ACTA HISTOCHEM. CYTOCHEM. Vol. 17, No. 4, 1984

ENZYMATIC RESPONSE TO HCL INHIBITION IN OXYNTIC CELLS OF FASTING RATS

F. J. MORENO, J. PIÑERO, R. COVEÑAS AND J. L. LÓPEZ-CAMPOS

Departments of Cytology and Histology, Faculty of Biology, Service of Electron Microscopy, Sevilla University, Sevilla, Spain

Received for publication February 8, 1983 and in revised form June 14, 1984

Morphological modifications and cytochemical changes in the acid phosphatase on oxintic cells of the gastric mucosa of rats deprived of food for varying lengths of time were studied. With long fasting periods a lysosomal qualitative increase and mitochondrial morphological alteration are caused. This last fact would make an ATP decrease and an alteration in the ATPase actuation, so preventing the interchange of H+ for K+ and thus blocking intravesicular HCl formation.

Of the different cell types which form the gastric mucosa, the oxyntic cells are those responsible for the secretion of hydrochloric acid. This cell type, described by Heidenhain in 1870 (7), has been studied frequently, on account of both its cytological structure (1, 9), its physiology (3, 16) and its cytochemical characterization (2).

Studies relating to the localization of active sites of enzymes belonging to the K^+/H^+ ATPase complex have been performed on different tissues at the cell level. Evidence of the presence of these enzymes in the cell membranes and in other organelles has partly established the relation between structure and function and there is ever increasing evidence of this.

The object of our study was to establish a functional comparison of the activity of acid phosphatase, using β-glycerophosphate as substrate, in gastric mucosa oxyntic cells of rats subjected to fasting and others with normal diets.

There have been several studies related to ultrastructural modification of oxyntic cells in different secretory states (4, 5, 15) and the physiological HCl secretory mechanism has been demonstrated with enzymatic, cytochemical and biochemical techniques (17, 18).

MATERIAL AND METHODS

Sprague dawley rats, born and raised in our laboratory, were used. One lot of animals was isolated and deprived of food for periods of 82 and 342 hr; gastrectomies were performed and the stomachs then sectioned, selecting the fundus region. This same zone was also obtained from animals whose diet had been normal.

Small pieces of gastric fundus were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for one hr. Thick frozen sections were cut on a cryostat

previously cooled to −20°C, and incubated in a medium containing β-glycerophosphate and lead nitrate in a 0.5 M tris-maleate buffer at pH 5 for one hr at 37°C (11). We also incubated sections without β-glycerophosphate.

After washing in cacodylate buffer sections were post-fixed in 1% osmium tetroxide in distilled water for 1 hr at 4°C. They were then embedded in Spurr's resin. Other fragments of gastric fundus were fixed and embedded using conventional electron microscopic techniques, for normal histological study.

RESULTS

Electron microscopic enzymatic studies of the activity of acid phosphatase carried out on oxyntic cells in gastric mucosa of animals deprived of food for different periods using β-glycerophosphate as substrate, gave different results when compared to this enzyme activity in normal mucosa at the oxyntic cell level.

Figure 1 shows thin section of normal mucosa oxyntic cell, in which the distribution of structural elements in a period of secretion can be observed. When compared with mucosa oxyntic cells under fast, we can see lysosomes present in the

