CORRELATION OF THE FINE STRUCTURE OF THE GASTRIC PARIETAL CELL (DOG) WITH FUNCTIONAL ACTIVITY OF THE STOMACH

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ABSTRACT

The fine structure of the parietal (oxyntic) cell in the gastric glands (corpus of the stomach) of the dog was examined under conditions of active gastric acid secretion and compared with cellular structure in the non-acid-secretory (basal) state. Animals, in both acute and chronic experiments, were equipped with gastric fistulae so that gastric juice could be collected for analysis of total acidity, free acidity, volume, and pH prior to biopsy of the gastric mucosa. The specimens of mucosa were fixed in buffered OsO_4 and embedded in *n*-butyl methacrylate and the thin sections were stained with lead hydroxide before examination in the electron microscope. A majority of parietal cells showed an alteration of fine structure during stimulation of gastric acid secretion by a number of different techniques (electrical vagal stimulation, histamine administration, or insulin injection). The changes in fine structure affected mainly the smooth surfaced vesicular elements and the intracellular canaliculi in the cytoplasm of the cell. The mitochondria also appeared to be involved to some extent. During acid secretion a greater concentration of smooth surface profiles is found adjacent to the walls of the intracellular canaliculi; other parietal cells exhibited a marked decrease in number of smooth surfaced elements. Intracellular canaliculi, always present in non-acid-secreting oxyntic cells, develop more extensively in cells of acid-secreting gastric glands. The surface area of these canaliculi is greatly increased by the elaboration of a large number of closely approximated and elongated microvilli. Still other parietal cells apparently in a different stage of the secretory cycle exhibit non-patent canaliculi lacking prominence; such cells have very few smooth surfaced vesicular elements. These morphological findings correlated with the acid-secretory state of the stomach provide evidence that the parietal cell participates in the process of acid secretion.

INTRODUCTION

Since the discovery by William Prout in 1824 (28) that the acidity in the stomach was due to hydrochloric acid, there has been a sustained interest in the mechanisms involved in the formation and secretion of gastric acid (5, 16, 18, 29, 9). It is generally accepted that the different cell types of the gastric glands proper (corpus of the stomach) in mammalian species each have a specific function and elaborate a particular cellular product (8). With few exceptions (30) most investigators agree that the parietal or oxyntic cells secrete all the hydrogen ions and most of the chloride ions of the gastric juice; the zymogenic cells produce pepsinogen granules; and the mucous neck and surface epithelial cells elaborate mucin.

A study of the fine structure of the parietal cell

under conditions of active acid production compared with the cellular structure in the non-acid-secretory (resting or basal) state should contribute to a better understanding of the mechanism of acid formation. The following is a report of an attempt to correlate the roles of the morphological elements of the parietal cell in the acid-secretory process. The continuous and spontaneous acid-secretory activity of the rat glandular stomach (11) makes studies such as those presented previously

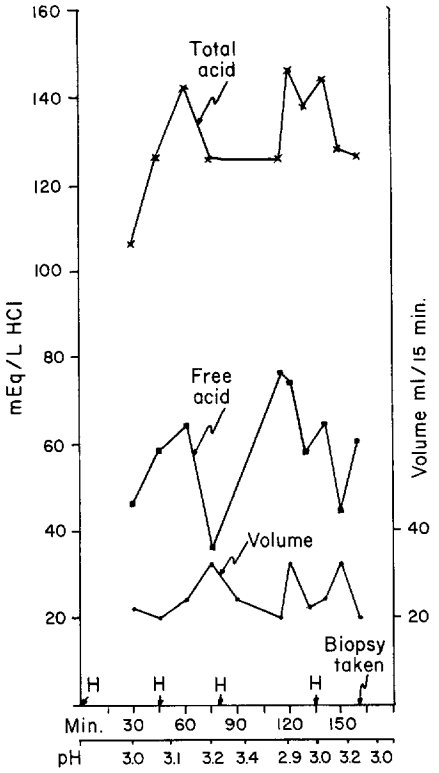


FIGURE 1

Gastric secretion in response to histamine (chronic experiment). H = 0.2 mg histamine.

(32, 33, 19) unsatisfactory for this purpose. The same objection (absence of a non-acid-secretory phase) holds also for the use of mouse stomach (2, 17, 12, 13). The requirements of a control period of a basal non-acid-secretory phase could be fulfilled by the bullfrog, reported earlier (35, 36), or by the dog, where gastric secretion is intermittent (1).

MATERIALS AND METHODS

Experiments were carried out on dogs (7 to 10 kg) fasted 24 hours but allowed water. Only animals showing absence of basal acid secretion during a 30 minute control period were studied.

In acute experiments, the animals were put under pentobarbital sodium anesthesia and a metal gastric cannula was sutured into the anterior wall of the corpus of the stomach; the pylorus and the cardiac region or esophagus were ligated in order to avoid contamination from extragastric sources. During the administration of the various gastric stimulants used in the study and collection of samples of gastric juice for analysis, the animal was maintained under

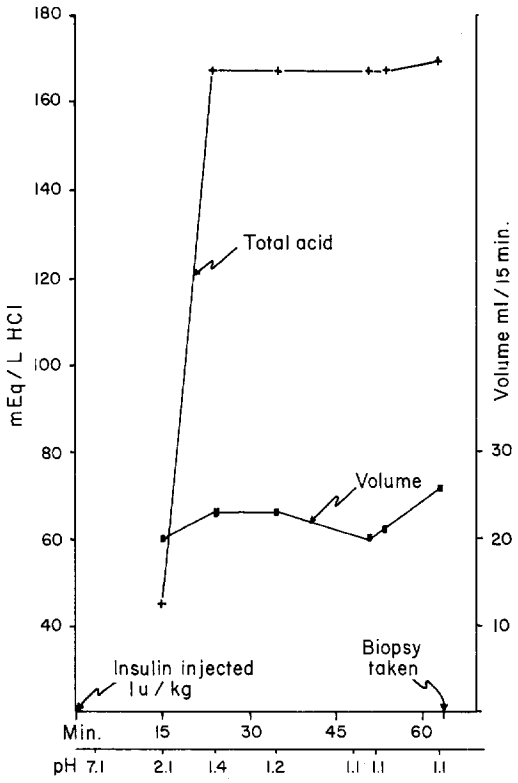


FIGURE 2

Gastric secretion in response to insulin (chronic experiment).

pentobarbital anesthesia, being placed in a stand and supported in an erect position. A femoral vein was cannulated to permit administration of saline and drugs.

Experiments on unanesthetized dogs were conducted on animals previously equipped with a permanent gastric cannula; these are referred to as chronic experiments. The dogs were trained to stand quietly in the collecting stand throughout the experimental period. Biopsy specimens of gastric mucosa could be obtained readily through the permanent gastric fistula without exciting the animal, presum-

ably because there are no afferent pain fibers from this region.

