Histol Histopathol (1997) 12: 739-754

Histology and Histopathology

Invited Review

Gastric oxyntic cell structure as related to secretory activity

T. Ogata
Department of Surgery, Kochi Medical School, Nankoku, Kochi, Japan

Summary. The oxyntic, or parietal cell has two characteristic membrane systems. The mammalian intracellular canaliculi are specialized networks of narrow channels lined with numerous microvilli. The other common to all oxyntic cells is the tubulovesicles, a system of tubules and vesicles. The tubulovesicular compartment is drastically depleted during maximal gastric acid secretion and this is coincident with an increase in the cell surface membrane area. A plausible explanation of this process is the fusion and transfer of tubulovesicular membranes to the plasma membrane. However, for many years there was no convincing evidence of the connections between these two membrane systems. How the tubulovesicular membranes transform into plasma membrane without demonstrable connections has been an enigma to electron microscopists. Recent ultra-high resolution scanning electron microscopic observations on the rat oxyntic cell treated with aldehyde-osmium-aldehyde method revealed that in the resting stage, the tubulovesicles were isolated spherical vesicles. But after tetragastrin stimulation, they were interconnected by slender connecting tubules forming a tubulovesicular network. Then this network was fused to the intracellular canaliculus at relatively few points. These connections between the tubulovesicles and luminal surface membrane was also demonstrated in the frog oxynticopeptic cells. In this review, these membrane transformations as well as changes of the H^+/K^+-ATPase, the lectin binding glycocalyx and the cytoskeleton during secretion will be illustrated and discussed.

Key words: Oxyntic cell, Parietal cell, Membrane system, Stomach, Scanning electron microscopy

Introduction

The most prominent cell in the gastric mucosa is the oxyntic or parietal cell that produces the hydrochloric acid in the gastric juice. Oxyntic, which refers to acid, is more appropriate than the parietal, but is used less commonly. The parietal cell was so named because the large cell often bulges outward from the gastric glands and is in parietal position, and this is commonly used to identify the mammalian oxyntic cell.

The oxyntic cell has two distinctive, characteristic membrane systems. One is the specialized luminal plasma membrane that is the presumptive site of gastric acid secretion and is lined with numerous microvilli or with cell folds which are also known as lingulae. The other feature is the extensive cytoplasmic tubulovesicles or tubulovesicular membrane system, the most likely reservoir of luminal membrane. In the resting oxyntic cell cytoplasm, there are many tubulovesicles, but few profiles of the intracellular canaliculi (Fig. 1).

Previous reports have shown that this tubulovesicular compartment was greatly depleted during gastric acid secretion and that this change was coincident with an increase in the cell surface membrane area (Fig. 2) (Helander and Hirshowitz, 1972; Ito and Schofield, 1974; Forte et al., 1977; Schofield et al., 1979; Vial et al., 1985; Ito, 1987). Stereological measurements indicated at least a fourfold increase in microvillar surface, as well as a 50% increase in lateral and basal cell membrane in mice (Ito and Schofield, 1974). Concomitantly, there was a 90% reduction in the tubulovesicular membranes of the cytoplasm. Other workers have found even greater increases in the microvillar membrane surface of secreting parietal cells (Helander and Hirschowitz, 1972, 1974; Zalewsky and Moody, 1977). The active mechanism underlying the virtual disappearance of the cytoplasmic membrane compartment and the rapid increase in surface membranes has, heretofore, not been unequivocally demonstrated.

In this review, the morphological changes of cell organelles, especially membrane systems during secretion, in mammalian and amphibian oxyntic cells, which have been extensively studied by many authors, are described and discussed. Recent scanning electron microscopy (SEM) evidence indicating continuity between the tubulovesicles and the plasma membrane published from our laboratory (Ogata, 1992; Ogata and Yamasaki, 1993, 1996; Ogata et al., 1995) will also be

Offprint request to: Prof. Takuro Ogata, Department of Surgery, Kochi Medical School, Nankoku, Kochi, 783, Japan

included.

Current hypotheses about oxyntic cell membrane redistribution at the onset of acid secretion

There are two hypotheses to explain oxyntic cell membrane redistribution when going from the inactive to secreting phase: membrane recruitment-and-recycling, and osmotic swelling (Forte et al., 1981).

1) The membrane-recruitment-recycling hypothesis

The membrane-recruitment-and-recycling hypothesis postulates that expansion of the apical surface occurs by recruitment from a pool of cytoplasmic H^+/K^+-ATPase-rich membranes to the apical plasma membrane (Forte et al., 1977; Forte and Yao, 1996).

The following is supporting evidence of the membrane recycling hypothesis. 1) Forte and Forte (1970) showed that the frog cytoplasmic tubular system and the apical plasma membrane share the same glycoproteins and chemical characteristics. 2) Bulk-phase markers placed in the luminal solution do not enter the tubulovesicles of fixed, resting cells; however, they do penetrate the tubulovesicles that reform when stimulated cells are brought back to rest (Sedar, 1969). 3) A study on toad gastric mucosa showed that lumenally applied [^{14}C]-inulin and horseradish peroxidase were incorporated into tubulovesicular membranes when secreting oxyntic cells were returned to the resting state. The markers were then released back into the lumen upon restimulation (Jiron, 1984). 4) Freeze-fracture replicas of resting oxyntic cells of piglet gastric mucosa have shown that membrane-associated particles (MAPs) for tubulovesicles were distinctly different from apical plasma membrane (Black et al. 1980). On the other hand, in stimulated cells the distribution of MAPs on the apical membrane was similar to that seen for tubulovesicles. This transitional substructure of the membranes is interpreted to be initial dilution of the apical plasma membrane by the massive recruitment of tubulovesicular membrane.

For this hypothesis the demonstration of the direct continuity between the membrane of tubulovesicles and that of intracellular canaliculus is essential. However, electron microscopy since the early 1950s, has resulted in no reports clearly demonstrating connections between these two membrane systems. Sugai et al. (1985) examined the resting mouse parietal cell by rapid-freeze-fixation/freeze-substitution technique. In their observations, most of the tubulovesicular system was composed of relatively equal diameter tubules, slightly thinner than microvilli (Fig. 3). Individual tubular components 50 to 70 nm in diameter were curved, branched, or tortuous and had few varicosities or swellings. The blunt ends were frequently arranged perpendicular to the plasmalemma of intracellular canaliculi and appeared as close as 10 nm apart, but continuities with the surface membrane were never observed (Fig. 3).

2. The osmotic swelling hypothesis

The osmotic swelling hypothesis proposes that the H^+/K^+-ATPase-rich membranes are always in physical continuity with the apical plasma membrane (Berglindh et al., 1980); thus, no process of fusion is required; rather, it envisages an expansion and extension of the highly involuted membrane system by using osmotic forces.

When there is no secretion or volume flow in the resting state, the tubulovesicular compartments are believed to remain in a supercollapsed state and open only when there is net ionic and volume flow into the intratubulovesicular space. Gibert and Hersey (1982) designed experiments of promote salt and water accumulation within tubulovesicles; morphological responses were confined to large vacuole swellings, but no apical surface increase occurred. Thus, experimental increase in luminal membrane area and decrease in the tubulovesicular compartment did not occur. Osmotic expansion, *per se*, did not lead to communication between tubulovesicles and the intracellular canaliculus. Although this hypothesis has interesting features, convincing experimental evidence is still lacking.

Fig. 1. TEM of a resting parietal cell of the rat. Transversely cut profiles of the intracellular canaliculi (I) and numerous tubular and round tubulovesicles (T) are seen in the cytoplasm. x 9,000

Fig. 2. A parietal cell 15 minutes after gastrin stimulation. Apical microvilli are extensive and greatly elongated, whereas cytoplasmic tubulovesicles (T) are drastically reduced. I: intracellular canaliculi. x 5,000

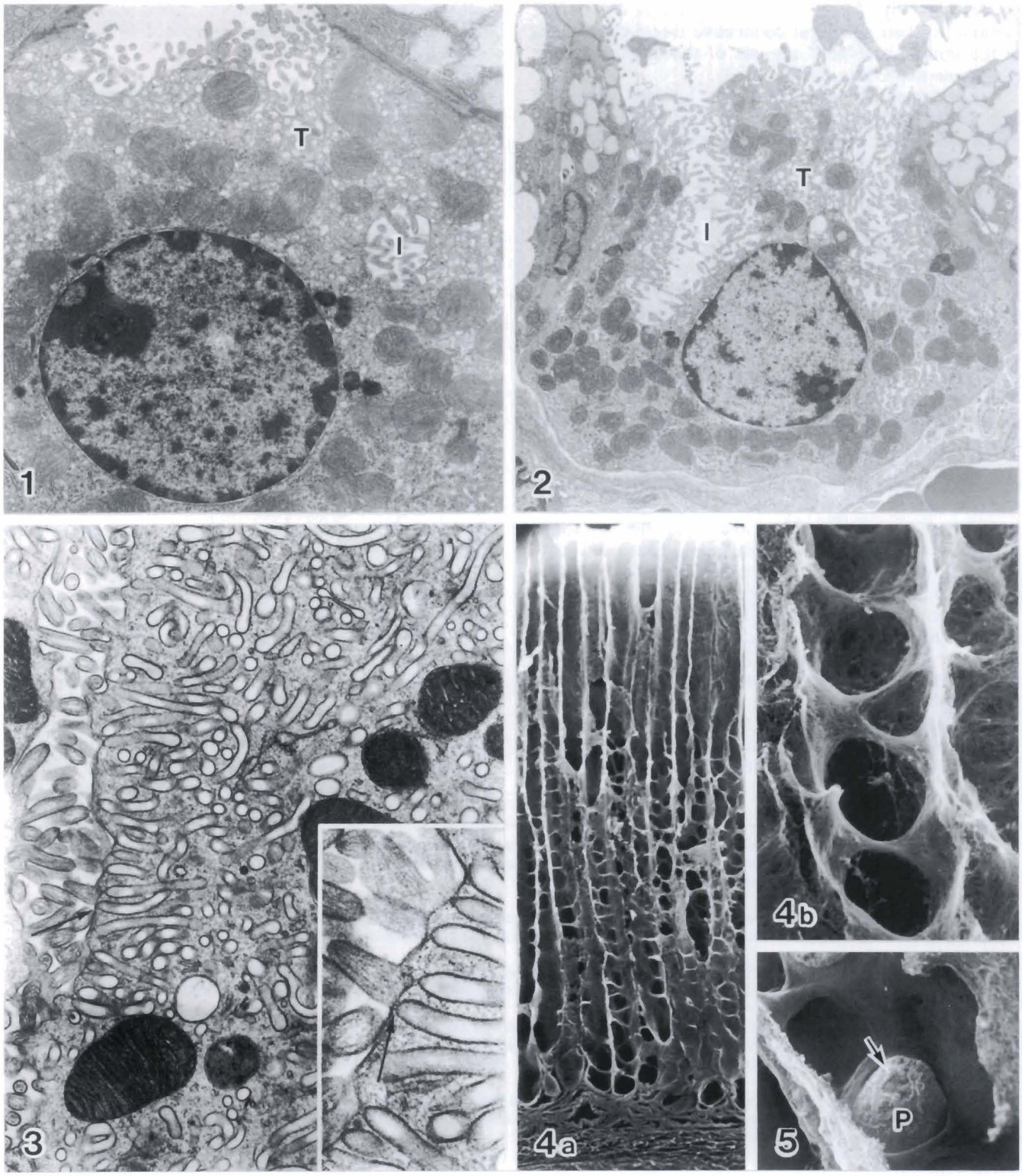
Fig. 3. A parietal cell of the fasted mouse fixed by a rapid freeze and substitution method. The tubular membrane profiles appear to be a collection of many tubules with rounded, closed ends. Note that some tubules are branched, and some tubule ends are adjacent to the intracellular canaliculi (arrow). x 20,000; Inset, x 42,000. (From Sugai et al., 1985. J. Electr. Microsc., Vol. 34, reproduced with permission).

