The Ultrastructural Localization of Gross Cystic Disease Fluid Protein (GCDFP-15) in Breast Epithelium

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GCDFP-15 is a major constituent protein of 15,000-dalton monomer size present in breast gross cystic disease fluid. Immunoperoxidase staining of GCDFP-15 has shown the protein to be present in normal apocrine epithelium, metaplastic apocrine epithelium of the breast, and breast carcinomas with apocrine features. To delineate ultrastructurally the localization of GCDFP-15 in benign breast epithelium, a low-temperature embedding colloidal gold technique was used. Metaplastic apocrine epithelium of the breast showed GCDFP-15 to be localized in Golgi vesicles and cytoplasmic granules. At the cell apices these granules were contained in vacuoles and appeared to be released by exocytosis. There was labeling of cyst fluid within microcyst lumens. This report is the first to ultrastructurally characterize this protein and its mode of secretion. (Am J Pathol 1984, 116:305–310)

CYSTIC DISEASE of the breast produces a fluid containing a unique protein designated gross cystic disease fluid protein-15 (GCDFP-15).[1,2] Previous studies with light microscopy utilizing the immunoperoxidase technique have shown this protein to be a specific marker of apocrine epithelium.[3] In cystic disease of the breast, this protein is localized to metaplastic apocrine epithelium with a granular, perinuclear pattern of staining. A similar pattern of staining is observed in apocrine epithelium throughout the body.

To define precisely the ultrastructural localization of GCDFP-15 in metaplastic apocrine epithelium of the breast, we employed a low-temperature embedding colloidal gold technique. This report is the first on ultrastructural localization of this protein and the morphologic demonstration of its mode of secretion.

Materials and Methods

Samples were obtained from breast biopsies with gross cystic disease immediately after surgical removal. The tissue was minced into 0.5-mm cubes and fixed in a mixture of 3% paraformaldehyde, 0.1% glutaraldehyde for 1 hour. Following fixation, the tissues were washed three times in phosphate-buffered saline, (PBS) pH 7.4. Immediately prior to embedding, the tissues were reacted with 0.5 M ammonium chloride to quench unreacted aldehyde groups. The tissues were again washed in PBS, dehydrated in alcohols, and embedded in Lowicryl K4M[4] (Balzer's, Hudson, NH). Following polymerization, 1-μ sections were cut and stained with toluidine blue for selection of areas for thin-sectioning. Thin (600–800 A) sections were cut and picked up on nickel grids for immunoelectron microscopy.

For immunoelectron microscopy, the grids were first incubated for five minutes at room temperature with 0.5% egg albumin. This was followed by a 1-hour incubation with anti-GCDFP-15 (dilution 1:200)[1] for 1 hour at room temperature. The grids were then washed three times with PBS and incubated with staphylococcal protein A colloidal gold for 1 hour at room temperature. The sections were again washed two times in PBS and then in distilled water. The grids were then stained with uranyl acetate for 5 minutes and then by lead citrate for 45 seconds. The grids were then examined on a JEOL JEM-100S electron microscope.

Accepted for publication March 5, 1984.

