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Reduction and Accumulation of Cell Surface Membrane and Changes of the Tubulovesicles in Secreting and Non-Secreting Oxyntic Cells in Frog Stomach

(gastric oxyntic cells/tubulovesicles/acid secretion)

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Oxyntic cells of the gastric mucosa in the resting state contain prominent tubulovesicles. Previous electron microscopic studies showed that the number of tubulovesicles was reduced and that the cell surface membrane was increased, in the secreting state.

To clarify the related mechanisms, tannic acid was used for the tracer of the cell surface and the acid secreting and non-acid-secreting oxyntic cells in frog stomach were studied. In the acid-secreting oxyntic cells induced by gastrin, fusion between the tubulovesicles and cell surface membrane was apparent.

After this fusion, the tubulovesicles may become attached to the cell surface membrane, and an increase in the area of the apical free surface would ensue.

Acid secreting cells of the gastric mucosa contain a system of abundant smooth-surfaced membranes, or tubulovesicles within the apical cytoplasm. Tubulovesicles are more prominent in resting cells. During the acid secretion, tubulovesicles decrease in number, and the cell free surface exhibits many elongated and tightly packed microvilli, thereby resulting in an increase in the area of the free cell surface.

Morphological and biochemical studies have been done to determine the mechanism of acid secretion, at the cell level.

It has been suspected that acid-secreting stimulation induces a fusion of tubulovesicles to the apical cell membrane after which contents of the tubulovesicles are discharged into the gastric lumen. The area of the cell surface then expands considerably (1–4).

In this present study, tannic acid was used for the tracer of the cell surface or its invagination into the cytoplasm, and morphological studies were done on the cell surface membrane change during the acid secreting cycle.

MATERIALS AND METHODS

Adult frogs (Rana catesbeiana) were fasted overnight but had free access to water. Acid secreting stimulation was induced 30 minutes after administration of tetragastrin given intraperitoneally (200 mg per animal).

Under ether anesthesia a laparotomy was carried out and small pieces of the mucosa in the ventral wall of the corpus ventriculi were excised and immediately reinsed in ice-cold fixative on a dental wax, cut into 1× 1 ×1 mm pieces and transferred into the fixative solution. The fixative consisted of 4% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4.

Some specimens were fixed in the same fixatives to which 2.5 % tannic acid was added (5, 6). Tissue specimens were all postfixed in cold 1 % osmium tetroxide in the same buffer for 2 hours and were then dehydrated in a graded series of ethanol and propylene oxide and embedded in epoxy resin. The samples were sectioned using a Porter-Blum ultra-microtome and glass knives. Thick sections (1—2 μ) were cut, stained with a 1 % solution of toluidine blue and examined under a light microscope. Thin sections, 500 — 900 Å thick, were cut and stained with lead citrate for 20 min.

These sections were then examined under a HU12A or 100B type electron microscope.

RESULTS

Under Basal Conditions

The apical cell surface of oxyntic cells in the frog stomach was covered by slender microvilli.

Intracellular canaliculi were absent. The microvilli bordering the luminal surface often had a central core that resembled fuzzy filamentous material invested in the intestinal absorptive cell (7). Intercellular canaliculi were well developed between the adjacent cell borders ; and, interdigitations between the neighbouring cells were numerous.

Distinct to the oxyntic cells were the smooth surfaced membrane structures in the cytoplasm. These membrane elements (tubulovesicles) were tightly packed just beneath the apical surface facing the lumen of the gastric gland. An accumulation was also seen along the opposing borders, near the cell membranes of the adjacent cells. Here most elements assumed the form of tubules, although vesicles were also to some extent apparent. These vesicles were usually seen as individual units, and branching or anastomosing profiles were not so common. The diameter of these vesicles ranged in size from 50 nm to 68 nm at the tubular area. In the vesicular areas, the diameter was in the range of 85.0 nm to 158 nm.

The lumen of the tubules and vesicles appeared empty but there was a fuzzy coating on the inner leaflet adjacent to the lumen. The membranes showed a trilamellar structure, that is so-called unit membrane structure, and the thickness was the same as that of the cell membrane (Fig. 1).

In this study, there was no evidence of continuity between the lumen of the tubulovesicles and the cell surface membrane, under basal conditions. In the tracer study, application of tannic acid produced a complete outline of the extracellular regions of the oxyntic cells. Tannic acid could be traced

along the apical surface of the cell membrane and lateral borders between the adjacent cells, but was absent at the site of tight junctions. The tannic acid was confined to the cell membrane and did not penetrate into the tubules or vesicles. Therefore, in the tracer study, the absence of continuity between the lumen of the tubulovesicles and the extracellular space was evident under the basal conditions.

