ELECTRON MICROSCOPY OF THE OXYNTIC CELL IN THE GASTRIC GLANDS OF THE BULLFROG, *RANA CATESBIANA*

II. The Acid-Secreting Gastric Mucosa

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ABSTRACT

The oxyntic cell in the gastric glands of the bullfrog was examined in lead hydroxide–stained sections of gastric mucosae fixed in buffered osmium tetroxide and embedded in *n*-butyl methacrylate. During gastric acid secretion (pH 1–2) induced by histamine administration in cannulated frogs, the pattern of fine structure in the oxyntic cell differs strikingly from that in the oxyntic cell of the non-acid-secreting stomach. The relative number of smooth surfaced profiles decreases and a greater concentration of these elements is associated with the apical region of the oxyntic cell facing the lumen of the gastric gland. Similar concentrations of these elements are found in those regions of cytoplasm surrounding intercellular canaliculi which lie between adjacent cells and communicate with the lumen of a gastric gland. In these regions, the smooth surfaced profiles (35 to 65 mμ in width) characteristically form a tubular network. The membrane-bounded contents appear to be continuous with the extracellular medium, both on the capillary side and at the apical surface of the cell adjoining the lumen of the gastric gland. Mitochondria are distributed randomly in the cytoplasmic matrix of the oxyntic cell.

INTRODUCTION

In the first paper of this series (16) the pattern of fine structure in the oxyntic cell of the gastric glands of the non-acid-secreting stomach of the bullfrog was established. This information provides a firm basis for a comparative study of the structure of this cell under experimental conditions in which the physiological state of the gastric mucosa has been altered. The present report contains a description of the changes in structure of the oxyntic cell which occur when gastric acid formation is stimulated by administration of histamine.

MATERIALS AND METHODS

The animals used in this study were collected locally during the late winter months and consisted of both male and female bullfrogs (*Rana catesbiana*) ranging in weight from 200 to 260 gm. They were maintained in the laboratory at approximately 10°C.

Under ether anesthesia, gastric fistulae were established by procedures which avoided contamination from extragastric sources (13). The cannula consisted of a single piece of lucite 17 mm. long, with a disc-shaped flange (16 mm. × 3 mm.) at either end. The diameter of the tube between the two flanges was 8 mm. and of the bore was 6 mm. The inner flange

of the cannula was sutured into the stomach along the mid-portion of the greater curvature by means of a purse string suture; the outer flange was exteriorized. The anterior body muscle and skin were sutured separately. Both the cardiac and pyloric areas of the stomach were ligated in the regions of the sphincters. Animals were allowed to recover for approximately 2 weeks after surgical procedures before being used in experiments.

Only frogs demonstrating absence of basal gastric acid secretion initially (tested with hydrion paper) were studied. Histamine phosphate, calculated as the base, was administered intraperitoneally at 30 minute intervals in a dose of 0.18 mg. per 200 gm. body weight to stimulate secretion of an acid gastric juice. Usually three separate injections of histamine were required to obtain a gastric juice with a pH between 1.0 and 2.0.

Under ether anesthesia, the stomachs were exposed and opened by an incision along the greater curvature. The pH of the gastric contents was determined with hydrion papers. Only frogs with gastric contents showing a pH between 1.0 and 2.0 were used for obtaining tissue specimens.

For electron microscopy, tissue specimens from the corpus of the stomach were fixed in cold (0°C.) 1 per cent osmium tetroxide buffered at pH 7.6 with 0.06 M KH_2PO_4—K_2HPO_4 buffer for 30 minutes. The tissue was then dehydrated in ethanol and embedded in *n*-butyl methacrylate. Sections 600 to 900 A thick (8) were cut from plastic blocks with the Porter-Blum microtome (9) and mounted on carbon-coated 150 mesh copper grids (21). These preparations were stained for 20 to 30 minutes with Watson's lead hydroxide solution (23) and sandwiched with a formvar film (22).

Microscopy was done with an RCA EMU 3D electron microscope containing a 1 mil platinum objective aperture. Micrographs were taken at original magnifications of 3,600 to 12,500 diameters and enlarged or reduced photographically as required.

OBSERVATIONS

All observations here were obtained on the gastric mucosae of bullfrogs demonstrating a gastric juice with a pH between 1.0 and 2.0.

Vesicular Component: An electron micrograph of an oblique section through a gastric tubular gland (Fig. 1) exhibits the characteristic cytoplasmic fine structure of portions of adjacent oxyntic cells facing the lumen of a gastric gland during acid secretion. In contrast to the randomly oriented and tightly packed system of smooth surfaced vesicular and tubular elements in the cytoplasmic matrix of the oxyntic cell in the non-acid-secreting stomach of the bullfrog (16), these vesicular and tubular elements in the oxyntic cells of the acid-secreting stomach are concentrated in the vicinity of the apical surfaces of the cells facing the lumen of the gastric gland (Fig. 1); they accumulate also along the apposing borders, near the cellular membranes of adjacent oxyntic cells (*arrows*, Figs. 1 and 4), where presumably the plane of section is close to an intercellular canaliculus. More peripheral areas of cytoplasm in the oxyntic cells contain fewer vesicles and tubules (Figs. 1 to 3).

