma membrane. Banded fibrillar complexes, which gave added support to the tissues in lactation (Radnor, 1972), were thickly distributed and looped from one part of the lamina densa to another; the filamentous nature of the basement membrane prevented the course of the fibrils being traced. Interdigitations were commonly found linking adjacent myoepithelial cells, as well as desmosomes and an occasional nexus or close junction (Fig. 2). The cytology of these cells was essentially similar to that of the alveolar myoepithelium, with an occasional profile of rough endoplasmic reticulum, small mitochondria which were indistinct against the myofilaments, and a sprinkling of ribosomes around the nucleus which was similar to that of the alveolar cells.

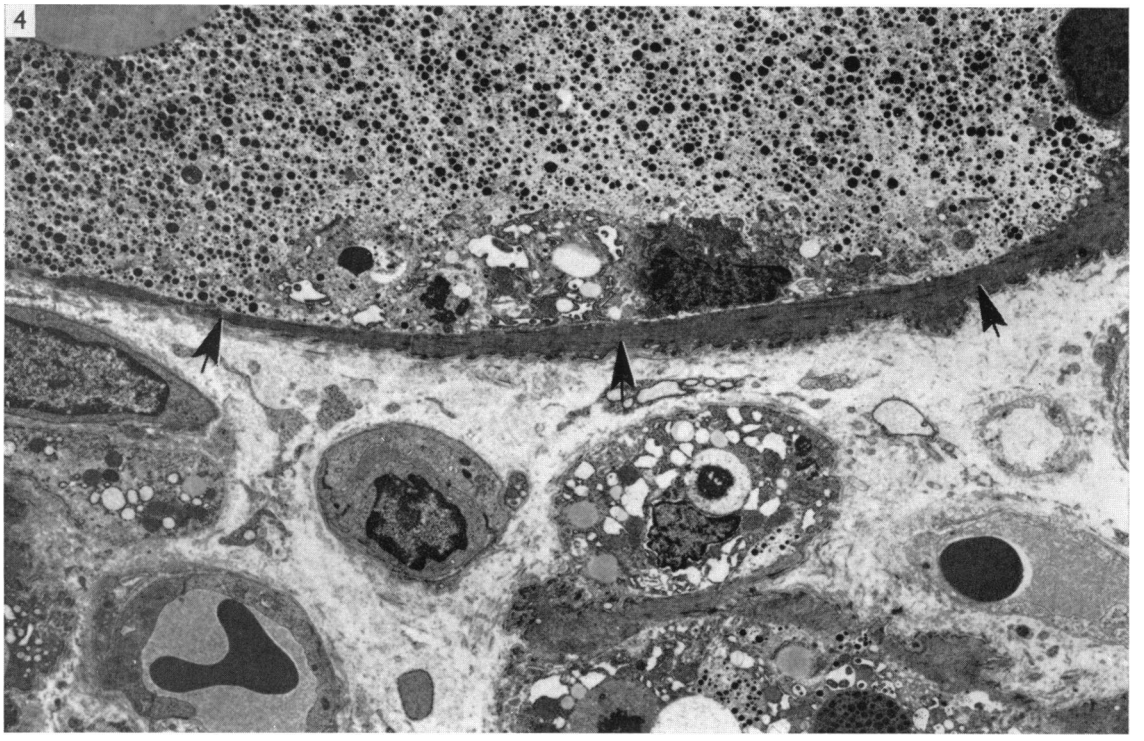

Fig. 4. Four days involution. A myoepithelial cell (arrows) acts as a bridge across acellular portions of an alveolus. × 5000.

After four days involution the alveolar contents were considerably condensed and distinct changes were seen in the myoepithelial cells. Their outline was highly irregular, with pronounced 'feet' on their basal face, the distal surfaces of which usually bore dense filament attachment areas (Figs. 3, 5, 7). In places where epithelial cells had degenerated or become detached, myoepithelial cell processes extended across the gap to act as bridges, thus probably preventing disruption of the entire alveolus (Fig. 4).