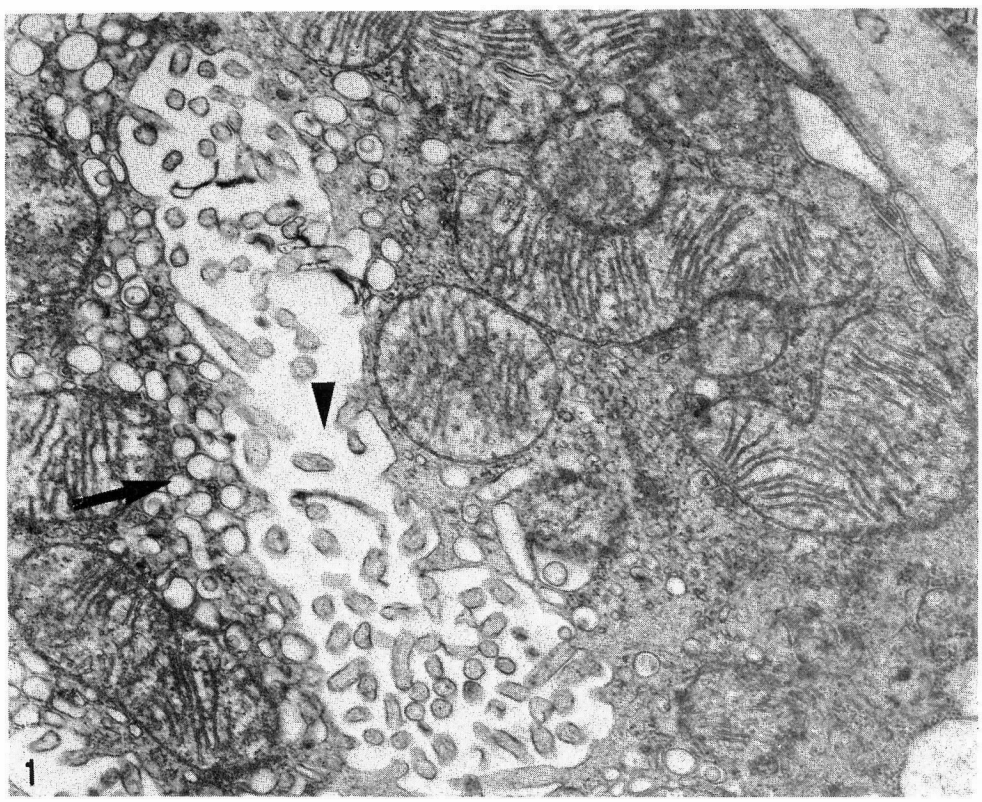


FIG. 1. Oxyntic cell fragment of normal gastric mucosa in secretion phase. Observe the dilated lumen of intracellular canaliculi (head arrow), the moderate tubulo-vesicular system (arrow) and mitochondria with conserved crests. ×30,000

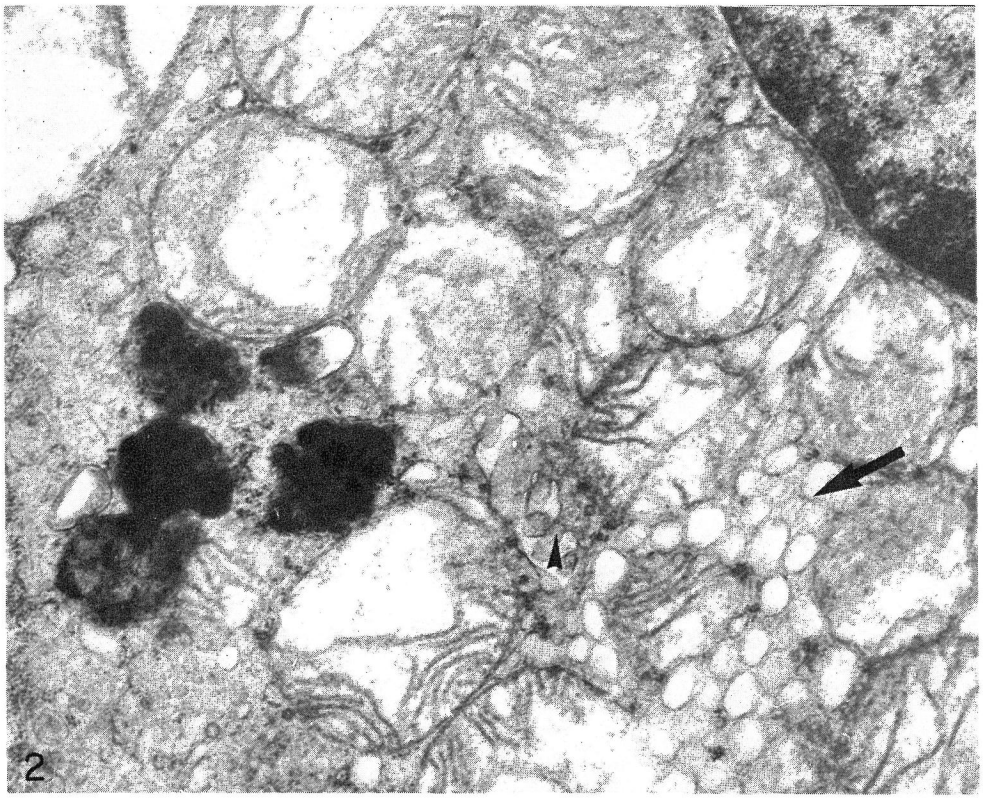


FIG. 2. Increase of tubulo-vesicular system (arrow) and obliterated intracellular channels of an oxyntic cell of gastric mucosa in fast. Note lysosomes next to obliterated intracellular canaliculi (head arrow). Mitochondria present altered crest. ×32,000

proximity of the intracellular canaliculi (Fig. 2), growth of the tubular vesicular system; obliteration and deformation of the intracellular canaliculi and mitochondrial alterations (Fig. 3).