The following techniques were employed to provoke the secretion of an acid gastric juice: (*a*) histamine, calculated as the base, was administered subcutaneously to dogs used in either acute or chronic experiments, in a dose of 0.05 mg/kg/hr; (*b*) insulin was injected subcutaneously into chronic animals in a dose of 1 unit per kg; (*c*) electrical stimulation of the peripheral ends of the sectioned cervical vagi was carried out, in acute experiments, with a square wave stimulator at a frequency of 90, pulse duration 1 msec, 60 to 85 v. (During the period of vagal stimulation, the nerves were used in alternate sequence of 5 minutes' duration.)

In a number of acute and chronic experiments the rate of secretion was measured at 10 to 15 minute intervals. The pH of the samples as well as the free and total acidities were determined by electrometric titration with 0.02 N NaOH. Accordingly the sample of gastric mucosa biopsied at a given time during the course of an experiment could be correlated with the acid-secretory state of the stomach (see Figs. 1 and 2).

Only experiments showing no acid secretion during the 30 minute basal control period are considered in this report. Control biopsy specimens were obtained from the gastric mucosa of dogs in both acute and chronic experiments.

Biopsy specimens from the corpus of the stomach were fixed in cold (0°C) 1 per cent OsO_4 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 A to 900 A thick (25) were then cut from plastic blocks with the Porter-Blum microtome (27) and mounted on carbon-coated 150 mesh copper grids (41). These preparations were stained 20 to 30 minutes with lead hydroxide solution (43) and sandwiched with a Formvar film (42). Specimens were examined with an RCA EMU-3D electron microscope containing a 1 mil platinum objective aperture.

OBSERVATIONS

Parietal Cells under Basal Conditions

Determination of the pH of the contents of the stomach either in acute experiments or in chronic experiments where unanesthetized dogs were already equipped with gastric cannula revealed that such animals were not actively producing acid.

Intracellular Canaliculi: Intracellular canaliculi, characteristic of the parietal cell, are always evident in micrographs of this cell type obtained from the non-acid-secreting gastric mucosa (Figs. 3 and 4). The canaliculus may be patent (Fig. 3) or collapsed (Fig. 4). The walls of the canaliculus are continuous with the plasma membrane of the cell, and the contents of the canaliculi communicate with intercellular canaliculi between adjacent mucous neck cells (32, 39). These intracellular canaliculi are lined with finger-like projections of cytoplasm, or microvilli (Figs. 3 and 4), which show some variation in shape and arrangement. Sometimes these projections are elongate but on other occasions they may be blunt. Regions in the walls of a canaliculus may be entirely devoid of microvilli. Areas of cytoplasmic matrix surrounding the intracellular canaliculi as well as the contents of the microvilli display a greater electron scattering than other areas of cytoplasmic matrix (Figs. 3 and 4). The significance of this cytoplasmic differentiation remains obscure.

Vesicular Component: The mammalian parietal cell typically exhibits a system of smooth surfaced elements in its cytoplasm that assume the forms of vesicles, tubules, or vacuoles (32, 33, 2, 38, 17, 13, 19, 40, 39). These elements show much variability in shape, and range in diameter from 40 mμ to 150 mμ (Figs. 3 and 4). In material obtained from dogs in chronic experiments, the elongated profiles predominate (Fig. 3), whereas in the cytoplasm of parietal cells obtained in acute experiments more nearly circular profiles are more abundant (Fig. 4). It is at present uncertain whether the effects of anesthesia could account for these differences. Some of the smooth surfaced vesicular and tubular elements appear interconnected in micrographs, but whether all of them form the reticulum described by Palade (22) remains without answer for the moment. The most characteristic feature of the parietal cell from the non-acid-secreting gastric mucosa is the random distribution of these smooth surfaced profiles. These elements show no preferred orientation with respect to an intracellular canaliculus and are observed in areas of the cytoplasm at some distance from canaliculi (Figs. 3 and 4).

Golgi Apparatus: Infrequently, in micrographs of parietal cells, groupings of membrane-bounded elements are distinguishable because of their characteristic arrangement. This configuration consists of a parallel array of several elongated profiles with associated vesicular elements (Fig. 4); this micrograph shows the grouping adjacent to the basal plasma membrane of the cell. The configuration described represents the Golgi apparatus of the parietal cell and is similar in fine structure

to the apparatus in other cell types (3, 4). However, in this cell type the Golgi complex is not localized in special regions of the cell, *i.e.* on the apical side of the nucleus close to the nuclear envelope, but is found in the cytoplasm in random positions. Occasionally, the Golgi apparatus is seen in a given section in more than one locus, suggesting that the organelle may consist of multiple units in the parietal cell. A Golgi complex consisting of a structure similar to that reported here has been described in the gastric parietal cell of the rat (32, 19), but other studies have failed to demonstrate it in the gastric parietal cell of the mouse (17, 13). Hally (14), using techniques of electron microscopy and postosmication methods, has identified the Golgi apparatus of the gastric parietal cell of the mouse with the agranular reticulum or vesicular component of the cytoplasm, because these elements blacken and become more electron opaque after postosmication as do the Golgi apparatuses in other cell types.

Mitochondria: Mitochondria are particularly numerous in the cytoplasm of the parietal cell of the dog's gastric glands. Characteristically, as reported for mammalian (32, 33, 2, 38, 17, 13, 19, 40, 39) and amphibian (35, 40, 36) oxyntic cells, the mitochondria display a dense matrix in which are embedded numerous and closely approximated cristae mitochondriales (Figs. 3 and 4). The mitochondrial profiles appear to be distributed at random in the cytoplasm of the cell. The mitochondria of the parietal cell have a fine structure similar to that already described for a number of animal cell types (21).

