UPTAKE OF PEROXIDASE INTO THE SMOOTH-SURFACED TUBULAR SYSTEM OF THE GASTRIC ACID-SECRETING CELL

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INTRODUCTION

The apical cytoplasm of the gastric oxyntic or acid-secreting cell contains a striking array of smooth-surfaced tubular elements (6, 7, 14, 16). These smooth-surfaced elements may assume various shapes depending on preparatory procedures, so that profiles of elongated elements and vesicles are observed (8, 15). The inclusive term "vesicotubules" has been used to describe such a system in the amphibian oxyntic cell (10).

During the secretion of gastric acid provoked by histamine, insulin, gastrin, or electrical vagal stimulation there is marked amplification of cell surface membrane adjacent to the lumen of the gastric gland (1, 5, 12, 13, 20–22, 24). The enhanced apical surface consists of numerous long and sometimes branching microvilli projecting into the lumen of the gastric gland (amphibian cell type) or intracellular canaliculus (mammalian cell type). This fine structural pattern which involves an increased surface area associated with acid secretion is thought to result at the expense of the "membrane reserve" which exists in the form of smooth-surfaced tubules and vesicles in the apical cytoplasm (2, 13, 16, 24). Occasional tubules or vesicles have been observed communicating with

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space between microvilli (13, 16); this supports the hypothesis that the tubules contribute to the elaboration of microvilli by a process of eversion, or turning inside out, associated with membrane flow. The fact that the number of tubules and vesicles decreases with the concomitant amplification of apical surface gives some additional support to this thesis. It has been shown also in metamorphosing frogs that the development of the smooth-surfaced tubules correlates well with the onset of hydrochloric acid secretion (3).

The recent introduction of the peroxidase cytochemical tracer technique (4) which yields an electron-opaque reaction product readily visualized in micrographs provides a means to reexamine the question of continuity or potential continuity of the tubular system of the oxyntic cell with the lumen of the gastric gland. The present study will present evidence that the peroxidase tracer can pass from the glandular lumen into the smooth-surfaced tubular or vesicotubular system of the amphibian oxyntic cell.

MATERIALS AND METHODS

The seven animals used in this study consisted of both male and female bullfrogs (*Rana catesbeiana*) ranging in weight from 200 to 260 g. They were maintained in the laboratory at approximately 10°C.

Under ether anesthesia, the pylorus was ligated and ~3 ml of an aqueous solution of peroxidase (Sigma, type II)[1] containing 30 mg/ml were injected into the lumen of the stomach via the esophagus. The volume of solution introduced varied with the size of the animal's stomach. The cardiac sphincter was then ligated. The frogs were exposed to the peroxidase solution for periods ranging from 0.5 to 1.5 hr during recovery from anesthesia.

For electron microscopy, tissue specimens were fixed in cold (4°C) 5% glutaraldehyde buffered at pH 7.6 with 0.12 M $Na_2HPO_4 \cdot 7H_2O$-$NaH_2PO_4 \cdot H_2O$ for 1–2 hr. Thin slices (40–50 μ) of gastric mucosa were washed in the phosphate buffer in the refrigerator at 4°C for either 1 hr (three changes) or overnight. The tissue slices were incubated for 0.5 hr at 37°C in freshly prepared Karnovsky medium containing diaminobenzidine and H_2O_2 (9). After incubation the slices of mucosa were washed in phosphate buffer for 0.5 hr (three changes) and postfixed for 0.5 hr in 1% osmium tetroxide buffered at pH 7.6 with the 0.12 M phosphate buffer. The tissue was then dehydrated in ethanol and propylene oxide and embedded in Epon 812 according to Luft (11), using a slow cure. Thin sections from Epon 812 embedments on naked copper grids (400 mesh) were examined, for the most part, without further staining to better visualize the peroxidase reaction product although some sections were stained with lead citrate for 3–6 min (23).

The sections were examined with a Siemens Elmiskop I at 80 kv with a pointed filament, double condenser containing a 250 μ aperture, and a 30 μ silver objective aperture at magnifications of 3,000–20,000.