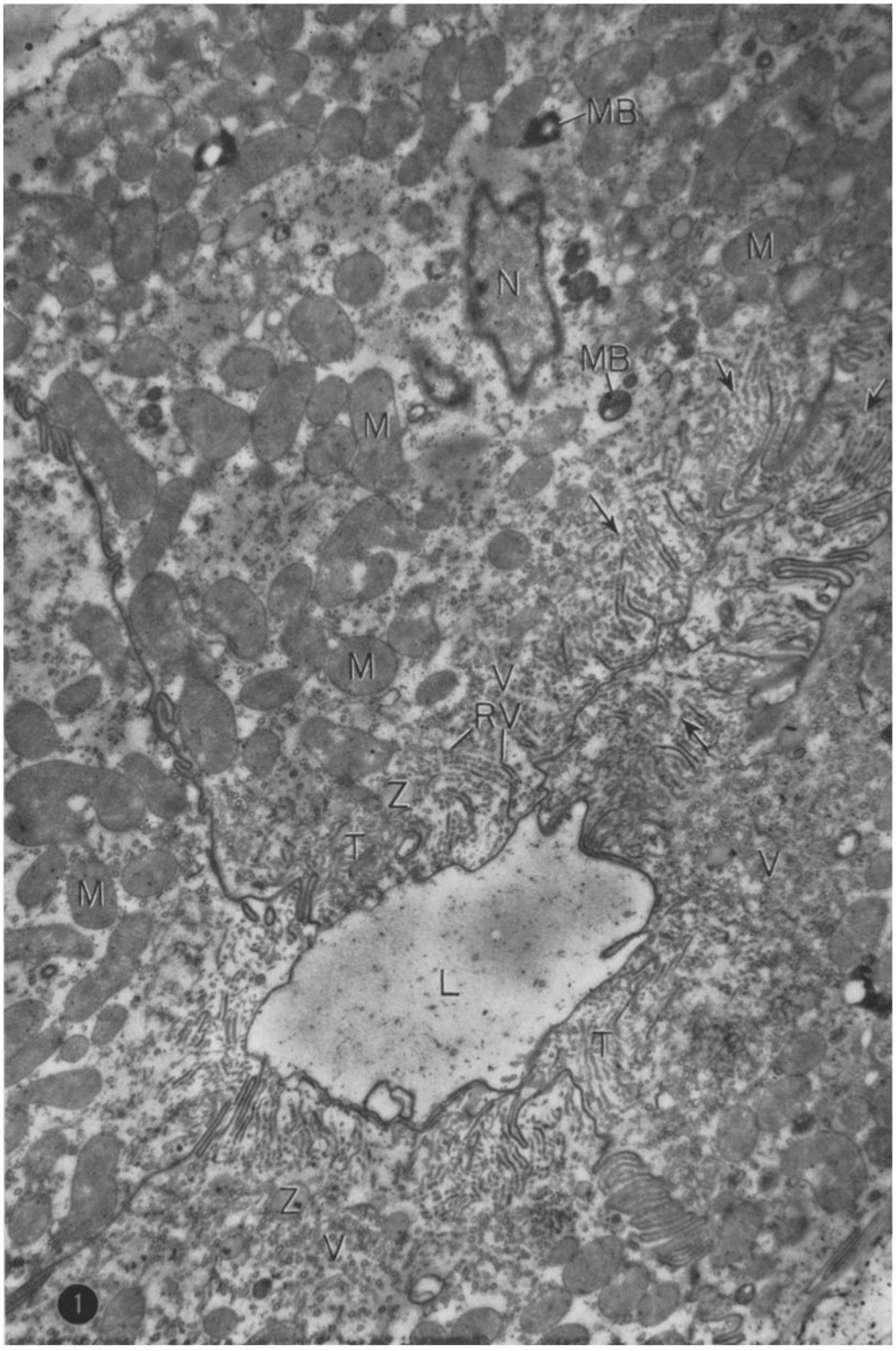
Apical Zone of Cytoplasm: In histamine-stimulated animals the apical region of cytoplasm of oxyntic

Explanation of Figures

All the electron micrographs included in the plates pertain to gastric mucosae (corpus portion) of bull-frogs stimulated with histamine. In all cases, sections were stained with lead hydroxide.

FIGURE 1

An electron micrograph of an oblique section through a gastric gland showing portions of adjacent oxyntic cells surrounding the lumen (*L*). Numerous mitochondria (*M*) are disposed randomly in the cytoplasm. Profiles of vesicles (*V*), rows of vesicles (*RV*), and elongated profiles (*T*) appear more concentrated in cellular zones adjacent to the lumen (*L*) of the gastric gland. More peripheral areas of cytoplasm contain fewer numbers of vesicles and tubules. The smooth surfaced elements also appear to be concentrated in the cytoplasm along apposing borders of adjacent oxyntic cells near the lumen of the gastric gland (*arrows*). Presumably, in such an instance, the plane of section is close to an intercellular canaliculus between adjacent oxyntic cells. Microbodies (*MB*), zymogen granules (*Z*), and a nucleus (*N*) are indicated. × 10,000.