Fig. 4. SEM images of the fundic glands of the rat stomach treated with NaOH. All gland cells are removed with NaOH. **a.** Low magnification. The surface of the subbasal lamina fibrous sheet of the upper one third of the gland is smooth, whereas that of the lower two thirds has numerous hemispherical concavities. x 170. **b.** The fibrous sheet of the deep gland. Deep hemispherical concavities, presumably occupied by a parietal cell, are seen. x 1,000

Fig. 5. Fundic glands subjected to ultrasonic vibration. The exposed basal lamina forms deep hemispherical concavities. A parietal cell (P) still present in a concavity. Arrow shows orifices of the intracellular canaliculi. x 1,600

Mammalian oxyntic (parietal) cell

Mammalian oxyntic cell or parietal cells are large oval or pyramidal cells measuring up to 25 μm in diameter with their bases bulging into the lamina propria of the gland (Ito, 1987). In this section, the term



"parietal cell" is used instead of the "oxyntic cell", because the former is in more common use for the mammalian acid secreting cell. SEM observation of the subepithelial tissue of the rat stomach treated by cell-maceration with a low temperature NaOH solution (Fig. 4) or by prolonged osmification followed by ultra-sonication (Fig. 5) revealed that parietal cells were harbored in hemispherical connective tissue concavities in the middle and basal regions of the oxyntic glands (Sugimoto and Ogata, 1989).

In the mammalian parietal cells, luminal membrane invaginations into the cell body are lined with numerous microvilli and are designated "intracellular canaliculi" or sometimes "secretory canaliculi". The abundant cytoplasmic membranes of parietal cells in the non-secreting stomach are the tubulovesicular membranes. These have also been described as the agranular endoplasmic reticulum, vacuoles, vesicles, vesico-tubules, or bulbotubules. During the early period of gastric mucosal electron microscopy, there were questions regarding the actual morphology of this system. Is it vesicular or is it tubular? This seems to depend on the method of specimen fixation or preparation. Vial and Orrego (1960) showed that with osmium tetroxide fixation, the image of the membrane system was critically dependent on the concentration of a solute-like sucrose to which the cell membrane is relatively impermeable. Recently Sugai et al. (1995) showed that when the stomach tissue was fixed with a fixative solution higher than 290 mOsm, the tubulo-vesicles were tubular. They were similar to mouse parietal cells prepared by rapid freeze fixation/freeze-substituion method, which resulted in mainly tubular tubulovesicles (Fig. 3) (Sugai et al., 1985).

Recently, Pettitt et al. (1995) showed that the cytoplasm of the unstimulated parietal cell of the rat prepared by rapid freeze fixation/freeze-substituion method contained numerous and densely packed helical coil of tubule, each having an axial core of cytoplasm. The helical coils are linked together by connecting tubules, which are relatively straight. They surmised that these straight connecting tubules also extended from coils adjacent to the apical and canalicular surfaces and ended at the apical canaliculus membranes, although they did not demonstrate the continuity between two membrane systems. Sugai et al. (1985) observed some twisting or bending of tubulovesicles in mice parietal cells prepared by rapid freeze-fixation/freeze-substitution method, but similar coiled structures were not found. Further studies are needed to clarify these discrepancies.

Scanning electron microscopic observation on stimulated parietal cell

Parietal cell structure in the early stage of secretion

Osawa and Ogata (1978) observed the fractured surface of the rat oxyntic cells with SEM and showed the changes of three-dimensional images of intracellular canaliculi and tubulovesicles after tetragastrin stimulation. However, they could not observe the cytoplasmic membrane obstructed by the cell matrix and cytoskeleton.

Tanaka and Mitsushima (1984) introduced the aldehyde-osmium-DMSO-osmium (A-ODO) method, which allowed visualization of the intracellular membranes by the SEM removing the cytoplasmic matrices. Using this method, Ogata (1992), Ogata and Yamasaki (1993) and Ogata et al. (1995) reported the changes of the membrane systems of the rat parietal cells induced by tetragastrin stimulation observed by an ultra-high-resolution Hitachi S-900 SEM with a resolving power of 0. 7 nm.

In SEM micrographs of resting parietal cells, the intracellular canaliculi were lined with numerous slender microvilli (Fig. 6). From the cytoplasmic side, the canaliculi appeared as an arborized system of cactus-like structures with numerous 100 nm holes, representing the macerated bases of microvilli. Numerous vesicles, 100-200 nm in diameter, were found in the cytoplasm (Figs. 6, 7). The spherical nature of tubulovesicles may be due to the initial weak fixative required by the A-ODO procedure. Stronger fixation does not permit complete cytoplasmic maceration. Most tubulovesicles were

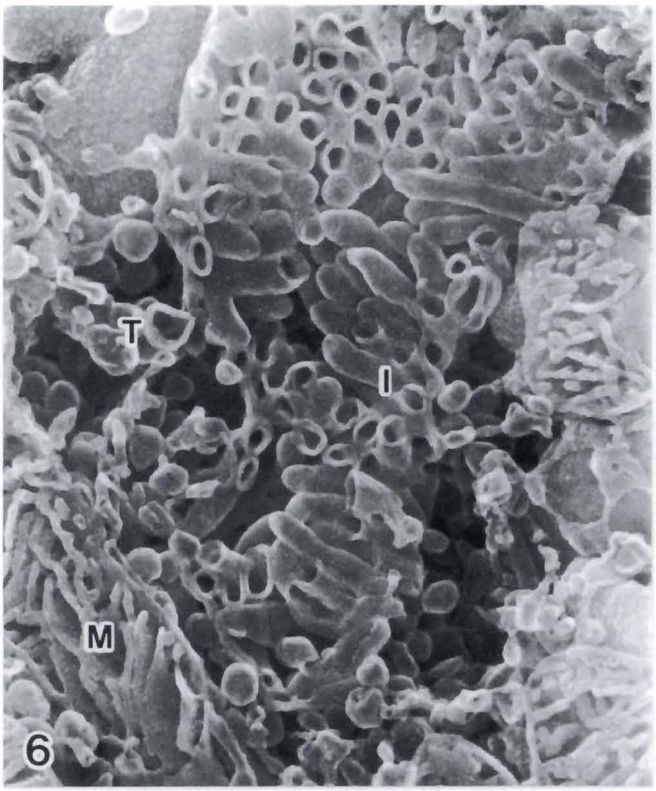
Fig. 6. SEM image of macerated specimens of the rat parietal cell. A resting parietal cell. The fractured intracellular canaliculus (I) is lined with numerous slender microvilli (M). Spherical tubulovesicles (T) are seen around the intracellular canaliculi. x 35,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).

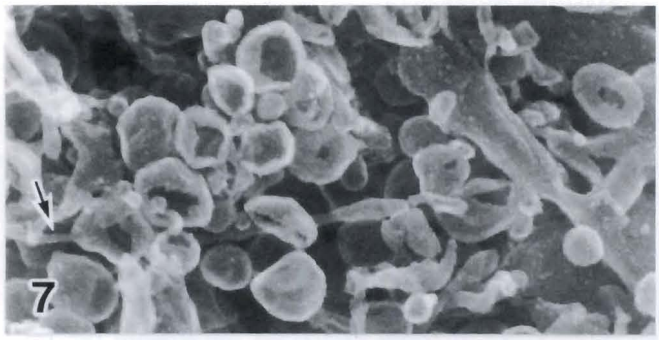
Fig. 7. SEM image of macerated specimens of the rat parietal cell. SEM images of tubulovesicles in a resting parietal cell. Numerous spherical isolated tubulovesicles (arrow) accumulate in the cytoplasm. x 45,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).

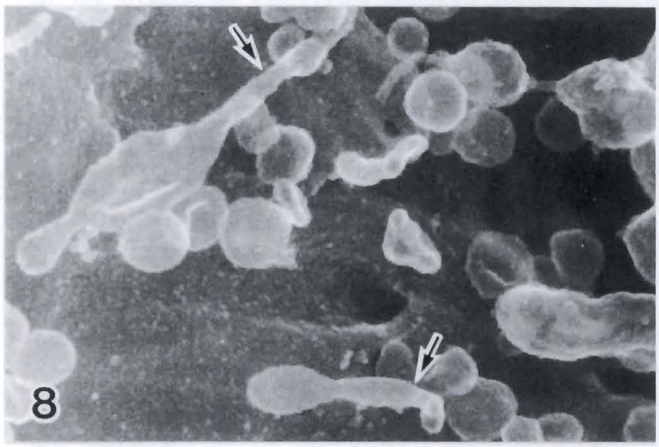
Fig. 8. SEM image of macerated specimens of the rat parietal cell. Tubulovesicles 7 minutes after gastrin stimulation. A blunt protrusion (arrows) extends from a tubulovesicle. x 54,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).

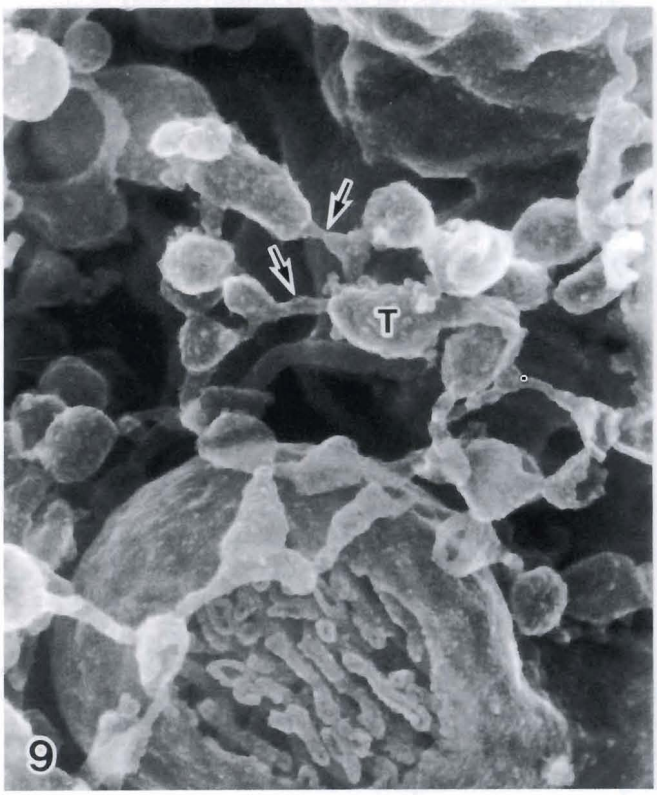
Fig. 9. SEM image of macerated specimens of the rat parietal cell. A parietal cell 15 minutes after gastrin stimulation. The tubulovesicles (T) are connected to each other by slender connecting tubules (arrows) to form a tubulovesicular network. Most tubulovesicles are spherical, but some are irregularly shaped. x 80,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).