Normal gastric mucosa oxyntic cells show acid phosphatase activity in lysosomes, which are localized in the basal zones of the cell (Fig. 4); however, the number of lysosomes increased qualitatively with longer periods of fasting, through the whole of the cytoplasm (Fig. 5); thus, the oxyntic cell, under these conditions of fasting, shows an increase in lysosomal enzymes.

In normal mucosal oxyntic cells, the existence of a moderate tubular vesicular system (1), which is increased when the animals are deprived of food, is observed.

With respect to ultrastructural modifications of the intracellular canaliculi of control animals, we observed that the acid phosphatase technique resulted in lead phosphate precipitate on the membranes which make up the microvilli of these canaliculi (Fig. 6).

Comparison of the different groups of animals under study showed a decrease in enzyme activity in the microvilli along with the structural modifications of the canaliculi. The loss of phosphatase activity on the canaliculi was parallely pro-

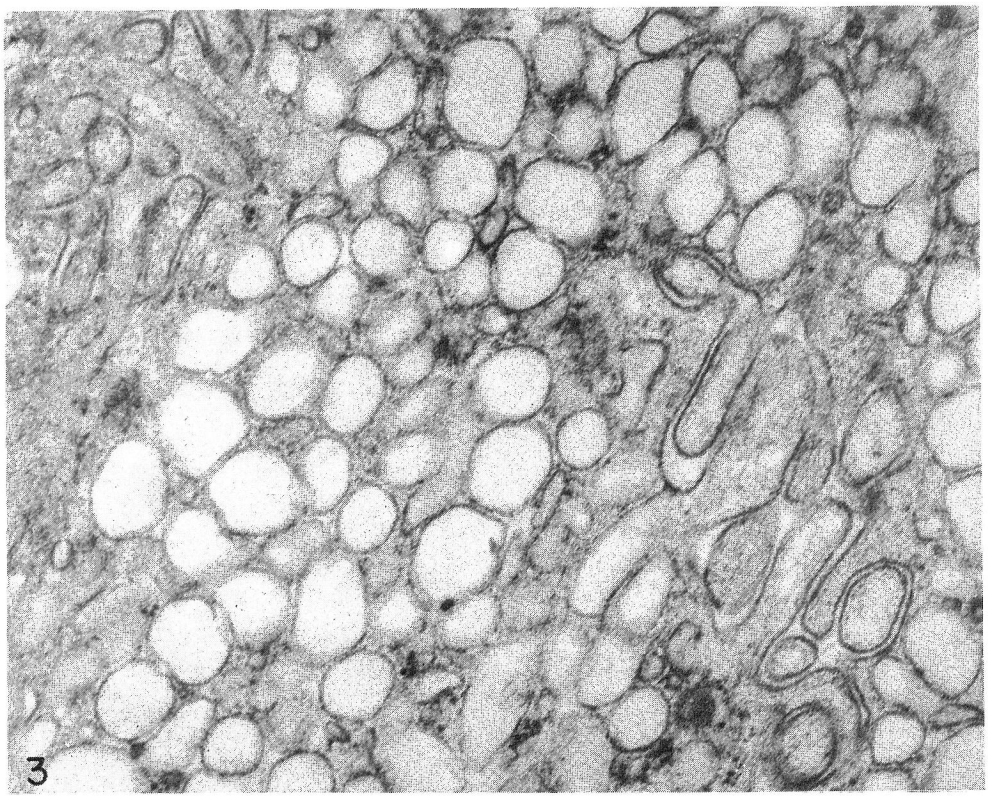


FIG. 3. Detail of tubulo-vesicular development and obliteration of oxyntic cell canaliculi of gastric mucosa in fasting period. ×35,000

portional to the increase of fasting time. Thus, we observed an increase of lysosomal phosphatase activity.

