ULTRASTRUCTURAL CHANGES AND CYCLIC AMP IN FROG OXYNTIC CELLS

K. S. CARLISLE, C. S. CHEW, and S. J. HERSEY

From the Department of Physiology, Emory University, Atlanta, Georgia 30322

ABSTRACT

In vitro frog gastric mucosa was employed as a model for a combined physiological, biochemical, and ultrastructural study of the morphological changes which accompany the onset of acid secretion by the oxyntic cell. The histamine H_2-receptor antagonist metiamide was used to provide a reproducible control state. Stimulation of acid production by theophylline resulted in a 10-fold increase in plasma membrane surface area and a distinct change in the conformation of mitochondrial cristae. Studies using the acid secretion inhibitors, thiocyanate and anoxia, demonstrated that neither acid production per se nor oxidative metabolism is essential for the theophylline-dependent changes in surface area. Increases in tissue cyclic AMP levels were observed under the conditions producing morphological changes. It is postulated that surface area changes induced by theophylline are controlled by cellular cyclic AMP levels.

KEY WORDS gastric mucosa · cyclic AMP · hydrochloric acid, gastric · oxyntic cells · cell membrane

Morphological changes accompanying the onset of gastric acid secretion have been well documented for the mammalian parietal cell (5, 9–11, 15) and the amphibian oxyntic cell (6, 21, 22, 24, 25). The most dramatic changes involve the membranous components of the cell, i.e., the tubulovesicles and surface membrane. It is generally agreed that, with the onset of secretion, there is a decrease in tubulovesicle membrane and an increase in surface membrane. This transformation is postulated to involve an interconversion of surface and tubulovesicle membranes although direct evidence has not been offered for such a conversion. Although it is likely that the morphological changes represent an essential event in the stimulus-secretion coupling for gastric mucosa, the exact role of the changes has not been demonstrated nor have the molecular events which underlie the transition been identified.

The present study was undertaken in an effort to further understand the relationship between the morphological changes and the process of acid secretion. To this end, the morphological changes are treated as an intermediate event in the stimulus-secretion coupling, and the relationship of this event to other intermediate reactions was examined. The results show that the actual production of acid is not required for the morphological changes, nor is oxidative metabolism. However, there does appear to be a correlation between morphology and the cellular cyclic AMP levels. The data suggest that secretory stimulants act via cyclic AMP to produce morphological changes. Preliminary results of the present study have been previously reported (2).

MATERIALS AND METHODS

Preparation

Bullfrogs (*Rana catesbeiana*) were obtained from Mogul Ed (Oshkosh, Wis.) and were kept unfed in running tap water until sacrifice. Stomachs were removed from

the animals and stripped of their outer muscle layer. Chambered, in vitro preparations of gastric mucosa were employed to permit continuous monitoring of acid secretion by the pH stat method (13) before fixation for electron microscopy. The standard bathing medium for the serosal side contained in millimoles/liter: Na, 102; K^+, 2.5; Ca^{++}, 1.0; Mg^{++}, 0.8; Cl^-, 80; HCO_3^-, 25; SO_4^{--}, 0.8; phosphate, 1.0; at an initial pH of 7.4. The medium was continuously gassed with 95% O_2 + 5% CO_2 at room temperature. The mucosal surface was bathed with an identical solution except that the solution contained Cl^- as the only anion and was gassed with 100% O_2. Additional agents were added to the serosal side only. All tissues, except the spontaneously secreting and the nontreated resting tissue used for comparison studies, were initially treated with 1×10^{-4} M metiamide (1) to reduce spontaneous secretion to zero (26). Metiamide was a generous gift of Dr. H. Green of Smith, Kline & French Laboratories. The tissues treated only with metiamide served as the control and are referred to as resting tissues. Additional controls were obtained for tissues treated with multiple agents as described in Results.

Morphological Studies

Tissues to be studied by electron microscopy were initially fixed in the chamber with the addition of 70% glutaraldehyde (Ladd Research Industries, Burlington, Vt.) to both the serosal and mucosal bathing media to give a final concentration of 2%. After 1 h of fixation, the chamber was dismounted; the stomach was cut into 1-mm^2 blocks and fixed for an additional hour in 2% glutaraldehyde in 0.1 M phosphate buffer at room temperature. The pieces of tissue were rinsed in buffer and postfixed in 1% osmium tetroxide in phosphate buffer at 4°C. Dehydration was carried out in a graded series of alcohols followed by Araldite embedding. Thick and thin sections were cut on a Sorvall Porter-Blum MT2-B ultramicrotome (DuPont Instruments, Sorvall Operations, Newton, Ct.) or LKB ultratome III (LKB Instruments, Inc., Rockville, Md.) and mounted on copper grids. Alcoholic uranyl acetate and lead citrate (20) stains were used. The grids were examined with a Philips EM 300 or Philips EM 400 microscope.

Morphometric studies were performed according to the methods of Weibel (27, 28). Grids were prepared from a minimum of two experiments for each of the five analyzed experimental groups. The grids were initially examined to assure that the tissue came from a region containing primarily oxyntic cells. Four grids from each of the experimental groups were chosen randomly, and photographs were taken of the first four cells on each grid which met the criteria of containing a cross section of the nucleus and no obvious section defect. A minimum of ten of these cells was used for the analysis. The lineal analyses to determine surface density were made on micrographs having magnifications of × 10,700. This magnification was chosen for two reasons: first, it allowed the negative to contain an entire cross section of an oxyntic cell; secondly, it was the lowest magnification that permitted adequate viewing of the surface membrane with reproducible surface intersect counts. The distances between grid lines on the transparent overlay used for counting were constructed to insure a minimum of 25 surface intersects per cell.

Cyclic AMP

Tissues to be assayed for cyclic AMP were treated in the same way as those used for the morphological studies up to the time of fixation. At this time, the tissues were frozen in the chamber by using metal tongs cooled to the temperature of liquid nitrogen. The frozen tissues were then removed from the chamber and pulverized with a stainless steel pestle while being maintained at liquid nitrogen temperature. A trace amount of [^{3}H]cyclic AMP was added for the calculation of recovery, and the frozen tissue powder was then extracted with 10 vol of 5% TCA which had been redistilled. After thawing to 0°C, the TCA precipitate was centrifuged at 15,000 *g* for 10 min and the precipitate was saved for analysis of protein by a modified biuret method (18). The supernate was acidified with 0.1 ml of 1 N HCl per ml, washed eight times with 2 vol of anesthesia-grade ethyl ether (J. T. Baker Instruments, Milford, Conn.) and evaporated to dryness on an Evapomix (Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.). The residuum was then redissolved in 0.05 M acetate buffer, pH 4.5, to yield 10 mg of original tissue protein per ml. Cyclic AMP was measured by the competitive protein-binding method of Gilman (7) as previously described (4). The results are expressed as picomoles of cyclic AMP per milligram of tissue protein.

