STUDIES ON THE DEPLETION AND ACCUMULATION OF MICROVILLI AND CHANGES IN THE TUBULOVESICULAR COMPARTMENT OF MOUSE PARIETAL CELLS IN RELATION TO GASTRIC ACID SECRETION

S. ITO and G. C. SCHOFIELD

From the Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115 and the Department of Anatomy, Monash University, Melbourne, Victoria, Australia 3168

ABSTRACT

Gastric parietal cells in mice present a spectrum of microscopic appearances due mainly to variations in the abundance of the tubular and vesicular component of the cytoplasm and in the size and number of microvilli lining the intracellular canaliculi. Differences in the range of forms among parietal cells of fasting versus fed mice were not especially striking, but cells with very numerous tubules and vesicles were more common after fasting. However, in mice treated with drugs or hormones that induce acid secretion, parietal cells were more uniform in appearance. There was a marked reduction of these cytoplasmic membranes and a concomitant increase in both the number and size of microvilli. Measurements of acid secretion in control animals and in animals treated with acid secretagogues indicated hydrogen ion secretion contemporaneous with depletion of the cytoplasmic tubulovesicular membranes and with increase of the microvilli. In mice with inhibited acid secretion, parietal cells showed an accumulation of cytoplasmic tubules and vesicles and reduction in the numbers of microvilli. Stereological methods were used to quantitate 10 different parietal cell compartments. Tracer studies with lanthanum did not reveal continuity between the tubules and the plasma membrane. However, there were regions of close apposition between the tubulovesicular membranes and the cell membrane of the canaliculus, and instances where cytoplasmic tubules extended from the cell into the core of enlarged microvilli.

Conspicuous morphological changes in the gastric gland associated with feeding, electrical stimulation, and hormone- or drug-induced gastric acid secretion are those affecting the parietal cells of mammals (1, 8, 13-16, 18-20, 25, 35) or oxyntic cells of amphibians (5, 27, 33, 37) or birds (35). The cellular components which distinguish parietal cells from other secretory cells in the gastrointestinal tract and which presumably are involved in hydrogen ion release are the intracellular canaliculi and a cytoplasmic membrane system variously designated as smooth surfaced endoplasmic reticulum, vacuoles, vesicles, tubulovesicles, vesicotubules, or bulbotubules. In this report we will use the term tubulovesicular system or membranes to describe this cytoplasmic component. In most

mammalian parietal cells the abundant tubulove-sicular membranes show considerable variation from cell to cell in both fasted and fed animals. However, Helander and Hirschowitz (15, 16) using stereological analysis have recently quantitated the marked reduction in the number of cytoplasmic membranes in parietal cells of dogs injected with histamine to produce maximal gastric acid secretion, and Frexinos et al. (8) also demonstrated a significant reduction in the number of cytoplasmic tubules and vesicles in parietal cells of human subjects treated with pentagastrin. In the present study insulin and reserpine were used as the major stimulatory or inhibitory reagents because pentagastrin, histamine, secretin, atropine, and prostaglandin E_2 did not produce changes in cell structure that were as consistent or extensive. Insulin stimulation of acid secretion is generally acknowledged (10, 22, 23, 30). Reserpine is known to cause increased acid secretion in rats (23), but in mice reserpine injection in high doses does not stimulate acid secretion (2).

This study describes the cytological effects on mouse parietal cells of insulin injection, which causes a rapid increase in microvilli and disappearance of cytoplasmic tubules and vesicles, and of administration of high doses of reserpine which results in the accumulation of the cytoplasmic membranes. These morphological changes apparently involving translocation of membrane from tubulovesicular elements in the cytoplasm to the surface of microvilli are correlated with measured hydrogen ion secretion.

MATERIALS AND METHODS

Adult male Swiss-Webster mice (20–35 g) were used and, unless indicated, were fasted overnight in wire-bottom cages with free access to water. Solutions administered were injected intraperitoneally and the animals were killed by cervical dislocation without prior anesthesia. The abdominal cavity was then rapidly opened and the stomach was fixed by injecting fixative into the stomach. The esophagus and duodenum were then clamped while distended with fixative, excised, and immersed in the same fixative. The fixative employed contained 3% formaldehyde, 4% glutaraldehyde, and 0.05% trinitrocreosol in 0.05 M cacodylate buffer pH 7.4 (21). After a period of 5–10 min the glandular part of the stomach in the region of the greater curvature was cut into 1 × 5-mm wedges with the narrow base adjacent to the junction of the forestomach and the glandular stomach. The tissues were fixed for 1–2 h at room temperature, rinsed in 0.1 M cacodylate buffer, pH 7.4, and postfixed for 1–2 h in 1% osmium tetroxide in the same buffer. The tissue was then treated with 1% uranyl acetate in maleate buffer for ½–1 h. After dehydration in a graded series of increasing concentrations of cold ethanol, the tissue was embedded in a mixture of Epon-Araldite in plastic capsules. The thin sections were stained with uranyl acetate and lead citrate and examined in the electron microscope. Some samples were treated with lanthanum hydrosol by the method of Revel and Karnovsky (29) and embedded in the usual manner.

For preparing replicas of freeze-fractured gastric mucosa, mouse stomachs were fixed for 20–30 min in the aldehyde mixture described above or in 2% paraformaldehyde, 2.5% glutaraldehyde fixative in cacodylate buffer at pH 7.4 and containing 2 mg/ml $CaCl_2$. All fixation was carried out at room temperature. The tissue was washed in cacodylate buffer, and infiltrated for 2 h with 20% buffered glycerol at room temperature, trimmed to appropriate size, frozen in liquid Freon 22 (chlorodifluoromethane), and stored in liquid nitrogen. Specimens were fractured in a Balzers apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.) and the replicas were cleaned in hypochlorite solution (Clorox) and collected on grids after several distilled water washes.

Crystalline reserpine (Sigma Chemical Co., St. Louis, Mo.) or a proprietory preparation Serpasil (Ciba Corp., Summit, N.J.) was diluted to appropriate concentrations. A maximum dose of 15 mg/kg body weight and lower concentrations of 10, 5, 2.5, 1, 0.5, 0.1, 0.05, and 0.01 mg/kg were used. Intervals between injection and fixation of the stomach varied from 10 min to 96 h.

The insulin used was Iletin (Eli Lilly and Company, Indianapolis, Ind.) U.S.P., 40 units/ml diluted with distilled water to appropriate concentrations and injected subcutaneously or intraperitoneally in doses of 5, 1, or 0.1 U/kg. The stomachs were fixed at intervals between 10 min and 11 h after injection and the tissues were prepared for electron microscopy as described above. Other drugs used to produce changes in the parietal cells included pentagastrin (Pentavlon, I. C. I. Organics, Inc., Stammford, Conn.), histamine phosphate, 5-hydroxytryptamine, atropine sulfate, secretin, and prostaglandin E_2.

Assessment of hydrogen ion secretion by the mouse stomach was determined by modifying the method of Gosh and Schild (9) for recording gastric acid secretion in the rat. The mice were anesthetized with 0.2–0.35 ml of a 25% solution of urethane injected intraperitoneally or subcutaneously. The trachea was cannulated with polyethylene tubing and a second polyethylene tube was inserted into the esophagus and ligated in the neck, taking precautions to exclude the vagus nerves. The abdomen was opened through a midline incision and a polyethylene tube was passed into the stomach through the duodenum and tied firmly in place at the pyloroduodenal junction. After closure of the abdomen, 250 mM glycine buffer solution (pH 6.15) or unbuffered 154 mM sodium chloride solution was perfused through the stomach at a constant rate of 9.12 ml/h, using a 20 ml
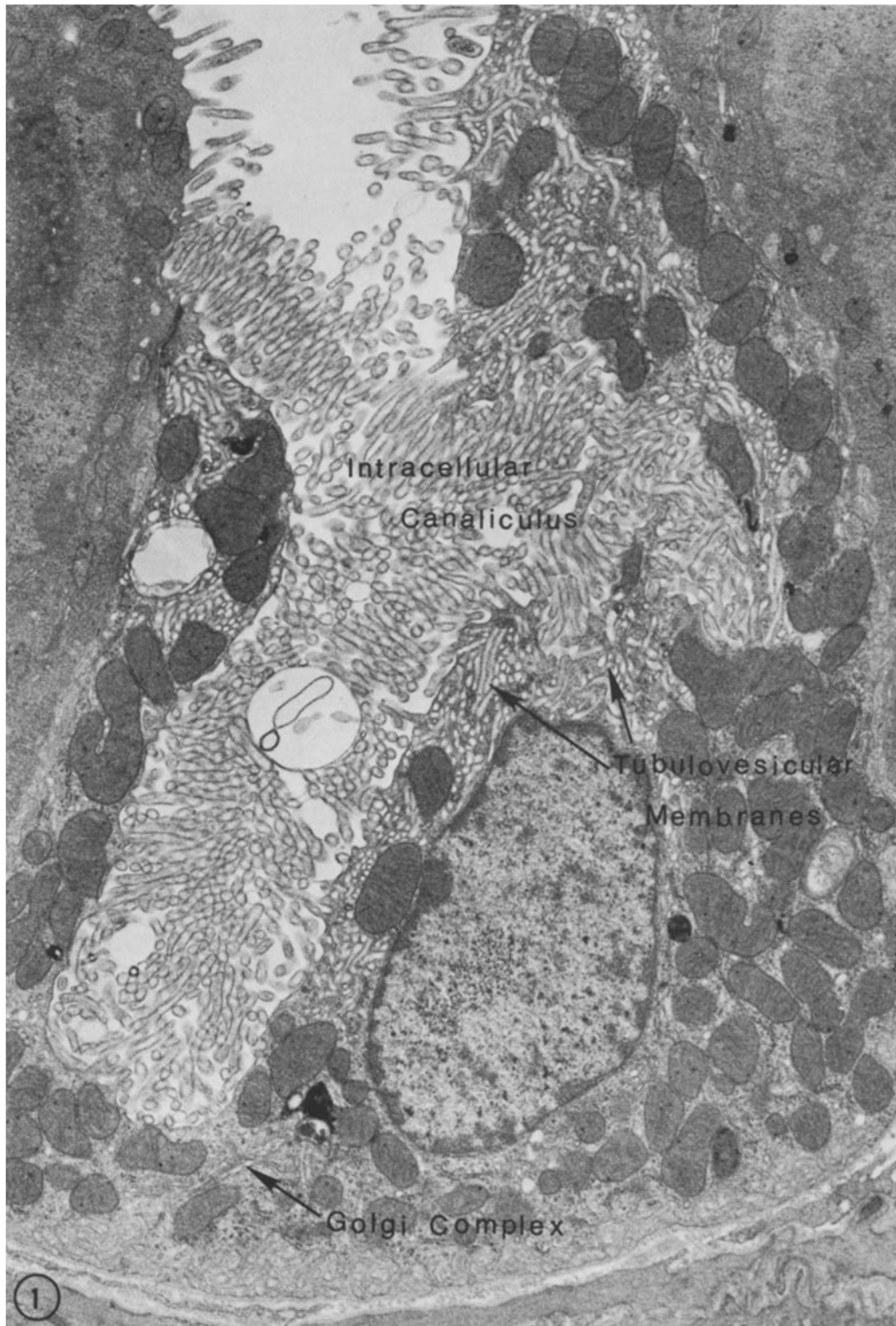
FIGURE 1 An electron micrograph of a parietal cell from a gastric gland of a normal nonfasted mouse. The cell depicts the general features found in parietal cells from a moderately actively secreting stomach. The intracellular canaliculi is extensive and is almost occluded with numerous, long microvilli. An area of the cytoplasm above and to the left of the nucleus and the apical cytoplasm is packed with moderate amounts of cytoplasmic tubulovesicular membranes. The remaining cytoplasm contains an abundance of large mitochondria, and two small Golgi complexes are present in the basal cytoplasm. Small clusters of rough-surfaced endoplasmic reticulum are also present. Between the mitochondria-rich cytoplasm and the basal lamina there is a zone occupied by closely interdigitated basal folds. × 9,000.

