

ULTRASTRUCTURAL LOCALIZATION OF ALKALINE PHOSPHATASE IN LACTATING
RAT MAMMARY GLAND

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Alkaline phosphatase is found in almost all nucleated mammalian cells as well as in many microorganisms (1). In animal tissues, it is often found associated with plasma membranes and also has been indirectly implicated in the accumulation of calcium-phosphate in calcifying tissue, osteoblasts and odontoblasts (2). In the secretion of milk, vesicles accumulate casein and colloidal calcium-phosphate (3), while in a separate pathway milk fat is secreted and bounded by the apical plasma membrane (4). Alkaline phosphatase is found in milk, and all milks contain both colloidal calcium-phosphate and fat globule membranes which are derived in part from the plasma membrane. Previous studies (5) had suggested that mammary alkaline phosphatase is limited to nonsecretory myoepithelial cells, but recent work (6) has indicated that intracellular alkaline phosphatase is cryptic. We therefore decided to investigate the cytochemical distribution of alkaline phosphatase in mammary secretory cells, and to determine if it could participate in calcium-phosphate accumulation in milk.

Tissue, obtained from lactating female Sprague-Dawley rats 8-10 days postpartum, was excised, placed into ice-cold glutaraldehyde (1% in 0.1M cacodylate, pH 7.2), minced and fixed for 1 hour. Specimens were rinsed with 0.1M cacodylate, followed by 0.1M tris-maleate, pH 8.0. Treatments, based on the procedures of Robinson and Karnovsky (7), included: complete reaction mixture using naphthol AS-MX phosphate as substrate; controls without substrate; inhibition studies using 500 μ M levamisole or dithiothreitol (DTT); and activation by the detergent saponin at 0.005% (6). Following incubation, samples were rinsed with tris-maleate followed by cacodylate, refixed in glutaraldehyde and rinsed again in cacodylate; half were postfixed in 1% OsO₄ in 0.1M cacodylate and the other half were not. All samples were then dehydrated through a graded ethanol series and 100% acetone, embedded in epoxy resin and sectioned.

We found that alkaline phosphatase was localized primarily on the basolateral membranes of secretory epithelial cells as well as on the myoepithelial cells, and to a lesser degree on collagen bundles, fibroblasts and endothelial cells. Osmicated tissues have defined intracellular lipid droplet membranes, whereas nonosmicated tissues do not. We therefore concluded that any stain seen on fat globule membranes may be due to OsO₄, not reaction product. Alkaline phosphatase was selectively inhibited by DTT and levamisole. Saponin serves to enhance the intracellular activity of secretory cells, but only in the rough endoplasmic reticulum adjacent to the myoepithelium. However, there was no evidence of intracellular tag on any other organelle, including Golgi-derived components which accumulate calcium-phosphate and casein.

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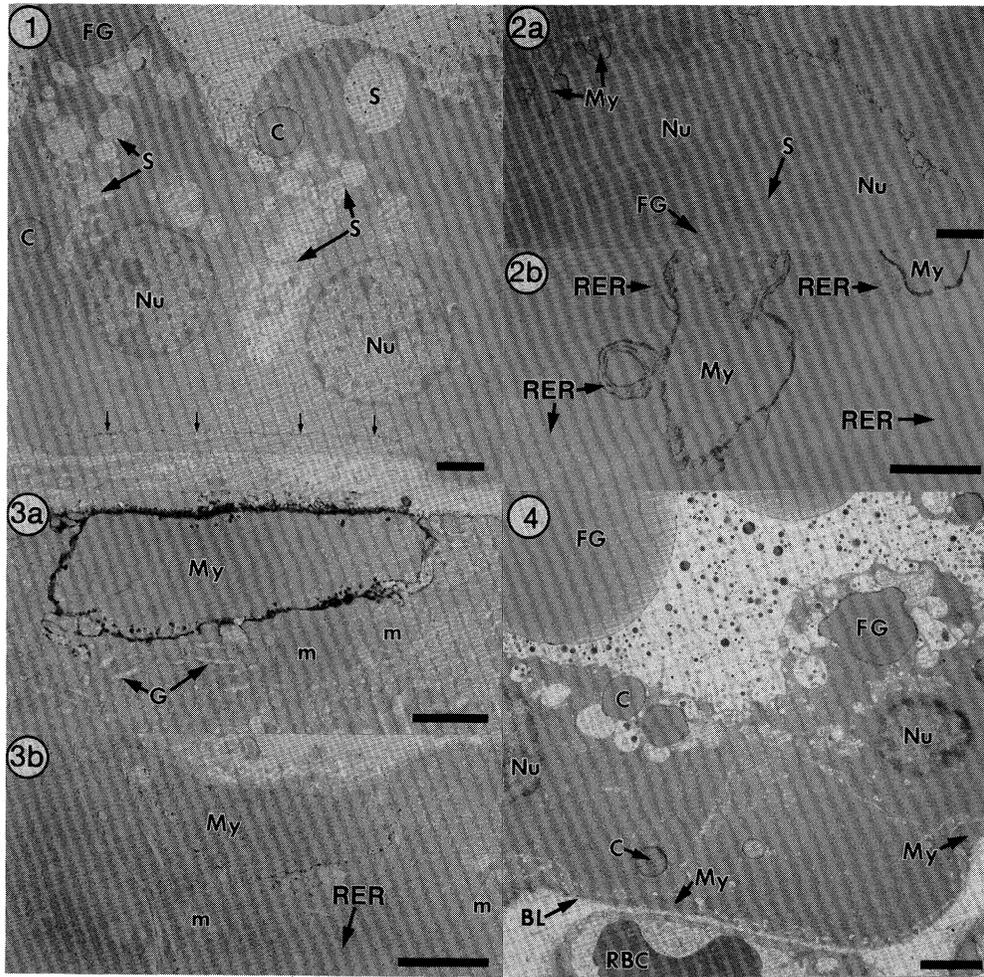


Fig. 1. Secretory epithelial cells following incubation with naphthol AS-MX phosphate and postfixation with OsO_4 . Note reaction product at the interface between the secretory epithelium and the myoepithelium (arrows). Lipid coats and fat globule membranes are stained with OsO_4 . No tag occurs on apical plasma membranes. Nu=nucleus, C=cytoplasmic lipid droplet, S=secretory vesicle, FG=fat globule. Bar = 2 μm .

Fig. 2. a. Effect of saponin on the location of reaction product in the secretory epithelium. Cells have not been postfixated with OsO_4 . Saponin removes lipids from membranes thereby freeing active sites. Bar = 2 μm .

b. Enlargement showing enhanced reactivity in rough endoplasmic reticulum cisternae adjacent to myoepithelial cells. My=myoepithelial cell, RER=rough endoplasmic reticulum. Bar = 1 μm .

Fig. 3. a. Myoepithelial cell incubated with naphthol AS-MX phosphate and postfixated with OsO_4 . Heavy accumulations of reaction product appear on the basolateral and myoepithelial membranes. Bar = 1 μm .

b. Tissue exposed to levamisole exhibits a reduction of reaction product. G=Golgi apparatus, m=mitochondrion. Bar = 1 μm .

Fig. 4. Selective inhibition of alkaline phosphatase by DTT. There is no evidence of reaction product on myoepithelial or basolateral membranes. Fat globule membranes remain dark due to their osmiphilic nature. BL=basolateral membrane, RBC=red blood cell. Bar = 2 μm .