ABSTRACT

Mammary gland pieces from lactating mice were incubated for various times up to 4 h in collagenase and then additionally for up to 1.5 h in a mixture of collagenase and hyaluronidase. Tissue then was fixed and prepared for scanning electron microscope viewing. Monitoring of time of enzymic hydrolysis allowed visualization of connective tissue sheets, secretory lobules, blood vessels, alveoli encased in and devoid of basement membrane, secretory epithelia, alveolar milk ducts, and myoepithelial cells. Scanning electron microscope viewing of alveoli in cross section showed good internal microanatomy including a valve-like arrangement of secretory epithelia at the point of milk duct attachment. Morphology of myoepithelial cells and their relationship to secretory cells and to each other was presented. There was no evidence that myoepithelial cells were interconnected continuously throughout the tissue, but they interlaced over an alveolus; thus, the concept of a limited physiological syncytium was advanced.

INTRODUCTION

My interest in mammary myoepithelial cells led to the necessity for their microscopic visualization. Silver staining of these cells is one such method for visualization (7). Scanning electron microscopy (SEM) is appealing because it offers three dimensional visualization for morphological study. The results and photographs from a study of the mammary gland by SEM (3) offered little hope that this would be a useful adjunct to light microscope visualization of silver stained myoepithelial cells. Nonetheless, various methods of tissue preparation for scanning electron microscopic visualization were pursued with limited success. A recent note on sublingual salivary gland myoepithelial cells (2) was responsible for the degree of success reported herein.

MATERIALS AND METHODS

Primiparous mice of the HaM/ICR strain were used. Illumination intensity at their cages was about 1160 lx. Lighting was controlled at 12 h of light and 12 h of dark. Room temperature was controlled at 24 ± 3°C. Mice were fed Purina Lab Chow 5015. Litter size was standardized to 10 mice on day 1 of lactation and maintained throughout lactation. Pups remained with their mother until she was killed (milking interval = 0 h) or pups were separated from their mothers for various times, i.e., 2 h before she was killed (milking interval = 2 h). Days in lactation and milking interval are noted in figure legends to denote completely experimental conditions.

Mice were killed with an i.p. injection of Nembutal. The fourth mammary gland was removed and cut into strips having the full thickness of the gland and measuring approximately 2 to 4 mm wide × 7 to 9 mm long. These pieces were placed in Tyrode solution (4) at 37°C, pH 7.2, containing collagenase, 200 U/ml (Worthing, NC; class 11, 130 U/mg), for various times up to 4 h. The tissue then was removed and placed in a fresh solution of Tyrode solution containing collagenase, 200 U/ml, and hyaluronidase, 5 NFU/ml (Sigma, St. Louis, MO; type 1-S, 270 NFU/mg) for times up to 1.5 h. The tissue then was rinsed in Tyrode solution and stored at 4°C in 2% gluteraldehyde in Sorenson's phosphate solution.
(5). The tissue was processed for SEM by dehydration in ethanol, and critical-point dried with Freon. The tissues were coated with gold by a Technics Hammer II sputter coater. Specimens were viewed in an ETEC Autoscan. The nomenclature of many structures referred to in the results is consistent with that used by others (8).

RESULTS AND DISCUSSION

There were considerable differences among mammary tissues from different mice in the extent of collagen hydrolysis for a given set of incubation conditions. Such tissue samples could be expected to contain different amounts of collagen; consequently, it was necessary to monitor the degree of hydrolysis individually and incubate for different times. No set of conditions guaranteed repeatable visualization of a given structure.

Figure 1 shows a collagen sheet (A) covering many alveoli; it has been hydrolyzed partially to uncover an alveolus still enclosed in its basement lamina (B). In Figure 2 the collagen sheet has been hydrolyzed completely, leaving alveoli fully enclosed in their basement lamina (A) and a chain of leucocytes (B).

The lobule, with its many alveoli and secretory epithelial cells, appears as if it had been dissected out in three dimensions in Figure 3. The lobular duct with associated blood vessels is at A, and adipocytes are at B in Figure 4.