RESULTS

When gastric mucosa is isolated from bullfrog and mounted in an in vitro chamber, it secretes acid spontaneously and the addition of various stimulants results in only small increases in secretory rate. Tissue that was allowed to spontaneously secrete for 25 min before fixation for electron microscopy showed a heterogeneous population of oxyntic cells. This ultrastructural spectrum included oxyntic cells with few microvilli arranged in glands with small lumens and cells with many microvillarlike projections extending into large lumens. The majority of cells were in states intermediate between the two extremes. This wide range in morphology is interpreted as reflecting oxyntic cells in various physiological secretory states.

To obtain maximal stimulation by test agents and to have a homogeneous population of oxyntic cells for controls, it is desirable to initially reduce the spontaneous secretion to zero. This is accom-

plished in the present study by treating tissues with the recently developed histamine H_2-receptor antagonist metiamide (1, 26). This agent inhibits spontaneous secretion, and the inhibition is readily overcome by the subsequent addition of various secretory stimulants including histamine and theophylline. Fig. 1 shows acid secretion changes during a typical experimental protocol: spontaneous acid secretion (*A*) is inhibited by metiamide to produce a resting tissue (*B*); subsequent addition of a stimulant then restores secretion (*C*). The use of metiamide as a pretreatment allows a reproducible starting point, or control, for subsequent comparisons with other physiological states. These studies thus differ from most previous investigations which employed spontaneous secretion as the control state.

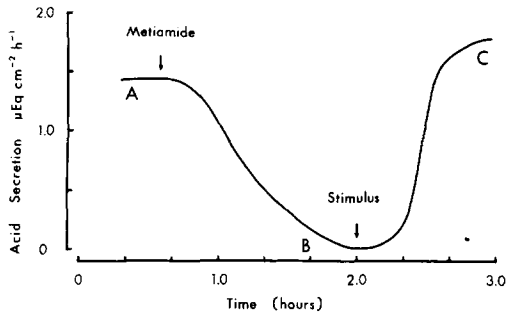


FIGURE 1 Experimental protocol for measuring acid secretion in relation to morphological and biochemical changes. The physiological states are identified as: *A*, spontaneous secretion; *B*, resting; *C*, stimulated secretion. Trace is redrawn from a typical experimental record. Ordinate: acid secretory rate in μEq $cm^{-2}h^{-1}$. Abscissa: time in hours.

Cross sections of a gastric gland containing oxyntic cells of resting mucosa (Fig. 2) are typified by closed lumens bordered by plasma membranes which at low magnification appear relatively smooth with few projections. At higher magnification (Fig. 3), the plasma membranes are observed to be continuous with enfoldings into the cytoplasm. Numerous vesicles and short tubules are found dispersed throughout the cytoplasm. The regular plasma membrane and distribution of small vesicles are the two most characteristic features of the metiamide-treated or resting state and to serve to distinguish it from other states. This finding contrasts with the appearance of closely packed tubules described for "nonsecret-

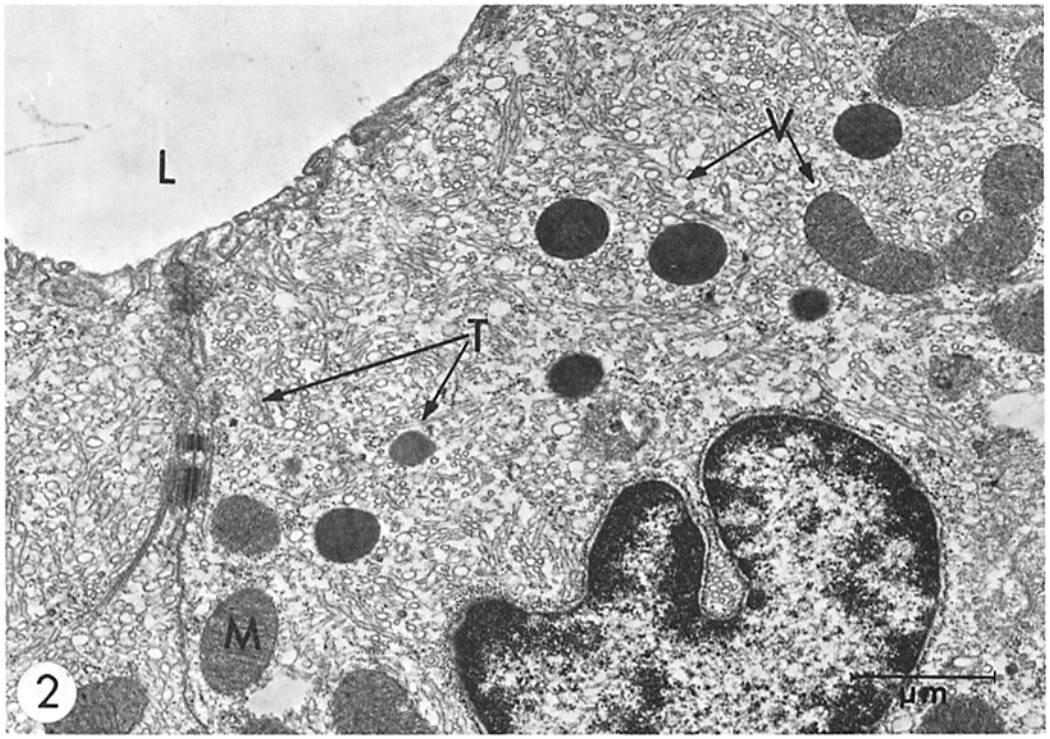


FIGURE 2 Oxyntic cells from metiamide-treated tissue. Section through a gastric tubule showing a small lumen (*L*) bordered by a relatively smooth-surfaced membrane with few extensions. Short tubules (*T*) and vesicles (*V*) are scattered through the cytoplasm. Mitochondria (*M*). × 17,500.

