

# EFFECT OF CHOLERA TOXIN ON MITOCHONDRIAL ULTRASTRUCTURE AND FUNCTION IN THE EPITHELIOCYTES OF THE RABBIT SMALL INTESTINE

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Ultrastructural changes in the mitochondria of the epitheliocytes of the rabbit small intestine under the influence of cholera toxin took the form of widening of the intracristal and intermembranous spaces, probably reflecting their transition from the mainly "orthodox" to the mainly "condensed" configuration. Similar changes were observed in isolated mitochondria in Chance's states 4 and 3 in a saline medium with 2% polyvinylpyrrolidone. The respiratory control coefficient of mitochondria isolated from the tissue of pathologically changed organs was the same as the normal control. It is suggested that the ultrastructural changes found in the mitochondria through the action of cholera toxin are due to transition of the mitochondria from state 4 into state 3 in connection with an increase in the ADP concentration in the cell as a result of activation of ATPases.

KEY WORDS: cholera toxin; small intestine; mitochondria.

The question of the role of mitochondria in the epitheliocytes of the small intestine in the development of the syndrome of rapid interspinal dehydration resulting from the action of cholera toxin arises because in this disease processes of active ion transport through the plasma membrane of the cells are disturbed [6, 11, 13]. The absence of data on the functional state of the epitheliocyte mitochondria and the contradictory information on their ultrastructure in cholerogenic poisoning [1, 5, 9], motivated the present investigation.

An attempt was made to determine the character of participation of mitochondria in the pathological process by comparing their ultrastructure in situ with the results of functional and ultrastructural investigations of isolated mitochondria in vitro.

## EXPERIMENTAL METHODS

Fourteen male rabbits weighing 600-800 g were used. An injection of 5 ml cholero-gen was given into the intestine of the experimental animals, and control animals received 5 ml of physiological saline. The animals were killed 3-4 h after the injection of cholera toxin or physiological saline, the small intestine was removed, and the mitochondrial fraction was isolated from the mucosa by the method described in [4].

The rate of oxygen utilization by the mitochondrial suspension was determined polarographically by means of a covered Clark's electrode. The incubation medium contained: 110 mM KCl, 10 mM  $\text{KH}_2\text{PO}_4$ , 5 mM each of glutamate+malate (or 10 mM succinate), 1 mM EDTA, and 10 mM Tris-HCl (pH 7.4, temperature 22-24°C).

For the parallel electron-microscopic investigation pieces of the wall of the jejunum were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 1 h at 4°C, and rinsed for 1 h in cacodylate buffer. The isolated mitochondria were fixed actually in the polarographic cuvette with 1% glutaraldehyde and sedimented at 5000g for 15 min. Material for electron-microscopic investigation was then postfixated in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in acetone of increasing concentration, and stained with 2% uranyl acetate, made up in 70° acetone, and then embedded in a mixture of Epon with Araldite. Ultrathin sections