Nuclei were variable in shape, generally possessing a smooth outline, but sometimes appearing to be incised and irregular, with electron-dense chromatin granules aggregated beneath the nuclear membrane. In the apical part of the cell, a Golgi apparatus was occasionally seen with very thin, flattened dictyosomes (Fig. 3). Vesicles up to 500 nm in diameter were quite common, and some of them had flocculent contents. Multivesicular bodies were also seen. Short profiles of rough endoplasmic reticulum and masses of free ribosomes were found in the perinuclear area and near the well-developed 'feet'. Mitochondria were less frequent than at earlier stages. Occasional membrane-bound dense bodies were found near the nucleus and at the edges of processes, with contents of varying electron density (Fig. 5).

In addition to the development of processes in the basal part of the cell, the upper plasma membrane was also convoluted and often formed interdigitations with similar

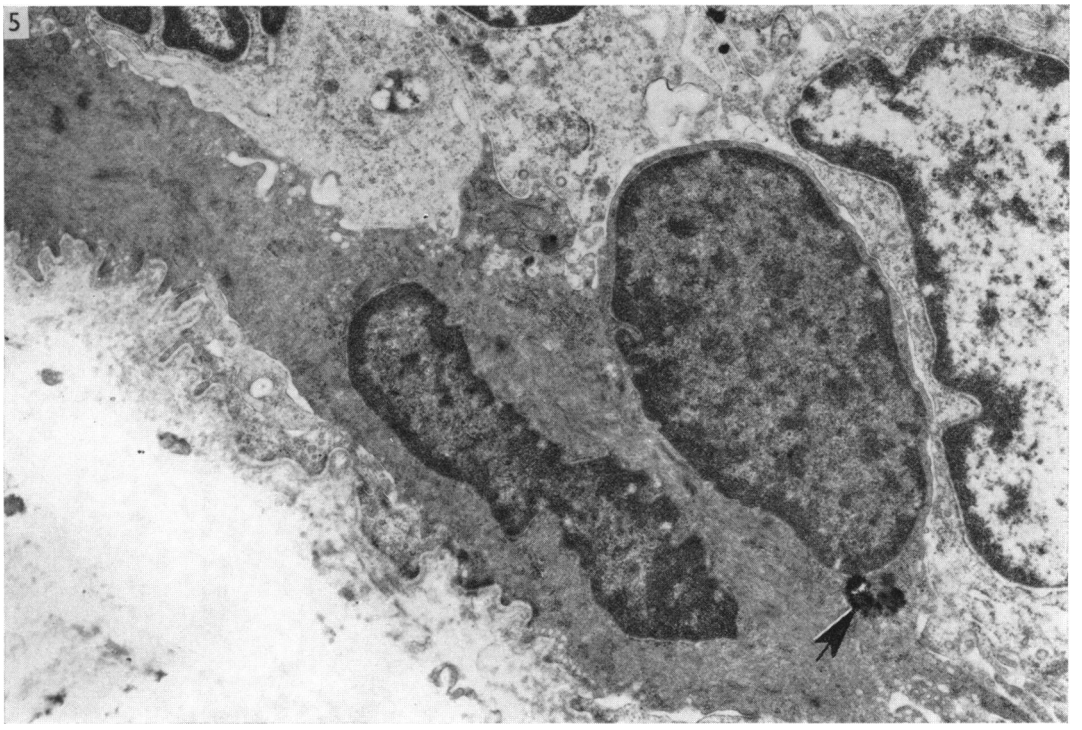

Fig. 5. Four days involution. A membrane-bound dense body (arrow), suggestive of a lysosome, seen in a myoepithelial cell. × 12500.

processes of epithelial cells. Desmosomal attachments were found, although they were generally small.