In the resting state, other membrane elements, such as lysosomes and multivesicular bodies were frequently found to be diffusely distributed over the entire cytoplasm.

Tannic acid was absent in lysosomes and multivesicular bodies.

After Tetragastrin-Treatment

Oxyntic cells showed strikingly remarkable changes and a homogeneous appearance 30 minutes after intraperitoneal administration of a high dose of tetragastrin. The prominence of the microvilli and reduction in number or almost complete absence of tubulovesicles in the cytoplasm were characteristic features after stimulation by tetragastrin (Figs. 2—4).

The glandular lumina were frequently occluded by microvilli which were tightly packed and interplicated. Favourable sections exhibited some of the tubular or vesicular elements communicated with the free surface of the cell facing the lumen of a gastric gland.

Oxyntic cells exposed to the tannic acid showed the reactive product in some tubulovesicles near the apical surface as well as in the luminal cell membranes. Some electron micrographs exhibited connections of the tubulovesicles containing the tannic acid and the free surface of the cells between adjacent interplications. The fusion of tubulovesicles connected directly with the apical free surfaces after gastrin stimulation (Figs. 2—4).

Lysosomes were reduced in number in the stimulated cells and the distribution pattern was altered. The lyososmes were found mostly in the apical portion. Multivesicular bodies were not notably affected by tetragastrin treatment in either number or distribution pattern. Lysosomes and multivesicles showed negative tannic acid reactions (Fig. 2).

DISCUSSION

Oxyntic cells are glandular structures composing the tubular gastric gland and these cells secrete hydrochloric acid (8).

The cells is characterized by ;

1. elaborated specialization of the cell surfaces 2. extensive tubulovesicular smooth surfaced structures in the cytoplasm 3. numerous mitochondria

These characteristics make up an elaborate membrane apecialization. The luminal surface membranes are lined with numerous and long microvilli, and the lateral surfaces are adjoined with neighbouring cells with a junctional complex (9). Below the junctional complex, interdigitation of the cell membrane is well developed. Basal surfaces also have interplications of cell mem-

branes that is called basal infolding. These cell surface arrangements are attributed to the enlargement of the area of the cell surface and may be adequate for secretion of hydrochloric acid at the apical surface, and uptake of water and ions at the basal and lateral membranes (10—13). In the cytoplasm, smooth surfaced membrane structures are observed in the acid secreting cells of the gastric gland in all species of vertebrates (14).

There seems to be a relationhsip between the development of tubulovesicles and the area of the apical surface in the oxyntic cells : in the cells provided with such a large surface area, the tubulovesicles are relatively poorly developed, whereas those in the cells with a small surface area well developed. In the latter, tubulovesicles appear to occupy the whole area of the apical cytoplasm. In the oxyntic cells in which the luminal surface is intermediate in area between the former and the latter, tubulovesicles show an intermediary development.

In the relationship between the cell surface and tubulovesicles, the unsolved problem is the communication between the tubulovesicles and cell surface. Tubulovesicles are similar to cell membrane in thickness and structure (2, 15, 16). There are, however different properties, as seen histochemically. The tubulovesicles and surface membranes are partly homogenous in nature (11, 17—19). In the resting state, tubulovesicles have no communication with the free luminal and Tan surface. Ling and Tan reported connections between the membrane structure in the coral fish (20). Leeson found that there was a continuity between membranes of cell surface and tubulovesicles in the specimen rapidly frozen by immersion in liquid nitrogen or Freon 22 before chemical fixation, in glutaraldehyde and osmium tetroxide. The related micrographs did not, however, clearly show the continuity (21).

Tracer studies done in the resting state did not elucidate the problem. Peroxidase increases the secretion of histamine and stimulates the acid secretion by the oxyntic cells. Using frogs and peroxidase as a tracer, Sedar observed that the peroxidase did enter the tubulovesicles (4). However Karpinski *et al.* reported negative findings (22). Using lanthanum, Forte *et al.* observed the communication of cell surface with the tubulovesicles in frog stomach (1, 15). On the other hand, Ito not find sufficient evidence for communication in mouse cells in similar experiments using lanthanum (3).

In this present study, using tannic acid as the cell surface tracer in the resting state, continuity of the cell surface with tubulovesicles could not be demonstrated in the frogs cells. At the acid secreting state, however, the continuity between them was observed. The marked reduction of the tubulovesicles in the cytoplasm, the increase of the microvilli and area of the cell surface were characteristic features of the acid-secreting cells (1, 3, 23). At a higher magnification, the fusion of tubular elements with the cell surface was observed. Using tannic acid, the connection between the tubulovesicles and the cell surface was evident in the secreting cells. These findings were much the same as reported by Sedar who used peroxidase as the tracer (4).