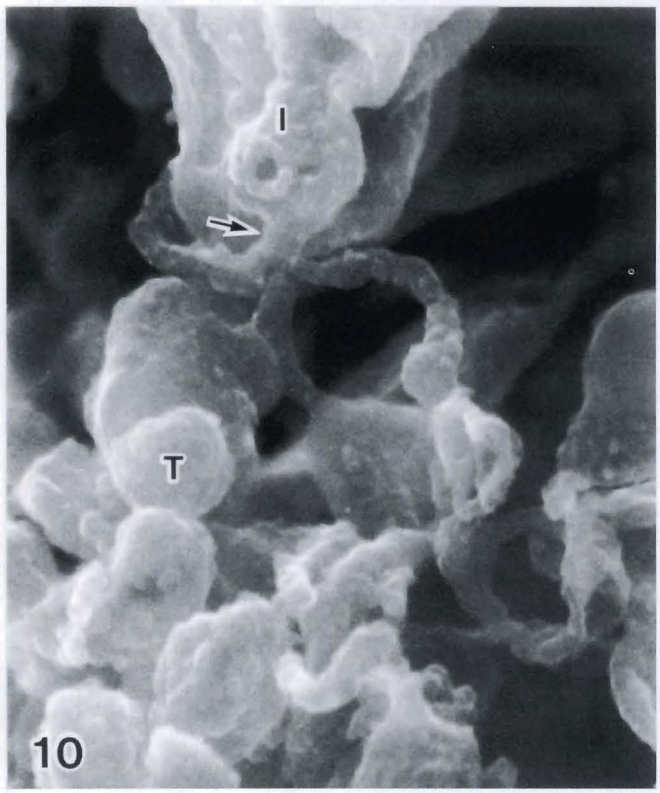
Fig. 10. SEM image of macerated specimens of the rat parietal cell. The tubulovesicular (T) network membrane is continuous with the intracellular canalicular (I) membrane (arrow). Note that tubulovesicles have become closer to each other. x 150,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).


T
I
M
6


7


8


T
9


I
T
10

isolated structures but some were connected by slender 30 nm tubules. These have been designated as connecting tubules. Some blunt protrusions were observed on the cytoplasmic side of the tubulovesicles in both resting and stimulated parietal cells, but they were more abundant in stimulated cells (Fig. 8). The number of interconnected tubulovesicles increased after gastrin stimulation and formed extensive tubulovesicular networks (Fig. 9). Using the TEM, Lawn (1960) reported that in addition to 200 nm vacuoles, a system of small 20 nm tubules ran in many directions throughout the rat parietal cell. These tubules occasionally joined vesicles and tubules. However, interconnections between two vacuoles were not shown. In our SEM observations, the formation of the tubulovesicular network is clearly evident after gastrin stimulation (Ogata, 1992; Ogata and Yamasaki, 1993).

After gastrin stimulation, the relatively rare sites of continuity of the tubulovesicular network with the intracellular canalicular membrane were observed (Fig. 10). These connections were visualized more distinctly in stereo SEM micrographs (Fig. 11). These connections only occurred at rare sites along extensive canalicular areas. The small connecting tubules were only about 30 nm in diameter and were frequently curved as they joined the two systems. The usual thickness of thin sections were much thicker than 30 nm. Therefore it is highly improbable to observe these connections in thin sections. This is probably the reason why TEM studies have failed to demonstrate connections between the intracellular canaliculus and the tubulovesicular membrane.

Biochemical studies have revealed that the apical cell membrane of the parietal cell contains H^+/K^+-ATPase, which is associated with HCl secretion (Forte et al., 1975; Sachs, 1977, 1987). Forte and Wolosin (1987) described that after secretagogue stimulation, KCl moves rapidly from the cell to canalicular lumen and that the H^+/K^+-ATPase recycles K^+ back into the cell in exchange for H^+, thus forming HCl. The exact nature and localization of K^+ and Cl^- pathways are, however, unknown. In SEM micrographs of parietal cells 15 minutes after gastrin stimulation, the tubulovesicles are of various sizes and shapes, some are markedly swollen and others shrunken or flattened (Ogata and Yamasaki, 1993). In addition, they are packed closer. It seems, therefore, reasonable to assume that upon gastrin stimulation the tubulovesicular content, probably K^+ and Cl^-, is transferred through the connecting tubules from one tubulovesicle to another and is secreted into the canalicular lumen, resulting in the exchange between K^+ and H^+ by H^+/K^+-ATPase. However, further studies are necessary to validate the precise sequence of events involved in HCl secretion.

After gastrin stimulation, the microvilli on the luminal surface of the intracellular canaliculi near the site of the intracellular canaliculus-tubulovesicular network connection are relatively scarce. Microvilli gradually increase in number and size with increased distance from this site (Ogata and Yamasaki, 1993). On the cytoplasmic side, the round holes of the intracellular canaliculi indicating the base of the microvilli are also fewer and smaller near the connection site (Fig. 12). When these observations are analyzed, they suggest that this part of the membrane is formed by fusion of the tubulovesicles to the intracellular canaliculus and that the transposed membrane becomes the microvillar membrane.

A summary of our analysis of the rat parietal cells in early stages after stimulation is as follows: 1) in the resting state, most tubulovesicles are isolated. After gastrin stimulation, the tubulovesicles become interconnected by slender tubules to form a tubulovesicular network; 2) the tubulovesicular network joins the intracellular canaliculus by one or a few slender connecting tubules; 3) after the connection is established, the tubulovesicles aggregate and diminish; 4) the increase in the canalicular membrane area and the depletion of tubulovesicles is most likely explained by the transfer of the tubulovesicular membrane to the intracellular canaliculus. The newly incorporated membrane thus becomes the membrane of microvilli. These morphological changes of the membrane system of the parietal cell after gastrin stimulation are schematically represented in Figure 13.

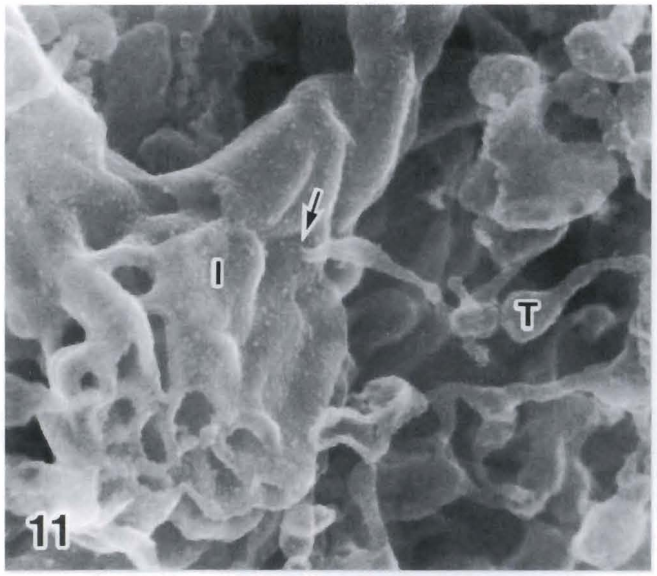
Return of secreting parietal cells to the resting stage

When active secretion ceases, parietal cells revert back to the nonsecreting stage and restore their tubulovesicular compartment. This process is poorly understood. Schofield et al. (1979) reported that after intense stimulation and secretory activity, a variety of vesicular elements, such as flattened vesicles, concentric membrane profiles, coated vesicles, and multivesicular bodies, might be involved in the movement of

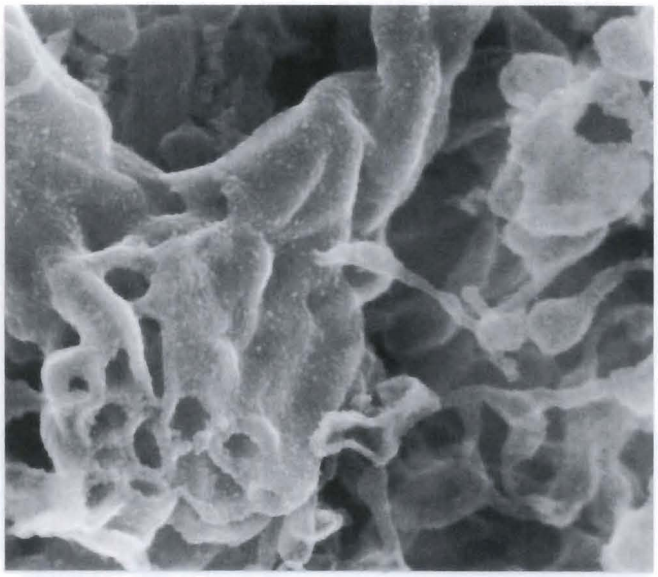
Fig. 11. SEM image of macerated specimens of the rat parietal cell. Stereo SEM view of a parietal cell 15 minutes after gastrin stimulation. A tubulovesicle network (T) connects to the fractured intracellular canaliculus (I) with a slender connecting tubule (arrow). x 67,000. (From Ogata et al., 1995, J. Anat. Embryol. Vol.100, reproduced with permission)

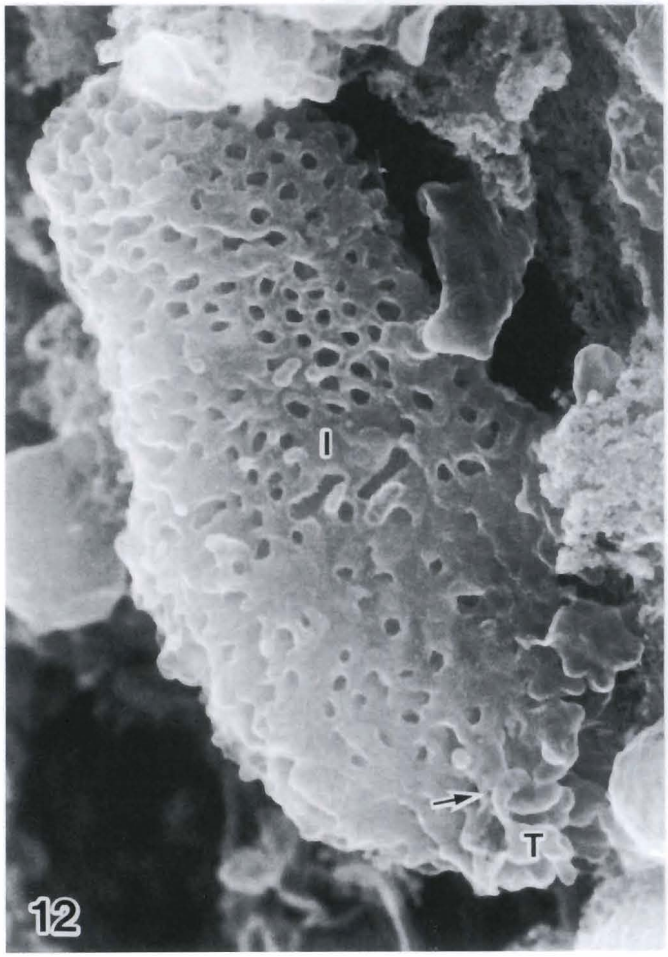
Fig. 12. SEM image of macerated specimens of the rat parietal cell. Cytoplasmic view of the intracellular canaliculus (I) in a parietal cell 7 minutes after gastrin stimulation. The round holes corresponding to the basal openings of the microvilli are less numerous and smaller in size at the lower part of the figure, where a tubulovesicle (arrow) is connected. x 31,000. (From Ogata and Yamasaki, 1993, Anat. Rec., Vol. 237, reproduced with permission).