syringe connected to the esophagus and driven by a Harvard Apparatus Co. Compact Infusion Pump Model 975 (Harvard Apparatus Co., Inc., Millis, Mass.). After an initial washout with 2 ml of perfusate, fluid was collected at 15-min intervals. The pH of the collected samples, which ranged in volume from 2.0 to 2.4 ml/15 min, was measured with a pH meter using combined electrodes. The total hydrogen ion concentration present in each sample was determined by titration against 0.001 N NaOH. Collections were made for periods of up to 6 h; the stomach was then fixed for electron microscopy.

To determine the relative volume occupied by the various parietal cell components, a modified Chalkley method (3) was employed. Using randomly selected electron micrographs, an overlay with plotted random points was used to score hits on 10 different compartments of parietal cells.

RESULTS

In Normal Animals

Parietal cells in normal adult mice with free access to food had prominent intracellular canaliculi lined by microvilli and surrounded by cytoplasm rich in mitochondria and membrane-limited tubular or tubulovesicular structures (Figs. 1 and 2). There was, however, considerable variation in the appearance of parietal cells in normal mice. These involved differences in the patency of the lumen of the intracellular canaliculi, the size and number of microvilli, and the amount of smooth surfaced tubules in the cytoplasm. Some cells were replete with tubules (Fig. 2), many cells had few and, in some cells tubulovesicular membranes were all but absent.

The variation in parietal cell morphology in mice given free access to food was interpreted to represent variations in secretory activity among different cells. In mice fasted up to 24 h there was an apparent increase in the numbers of parietal cells containing numerous cytoplasmic tubules, but the range of morphological appearances at any stage during fasting was no less than that seen in fed mice. Furthermore, variations among similarly treated animals made it difficult to obtain consistent observations.

Stereological measurements of various cell components were made on micrographs of tissues taken from mice in various nutritional states, but it was found that the differences were not consistent. To establish a control value for this study, mice were fasted for 4, 8, 12 and 24 h; and pooled data are presented in Table I. The cytoplasmic tubulovesicular system (18.1%), microvilli (14.7%), and

cytoplasmic matrix (21.6%) are the structures of primary relevance to this study. Values for nuclei, mitochondria, and other organelles are also listed, but they are of less interest because they do not change significantly in the experimental animals. The relative volumes of various parietal cell compartments in an anesthetized control animal subjected to gastric perfusion, with monitoring of acid secretion over a period of 4 h but not otherwise treated, are also included in Table I.

The large, prominent mitochondria had numerous cristae and varying numbers of intramitochondrial granules, but there seemed to be no clear correlation between the physiological activity of the stomach, varied morphology of the parietal cell, and mitochondrial configuration. In some cells the mitochondria were in the typical orthodox configuration, while in others they were in the condensed or activated state (11). The mitochondria were found adjacent to all plasma membranes but were most closely and consistently associated with the intracellular canaliculi. Some parietal cells had a number of dense bodies, presumed to be lysosomes. A structure regularly encountered in the parietal cell cytoplasm was the multivesicular body. These bodies varied from very small vacuoles containing a few vesicles to larger bodies that contained a dozen or more vesicles. The multivesicular bodies were usually found between the central mass of cytoplasmic tubules and the mitochondria-rich peripheral cytoplasm. They were in close association with the tubulovesicular system and sometimes contained some of these tubules. Occasionally, distorted mitochondria were also found in the multivesicular bodies.

The nuclei of parietal cells were not distinctive and they constituted about 6% of the cell volume. The multivesicular bodies and the dense lysosome-like bodies each occupied less than 1% of the cell volume. Golgi complexes of parietal cells were small and relatively inconspicuous. They were located near the lateral or basal cell membrane and consisted of four to five flattened parallel cisternae with few associated vesicles. Several, widely separate Golgi complexes were often seen in a section of a single cell. The cytoplasmic matrix had a moderate number of clustered polyribosomes and short arrays of rough-surfaced cisternae of endoplasmic reticulum. Glycogen granules were not abundant in mouse parietal cells. Fine cytoplasmic filaments were regularly encountered, but microtubules were only infrequently seen.

Typical junctional complexes were found be-
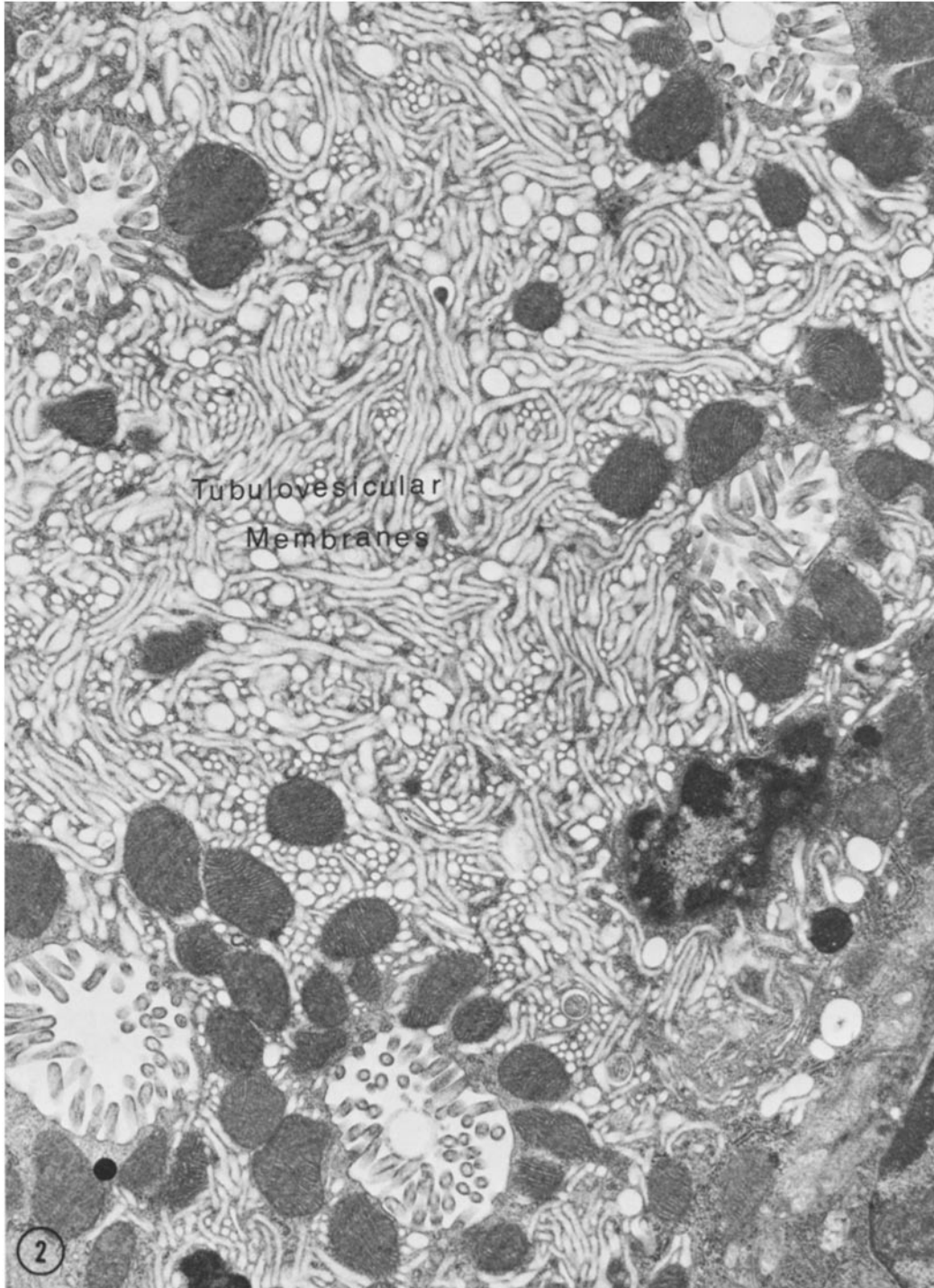
FIGURE 2 An example of extensive tubulovesicular membrane accumulation in a parietal cell from a normal mouse fasted for 24 h. Cells with this morphology are intermixed with others resembling the parietal cell in Fig. 1 as well as intermediate forms. Although fasting seems to enhance the accumulation of tubules in cells, both extremes may be found in fasted or nonfasted mice. This particular section illustrates clearly the tubular and vesicular forms of these cytoplasmic membranes. The major part of the membranes are arrayed as a system of irregular bundles of up to a dozen tubules that are closely intermeshed. Minor focal dilatations and branching of the tubules are common. In this field there are some 110 sections of vesicular profiles that are at least 200 nm in diameter, and about 25% of these show indications of continuity with the tubular system. In cells with abundant tubules the intracellular canaliculi are small and form only a minor part of the cell. × 13,000.

