CORRELATION BETWEEN FUNCTIONAL STATE AND ELECTRON-MICROSCOPIC MORPHOLOGY OF MITOCHONDRIA

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Comparison of the structure and function of isolated mitochondria in various functional states showed that the configuration of the mitochondria may reflect the state in situ and their ability to accumulate energy. Condensed mitochondria have a lower respiratory control and a lower ADP/O ratio than orthodox. The ultrastructure of the mitochondria in the tissues is of either the low-energy or the high-energy type.

The two ultrastructural configurations of isolated liver mitochondria (condensed and orthodox) were first described by Hackenbrock in 1966 [5]. Because of the contradictory findings he was unable to establish any correlation between the configuration of the mitochondria and their metabolic state. He postulated on the basis of his investigation [5, 6] that transformation of mitochondria from condensed to orthodox takes place with the conversion of chemical energy of electron transport directly into conformation energy and that it is evidently unconnected with phosphorylation.

This conclusion was questioned by other workers [9, 11, 12]. It was shown that the ultrastructure of isolated mitochondria may depend on the medium in which they are isolated [1]. Mintz and co-workers [9] also showed that mitochondria from tumors, with a very low respiratory control, are generally unable to undergo ultrastructural transformation.

The object of the investigation described below was to determine whether the configuration of isolated mitochondria is a manifestation of their original functional state (their state in situ) and whether it can characterize their metabolic state. This would help to show whether a parallel exists between the configuration of the isolated mitochondria and the ultrastructure of mitochondria in liver tissue.

EXPERIMENTAL METHOD

Rat liver mitochondria were isolated by the method of Schneider and Hogeboom [10] with small modifications. The isolation medium contained 0.32 M sucrose solution and 0.001 M EDTA solution. Respiration of the mitochondria was recorded on a polarograph.* The incubation medium contained the following solutions: 0.25 M sucrose, 20 mM KH_2PO_4, 15 mM KCl, 10 mM $MgCl_2$, 15 mM succinate; pH 7.4.

The ultrastructure of the mitochondria was studied in the 2nd, 3rd, and 4th metabolic states. The second state was obtained by addition of oxidation substrate to the polarographic cell. The 3rd and 4th states were simulated by the method of Chance and Williams [4]. The rate of O_2 consumption in the 2nd, 3rd, and 4th states, the respiratory control ($RC = U_3/U_4$), the phosphorylation time, and the ADP/O coefficient were determined by a polarographic method. All investigations were carried out at 27°C. The time of taking the material for electron-microscopy was based on the polarographic findings. By contrast with Hackenbrock's method, samples of the preparation from the polarographic cell were immediately placed in fixing

*The polarographic investigations were carried out by S. V. Eliseeva and E. N. Kotova.

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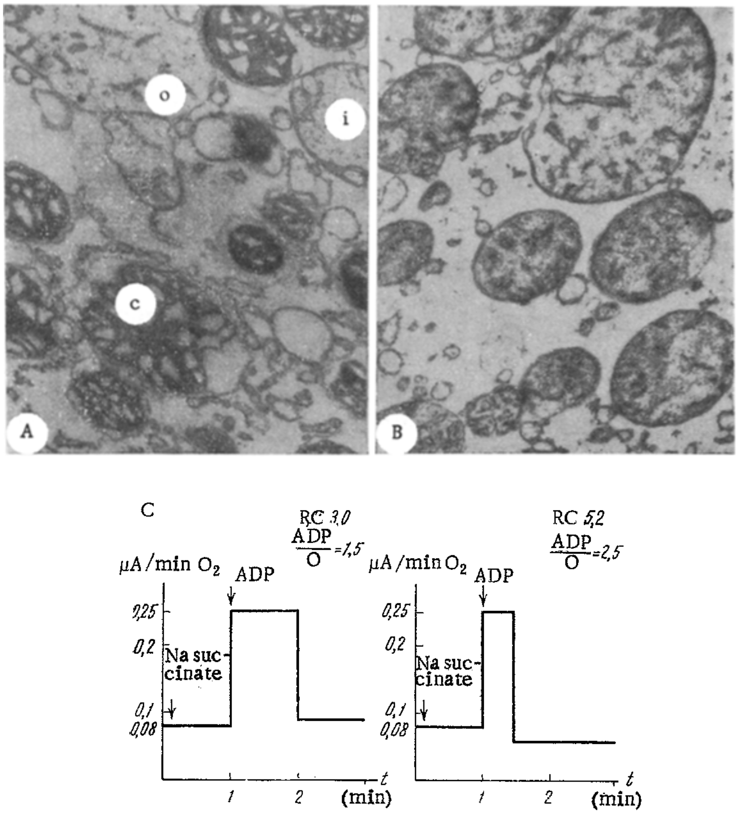