RESULTS AND DISCUSSION

Electron micrographs of oxyntic cells exposed to the peroxidase solution showed the enzyme reaction product within the lumina of a number of smooth-surfaced tubules in the apical cytoplasm (Figs. 1 and 3) as well as within some tubules adjacent to the lateral surface of the nucleus (Fig. 2). In any given cell only a portion of the smooth-surfaced tubules contained the reaction product, and these, for the most part, tended to be congregated near the free surface. Some electron micrographs (Fig. 3, arrow) exhibited connections between the lumina of cytoplasmic tubules containing peroxidase and the free surface of the cell between adjacent plications. The rough-surfaced endoplasmic reticulum (arrows) found more basally in the cell, zymogen granules (*Z*), and mitochondria (*M*) showed negative peroxidase reactivity (Fig. 4). Occasionally, perhaps due to the toxicity of the enzyme in certain cells, peroxidase reactivity was found within the cristal spaces of mitochondria. Similar results have been reported for other cell types and isolated rat liver mitochondria exposed to peroxidase (17). Profiles interpreted to be lysosomes (*LY*) showed heavy staining for peroxidase, especially in those animals exposed to the solution for longer periods (Fig. 1). It was uncertain how much reactivity in the lysosomes was due entirely to exogenous peroxidase since control sections were not incubated for peroxidase activity. No staining was observed within the components of the junctional complex (*JC*) or intracellular clefts (Fig. 3).

These data provide evidence that some of the smooth-surfaced tubules can communicate directly with the apical free surface of the amphibian oxyntic cell. The findings suggest that the lumen of the tubules and the gastric gland lumen were linked directly through membrane-limited channels and not indirectly via shuttle vesicles. Whether these connections were stable or sporadic cannot be determined from the experimental data. Since

[1] Sigma Chemical Co., St. Louis.

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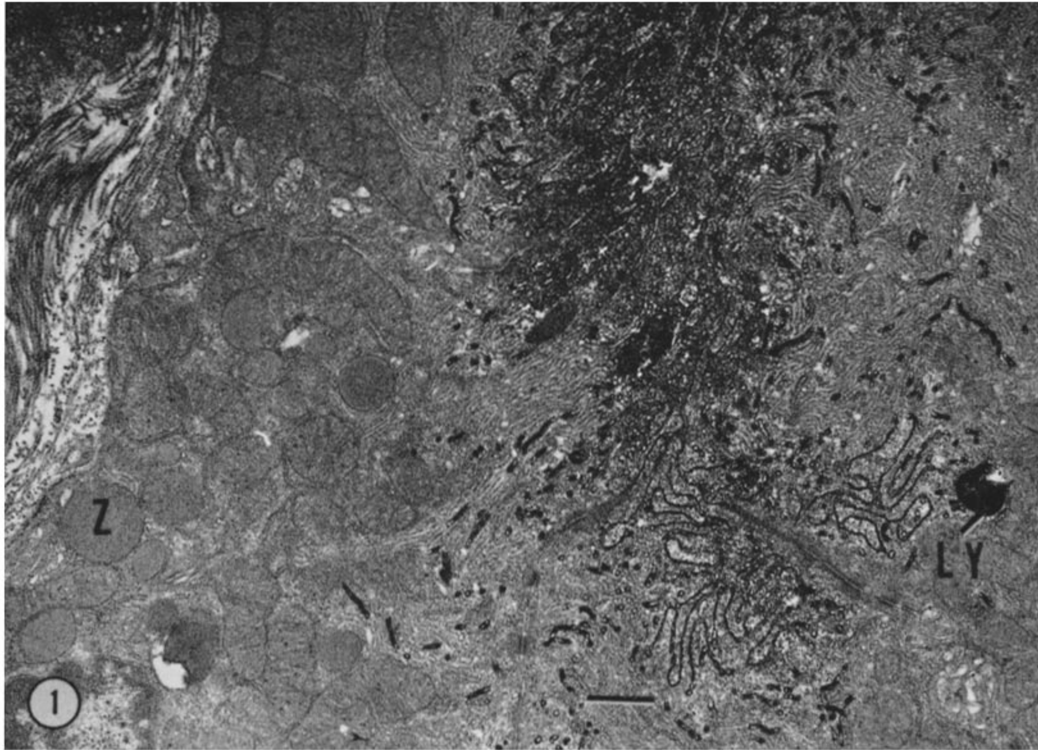