Thus, under the influence of an appropriate stimulus for hydrochloric acid

production, membrane translocation of fusion and eversion of tubulovesicle membrane can be demonstrated. This phenomenon suggests that the cell surface membrane increases by exteriorization of tubulovesicles by a process of membrane fusion and eversion.

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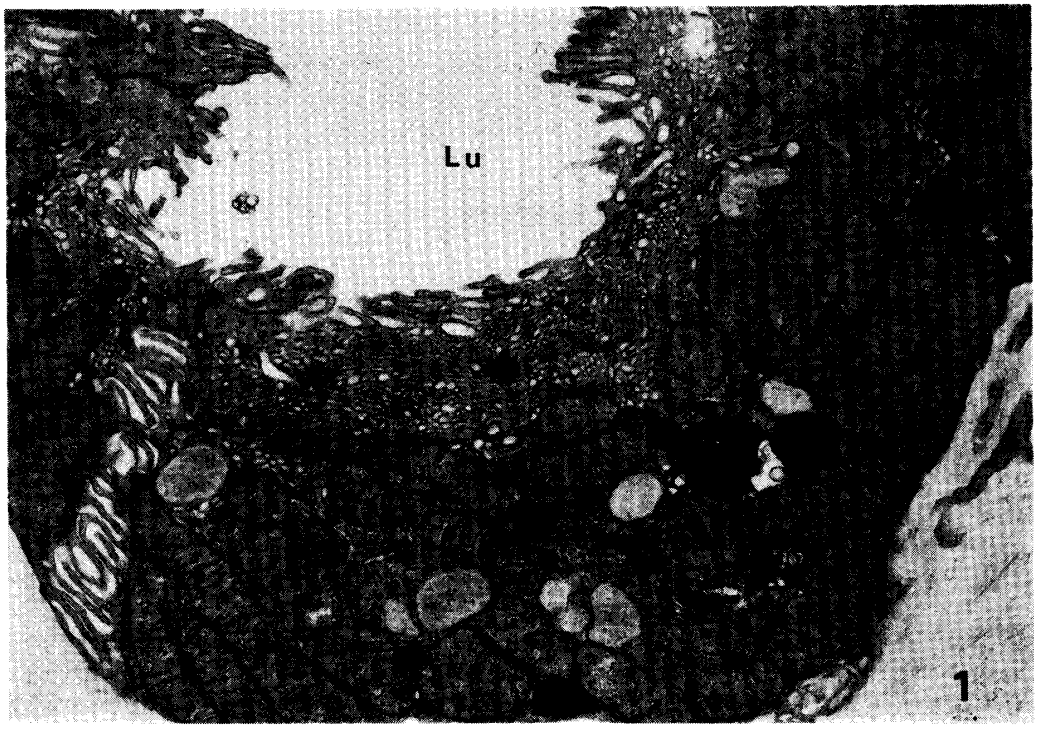
LEGENDS

Fig. 1. An electron micrograph of an oxyntic cell adjacent to the glandular lumen (Lu) from a stomach of a healthy fasting frog. The free surface of the cell is lined with microvilli.
An extensive tubulovesicular membrane (TV) accumulation in the upper portion of the cell.
In this field some vesicular profiles show a continuity with tubular profiles.
Large and numerous mitochondria are packed in the lower half the cell, and contain the dense mitochondorial granules and tightly packed cristae.
Lysosomes (Ly) and junctional complex (JC) are also observed. ×10,000.

Fig. 2. Tannic acid-stained section of the gastric gland during the acid secretion induced by administration of tetragastrin.
The luminal space is completely occluded by elongated and tightly packed microvilli. Tubulovesicles (TV) are remarkedly reduced in the cytoplasm. Some tubulovesicles as well as the surface membrane are dense due to the penetration and presence of the dense tracer. A probable continuity between the tubulovesicles and free surface membrane is illustrated. Note the lack of dense tracer in the multivesicular bodies (MB) and lysosomes. ×23,000.

Fig. 3. Tannic acid-stained section of the apical portion of an oxyntic cell during the acid secretion. Tannic acid staining is demonstrated on interdigitations of the lateral cell surfaces. Connection between the tubulovesicles and the free surface are seen easily. ×25,000.

Fig. 4. An enlarged view of an area showing continuity of the tubulovesicles with the cell surface membrane. ×55,000.


Lu
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