Fig. 13. Schematic representation of the author's interpretation of the morphological changes of the parietal cell membranes in the early stage of secretion after gastric stimulation. I: intracellular canaliculus; T: tubulovesicle.

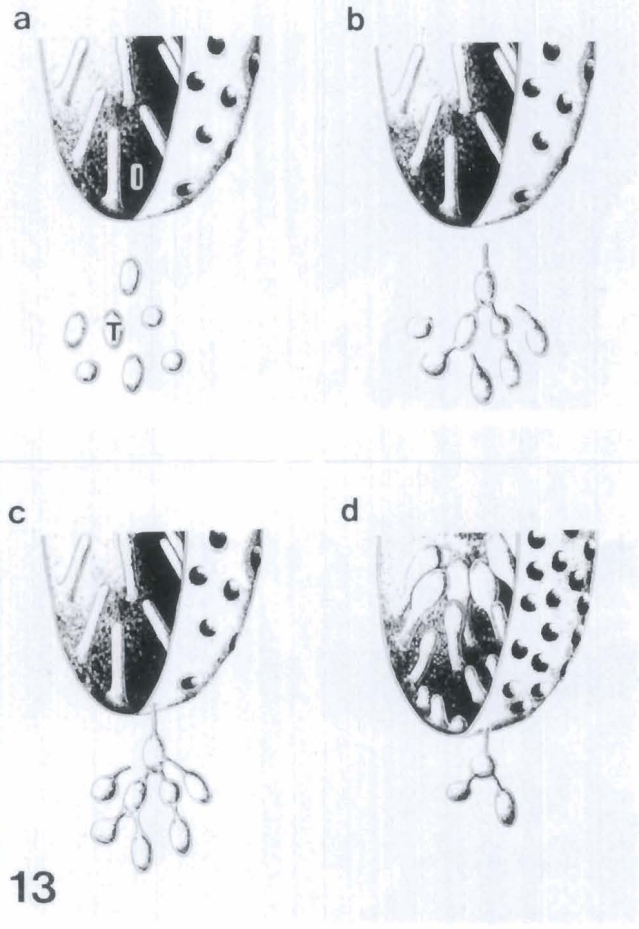


11





12



13

microvillar membrane to the tubulovesicular system in the mouse parietal cell. In dog parietal cells after stimulation with histamine, Helander and Hirshowitz (1972) observed the apparent invagination of microvilli and the merging of their membranes to form a five-layer structure after acid secretion had ceased. Studies with piglet stomachs by Forte et al. (1977) revealed that microvillar membranes folded upon each other and formed pentalaminar structures, which were then endocytosed. After the endocytic uptake of these pentalaminar structures, the membrane components were believed to be recycled back into the tubulovesicular membranes. Our SEM observations on gastrin-stimulated rats reveal that in the early phase of the return to resting state, observed 20-30 minutes after stimulation, microvilli are swollen, and sometimes branched (Ogata and Yamasaki, 1993). Close approximation of adjacent microvilli is also seen. In the late phase return to the resting state, observed 60 minutes after the stimulation, the number of microvilli decrease and the wide areas of the intracellular canaliculus have no microvilli (Fig. 14a). From the cytoplasmic side of the intracellular canaliculus, the number of the holes, corresponding the basal openings of the microvilli, also decreases and there are large protuberances of the membrane without holes which protrude into the cytoplasm (Fig. 14b). Swellings of membrane without openings of the microvilli cores frequently protrude into the cytoplasmic side (Fig. 14b,c). Some are joined to the intracellular canaliculus by slender stalks (Fig. 14c). These observations strongly suggest that the membrane of the intracellular canaliculi returns to the tubulovesicles by a process resembling endocytotic uptake. The process of membrane transfer in parietal cells reverting to the resting state is complex and unclear. We are making a

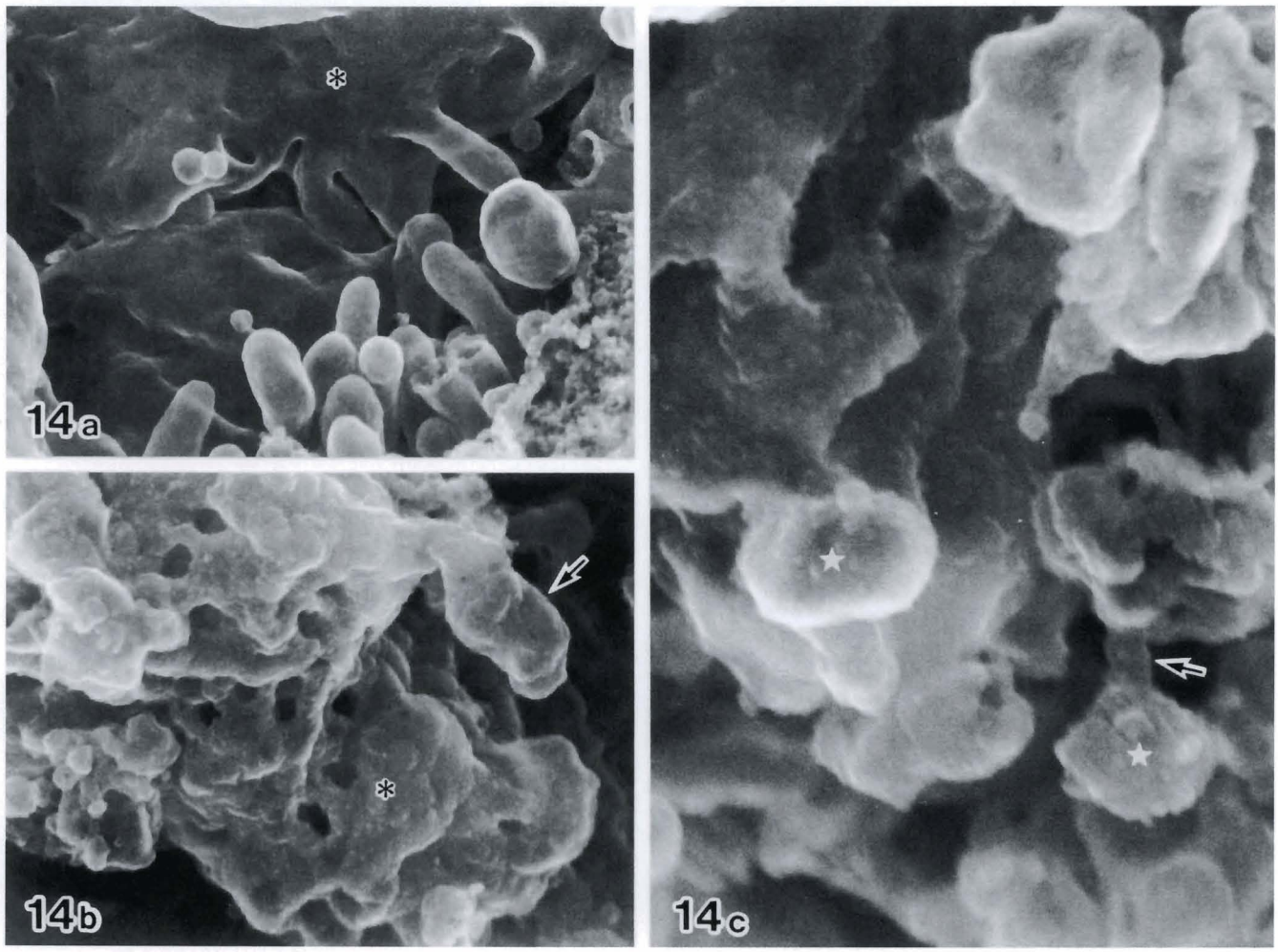


Fig. 14. SEM image of macerated specimens of the rat parietal cell. A parietal cell 60 minutes after gastrin stimulation. **a.** Luminal view of the intracellular canaliculus. A wide area without microvilli (*) is seen. x 50,000. **b**. Cytoplasmic side of the intracellular canaliculus. Area without the holes (*), indicating absence of microvilli. An arrow shows protrusion of the membrane. x 67,000. **c.** Membrane protrusions (stars) are seen. One is connected by a small stalk (arrow). x 175,000

more detailed analysis of this process.

H^+/K^+-ATPase localization in parietal cells

H^+/K^+-ATPase, which exchanges H^+ and K^+ ions, is closely associated with gastric HCl secretion. Immunocytochemical localization of H^+/K^+-ATPase in the parietal cell by several investigators (Smolka et al., 1983; Hanzel et al., 1991; Pettitt et al., 1995) showed that H^+/K^+-ATPase was heavily stained in tubulovesicular as well as luminal membranes.

In earlier studies Rubin and Aliasgharpour (1976) demonstrated adenosine triphosphatase (ATPase) activity by the method of Wachstein-Meisl and p-nitrophenyl-phosphatse (NPPase) of Ernst in rat and human gastric mucosa. The ATPase reaction was localized on the plasmalemma and mitochondrial cristae and the NPPase reaction was restricted to the plasmalemma. Kobayashi and Seguchi (1990) used a cytochemical cerium salt method for H^+/K^+-ATPase and observed a positive reaction product exclusive to microvilli, but not on the tubulovesicular membranes. In a more recent study, Ando et al. (1995) compared the staining of resting rat parietal cells with H^+/K^+-ATPase by immunocytochemical and cerium salt cytochemical methods. Immunocytochemical staining showed that monoclonal antibodies against α- and ß-subunits of the H^+/K^+-ATPase were positive on both the luminal and tubulovesicular membranes (Fig. 15), but the cerium salt method showed that H^+/K^+-ATPase was positive only on the luminal membrane while the tubulovesicular membrane was negative (Fig. 16). These results suggest that the H^+/K^+-ATPase exists in both membranes of the tubulovesicles and the microvilli. However, H^+/K^+-ATPase in the tubulovesicles may be in a masked form. After transposition to the luminal membrane, the H^+/K^+-ATPase may be unmasked or activated by some unknown process. The lectin binding studies described below also indicate similar differences.

Carbonic anhydrase localization in parietal cells

Carbonic anhydrase catalyzes the hydration of carbon dioxide to form carbonic acid as well as the reverse reaction. This zinc-containing enzyme is particularly abundant in red blood cell and in the gastric mucosa. Using Hansson's carbonic anhydrase technique, Sugai and Ito (1980) studied the cytochemical localization of carbonic anhydrase in mouse parietal cells. By this technique this enzyme appears to be in the microvillar cores and on the cytoplasmic surface of the plasma membrane and is not consistently associated with the tubulovesicular system.