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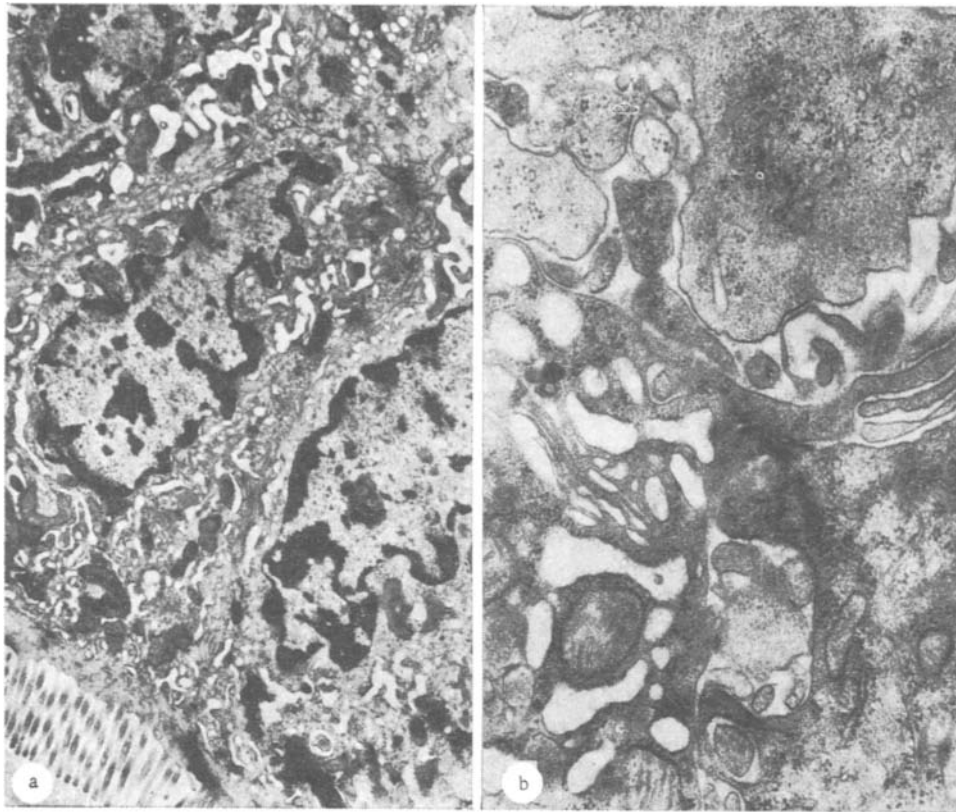


Fig. 1. Changes in epitheliocyte ultrastructure in the rabbit small intestine following injection of cholera toxin: a) 8000 $\times$ , b) 30,000 $\times$ .

were counterstained with lead citrate and examined in the IEM-100B electron microscope with an accelerating voltage of 80 kV.

#### EXPERIMENTAL RESULTS

A typical picture of cholerogetic poisoning with well-marked hyperemia of the wall and accumulation of fluid in the lumen of the bowel was observed macroscopically in the rabbit's small intestine 3-4 h after injection of the cholera toxin. Electron-microscopic investigation of the epithelial layer of the small intestine at this time revealed considerable widening of the cisternae of the granular endoplasmic reticulum of the epitheliocytes (Fig. 1). The difference between the configuration of the epitheliocyte mitochondria of the experimental animals and the control (Fig. 2c) was that most of them had widened intracristal and intermembranous spaces (Fig. 2a, b). The degree of widening of the intracristal spaces varied considerably. This phenomenon is demonstrated to an extreme degree in Fig. 2a, whereas the typical picture of widening of the intracristal spaces is shown in Fig. 2b. The other morphological features characterizing the mitochondria (the degree of density of the matrix, incorporation, etc.) did not differ significantly in the experimental and control animals.

Investigation of the respiratory control coefficient (RCC) of mitochondria isolated from the intestinal mucosa of the experimental and control animals showed no statistically significant differences. For instance, when a mixture of glutamate and malate was used as the substrate in the experimental group RCC was  $2.1 \pm 0.14$ , whereas when succinate was used it was  $1.8 \pm 0.28$ ; the corresponding values in the control group were  $2.2 \pm 0.23$  and  $1.8 \pm 0.27$  ( $P < 0.05$ ,  $n = 4$ ). The absence of changes in the rate of oxygen utilization by the mitochondrial suspensions, despite the ultrastructural changes demonstrated above, suggested that the morphological differences observed do not extend beyond functional changes in the configuration of the mitochondria of the "orthodox"-"condensed" type [7, 8].

