TRANSPORT OF CHLORINE IONS IN PARIETAL CELLS OF THE GASTRIC MUCOSA: ELECTRON-HISTOCHEMICAL AND AUTORADIOGRAPHIC INVESTIGATION

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The location of chlorine ions in the parietal cells of the mouse and rat gastric mucosa was studied by electron-histochemical and autoradiographic methods together with the direct method of detection of radioactive isotopes in tissue. On the basis of the results a scheme of specific transport of chlorine ions is proposed and envisages the transposition of individual layers of the cell membrane, double folds of the basal plasmalemma, tubulovesicles, and intracellular tubules. This interconversion takes place in the course of the secretory cycle of the cell and is directly dependent on it. A nonspecific pathway for the transport of the total chlorides of the gastric juice across intercellular spaces is postulated.

KEY WORDS: *ultrahistochemistry; autoradiography; parietal cells; transport of chlorine ions.*

The parietal cells of the chief glands of the stomach produce hydrochloric acid. No morphological methods yet exist for the identification of hydrogen ions and the determination of the pathway whereby (in the bound state) they are transported from the site of their production in the mitochondria [3, 4] to the lumen of the gland. The other component of hydrochloric acid, namely chlorine, which enters the body from outside and is transported by the parietal cell as a specialized channel, can be detected histochemically [8] and autoradiographically. A method of direct detection of radioactive isotopes with the aid of halides of the heavy metals, developed by Caro [5] and improved by Normandin [10], also exists and has certain advantages (it is faster and has high resolution) over the ordinary autoradiographic method. However, this method has not so far been used in the USSR.

The object of the present investigation was, with the aid of electron-histochemical and electron-autoradiographic methods and also by the direct method of detection of radioactive isotopes in the tissue, to determine the localization of chlorine ions in the mucosa of the fundal part of the stomach, to identify the pathways of migration of these ions in the parietal cells, and to discover the structures which participate in this transport.

EXPERIMENTAL METHOD

The electron-histochemical determination of chlorine in the gastric mucosa of Wistar albino rats was carried out by Komnick's method [8] using silver lactate. To minimize the disadvantages of this method [9, 12], the volume of the fixing and incubation solution and of all other fluids containing water was reduced to the minimum. Dehydration also began with 100% acetone immediately, using the rapid method [1] for the purpose.

For the autoradiographic investigation and the direct method of detection of radioactive isotopes, ^{36}Cl with relatively low specific radioactivity (140 μCi/g) was used. In order that the animal should receive the required dose, it was therefore necessary either to inject the labeled sodium chloride as a hypertonic solution or to reduce the dose.

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In the experiments of series I, ^{36}Cl was injected intraperitoneally into (CBA × C57BL)F_1 mice, as 1 ml of a solution of the sodium salt in different concentrations (3-6%). The dose of the isotope was 0.5 μCi/g body weight. The result depended on the animal's individual sensitivity, for in some cases the solution was not absorbed from the peritoneal cavity. Gastric tissues from eleven mice, in which no fluid was found in the peritoneal cavity after 30 min-2 h, were investigated.

In the experiments of series II, five albino rats were kept on a balanced chloride-free diet for one week. After starvation for two days, the whole of the required dose of chlorine isotope (2 μCi/g body weight) was mixed with the food and administered over a period of 24 h, after which the animals were sacrificed.

Pieces of tissue from the fundal part of the stomach were quickly washed in three changes of cold Hanks' solution, fixed in 4% paraformaldehyde solution and, after postfixation with 2% osmium tetroxide solution and rapid dehydration in acetone, they were embedded in a mixture of Epon and Araldite.

To verify the presence of radioactivity, the homogenate of the washed tissue from the fundal part of the stomach was tested on an Isocap-300 automatic scintillation counter, which revealed high radioactivity of the material (16,000 cpm).

Sections cut on the LKB Ultratome were mounted on a grid with Formvar backing. After spraying with carbon, some grids were coated with a film of type M emulsion and after an exposure of 1-3 months were developed in D-19 developer and examined in the JEM-7a/120 electron microscope.

To detect the radioactive isotope directly in the tissues, the grid was placed with the sections on a drop of 0.000,001% silver chloride solution made up in a 30% solution of NH_4OH. After an exposure of 20-60 sec the grids were dried on a filter, placed for 1 min on a drop of solvent, and then washed on several drops of bidistilled water. Flotation of the sections of experimental material on a drop of distilled water and incubation of the material from animals not receiving radioactive isotope on a drop of the working solution (AgCl) were used as the control. The sections were unstained.