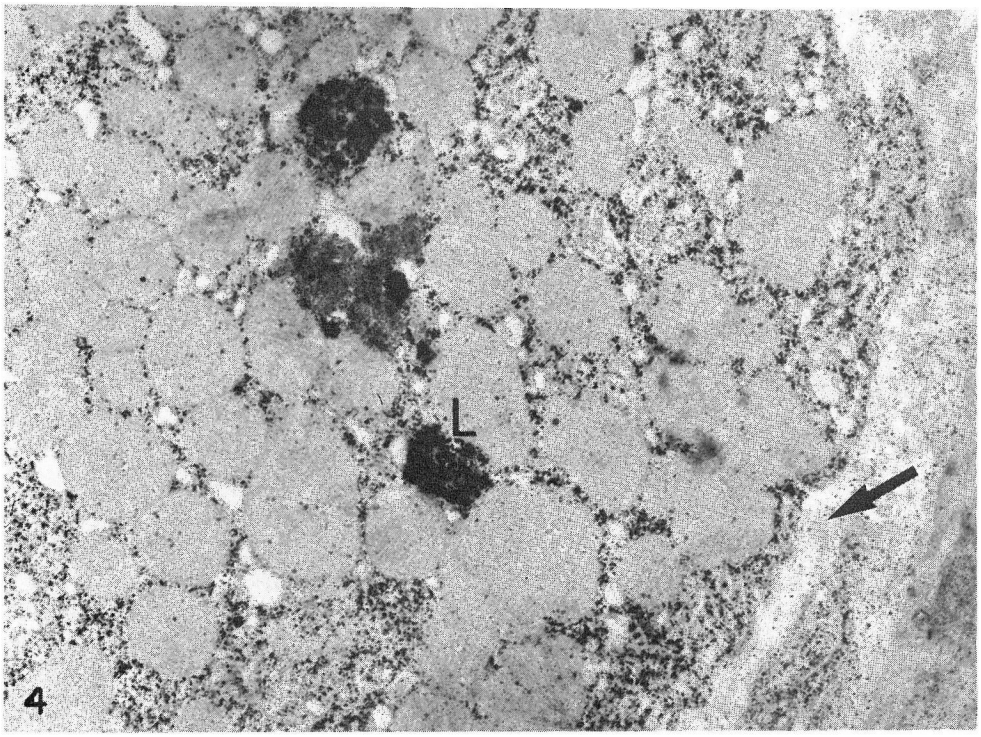
DISCUSSION

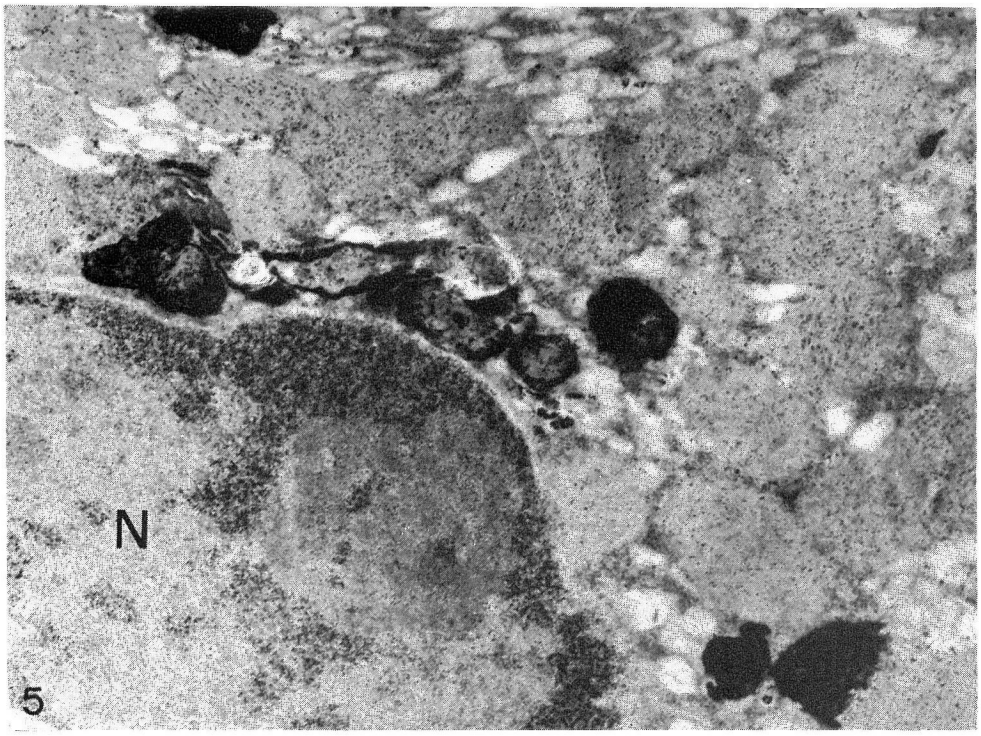
Ito and Schofield (10) described how, in gastric parietal cells, one of the most notable details in connection with secretion inhibition is the presence of a great number of intracellular vesicles and the scarcity of surface microvilli in the canaliculi. However, the presence of lysosome-type elements and multivesicular bodies has been related to inhibitory action of secretory activity in oxyntic cells (6, 14).