The myoepithelium around the ducts had blunt processes extending into the stroma (Fig. 6). At this stage the distribution of myofilaments had changed and they were now arranged in isolated, coarse clumps. Overlying processes were linked by desmosomes and interdigitations. Hemi-desmosomes were numerous along the basal surface, and some pinocytotic vesicles were found between them. The basement membrane had a well-developed lamina densa, against which banded fibrillar complexes were still found. Mitochondria were numerous in these processes, being round or rod-shaped with rather indistinct cristae. An occasional profile of rough endoplasmic reticulum and various vesicles were seen, and ribosomes were found between myofilaments. Occasional fat droplets, membrane-bound dense bodies and multivesicular bodies were present in these cells. Apical to the nucleus, Golgi dictyosomes were sometimes found as in the alveolar cells, together with smooth and coated vesicles.

Fine, filamentous material was seen to have accumulated around the irregularly shaped basal processes, filling the spaces between adjacent projections. In masses it appeared amorphous and was similar to basement membrane substance. In a few places, the basement membrane itself was laminated, and consisted of alternating

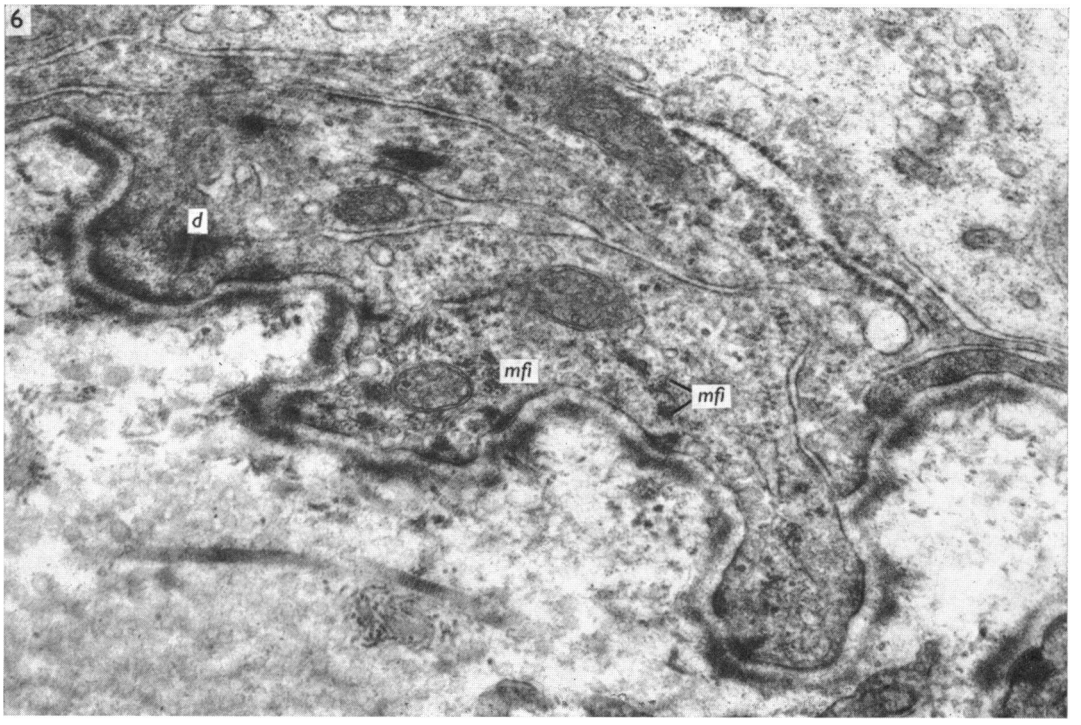

Fig. 6. Four days involution. Duct myoepithelium with clumped myofilaments (*mfi*) and blunt processes linked by desmosomes (*d*) and interdigitations. × 35000.

layers of lamina lucida and lamina densa, following the contours of the myoepithelial cells (Fig. 7).

Thirteen days after weaning the gland appeared macroscopically to have returned to the resting state; however, examination of the ultrastructure showed that this was not so. Myoepithelial cells assumed bizarre forms with branched processes protruding into the stroma (Fig. 8). Myofilaments were not so thickly distributed as previously and the dense bodies associated with them were less in evidence, the myofilaments having a more uniform appearance. Dense filament attachment areas were still found, with pinocytotic vesicles nearby which were easily seen against the clear background. Hemi-desmosomes were not as frequent as previously.