Lectin binding sites in parietal cells

Lectins are a group of carbohydrate-binding proteins which have selective capacities to bind specific polysaccarides or glycoproteins. The presence of glycoconjugates in parietal cell membranes has been studied numerous investigators (Kuhlman and Peschke, 1984; Albedi et al., 1985; Kessimian et al., 1986; Okamoto and Forte, 1988; Ito et al. 1989; Callaghan et al., 1990; Ihida et al., 1990). Kessimian et al. (1986) examined 12 different biotinylated lectin receptors on parietal cells from human fundic mucosa. Parietal cells reacted strongly with *Bandieraea simplicifolia*, *Dolichos biflorus*, peanut agglutinin, and soybean agglutinin (all specific for galactosyl/galactosaminyl groups) and weakly with *Ulex europaeus* (specific for fucose). The membranes of the intracellular canaliculi were characterized by an abundance of galactosyl residues, a paucity of fucosyl groups, and lack of mannosyl and glucosyl residues. However, galactosyl residues were absent on the membrane of the tubulovesicles.

Ito et al. (1989) cytochemically studied the *Dolichos biflorus* agglutinin (DBA), which specifically binds to N-acetyl-D-galactosamine, in human parietal cells. In the resting state, DBA-positive reaction was positive on the membrane of the intracellular canaliculi and of the Golgi apparatus, but negative for the tubulovesicular membranes. After stimulation with tetragastrin or betazol hydrochloride, the intracellular canaliculi expanded and tubulovesicles decreased, but the staining pattern was the same as in the resting state.

Ihida et al. (1990) studied the development of rat fundic glands using *Griffonia simplicifolia* agglutinin-IB_4 (GSA-IB_4). In the adult rats, GSA-IB_4 specifically labeled the intracellular canaliculi, whereas the sites of the cells were very weakly labeled. At 18 days of gestation, a GSA-IB_4-positive reaction was found along the entire cell surface of fundic gland cells and immature intracellular canaliculi of immature parietal cells. One day after birth, the positive reaction was restricted to the parietal cells. Madrid et al. (1990) studied the glycoconjugate distribution of the human fundic mucosa at the ultrastructural level by means of direct and indirect lectin techniques. The surface of the intracellular canaliculi of matured parietal cells were strongly labeled by *Helix pomatia* agglutinin (HPA) and wheat germ agglutinin (WGA) while Concanavalin A (ConA) showed a weak reactivity. In the neck region of the gastric glands, immature parietal cells exhibiting mucous granules were observed. These granules were reactive to HPA, WGA and ConA. The tubulovesicular system of the immature parietal cells was labeled by HPA and occasionally by WGA. ConA also labeled the nucleus.

Naoki et al. (1995) stained adult rat parietal cells with GSA-IB_4 after gastrin or omeprazole administration. In the resting state, the reaction was positive in the intracellular canaliculus but negative in the tubulovesicles (Fig. 17). After stimulation the extent of the intracellular canaliculi increased and the tubulovesicles decreased, but the reaction pattern was the same as in the resting parietal cell (Fig. 18). After omeprazole administration, the membrane compartments reverted to the resting configuration, but GSA-IB_4 was positive on the intracellular canaliculi, and negative in the tubulovesicles. Okamoto and Forte (1988)

determined distribution of lectin-binding sites in isolated rabbit gastric glands with 7 fluoresceinated lectins. Intracellular canaliculi were labeled by wheat germ, *Helix pomatia*, and peanut lectins, suggesting a predominace of N-acetylhexosamines. Tubulovesicules were heavily stained by wheat germ, *Helix pomatia*, and *Ricinus communis* I lectins, indicative of N-acetyhexosamine- and galactose-containg glycoconjugates. It is interesting that the reaction pattern of the lectins differs between the tubulovesicles and the intracellular canaliculi. These differences may be related to the difference of the cytochemical reaction for H^+/K^+-ATPase between these two membrane systems (Kobayashi and Seguchi, 1990; Naoki et al., 1995). However, further studies are necessary to confirm the significance of these differences.

Changes of the cytoskeleton after stimulation

The cytoskeletal organelles, especially actin microfilaments and actin-binding proteins of the parietal cells play an important role in cellular alterations during the process of gastric acid secretion (Urushidani et al., 1987; Hanzel et al.,1989; Mercier et al., 1989; Mizuno et al., 1989a,b). Black et al. (1982) have suggested a correlation between the disorganization of parietal cell microfilaments by cytochalasin B, as a microfilament disrupting agent, and the inhibition of acid secretion.

Vial and Garrido (1976) have demonstrated a change in the orientation of actin microfilaments during acid secretion. Forte et al. (1977) have drawn attention to the presence of microtubules just beneath the secretory surface of parietal cells. They have also demonstrated cyclical changes in the microfilament orientation that parallel the changes in microvilli and tubulovesicular elements with secretion.

Yao et al. (1995) examined the actin isoforms in gastric parietal cells. They showed that α- and γ-actin isoforms were differentially distributed in parietal cells. Furthermore, their data suggest a preferential, but not exclusive, interaction between ß-actin and ezrin in parietal cell. In addition they suggest that the ß- and γ-actin-based cytoskeleton network might function separately in response to stimulation of acid secretion.

Identification of heavy meromyosin (HMM) by the method of Ishikawa et al. (1969) is suitable for localizing the course of actin filaments in parietal cells. Actin filaments decorated with HMM are present beneath the cell membrane, in the cytoplasm around the secretory canaliculi (Fig. 19) (Namikawa et al. 1995). In parietal cells 15 minutes after gastric simulation, the microvilli are swollen and many tubulovesicules are in close association with the intracellular canaliculi. Parallel arrays of the actin filaments in the microvilli remain, but actin filaments around the intracellular canaliculi are markedly reduced (Namikawa et al. 1995). Considered together the accumulated data not surprisingly suggest that the parietal cell cytoskeletons play a role in the change in cell configurations which coincide with the process of acid secretion.

Actin binding proteins

Several types of actin binding proteins play an important role for structural changes of parietal cells during secretion (Mercier et al., 1989; Mizuno et al., 1989a,b; Smith et al., 1993). Mizuno et al. (1989a) immunocytochemically examined fodrin (spectrin) and ankyrin in rat parietal cell after stimulation with gastrin or inhibition with famotidine. These two proteins were distributed along the basolateral cell membrane. Dense fodrin accumulation was also seen around the intracellular canaliculi of resting cells, but became sparser in stimulated cells. Neither fodrin nor ankyrin was found in or around the tubulovesicles.

Immunocytochemical localization of α-actinin in resting and stimulated parietal cell of the rat was studied by Mizuno et al. (1989b). Labeling for α-actinin was found adjacent to the zonula adherentes around the secretory canaliculi, in the microvillus, and along the basolateral infoldings. Scarce labeling was seen around tubulovesicles. After stimulation, localization of α-actinin was not altered.

Ezrin is a minor component with an apparent molecular

Fig. 15. A resting parietal cell immunocytochemically stained with 1H9, a monoclonal antibody against α-subunit of H^+/K^+-ATPase. Gold particles are seen on the membrane of intracellular canaliculus as well as on the tubulovesicles. x 40,000. (Courtesy of T. Ando.)

Fig. 16. A resting parietal cell stained with cerium method for H^+/K^+-ATPase. The reaction is positive on the membrane of intracellular canaliculus but negative in the tubulovesicles. x 35,000. (Courtesy of T. Ando.)

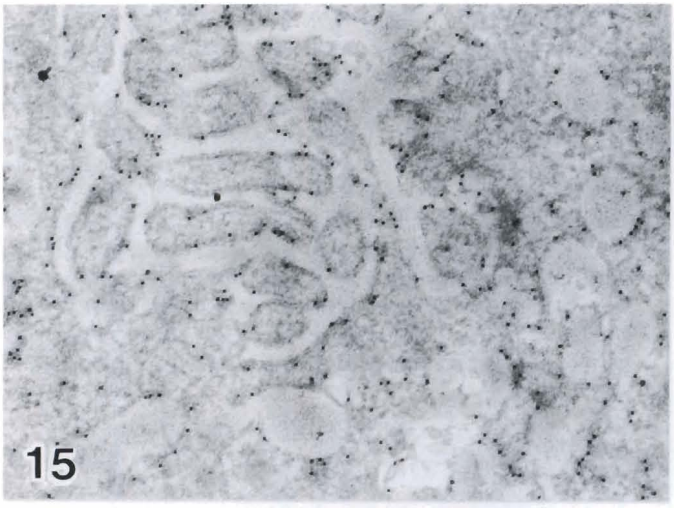
Fig. 17. A resting parietal cell stained with a lectin, GSA-IB_4. The reaction is positive on the membrane of the intracellular canaliculi, but negative on the tubulovesicles. x 40,000. (Courtesy of I. Naoki.)

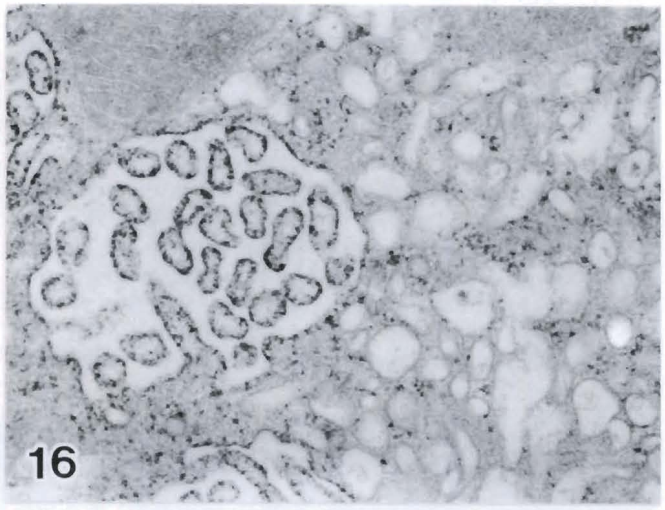
Fig. 18. A parietal cell stained with GSA-IB_4 15 minutes after gastrin stimulation. The number of tubulovesicles is diminished. The reaction pattern is the same as in the resting parietal cell. x 40,000. (Courtesy of I. Naoki.)

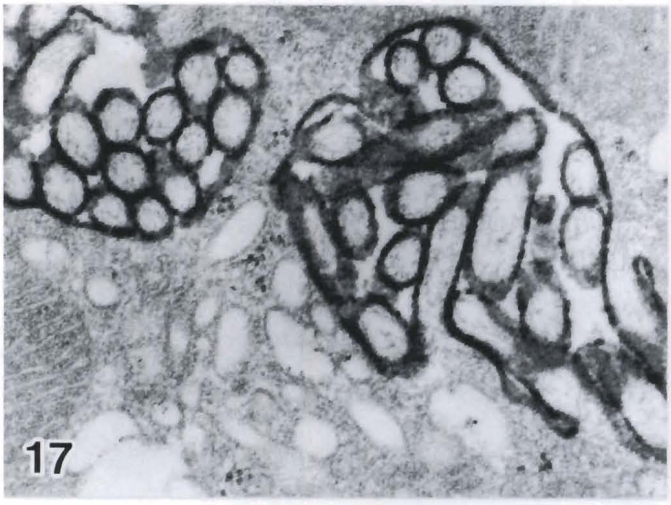
Fig. 19. A resting parietal cell decorated HMM. Actin filaments are parallel in the microvilli, then radiate into the cytoplasm around the intracellular canaliculus. A few filaments are seen around the tubulovesicles. x 30,000. (Courtesy of T. Namikawa.)