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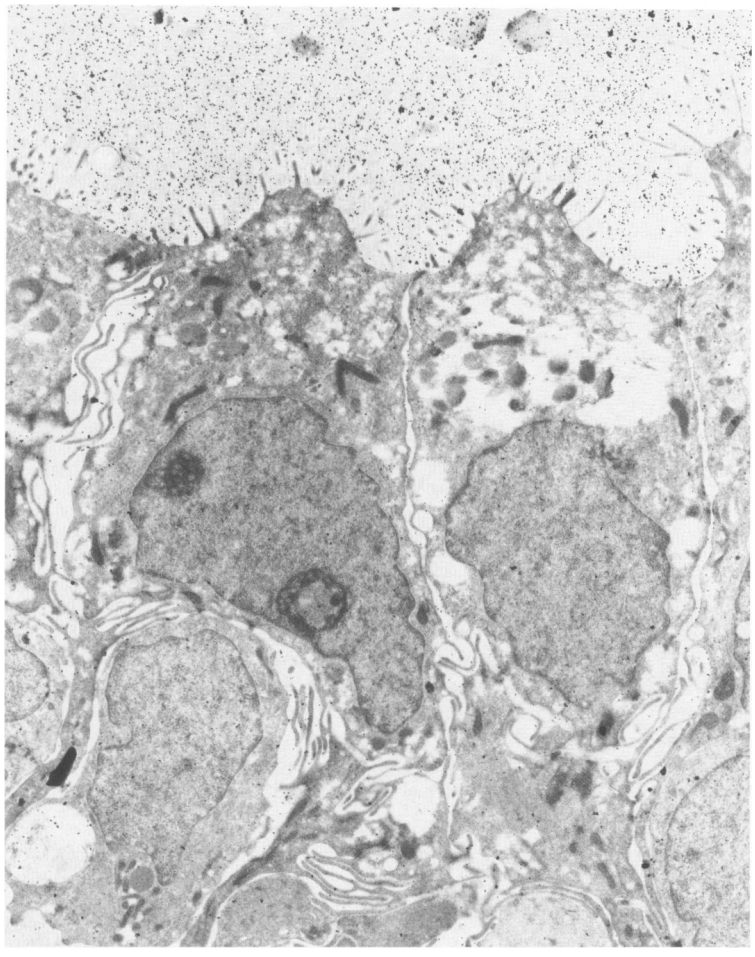

Figure 1 — Apocrine epithelium, embedded in Lowicryl and stained with anti GCDFP-15 (dilution 1:200). There are prominent apical "snouts" and microvilli. The cyst fluid is heavily labeled with colloidal gold. (Uranyl acetate and lead citrate counterstain, × 2000)

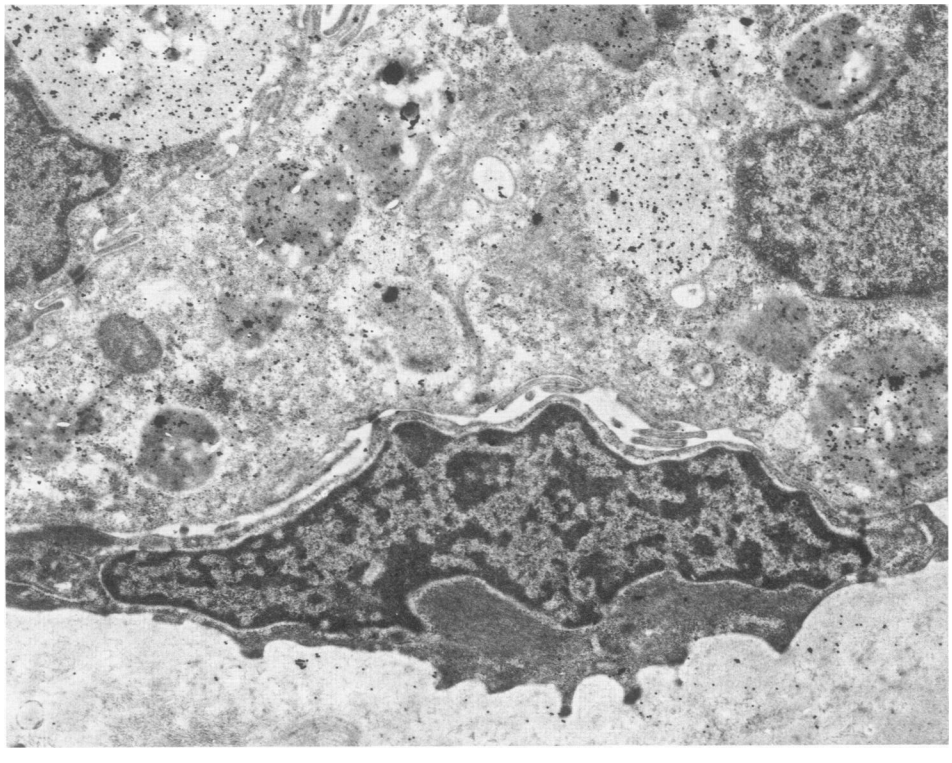

Figure 2 — Apocrine epithelial cells and a myoepithelial cells. The myoepithelial cell is unlabeled (*bottom*). The granules in the apocrine cells are heavily labeled. (Anti-GCDFP-15 1:200, uranyl acetate and lead citrate counterstain, × 4000)

