EQUIVALENTS OF MITOCHONDRIA IN CELLS OF E. coli

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Presented by Active Member of AMN SSSR V. L. Troitskii
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 53, No. 3, pp. 65-67, March, 1962
Original article submitted July 4, 1961

At present, the existence of mitochondria in bacteria is not definitely established. It was demonstrated by us [3] that succinic dehydrogenase activity, which is specific for mitochondria, is located in defined areas of E. coli (at one of the ends or in the middle). The formation of formasan in the intestinal rod was depressed and activated by specific succinic dehydrogenase reactions. In the absence of succinate, there was no reduction of tetrazolium. A similar localization of general dehydrogenating activity of the intestinal cell was reported by Niklowitz [12] and V. I. Biruzova [1].

In 1945, M. N. Meisel' and T. I. Kondrat'eva [4] pointed out the existence in bacteria of structures similar to mitochondria. Electron microscopy of thin sections of bacteria allowed one to establish that, in locations corresponding to the dehydrogenating activity, there exist definite structures which were named mitochondrial equivalents [6, 7, 9, 14, 16, 18]. Certain authors pointed out the membraneous nature of these structures [1, 6, 7].

Inasmuch as succinic dehydrogenase activity in cells of higher organisms is ascribed entirely to the mitochondria – to be more specific, to the cristae of mitochondria [13] – it was of interest to study the mitochondrial equivalents of bacteria under conditions of specific reactions with succinic dehydrogenase. We assumed from Nelson's studies [11], investigating ultra-thin sections of spermatozoa treated with malonate, a specific inhibitor of succinic dehydrogenase, that it has been concluded that it was possible to localize the enzyme in the cell and that succinate in minute concentration may "contrast" those structures which contain succinic dehydrogenase.

In the present communication, we reported the results of the study of ultra-thin sections of the intestinal cell, incubated with triphenyl tetrazolium chloride in the presence of sodium succinate.

Materials and Methods

Two strains of E. coli (894 and 734-47) were used in this study. Both strains are typical morphologically, culturally, and biochemically. Cells of E. coli 734-47 are more pleomorphic than those of E. coli 894. Prior to incubation, bacterial sediment was washed 6 times by physiological saline which removed completely the endogenous activity of succinic dehydrogenase. One-tenth per cent triphenyl tetrazolium chloride and sodium succinate in 0.001 and 0.05 M concentrations were added to microbial sediment in test tubes. Incubation with tetrazolium lasted for 30 minutes at 37°. In the test tube with 0.004 M solution of succinate, the dye did not change after 30 minutes; and in the test tube with 0.05 M solution, it turned dark-rose in color.

The method of preparation of ultra-thin sections was previously described by us [3]. Electron microscopy of ultra-thin sections was carried out using microscope JEM-54 at 80 kv. Final magnification was 8,000-15,000×.

Results

On Fig. 1 are shown sections of cells from bacterial cultures not subjected to any action. The cell wall and the cytoplasmic membrane are clearly seen. The central part of the cell is occupied by the transparent nuclear vacuole. In the cells are seen small, parallel canaliculi consisting as through of small granules. The direction of canaliculi varies in different parts of the cell: in certain cases it is possible to trace the connection of these formations with cytoplasmic membrane. It was not possible to uncover clearly defined mitochondria-like structures, separated by a membrane from the cytoplasm, as are seen on Fig. 2.

Cells of E. coli 894, after treatment with 0.1% triphenyl-tetrazolium chloride in the presence of 0.05 M solution of succinate are seen on Fig. 2. Cytoplasmic condensations are clearly indicated in some cells in approximately the same locations where one finds the activity of succinic dehydrogenase (by means of separation of formasan

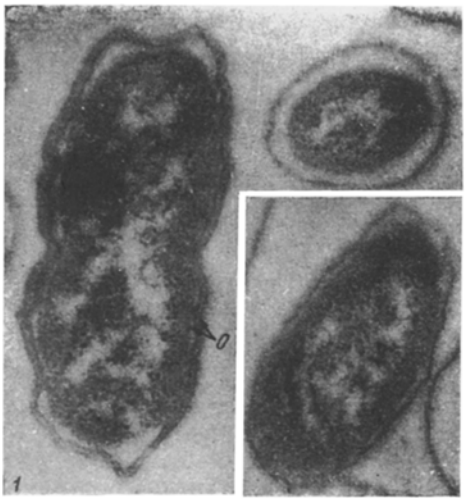

Fig. 1. 17.5 hr. culture of E. coli 894. Control (1) 0 = cell membrane.

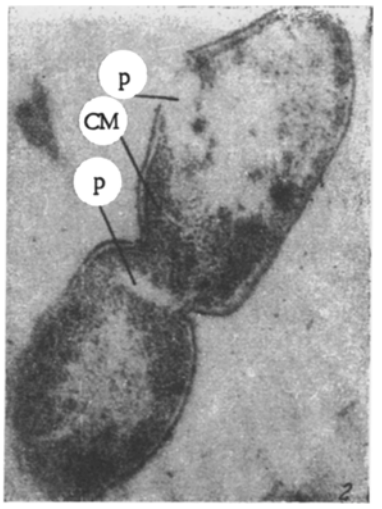

Fig. 2. 17.5 hr. culture of E. coli 894, treated for 30 minutes with 0.05 M solution of sodium succinate and 0.1% solution of triphenyl-tetrazolium chloride (2). P = location of formazan deposits; CM = cytoplasmic membrane.

crystals). In certain cases, it is possible to trace a membrane around such a condensation, as well as to observe quite clearly depicted cristae. In addition to the condensed equivalents of mitochondria, there are also found structures with cristae clearly delimited by a membrane morphologically, indeed, resembling mitochondriae.

