reactions of the animals at the level of the cerebral circulation is probably attributable to the fact that during adaptation to hypoxia the capilarization of the tissues (especially in the brain) increases, as also does the permeability of the vessel walls. All these effects inevitably lead to an increase in the oxygen supply to the nerve cells, both on account of the increased volume of the blood flow and as a result of increased diffusion of oxygen from the blood into the tissue. Conditions favoring the earlier onset of seizures are thereby created. Consequently, despite the increased resistance of the animals to various extremal factors after adaptation to hypoxia, in the case of exposure to HO the negative effect of inadequacy of the protective reactions at the level of the cerebral circulation predominates.

From the practical point of view it is important to emphasize the need to allow for the negative effect of preliminary adaptation to hypoxia when individual sensitivity to the paroxysmal action of HO is assessed. In clinical practice, during the treatment of persons exposed for long periods to hypoxia, milder conditions of hyperbaric oxygenation should probably be recommended.

LITERATURE CITED

1. Z. I. Barbashova, Usp. Fiziol. Nauk, 1, No. 3, 70 (1970).
2. Z. I. Barbashova and O. I. Tarakanova, Fiziol. Zh. SSSR, No. 3, 434 (1974).
3. A. G. Zhironkin, in: Oxygen Insufficiency [in Russian], Kiev (1963), p. 353.
4. A. I. Selivra, in: The Physiology of Man and Animals [in Russian], Vol. 14, Moscow (1974), p. 63.
5. R. W. Brauer, D. E. Parris, R. O. Way, et al., J. Appl. Physiol., 28, 474 (1970).
6. M. J. Purves, The Physiology of the Cerebral Circulation, Cambridge (1972).

EFFECT OF CHOLERAGEN ON STRUCTURE AND OXIDATIVE PHOSPHORYLATION OF ISOLATED MITOCHONDRIA IN EPITHELIUM OF RABBIT SMALL INTESTINE

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UDC 612.33.014.21:612.26].0.14
46:576.851.315.097.29

The mitochondrial fraction was isolated from the epithelium of the rabbit small intestine by differential centrifugation in isotonic sucrose. When a malate-glutamate mixture was used as the substrate the respiratory quotient of these mitochondria was 3-5. Changes in the functional state of the mitochondria were accompanied by stereotyped structural changes of configuration of the "orthodox–condensed" type. Addition of unpurified choleragen to the incubation medium of the mitochondria caused no change in the rate of oxygen utilization in Chance's third or fourth state.

KEY WORDS: mitochondria; structure; oxidative phosphorylation; intestine; choleragen.

The mitochondria are among the most vulnerable organelles of the cell and they undergo functional and structural changes in a variety of pathological processes, including in cholera intoxication, for under these circumstances active ion transport, for which these organelles supply energy, is significantly disturbed [5, 14]. Involvement of the mitochondria in the pathological process could be the result either of direct interaction between the penetrating subunit of choleragen [7] or of activation of nucleotide-cyclase systems [4, 12, 13]. The available data on the direct action of choleragen on isolated mitochondria in vitro are contradictory. Mitochondria isolated from the epithelium of the alimentary tract are rarely used as a test object, for hydrolases and mucus, which could injure or contaminate the mitochondrial fraction, are present in the initial homogenate.

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 3, pp. 267-270, March, 1978. Original article submitted July 14, 1977.

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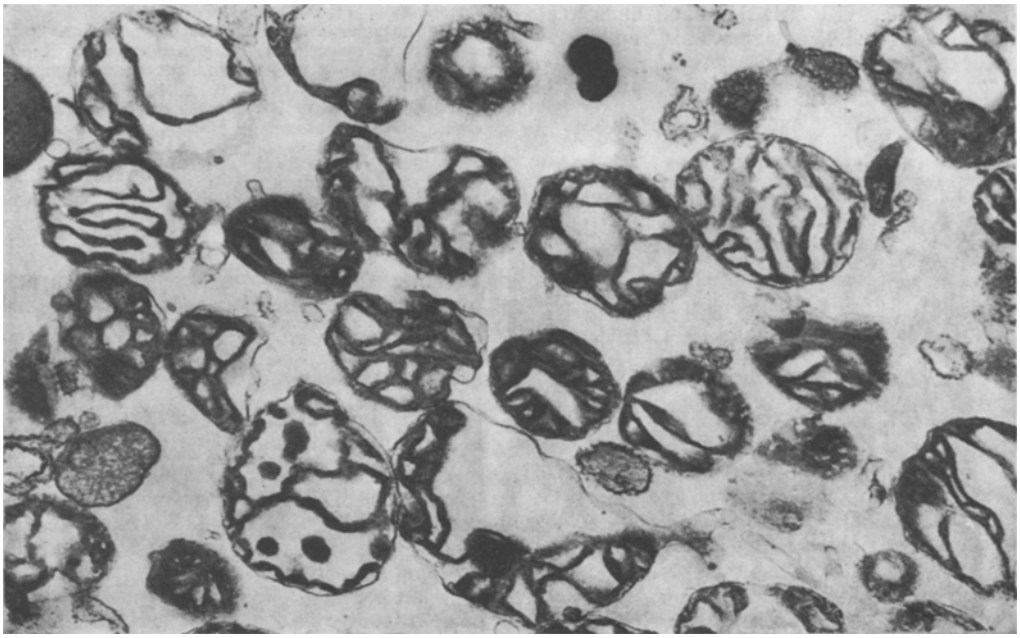