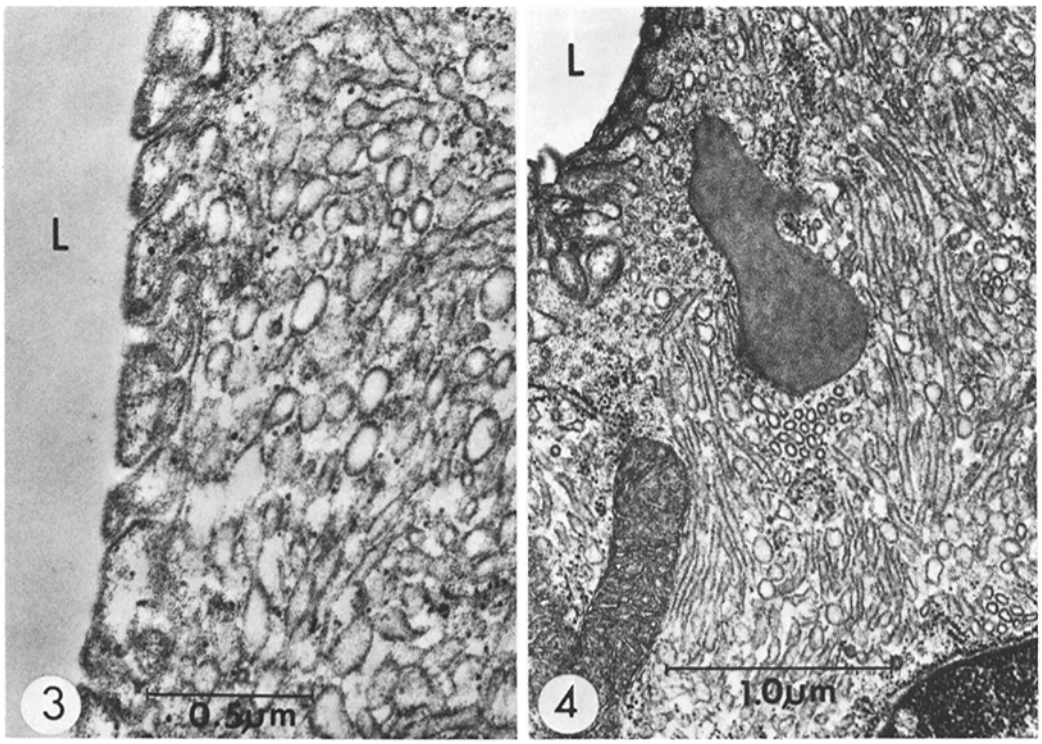


FIGURE 3 Apical surface area of metiamide-treated oxyntic cell. At this magnification, the plasma membrane is observed to be continuous with short enfoldings of the cell surface. The outer portion of the cell membrane consists of a dense coating. × 43,000.

FIGURE 4 Nontreated, resting oxyntic cell bordering lumen (*L*). Spontaneously secreting gastric mucosa was mounted in a chamber and reached zero secretion 15 h later. The ultrastructure of this tissue is indistinguishable from that of tissue treated with metiamide (Fig. 2) to shorten the time required to attain the zero secretory or resting state. × 29,000.

ing" oxyntic cells in previous publications (6, 21). The difference might be due to the production of a true zero secretory rate in the present study or possibly to an action of metiamide per se.

To test the latter possibility, metiamide-treated tissue was compared to tissues which reached a zero secretory rate spontaneously. This was accomplished by preincubating the tissues in the in vitro chamber until the spontaneous secretion declined to zero (~15 h later) (16). The ultrastructure of this tissue (Fig. 4) is indistinguishable from that of gastric mucosa brought to zero secretory rate with metiamide (Figs. 2 and 3). Both tissues exhibit small glandular lumens, plasma membranes with few projections, and short, scattered tubulovesicular systems. Addition of metiamide to the preincubated resting tissues produced no observable morphological changes, again indicating that this agent acts via inhibition of secretion rather than directly. Since no difference was observed, it is readily apparent that it is advantageous to use metiamide rather than a 15-h preincubation to obtain the resting state.

The ultrastructure of oxyntic cells in which acid secretion has been restimulated with theophylline (10^{-2} M) (Fig. 5) is in striking contrast to that of the resting cell. Cross section of a gastric gland shows a greatly enlarged lumen bordered by cells which exhibit many filamentous projections. These projections are generally referred to as microvilli but are quite different in appearance from the more regular microvilli of intestinal or renal epithelial cells. In addition to having filamentous projections, the stimulated cells are characterized by the presence of an extensive "tubulovesicular" system which occupies most of the apical region of the cell. In contrast to those of the resting cell, the tubular profiles are more

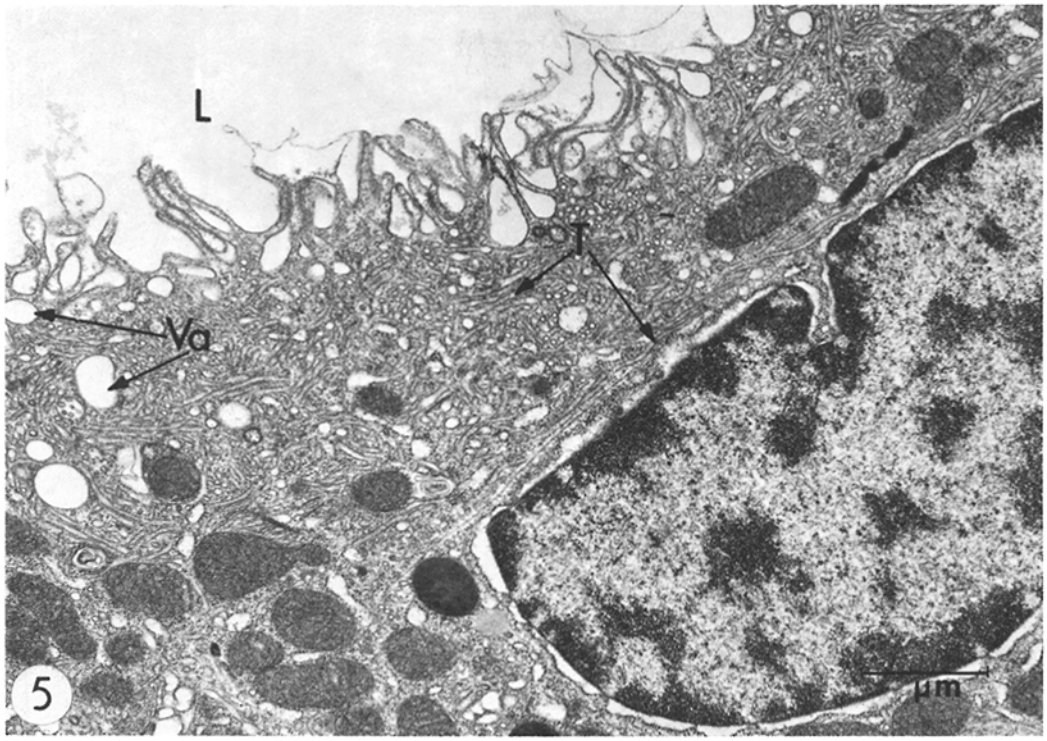


FIGURE 5 Oxyntic cells from theophylline-stimulated tissue. Note increased lumen size and surface elaboration compared to Fig. 2. Extended tubules (*T*) and smooth-surfaced vacuoles (*Va*) are observed. × 14,000.

numerous and appear to exist in longer segments. Another distinguishing feature of the stimulated cells is the presence of smooth-surfaced vacuoles ranging in diameter from 200 to 700 nm. These vacuoles are observed throughout the cell but appear most numerous in the apical region.