TABLE I

Morphometric Data on Components of the Mouse Parietal Cell

	Tubulo-vesicular system*	Microvilli*	Canalicular lumen‡	Cytoplasmic matrix	Nucleus	Mitochondria	Lateral basal folds*	Golgi complex	Multi-vesicular bodies	Dense bodies
	% relative volume									
Control	18.1 ± 1.1	14.7 ± 1.0	5.2 ± 0.6	21.6 ± 1.2	4.9 ± 0.6	27.8 ± 1.3	6.0 ± 0.7	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Control urethane anesthesia	30.5 ± 1.1	9.1 ± 0.7	0.8 ± 0.2	14.1 ± 0.8	6.6 ± 0.6	34.0 ± 1.0	7.4 ± 0.6	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
Insulin 5 U/kg	5.6 ± 0.6	20.6 ± 1.1	2.9 ± 0.5	28.6 ± 1.2	6.7 ± 0.7	26.5 ± 1.2	7.3 ± 0.7	0.4 ± 0.2	1.0 ± 0.3	0.7 ± 0.2
Insulin 5 U/kg urethane anesthesia	3.4 ± 0.3	21.4 ± 0.8	5.8 ± 0.4	29.6 ± 0.9	5.8 ± 0.4	27.5 ± 0.8	4.3 ± 0.4	0.7 ± 0.2	0.8 ± 0.2	0.4 ± 0.1
Reserpine 5 mg/kg	38.5 ± 0.1	4.3 ± 0.7	6.0 ± 0.7	13.4 ± 0.1	7.1 ± 0.8	22.2 ± 0.1	6.2 ± 0.7	0.7 ± 0.2	0.8 ± 0.3	0.4 ± 0.2
Reserpine 5 mg/kg urethane anesthesia	34.2 ± 1.3	2.5 ± 0.4	1.6 ± 0.3	25.4 ± 1.2	7.1 ± 0.7	24.2 ± 1.1	4.4 ± 0.5	0	0.3 ± 0.1	0.8 ± 0.2

Percent relative volume of 10 compartments of mouse parietal cells. In each group 13–20 representative electron micrographs of one or parts of several parietal cells were printed at magnifications of 10,800 or 12,600 and scored by using an overlay with random points. The total number of counts ranged from 4,400 to 10,700 for each group. The values are expressed as means ± 95% confidence limits of the binomial distribution. Values for the control mice were obtained from intact animals fasted for 4, 8, 12, or 24 h, and the data were pooled. All other mice were fasted overnight. The three urethane-anesthesized mice were cannulated to monitor acid secretion after injecting insulin, reserpine, or saline (control). Individual secretion rates for these animals are shown in Fig. 13. The values obtained after insulin treatment (without anesthesia) represent pooled data from mice sacrificed ¾, 1, 1½, and 2 h after injection. The reserpine (without anesthesia) data were obtained from mice treated for 4, 8, and 12 h and pooled.

* Values for the tubulovesicular system, microvilli, and lateral and basal folds include their membranes as well as their contents.

‡ The canalicular lumen is that portion of the intracellular canaliculus not occupied by microvilli.
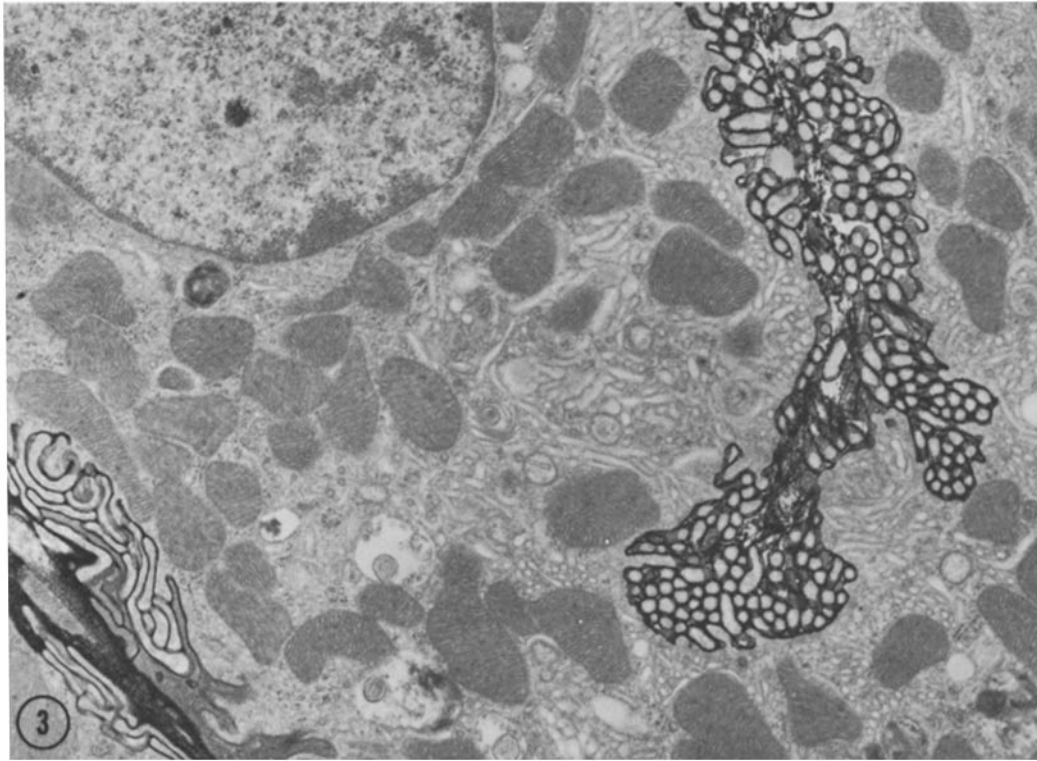
FIGURE 3   Mouse parietal cells from a nonfasted mouse treated with lanthanum hydrosol. The luminal space between microvilli lining the intracellular canaliculi as well as the extracellular space at the base and between the basal folds is dense due to the penetration and presence of the dense tracer. The tubulovesicular system does not contain any of the dense tracer. × 13,000.

tween parietal and adjacent cells, but intercellular canaliculi or extensions of the lumen between cells were rare. The lateral cell borders were straight or plicated and interdigitated. Desmosomes and some gap junctions were found on these surfaces. The basal cell membranes were either smooth or more often thrown into a series of folds which in section resemble microvilli compressed between the basement lamina and the cell body.

Distinctive specializations of the parietal cells were the intracellular canaliculi. In some cells the lumens of the canaliculi were patent, but in other cells numerous packed microvilli obliterated the lumens. The microvilli were 0.1–0.2 μm in diame-

FIGURE 4   A freeze-fracture preparation of mouse parietal cell microvilli from the intracellular canaliculi. The A or cytoplasmic face of the microvillous membrane is studded with numerous particles, while the B or lumenal face is relatively smooth and has numerous small depressions. Note the bifurcating microvilli near the center of the field. × 55,000. Inset: an enlarged view of microvilli near the left margin which shows the small pits on the B face. × 125,000.

FIGURE 5   A freeze-fracture replica of a mouse parietal cell and an adjacent epithelial cell. The microvilli project into the gland lumen and their membrane fracture faces resemble those of the microvilli illustrated in Fig. 4. The tubulovesicular membrane faces are also similar. The lumenal or B faces of the tubules are convex and smooth with small depressions, while the cytoplasmic or A faces are packed with particles. Note the zonula occludentes and the A face of the adjacent cell in the upper part of the micrograph. × 42,000.