Fig. 1. Electron-microscopic picture of isolated mitochondrial fraction from mucous membrane of rabbit small intestine; 37,000 ×.

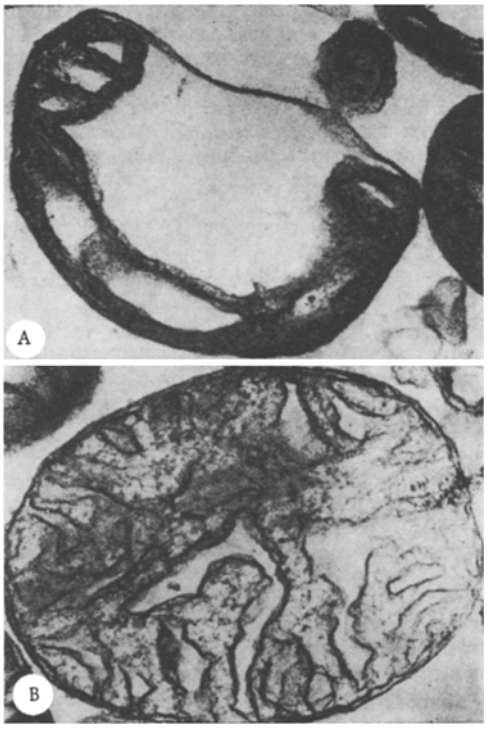

Fig. 2. Condensed (A) and orthodox (B) configurations of mitochondria isolated from mucous membrane of rabbit small intestine; 100,000×.

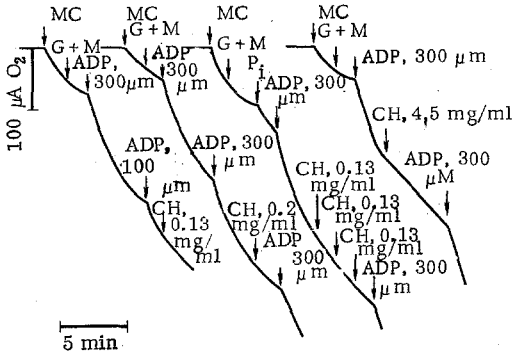

Fig. 3. Oxygen consumption of mitochondria from mucous membrane of small intestine in different functional states after addition of choleragen. Arrows indicate times of addition of mitochondria (MC), malate–glutamate mixture (G+M), choleragen (CH), and inorganic phosphate (P_i).

In the accessible literature only a few methods are described, and these differ substantially from each other [2, 3, 6, 15].

The object of this investigation was to develop a method of obtaining isolated native mitochondria from the epithelium of the rabbit small intestine and to study the effect of choleragen on their oxidative phosphorylation.

EXPERIMENTAL METHOD

Rabbits weighing 600-800 g, deprived of food for 12-24 h, were decapitated, and loops of small intestine were removed and washed in 200-300 ml of a solution containing 250 mM sucrose, 5 mM EDTA, 2 mM HEPES, and 0.1% trypsin inhibitor, pH 7.4, at 0-2°C. The washed intestinal loops were minced, passed through a blender to remove muscle and connective tissue, homogenized manually in a glass homogenizer with Teflon pestle in 50 ml of the same sucrose solution, filtered through glass wool, and centrifuged at 600g for 10 min. The supernatant was centrifuged at 5000g for 15 min and the resulting sediment was twice resuspended and centrifuged at 5000g for 12 min in a solution of 250 mM sucrose with 2 mM HEPES, and 0.2 mM EDTA(pH 7.4).

The rate of oxygen uptake by the mitochondrial suspension was determined polarographically with a closed Clark's electrode with a voltage of 0.65 V and in a 1.5-ml cuvette. The incubation medium had the following composition: 200 mM sucrose, 25 mM KCl, 10 mM KH_2PO_4, 10 mM Tris-HCl buffer; temperature 22-26°C. A mixture of malate and glutamate, each 5 mM, was used as the respiration substrate.