The blood capillary network interlaces over the basal surface of alveoli in Figure 5. Capillaries were hydrolyzed readily by the enzymes and were often virtually absent in conditions necessary to visualize clearly some of the cell structures.

Individual secretory epithelia of an alveolus varied in size and shape. Some were round; others were crescent shaped, whereas still others were oval (Figure 6 and 7). Blood vessels fairly well encircle an alveolus in traversing the basal surface of the secretory epithelia, Figure 6B.

Myoepithelial cells varied in size, shape, and degree of branching. The myoepithelial cell bodies varied in size and shape; some were short and readily distinguished from the processes, and others were elongated and almost confluent with their processes (Figures 7C, 8A, 9A). The myoepithelial cell processes varied in number, size, shape, and degree of branching (Figures 7 and 8B). Different degrees of branching were on different processes of the same myoepithelial cell (Figure 9B). Myoepithelial cells lay in contact with the basal surface of the secretory epithelial cells (Figure 8). Figure 10 shows this association under conditions more like in vivo due to less enzymic hydrolysis. Attachment of the myoepithelial cell processes to the secretory epithelial cells was quite firm as evidenced by the deep basal indentations on the epithelial cell caused by myoepithelial cell processes (Figure 9C). This attachment has been noted on transmission electron micrographs as hemidesmosomal and desmosomal (6). A point of attachment appears to be at A, Figure 11, where the end of the process fans out into the basal surface of an epithelial cell.

At times the myoepithelial cells appeared to separate secretory epithelia as in Figure 8C. A word of caution should be interjected here, in that enzymic hydrolysis does affect cell to cell attachment and shrinkage may occur during dehydration in preparation of the tissue for SEM viewing. Nonetheless, the distinct impression was that processes do follow adjacent secretory cell boundaries. Occasionally, myoepithelial cell processes appeared to course between adjacent secretory cells as in A, Figure 10. Processes could be displaced if the attachments were loosened during tissue preparation, but a process runs between adjacent cells under a still-existing basal lamina bridge in Figure 8D. This was noted with transmission electron microscopy — a technique best suited to answer questions like this — where it was reported that "in-tucking myoepithelial cells could often be seen with epithelial cells balloon ing out around them so that the sides of neighboring cells almost met under the basal surface of the myoepithelium" (6).

Many different preparations have been used to show specific structures. Because different

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Figure 1. The SEM of tissue incubated 2 h in collagenase, 0.5 h in collagenase and hyaluronidase; 23 days of lactation, 0-h milking interval. Collagen sheet at A, basement lamina surrounding alveolus at B.

Figure 2. The SEM of tissue incubated 2 h in collagenase, 0.5 h in collagenase and hyaluronidase; 23 days of lactation, 0-h milking interval. Alveoli encased in basement lamina at A; leucocytes at B.

structures have varying degrees of susceptibility to enzymic hydrolysis, it was not possible to show clearly each structure simultaneously. By much trial and error, close to in vivo associations of the myoepithelial cells are in Figure 12. Blood vessels bridge between two alveoli at A. Myoepithelial cell bodies and some of their processes are in direct contact with epithelial cells at B, and processes of different myoepithelial cells appear to be interlaced at C.

The question of how a contraction response is propagated throughout the mammary gland has been considered by others. Both Richardson (7) and Linzell (1) used light microscope visualization of silver-stained myoepithelial cells. Richardson pointed out that processes from different myoepithelial cells came close together, but he was not able to visualize contact. Linzell noted that processes met and crossed over, but he concluded that a true syncytium was not formed. Radnor (6) used transmission electron microscopy and noted that myoepithelial cells formed desmosomal attachments with each other. In the present study, myoepithelial processes covering each alveolus often interlaced with each other (Figures 13 and 14), and processes also ran between alveoli but not as a continuous network throughout the tissue. In a true or physiologically functional syncytium, all of the myoepithelial cells in contact with each other would contract following stimulation of any one cell. The anatomical evidence from this study, supported by (1, 6), suggests that stimulation of a myoepithelial cell could result in contraction of other cells on the same alveolus and, to a limited degree, probably in contraction of other cells on an adjacent alveolus.
Figure 7. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 0-h milking interval. Differently shaped secretory epithelial cells at A and B, myoepithelial bodies at C, basal lamina bridging two secretory epithelial cells with underlying myoepithelial process at D.