B Faces

A Faces

Microvilli

Tubulovesicular
membranes

A Faces

B Faces
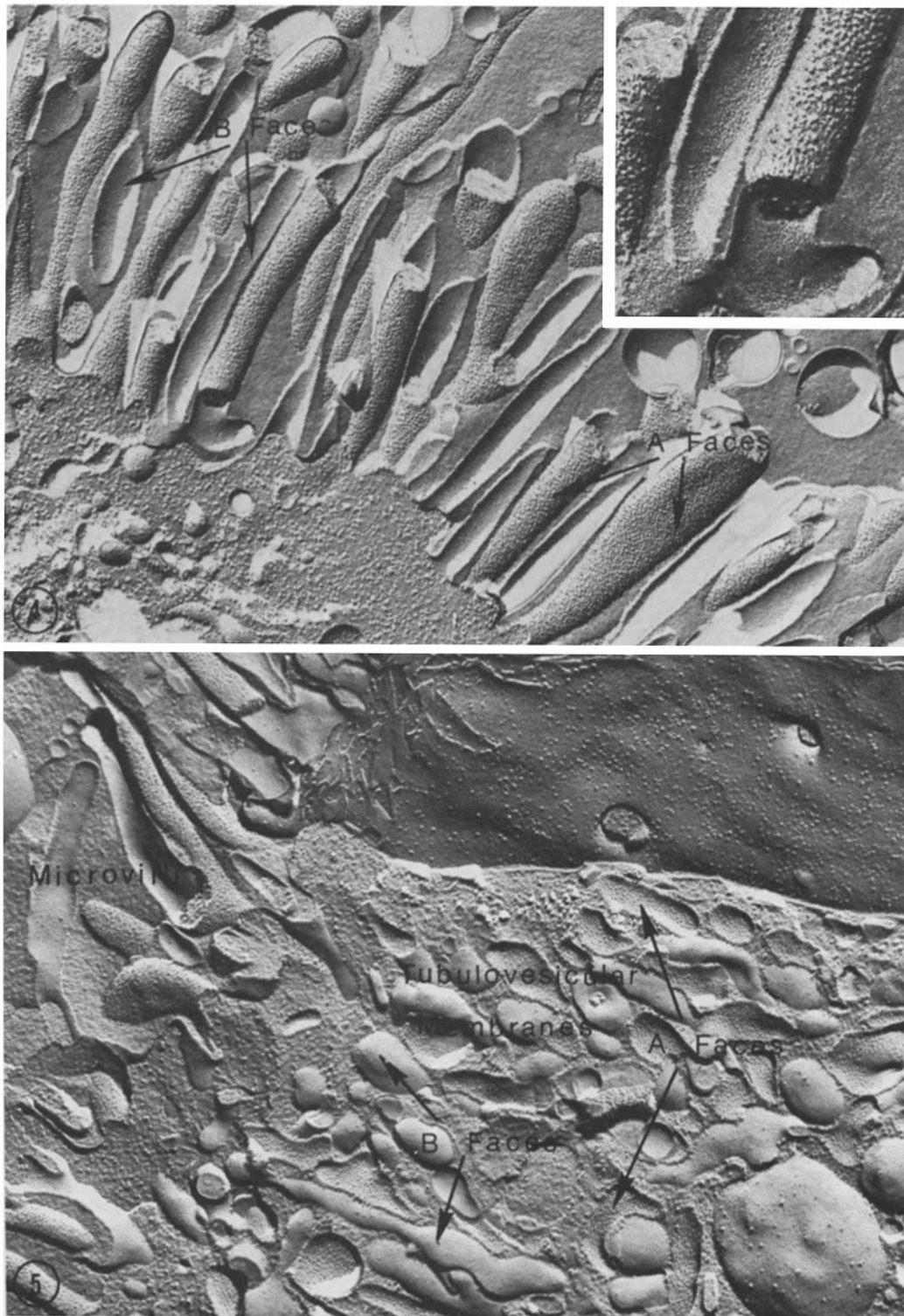
ter, 0.5–1.5 μm in length and regular in form. A few microvilli were bifid and others were slightly bulbous at their tips. The matrix of the microvilli contained longitudinally oriented filaments around a central core of low electron density. Our routine method of preparation of tissues did not enable us to establish the presence or absence of a glycocalyx or fuzzy surface coat on the outer surface of microvilli, but the results on lanthanum-treated tissues described below have some bearing on this question.

Most parietal cells contained cytoplasmic tubules 0.06–0.10 μm in diameter, some bulbous enlargements, and larger vesicles (Figs. 2, 5). The tubules which sometimes branched and anastomosed were closely packed and very conspicuous in the central part of the cell in a zone immediately surrounding the intracellular canaliculi but were absent in the cytoplasm adjacent to the lateral and basal cell membranes. In some areas where these tubule lumens were enlarged there were tiny vesicles within the tubules. Some of these vesicles were interpreted as pedunculated invaginations of the membrane.

There is a persisting question as to whether these tubular structures are continuous infoldings of the cell membrane or elements of smooth reticulum. In this study no evidence of continuity between the lumen of the cytoplasmic tubulovesicular membrane and that of the intracellular canaliculi was found. However, close approximation of the membranes and the appearance of images which might suggest intermittent, transient continuity were not uncommon. Attempts were made to resolve this question by using lanthanum hydrosol as a tracer to outline the extracellular space and locate sites of possible continuity between the lumen of tubulovesicular system and the lumen of intracellular canaliculi. The results of these efforts were consistently negative (Fig. 3). Although lanthanum was readily demonstrated between the microvilli and

filling the extracellular clefts between the basal and lateral folds, no evidence of penetration of the marker into the tubulovesicular system was found. Numerous attempts and modifications of this tracer technique yielded the same negative results.

In replicas of freeze-fractured mouse gastric parietal cells (Figs. 4, 5) the microvillous membrane had numerous particles or globules on the A fracture face which is the cytoplasmic or inner membrane face. The corresponding B fracture face or outer membrane face was relatively smooth, and had few particles and fine depressions presumed to be complementary to the A face particles. The array of particles was abundant but not ordered. Freeze-fractured membranes of cytoplasmic tubules and vesicles appeared very much like the microvillous membrane. The primary difference was that the tubulovesicular membrane had a concave A fracture face and a convex B fracture face (Fig. 5).

After Treatment with Insulin or Pentagastrin

Parietal cells showed a strikingly homogeneous appearance 1 h after intraperitoneal injection of insulin (0.1–5 U/kg) or pentagastrin (125 μg/kg) (Figs. 6, 11). The effects were apparent within 40 min and persisted for 2 h after injection. Cytoplasmic tubules were either completely or almost completely depleted so that intracellular canaliculi and mitochondria stood out clearly in a relatively homogeneous cytoplasmic matrix containing many ribosomes frequently arranged in rosettes. Intracellular canaliculi were usually occluded by microvilli which were now markedly elongated and often sinuous in form. The electron-transparent core in some of the elongated microvilli was conspicuous and in some cases was limited by an interface which resembled the microvillous membrane (Figs. 8, 9, 10). In favorable sections both

FIGURE 6 A parietal cell from a mouse stimulated with insulin to induce acid secretion. The secretion rate is shown in Fig. 13, and the stereological data on parietal cells from this animal are shown in Table I. The prominence of the microvilli bordering the intracellular canaliculi and the absence of the tubulovesicular system in the cytoplasm are characteristic features of parietal cells from secreting stomachs. × 12,600.

FIGURE 7 A parietal cell from a mouse injected with high dose (5 mg/kg) of reserpine whose stomach was perfused with saline and monitored for acid secretion as shown in Fig. 13. The stereological data on these parietal cells are included in Table I. The abundance of tubules and the reduced area occupied by the intracellular canaliculi are consistent and characteristic features. × 8,400.

Intracellular
Canaliculus

6'