MB
N
M
MB
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V
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V
1

cells in gastric glands shows a high concentration of smooth surfaced profiles (Figs. 1 to 3, and 5). These profiles are in most cases arranged in rows of vesicles or tubules (35 to 65 mμ in width) (Figs. 2 and 5). In some micrographs (Fig. 3) the tubules appear to interconnect with one another and with cisternae. Fig. 3 shows a tightly packed, smooth surfaced network of tubules. This network represents a portion of the smooth surfaced endoplasmic reticulum that is characteristic for the oxyntic cell in the acid-secreting gastric mucosa. Presumably, rows of such profiles could result from sectioning through tubules disposed in a serpentine course. The fact that adjacent profiles in a given row (*VT*, Fig. 2) assume both elongated and nearly circular outlines gives support to this possibility. The contents of the tubular and vesicular profiles are in direct contact with the lumen of the gastric gland (*arrow*, Fig. 5) and exhibit a greater electron scattering than the surrounding cytoplasmic matrix (see also Figs. 2 to 4).

Basal Zone of Oxyntic Cell: The basal region of the oxyntic cell in the histamine-stimulated frog shows only a few smooth surfaced vesicular or tubular elements (Figs. 2 and 3). Some electron micrographs demonstrate that a possible connection exists between invaginations of the basal plasma membrane and smooth surfaced profiles (Fig. 6).

Golgi Apparatus: The Golgi complex observed in the cytoplasm of oxyntic cells in the gastric mucosae of animals secreting an acid gastric juice (Fig. 7) exhibits morphological characteristics similar to those described for this organelle in oxyntic cells in non-acid-secreting stomachs (16). It consists of an array of several elongated profiles and associated vesicular and tubular elements (Fig. 7); all elements included in the grouping are smooth surfaced.

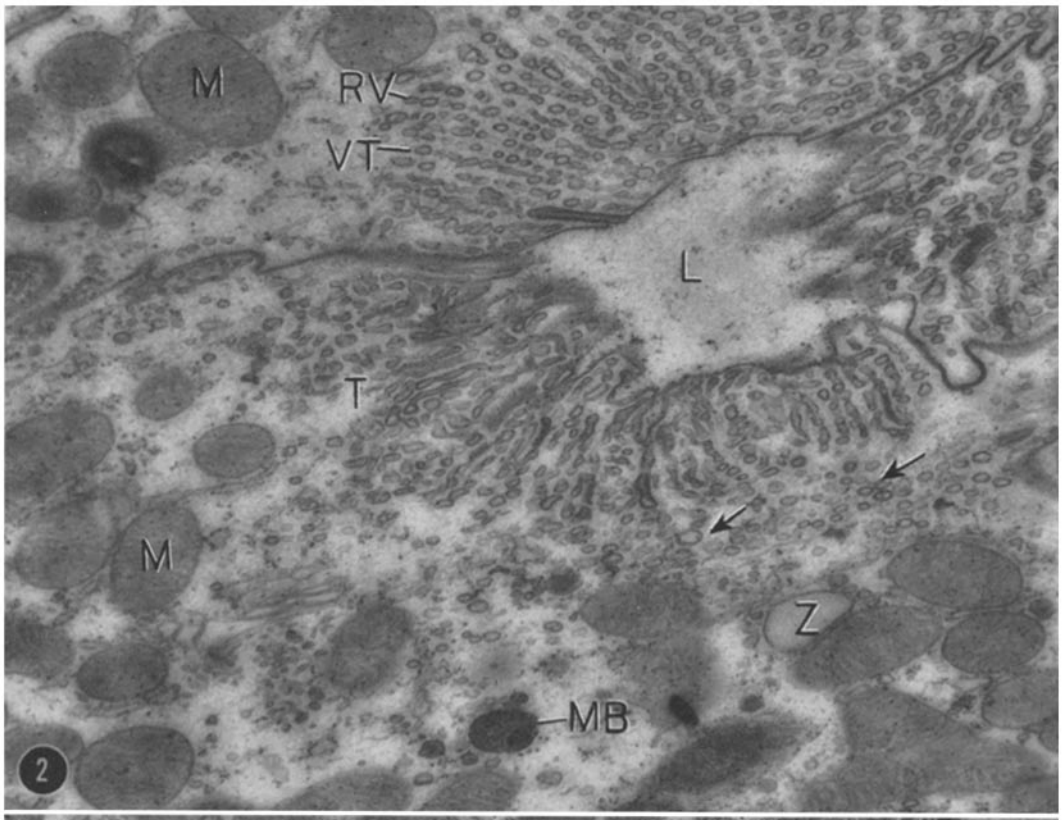
Mitochondria: It was previously reported that mitochondrial profiles were more concentrated in the basal regions of oxyntic cells in the non-acid-secreting gastric mucosae of the bullfrog. Under conditions in which gastric acid production has been stimulated by administration of histamine, however, the distribution of mitochondria in the oxyntic cell is modified and a greater number of mitochondrial profiles is found near the apical surface and middle zone of the cell (Figs. 1 to 3, and 5). There are, however, many mitochondria in the basal zone of the cell (Fig. 1). The fine structure of the mitochondria in these