The mitochondria also differ between the resting and stimulated states. In the resting state the cristae are relatively straight and quite regular (Fig. 6*a*), whereas in the stimulated state the cristae exhibit a reticulated conformation (Fig. 6*b*). Presumably, this difference reflects a difference in the metabolic rates observed under the two conditions (14).

The transition between the resting and secreting states has been postulated to involve an interconversion of tubular vesicular membrane with surface membrane (5, 10, 15). Further support for this idea is obtained by the observation of various structures which may be interpreted as transitional structures. Fig. 7*a* shows a high magnification of a constricted tubule. These structures are very common and may well represent a transition between the tubules and vesicles. The direction of this transition cannot be determined from these studies, and it is possible that the transition is a reversible process which continues even in the resting state. Further support for vesicle fusion and/or fission is provided in Fig. 7*b* which shows two vesicle profiles having fused membranes. The possibility of interconversion between surface membrane and tubulovesicles is supported by the observation that the luminal spaces between adjacent filamentous projections frequently terminate in structures which are indistinguishable from the bulbous terminals of cytoplasmic tubules (Fig. 8*a*). Bulbous smooth-surfaced vacuoles unique to secreting tissue are observed (Fig. 8*b*) adjacent to the luminal surface and scattered throughout the apical portion of the cell. These transitional structures may play a role in membrane turnover or a recycling process between tubules and microvillar membranes. Here again, the direction of the possible interconversion cannot be determined.

Although the above results confirm previous

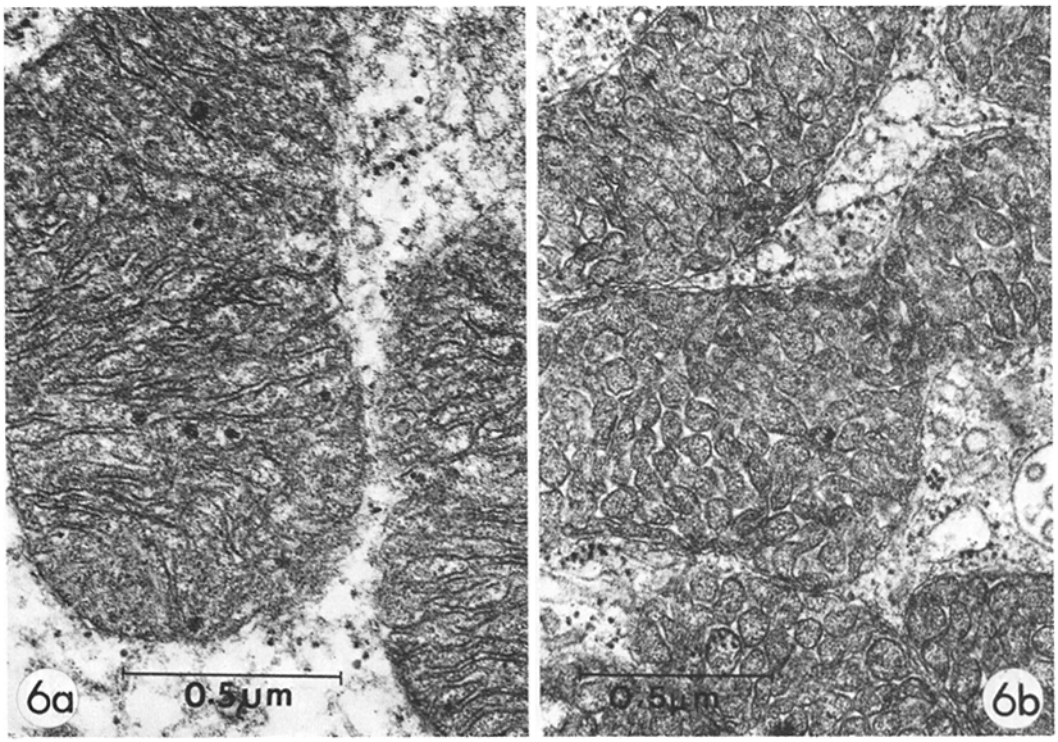


FIGURE 6 Comparison of mitochondria from resting and stimulated tissues. (*a*) Resting tissue exhibiting regular plate-like cristae. × 59,000. (*b*) Stimulated tissue mitochondria include cristae with a reticulated conformation. × 50,000.

investigations and are consistent with the membrane-conservation hypothesis for the transition from the resting to secretory state, it is desirable to gain more insight into the mechanisms underlying this transition. A major question regarding the morphological transition is whether it requires the actual production of acid or whether it occurs as a direct result of the stimulating agent. To test these possibilities, tissues were examined for morphological changes under two conditions in which acid production does not occur upon addition of theophylline. The first condition involves the simultaneous addition of theophylline and thiocyanate (SCN), a potent inhibitor of acid secretion. Under this condition, it has been shown that the tissue responds metabolically (increased oxygen consumption, cytochrome reduction) but does not secrete acid (12). The second condition tested is that of nitrogen anoxia. Under anoxic conditions, the addition of theophylline or other stimulants fails to result in acid production, presumably due to the lack of metabolic energy. Despite the lack of acid production, both test conditions were found to result in obvious morphological changes (Figs. 9 and 10). The morphological transitions were found to be similar to, but not identical with, those observed with secreting tissues. The theophylline plus SCN condition results in the formation of filamentous surface projections but differs from the secreting state in the appearance of tightly packed tubules and an absence of smooth-surfaced vacuoles. Although some mitochondria exhibit a reticulated conformation, most of them have straight, regular cristae similar to those of the resting state. The anoxia-stimulated tissues also show surface elaborations and increased numbers of tubules, but the tubules are not densely packed. Smooth-surfaced vacuoles are absent, and the mitochondrial conformation resembles that of the resting state. Despite the detailed differences between the various conditions, it is clear that both the theophylline plus SCN- and the anoxia-stimulated tissues have undergone significant morphological changes compared to the resting state. Control studies using SCN alone or anoxia alone (Figs. 11 and 12)