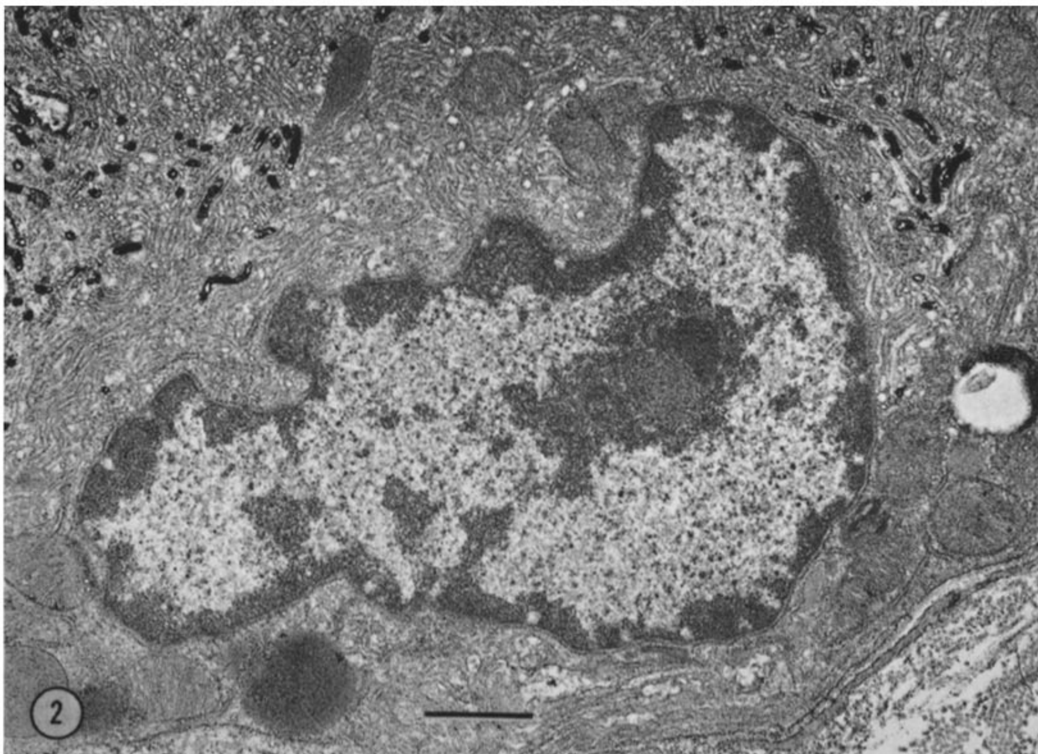

All figures are from frogs exposed to peroxidase solutions placed in the lumen of the stomach for a period of 1 hr. The sections were not stained with lead citrate. Bar length equals 1 μ in all electron micrographs.

FIGURE 1 Portions of oxyntic cells adjacent to the lumen of the gastric gland. Peroxidase reactivity is demonstrated in the lumen and in a number of tubular elements found in the apical cytoplasms. A body (*LY*) interpreted as being a lysosome also stains heavily for peroxidase. Intercellular clefts, mitochondria, and zymogen granules (*Z*) show no reactivity. × 9,000.

FIGURE 2 Part of an oxyntic cell showing peroxidase reaction product within a number of tubules near the lateral surface of a nucleus. × 14,000.