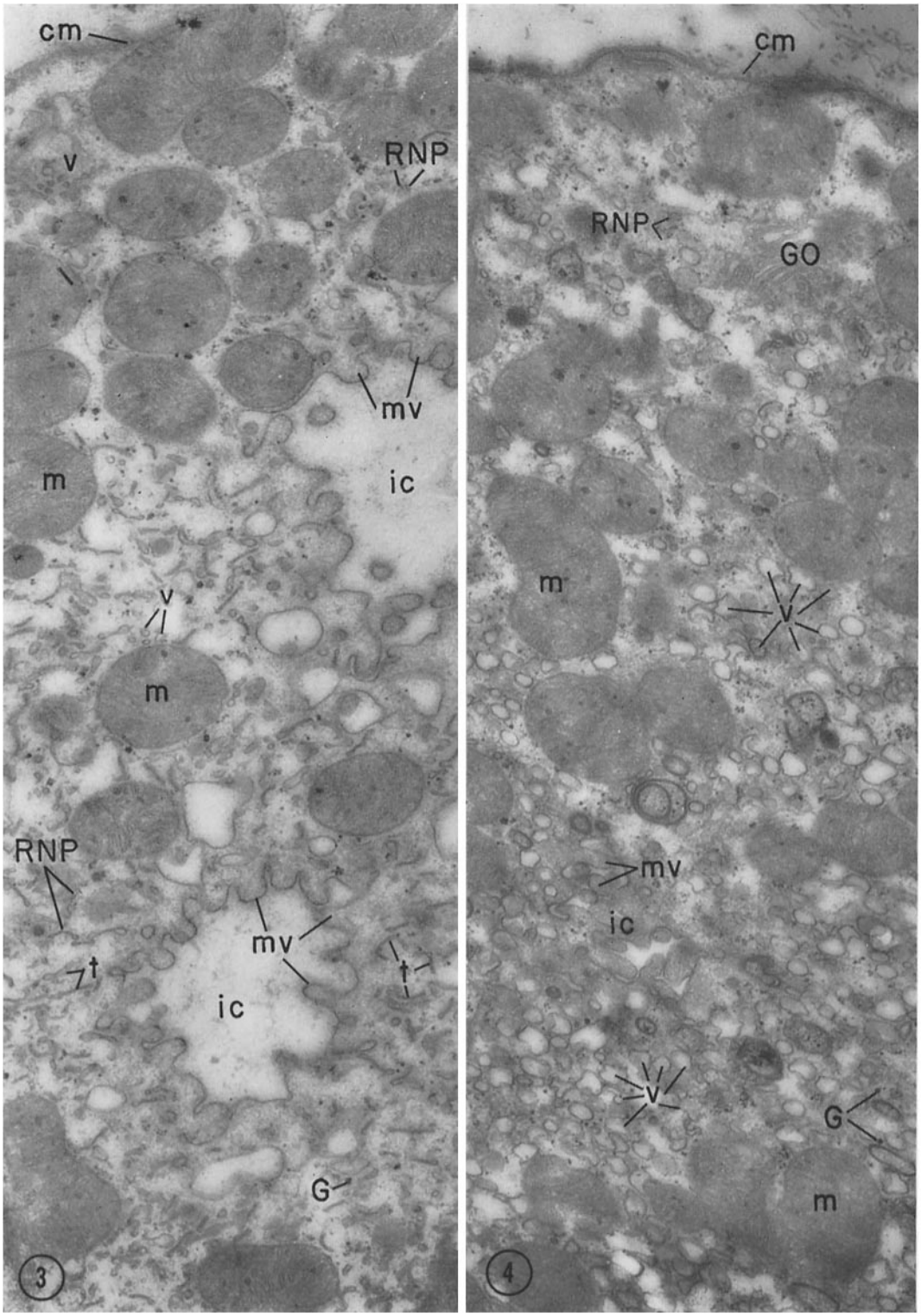
Rough Surfaced Profiles, RNP Granules, and Presumptive Glycogen Granules: These elements are always

FIGURE 3

An electron micrograph of part of a parietal cell from a gastric gland of a dog equipped with a permanent gastric cannula. The biopsy specimen of gastric mucosa was obtained under conditions of no anesthesia and absence of basal acid secretion. Sections through intracellular canaliculi (*ic*) characteristic of the parietal cell are depicted. These canaliculi are lined with projections of cytoplasm, or microvilli (*mv*), which show little regularity in spacing or shape. The cytoplasmic matrix contained within the microvilli exhibits greater electron scattering than other areas of cytoplasmic matrix. A system of smooth surfaced tubules (*t*) and vesicles (*v*) is seen scattered randomly in the cytoplasmic matrix. Mitochondria (*m*), particularly abundant in this cell type, contain a dense matrix in which are embedded numerous closely approximated cristae mitochondriales. In addition, groups of isolated RNP granules (*RNP*) and larger dense granules (400 A) identified as glycogen (*G*) are observed. The basal cell membrane is indicated (*cm*). × 22,000.

FIGURE 4

Micrograph of part of a parietal cell obtained from the non-acid-secreting gastric mucosa. The dog used in this experiment was under pentobarbital anesthesia. The biopsy specimen of gastric mucosa was taken through a gastric fistula after determining an absence of basal acid secretion during a 30 minute control period. An intracellular canaliculus is indicated at *ic*; cytoplasmic projections or microvilli (*mv*), containing material of some density, partially occlude the lumen of the canaliculus. Mitochondria (*m*) contain closely packed cristae embedded in a dense matrix; occasional dense intramitochondrial granules are also seen. A large number of smooth surfaced vesicular elements (*v*) are distributed in a random fashion in the cytoplasm. Nearly circular profiles predominate in this specimen in contrast to Fig. 3, where there is a greater number of tubular elements. In this cell type the Golgi apparatus occupies an unpolarized position and is found in multiple units in the cytoplasm. Here one such unit is observed (*GO*) in the vicinity of the basal cell membrane (*cm*). The unit of the Golgi complex consists of a grouping of a parallel array of smooth surfaced profiles and associated smooth surfaced vesicles. Freely scattered RNP particles (*RNP*) as well as presumptive glycogen granules (*G*) are also shown. × 22,000.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found in the cytoplasm of the parietal cell. The rough surfaced profiles studded with RNP granules are not numerous and are found scattered randomly in the cytoplasm. Although the RNP component displays little prominence in the cytoplasm of the parietal cell, occasional groupings of RNP granules are distributed freely (Figs. 3 and 4). The paucity of RNP granules correlates well with the acidophilic staining reaction of the cytoplasm seen in light microscope preparations. Larger granules (400 A; *G*, Figs. 3 and 4) are identified as glycogen deposits since granular glycogen deposits are stained with lead in other cell types (43).

Parietal Cells during Secretion of Gastric Acid

Stimulation with Histamine: CHRONIC EXPERIMENTS. The results of a typical experiment using histamine as a stimulus for the production of an acid gastric juice are indicated in Fig. 1. The data show the values for the total acidity, free acidity, and volume of gastric juice at intervals of 15 minutes during the course of the experiment, which lasted 155 minutes. A biopsy specimen of gastric mucosa (to be described below) was obtained through the cannula immediately after the collection of the last sample of gastric juice. It can be observed that during the period prior to biopsy, the animal was secreting gastric juice at a rate that varied between 20 and 30 ml per 15 min. The titration data in Fig. 1 show that this gastric juice was reasonably high in both free and total acidity. Accordingly it seems justifiable to assume that a large number of the parietal cells studied in electron micrographs of the biopsied mucosa represent cells engaged in secretory activity. Figs. 5 and 6 demonstrate parts of parietal cells observed in thin sections of this biopsy specimen.