7

Tubulovesicular
Membranes
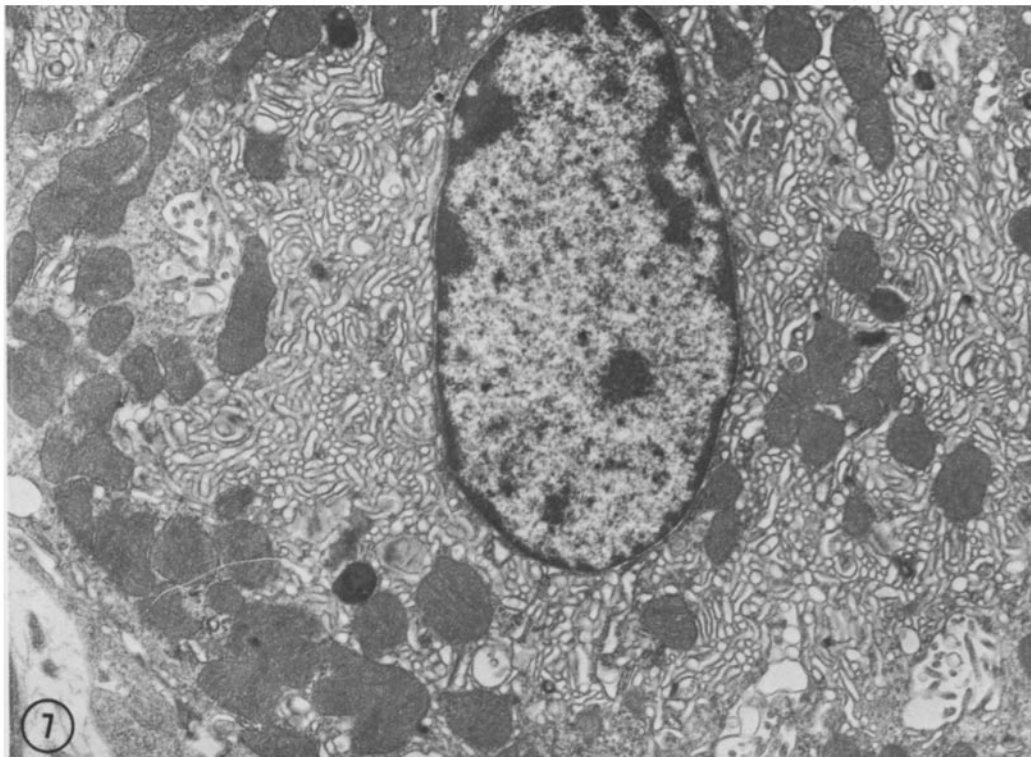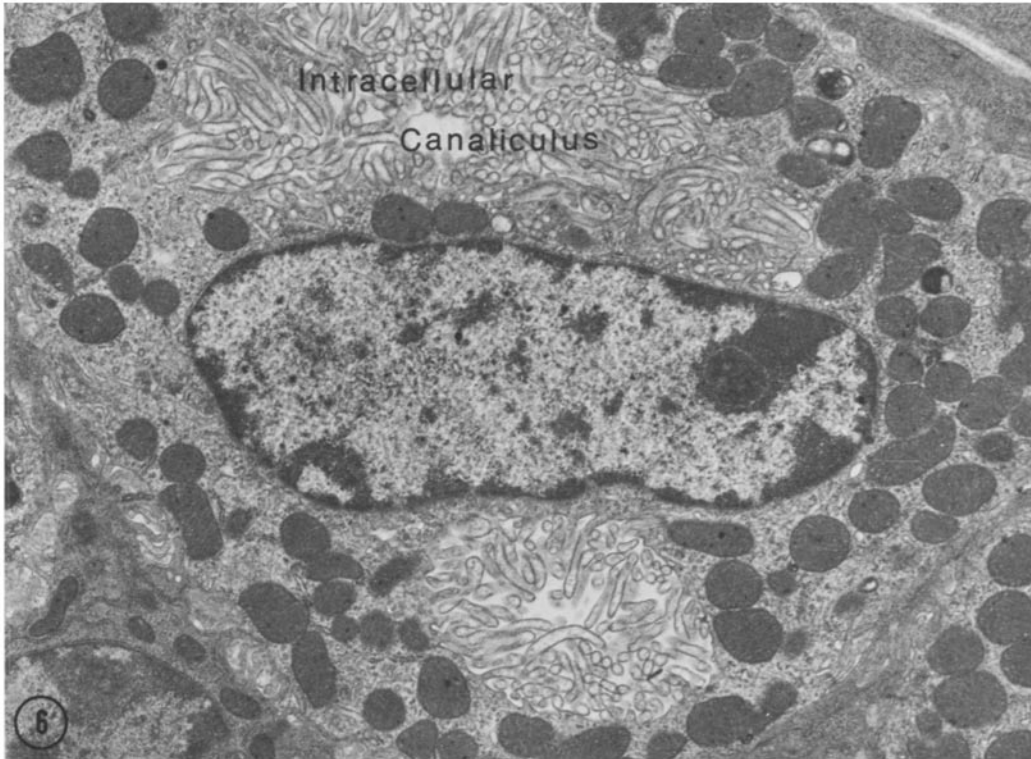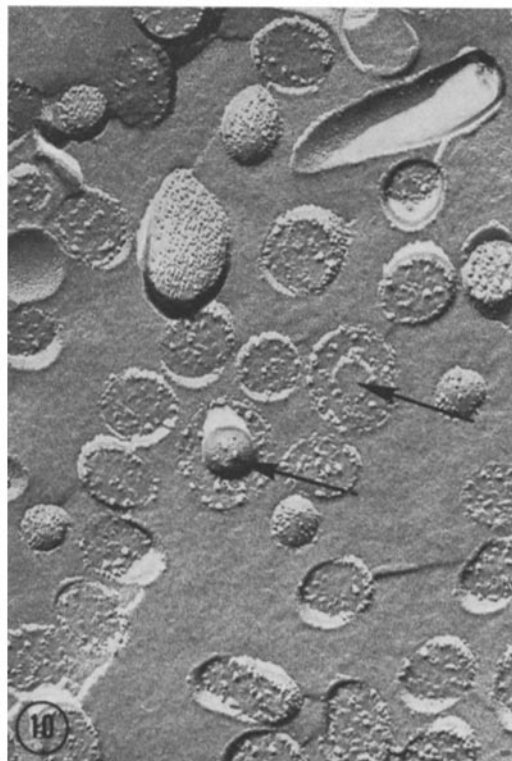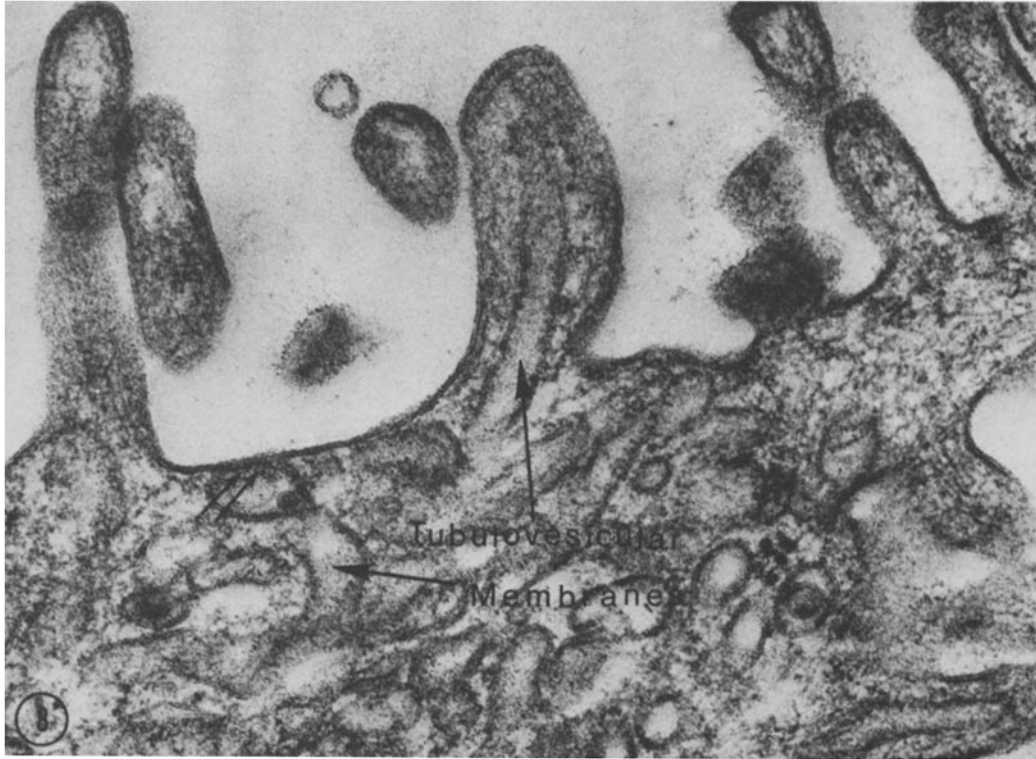
the electron-transparent core and the membrane which limited it extended beyond the base of the microvilli into the cytoplasm. Such an arrangement is shown in Fig. 8 taken from a parietal cell in an animal treated with secretin before injection with insulin.

In animals sacrificed at intervals between 10 min and 1 h after insulin injection there was a progressive disappearance of cytoplasmic tubules. This occurred first in peripheral parts of the cell and only later in the area between the nucleus and the intracellular canaliculi. During these changes there was no obvious continuity between the lumen of cytoplasmic tubules and the lumen of intracellular canaliculi although membrane-to-membrane apposition between tubules and the plasma membrane was seen occasionally. No evidence of penetration of lanthanum into the cytoplasmic tubules was seen in tissues from insulin-treated animals during depletion of tubulovesicular elements. In certain cells with intermediate amounts of cytoplasmic tubules and vesicles (Fig. 11) there were small circular areas of cytoplasm about 0.25 μm in diameter. These profiles were surrounded by a membrane-limited, clear zone formed by invagination of cytoplasmic matrix into clear cytoplasmic vesicles. The infrequent occurrence of these invaginated vesicles suggests their transient existence. Multivesicular bodies, lysosomes, and other cell organelles were not notably affected by insulin or pentagastrin treatment (Table I).

The major changes occurring in parietal cells of insulin-treated animals are summarized in Fig. 14 which presents stereological data for cell compartments in parietal cells from animals treated with insulin for periods of ¾–2 h. A more comprehensive list of values is presented in Table I. Compared with control animals, there is a pronounced reduction in the cell area occupied by cytoplasmic tubules and vesicles, accompanied by an increase in the area occupied by microvilli and cytoplasmic matrix. Values for nuclei and other cell components are not significantly different from those of control animals.

The tubule-depleting effects of both insulin and pentagastrin were maintained for periods up to 3 h. In animals sacrificed 4 h after injection the range of appearances of parietal cells was indistinguishable from that in control animals.

After Treatment with Reserpine

Two distinct sets of dose-dependent changes were seen to occur in fasting mice treated with reserpine.

AFTER LOW-DOSE RESERPINE TREATMENT: At 1 h after injection of reserpine (0.1–0.5 mg/kg), parietal cells were similar in appearance to those in animals stimulated with insulin or pentagastrin. Microvilli were elongated and cytoplasmic tubules were almost entirely absent from the cytoplasmic matrix. The changes observed occurred as early as 30 min after injection and persisted for as long as 5 h, which was the longest survival period used with this procedure. The effects were more sustained than with either insulin or pentagastrin.

AFTER HIGH-DOSE RESERPINE TREATMENT: The effects seen after administration of reserpine in high doses were the opposite of changes induced by insulin, pentagastrin, or low doses of reserpine. At 4 h after injection of reserpine (5 mg/kg) there was a striking increase of cytoplasmic tubules in almost all parietal cells. The accumulation of cytoplasmic tubules was so pronounced as to obscure most of the cytoplasmic matrix except at the extreme periphery of the cells.

FIGURE 8 A microvillus in longitudinal profile with a cytoplasmic tubule extending into the core. These images are only found in a few cells from certain animals. This example was taken from a mouse stimulated with secretin followed by insulin. To the left of the tubule-containing microvillus, there is a close apposition of a cytoplasmic tubule with the plasma membrane (large arrow). × 120,000.

FIGURE 9 Profiles of parietal cell microvilli with transverse sections of tubules within the core. Note that the thickness and appearance of the membranes are very similar. An oblique profile of another tubule is shown in the microvillus at the lower left. × 80,000.