FIGURE 2

This micrograph, at a higher magnification than Fig. 1, depicts in more detail the arrangement of the smooth surfaced elements in the oxyntic cells in the acid-secreting gastric mucosa. Portions of the apical regions of three oxyntic cells adjacent to the lumen (*L*) of an obliquely sectioned gastric gland are seen. The elements concentrated near the luminal surfaces of the cells are arranged in rows of vesicles (*RV*), rows containing both vesicles and tubules (*VT*), or, in some cases, as elongated and undulating tubules (*T*). The fact that it is possible to observe continuity among some of the smooth surfaced profiles in this area suggests that the structural arrangement here consists of a series of undulating tubules located at right angles to the luminal surface of the cell. The elliptical profiles (*arrows*) located beneath this zone just described have a more random distribution with no preferred orientation. More peripheral areas in these cells contain fewer numbers of vesicles and tubules. A zymogen granule (*Z*), microbody (*MB*), and mitochondria (*M*) are also seen. × 19,000.

FIGURE 3

This micrograph shows a portion of an oxyntic cell and a tiny part of an adjacent cell facing the lumen (*L*) of a gastric gland. Here, in the apical region of the cell adjacent to the lumen (*L*), the tubular disposition of the smooth surfaced elements can be seen more clearly than in Fig. 2. A cisterna (*arrow*) is connected into a tubular network. Only a few smooth surfaced profiles are observed in the more peripheral cytoplasm. Occasional rough surfaced profiles (*RS*) are encountered. Mitochondria (*M*) contain usually a large proportion of cristae but occasionally show zones that are devoid of cristae (*X*). × 19,000.



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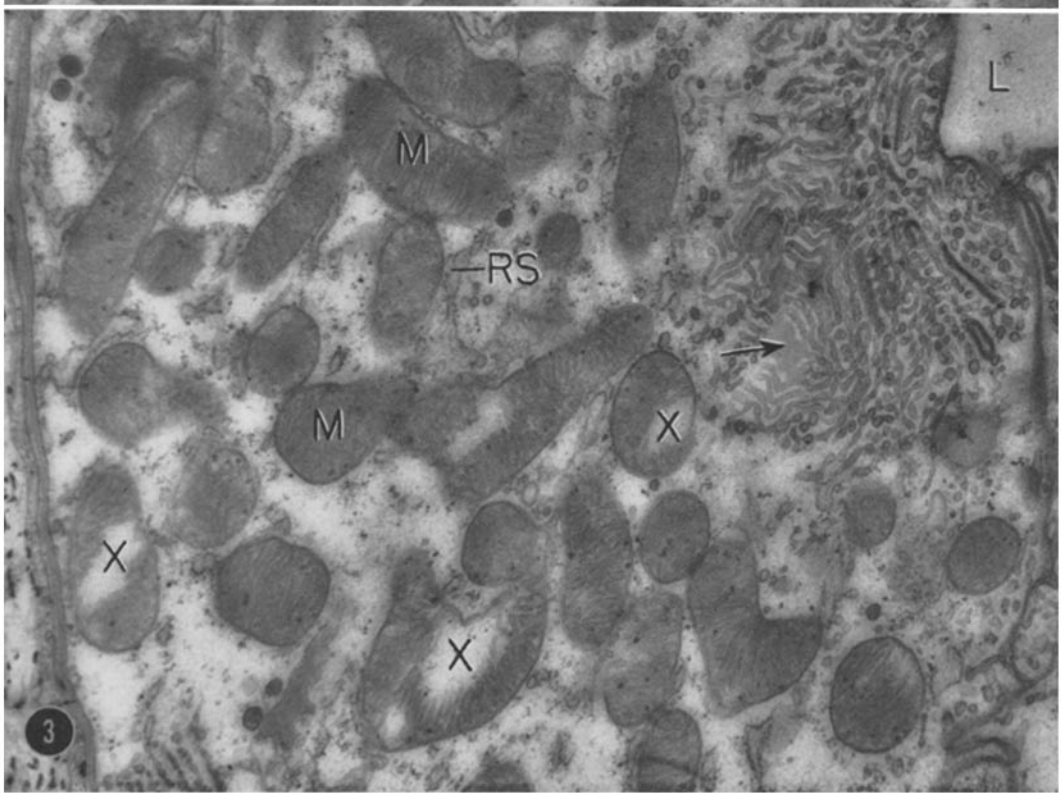

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cells does not differ appreciably from that of mitochondria observed in oxyntic cells in the non-acid-secreting stomach of the bullfrog (16). However, in a number of instances, areas exhibiting less electron scattering than adjacent regions are seen within mitochondrial profiles (Fig. 1, 3, and 4); these areas are devoid of cristae mitochondriales. The significance of this observation remains unknown at present.

