# ULTRACYTOCHEMICAL INVESTIGATION OF ATPase ACTIVITY IN MUCOSAL PARIETAL CELLS AND IN ADENOCARCINOMA CELLS OF THE HUMAN STOMACH

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UDC 616.33-006.66-008.931-092.18

An electron-histochemical investigation was made of ATPase in the parietal cells of the mucous membrane and in tumor cells of an adenocarcinoma of the human stomach, with similar ultrastructure. The reaction product for ATPase in the parietal cells was found on membranes of microvilli of the intracellular tubules, on membranes bounding the lateral intercellular spaces, on the basal plasmalemma, and in the nucleoli. No reaction product was found on membranes of the tubulovesicles or on the apical surface of the plasmalemma. The reaction product was observed in the tumor cells on membranes of the villi of the intracellular tubules, on the basal plasmalemma, and in the nucleoli. Comparison of ATPase activity in these cells showed that the part of the mechanism of hydrochloric acid secretion that is connected with $H^+$ and $Cl^-$ transport is preserved in the tumor cells. The permanent decrease in hydrochloric acid secretion by the gastric mucosa in the presence of cancer and by the cancer itself is probably attributable to other mechanisms.

KEY WORDS: adenocarcinoma of the stomach; parietal cells; adenosine triphosphatase; electron histochemistry.

An early and important symptom of the development of gastric cancer may be a reduction in, or the total disappearance of hydrochloric acid in the gastric juice. The mechanisms of this phenomenon have not yet been explained. The parietal cells of the glands of the gastric mucosa are known to play the principal role in hydrochloric acid formation.

Hersey et al. [7] concluded from their investigations that $H^+$ ions in the parietal cells are formed in the mitochondria, whereas $Cl^-$ ions enter the parietal cells from the tissue fluid and blood serum through the basal plasmalemma of the cell.

The principal mechanism of the subsequent transport of $Cl^-$ and $H^+$ ions within the parietal cells, then into the lumen of the glands, and finally into the lumen of the stomach, where the hydrochloric acid is evidently formed directly, is connected with the enzymic hydrolysis of ATP, which leads to the liberation of the energy required for the transport of these ions against the existing electrochemical concentration gradient. Two stages can thus be distinguished in the mechanism of secretion of hydrochloric acid by the parietal cells: The first is connected with the formation of the original substances ($H^+$ and $Cl^-$), the second with their transport. The ultrastructures concerned with the transport of $H^+$ and $Cl^-$ ions are the tubulovesicles, intracellular tubules, basal plasmalemma, and microvilli of the apical plasmalemma [2, 6, 9-12]. The most important indicator of the function of the ATPase mechanism of secretion is the presence of the enzyme ATPase in the structures concerned. However, no investigations have been undertaken of the activity of this enzyme at the electron-histochemical level in cancer of the stomach. Accordingly the object of this study was to examine the ATPase mechanism of transport of $H^+$ and $Cl^-$ ions in the parietal cells of the gastric mucosa and in cancer cells, with a similar type of ultrastructure, of an adenocarcinoma of the stomach.

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0007-4888/78/8607-0902 $07.50 