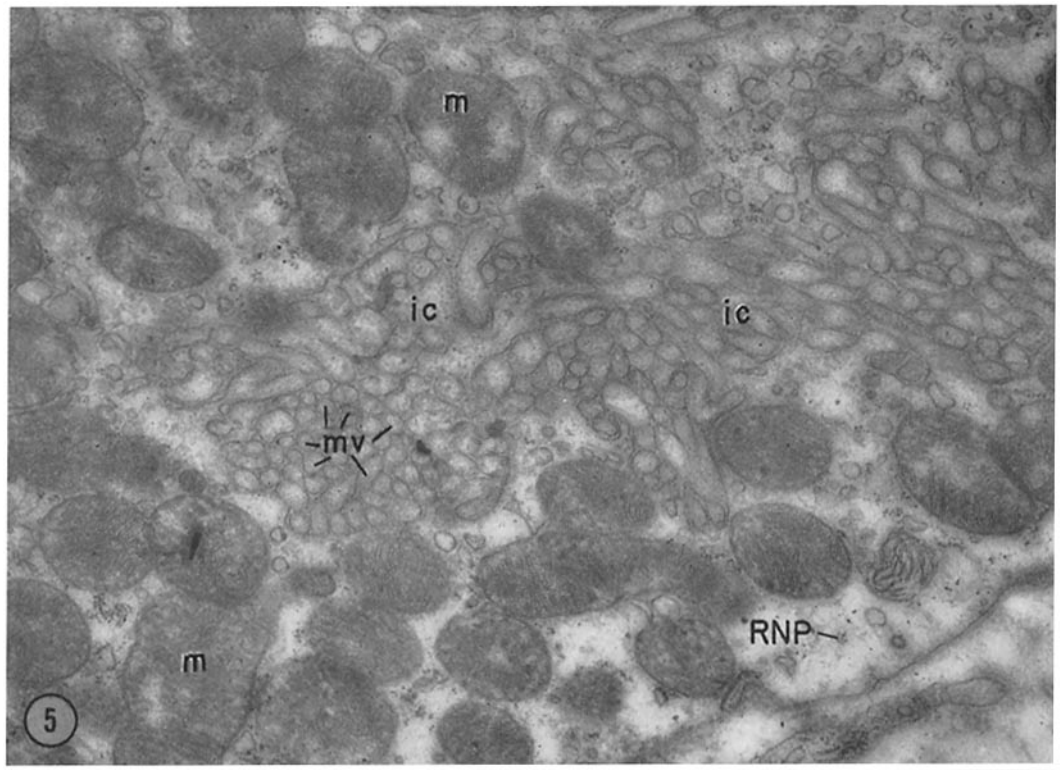
It should be emphasized that in such biopsy material all parietal cells do not exhibit the same pattern of fine structure. Our observations are based on ~65 to 75 per cent of the parietal cells which showed a marked departure from the basic pattern of fine structure observed in oxyntic cells from the non-acid-secreting gastric mucosa.

FIGURE 5

This micrograph shows part of a parietal cell from the gastric mucosa biopsied during the secretion of an acid gastric juice induced by administration of histamine. An unanesthetized dog equipped with a permanent gastric fistula was used in this experiment. The physiological data (total acidity, free acidity, and volume of gastric juice secreted per unit time) which correlate with this specimen are shown in Fig. 1. Examination of these data shows that gastric acid was being produced at the time the sample of gastric mucosa was taken. The number of smooth surfaced vesicles has decreased in the cytoplasmic matrix as compared with that in the parietal cells obtained from the non-acid-secreting gastric mucosa (Figs. 3 and 4). An intracellular canaliculus (*ic*) is particularly prominent in the micrograph since the surface area of this canaliculus has been increased by the formation of a large number of closely apposed microvilli (*mv*). Mitochondrial profiles (*m*) in some instances show areas of less density. Scattered groupings of RNP particles (*RNP*) are present in the cytoplasmic matrix. × 22,000.

FIGURE 6

Part of a parietal cell and a tiny part of an adjacent mucous neck cell (*mu*) are shown in this micrograph. The specimen of gastric mucosa was obtained from a chronic animal stimulated with histamine to produce an acid gastric juice. The physiological data recorded prior to removal of the tissue are given in Fig. 1. An intracellular canaliculus (*ic*) shows many closely spaced microvilli (*mv*) projecting into its lumen; some of these finger-like projections of cytoplasm are sectioned end-on. Elaboration of membrane lining intracellular canaliculi appears to be a constant feature of the stimulated parietal cell. Note that the cytoplasmic matrix peripheral to the canaliculus contains very few smooth surfaced vesicular elements. Compare this with Fig. 7, which probably represents a cell at a less advanced stage of secretion. Mitochondrial profiles (*m*) and groups of RNP particles (*RNP*) are also indicated in the micrograph. × 22,000.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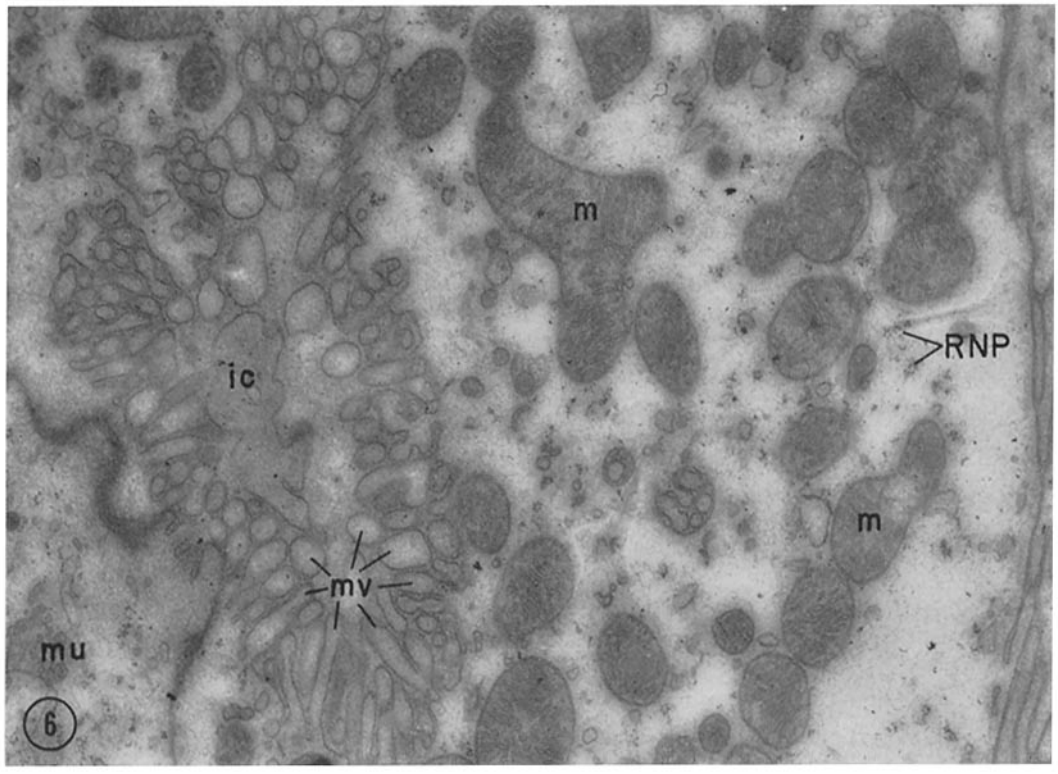
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The changes in fine structure in the cytoplasm of the parietal cell during stimulation of acid secretion with histamine affect mainly two systems, the vesicular component and the intracellular canaliculi, although some subtle changes appear also in the mitochondria. The number of smooth surfaced vesicles and tubules decreases markedly in the cytoplasmic matrix (Fig. 5). Cytoplasmic smooth surfaced profiles are observed in greater numbers adjacent to intracellular canaliculi as compared with more peripheral regions of cytoplasm. Characteristically the intracellular canaliculi become more prominent in the micrographs of parietal cells; the surface area of these canaliculi is increased by the development of a large number of closely approximated microvilli or finger-like extensions of cytoplasm (compare Figs. 5 and 6 with Figs. 3 and 4). In a number of instances areas of decreased electron scattering are seen within the matrix of mitochondrial profiles (Figs. 5 and 6). Groupings of RNP particles are observed scattered freely in the cytoplasm (Figs. 5 and 6).