Examination of the material showed that the results of the experiments of series I and II were identical.

EXPERIMENTAL RESULTS

In the histochemical reaction for chloride, the largest quantity of reaction product, namely silver chloride, was found in the lamina propria of the mucosa. Its distribution was uniform in the mucosa itself, in the walls of the blood vessels, and in the plasma protein (Fig. 1a). Only on the basal plasmalemma of all the epithelial cells was there an increased concentration of reaction product. The precipitate was equally abundant in the intercellular space. In the parietal cells crystals of silver chloride (measuring 100-500 nm) were found in double folds of the basal plasmalemma (Fig. 1b). However, because of the abundance of the precipitate and the large size of the crystals it was impossible to determine accurately the location of the chlorine ions relative to the membranes. Inside the cell the reaction product was found in the tubulovesicles (most frequently on the inner aspect of the membrane; Fig. 1c) and very rarely it lay freely in the cytoplasm, which corresponded to a low intracellular chloride concentration. A similar picture was observed by Pipan [11] in the parietal cells of embryonic and newborn mice. Sometimes small crystals of silver salt were found in the mitochondria, possibly indicating either the presence of chlorine ions in them or the formation of silver phosphate, which also is insoluble. Solitary crystals also were found in the cytoplasm of the other epithelial cells. The reaction product was washed out of the lumen of the glands during fixation and rinsing of the material.

Autoradiographic detection of chlorine ions had no advantage over the histochemical reaction. The arbitrary form of the label was found only above the parietal cells. No label was found in any other cells, whether in the lamina propria or in the lumen of the glands. This fact is undoubted evidence that the radioactive isotope ^{36}Cl in the parietal cells is in the bound state, and it is eluted from the other structures of the mucosa during processing of the material. Because of the large size of the label (0.2-0.4 μ) it is difficult to judge the precise disposition of the radioactive isotope relative to the membranous structures of the cell (Fig. 2a).