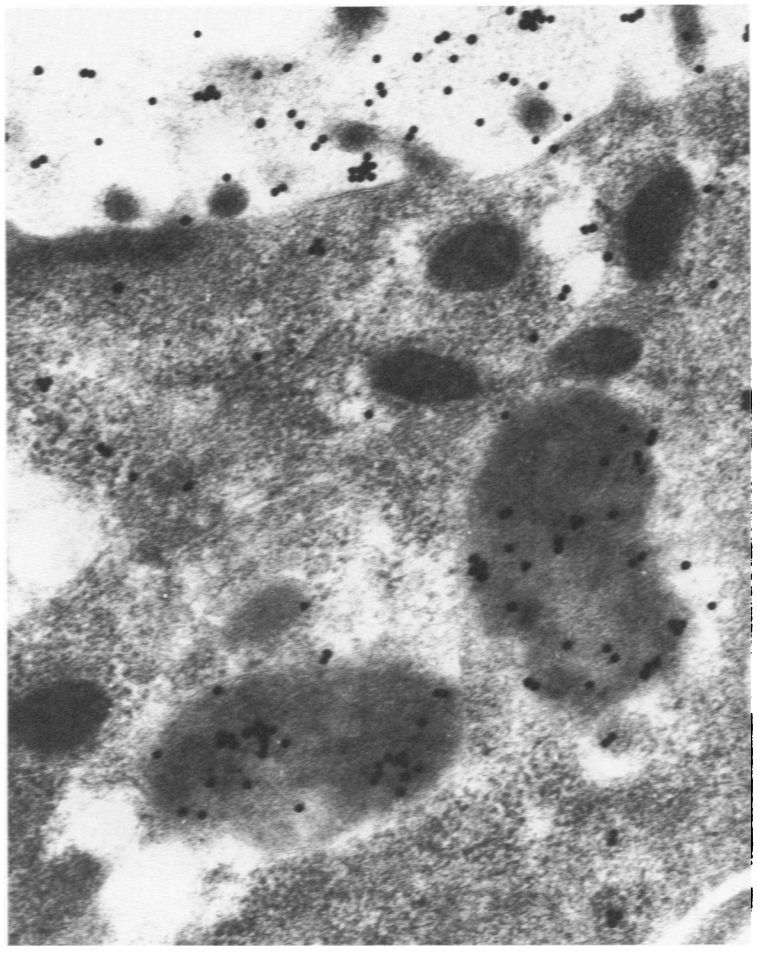

Figure 3—Apocrine epithelium showing cytoplasmic granules and vacuoles beneath the apical plasma membrane. There is labeling of both the granules and the vacuoles with colloidal gold. The luminal fluid is also intensely labeled. (Anti-GCDFP-15 1:200, uranyl acetate and lead citrate counterstain, × 10,000)

The protein A colloidal gold was prepared by reducing a boiling solution of 100 ml of 0.1% tetrachloroauric acid (Aldrich, Milwaukee, Wis) with 5 ml of sodium citrate.[5,6] The pH of the resulting solution was adjusted to 5.9 and protein A (E-Y Laboratories, Inc., San Mateo, California) added in a ratio of 5 μg/ml. The protein A colloidal gold was collected by centrifugation at 65,000 for 45 minutes. The pellet was resuspended in 1.5 ml of PBS containing 1% sodium azide and 0.1% polyethylene glycol. One to 25 dilutions of this stock solution were utilized.

Control grids include grids reacted with PBS alone, nonimmune serum, and antisera adsorbed with antigen.