FIGURE 10 A preparation of mouse parietal cell microvilli freeze-fractured transversely. Most of the microvilli have the usual appearance, but two microvilli contain tubules. One tubule is fractured and appears as a circular profile and corresponds to the thin-sectioned image seen in Fig. 7. The other is interpreted as the fractured apical B or lumenal surface of a tubule membbrane. × 80,000.
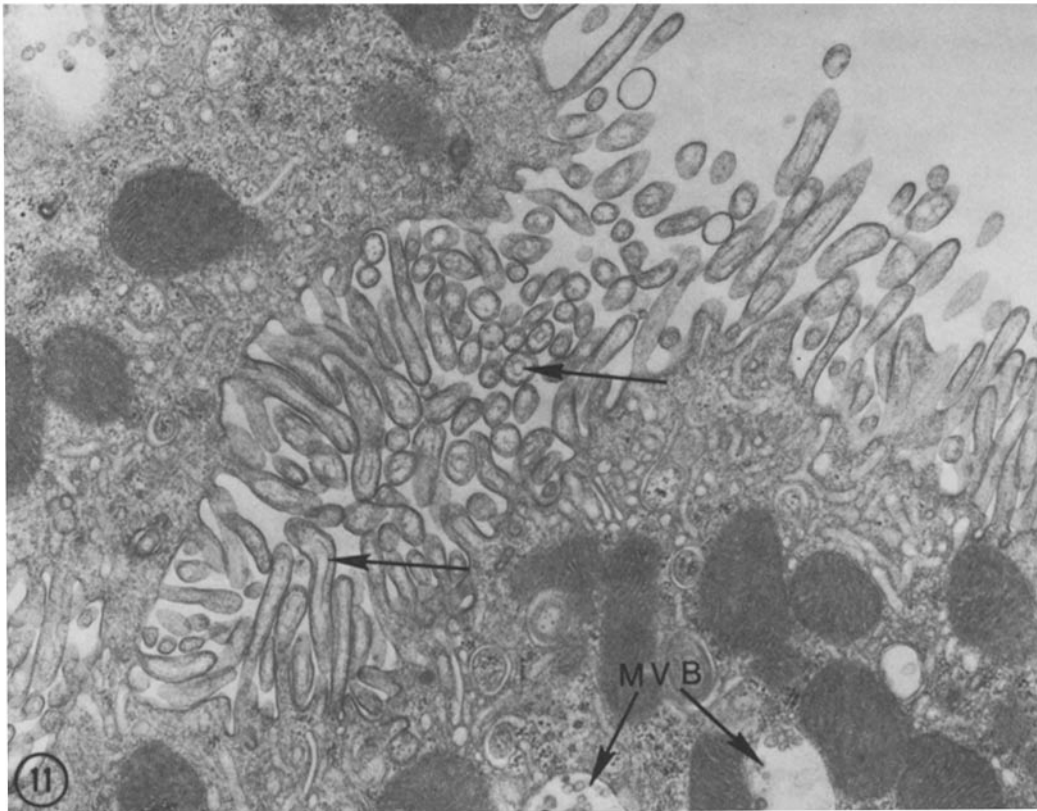
FIGURE 11 The apical part of a parietal cell from a mouse stimulated 1 h earlier with pentagastrin. The thin cell which still contains some cytoplasmic tubules has a number of large microvilli containing profiles of tubules (arrows). The cytoplasm contains a number of circular profiles of membrane whorls. × 23,000.

Microvilli lining the canaliculi were shorter and less prominent than in either control animals or animals treated with insulin, with pentagastrin, or with low doses of reserpine. Extensive areas of intervillous cell membrane were evident due to the reduction in the number of microvilli which occupied a relatively small proportion of the lumen of the canaliculi. This appearance persisted in animals killed at 8, 12 and 24 h after treatment with high doses of reserpine. During this time the animals were heavily sedated by the reserpine treatment but usually recovered with normal activity in 2–3 days if allowed to recover.

Values for cell compartments in parietal cells from mice treated with reserpine (5 mg/kg) for periods of 4–12 h are presented in Fig. 14 and listed in Table I. The tubulovesicular system in animals treated with high doses of reserpine occupies over twice the volume of the corresponding compartment in control animals and more than ten

times that of mice injected with insulin. In contrast, microvilli and cytoplasmic matrix are reduced in comparison with corresponding compartments in control and insulin-treated mice.

After Treatment with other .Hormones and Drugs

Less extensive studies were carried out to ascertain the effects of secretin (125–300 U/kg), atropine (6–20 mg/kg), 5-hydroxytryptamine (60–300 mg/kg), and prostaglandin (PG_{E2}, 1 mg/kg) when administered to mice for periods of 1–6 h. The great majority of parietal cells in animals treated with these substances contained numerous cytoplasmic tubules and resembled those cells in fasted control animals in which accumulation of cytoplasmic tubules was pronounced. Histamine treatment by either intraperitoneal injection (0.25–1 mg/kg) or tail-vein infusion (0.25–1 mg/ml/h)

also led to some reduction in the numbers of tubules, but the effects were less pronounced than with insulin, pentagastrin, or low doses of reserpine.

Some mice were treated with secretin (300 U/kg) 1–3 h before injection with insulin (5 U/kg) and killed after a further period of 35–45 min in an attempt to visualize intermediate stages of tubule depletion after insulin treatment. Tissues from these animals contained many parietal cells in which the disappearance of tubules was only partial, and it was possible to identify well-defined tubulovesicular membranes located in the cytoplasmic matrix and extending into the interior of microvilli (Fig. 8). Transverse sections of such microvilli revealed a clear tubule lumen and microvillous matrix containing filaments (Fig. 9). In some regions the tubules within microvilli were closely apposed to the membrane of the microvillus or to the plasma membrane between microvilli (Figs. 8, 11).

Appearance of Parietal Cells in Animals of Known Secretory Status

Gastric perfusion and monitoring of acid secretion in control animals and animals treated with acid secretagogues was carried out on 29 mice for periods of up to 6 h. These stomachs were fixed for electron microscopy when the level of secretory activity was being monitored.

In control animals and in experimental animals before injection of drugs or hormones, the hydrogen ion content of the initial gastric perfusate varied from 0.4 to 5.5 μeq. In the subsequent 15-min samples taken over the first hour, a hydrogen ion content above 1 μeq/15 min was rare.

An example of secretory inactivity of an animal in which the hydrogen ion in the gastric perfusate dropped to very low levels during a 4-h period is included in Fig. 13. An electron micrograph of a parietal cell from the same preparation is shown in Fig. 12. Parietal cells from animals which released little or no titratable hydrogen ion into the gastric lumen for several hours showed a pronounced accumulation of cytoplasmic tubular and vesicular profiles, and a reduction in the size and number of microvilli.

The insulin stimulation of acid secretion produced a maximal response ¾ h–2 h after insulin injection (Fig. 13). The peak output varied from animal to animal and ranged between 3 and 6

μeq/15 min, but the rise and the drop in secretion were always sharp. Tissues fixed during or soon after maximal secretion consistently contained parietal cells with much reduced or depleted tubulovesicular membranes. Fig. 6 illustrates such a parietal cell and Table I indicates the results of morphometric analysis on a sample of cells from one of these stimulated mice.

When secretion rates were determined in our mouse preparations treated with low doses (0.5 mg/kg) of reserpine, a moderate but sustained release of acid was found (Fig. 14). However, when a high dose (5 mg/kg) was injected into mice, the typical response was either a gradual drop in secretion to no detectable acid secretion or a low level secretory response just above control values as shown in Fig. 14. A determination of membrane distribution in parietal cells from a preparation in which the response to a high dose of reserpine was recorded (Fig. 13) is shown in Table I.

DISCUSSION

The present study attempts to extend our understanding of parietal cell structure and function by the following approaches and observations. (a) To reduce the wide spectrum of parietal cell configurations found in fasting or fed mice, drugs and hormones were used to produce a more uniform appearance of parietal cells. (b) A depletion of cytoplasmic tubulovesicular membranes and an increase in the size and number of microvilli in parietal cells of mice stimulated to secrete acid were noted. The converse was demonstrated after inhibition of acid secretion. These changes were measured by stereological techniques. (c) No evidence for continuity of the plasma membrane and the cytoplasmic tubular system was found by the lanthanum or peroxidase tracer technique or in freeze-fracture replicas. (d) Parts of the tubulovesicular system were found extending into the microvillous core. (e) A system for perfusing the stomachs of rats was adapted for mice, and morphological studies were made on animals with determined rates of acid secretion. The present studies are in general agreement with a number of earlier reports (8, 15, 16, 31–37) which have shown that hydrogen ion release is associated with a reduction in the tubulovesicular membrane content of parietal cells and an increase in the area occupied by intracellular canaliculi and their microvilli.
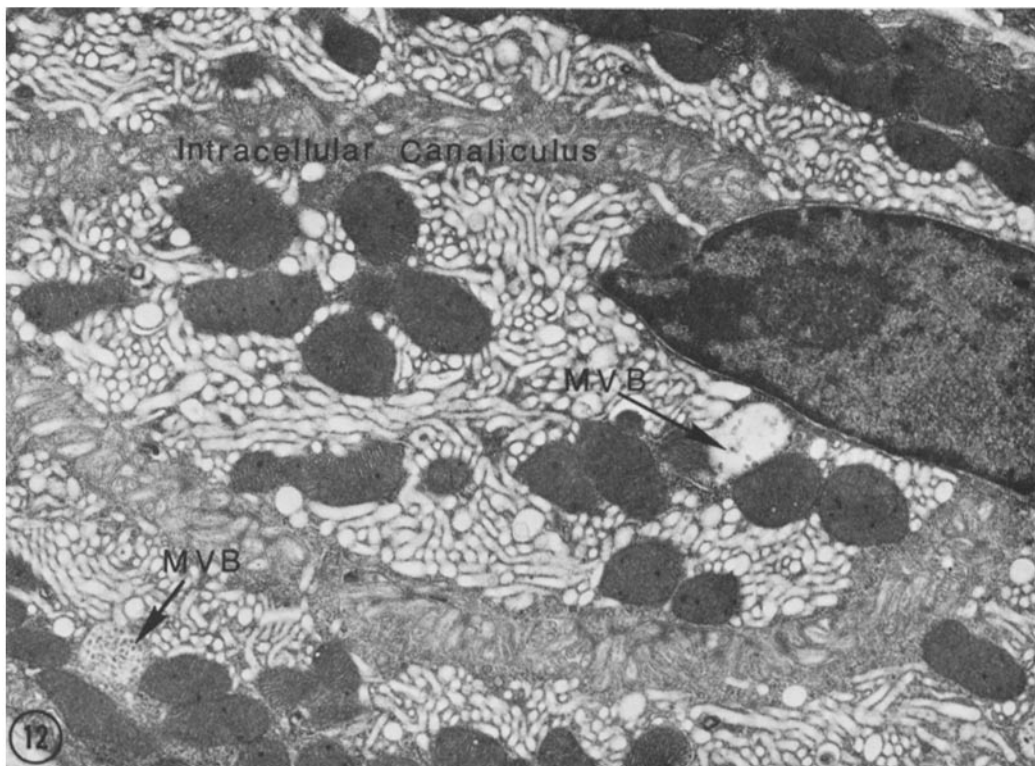
In perfused mouse stomachs where a virtual