Myoepithelial cell nuclei were less osmiophilic than at earlier stages and were often difficult to distinguish from those of epithelial cells on density alone (Fig. 8). The granular chromatin was slightly aggregated beneath the nuclear membrane, which was covered by a clearly defined envelope of rough endoplasmic reticulum, although the system itself was not well developed in the cell and very few profiles could be found. Ribosomes were plentiful, especially in the apical part of the cell, around the nucleus and between other organelles. Mitochondria were also numerous and many vesicles, some with flocculent contents, were found; the pinocytotic vesicles occasion-

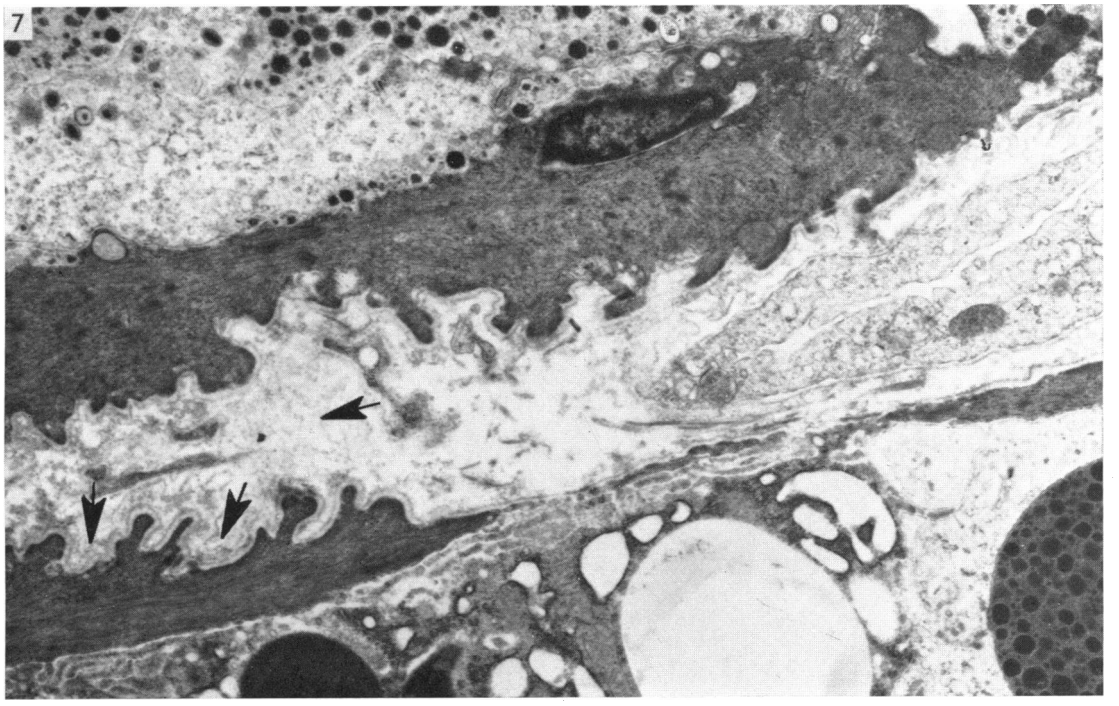

Fig. 7. Four days involution. Basement membrane material (arrows) can be seen around myoepithelial cell 'feet' which contain dense filament attachment areas on their distal surfaces. ×12500.

ally seen in contact with them (Fig. 9) may have been an artifact created by the plane of section. Multivesicular bodies were present.

To some extent, myoepithelial cells interdigitated with neighbouring epithelial cells, although the processes that they extended were blunt rather than villiform. There was some degree of overlap between myoepithelial cell processes around the ducts, with desmosomal junctions and interdigitations between them (Fig. 10).

The stroma around the alveoli and ducts was filled with masses of material similar in appearance to that forming the basement membrane (Fig. 9). This was seen to some degree at four days involution but now the process of deposition was far more advanced, and in places the basement membrane formed complex patterns with alternating layers of lamina lucida and lamina densa material. Such deposits exaggerated the form of the cell processes and made them appear more extensive than they were. Within the laminations, occasional collagen fibres were found and elastic tissue and collagen bundles could be seen in the loose material.