Results

The ultrastructural appearance of the apocrine epithelium fixed and embedded in Lowicryl K4M was similar to that previously described. It was characterized by apial protrusions or "snouts" with microvilli, cytoplasmic granules, and numerous mitochondria (Figure 1). Background staining over mitochondria, nuclei, and connective tissue was slight (Figure 2). Labeling greater than background was interpreted as positive localization of the antigen. There was strong labeling of the material within the cyst lumen (Figure 1) as well as the dark granules and vacuoles within the cytoplasm of the epithelial cells (Figure 3). Myoepithelial cells (Figure 2) showed no evidence of GCDFP-15 localization.

Vesicles and granules within the perinuclear Golgi region contained strong labeling with colloidal gold (Figure 4). The perinuclear vesicles labeled are presumably part of the Golgi complex. The absence of osmium as a fixative precludes distinct visualization of membrane structures. Vacuoles present in the apical cytoplasm were also often labeled (Figures 3 and 5).

Nonapocrine epithelium contained rare GCDFP-15 localized to cytoplasmic granules (Figure 6). Although nonapocrine epithelium exhibited sparse labeling, individual labeled granules within nonapocrine epithelium were a frequent observation.

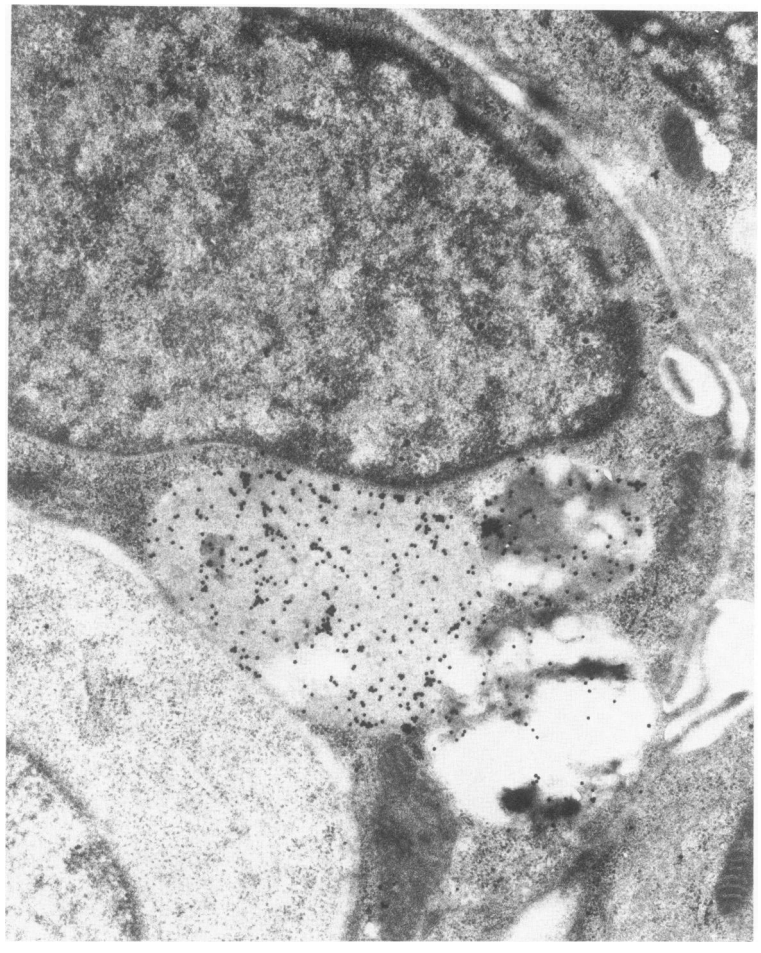

Figure 4—Apocrine epithelium with a perinuclear Golgi area. Granules can be seen forming within vesicles with strong staining of the granular material. (Anti-GCDFP-15 dilution 1:200, uranyl acetate and lead citrate counterstain, ×8000)