Rough Surfaced Reticulum, RNP Granules, Glycogen Granules, and *Zymogen Granules:* These components are always observed in the cytoplasm of the oxyntic cell. Profiles of rough surfaced reticulum, though not numerous, are seen within the middle (Fig. 7) and basal (Fig. 6) zones of cytoplasm. The elements that comprise this reticulum are studded with granules (140 A) identified as the ribonucleoprotein component of the cytoplasm. Some of the RNP granules are distributed freely in the cytoplasm (Figs. 6 and 7). Larger granules (260 A) (*G*, Figs. 6 and 7) are tentatively identified as glycogen deposits since they resemble granular glycogen deposits which are stained with lead in other cell types (23). Zymogen granules described previously in the oxyntic cell (16) are found scattered throughout the cytoplasm (Figs. 1 and 2); these granules are similar in structure to those of the peptic cell in the oesophageal glands of the bullfrog (16).

DISCUSSION

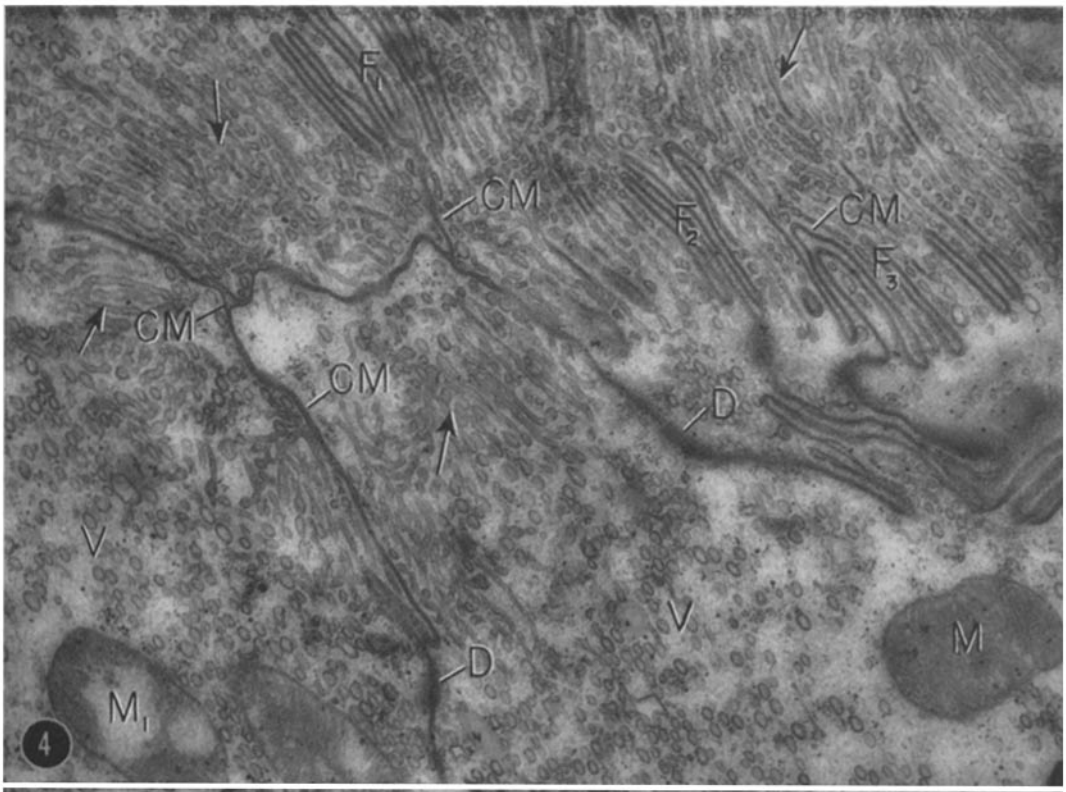
The observations reported in this paper show that the pattern of fine structure in the oxyntic cell of the gastric glands of the acid-secreting stomach of the bullfrog differs strikingly from that in the oxyntic cell of the non-acid-secreting stomach. Under basal conditions, when little if any hydrochloric acid is being produced, the smooth surfaced tubular and vesicular elements present a randomly oriented and tightly packed system in the cytoplasmic matrix of the oxyntic cell (16). During acid secretion induced by histamine administration, the volume and distribution of these elements change markedly. The relative number of smooth surfaced profiles decreases and a greater concentration of these elements is associated with the apical surface of the cell. Areas of cytoplasm along the apposing borders of adjacent oxyntic cells near the lumen of a gastric gland also show an increased concentration of these elements, presumably related to intercellular canaliculi that communicate with the lumen of a gastric gland. These findings are in agreement with those reported for the oxyntic cells in the histamine-stimulated gastric glands of *Bufo spinulosis* (20) and *Rana catesbiana* (15). Similar electron microscopic data on changes in distribution and volume of the smooth surfaced component have