On Fig. 4 some cells are shown in the state of partial lysis. This allowed clearer observation of membrane formation on the background of the lysed cytoplasm, although other structural elements are absent (Figs. 1-4, see insert).

In E. coli 894, incubated in 0.001M solution of succinate, there is almost no deposition of formazam crystals. Where they are found, they are very small (Fig. 4). Small crystals destroy the cytoplasm imperceptibly, leaving intact the membrane structures resembling sections of mitochondrial cristae.

Succinic dehydrogenase activity in plant and animal cells is related entirely to mitochondria [5]. Green [8] stated that succinic dehydrogenase is localized in granules 100-200A° in diameter, from which are made mitochondrial christae.

At present the question of the presence in bacteria of formations identical to mitochondria is not solved. Many investigators [15, 17] consider that function of mitochondria (oxidation activity, in particular) is carried out by granules (100-200A° in diameter), distributed in cytoplasm. Other authors [2, 4, 6, 7, 9, 10, 14, 16, 18] consider that bacteria possess formations analogous to mitochondria. In this connection it becomes important to demonstrate in bacteria structures characterizing mitochondria as an isolated formation. Such structures were observed by Giesbrecht [7] in B. megaterium.

We were able to demonstrate the presence of special envelope-membrane surrounding the structures in a cell shadowed by specific "staining" with sodium succinate. Localization of succinic dehydrogenase activity in the structures demonstrated in our experiments, confirms that these structures carry out mitochondrial functions.

The structures discovered by us differ clearly from the nuclear elements of bacteria of the intestinal group, representing electron dense or transparent mesh on the background more penetrating for electrons of nuclear vacuole. In cells of control preparations not "stained" by the succinate, the above described cultures are not observed. It is possible that with the change in fixation and imbedding procedures, bacterial mitochondria will be observed without shadowing. However, shadowing with specific reagents offers a possibility of judging about physiological function of the uncovered structures.

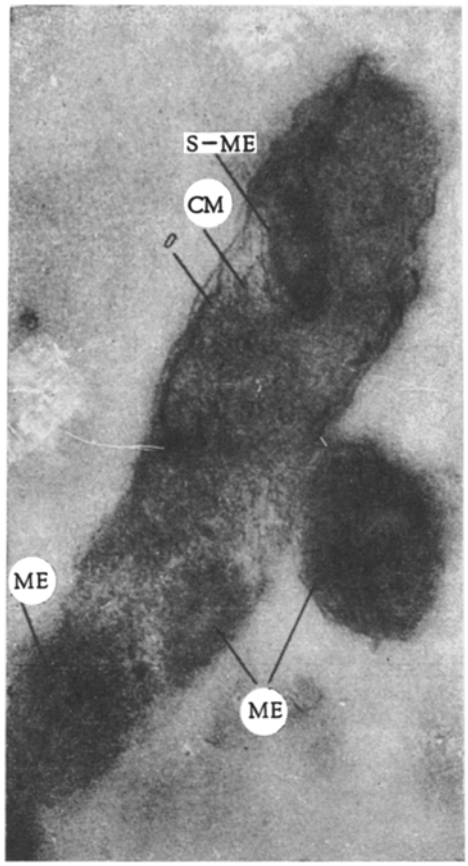

Fig. 3. 17.5 hr. culture of E. coli 734-47, treated for 30 minutes with 0.001 M solution of sodium succinate and 0.1% solution of triphenyl-tetrazolium chloride. ME = mitochondrial equivalents S.ME = mitochondrial equivalents, shadowed by succinate; O,CM = the same as on Figs. 1, 2.

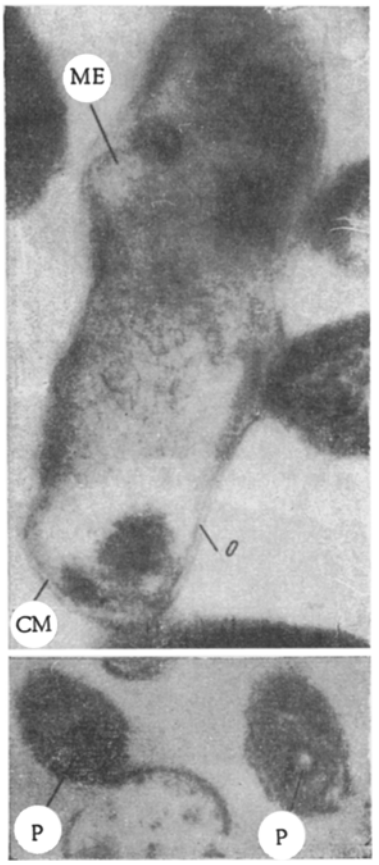

Fig. 4. 17.5 hr. culture of E. coli 894, treated for 30 minutes in 0.001 M solution of sodium succinate and 0.1% solution of triphenyl-tetrazolium chloride. For legend see Figs. 1, 2, 3.

As was shown by Giesbrecht using B. megatherium [7], the structure of mitochondria of the sporebearing bacteria may be highly complex in nature; it is probable that in Gram-negative bacteria (E. coli) they are less complex.

The results obtained in the present study offer a basis for consideration that the mitochondrial equivalents of the intestinal rod correspond in function to mitochondrial membranes (the terminal stages of electron transfer) and are morphologocally specialized formations.

I wish to acknowledge with thanks the help given by V. I. Biruzova in carrying out this investigation.

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