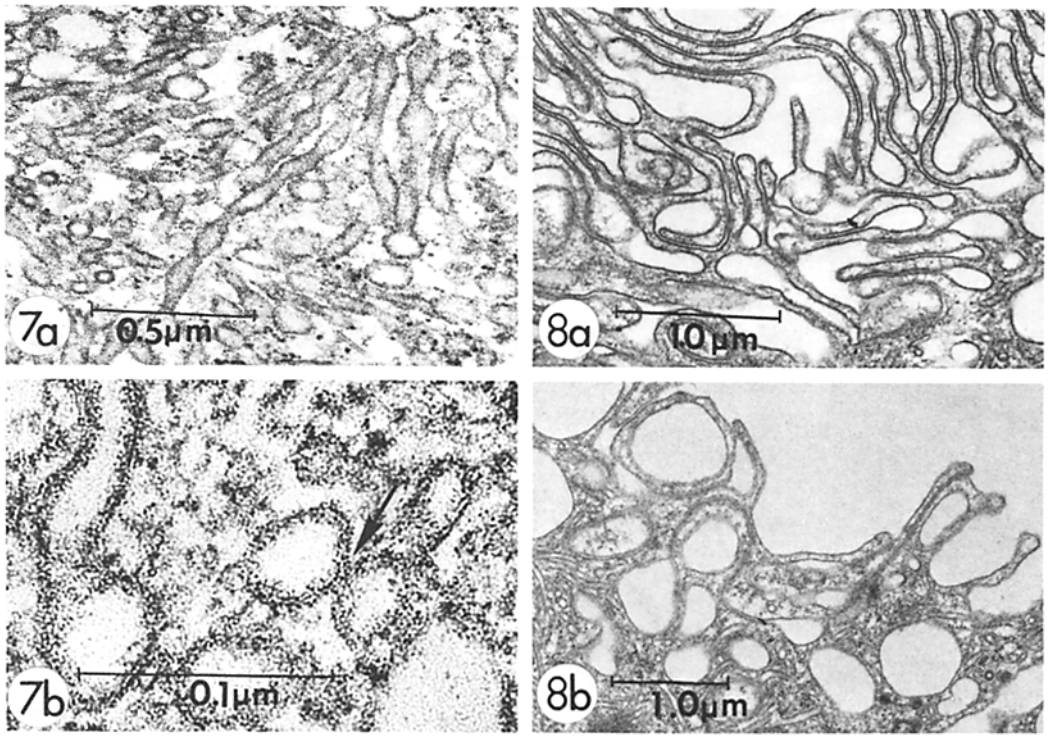


FIGURE 7 Tubular transition figures. (*a*) A tubule exhibiting constrictions possibly indicating a tubule-vesicle interconversion. × 41,000. (*b*) Vesicles exhibiting a fusion of membranes (arrow) possibly indicating a mechanism for tubule formation or dissolution. × 333,500.

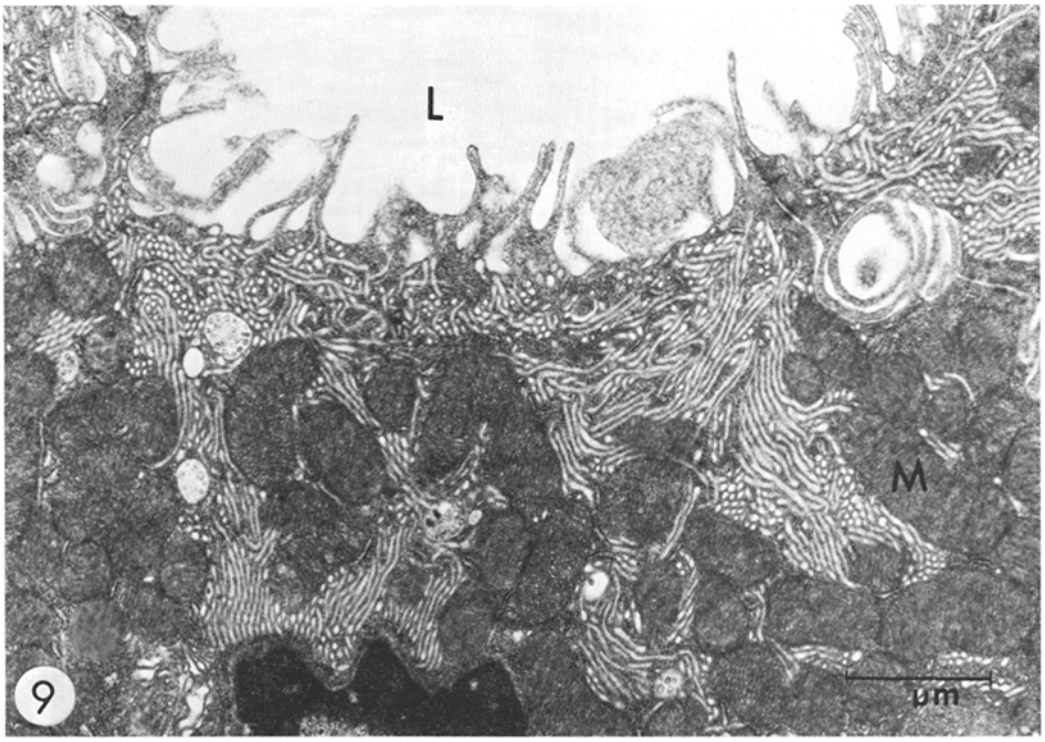
FIGURE 8 Surface transition figures. (*a*) Intermicrovillar space terminating in bulbous structure. × 20,000. (*b*) Smooth-surfaced vacuoles adjacent to luminal surface and in apical area of secreting cell. Note similar transitional structures in Fig. 5. × 15,500.

show that the transitions are due to the theophylline treatment rather than to the SCN or anoxia per se.

The most conspicuous change among the transitional states of the oxyntic cells is that of the apical surface membrane. For this reason, reliable quantitative measurement of morphological change is most easily accomplished by morphometric lineal analysis (27, 28) of the apical surface density. The results of such an analysis are presented in Table I. The values reveal approximately a 10-fold increase in surface area after theophylline stimulation compared with metiamide controls. Spontaneously secreting tissues show a value intermediate between that of the resting state and that of the theophylline-stimulated group. This supports the qualitative observation that spontaneously secreting tissue contains a nonhomogeneous population of cells.

A value similar to that for the theophylline-stimulated tissue was found for the nonsecreting state after treatment with theophylline plus SCN. The anoxia-stimulated tissue shows a significant increase in surface area compared to metiamide-treated tissue, but the increase is less than that observed with theophylline or theophylline plus SCN.