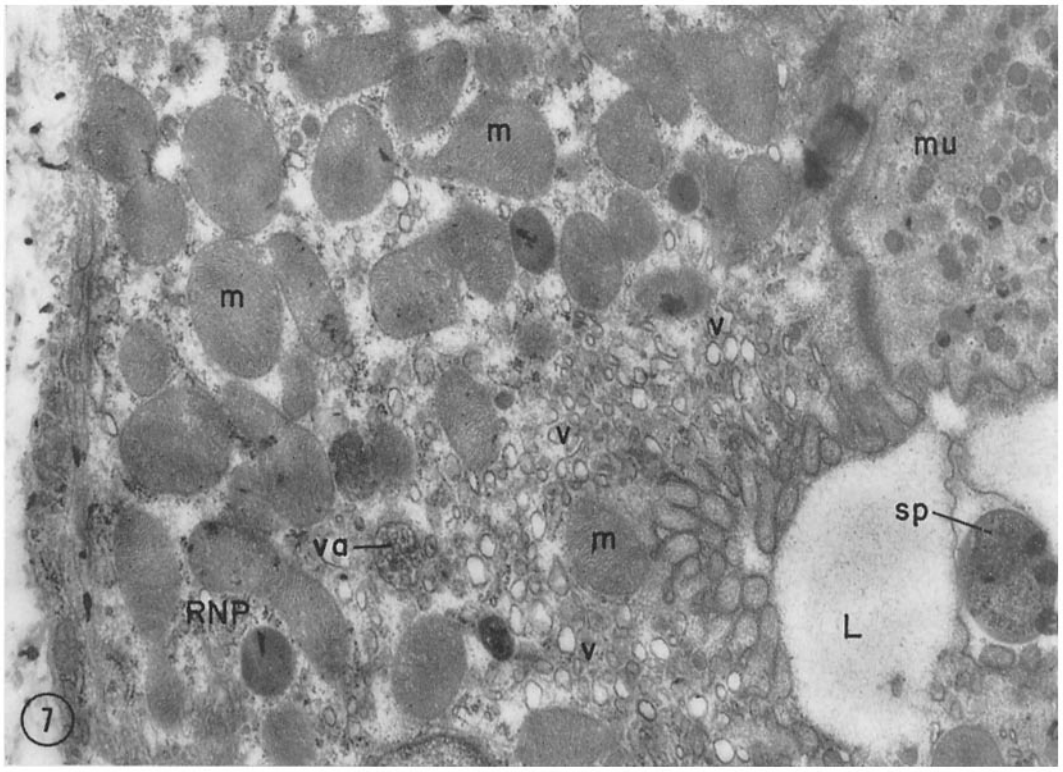
ACUTE EXPERIMENTS. The results obtained with animals used in acute experiments are similar to those described above for chronic experiments. Changes in fine structure also involve the intracellular canaliculi and the volume of the vesicular component. Fig. 7 represents a micrograph of part of a parietal cell and an adjacent mucous neck cell from a biopsy of gastric mucosa taken during the course of an acute experiment. Titration analysis of the gastric juice sample collected just before biopsy shows a total acidity of 70 mEq/L. In this instance the majority of parietal cells of the biopsy show less pronounced alterations in the vesicular component (Fig. 7). Here (Fig. 7) a greater number of smooth surfaced elements are

FIGURE 7

An electron micrograph of part of an oxyntic cell and an adjacent mucous neck cell (*mu*) facing the lumen (*L*) of an intercellular canaliculus which would communicate with the lumen of a gastric gland. The biopsy specimen of gastric mucosa was obtained from an animal stimulated with histamine in an acute experiment. Titration analysis of the gastric juice collected just prior to biopsy demonstrated a total acidity of 70 mEq/L, indicating that the dog was not producing a secretion particularly high in acid content. Examination of the parietal cells in this biopsy specimen shows that the majority of cells exhibit less obvious fine structural changes than do parietal cells of dogs producing gastric juice of higher acid content (compare with Fig. 6). A greater number of smooth surfaced vesicles (*v*) is seen concentrated in the cytoplasm adjacent to the wall of a canaliculus at the apical surface of the cell. More peripheral regions of cytoplasm show fewer vesicles. Mitochondria (*m*) in large numbers, clusters of RNP particles (*RNP*), and a vacuole containing a number of vesicles (*va*) are also observed in the cytoplasm. Within the intercellular canaliculus is observed a profile of what is presumed to be a spirillum (*sp*), since such organisms have been reported in the stomach of the dog and cat (40); occasionally such profiles of spirilla are found within the lumina of intracellular canaliculi. × 22,000.