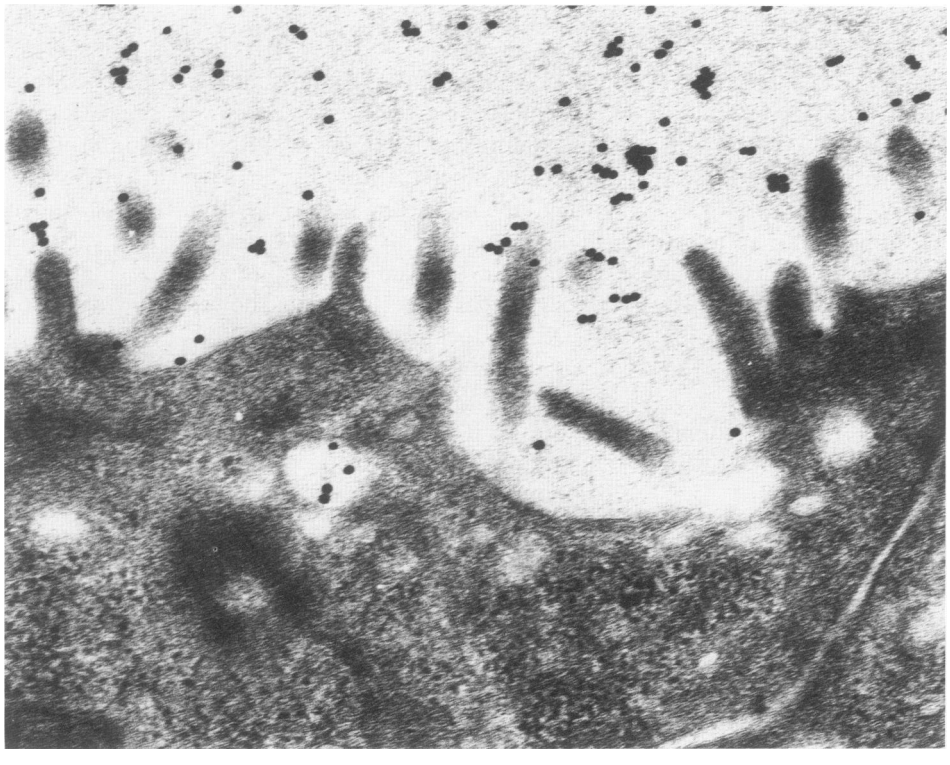

Figure 5—The luminal surface of the apocrine epithelium showing cytoplasmic vacuoles beneath the apical plasma membrane. A vacuole is labeled with colloidal gold, as is the luminal material. (Anti-GCDFP-15 1:200, uranyl acetate and lead citrate counterstain, ×15,000)