The use of theophylline as a secretory stimulant immediately suggests a role for cyclic AMP in the morphological transitions as has been suggested for acid secretion per se (8). To test the possible role of cyclic AMP in promoting morphological changes, tissue cyclic AMP content was measured for the various experimental conditions. Table II shows the results of these studies as being consistent with cyclic AMP mediating the theophylline-induced morphological changes. Under the conditions tested, a significant increase in cyclic AMP was observed after theophylline, whether or not acid secretion was stimulated. Furthermore, the



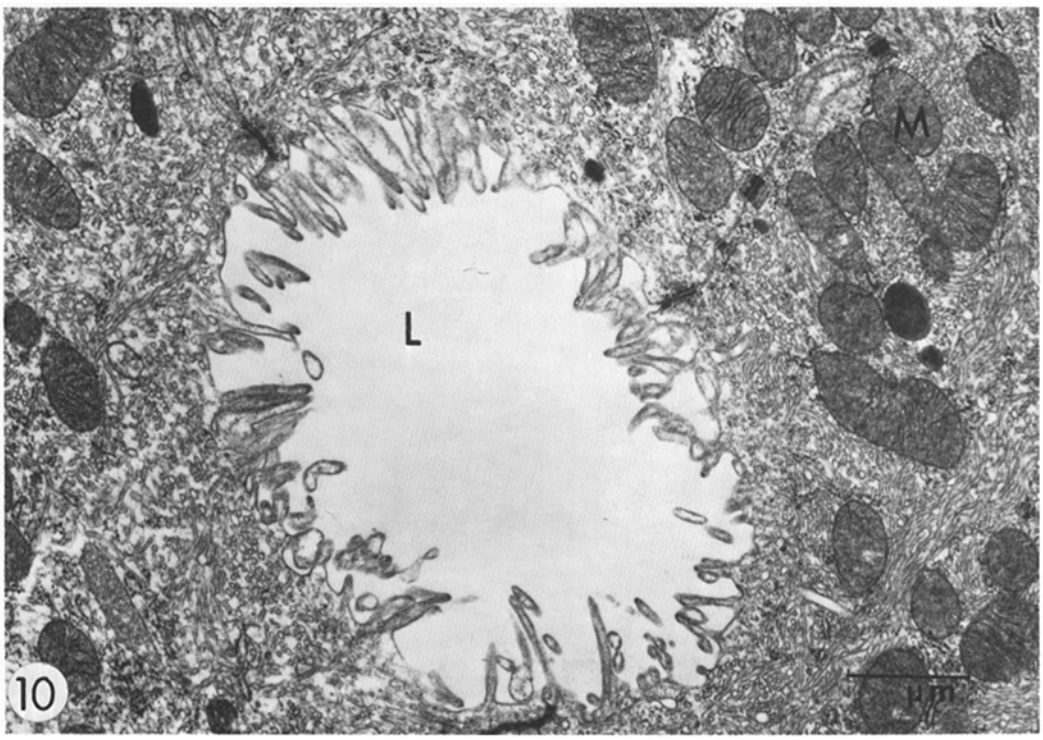


FIGURE 9 Oxyntic cell treated with theophylline plus SCN. Note surface elaboration and exaggerated tubule system compared to that of nonsecreting tissue in Fig. 2. × 17,500.

FIGURE 10 Oxyntic cell treated with theophylline under anoxic conditions. Note surface elaboration compared to that of resting tissue in Fig. 2. × 14,000.

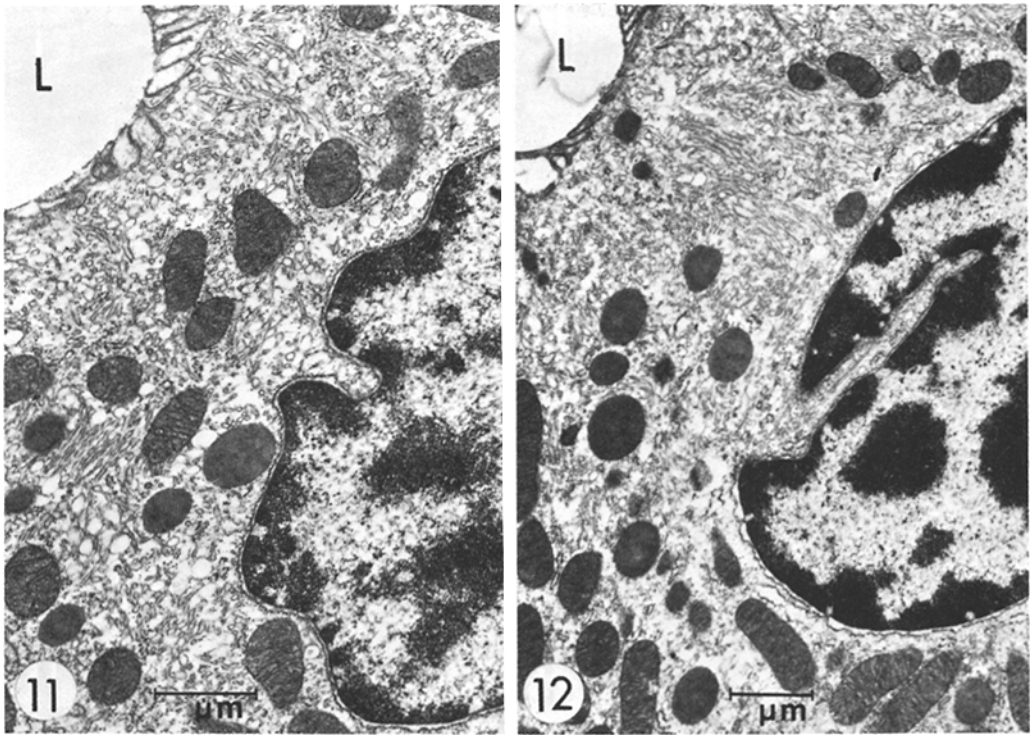


FIGURE 11 Control tissue for SCN. A tissue was treated with SCN (10^{-2} M) only after metiamide. Compare with Figs. 2 and 9. × 12,000.

FIGURE 12 Resting tissue made anoxic for 40 min. Control anoxic tissues are similar to those treated with metiamide alone (Fig. 2). Compare with Fig. 10. × 10,000.

TABLE I
Morphometric Analysis of Surface Area

Experimental group	N*	Surface density
		$m^2\ cm^{-3}$
Metiamide	13	0.122 ± 0.014
Spontaneous secretion, 25 min	15	0.442 ± 0.072‡
Theophylline	9	1.130 ± 0.124‡
Theophylline + SCN	11	0.970 ± 0.136‡
Anoxia + theophylline	10	0.525 ± 0.058‡

* N = number of cells measured.
‡ $P < 0.001$ compared to metiamide.

increases in cyclic AMP found with SCN and anoxia were as great as that with theophylline alone, indicating that these treatments do not interfere with the production of cyclic AMP. The spontaneously secreting mucosa showed a cyclic AMP level slightly higher than that of metiamide-treated tissues, but the difference is not significant.