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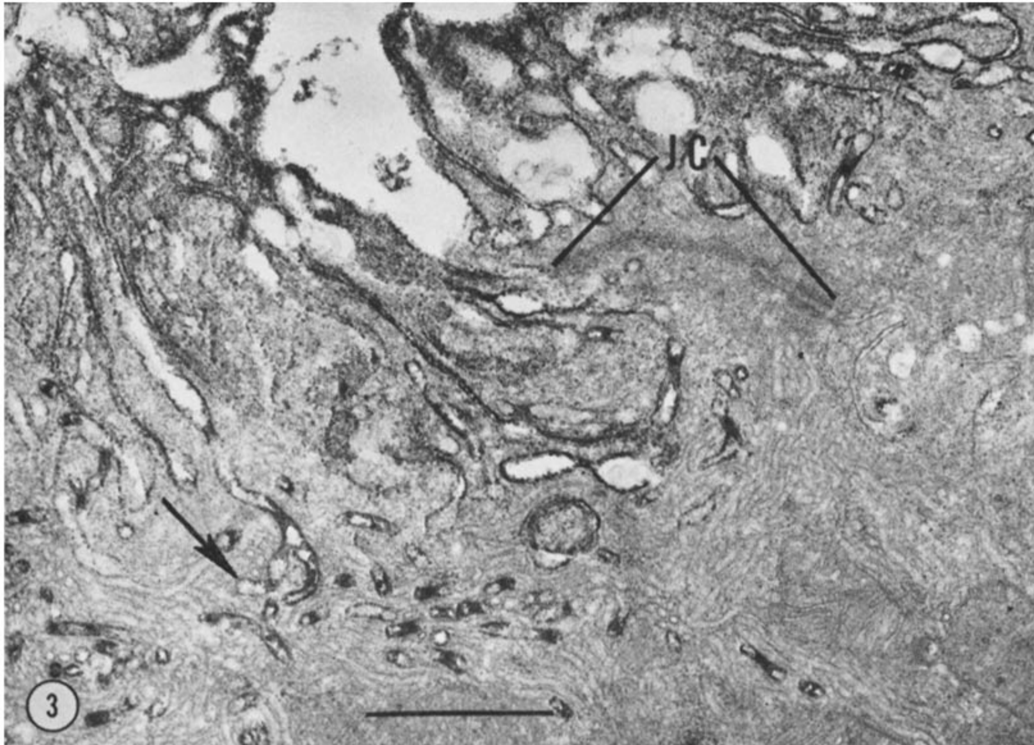