Occasionally, myoepithelial cells were almost detached from the main body of cells and extended into the stroma, still surrounded by basement membrane (Fig. 10), although this appearance may have been due to the plane of section skimming the edge of a duct.

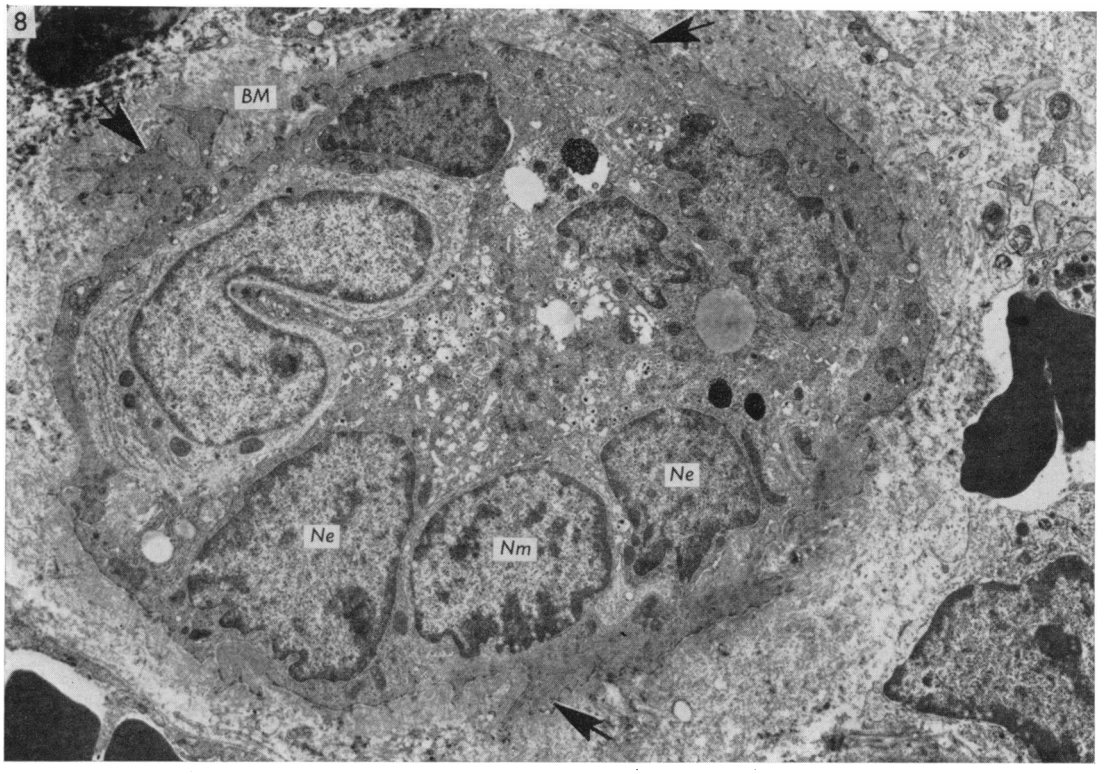

Fig. 8. Thirteen days involution. Low power view of a duct showing branching processes of myoepithelial cells (arrows) surrounded by a thick basement membrane (*BM*). Note the similarity between the nucleus of the myoepithelial cell (*Nm*) and that of neighbouring epithelial cells (*Ne*). × 6250.

DISCUSSION

This study has demonstrated the ultrastructural changes seen in myoepithelial cells accompanying the involution of the mammary gland and has shown that, as stressed by Hollman & Verley (1967) and Verley & Hollman (1967), they are relatively resistant to the process of glandular involution and probably help to maintain the integrity of the glandular tree by acting as bridges and holding surviving cells together.

Whether or not all the myoepithelial cells are retained is not known. One or two were seen during involution with dense bodies within them which may have been lysosomes; however, no signs of cellular disruption were seen in the myoepithelium. Possibly surplus cells become detached in the later stages of involution, when the epithelial elements have shrunk in size.