TABLE II
Tissue Cyclic AMP Content

Experimental group	N‡	Cyclic AMP	Acid secretion
		pmol/mg protein	$\mu Eq\ cm^{-2}h^{-1}$
Metiamide	12	0.9 ± 0.09	0.24 ± 0.06
Spontaneous secretion, 25 min	8	1.3 ± 0.12	1.44 ± 0.12*
Theophylline	12	4.8 ± 0.40*	2.82 ± 0.43*
Theophylline + SCN	8	6.3 ± 0.60*	0.54 ± 0.12
Anoxia + theophylline	10	4.3 ± 0.40*	0

* $P < 0.001$ compared to metiamide.
‡ N = number of tissues measured.

DISCUSSION

The studies reported here have utilized in vitro frog gastric mucosa as a model system for studying the morphological changes that are concomitant with varying physiological activity. This preparation allows considerable latitude in our ability to manipulate the physiological state of the tissue and permits the simultaneous measurement of morphological, physiological, and biochemical parameters. Thus, it is an ideal preparation for studying the mechanisms which underlie the observed morphological changes.

Pretreatment of the tissue with the histamine H_2-receptor antagonist metiamide results in an easily obtainable, reproducible control state in which the oxyntic cells have a uniform appearance characterized by a smooth surface and no apparent organization of the tubulovesicular system. This appearance contrasts with the heterogeneous ultrastructure of the oxyntic cells observed in the spontaneously secreting tissue. The morphological effects of metiamide appear to be related to the inhibition of secretion rather than to a direct action of this agent on oxyntic cell morphology. Tissues brought to zero secretion by preincubation showed a morphology similar to that after metiamide treatment, and subsequent addition of metiamide resulted in no further change. Therefore, it appears that metiamide does not alter the ultrastructure of the true resting oxyntic cells and that it can be used to produce a reliable, reproducible control state which is readily distinguished from the secreting state.

The metiamide-treated tissues differ from the nonsecreting controls reported by other investigators (6, 21), particularly with regard to the appearance of the tubulovesicular system which seems to be much more organized in their preparations. The explanation for this difference may lie in the true zero secretory rate of our preparations.

The morphology of the theophylline-stimulated tissue is consistent with that reported by other investigators (5, 6, 9–11, 15, 20–24) in showing a marked increase in surface area primarily due to the appearance of filamentous projections or microvilli. This large, 10-fold change, compared to the metiamide control, is readily quantitated by lineal analysis as is shown in Table I. These morphometric results are consistent with changes observed in mammalian parietal cells (9, 15), and the values are similar to those reported by Helander et al. (11) in frog. The formation of these microvilli has been postulated to involve incorporation of tubulovesicle membrane into the cell surface, i.e., the membrane-conservation hypothesis (9, 15, 23). This hypothesis is supported by the observations that inhibition of protein synthesis does not block the transition (3) and that the total phospholipid content does not change (17). Thus de novo membrane synthesis does not seem to be required. This would not rule out the possibility that membrane dissolution to the molecular level may be involved, but this seems unlikely. The observation of various structures which are interpreted as "transition figures" also lends support to the membrane-conservation hypothesis, and we have presented several structures that seem to fit this category (Figs. 7 and 8). Despite the circumstantial evidence, no definite proof of transitions between vesicles, tubules, and surface membrane is yet available. Perhaps the most convincing evidence, though not proof, is the morphometric analysis showing a reciprocal relationship between surface membrane and subsurface membrane (10, 15).

Accepting the observation that morphological changes accompany secretory stimulation, two general questions arise. First, what mechanisms are responsible for the transition and, second, what role does the morphological change play in the production of acid? The present study represents an initial attempt to correlate morphological, secretory, and biochemical evidence in order to gain insight into these two problems. The morphometric results obtained with SCN and anoxia demonstrate that the ultrastructural changes can be dissociated from the secretory response and thus can be assumed to be a direct result of the stimulating agent rather than secondary to acid production. This would be consistent with the observation of Helander and Hirschowitz (10) that morphological changes precede the onset of acid secretion. It is also consistent with the recent observation that secretory inhibition by anoxia does not reverse the morphological changes (5). The observation that morphological changes occur under anoxic conditions demonstrates that they are not dependent upon oxidative metabolism and probably do not require excessive metabolic energy. The fact that acid secretion itself is very dependent upon oxidative metabolism (12) further emphasizes the independence of these two phenomena. The dissociation of morphological changes from acid production does not indicate that acid secretion can occur in the absence of the

morphological transition. Indeed, all of the evidence would indicate that the morphological changes are a prerequisite to acid secretion, although the exact relationship is unknown.

The demonstration that theophylline stimulates acid secretion and morphological changes suggests a role for cyclic AMP in these processes. The observations of Harris et al. (8) that theophylline increases tissue cyclic AMP levels and that cyclic AMP stimulates acid production and respiration provide a basis for the assumption that cyclic AMP mediates the action of theophylline on the secretory and metabolic processes. Moreover, cyclic AMP has been implicated in morphological changes in other tissues (19, 29, 30). Consistent with this view, the present results show a correlation between the theophylline-dependent morphological changes and tissue cyclic AMP content. Although these conditions do not represent an exhaustive comparison of cyclic AMP and morphological changes, they do represent four distinct physiological states. The metiamide-treated tissue has a low cyclic AMP content, zero secretion, low metabolic rate, and resting morphology. The theophylline treatment results in secretory, metabolic, and morphological responses, whereas the theophylline plus SCN treatment results in only metabolic and morphological responses. Finally, the anoxia-stimulated tissue exhibits only the morphological response. The last three conditions have only morphological changes and increased cyclic AMP content in common. Ideally, this sequence should be completed by identifying a condition in which the addition of theophylline did not result in morphological changes; however, we are not aware of such a condition. The spontaneously secreting tissue has a secretory rate and surface area which is intermediate between that of the resting condition and that of the theophylline condition. The cyclic AMP content, however, is low in proportion to the other parameters, being essentially the same as for metiamide treatment. Several explanations for this quantitative discrepancy may be envisioned. The temporal relations between morphology and cyclic AMP may not be exact, i.e., the cyclic AMP level may rise and then return to basal level, while the morphological changes and secretory rate persist for some time. Alternatively, the increase in cyclic AMP seen with theophylline may represent an overproduction, and thus no strict quantitative relationship between cyclic AMP and morphology would be expected. Finally, it is possible that while theophylline acts via cyclic AMP, other stimulants (including the one which produces spontaneous secretion) may act by a mechanism which does not involve this cyclic nucleotide. Distinguishing among these possibilities is clearly beyond the scope of the present study. We can conclude only that for theophylline there is at least a qualitative relationship between surface area and cyclic AMP.

The present study demonstrates a certain independence concerning the role of morphology in the process of acid secretion. Previous investigators have postulated that the morphological changes serve to increase secretory surface area. However, this postulate only states the obvious and does not focus on the important question of the physiological significance of membrane transformation and turnover. The observation of membrane transition figures which are interpreted as membrane recycling suggests that the surface elaboration is not static but continually turning over. This would imply a system which requires surface turnover rather than just increased surface area. Thus, the raison d'etre of the morphological transition most likely involves more than surface increase, and future studies should be directed to this important question.