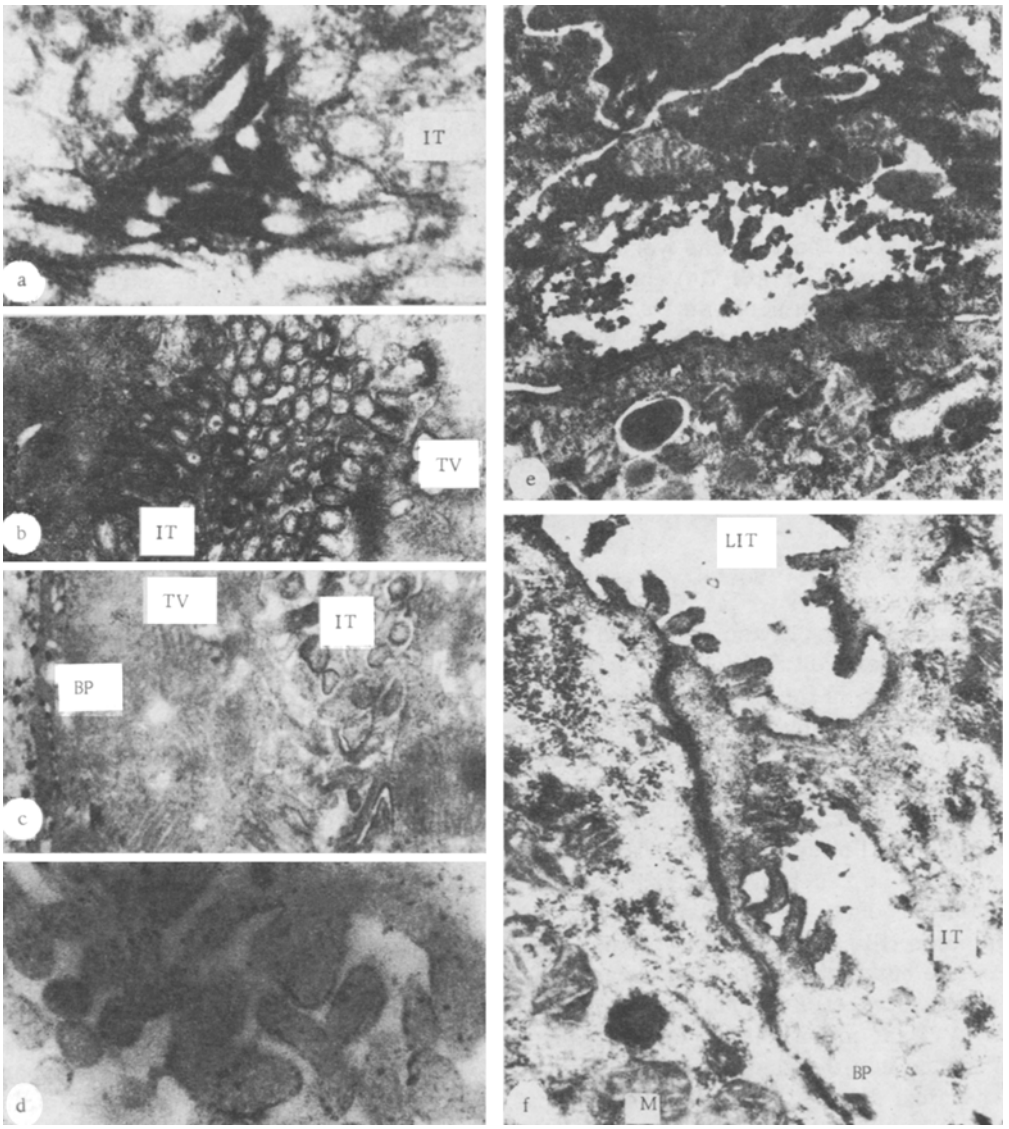


Fig. 1. ATPase in parietal cells of mucous membrane (a, b, c, d) and in tumor cells (e, f) of adenocarcinoma of the human stomach with similar ultrastructure. a) Parietal cell with high ATPase activity, connected with intracellular tubules. Unstained sections, 82,500×; b) moderate ATPase activity connected with intracellular tubules. No reaction product present in tubulovesicles. Unstained sections, 26,400×; c) parietal cell with reaction product located on folds of basal plasmalemma. Intracellular tubules inactive. Reaction product not present in tubulovesicles. Unstained sections, 27,900×; d) distribution of reaction product on inside of membranes of microvilli of intracellular tubules in normal parietal cells. Unstained sections, 80,000×; e) tumor cells of adenocarcinoma of human stomach with reaction product on villi of intracellular tubules. Relatively high enzyme activity. Sections stained with uranyl acetate and lead citrate, 19,000×; f) tumor cells with reaction product connected with villi of intracellular tubules and plasmalemma. Cell with low enzyme activity. Sections stained as in Fig. 1e, 27,000×. IT) Intracellular tubule, LIT) lumen of intracellular tubule, TV) tubulovesicles, BP) basal plasmalemma, M) mitochondrion.

## EXPERIMENTAL METHOD

Biopsy material taken for diagnostic purposes from tumors in nine patients with cancer of the stomach and from the mucous membrane of three persons in whom no tumors were found and who had no gastric symptoms was investigated.

Pieces of the mucous membrane and gastric carcinoma were prefixed in 2.5% glutaraldehyde solution in 0.2M cacodylate buffer (pH 7.4) with 0.25M sucrose for 1 h at 4°C. They were then rinsed in 0.25M sucrose solution successively for 10, 20, and 90 min. Sections were cut to a thickness of 50-60 $\mu$ in a cryostat and incubated on a waterbath at 36°C for 45 min in medium of the following composition: 0.08M Tris-maleate buffer, pH 7.2, 2 mM ATP (from Reanal, Hungary), 2 mM magnesium acetate, 2 mM lead acetate, and 0.25M sucrose [1]. After incubation the sections were rinsed in 0.25M sucrose and postfixed for 1 h in 1% $OsO_4$ solution in 0.1M cacodylate buffer (pH 7.4) containing 0.25M sucrose. The sections were then dehydrated and embedded in Epon-812. Semithin sections 1 $\mu$ thick were cut from all the blocks, stained with toluidine blue, and examined in the light microscope; the blocks were then trimmed to a point. Ultrathin sections were examined in the IEM-100C electron microscope. Some of the ultrathin sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate. As a control of the specificity of the histochemical reaction for ATPase sections were incubated in medium without substrate, after the addition of 0.01M sodium fluoride as inhibitor to the incubation medium, and after replacement of the ATP by $\beta$-glycerophosphate or AMP in equimolar concentrations. All these controls confirmed the specificity of the results of ATPase determination.