FIGURE 8

Micrograph of part of a parietal cell obtained from a biopsy specimen of gastric mucosa from a chronic animal stimulated with insulin to produce an acid gastric juice. Data on the total acidity, volume, and pH of the gastric juice collected prior to biopsy of the specimen are indicated in Fig. 2. A section of a prominent intracellular canaliculus (*ic*) lined with many closely approximated microvilli (*mv*) is evident in the micrograph. Very few smooth surfaced profiles are seen in the cytoplasmic matrix (compare with Figs. 3 and 4) although clusters of RNP particles (*RNP*) and an occasional rough surfaced profile (*rs*) are observed. Mitochondrial profiles (*m*) are numerous; some of these show areas lacking cristae. A small part of a nucleus (*N*) is seen in the lower right corner of the figure. The basal plasma membrane is indicated (*cm*); its surface is irregular, being composed of a number of cytoplasmic projections. Such configurations are encountered also in the non-acid-secreting parietal cell. × 22,000.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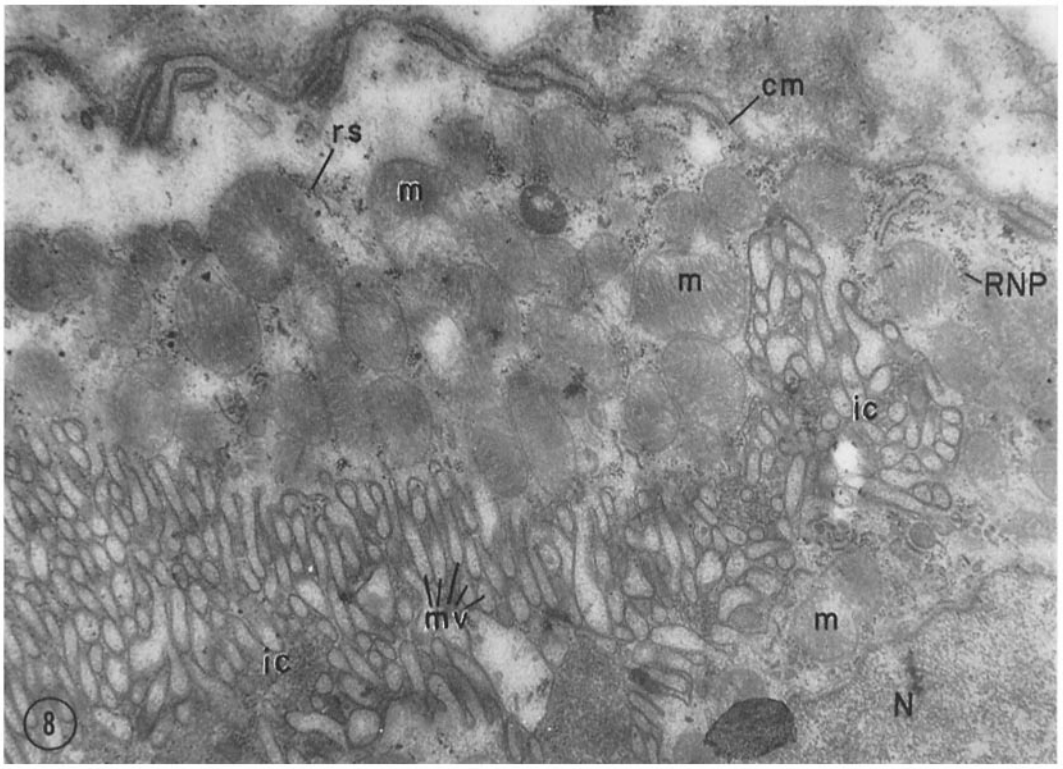
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seen adjacent to an intracellular canaliculus than in Fig. 6. It seems reasonable to suppose that the cell depicted in Fig. 7 represents a less advanced stage in the secretory cycle.

Stimulation with Insulin: It is well known that administration of insulin provokes the secretion of an acid gastric juice (8). Presumably insulin exerts its action by producing a hypoglycemic condition which affects the gastric secretory vagus center in the medulla.

The results of a typical experiment involving insulin stimulation of gastric secretion in a chronic animal are shown in Fig. 2. Here the total acidity (mEq/L) and the volume of the gastric juice are plotted against time (minutes). The titration data show that the output of acid during the 45 minute period preceding biopsy of the gastric mucosa was even higher than after histamine stimulation (Fig. 1); the volume of gastric juice (25 ml per 15 min.) is similar to that in the histamine experiment. On the basis of the data in Fig. 2, it is reasonable to assume that the majority of the parietal cells in the biopsy specimen were in the stage of active secretion.

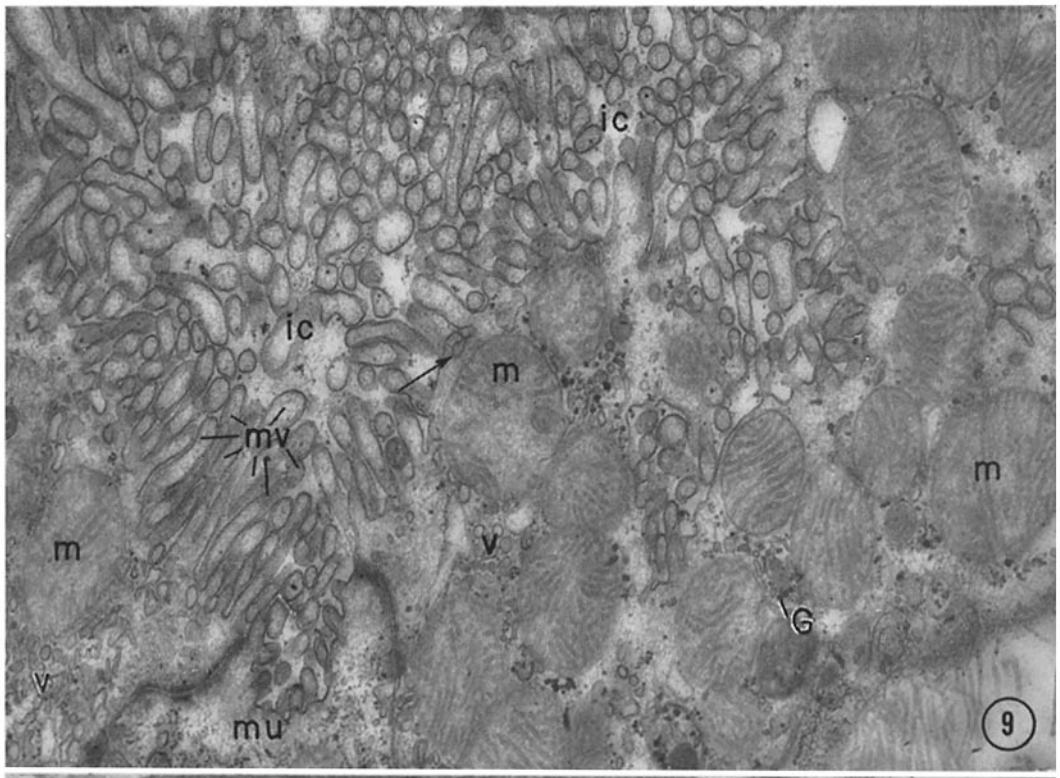
Examination of electron micrographs of the gastric glands from the biopsy specimen shows that ~70 to 80 per cent of the parietal cells demonstrate a definite alteration of fine structure. These changes (Fig. 8), although similar in general respects to those observed in histamine-stimulated dogs, are even more obvious. Very few smooth surfaced vesicular elements are evident in the cytoplasmic matrix of the parietal cell, even in the vicinity of an intracellular canaliculus (Fig. 8). Intracellular canaliculi become more obvious in the micrographs evidently because of their more

FIGURE 9

Part of a parietal cell and a tiny part of the apical region of a mucous neck cell (*mu*) are seen in this micrograph. The thin section was obtained from a biopsy specimen of gastric mucosa of a dog producing gastric acid in response to electrical vagal stimulation; the sample of gastric juice collected just prior to biopsy indicated a total acidity of 130 mEq/L. Note the extensive development of an intracellular canaliculus (*ic*) seen in the figure. Many closely approximated microvilli (*mv*) line the lumen of this channel within the cytoplasm of the cell, increasing its surface area. A number of smooth surfaced vesicles (*v*) are present in the cytoplasm adjacent to the canaliculus (*ic*); sometimes a vesicle is observed to communicate with an area of the canalicular wall between adjacent microvilli (see arrow). Mitochondrial profiles (*m*) appear to contain a less dense matrix and are devoid of cristae in some areas. Compare with the mitochondria of control preparations (Figs. 3 and 4). Large dense granules (*G*) are presumed to be glycogen deposits. Clusters of smaller dense granules represent the ribonucleoprotein component of both the parietal and the mucous neck cell. × 22,000.