The development of the Golgi complex in oxyntic cells has been related to HCl secretion inhibition (12) and to the development of new lysosomes in gastric mucosa of animals subjected to the action of the drug.

FIG. 4. Oxyntic cell of normal gastric mucosa acid phosphatase reaction in basal lysosomes (L). Basal membrane (arrow). ×8,000

FIG. 5. Intense acid phosphatase reaction in lysosomal bodies of oxyntic cells of gastric mucosa under fast. Unspecific precipitate on mitochondria. Nucleus (N). ×15,000


L
4


N
5

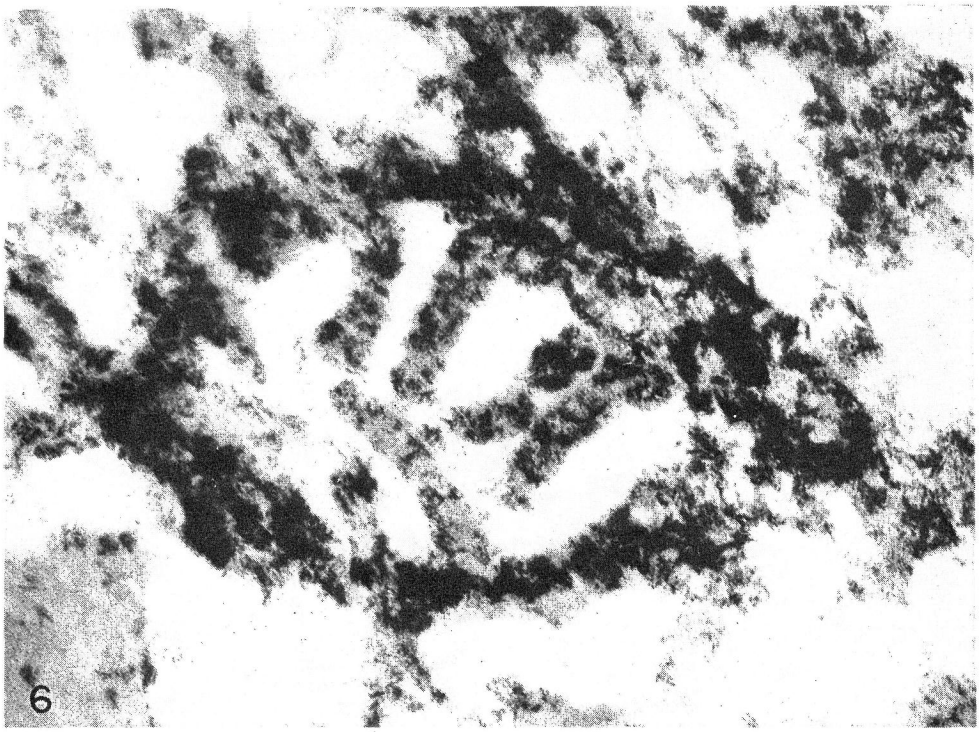


FIG. 6. Intracellular microvilli with enzymatic reaction to acid phosphatase in oxyntic cells of normal gastric mucosa. ×40,000

We have not observed any considerable increase in the development of the Golgi complex in fasting animals and for this reason we belive that the origin of the lysosomes and therefore the synthesis of lysosomal hydrolase, takes place in the ribosomes associated with the membranous cisterna of the endoplasmatic reticulum, continuing towards the GERL which produces (13), by genmation, vesicles which form lysosomes.