Fig. 1. Ultrastructure of isolated mitochondria and their functional state: A) isolated mitochondria from rat liver in experiment of group 1, taken from residue immediately after centrifugation. Most mitochondria have a condensed configuration (o – orthodox mitochondria, i – intermediate, c – condensed); B) isolated mitochondria from rat liver in experiment of group 2, taken from residue immediately after centrifugation. Most mitochondria have orthodox configuration. Magnification here and in Figs. 2 and 3, 2400 ×; C) diagram demonstrating respiration of mitochondria in 2nd, 3rd, and 4th states in experiments of groups 1 (on left) and 2 (on right).

solution for 30 min (buffered 4% formaldehyde solution, pH 7.4, containing 0.44 M sucrose) and then centrifuged in a clinical centrifuge. Fixation of the residue was completed in buffered 1% OsO_4 solution, pH 7.4, with 0.44 M sucrose, and it was then dehydrated in alcohols of increasing concentration, stained with 3% uranyl acetate solution in 100° ethanol, and embedded in methacrylates and Araldite. Sections were cut on the ZKB-480A ultratome, stained by Reynolds' method, and examined in the UMV-100B electron microscope at 75 kW. The percentage of condensed mitochondria was determined in the electron micrograph. Altogether 15 experiments were carried out.

EXPERIMENTAL RESULTS

The electron-microscopic study of the isolated liver mitochondria revealed three main configurations of the mitochondria (Fig. 1A): orthodox, intermediate, and condensed.

Depending on the results of the morphological study all the experiments were divided into two groups: in group 1 condensed mitochondria were predominant, while in group 2 most mitochondria were orthodox and intermediate. From each group the most typical experiment was chosen and used as an example for comparing the structure and function of the mitochondria.

TABLE 1. Relative Number of Condensed Mitochondria Correlated with RC and ADP/O Coefficients

Serial No.	RC	ADP/O	Number of condensed mitochondria (in %)
1	2,8	1,5	88
2	3,0	1,5	86
3	3,4	1,6	79
4	5,1	2,5	14

Mitochondria from the experiments of group 1 are shown in Fig. 1A (here and in Fig. 1B the mitochondria were taken from the residue immediately after centrifugation). Most mitochondria have the condensed configuration. Solitary mitochondria of orthodox type with dense, narrow cristae adjoin intermediate mitochondria with greatly widened intermembraneous spaces. In the preparation from the experiments of group 2 (Fig. 1B), few mitochondria have a condensed configuration. The whole field of vision is scattered with mitochondria with a clearly marked orthodox or intermediate configuration. The diagram (Fig. 1C) shows that in both cases the mitochondria have identical rates of O_2 consumption in the second and third states and differ in their respiratory control (RC) and ADP/O coefficient: in the first case RC = 3.0 and ADP/O = 1.5,and in the second case RC = 5.1 and ADP/O = 2.5. The decrease in the number of condensed mitochondria with an increase in the respiratory control and ADP/O coefficient is clearly shown in Table 1.

Mitochondria with the orthodox configuration are thus highly productive from the point of view of energy liberation. Condensed mitochondria, on the other hand, i.e., mitochondria with grossly dilated intermembranous spaces, accumulate ADP slowly and with considerable oxygen utilization. The possibility is not ruled out that the configuration of the isolated mitochondria characterizes their original state (their state in situ).

In all the experiments the ultrastructures of the mitochondria were studied in the 2nd, 3rd, and 4th functional states.