FIGURE 4

Portions of adjacent oxyntic cells are shown. The cell membranes (*CM*) separating the cells form a number of foldings (F_1, F_2, F_3). Within the cytoplasm adjoining the zones of contact among the cells, rows of elongated profiles and/or vesicles (arrows) are seen. These elements are smooth surfaced and display a content with more density than the surrounding cytoplasmic matrix. Farther inside the cytoplasm (*V*) the smooth surfaced profiles show no preferred orientation and are dispersed randomly. It has been observed in oxyntic cells from acid-secreting gastric mucosa that alignment of tubular or vesicular elements is typical in the apical cytoplasm adjacent to either the lumen of a gastric gland or an intercellular canaliculus. In this micrograph, it is presumed that the plane of section is close to such lumina. Mitochondria (*M*) are also depicted; one of them (M_1) shows two areas which are devoid of cristae. Desmosomes are indicated at *D*. × 22,000.

FIGURE 5

The apical cytoplasms of two oxyntic cells adjacent to the lumen (*L*) of a gastric gland are demonstrated. The cell membrane (arrow) in contact with the lumen (*L*) of the gastric gland forms a number of infoldings. Some of these are continued inside the cytoplasm by rows of vesicles and tubules (R_1, R_2, and R_3). Such appearances suggest that the contents of the smooth surfaced profiles could be added to the gastric juice by such a pathway. Mitochondria (*M*) and some rough surfaced profiles (*RS*) are also evident. × 38,000.



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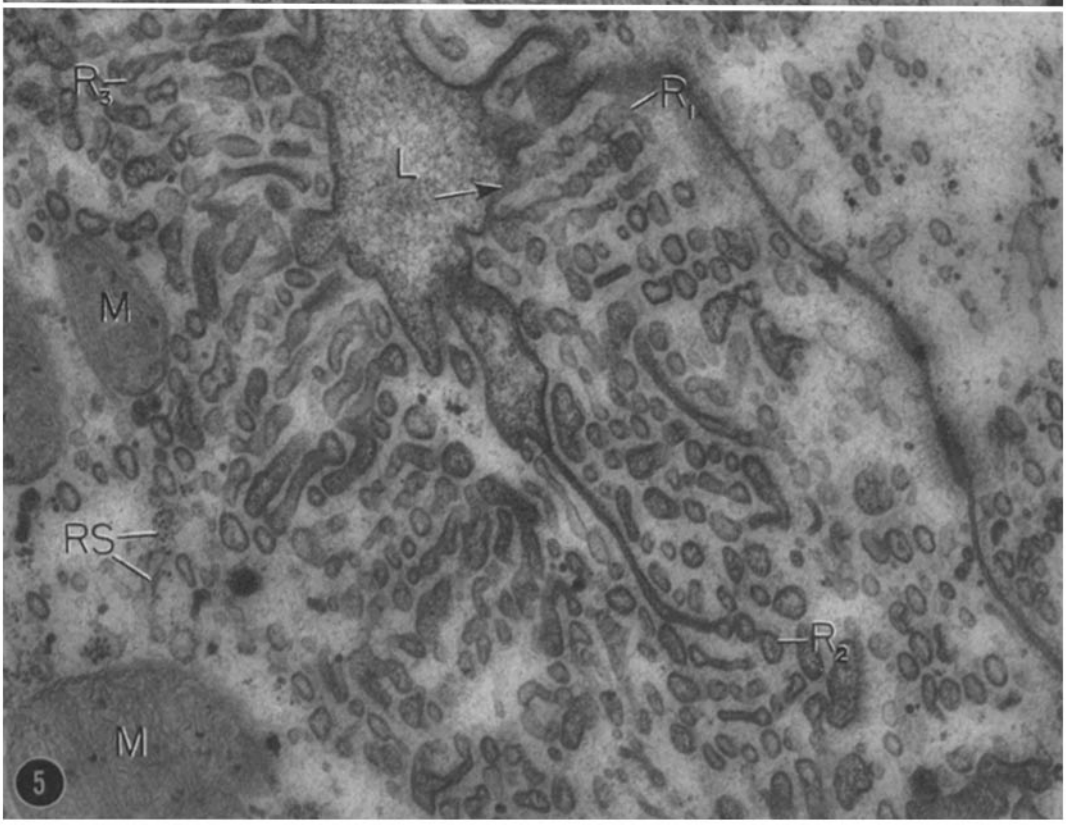

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been provided for the mammalian oxyntic cell in experiments where gastric acid secretion was provoked by insulin in the rat (14), by histamine in the dog (14, 17) or cat (14, 20) and by electrical vagal stimulation in the dog (17, 18).

Hally (5) has reported that vacuole-containing bodies within the cytoplasm of the gastric parietal cell of the mouse became more numerous, larger in diameter, and containing a larger number of vacuoles after repeated injections of pilocarpine nitrate. Although occasional profiles containing vesicles are encountered in the cytoplasm of the oxyntic cell of the histamine-stimulated bullfrog, no obvious morphological changes are observed in these elements.