In an exhaustive study on structural and functional changes during the HCl secretory cycle in oxyntic cells (15), the stated morphological changes are corroborated, and at the same time a functional interpretation is given of the HCl secretory mechanism, which takes place in the tubular vesicular system; H^+ originating in ATP hydrolisis are produced, and interchange with K^+ reducing the concentration of K^+ ions in the interior of the vesicles, which, in the inhibitory state of cell synthesis are abundant in the cytoplasm and in whose there is a high concentration of potassium ions.

Forte *et al.* (4) also point out the presence of lysosomes in the proximity of these vesicles, and suggest that the localization at these cell levels could have an accidental or functional significance.

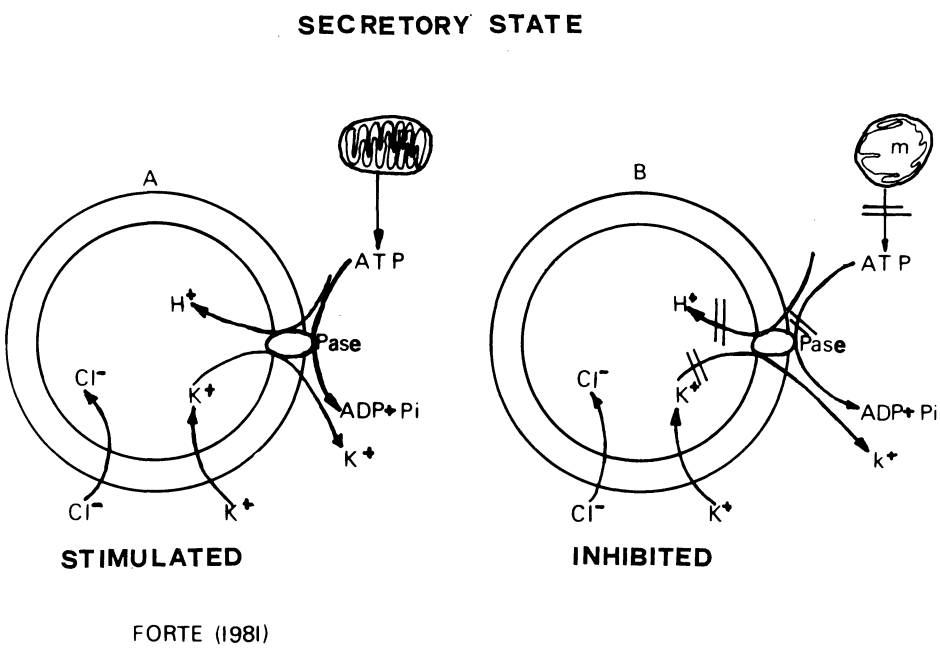
We have observed that oxyntic mitochondria degenerate with an increase in fasting time; this could be attributed initially to an artifact in the tissue fixation technique, however, in control groups under the same conditions no mitochondrial

degeneration took place. On the other hand, studies carried out by Helander and Hirschowitz (8) from a morphologic and stereologic viewpoint have shown that with the use of chlorhydric secretion inhibition drugs a decrease of mitochondrial volume and an increase of the tubulo-vesicular membrane can be observed. Thus we could establish a functional correlation between the degree of fasting, mitochondrial alterations and modifications of the tubulo-vesicular system and subsequent decrease of chlorhydric secretion.

Since in our study we obtained an increase of acid phosphatase and taking into account the above mentioned data, we suggest a functional correlation between ultrastructural alterations and localization of enzyme activity. Thus, as a consequence of mitochondrial degeneration, along with the prolongation of fasting times, there is a decrease of ATP concentration in the intracellular medium. This ATP decrease implies that the hydrolytic enzymes of the K^+/H^+-ATPase complex sequentially inhibit their activity as fasting time increases.

On the other hand the synthesis of lysosomal enzymes acting on the K^+/H^+-ATPase complex would be retained at the lysosomal level, thereby increasing the number of enzymes which would be distributed through the apical zones of the cell next to the occluded intracellular canaliculi.