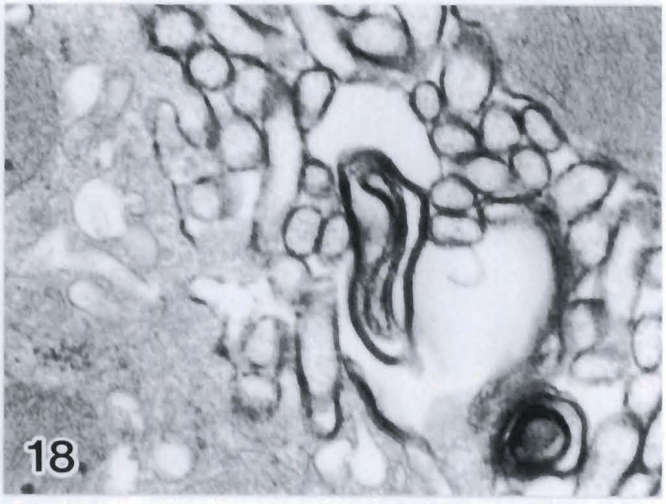
Fig. 20. Immuncytochemical staining of ezrin in a resting parietal cell. Note the gold particles only localized on the membrane of the microvilli and absent on the tubulovesicles. x 50,000. (Courtesy of T. Namikawa.)

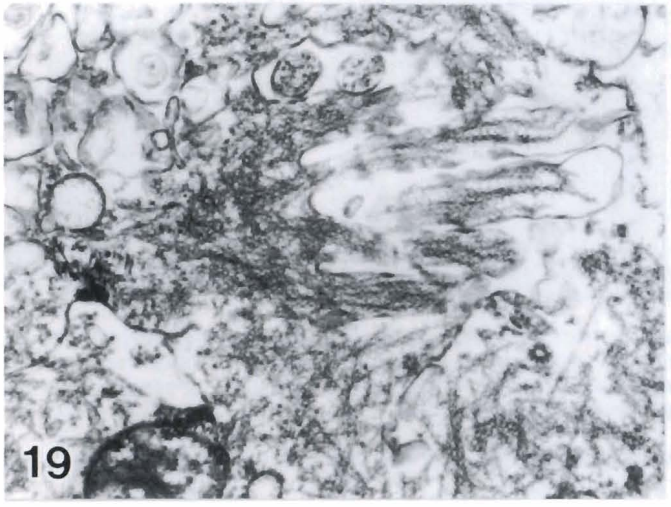
mass of 80 kDa which was purified from among the proteins of the microvillar cytoskeleton of chicken intestinal epithelial cells (Bretscher, 1986). Urushidani et al. (1989) have characterized an 80 kDa phosphoprotein which was mediated by cAMP-dependent protein kinase in the gastric parietal cell. Later, the homology of this protein was demonstrated with intestinal ezrin by the biochemical, physical and immunological properties of

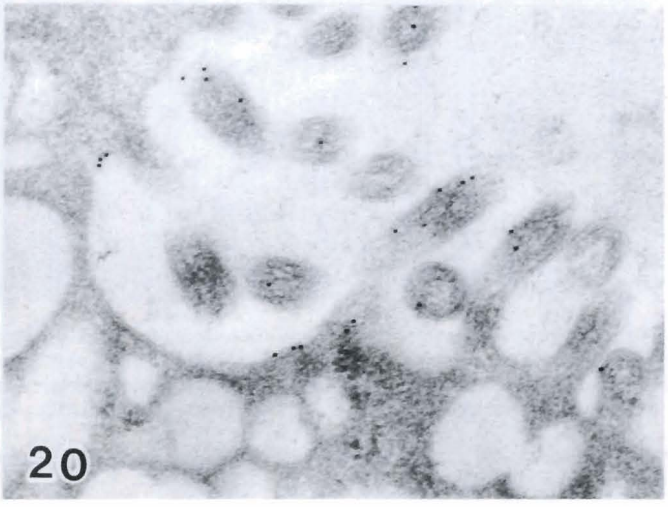












the gastric protein (Hanzel et al., 1991). Immunohistological colocalization of ezrin and actin by light microscopy suggests that ezrin is an apical membrane protein of the parietal cell (Hanzel et al., 1989). In the resting parietal cell immunoelectron microscopy of ezrin showed their localization on the microvillar membranes of the secretory canaliculi and to a lesser extent in basolateral membrane infoldings. It was absent in the tubulovesicules (Fig. 20) (Hanzel et al., 1991). In stimulated parietal cell, ezrin antibody reaction was positive on the microvillar plasma membrane in the expanded secretory canaliculi (Hanzel et al., 1991). The localization of actin and ezrin in the secretory canaliculi of parietal cell suggests that ezrin is a cytoskeletal protein which links the actin cytoskeleton to the plasma membrane of the microvilli (Algrain et al., 1993; Yao et al., 1993). Furthermore, it is postulated that this protein may play a key role in the translocation of the tubulovesicular membrane containing H^+/K^+-ATPase to the luminal membrane.

Amphibian oxynticopeptic cell

Nonmammalian vertebrates do not have separate cell types secreting acid and digestive enzymes (Ito, 1987). Instead, a single cell type is found; the oxynticopeptic cell, which secretes both hydrochloric acid and pepsinogen. Frog and toad oxynticopeptic cells have been extensively studied because they are well suited for experimental studies (Vial and Orrego, 1960; Sedar, 1961a,b, 1965, 1969; Lillibridge, 1968; Forte and Forte, 1971; Carlisle et al., 1978; Logsdon and Machen, 1982). The luminal surface of the amphibian oxynticopeptic cells are highly convoluted with apical projections or folds and some microvilli. The cytoplasm is packed with tubules of 20-60 nm in diameter and a few 200-500 nm vesicles. Typical intracellular or secretory canaliculi are not present in oxynticopeptic cells. During acid secretion

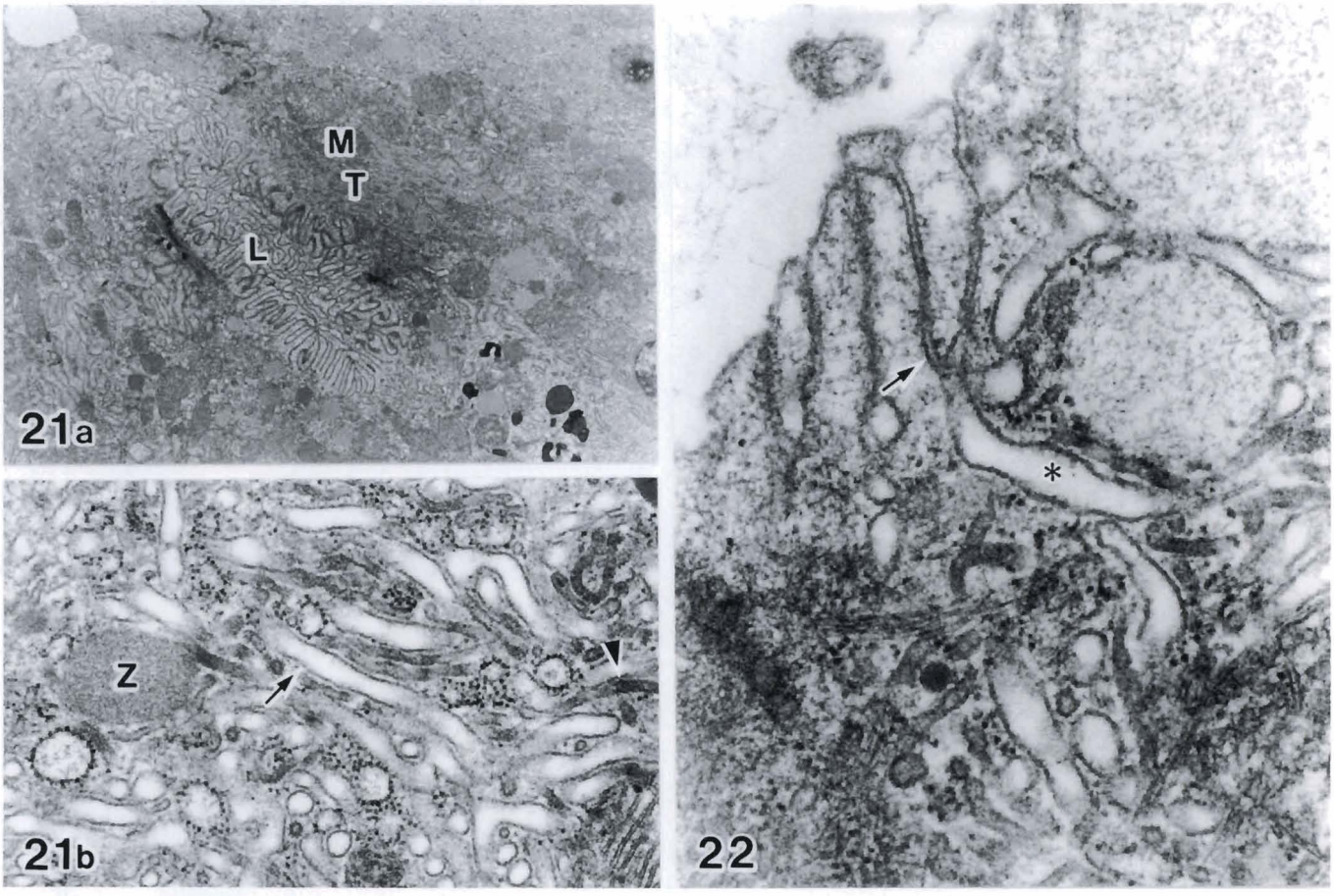


Fig 21. Electron micrographs of oxynticopeptic cells of the Japanese meadow frog. TEM of the resting oxynticopeptic cells. **a.** The lumen of the gland is occluded with many closely packed lingulae. Numerous tubulovesicles (T), predominantly tubular in shape, fill the cytoplasm. L: lumen; M: mitochondrion. x 4,000. **b.** Higher magnification of tubulovesicles. There are small diameter tubules which appear dense (arrowhead), and larger tubules with lucent lumens (arrow). Z: zymogen granule x 27,000. (From Ogata and Yamasaki, 1996, Anat. Rec., Vol. 245 reproduced with permisson).

Fig 22. Oxyntic cell 15 minutes after histamine stimulation. The tubulovesicular membrane, with a clear lumen (*) is continuous with the lingular membrane (arrow). x 50,000

the luminal surface membrane area increases with a simultaneous decrease in the tubulovesicular components as in mammalian parietal cells. As in the case of the mammalian parietal cell, there was no convincing morphological evidence for the continuity between the two membrane systems.