## EXPERIMENTAL RESULTS

The reaction product for ATPase was found in the parietal cells of the normal gastric mucosa on membranes of the villi of the intracellular tubules, on membranes bounding the lateral intercellular spaces, on the basal plasmalemma, and in the nuclei; no reaction product was found on the membranes of the tubulovesicles or on the apical surface of the plasmalemma. Parietal cells with different degrees of enzyme activity connected with the intracellular tubules were found in the glandular epithelium of the stomach (Fig. 1a, c). This variation of activity can evidently be explained by differences in the functional state of the parietal cells, possibly depending on the phase of hydrochloric acid secretion by these cells. Rubin et al. [12], who studied the ultrastructural localization of ATPase in the parietal cells of the normal gastric mucosa in rats and man observed a similar distribution of the reaction product to that in the present investigation. However, in some cells we found that the reaction product was distributed solely on the inner side of the membranes of the microvilli of the intracellular tubules (Fig. 1d), unlike in the experiments of Rubin et al., who describe the distribution of reaction product on the outer side of the membranes of the microvilli. The distribution of reaction product in the microvilli of the intracellular tubules is in harmony with the concept that the energy liberated on hydrolysis of ATP by ATPase maintains the active transport of $H^+$ and $Cl^-$ ions into the lumen of the intracellular tubules, and from thence into the gastric glands, against the electrochemical gradient. This hypothesis is confirmed by biochemical data [4, 5, 8, 13], showing that the rate of secretion of hydrochloric acid by the gastric mucosa depends on the ATP concentration. The presence of ATPase in the lateral and basal plasmalemma is evidence that energy is required to maintain the "membrane flow" which, as Morozov [2] has shown, lies at the basis of the specific function of the parietal cells: The repeated transposition of the membranous structures of the plasmalemma (folds of basal plasmalemma – tubulovesicles – membranes of the intracellular tubules and apical surface – folds of lateral plasmalemma – folds of basal plasmalemma) in the process of transport, in particular of $Cl^-$. The absence of ATPase in tubulovesicles formed from folds of the basal plasmalemma, and responsible for $Cl^-$ transport as far as the intracellular tubules [2, 10, 11], indicates that whereas the passage of $Cl^-$ through the basal membrane requires the expenditure of energy (the presence of ATPase), its onward movement through the cytoplasm within the tubulovesicles evidently takes place on account of other mechanisms.

The electron-microscopic study of gastric tumors with the histological structure of differentiated adenocarcinomas showed that besides undifferentiated cancer cells, with a large nucleus occupying nearly all the cytoplasm, many polysomes, and solitary mitochondria, other cells were present which, in their ultrastructure, resembled the parietal cells of the normal gastric mucosa. They had intracellular tubules, tubulovesicles, fragments of smooth endoplasmic reticulum, and many mitochondria. The number of cancer cells with this type of ultrastructure varied in different tumors. For instance, some tumors had many such cells, whereas others had only single cells which retained the features of the normal type to a certain degree. Cancer cells with the characteristic ultrastructure of normal parietal cells have also been found in gastric tumors by Rottenberg [3] and by Sasano Nobuaki et al. [14].

The electron-histochemical investigation of ATPase of cancer cells with this type of ultrastructure showed that the reaction product was connected with the membranes of the villi of the intracellular tubules, the basal plasmalemma, and the nucleoli (Fig. 1e, f). Some cancer cells with different enzyme activity on the membranes of the microvilli were found. For instance, some cells had relatively high enzyme activity (Fig. 1e), whereas others had low activity, reflected in some cases in the almost complete disappearance of reaction product (Fig. 1f).

This comparative study of the distribution of ATPase activity in normal parietal cells and cancer cells with a similar ultrastructure thus shows that in cells of the latter type the part of the mechanism of hydrochloric acid secretion which is connected with $H^+$ and $Cl^-$ transport remains intact. The observed decrease in hydrochloric acid production by the gastric mucosa in the presence of cancer and by the cancer itself is evidently attributable to other mechanisms.

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# EFFECT OF CHLORAMPHENICOL ON THE CARCINOGENICITY OF N-NITROSOMETHYLUREA


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UDC 615.277.4.015.23:615.332 (CHLORAMPHENICOLUM)



Chloramphenicol was shown to reduce the carcinogenic action of N-nitrosomethylurea on CBA mice. The mean latent period of onset of the tumors was increased but the number of tumors was reduced both at the site of injection of the carcinogen and in remote organs during observation for 53-66 weeks.



KEY WORDS: chloramphenicol, nitrosomethylurea, carcinogenesis.


The possible role of nitroso compounds in the etiology of human malignant neoplasms is being widely discussed at the present time. These substances have also become widely used for chemotherapy in clinical oncology. Attempts to discover substances which can reduce the toxic and carcinogenic action of these compounds are therefore natural and essential.

In this investigation the possibility of reducing the carcinogenicity of N-nitrosomethylurea (NMU), which has carcinogenic properties, by means of the antibiotic chloramphenicol was studied; chloramphenicol has a


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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 7, pp. 61-63, July, 1978. Original article submitted January 30, 1978.