Figure 7 shows HCl production in the vesicles of the tubular vesicular system of



FIGS. 7A, B. A. HCl production mechanism in the tubulat-vesicular system of stimulated oxyntic cells (according to Forte *et al.* (4)).

B. Morphological alterations during long fasting periods suggest that HCl production is inhibited. Hyaloplasmic ATP concentration decreases owing to mitochondrial alteration, and therefore ATP hydrolysis carried out by the ATPase complex enzymes decreases, preventing the interchange of H^+ for K^+, and thus blocking intravesicular HCl formation.

stimulated oxyntic cells, according to the model proposed by Forte *et al.* (4). On the right is the proposed HCl secretion inhibitory mechanism, probed by extreme periods of fasting. In order to confirm the above mentioned data, biochemical and cytochemical studies of mitochondrial ATPase activity are at present being carried out.

REFERENCES

1. Beams, H. W. and King, R. L.: Notes on the cytology of the parietal cell of the stomach of the rat. *Anat. Rec.* 53; 31, 1932.
2. Forte, T. M. and Forte, J. G.: Histochemical staining and characterization of glycoproteins in acid secreting cells of frog stomach. *J. Cell. Biol.* 47; 437, 1970.
3. Forte, J. G. and Machen, T. E.: Transport and electrical phenomena in resting and secreting piglet gastric mucosa. *J. Physiol.* London. 244; 33, 1975.
4. Forte, J. G., Black, J. A., Forte, T. M., Machen, T. E. and Wolosin, J. M.: Ultrastructural changes related to functional activity in gastric oxyntic cells. *Am. J. Physiol.* 241; 5, G349, 1981.
5. Frexinos, J. M., Carbadillo, M., Louis, A. and Ribet, A.: Effects of pentagastrin stimulation on human parietal cells. *Digestive Diseases* 16; 12, 1065, 1971.
6. Hally, A. D.: The fine structure of the gastric parietal cell in the mouse. *J. Anat.* 93; 217, 1959.
7. Heindenhain, R.: Untersuchungen über den Bau der Labdrüsen. *Arch. Mikr. Anat.* 6; 368, 1870.
8. Helander, H. F. and Hirschowitz, B. I.: Quantitative ultrastructural studies on inhibited and on partly stimulated gastric parietal cells. *Gastroenterology* 67; 447, 1974.
9. Helander, H. F.: The cells of the gastric mucosa. *Inter. Rev. Cytol.* 70; 217, 1981.
10. Ito, S. and Schofield, G. C.: Ultrastructural changes in mouse parietal cells after high H^+ secretion. *Acta Physiol. Special Suppl.*; 25, 1978.
11. Lewis, P. R. and Knight, D. P.: Staining methods for sectioned material. In Practical Methods in Electron Microscopy, ed. by A. M. Glaevert, vol. 5, Part 1, North Holland Publishing Company, Amsterdam-London, 1977, p. 1.
12. López-Campos, J. L., Moreno, F. J. and Piñero, J.: Effect of pirenzepine on mucus histochemistry and on gastric secretory ultrastructure. Excerpta Medica Symposium Advances in Gastroenterology with the Selective Antimuscarinic Compound-Pirenzepine, Stockholm, Excerpta Medica, 1982, p. 105.
13. Novikoff, A. B.: GERL, its form and function in neurons of rat spinal ganglia. *Biol. Bull.* 127; 358, 1964.
14. Pfeiffer, C. J., Weibel, J. and Roth, J. L. A.: Unusual ultrastructural variants in the ferret parietal cell. *Experientia* 26; 395, 1970.
15. Rosa, F.: Ultrastructurea of the parietal cell of the human gastric mucosa in the resting state and after stimulation with Histalog. *Gastroenterology* 45; 354, 1963.
16. Villegas, L.: Cellular location of the electrical potencial difference in frog gastric mucosa. *Biochim. Biophys. Acta* 64; 354, 1962.
17. Wolosin, J. M. and Forte, J. G.: Funtional differences between K^+-ATPase rich membranes isolated from resting or stimulated rabbit fundic mucosa. *FEBS Lett.* 125; 203, 1981.
18. Wolosin, J. M. and Forte, J. G.: Changes in the membrane environment of the (K^+/H^+)-ATPase following stimulation of the gastric oxyntic cell. *J. Biol. Chem.* 256; 3149, 1981.