Accordingly, the ultrastructure of the mitochondria was studied in different states, as described by Chance *in vitro* under conditions as close to real as possible, i.e., not only in an isosmotic saline medium, but also in an isooncotic medium, prepared by adding 2% polyvinylpyrrolidone to the medium. As Fig. 3a

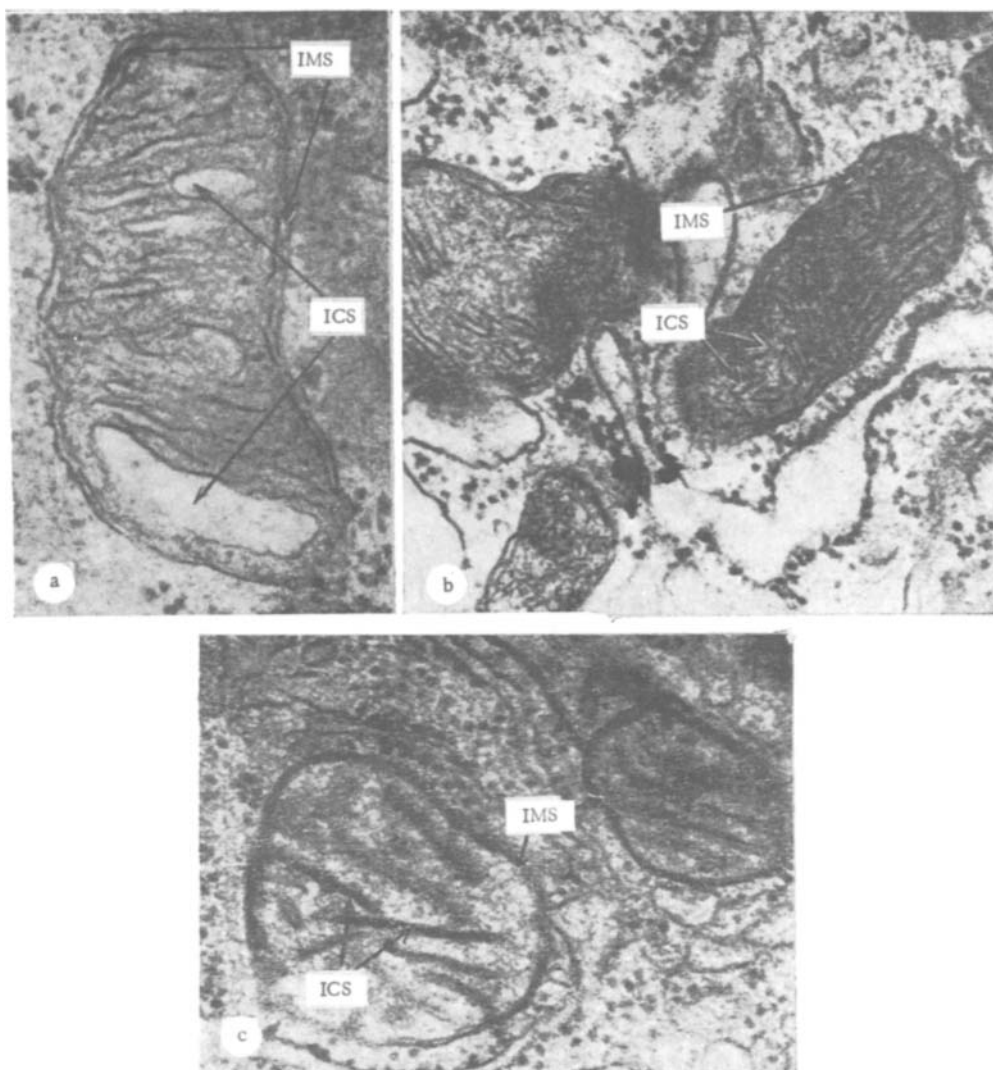


Fig. 2. Degree of widening of intermembranous and intracristal spaces of epitheliocyte mitochondria of rabbit small intestine following injection of cholera toxin (a, b) and under normal conditions (c) in situ. IMS) Intermembranous space; ICS) intracristal space. 80,000 $\times$ .

shows, the configuration of the mitochondria in metabolic stage 3 was similar to that observed in situ during cholerogetic poisoning (Fig. 2b), whereas the configuration of mitochondria in metabolic state 4 (Fig. 3b) was similar to that found in normal tissue (Fig. 2c).

It can thus be concluded that most mitochondria of the epitheliocytes, under the influence of cholera toxin, are evidently in a "condensed" state, whereas under normal conditions they are mainly in the "orthodox" state.