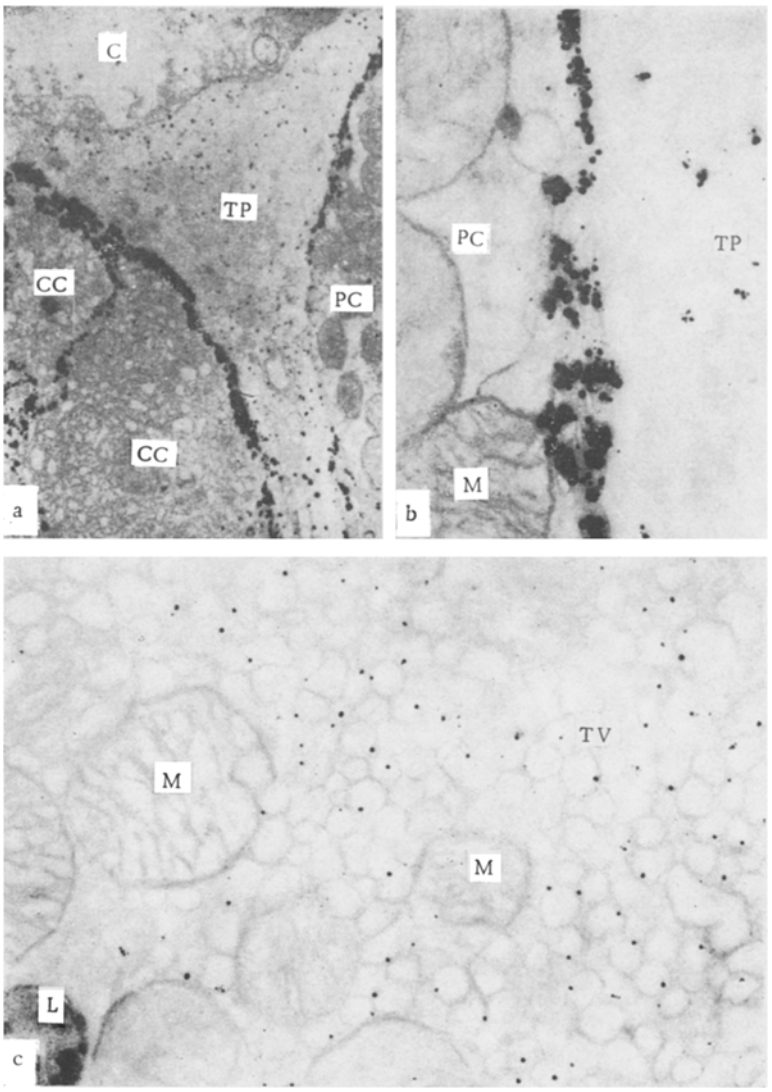

Fig. 1. Distribution of product of electron-histochemical reaction for chloride in gastric mucosa: a) uniform distribution of silver chloride crystals in tunica propria and its concentration on basal plasmalemma of glandulocytes and in intercellular spaces (5000×); b) concentration of silver chloride crystals in double folds of basal plasmalemma (30,000×); c) silver chloride crystals in tubulovesicles of a parietal cell; absence of reaction product in mitochondria (30,000×). Here and in Fig. 2: PC) parietal cell; CC) chief cell; TP) tunica (lamina) propria; C) capillary; M) mitochondria; TV) tubulovesicles; L) lysosome.

By contrast with autoradiography, the direct method of detection of radioactive elements in tissues with the aid of halides of the heavy metals enables the relationships of the ^{36}Cl isotope to the membrane structures of the parietal cell to be identified precisely. Crystals of silver measuring 20-100 nm were found only above the parietal cells. They were located on the outer (extracellular) side of the basal plasmalemma (Fig. 2b, c(in the layer of the mucopolysaccharides lining this membrane. Label was found between the double membranous folds of the basal plasmalemma and in vesicles detached from these folds (Fig. 2b). Sometimes vesicles bounded by double membranes became separated by a constriction ring and detached (Fig. 2c). In that case the silver crystals were located between the two membranes. Similar double vesicles could be seen also in the cytoplasm of the cell (Fig. 2d). Helander and