FIGURE 10

Micrograph of part of a parietal cell from the gastric mucosa of a dog secreting gastric acid in response to electrical vagal stimulation. The total titratable acidity of the gastric juice sample collected before biopsy showed a value of 130 mEq/L. The fine structural pattern represented here indicates that probably not all parietal cells within the gastric glands of the mucosa are in the same stage of secretory activity during secretion of an acid gastric juice. Intracellular canaliculi (*ic*) depicted are collapsed, resulting in the close approximation of cytoplasmic projections; the adjacent membranes are separated by a distance of only 250 A. In general, the cytoplasmic matrix contains fewer smooth surfaced vesicles (compare with Figs. 9 and 4). A grouping of smooth surfaced profiles and associated vesicles representing the Golgi complex (*GO*) in this cell type demonstrate no apparent change in structure when compared with the organelle in the non-acid-secreting parietal cell (see Fig. 4). Mitochondria (*m*) are similar to those illustrated in Fig. 9, showing areas of decreased electron scattering within the mitochondrial matrix, areas lacking cristae, and a slightly swollen appearance (compare with Figs. 3 and 4). Other items depicted in the micrograph include: a vacuole containing vesicles (*va*), RNP granules (*RNP*), and an occasional rough surfaced profile (*rs*). × 22,000.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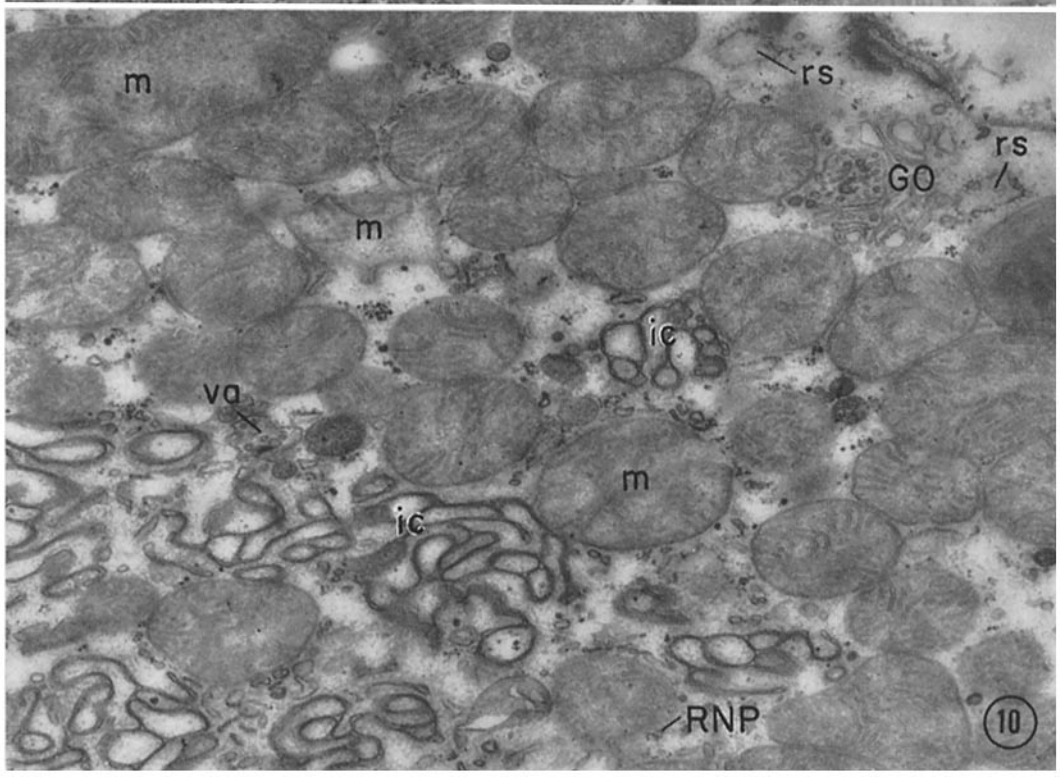
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extensive development. The surface area of the walls of these canaliculi is greatly increased by the formation of a large number of closely apposed microvilli (Fig. 8; compare with Fig. 3). These finger-like projections of cytoplasm, delimited by the plasma membrane of the cell, are more elongated (Fig. 8) than those observed in the non-stimulated parietal cell (Figs. 3 and 4). Some mitochondrial profiles show areas of less density within the mitochondrial matrix (Fig. 8). Freely scattered groupings of RNP granules and occasional rough surfaced profiles are also evident in the cytoplasm (Fig. 8).

Electrical Vagal Stimulation: During acid secretion induced by electrical vagal stimulation in acute experiments, parietal cells examined from biopsy specimens of gastric mucosa show changes similar to those observed in either histamine or insulin stimulation experiments. Fig. 9 and 10 illustrate parts of parietal cells from such a biopsy preparation of gastric mucosa. In this case analysis of the gastric juice collected just before biopsy showed a total acidity of 130 mEq/L. These micrographs demonstrate that parietal cells within the gastric glands of the stomach can exhibit different fine structural patterns during active stimulation of an acid gastric juice. Fig. 9 depicts the extensive development of microvilli lining the walls of a prominent and patent intracellular canaliculus. On the other hand, the part of the parietal cell shown in Fig. 10 demonstrates that in another stage of the secretory cycle the intracellular canaliculi lose their prominence. Here (Fig. 10) the walls of the canaliculi appear collapsed so that the projections of cytoplasm from the walls of the canaliculi closely approximate one another. The number of vesicular profiles in the cytoplasm adjacent to the walls of the canaliculus is greater in Fig. 9 than in Fig. 10. A percentage of the parietal cells (~20 per cent) does not demonstrate such marked changes in fine structure, and resembles more closely examples of parietal cells obtained from non-acid-secreting stomachs.

Mitochondria in oxyntic cells under the influence of electrical vagal stimulation show, in a number of instances, a decrease in matrix density as well as areas that are devoid of cristae (Figs. 9 and 10). Mitochondrial profiles exhibit a somewhat swollen appearance as compared with mitochondria in non-acid-secreting parietal cells (Figs. 3 and 4). Since this swelling effect was not especially pronounced in either the histamine or the insulin stimulation experiments, its significance remains obscure. The Golgi apparatus (Fig. 10) occasionally seen in secreting parietal cells appears to maintain a structure similar to that of non-secreting cells. Scattered RNP granules and occasional rough surfaced profiles are evident in the cytoplasm of the secreting cell (Figs. 9 and 10).