These results indicate that the structural changes observed in the mitochondria are the result not of direct, but of indirect (through changes in cell metabolism) involvements of the mitochondria in the pathological process in cholerogetic poisoning. This conclusion is confirmed by the writers' experiments to study the direct action of cyclic AMP in concentrations of  $10^{-4}$  to  $10^{-7}$  M on RCC of isolated mucosal mitochondria in KCl medium, for it is known that the cyclic AMP concentration in the epitheliocytes is increased by the action of cholera toxin [10]. The results of these experiments showed that this nucleotide has virtually no effect on mitochondrial function; this is in agreement with observations of Schotland and Mela [12].

The results are evidence that activation of adenylate cyclase by choleroegen probably leads to activation of intracellular ATPases and to a corresponding increase in the ADP concentrations in the cytoplasm. This last effect, in turn, stimulates the activity of the mitochondrial respiratory chain, i.e., converts mitochondria

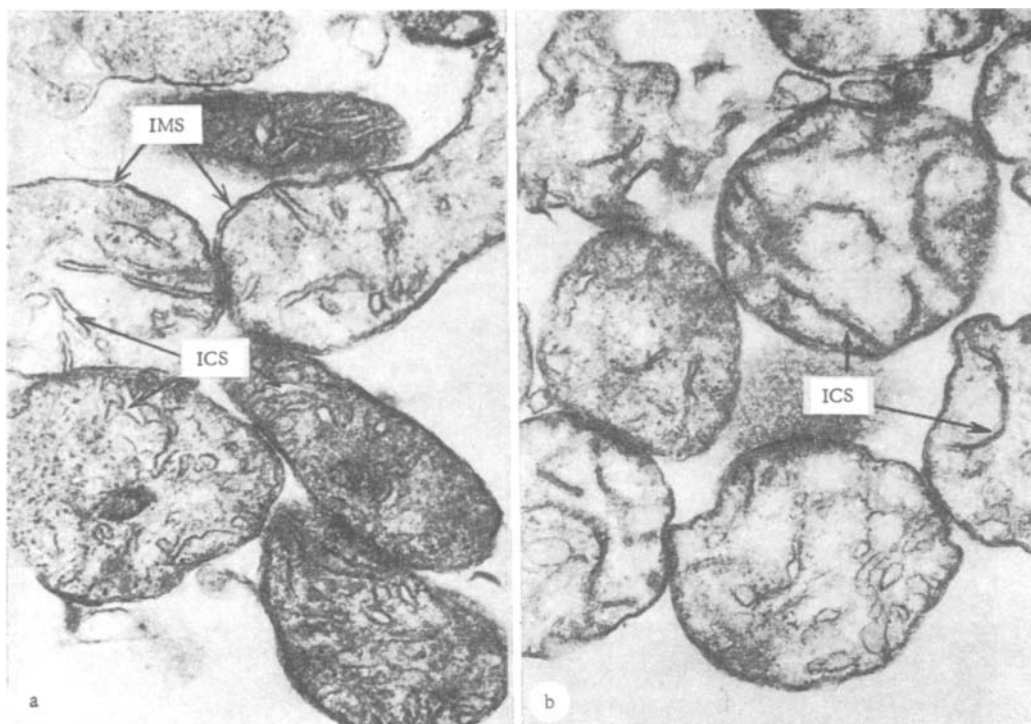


Fig. 3. Configurational changes in isolated epitheliocyte mitochondria in different metabolic states, in the presence of 2% polyvinylpyrrolidone (mol. wt. 40,000). a) "Condensed" configuration (metabolic state 3); b) "orthodox" configuration (metabolic state 4). IMS) Intermembranous space; ICS) intracristal space. 40,000 $\times$ .

predominantly in Chance's state 4 into mitochondria predominantly in state 3. This transition is accompanied by configurational changes in the mitochondria from the "orthodox" to the "condensed" state. This suggestion is confirmed by investigations by other workers who found similar changes in the mitochondria during intensification of metabolism in the cells of various organs [2, 3].

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