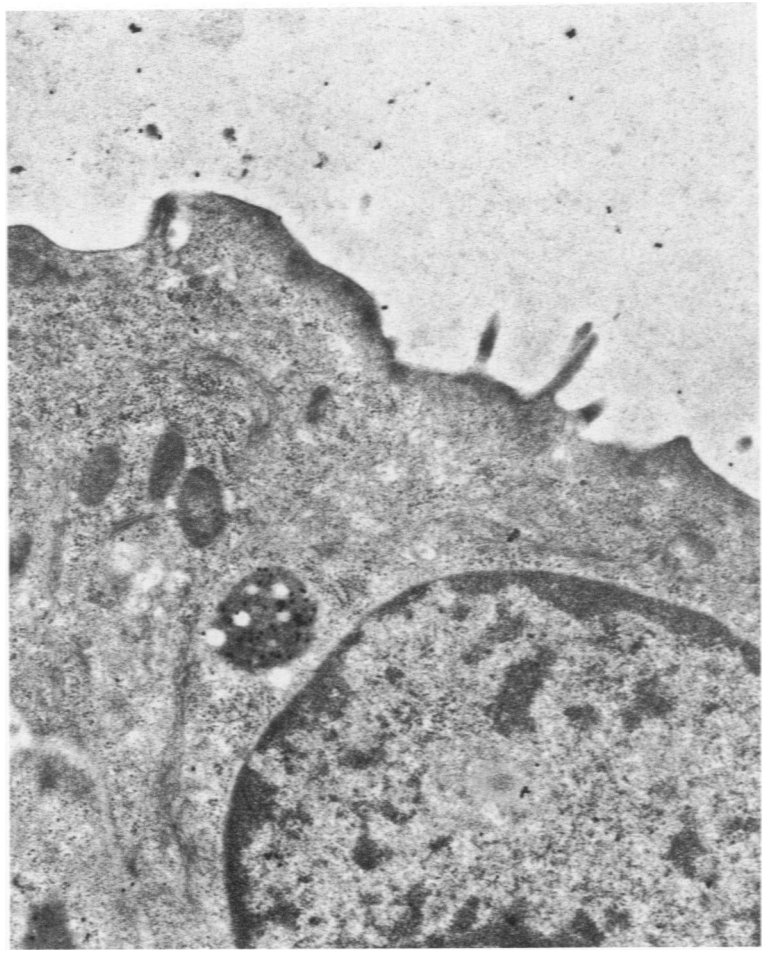

Figure 6—A nonapocrine epithelial cell containing a cytoplasmic granule labeled with colloidal gold. The luminal contents are unlabeled. (Anti-GCDFP-15 1:200, uranyl acetate and lead citrate counterstain, ×8000)

Discussion

Our study demonstrates discrete localization of GCDFP-15 to Golgi vesicles and cytoplasmic granules in metaplastic apocrine epithelium of the breast. Beneath the luminal surface of the cells, these granules appeared to merge with vacuoles. There was also intense labeling of cyst fluid within microcyst lumens. These observations provide evidence for the specific packaging, storage, and secretion of GCDFP-15 by metaplastic breast apocrine epithelium.

The ultrastructural characteristics of metaplastic apocrine breast epithelium have been well described.[7-12] These cells are characterized by prominent apical cytoplasm lined by microvilli, vesicles beneath the luminal border, well-developed Golgi regions, numerous mitochondria, dilated rough endoplasmic reticulum, and elaborate infolding and interdigitations of the plasma membrane. These features are similar to normal apocrine epithelium in the skin.[13-18] The presence of both cytoplasmic granules and microvesicles have suggested that this epithelium has a secretory function.

Our results support the concept of metaplastic breast apocrine epithelium having a secretory function and suggest that the secretion occurs by exocytosis. Our evidence for this is the presence of GCDFP-15 in vacuoles beneath the apical plasma membrane and localization of the protein within the cyst lumen. This method of secretion of a specific protein in metaplastic apocrine epithelium is similar to that reported for normal apocrine epithelium.[13-18]

The mechanism(s) of normal apocrine secretion remains controversial. Although exocytosis has been reported as a means of secretion in apocrine epithelium,[8,10,14-16] other authors have reported both merocrine and apocrine secretion.[13,17,18] Hashimoto and co-workers and Schamberg-Lever and co-workers have presented evidence that the mechanism of apocrine secretion is by decapitation.[17,18] Whether apocrine epithelium possesses two modes of secretion, a

slow, gradual, or merocrine type and a rapid amputation type, remains to be determined.

Our results also demonstrate the suitability of low-temperature embedding in Lowicryl K4M for the ultrastructural localization of intracellular antigens. The morphology by this technique is comparable to conventional Epon sections; yet there is excellent preservation of antigenicity. Not only can antigens be localized by this technique, but this method provides an understanding of the biology and fate of certain substances. Our observations indicate that GCDFP-15 is synthesized by metaplastic breast apocrine epithelium, packaged in the Golgi apparatus, and excreted into the cyst lumen by exocytosis. This type of information is not provided by any other ultrastructural immunocytochemical technique.

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Acknowledgments

The authors gratefully acknowledge the skilled secretarial assistance of Ms. Ann Benoit, the expert technical assistance of Ms. Christine Ridolfi and Nora Galins, and the photographic work of Mr. Anthony Merola.