It is generally assumed, on the basis of exclusion of other cell types, that the oxyntic cell secretes the hydrogen and chloride ions of the gastric juice (4). However, it has been suggested that chloride ions are secreted by the surface epithelial cells and hydrogen ions by the oxyntic cells (10). On the other hand, investigators have also hypothesized that hydrogen ions are secreted by the surface epithelial cells and chloride ions by the oxyntic cells (11). Unfortunately, there is no direct method of determining whether the oxyntic cells take part in the secretion of hydrochloric acid. The use of indicator dyes to localize the site of acid formation in the gastric tubules (1) has been severely criticized (24). Observations that hydrochloric acid secretion was maintained after the surface epithelium of the frog gastric mucosa had been scratched off (19) would implicate the oxyntic cell in gastric acid production since there is mainly one cell type comprising the body of the gastric gland in this species (16). Additional supporting evidence that the oxyntic cell is involved comes from the pronounced fine structural changes reported here in the amphibian oxyntic cell during gastric acid production induced with histamine.

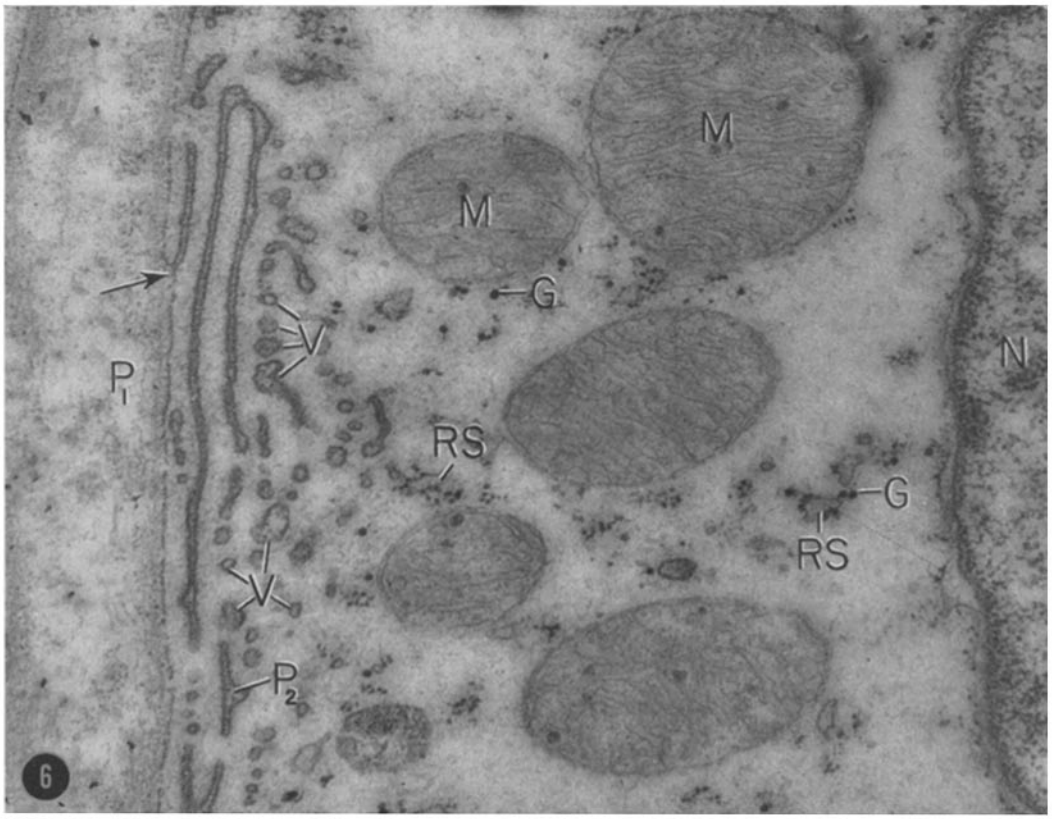
There is, at the moment, no information available to permit evaluation of the importance of the system of tubules and vesicles within the cytoplasm of the oxyntic cell in the production of hydrochloric acid. The membrane delimiting the system separates two phases in the cytoplasm so that the material within the cavities is isolated from the surrounding cytoplasmic matrix. As such, substances normally foreign or injurious to the cytoplasm could be sequestered. It may be significant that some of the membrane-bounded cavities of the system within the cytoplasm are in continuity with the extracellular medium, both on the capillary side and at the apical surface of the cell adjacent to the lumen of the gastric gland. Such a relationship would provide means both to incorporate substances into the cytoplasm (pinocytosis) and also to provide a pathway for substances leaving the cell at its apical surface. It