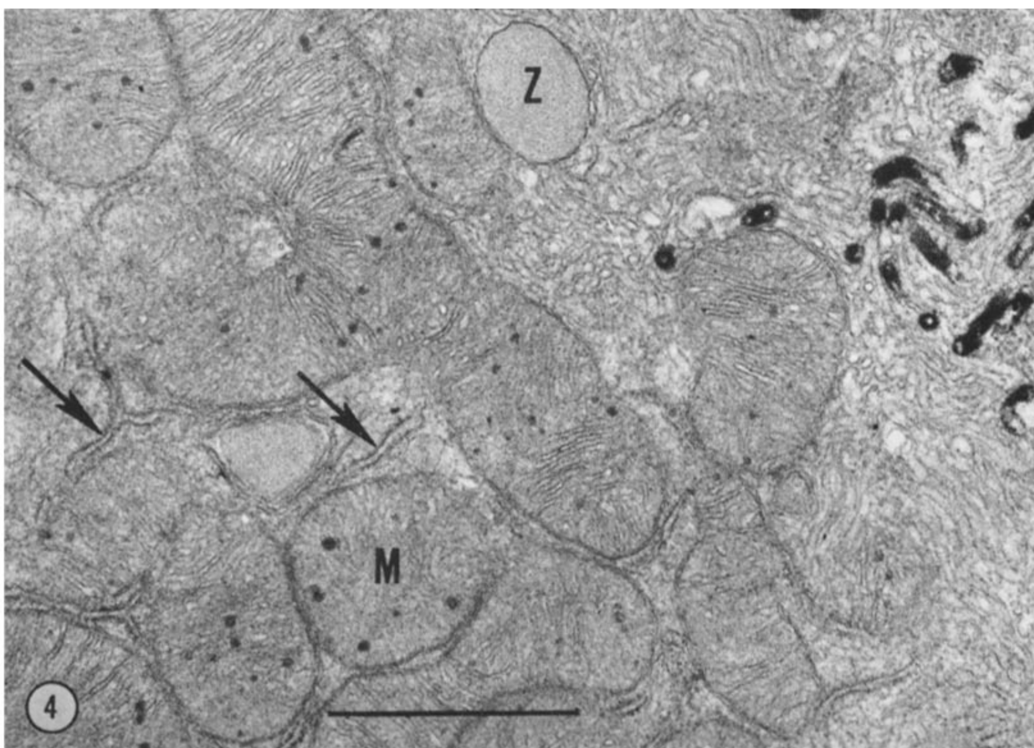

FIGURE 3 Apical portions of two oxyntic cells adjacent to the lumen of a gastric gland. Peroxidase staining is seen on the plasmalemma of the surface plications and within the smooth-surfaced tubules in the cytoplasm. A connection (arrow) between the lumina of the cytoplasmic tubules containing peroxidase and the free surface is indicated. The junctional complex (*JC*) between cells shows no peroxidase reactivity. × 25,000.

FIGURE 4 This micrograph shows the absence of peroxidase reactivity within rough-surfaced profiles of the endoplasmic reticulum (arrows). Mitochondrial profiles (*M*) and zymogen granules (*Z*) are also unreactive. Peroxidase staining is seen within a number of tubular elements. × 33,600.

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peroxidase is known to be a histamine releaser in some species, it is possible that these cells were secreting some hydrochloric acid at the time the tissue was fixed. A number of cells demonstrated elaborate surface plications similar to those reported in experiments where the Q_{HCl} was correlated with the fine structural pattern (16, 20). The fact that a greater proportion of smooth-surfaced tubules did not contain reaction product suggests that the smooth-surfaced tubules are not always in continuity with the gastric luminal surface of the cell. Presumably, under the influence of an appropriate stimulus for hydrochloric acid production, change in membrane distribution is accomplished by a dynamic process involving (*a*) fusion of the luminal plasmalemma and tubular (vesicotubular) membrane followed by (*b*) eversion of the tubular elements to form extensive surface plications or microvilli.

The literature contains evidence and suggestions that in their structure the membranes of the smooth-surfaced tubules found in the cytoplasm of the oxyntic cell resemble the plasmalemma more closely than the membranes of the rough-surfaced endoplasmic reticulum. Lillibridge (10) found in the frog oxyntic cell that the dimension of "unit membrane" composing the vesicotubules was indistinguishable from that of the plasmalemma at the glandular lumen. Moreover, the dimensions of the unit membrane of ribosome-coated endoplasmic reticulum and of mitochondrial membranes were always smaller than those of the plasma membrane of the membranes of vesicotubules. Similar observations have been made on the rat oxyntic cell (19). A polysaccharide coating has been identified on the free surface of the plasmalemma, surface projections, and on the inner surface of the smooth-surfaced tubular (vesicotubular) system of the acid-secreting cell in the stomachs of both the frog and rat (18, 19).

The fusion of tubular elements with plasmalemma and the eversion of these elements to form elaborate surface plications during acid secretion would present a large polysaccharide surface. This polysaccharide surface, in holding a relatively large volume of water, would provide an environment for the ions involved in hydrochloric acid secretion. Whether the polysaccharide facilitates the movement of ions across membranes would depend on such factors as the net charge of the particular ion under consideration and the net charge of the polysaccharide itself.

The significance of the findings that the exogenous peroxidase tracer can enter the smooth-surfaced tubular system of the oxyntic cell is that the apical cell membrane and the limiting membrane of the tubular elements are continuous with each other at times. Therefore the membranes are potentially capable of being translocated from one site to another to provide free-surface membrane for exchange of ions during secretory activity.

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