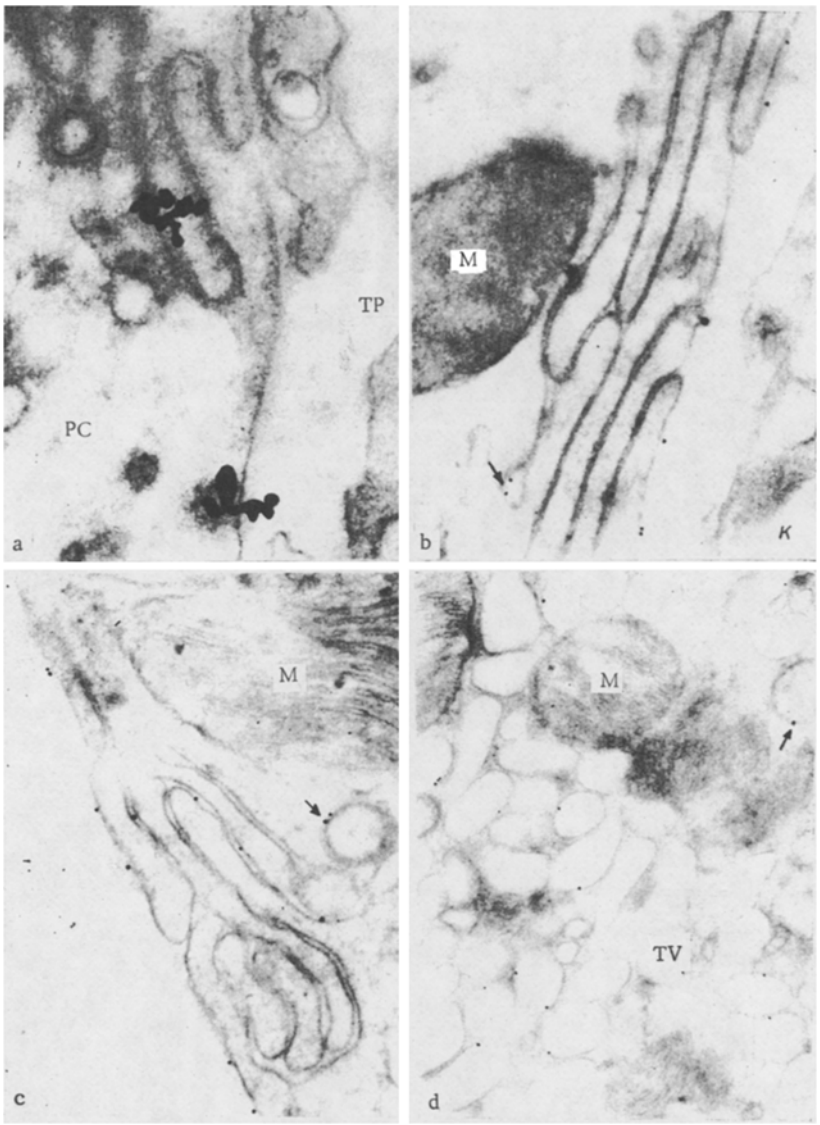

Fig. 2. Radioisotope investigation of distribution of ^{36}Cl in parietal cells of gastric mucosa: a) electron-microscopic autoradiography: label on basal plasmalemma and its double fold (80,000×); b) direct method of determination of radioactive isotope in tissue: silver chloride crystals on basal plasmalemma and between membranes of double folds, "label" in detached vesicle indicated by arrow (60,000×); c) direct method of determination of radioactive isotope in tissue: silver chloride crystals in double vesicle detached from fold of basal plasmalemma (arrow) (80,000×); d) direct method of determination of radioactive isotope in tissue: silver chloride crystals on inner surface of membranes of tubulovesicles and in double vesicle (arrow) in cytoplasm of parietal cell (50,000×).

Hirschowitz [7] suggested that formations such as these arise by "reserve pinocytosis" and invagination of the microvilli on the apical membrane of the parietal cells. Evidently the double vesicles are not an independent entity but belong to the system of tubulovesicles, as confirmed by the fact that Kiseleva et al. [2] observed their disintegration into single elementary tubulovesicles. As well as in the locations described above, crystals of silver also were found on the inner surface of the membranes of the tubulovesicles (Fig. 2d) and extremely rarely on the outer aspect of the apical surface of the cell and on the membranes of the intracellular tubules.

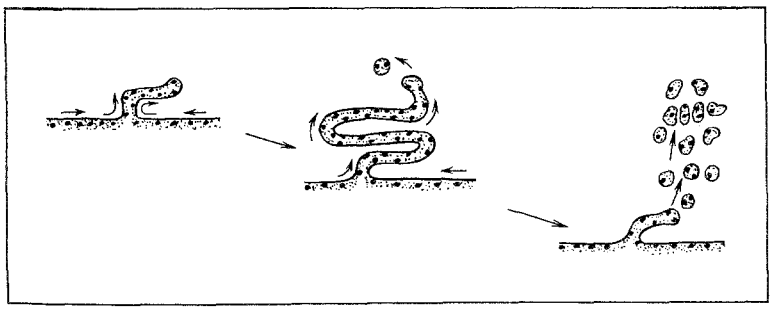

Fig. 3. Scheme of processes taking place on basal plasmalemma of parietal cell. Large dots denote chlorine ions, small dots specific acceptor of chlorine ions. Arrows indicate direction of "membrane current."

Histochemical investigation revealed adsorption of chlorine ions on the basal plasmalemma of all glandulocytes. After injection of the radioactive isotope ^{36}Cl, however, adsorption in this way was observed only on the membrane of the parietal cells. On this basis it can be concluded that the parietal cell has a specific acceptor on its plasmalemma with which the chlorine ion binds; moreover, it binds so firmly that it is not eluted from the tissue during fixation and processing of the material.

The pattern of distribution of the isotope discovered in the parietal cells suggests the following specific pathway of transport of chlorine ions. On leaving the bloodstream these ions bind to the side of the lamina propria with an acceptor located on the basal plasmalemma of the cell. The chlorine ions are then attracted by the "membrane current" [6], which arises in the course of secretion, into the numerous invaginations (double folds) of the cell membrane. From these folds there is a constant budding and detachment of vesicles, in which the chlorine ions lie together with the acceptor on the inner side (Fig. 3). In the absence of a stimulus to secretion, tubulovesicles are formed until the membrane reserves of the plasmalemma are exhausted. The tubulovesicles accumulating in the cell account for the whole stock of secretory membranes. At that moment the cell is ready to secrete chlorine ions into the lumen of the glands.

Besides this specific pathway for the transport of chlorine ions taking part in the formation of hydrochloric acid, there is another pathway along the intercellular channels (detectable histochemically), along which the total chlorides of the gastric juice and also other substances needing to be excreted are evidently transported into the lumen of the gland.

With the aid of different methods of identifying the chlorine ions in the tissues, nonspecific and specific pathways for its transport were thus identified. The specific pathway of transport of chlorine ions in the parietal cells producing hydrochloric acid is connected with transposition of the component layers of the cell membrane, double folds of the basal plasmalemma, tubulovesicles, and intracellular tubules. This interconversion takes place during the secretory cycle and is directly dependent on it. Nonspecific transport of the total chlorides of the gastric juice takes place along intercellular spaces.

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