For the electron-microscopic investigation the suspension of mitochondria was fixed actually in the cuvette with 1.5% glutaraldehyde and sedimented by centrifugation at 5000g for 15 min. The residue was post-fixed with 1% OsO_4 in the same medium, dehydrated with increasing concentrations of acetone, stained with 2% uranyl acetate, made up in 70° acetone, and embedded in a mixture of Epon and Araldite. Ultrathin sections were counterstained with lead citrate and examined in the IEM-100B electron microscope. Unpurified choleragen containing exo- and endotoxins was used.

EXPERIMENTAL RESULTS

The electron-microscopic study of the fraction showed that it consisted almost entirely of mitochondria with the addition of solitary other organelles and fragments of microvilli (Fig. 1). Mitochondria fixed immediately after isolation were characterized by a predominantly "condensed" configuration [8]. They had an intact outer membrane and well-developed cristae, they were ellipsoidal in shape, and they varied in size within wide limits. The isolated mitochondria measured between 0.5 and 1.4μ along their long axis and between 0.35 and 0.9μ along their short axis. The mean size based on the results of 15 random measurements was $0.82 \pm 0.06 \times 0.59 \pm 0.04\mu$.

The respiratory control of the mitochondria in the absence of EDTA was 2-3, it increased to 3-5 on the addition of 1-4 mM EDTA, and fell again after addition of Mg^{2+}, indicating the presence of Mg^{2+}-activated ATPase activity in the suspension.

During changes in their functional state mitochondria undergo stereotyped morphological changes [8-10]. In Chance's third state (oxidative phosphorylation) many mitochondria are in a "condensed" [8] or "de-energized" [9, 10] configuration. These mitochondria had a dense matrix and greatly widened intermembranous space (Fig. 2A). In the fourth state most mitochondria were in the "orthodox" [8] or "energized" configuration [9, 10]. The matrix of these mitochondria was less dense and the intermembranous space was only very slightly widened (Fig. 2B).

The results of the experiments to study the effect of choleragen on oxidative phosphorylation of the mitochondria (Fig. 3) showed that even in very high concentrations the choleragen caused no change in the rate of oxygen consumption of the mitochondria whether in Chance's third or fourth states. The results are also evidence that unpurified choleragen evidently does not interact with the membranes of the epithelial mitochondria of the small intestine, just as also with those of the liver [11]. These findings do not agree with the previously observed [1, 2] marked inhibition of mitochondrial respiration in the rat liver or guinea pig intestinal mucosa. In the latter case, however, the mitochondria were isolated from the intestine after freezing to −25°C for 15 min, the respiratory control was not determined, and the fraction was not subjected to morphological investigation, so that it is impossible to judge the integrity of the isolated organelles.

The results of the present experiments thus suggest that the mitochondria of the epithelium of the small intestine are involved in the pathological process in cholera intoxication only indirectly – through activation of nucleotide-cyclase systems.

LITERATURE CITED

1. M. N. Dzhaparidze, L. A. Martens, et al., Byull. Éksp. Biol. Med., No. 4, 66 (1970).
2. N. B. Ogarenko, Probl. Osobo Opasnykh Infektsii, No. 4 (26), 92 (1972).
3. A. A. Pokrovskii, M. M. Gapparov, et al., Tsitologiya, No. 2, 175 (1975).
4. J. G. Banwell, Gastroenterology, 65, 467 (1973).
5. M. Field, D. Fromm, et al., J. Clin. Invest., 51, 796 (1972).
6. T. Gaginella, P. Bass, et al., FEBS Lett., 53, 347 (1975).
7. D. M. Gill, Bull. Inst. Pasteur, 74, 65 (1976).
8. C. R. Hackenbrock, J. Cell Biol., 30, 269 (1966).
9. R. A. Harris, M. A. Asbell, et al., Arch. Biochem., 132, 545 (1969).
10. R. A. Harris, J. T. Penninston, et al., Proc. Nat. Acad. Sci. USA, 59, 830 (1968).
11. G. T. Keusch, J. J. Rahal, et al., Am. J. Physiol., 218, 703 (1970).
12. D. V. Kimberg, M. Field, et al., J. Clin. Invest., 50, 1218 (1971).
13. J. Leitch and M. S. Amer, Invest. Immun., 11, 1038 (1975).
14. D. W. Bowell, H. J. Binder, et al., Am. J. Physiol., 225, 781 (1973).
15. E. Slinde, K. J. Andersen, et al., Anal. Biochem., 70, 86 (1976).