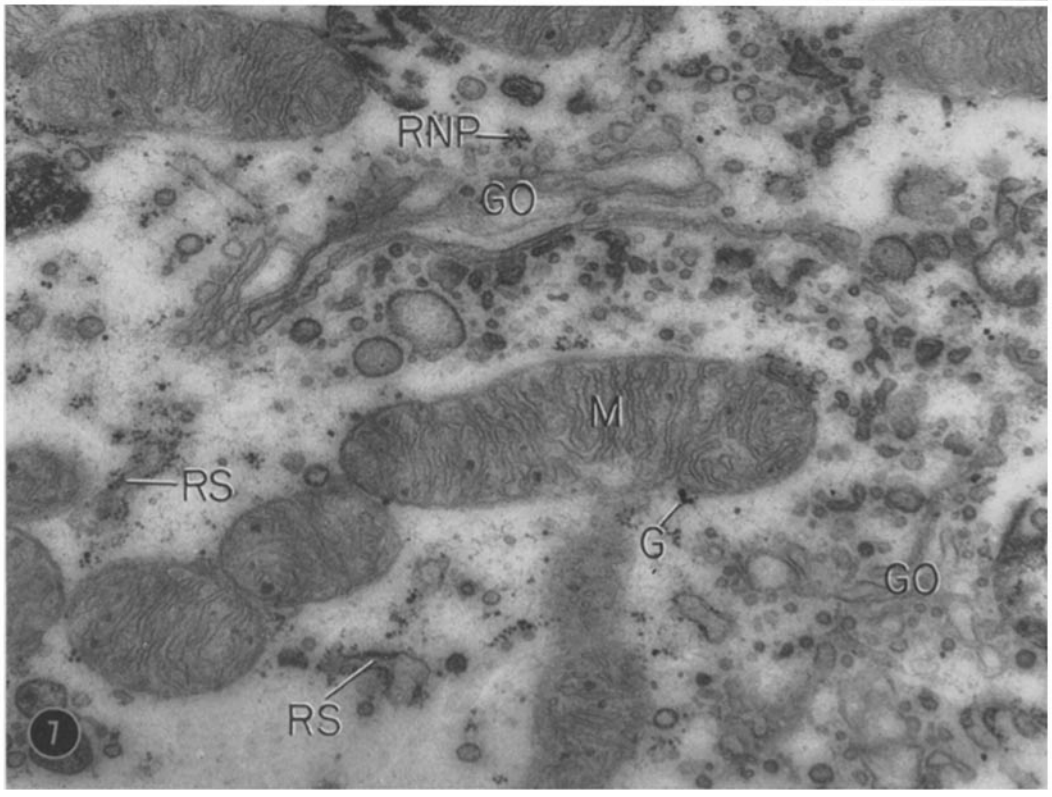
FIGURE 6

A small area of the basal portion of the cytoplasm of an oxyntic cell is seen in this micrograph. An infolding of the plasma membrane (*arrow*) as well as a number of elongated profiles (P_1, P_2) having a unit membrane structure (12) are observed. A number of smooth surfaced vesicular elements are located in the vicinity (*V*), whereas farther inside the cytoplasm only a few such profiles are evident. Micrographs such as this suggest that there is continued activity in the basal portion of the cytoplasm involving, perhaps, the incorporation of substances from the extracellular space. Part of a nucleus (*N*), mitochondrial profiles (*M*), rough surfaced profiles (*RS*), and scattered RNP particles are also demonstrated. × 38,000.

FIGURE 7

Part of the cytoplasm of an oxyntic cell showing the Golgi apparatus (*GO*). Arrays of smooth surfaced, elongated profiles with associated vesicles and tubules comprise the structure of the organelle. Since the structure of this Golgi complex is not noticeably different from the Golgi apparatus in oxyntic cells of the non-acid-secreting mucosa (16), it is uncertain what role, if any, may be assigned to this organelle in the production of gastric juice. The micrograph also shows several mitochondrial profiles (*M*), rough surfaced profiles (*RS*), clusters of RNP particles (*RNP*), and particulate glycogen (*G*). × 31,000.


M
M
G
V
P1
N
RS
G
RS
V
P2
6


RNP
GO
M
RS
G
GO
RS
7

seems reasonable to assume that the contents of the large population of tubules and vesicles observed in the cytoplasm of the oxyntic cell in the non-acid-secreting gastric mucosa are contributed to gastric juice, since the number of these elements decreases markedly during acid secretion and they become concentrated near the apical surface of the cell. The mechanism by which vesicles could move from one region of the cell to another is unknown.

It is well established that the secretion of hydrochloric acid against an electrochemical gradient leads to an equivalent formation of alkali which is liberated into the blood (3). Thus, bicarbonate ions must then leave the capillary side of the cell where they are exchanged for chloride ions (3). In studies of the flux of chloride in the frog mucosa (6, 7), it has been found that chloride movement occurs in two directions in the gastric epithelium: from the nutritive to the secretory surface and also from the secretory to the nutritive surface, although the net chloride movement is in the direction of the stomach lumen. Accordingly, in assigning a function to such a proposed vesicle transport system, one must take into account the movement of ions, namely, bicarbonate and chloride, in a direction opposite to the secretory surface of the cell. Water is another component of the gastric juice that could be handled by a vesicle transport system moving in the direction of the secretory surface. Some support is given to this possibility by the statements of Davies (2): "The rate of transport of water by the oxyntic cells is so enormous that it could not be handled molecule by molecule by any known enzyme systems. The water must be moved in bulk, and probably flows osmotically as a result of the secretion of the hydrogen and chloride ions by the oxyntic cells."

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