FIGURE 12 An electron micrograph of a parietal cell from an experimental mouse whose stomach was perfused with saline and no titratable acid was found. The mitochondria predominate in the peripheral cytoplasm while the tubules are conspicuous in the central area. A collapsed intracellular canaliculus with short microvilli forms a ring around the central mass and approaches the nucleus. In addition to the numerous vesicular profiles among the tubules, there are at least two clearly recognizable multivesicular bodies in this cell. × 9,200.

absence of hydrogen ion secretion was demonstrated for periods of up to 3 h, the parietal cells have a striking accumulation of cytoplasmic tubules and relatively small amounts of surface area represented by intracellular canaliculi and their microvilli. In other animals where hydrogen ion is known to have been released into gastric contents for periods of up to 3 h, with peak secretion of up to 5 μeq/15 min, parietal cells show a marked reduction in the number of tubules and a striking increase in the cell surface membrane of the microvilli in the canaliculus. The morphometric data have not been directed specifically at enabling us to establish whether any loss of tubulovesicular membrane is matched by an increase in microvillous membrane. It is clear, however, that any increase in the area occupied by microvilli, as a consequence of their proliferation and enlargement, is similar to the area previously taken up by tubules.

There is considerable interest in determining possible mechanisms for the rapid disappearance of cytoplasmic tubulovesicles. Helander and Hirschowitz (15), Helander et al. (16), the recent report of Leeson (27), as well as several earlier studies (8, 19) have suggested that the area occupied by plasma membrane is increased by exteriorization of tubulovesicular membranes by membrane flow or exchange and that continuity is established between the lumen of tubulovesicles and the extracellular lumen. However, there is as yet no conclusive evidence that such a process occurs in mammalian parietal cells; this lack of morphological evidence may be due in part to technical limitations in fixing membranes quickly or, alternatively, it may be due to the extreme rapidity with which these tubules undergo either modification or translocation to a new site.

A number of morphological observations appear

to be relevant to any consideration of the relationship which exists between cytoplasmic tubules and surface membrane, during or possibly just before the process of hydrogen ion release into the gastric lumen. (a) An increase in the area of the cell surface represented by microvilli lining intracellular canaliculi occurs progressively and contemporaneously with disappearance of these cytoplasmic tubules. (b) It is possible to demonstrate close membrane apposition between cytoplasmic tubules and the plasma membrane where it lines canalicular lumen between adjacent microvilli. (c) Tubular profiles are found extending into the microvillous cores in experimental animals during acid secretion when the tubules become reduced in number, and in some areas apposition can be seen between the membrane of intramicrovillous tubules and the surface membrane covering the microvilli. (d) The freeze-fracture images of the respective faces of the microvillous and tubulovesicular membranes are

indistinguishable with present techniques, other than by differences in location as noted by Leeson (26) in the rat, and Forte and Forte in the frog (7, 5). Nevertheless, there may well be cogent and important differences between tubulovesicular and microvillous membranes in parietal cells. For example, histochemical localization of carbonic anhydrase in the rat parietal cell was shown by Cross (4) to be limited to the microvillous surface and not present in the tubulovesicular membranes, and immunohistochemical localization of parietal cell antibody was found to be restricted to the microvilli and not present in the tubulovesicular system (17). Studies by Rubin et al. (32) detected histochemical differences between the endoplasmic reticulum and the tubulovesicles, but no inosine diphosphatase, alkaline phosphatase, or adenosine triphosphatase activity was detected in the plasma membrane or tubulovesicular membrane. In toad oxyntopeptic cells, differences in ATPase activity
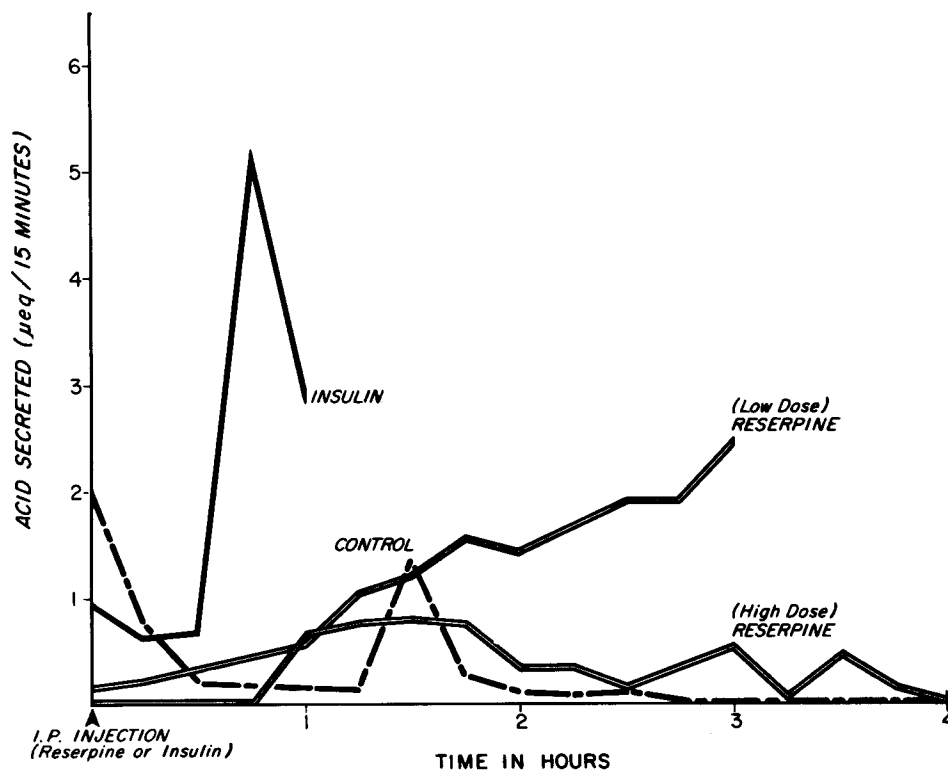

FIGURE 13 Gastric acid secretions by urethane-anesthesized mice prepared as described by Gosh and Schild (8) for the rat. The responses to insulin (5 U/kg), reserpine (low dose, 0.1 mg/kg; high dose, 5 mg/kg), and the response of a control mouse are plotted. The initial washout of residual acid is not included for the drug-treated animals but is shown for the control animal. Immediately after the final collection, the mice were sacrificed and the gastric mucosa fixed for electron microscope study.
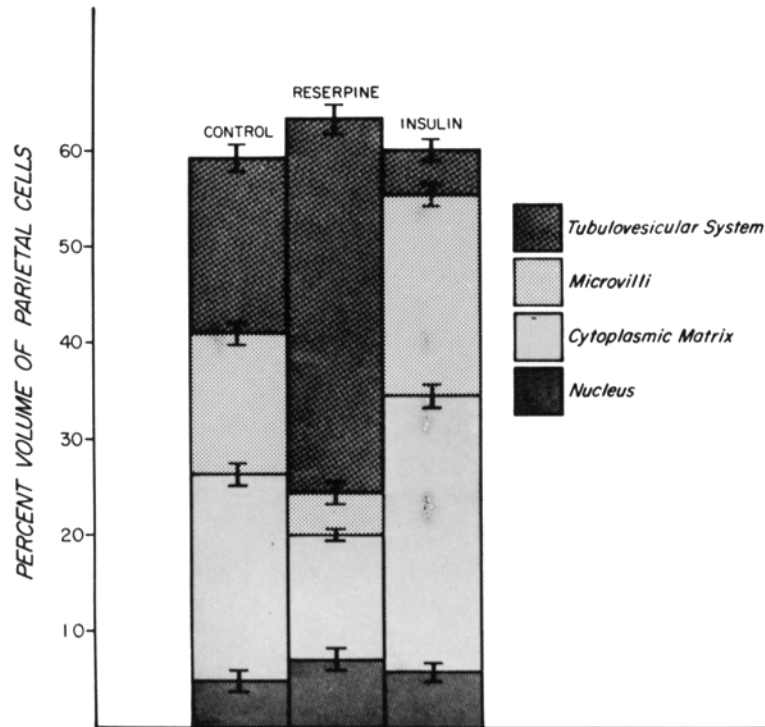
FIGURE 14 Fractional relative volumes of parietal cells occupied by the tubulovesicular system, microvilli, cytoplasmic matrix, and nucleus in control, reserpine- and insulin-treated mice. The graph is based on data given in Table I. The values were obtained by combining the respective components of both control groups. The two samples from the reserpine- as well as the insulin-treated mice are also combined values. The relative volumes are expressed as the mean ±95% confidence limits of the binomial distribution.

were demonstrated between the apical and basal or lateral cell membranes (24).