In addition to the elucidation of the acid secretory mechanism, the ability to manipulate the physiological, biochemical, and ultrastructural states of this tissue gives this system the potential of answering some basic questions in the field of cell biology concerning the function of mitochondria and the recycling processes of cytoplasmic and plasma membranes.

This study was supported by U. S. Public Health Service grants, AM14752 and AM29899.

Received for publication 9 December 1976, and in revised form 9 September 1977.

REFERENCES

1. BLACK, J. W., W. A. M. DUNCAN, G. J. DURANT, C. R. GANELLIN, and M. E. PARSONS. 1972. Definition & antagonism of histamine H_2-receptors. *Nature* (*Lond.*). **236**:385–390.
2. CARLISLE, K. S., C. S. CHEW, and S. J. HERSEY. 1976. Morphological changes and cyclic AMP in frog gastric mucosa. *J. Cell Biol.* **70**(2, Pt. 2):88*a*. (Abstr.).
3. CARLISLE, K. S., C. R. REAGAN, and S. J. HERSEY. 1977. Effect of protein synthesis inhibitors on gas-

tric acid secretion. *Texas Society of Electron Microscopy Newsletter.* **8:**25.

4. CHEW, C. S., and G. A. RINARD. 1974. Estrogenic regulation of uterine cyclic AMP metabolism. *Biochim. Biophys. Acta.* **362:**493-500.
5. FORTE, T. M., T. E. MACHEN, and J. G. FORTE. 1975. Ultrastructural & physiological changes in piglet oxyntic cells during histamine stimulation & metabolic inhibition. *Gastroenterology.* **69:**1208-1222.
6. FORTE, J. G., T. M. FORTE, and T. K. RAY. 1972. Membranes of the oxyntic cell: their structure, composition & genesis. *In* Gastric Secretion. G. Sachs, E. Heinz, and K. J. Ullrich, editors. Academic Press, Inc., New York. 37-67.
7. GILMAN, A. G. 1970. A protein binding assay for adenosine 3',5' cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67:**305-312.
8. HARRIS, J. B., K. NIGON, and D. ALONSO. 1969. Adenosine 3',5' monophosphate: intracellular mediator for methyl xanthine stimulation of gastric secretion. *Gastroenterology.* **57:**377-384.
9. HELANDER, H. F., and B. I. HIRSCHOWITZ. 1972. Quantitative ultrastructural studies on parietal cells. *Gastroenterology.* **63:**951-961.
10. HELANDER, H. F., and B. I. HIRSCHOWITZ. 1974. Quantitative ultrastructural studies on inhibited & on partly stimulated gastric parietal cells. *Gastroenterology.* **67:**447-452.
11. 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. Ullrich, editors. Academic Press, Inc., New York. 69-88.
12. HERSEY, S. J. 1974. Interactions between oxidative metabolism & acid secretion in gastric mucosa. *Biochim. Biophys. Acta.* **344:**157-203.
13. HERSEY, S. J., W. L. HIGH, and F. F. JOBSIS. 1972. Optical measurements of intracellular reactions in gastric mucosa. *In* Gastric Secretions. G. Sachs, E. Heinz, and K. J. Ullrich, editors. Academic Press, Inc., New York. 239-272.
14. HIGH, W. L., and S. J. HERSEY. 1974. Mechanism of theophylline stimulation of acid secretion by frog gastric mucosa. *Am. J. Physiol.* **226:**1408-1412.
15. ITO, S., and G. C. SCHOFIELD. 1974. Studies on the depletion & accumulation of microvilli & changes in the tubulovesicular compartment of mouse parietal cells in relation to gastric acid secretion. *J. Cell. Biol.* **63:**364-382.
16. KASBEKAR, D. K. 1967. Studies of resting isolated frog gastric mucosa. *Proc. Soc. Exp. Biol. Med.* **125:**267-271.
17. KASBEKAR, D. K., G. M. FORTE, and J. G. FORTE. 1968. Phospholipid turnover & ultrastructural changes in resting & secreting bullfrog gastric mucosa. *Biochim. Biophys. Acta.* **163:**1-13.
18. KOCH, A. L., and S. L. PUTNAM. 1971. Sensitive biuret method for determination of protein in an impure system such as whole bacteria. *Anal. Biochem.* **44:**239-245.
19. PORTER, K. R., T. T. PUCK, A. W. HSIE, and D. KELLY. 1974. An electron microscopic study of the effect of Bt_2 cAMP on CHO cells. *Cell.* **2:**145-162.
20. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17:**208-212.
21. SEDAR, A. W. 1961. Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbeiana*). I. The non-secreting gastric mucosa. *J. Biophys. Biochem. Cytol.* **9:**1-18.
22. SEDAR, A. W. 1961. Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbeiana*). II. The acid-secreting gastric mucosa. *J. Biophys. Biochem. Cytol.* **10:**47-57.
23. SEDAR, A. W. 1965. Fine structure of the stimulated oxyntic cell. *Fed. Proc.* **24:**1360-1367.
24. SEDAR, A. W., and M. H. F. FRIEDMAN. 1961. Correlation of fine structure of gastric parietal cell (dog) with functional activity of the stomach. *J. Biophys. Biochem. Cytol.* **11:**349-363.
25. SEDAR, A. W., and V. WIEBELHAUS. 1972. K^+ effects on acid secretion & ultrastructure of the amphibian oxyntic cell. *Am. J. Physiol.* **223:**1088-1092.
26. SHOEMAKER, R. L., E. BUCKNER, J. G. SPENNEY, and G. SACHS. 1974. Action of burimamide, a histamine antagonist, on acid secretion in vitro. *Am. J. Physiol.* **226:**898-902.
27. WEIBEL, E. R. 1963. Principles & methods for the morphometric study of the lung & other organs. *Lab. Invest.* **12:**131-154.
28. WEIBEL, E. R. 1973. Stereological techniques for electron microscopic morphometry. *In* Principles and Techniques of Electron Microscopy. M. A. Hayat, editor. Van Nostrant Reinhold, New York. **3:**239-291.
29. WILLINGHAM, M. C., and I. PASTAN. 1975. Cyclic AMP and cell morphology in cultured fibroblasts. Effects on cell shape, microfilament and microtubule distribution, and orientation to substratum. *J. Cell. Biol.* **67:**146-159.
30. WILLINGHAM, M. C., and I. PASTAN. 1975. C AMP modulates microvillus formation & agglutinability in transformed & normal mouse fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **72:**1263-1267.