Ogata and Yamasaki (1996) observed the changes of membrane system of oxynticopeptic (oxyntic) cells of the Japanese meadow frog after histamine stimulation. In TEM micrographs of the resting oxyntic cell, the lumen of the gastric gland was lined by packed surface folds or lingulae which were narrow and usually occluded (Fig. 21a). These surface folds were about 100-200 nm thick and had closely apposed membranes leaving little extracellular space. The cytoplasm, contained numerous tubulovesicles, which were predominantly tubular in

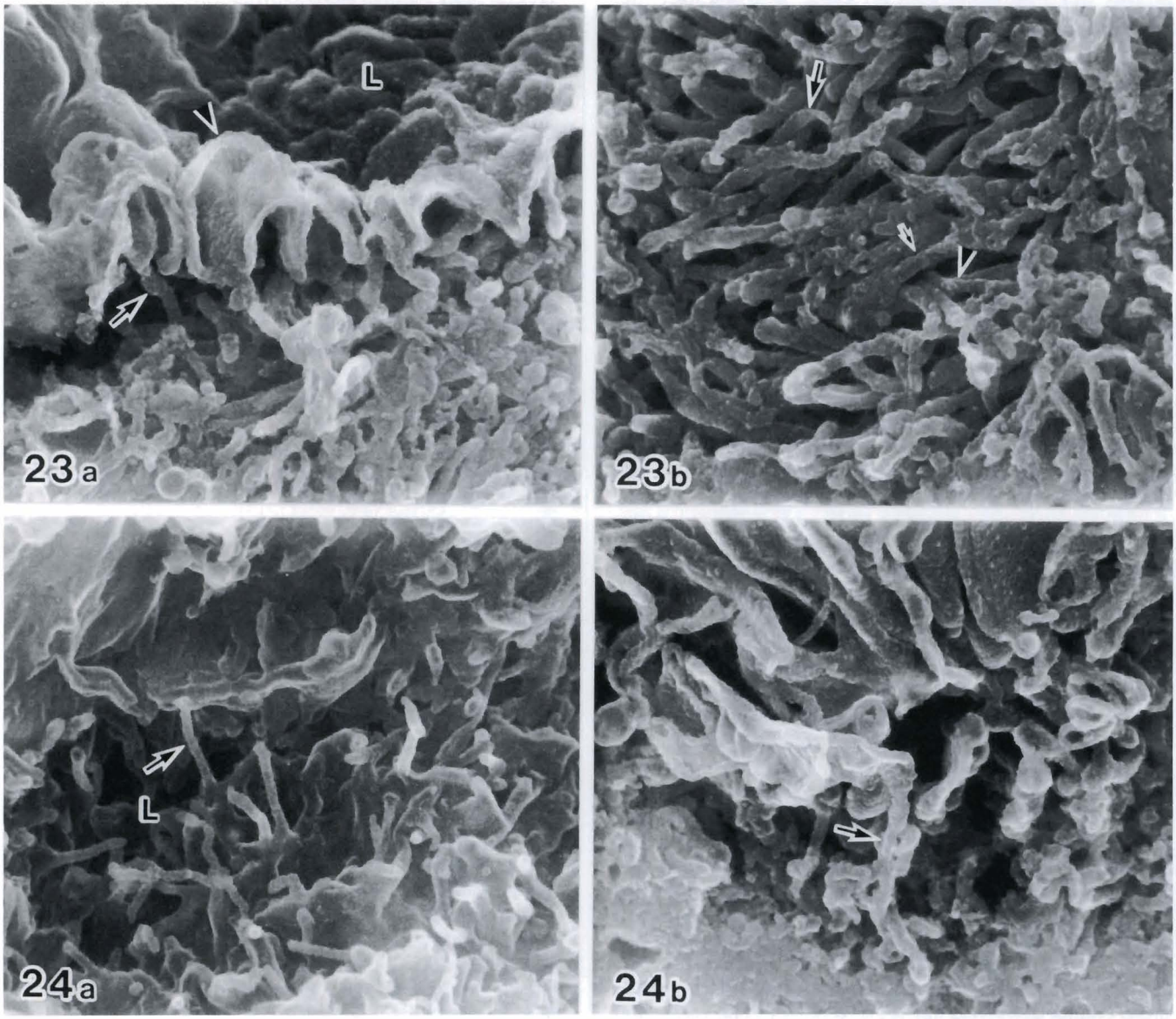


Fig. 23. Electron micrographs of oxynticopeptic cells of the Japanese meadow frog. SEM images of resting oxyntic cells. **a.** Luminal membrane is tightly ruffled with a few microvilli (arrowhead). The tubulovesicles are almost entirely tubular but a few of them are vesicular. A connection between a tubulovesicular membrane and luminal membrane is seen (arrow). L: lumen. x 38,000. **b.** Most of the tubular tubulovesicles of this area are connected to form a network. Some are large (small arrow), and others are thinner (arrowhead). Branching tubules are occasionally seen (large arrow). L: lumen. x 50,000. (From Ogata and Yamasaki, 1996, Anat. Rec., Vol. 245 reproduced with permission).

Fig. 24. An oxyntic cell 15 minutes after histamine stimulation. **a.** Luminal surface of the intracellular canaliculus. The surface is more ruffled than the resting cell and many slender microvilli (arrow) project into the lumen. x 17,000. **b.** The tubulovesicular network is connected to the convoluted luminal membrane (arrow). x 34,000

form. However, some were bulbs or vesicules. Continuity between tubules and vesicles was seen only occasionally in thin sections. Some of the tubules were about 30 nm in diameter and appeared darker than the larger 60 nm tubules with clear lumens (Fig. 21b). Mitochondria and zymogen granules were scattered in the cytoplasm (Fig. 21).

Logsdon and Machen (1982) examined the cryo-fractured surface of the oxynticopeptic cells of the resting and stimulated frog by SEM. Resting cells had apical surfaces which were relatively smooth with some short protruding microvilli. The apical cytoplasm was filled with smooth membrane tubulovesicles. After stimulation, the luminal surface was formed by flattened microplicae or lingulae and a decrease in number of tubulovesicles. Recently Ogata and Yamasaki (1996) examined macerated A-ODO specimens of frog oxyntic cell by ultra-high resolution SEM. In the resting oxyntic cells, the luminal surface was rather flat (Fig. 23a) whereas the lingulae were of uniform height and tightly packed, or appeared ruffled when separated. Short projecting microvilli were occasionally seen on the lingulae (Fig. 23a). The empty, macerated cytoplasm clearly revealed the cytoplasmic face of the luminal plasma membrane of the lingulae. The cytoplasm contained numerous 30-60 nm tubules and spherical or polygonal 200-500 nm vesicles (Fig. 23). Continuity of the tubulovesicular membrane and the luminal plasma membrane was occasionally recorded in resting cells (Fig. 23a).

In the oxynticopeptic cells 15 minutes after histamine stimulation, the luminal surface area was markedly increased and slender microvilli appeared on the elongated and irregularly separated lingulae. The amount of tubulovesicles was greatly reduced, but their basic arrangement did not change. Rare continuity of the tubulovesicular membrane to the luminal surface was observed in TEM (Fig. 22). In the SEM micrographs of oxynticopeptic cells 15 minutes after stimulation, the luminal surface folds were more extensively ruffled and had many more slender microvilli than on the surface in resting oxyntic cells (Fig. 24a). The arrangement of the tubulovesicles did not change but was drastically decreased and connecting tubules to the luminal membrane were much more evident (Fig. 24b). In the rat, such connections between the tubulovesicles and the luminal surface area are quite rare and occur infrequently. Although the frog oxyntic cell connections are observed more frequently, they are by no means numerous. The small tubule diameter of 20-60 nm makes these connections very difficult to detect. The rarity and small size of the connecting tubules no doubt contributed to their oversight in previous electron microscopy studies of thin sectioned amphibian oxynticopeptic cells.

In oxynticopeptic cells examined 30 minutes after stimulation, the luminal surface has lingual processes with very long and often branched thin microvilli. These changes markedly increase the surface area and make its membrane configuration very complex.

In many respects both mammalian parietal and amphibian oxynticopeptic cells behave similarly during the transformation to the secretory state or return to quiescense. However, some distinct differences in their morphology is present.

Concluding remarks

Conspicuous morphological changes occur when oxyntic cells change their morphology to the active acid secreting configurations. Observation of specimens of various secretory stages treated with A-ODO method and examined by ultra-high-resolution SEM provided a detailed three-dimensional structure of the membrane systems, especially the membrane connections by slender connecting tubules, which are difficult to demonstrate in routine thin sections by TEM. Future SEM observations on the specimens prepared by the immunocytochemical procedure of H^+/K^+-ATPase or for cytoskeleton-linked proteins will provide more detailed information of changes in oxyntic cells.

Although much has been learned about oxyntic cell structure and its relation to acid secretion in the past half century, many details remain unclear or unanswered. It is hoped that the next 50 years will provide us with a much better understanding of how this cell performs its amazing secretory activity.

Acknowledgments. The author is grateful to Dr. S. Ito, Department of Neurobiology, Harvard Medical School, for his critical reading of this manuscript, and to Mr. Y. Yamasaki, Dr. Y. Furuya, Ms. M. Miyata, M. Inoue and M. Maeda for their technical assistance throughout this study.

References

Albedi F.M., Barsotti P., Mingazzini P. and Marinozzi V. (1985). Visualization of the secretory canaliculi of human parietal cells with a peroxidase-labeled peanut lectin. Light- and electron-microscopic observations. Cell Tissue Res. 239, 447-450.

Algrain M., Turunen O., Vaheri A., Louvard D. and Arpin M. (1993). Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. J. Cell Biol. 120, 129-139.

Ando T., Araki K., Matsuura K., Kobayashi M., Namikawa T., Naoki I., Yamasaki Y. and Ogata T. (1995). Distribution of H^+/K^+-ATPase in parietal cell of the rat after stimulation with tetragastrin. Proc. of 27th Jpn. Clinic. Electron Microsc. Meeting, S132 (In Japanese).

Berglindh T., Dibona D.R., Ito S. and Sachs G. (1980). Probes of parietal cell function. Am. J. Physiol. 238, G165-176.

Black J.A., Forte T.M. and Forte J.G. (1980). Structure of oxyntic cell membranes during conditions of rest and secretion of HCl as revealed by freeze-fracture. Anat. Rec. 196, 163-172.

Black J.A., Forte T.M. and Forte J.G. (1982). The effects of micro-filament disrupting agents on HCl secretion and ultrastructure of piglet gastric oxyntic cells. Gastroenterology 83, 595-604.

Bretscher A. (1986). Purification of the intestinal microvillus cytoskeletal proteins villin, fimbrin and ezrin. Methods Enzymol. 134, 24-27.

Callaghan J.M., Toh B.-H., Pettitt J.M., Humpheris D.C. and Gleeson

P.A. (1990). Poly-*N*-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells. Identification of a tomato-lectin binding 60-90 x 10^3 M_r membrane glycoprotein of tubulovesicles. J. Cell Sci. 95, 563-576.

Carlisle K.S., Chew C.S. and Hersey S.J. (1978). Ultrastructural changes and cyclic AMP in frog oxyntic cells. J. Cell Biol. 76, 31-42.

Forte T.M. and Forte J.G (1970). Histochemical staining and characterization of glycoproteins in acid-secreting cells of frog stomach. J. Cell Biol. 47, 437-452.

Forte T.M. and Forte J.G. (1971). A freeze-fracture study of bullfrog gastric oxyntic cells. J. Ultrastruct. Res. 37, 322-334.

Forte J.G. and Wolosin J.M. (1987). HCl secretion by the gastric oxyntic cell. In: Physiology of the gastrointestinal tract. Chapter 27. 2nd Edition. Johnson L.R. (ed). Raven Press. New York. pp 853-863.

Forte J.G. and Yao X. (1996). The membrane recruitment-and-recycling hypothesis of gastric HCl secretion. Trends Cell Biol. 6, 45-48.

Forte J.G., Ganser A.L., Beesley R.C. and Forte T.M. (1975). Unique enzymes of purified microsomes from pig fundic mucosa. Gastroenterology 69, 175-189.