Smooth muscle cells are known to secrete connective tissue substances both in culture and *in vivo* (Ross, 1971; Ross & Klebanoff, 1971) and, in breast tumours, cells intermediate between smooth muscle (or myoepithelial) cells and fibroblasts have been seen (Ahmed, 1971). Similar cells have also been found in the lungs from cases of mitral stenosis (A. W. Jones, 1971, personal communication). Hübner, Klein,

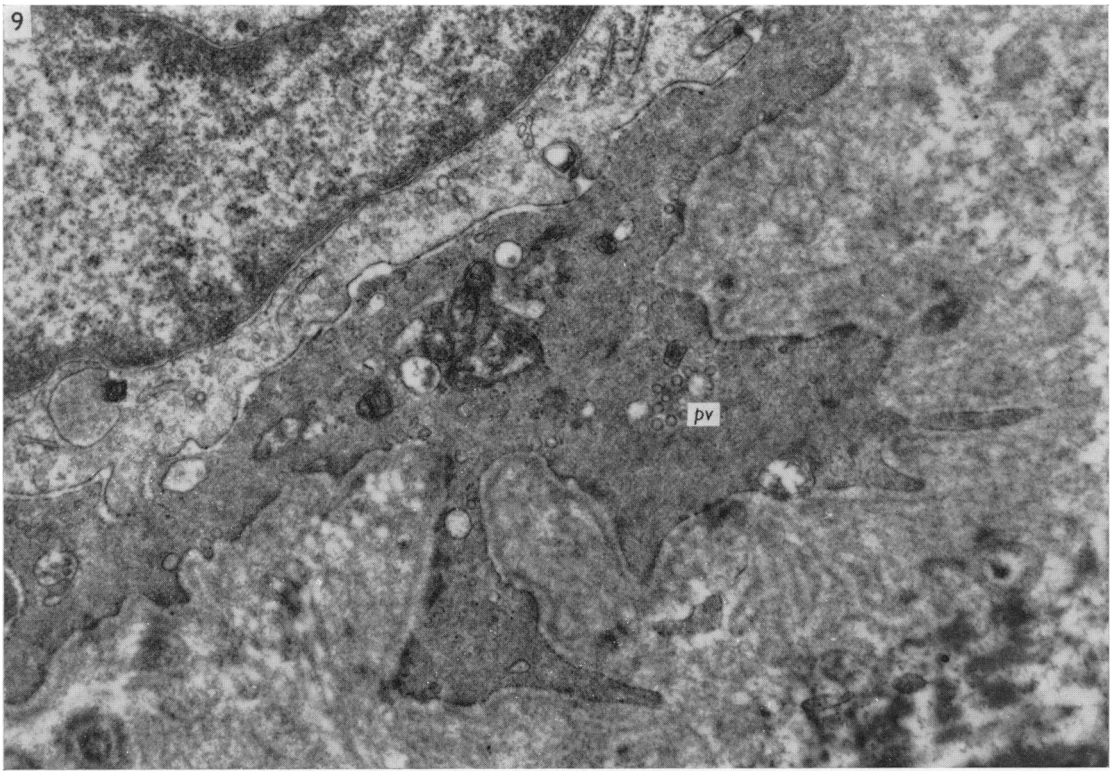

Fig. 9. Thirteen days involution. The laminated appearance of the basement membrane is shown. Note the clusters of pinocytotic vesicles (*pv*) and the uniform distribution of myofilaments. × 22 500.