DISCUSSION

The observations presented in this paper demonstrate that the pattern of fine structure in the parietal cell of the gastric glands (corpus of stomach) from the acid-secreting dog's stomach differs markedly from that in the non-acid-secreting stomach. Our attention has been focused in particular on the smooth surfaced vesicular component, the intracellular canaliculi, and the mitochondria in the cytoplasm of the cell. During minimum secretory activity, when little, if any, hydrochloric acid is being produced, the smooth surfaced profiles are randomly distributed in the cytoplasmic matrix. Under conditions of gastric acid secretion induced by administration of histamine or insulin or by electrical stimulation of the vagus nerves, the distribution and volume of the vesicular component change. A greater concentration of smooth surfaced profiles is found adjacent to the walls of intracellular canaliculi; other parietal cells in the same specimen show a marked decrease in the number of smooth surfaced elements, so that this component loses prominence in the cytoplasmic matrix. Intracellular canaliculi, although obvious in non-acid-secreting parietal cells, appear to develop more extensively in many of the cells from the acid-secreting gastric glands. At the same time, the surface area of these canaliculi is greatly increased by the elaboration of a large number of closely approximated and elongated microvilli. In the same specimen, other parietal cells from the acid-secreting gastric mucosa may exhibit non-patent canaliculi, lacking in prominence. Usually such cells have very few smooth surfaced profiles. Changes in mitochondrial structure in the parietal cell during acid secretion appear less obvious than the alterations in either the canaliculi or the vesicular components of the cell. During secretory activity mitochondrial profiles exhibit a decrease in density of the mitochondrial matrix, less closely approximated cristae, and areas within the profiles where cristae are lacking. Some of the preparations show

slightly swollen mitochondria. A small percentage of parietal cells observed in specimens obtained from acid-secreting stomachs show less marked structural alteration, and resemble more closely the cell type from the non-acid-secreting gastric glands. On the basis of their fine structural characteristics it appears, therefore, that parietal cells can exhibit more than one stage of secretory activity. Since the majority of parietal cells show an alteration of fine structure during stimulation of gastric acid secretion by a number of different techniques (electrical vagal stimulation, histamine administration, and insulin injection), it can be assumed that the cell plays a role in the secretion of hydrochloric acid.

The structural findings in the mammalian gastric parietal cell during secretion of gastric acid described above are in agreement with those already reported in the literature. Similar data were obtained where gastric secretion was provoked with insulin in the rat (34), histamine in the dog (34, 38) or cat (40), and electrical vagal stimulation in the dog (39). Vial and Orrego (40), using the cat, waited only a maximum of 30 minutes after administration of histamine before biopsy of the gastric mucosa; the pH of the mucosal surface was used as an indicator of functional activity. These workers noted an increase in the surface area of the canaliculi and a decrease in the number of vesicles in the cytoplasm of the parietal cell. These authors failed to report observations on cells demonstrating a concentration of vesicular components adjacent to canaliculi or collapsed canaliculi with few vesicular elements. Although the acidity of the gastric juice was not reported, Hally (12) has shown that vacuole-containing bodies within the cytoplasm of the gastric parietal cell of the mouse become more numerous and larger in diameter and contain a larger number of vacuoles after repeated injections of pilocarpine nitrate. Infrequently, profiles containing vesicles are encountered in the cytoplasm of the stimulated gastric parietal cell (see Fig. 10), but no obvious fine structural changes are seen in these elements in our specimens.

Experiments designed to show the effect of histamine stimulation on fine structural pattern of the oxyntic cell in amphibia (35, 40, 37) provide further evidence that this cell type is involved in the secretion of gastric juice. Sedar (35, 37) using the bullfrog (*Rana catesbiana*) and Vial and Orrego (40) using the toad (*Bufo spinulosus*) both have demonstrated during acid secretion a decrease in the number of smooth surfaced profiles and an increase of the surface area in the apical region of the cell as the result of extensive development of a network of tubules which communicate with the lumen of the gastric gland. Sedar (37) showed that in a number of oxyntic cells the cytoplasmic vesicular elements were concentrated in the vicinity of this tubular network in the apical surface of the cell. Although the amphibian oxyntic cell does not have intracellular canaliculi, the modification of its apical surface, as compared with that of the non-acid-secreting cell (36), to provide a greater surface area evidently fulfills the same function as the closely approximated microvilli lining the intracellular canaliculi in the stimulated mammalian parietal cell.

The literature on electron microscopy has provided evidence of a variety of morphological patterns which increase effectively the surface area of different cell types (10). The striated border of the intestinal epithelial cell (23) and the brush border of the proximal convoluted tubule cell (31) are examples of closely spaced projections of cytoplasm (microvilli) on the apical surface of the cells. Such configurations are thought to increase the absorptive capacity of these epithelia. Supporting evidence for this comes from the marked degeneration of the microvillous border of the jejunal cell in sprue or coeliac disease (15), where patients exhibit a malabsorption syndrome. Cells involved in fluid transport or secretion show, characteristically, an elaboration of their cell membranes to increase the surface area: The basal parts of the cells of the proximal convoluted tubule (31) and the distal convoluted tubule (26) show a complicated infolding of basal plasma membrane; the ependymal cells of the choroid plexus also show this type of configuration (20). The epithelium of the ciliary body involved in the secretion of aqueous humor exhibits devices for increasing its surface area. These consist of infoldings of the free surface as well as complicated interdigitations of cell membrane between adjacent cells (24). Experimental blockage of aqueous humor secretion produces an alteration of fine structure in the ciliary epithelium involving the disappearance of the cell membrane infoldings and interdigitations (24). Here the structural change can be correlated with a disturbance of normal aqueous humor secretion. The present study on the gastric parietal cell provides further evidence of the importance of

membrane surfaces in relation to physiological activities.

The system of smooth surfaced tubules and vesicles observed in the cytoplasmic matrix of the gastric parietal cells is involved in secretory activity. The fact that the volume and distribution of these vesicular elements change during the production of an acid gastric juice presents morphological evidence for such a possibility. Occasional connections of these vesicles with the free surface of the cell adjacent to the lumen of an intracellular canaliculus suggest that the contents of the vesicles are contributed to the gastric juice. Ingredients of gastric juice, perhaps in bound form attached to a carrier substance, could be compartmentalized within the vesicles or tubules for transport to the secretory surface of the cell. On the other hand, it should be borne in mind that some ions, for example the bicarbonate ion, must leave the capillary side of the cell, so that ions in either the free or the bound form are moving in opposite directions within the cytoplasm of the oxyntic cell during secretory activity. Vial and Orrego (40) have suggested that "the abundant membranous material lining the vesicles is used by the cell to increase its external surface during activity," which could account for the marked increase of surface cell membrane in a short period of time.

The energy for the secretion of hydrochloric acid by the gastric epithelial cells come from aerobic metabolism (8). The rate of respiration of the stimulated parietal cell appears to be among the highest shown by any cell in the body (7). Large numbers of mitochondria containing a high proportion of closely approximated cristae correlate with the need of the oxyntic cell to provide energy for the secretion of the ions in the gastric juice for long periods of time. It has been suggested that mitochondria are associated with the transport of inorganic ions (6). The observed changes in the fine structure of the mitochondria in gastric parietal cells during secretory activity may be related to transmembrane flux of ions or ion-bound substances, but further experimental analysis is needed to establish this possibility.

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