Sedar (34) and Forte and Forte (6) have shown that there is demonstrable continuity between gland lumen and tubulovesicular lumen in the amphibian oxyntic cell, but if in mouse parietal cells a similar arrangement exists between intracellular tubulovesicular lumen and extracellular lumen, it has so far escaped our detection. The absence of such a communication, even in stimulated animals, suggests that a role for cytoplasmic tubules as reservoirs for the storage of a secretory product is unlikely. This conclusion, together with the observation that cytoplasmic tubules are depleted during the process of acid secretion, invites consideration of the mechanism by which the identity of tubulovesicular membrane in ultrastructural preparations is lost. While it seems reasonable to link tubulovesicular and microvillous membranes as part of a system of membranes which moves rapidly between cytoplasm and sur-

face on appropriate stimulation, a view which has been repeatedly proposed by Sedar (33), Vial and Orrego (37), and by subsequent investigators, the evidence in support of this concept of membrane continuity and conservation is by no means conclusive. The possibility that dissolution of tubulovesicular membrane may in involved in some aspects of the reconstitution of new microvillous membrane should not be overlooked.

If tubulovesicular membrane is subsequently incorporated into preexisting canalicular and microvillous membrane, this process does not seem to be accompanied by structural evidence for fusion or intermingling of the respective membrane elements. However, if loss of tubulovesicular membrane is due to membrane disintegration, with redistribution of its molecular constituents either to surface membrane or to other areas of the cell, the process involved must be remarkably rapid and is unlikely to be susceptible to ultrastructural study without the use of membrane markers or other

means of identifying membrane components. Migration of tubulovesicular membrane towards the cell surface is consistent with the view that it may represent a reserve of membrane which, under conditions of inactivity of parietal cells, is resident in cytoplasmic matrix, possibly here lacking enzymatic and immunological characteristics of surface membrane but providing reserve capacity which can be drawn on with remarkable rapidity before or during hydrogen ion release.

In the return to the inactive state, the parietal cells certainly alter their membrane distribution rapidly so that now the cytoplasm contains numerous tubules and the size and number of microvilli are greatly reduced. The mode of reformation of these tubules in the dog was interpreted by Helander and Hirschowitz (15) as being due to invagination of entire microvilli into the cytoplasm and transformation of invaginating membranes into cytoplasmic tubules. In the mouse we have not observed this process nor have we been able to interpret membrane whorls, as seen in Fig. 11, as representing part of such a process. The cytoplasmic tubulovesicular system defines a cell compartment which occupies over 30% of the volume of inactive parietal cells. It seems probable that during secretion there is redistribution rather than complete loss of this membrane from the cells so that, during reconstitution of the tubules, some part of the membrane or membrane constituents is available for recycling. Multivesicular bodies in mouse parietal cells, recognized by Hally (12) as vacuole-containing bodies, were found in great abundance after pilocarpine stimulation. Although their significance is still obscure, they may yet prove to be involved in the activation or deactivation of parietal cell secretions. However achieved, the process of membrane redistribution or reconstitution is a rapid one, and if it were possible to witness the changing ultrastructural organization in the living state, the process probably would be one of great dynamism.

REFERENCES

1. ADKINS, R. B., N. ENDE, and W. G. GOBBEL. 1967. A correlation of parietal cell activity with ultrastructural alterations. *Surgery (St. Louis).* **62:**1059.
2. BLACKMAN, J. G., D. S. CAMPION, and F. N. FASTIER. 1959. Mechanism of action of reserpine in producing gastric haemorrage and erosion in the mouse. *Br. J. Pharmacol. Chemother.,* **14:**112.
3. CHALKLEY, H. W. 1944. Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst. **4:**47.
4. CROSS, S. A. M. 1970. Ultrastructural localization of carbonic anhydrase in rat stomach parietal cells. *Histochemie.* **22:**219-225.
5. FORTE, J. G., T. M. FORTE, and T. K. RAY. 1972. Membranes of the oxyntic cell: their structure, composition and genesis. *In,* Gastric Secretion. G. Sachs, E. Heinz, and K. J. Ulrich, editor. Academic Press, Inc., New York 37.
6. FORTE, T. M., and J. G. FORTE. 1970. Histochemical staining and characterization of glycoproteins in acid-secreting cells of frog stomach. *J. Cell Biol.* **47:**437.
7. FORTE, T. M., and J. G. FORTE. 1971. A freeze-fracture study of bullfrog gastric oxyntic cells. *J. Ultrastruct. Res.* **37:**327.
8. FREXINOS, J., M. CARBALLIDO, A. LUIS, and A. RIBET. 1971. Effects of pentagastrin stimulation on human parietal cells. An electron microscopic study with quantitative evaluation of cytoplasmic structures. *Am. J. Dig. Dis.* **16:**1065.
9. GOSH, M. N., and H. O. SCHILD. 1958. Continuous recording of acid gastric secretion in the rat. *Br. J. Pharmacol.* **13:**54.
10. GROSSMAN, M. L. 1968. Neural and hormonal stimulation of gastric secretion of acid. *In* Handbook of Physiology. Section 6, Alimentary Canal. C. F. Code, Section editor. American Physiological Society, Washington, D. C. 835.
11. HACKENBROCK, C. R. 1968. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. II. Electron transport-linked ultrastructural transformations in mitochondria. *J. Cell Biol.* **37:**345.
12. HALLY, A. D. 1959. Functional changes in the vacuole-containing bodies of the gastric parietal cell. *Nature (Lond.).* **183:**408.
13. HELANDER, H. F. 1962. Ultrastructure of fundus glands of the mouse gastric mucosa. *J. Ultrastruct. Res. Suppl.* **4:**1.
14. HELANDER, H. F. 1964. Ultrastructure of gastric fundus glands of refed mice. *J. Ultrastruct. Res.* **10:**160.
15. HELANDER, H. F., and B. I. HIRSCHOWITZ. 1972. Quantitative ultrastructural studies on gastric parietal cells. *Gastroenterology.* **63:**951.

16. HELANDER, H. F., S. S. SANDERS, W. S. REHM, and B. I. HIRSCHOWITZ. 1972. Quantitative aspects of gastric morphology. In Gastric Secretion. G. Sachs, E. Heinz, and K. J. Ulrich, editors. Academic Press, Inc., New York. 69.

17. HOLDEMAEKER, P. J., and S. ITO. 1970. Ultrastructural localization of gastric parietal cell antigen with peroxidase-coupled antibody. Lab. Invest. 22:184.

18. HUBNER, G., H. J. KLEIN, and M. EDER. 1969. Feinstrukturelle Utersuchungen an der Fundussacheimhaut des Rattenmagens nach Stimulierung mit Pentasgastrin und Betazol. Verh. Dtsch. Ges. Pathol. 53:247.

19. ITO, S. 1961. The endoplasmic reticulum of gastric parietal cells. J. Biophys. Biochem. Cytol. 11:333,

20. ITO, S. 1968. Anatomic structure of the gastric mucosa. In Handbook of Physiology. Section 6, Alimentary Canal. C. F. Code, Section editor. American Physiological Society, Washington, D. C. 705.

21. ITO, S., and M. J. KARNOVSKY. 1968. Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. J. Cell Biol. 39(2, Pt. 2): 168 a. (Abstr.).

22. JONES, R. S., R. E. GEIST, and A. D. HALL. 1970. Comparison of dose-response relations of insulin and 2-deoxy-D-glucose for biliary and gastric secretion in dogs. Gastroenterology. 59:665.

23. KIM, K. S., and P. A. SHORE. 1963. Mechanism of action of reserpine and insulin on gastric amines and gastric acid secretion, and the effect of monoamine oxidase inhibition. J. Pharmacol. Exp. Ther. 141:321.

24. KOENIG, C. S., J. D. C. VIAL. 1970. A histochemical study of adenosine triphosphatase in the toad (Bufo spinulosus) gastric mucosa. J. Histochem. Cytochem. 18:340.

25. KOMNICK, H. 1963. Zur funktionellen Morphologie der Salzsaureproduktion in der Magenschleimhaut. Histochemie. 3:354.

26. LEESON, T. S. 1972. Parietal cell canaliculi: a freeze-etch study. Cytobiologie. 5:352.

27. LEESON, T. S. 1973. Canaliculi and tubulovesicles of rat parietal cells. Am. J. Anat. 136:541.

28. LILLIBRIDGE, C. B. 1968. Electron microscopic measurements of the thickness of various membranes in oxyntic cells from frog stomachs. J. Ultrastruct. Res. 23:243.

29. REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. 33:C7.

30. RIDLEY, P. T., J. N. SCHLOSSER, and W. G. GROVES. 1969. Acid secretory responses to insulin and 2-deoxy-D-glucose in anesthetized and unanesthetized rats. Physiologist. 12:340. (Abstr.).

31. ROSA, F. 1963. Ultrastructure of the parietal cell of the human gastric mucosa in the resting state and after stimulation with histalog. Gastroenterology. 45:354.

32. RUBIN, W., L. L. ROSS, M. N. SLEISENGER, and G. H. JEFFRIES. 1968. The normal human gastric epithelia. Lab. Invest. 19:598.

33. SEDAR, A. W. 1961. Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (Rana catesbiana) II. The acid-secreting gastric mucosa. J. Biophys. Biochem. Cytol. 10:47.

34. SEDAR, A. W. 1962. The fine structure of the oxyntic cell in relation to functional activity of the stomach. Ann. N. Y. Acad. Sci. 99:9.

35. SEDAR, A. W., and M. H. F. FRIEDMAN. 1961. Correlation of fine structure of the gastric parietal cell (dog) with functional activity of the stomach. J. Biophys. Biochem. Cytol. 11:349, 1961.

36. TONER, P. G. 1963. The fine structures of resting and active cells in the submucosal glands of the fowl proventriculus. J. Anat. 97:575.

37. VIAL, J. D., and N. ORREGO. 1960. Electron microscope observations on the fine structure of parietal cells. J. Biophys. Biochem. Cytol. 7:367.