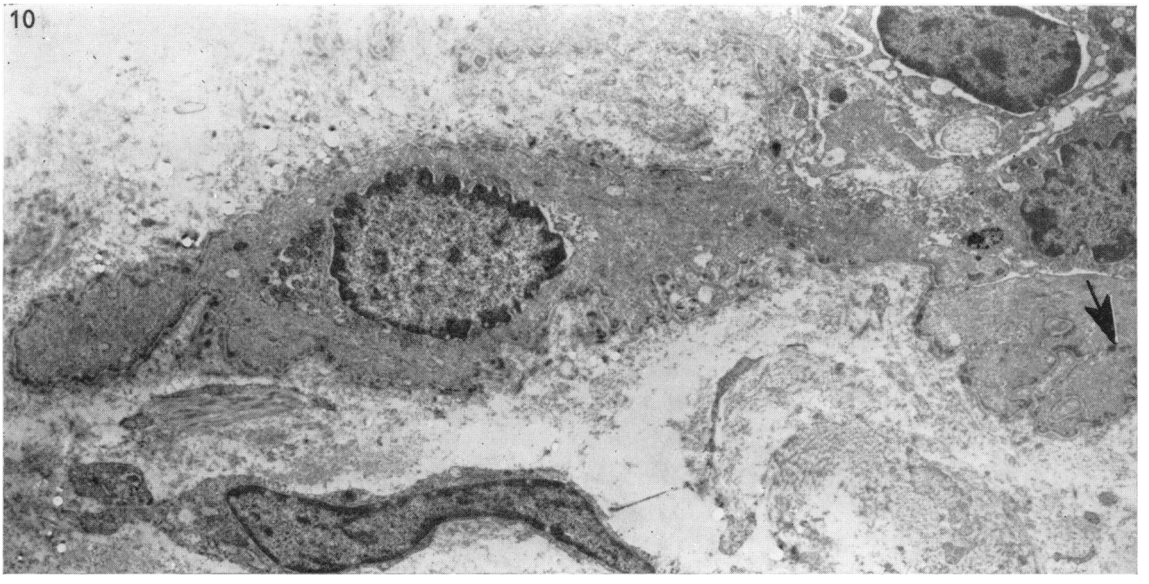

Fig. 10. Thirteen days involution. Duct myoepithelium with an extended myoepithelial cell and a desmosomal junction (arrow) is shown. × 6250.

Kleinsasser & Schieffer (1971) described how, in salivary gland tumours, myoepithelial cells lose their connexions to the epithelial cell complexes and become surrounded by basement membrane material. Whether myoepithelial cells, albeit epithelial and not mesenchymal in origin, are also capable of transforming into fibroblasts has yet to be shown. However, the basement membrane material seen in involution is probably secreted by withdrawing myoepithelial cells, and may act as a supporting matrix while connective tissue elements are laid down around the regressing parenchyma. The development of cell processes during involution does not seem to be related directly to secretion but is more likely due to the decrease in size of the alveoli, for dark attachment areas and hemi-desmosomes are frequently found in the most distal parts of the processes as if they were being retained in their original positions while the rest of the cell withdrew.

Luft (1966) suggested that, in capillary endothelial cells, it was the pinocytotic vesicles which secreted basement membrane material. It is unlikely that those of myoepithelial cells secrete this substance as it is found equally well developed under epithelial cells which have fewer vesicles. At involution, when basement membrane secretion is at its greatest, the pinocytotic vesicles are not as conspicuous as those seen during the lactating period (Radnor, 1972) when the myoepithelial cells themselves are most highly developed.

The decrease in the number of myofilaments towards the end of involution may be due to their depolymerization in an endeavour to return to the prelactating state; the occasional lysosome-like bodies may therefore be autophagic vacuoles. The myofilaments also appeared to be more randomly orientated at this time.

The banded fibrillar complexes which were prominent in the early stages of involution could no tbe detected in the material taken at thirteen days and it is possible that they are resorbed during the period of reorganization of the gland.

SUMMARY

1. The myoepithelium of involuting mammary glands of the rat 44 hours, 4 and 13 days after weaning was examined using the electron microscope.

2. During the early stages of involution, long processes of myoepithelial cells held existing cells of the disrupting alveoli together and probably prevented disorganization of the glandular tree.

3. As alveoli shrank in size, the myoepithelium developed an irregular outline, with bizarre processes protruding into the stroma, surrounded by laminations of basement membrane material. Myofilaments assumed a more homogeneous appearance, with few dense bodies amongst them.

4. There was no evidence of degeneration of the myoepithelium and the possibility of a subsequent detachment from the parenchyma and transformation into fibroblasts was discussed.

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