Forte T.M., Machen T.E. and Forte J.G. (1977). Ultrastructural changes in oxyntic cells associated with secretory function: a membrane-recycling hypothesis. Gastroenterology 73, 941-955.

Forte J.G., Black J.A., Forte T.M., Machen T.E. and Wolosin J.M. (1981). Ultrastructural changes related to functional activity in gastric oxyntic cells. Am. J. Physiol. 241, G349-G358.

Gibert A.J. and Hersey S.J. (1982). Morphometric analysis of parietal cell membrane transformations in isolated gastric glands. J. Membr. Biol. 67, 113-124.

Hanzel D., Urushidani T., Usinger W.R. and Smolka A. and Forte J. G. (1989). Immunological localization of an 80-kDa phosphoprotein to the apical membrane of gastric parietal cells. Am. J. Physiol. 256, G1082-G1089.

Hanzel D., Reggio H., Bretscher A., Forte J.G. and Mangeat P. (1991). The secretion-stimulated 80K phosphoprotein of parietal cells is ezrin, and has properties of a membrane cytoskeletal linker in the induced apical microvilli. EMBO J. 10, 2363-2373.

Helander H.F. and Hirschowitz B.I. (1972). Quantitative ultrastructural studies on gastric parietal cells. Gastroenterology 63, 951-961.

Helander H.F. and Hirschowitz B.I. (1974). Quantitative ultrastructural studies on inhibited and on partly stimulated gastric parietal cells. Gastroenterology 67, 447-452.

Ihida K., Tsuyama S. and Murata F. (1990). The use of *Griffonia simplicifolia* agglutinin-IB_4 in staining the developing rat fundic gland. Acta Histochem. Cytochem. 23, 475-486.

Ishikawa H., Bischoff R. and Holtzer H. (1969). Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. J. Cell Biol. 43, 312-328.

Ito M., Kawakami H., Saito S., Aoyagi T. and Hirano H. (1989). A morphometric study of changes in *Dolichos biflorus* agglutinin (DBA)-binding pattern of the human gastric parietal cell in association with acid secretion. Okajimas Folia Anat. Jpn. 66, 277-282.

Ito S. and Schofield G.C. (1974) Studies on the depletion and accumulation of microvilli and changes in the tubulovesicular compartment of mouse parietal cells in relation to gastric acid secretion. J. Cell Biol. 63, 364-382.

Ito S. (1987). Functional gastric morphology. In: Physiology of the gastrointestinal tract. Chapter 26. 2nd Edition. Johnson L.R. (ed). Raven Press. New York. pp 817-851.

Jiron C., Romano M. and Michelangeli F. (1984). A study of dynamic membrane phenomena during the gastric secretory cycle: fusion, retrieval and recycling of membranes. J. Membr. Biol. 79, 119-134.

Kessimian N., Langner B.J., McMillan P.N. and Jauregui H.O. (1986). Lectin binding to parietal cells of human gastric mucosa. J. Histochem. Cytochem. 34, 237-243.

Kobayashi T. and Seguchi H. (1990). Detection of Ouabain-insensitive H^+-transporting, K^+-stimulated *p*-nitrophenylphosphatase activity in rat gastric glands by cerium-based cytochemistry. J. Histochem. Cytochem. 38, 1895-1905.

Kuhlmann W.D. and Peschke P. (1984) Comparative study of procedures for histological detection of lectin binding by use of *Griffonia simplicifolia* aggulutinin I and gastrointestinal mucosa of the rat. Histochemistry 81, 265-272.

Lawn A.M. (1960). Observations on the fine structure of the gastric parietal cell of the rat. J. Biphys. Biochem. Cytol. 7, 161-166.

Lillibridge C.B. (1968). Electron microscopic measurements of the thickness of various membranes in oxyntic cells from frog stomachs. J. Ultrastruc. Res. 23, 243-259.

Logsdon C.D. and Machen T.E. (1982). Ultrastructural changes during stimulation of amphibian oxyntic cells viewed by scanning and transmission electron microscopy. Anat. Rec. 202, 73-83.

Madrid J.F., Ballesta M.T., Castells M.T. and Hernández F. (1990). Glycoconjugate distribution in the human fundic mucosa revealed by lectin- and glycoprotein-gold cytochemistry. Histochemistry 95, 179-187.

Mercier F., Reggio H., Devilliers G., Bataille D. and Mangeat P. (1989). Membrane-cytoskeleton dynamics in rat parietal cells: mobilization of actin and spectrin upon stimulation of gastric acid secretion. J. Cell Biol. 108, 441-453.

Mizuno M., Fuzimoto T. and Ogawa K. (1989a). Distribution of F-actin, fodrin, and ankyrin in gastric parietal cells of the rat. Acta Histochem. Cytochem. 22, 593-603.

Mizuno M., Fuzimoto T. and Ogawa K. (1989b). Immunocytochemical localization of α-actinin in gastric parietal cells of the rat. Acta. histochem. cytochem. 22, 639-650.

Namikawa T., Araki K., Matsuura K., Kobayashi M., Naoki I., Ando T., Yamasaki Y. and Ogata T. (1995). Cytochemical studies on the cytoskeletons and actin-binding proteins in the rat gastric parietal cells related to gastric acid secretion. Proc. of 27th Jpn. Clinic. Electron Microsc. Meeting, S134 (In Japanese).

Naoki I., Araki K., Matsuura K., Kobayashi M., Namikawa T., Ando T., Yamasaki Y. and Ogata T. (1995). Distribution of lectin-binding sites in parietal cell of the rat after stimulation and inhibition. Proc. of 27th Jpn. Clinic. Electron Microsc. Meeting, S133 (In Japanese).

Ogata T. (1992). Electron microscopic studies of the normal and pathological stomach. Part I. Ultra-high resolution SEM study of the rat parietal cell. J. Clin. Electr. Microsc. 25, 323-324.

Ogata T. and Yamasaki Y. (1993). Ultra-high resolution scanning electron microscopic studies on the membrane system of the parietal cells of the rat in the resting state and shortly after stimulation. Anat. Rec. 237, 208-219.

Ogata T. and Yamasaki Y. (1996). Ultra-high-resolution scanning electron microscopy of the continuity of cytoplasmic and luminal membranes in frog oxyntic cells. Anat. Rec. 245, 559-567.

Ogata T., Araki K. and Yamasaki Y. (1995) Ultra-high-resolution scanning electron microscopic studies on the membrane system of the rat parietal cells after tetragastrin stimulation. It. J. Anat. Embryol. 100, 393-401.

Okamoto C.T. and Forte J.G. (1988) Distribution of lectin-binding sites in oxyntic and chief cells of isolated rabbit gastric glands. Gastroenterology 95, 334-342.

Osawa W. and Ogata T. (1978). A scanning electron microscopic study on the fractured rat parietal cells in resting state and after stimulation with tetragastrin. Arch. Histol. Jpn. 41, 141-155.

Pettitt J.M, Humphris D.C., Barrett S.P. Toh B-H. van Driel I.R. and Gleeson P.A. (1995). Fast freeze-fixation/freeze-substitution reveals the secretory membranes of the gastric parietal cell as a network of helically coiled tubule. J. Cell Sci. 108, 1127-1141.

Rubin W. and Aliasgharpour A.A. (1976). Demonstration of a cytochemical difference between the tubulovesicles and plasmalemma of gastric parietal cells by ATPase and NPPase reactions. Anat. Rec. 184, 251-264.

Sachs G. (1977). H^+ transport by a non electrogenic gastric ATPase as a model for acid secretion. Rev. Physiol. Biochem. Pharmacol. 79, 133-162.

Sachs G. (1987). The gastric proton pump: The H^+/K^+-ATPase. In: Physiology of the gastrointestinal tract. Chapter 28. 2nd Edition. Johnson L.R. (ed). Raven Press. New York. pp 865-881.

Schofield G.C., Ito S. and Bolender R.P. (1979). Changes in membrane surface areas in mouse parietal cells in relation to high levels of acid secretion. J. Anat. 128, 669-692.

Sedar A.W. (1961a). Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbiana*) I. The non-acid-secreting gastric mucosa. J. Biophys. Biochem. Cytol. 9, 1-18.

Sedar A.W. (1961b). Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog, (*Rana catesbiana*). II. The acid-secreting gastric mucosa. J. Biophy. Biochem. Cytol. 10, 47-57.

Sedar A.W. (1965). Fine structure of the stimulated oxyntic cell. Fed. Proc. 24, 1360-1367.

Sedar A.W. (1969). Uptake of peroxidase into the smooth surface tubular system of the gastric acid-secreting cell. J. Cell Biol. 43, 179-184.

Smith P.R., Bradford A.L. Joe E.-H., Angelides K.J., Benos D.J. and Saccomani G. (1993) Gastric parietal cell H^+-K^+-ATPase microsomes are associated with isoforms of ankyrin and spectrin. Am. J. Physiol. 264, C63-70.

Smolka A., Helander H.F. and Sachs G. (1983). Monoclonal antibodies against gastric H^+/K^+ ATPase. Am. J. Physiol. 245, G589-596.

Sugai N. and Ito S. (1980) Carbonic anhydrase, ultrastructural localization in the mouse gastric mucosa and improvements in the technique. J. Histochem. Cytochem. 28, 511-525.

Sugai N., Ito S., Ichikawa A. and Ichikawa M. (1985) The fine structure of the tubulovesicular system in mouse gastric parietal cell processed by cryofixation method. J. Electron Microsc. 34, 113-122.

Sugai N., Okamura H., Tsunoda R.,Terashima H. and Suzuki K. (1995). Morphological changes of tubulovesicles of rat parietal cells according to the different fixative osmolarietes. Proc. of 100th Ann. Meet. of Jpn. Anat. Soc. p 115 (In Japanese).

Sugimoto K. and Ogata Y. (1989). Scanning electron microscopic studies on the subepithelial tissue of the gastrointestinal mucosa of the rat. Arch. Histol. Cytol. 52, 257-265.

Tanaka K. and Mitsushima M. (1984) A preparation method for observing intracellular structures by scanning electron microscopy. J. Microsc. 133, 213-222.

Urushidani T., Hanzel D.K. and Forte J.G. (1989). Characterization of an 80-kDa phosphoprotein involved in parietal cell stimulation. Am. J. Physiol. 256, G1070-G1081.

Vial J.D. and Garrido. J. (1976). Actin-like filaments and membrane rearrangement in oxyntic cells. Proc. Natl. Sci. USA 73. 4032-4036.

Vial J.D. and Orrego H. (1960). Electron microscope observations on the fine structure of parietal cells. J. Biophys. Biochem. Cytol. 7. 367-372.

Vial J.D., Garrido J. and González A. (1985) The early changes of parietal cell structure in the course of secretory activity in the rat. Am. J. Anat. 172, 291-306.

Yao X., Thibodeau A. and Forte J.G. (1993). Ezrin-calpain I interactions in gastric parietal cells. Am. J. Physiol. 265, C36-C46.

Yao X., Chaponnier C., Gabbiani G. and Forte J.G. (1995). Polarized distribution of actin isoforms in gastric parietal cells. Mol. Biol. Cell 6, 541-547.

Zalewsky C.A. and Moody F.G. (1977). Stereological analysis of the parietal cell during acid secretion and inhibition. Gastroenterology 73, 66-74.