Figure 8. The SEM of tissue incubated 2.5 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 0-h milking interval. Field of alveoli with myoepithelial cells of different sizes and shapes: bodies at A, processes at B.
Figure 9. The SEM of tissue incubated 2.2 h in collagenase, .5 h in collagenase and hyaluronidase; 10 days of lactation, 0-h milking interval. Myoepithelial cell body at A, process with many branches at B, indentation of myoepithelial attachment at C.

Figure 10. The SEM of tissue incubated 1 h in collagenase, .5 h in collagenase and hyaluronidase; 4 days of lactation, 2-h milking interval, showing the closer to in vivo-like relationship of myoepithelial cells to the basal surface of the alveolar secretory cells. Process running between adjacent secretory cells (insert A).
Figure 11. The SEM of tissue incubated 1.7 h in collagenase, .7 h in collagenase and hyaluronidase; 4 days of lactation, 0-h milking interval. Myoepithelial cell process attaching to basal surface of secretory cell at A, insert.

Figure 12. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 5-h milking interval. Blood vessel at A, myoepithelial cell bodies at B, and interlacing processes at C.
Figure 13. The SEM of tissue incubated 1.7 h in collagenase, 7 h in collagenase and hyaluronidase; 20 days of lactation, 0-h milking interval. Myoepithelial cells covering an alveolus where the processes from different cells show considerable contact with each other.

Figure 14. The SEM of tissue incubated 1.7 h in collagenase, 7 h in collagenase and hyaluronidase; 20 days of lactation, 0-h milking interval. Myoepithelial cell processes forming almost a continuous network over an alveolus.
Figure 15. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 5-h milking interval. Alveolar lumen at A, microvilli covering apical portion of secretory cell at B, fat globule membrane at C.

Figure 16. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 0-h milking interval. Polygonal cell boundaries at A, crater-like structures at B, alveolar duct opening at C.
Figure 17. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 0-h milking interval. Milk duct in cross section from top. Duct lumen at A, epithelial cell at B, blood vessel with wall bulging due to blood cell at C, myoepithelial cell process at D.

Figure 18. The SEM of tissue incubated 2 h in collagenase, .5 h in collagenase and hyaluronidase; 8 days of lactation, 5-h milking interval. Alveolar duct has been hydrolyzed, leaving valve-like epithelial cells at exit of alveolus at A, myoepithelial cell process at B, and fat globule membrane at C.
alveolus. But no evidence is yet available to indicate that a stimulus applied to one myoepithelial cell would be propagated throughout the entire mammary gland or even throughout a lobule via myoepithelial cell-to-cell contact.

The alveolus viewed in three dimensional cross section (Figure 15) had secretory epithelial cells projecting toward the lumen (A). Microvilli cover the apical surfaces of the secretory cells (B), and fat globule membranes still are attached to the secretory cell apices as in (C). In Figure 16, polygonal cell boundaries are visible (A) as are the crater-like structures (B) that probably represent fat secretion. The opening from the alveolus of the milk duct is at C. A cross section of one of the larger milk collecting ducts is in Figure 17 where the lumen is at A and an epithelial cell is at B. Alveolar secretory cells located where the milk duct exits from the alveolus well may serve some passive valve-like function in controlling milk translocation (Figure 18A). A myoepithelial cell process courses the periphery of these cells at B, and a fat globule membrane comes from the duct lumen at C.

Three dimensional visualization of the microanatomy of the lactating mammary gland confirmed most of our three dimensional concepts based on evidence gathered from light microscope and transmission electron microscope. The work of Richardson (6) and Linzell (1), which visualized myoepithelial cells by silver staining, was responsible in great part for our concepts of the involvement of the myoepithelial cell in milk ejection. Visualization of these cells by SEM promises to be instrumental in future elucidation of the myoepithelial cells' role in milk ejection. Further, interpretation of three dimensional visualization of mammary gland microstructure will be essential in studies which involve normal and diseased states.

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REFERENCES