In the second state the matrix of the condensed mitochondria was even more compressed than in the residue after centrifugation, and some intermediate forms were becoming condensed (Fig. 2A). This led to an increase in the relative percentage of condensed forms in the specimen. The orthodox mitochondria in the second state had the identical ultrastructure both when they were predominant in the specimen (Fig. 2B) and also when condensed forms were predominant. Most of their cristae were widened to 400 Å. The inner intermembranous space was becoming electron-transparent and the matrix was finely granular, of average electron density. Mitochondria with the intermediate configuration had grossly widened intermembranous spaces.

As a rule the mitochondria were in the third state 5 sec after addition of ADP. The number of mitochondria with the condensed configuration was smaller than in the second state (Fig. 2C). Mitochondria with the orthodox configuration had become more numerous, probably through transformation of intermediate and condensed forms into orthodox. At increased O_2 concentrations, five successive additions of ADP led to a further decrease in the number of condensed mitochondria. Often whole fields of the electronmicrograph consisted entirely of orthodox mitochondria (Fig. 2D). After a single addition of ADP this picture was not observed. Most cristae of the orthodox mitochondria in both groups of experiments appeared dense and narrow. Their thicknesses, excluding the lumen, was about 200 Å (Fig. 2E). The relative increase in the number of orthodox mitochondria in the third state suggests that some "straightening of the matrix" is a characteristic feature of the third state.

In the fourth state the condensed mitochondria were fewer in number than in the third state. Intermediate forms were very rare. The number of orthodox mitochondria had increased. Most of their cristae were narrow and dense (Fig. 2F).

Hence, as the mitochondria accumulate energy (third and fourth states) condensed forms are converted into intermediate and orthodox forms. In the orthodox mitochondria the widening of the intermembranous spaces disappears. The cristae become narrow and dense, i.e., the orthodox mitochondria come to resemble ordinary tissue mitochondria in their appearance.

To compare the ultrastructure of the isolated mitochondria and of mitochondria in the tissues pieces of liver were fixed and treated in the same way as for investigation of the isolated mitochondria.

Electron-microscopic study of sections of liver tissue from experiments in which most mitochondria were condensed showed that most mitochondria in the cells resembled the condensed type and only isolated mitrochondria were of the orthodox configuration (Fig. 3). An interesting conclusion can thus be drawn: mitochondria of relatively low productivity from the point of view of energy supply for intracellular

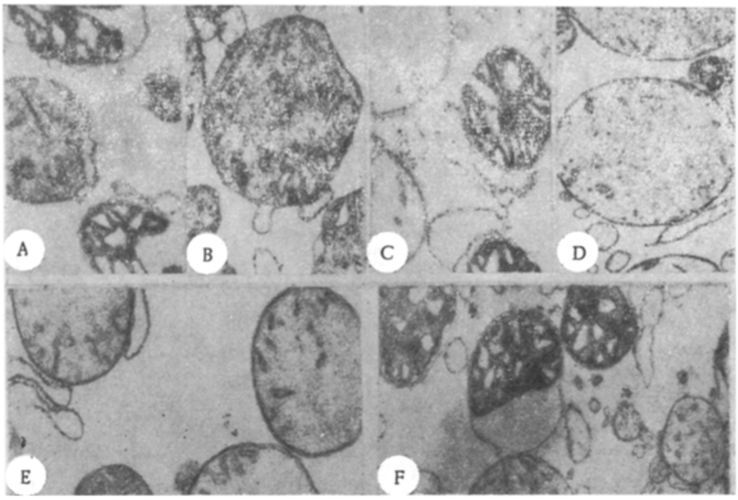

Fig. 2. Ultrastructure of isolated mitochondria in various metabolic states: A) isolated rat liver mitochondria from experiment of group 1 in second metabolic state (after addition of succinate). Intermembranous spaces of orthodox mitochondria are dilated. B) Isolated rat liver mitochondria from an experiment of group 2 in the second state. Intermembranous spaces of mitochondria are considerably dilated. C) Isolated rat liver mitochondria from an experiment of group 1 in the third state (after addition of succinate and ADP). D) Isolated rat liver mitochondria from an experiment of group 1 after fifth addition of ADP. Cristae of orthodox mitochondria are dense and narrow. E) Isolated rat liver mitochondria from an experiment of group 2. Membranes of mitochondrial cristae are in close apposition with each other. F) Isolated rat liver mitochondria from an experiment of group 1 in the fourth state.

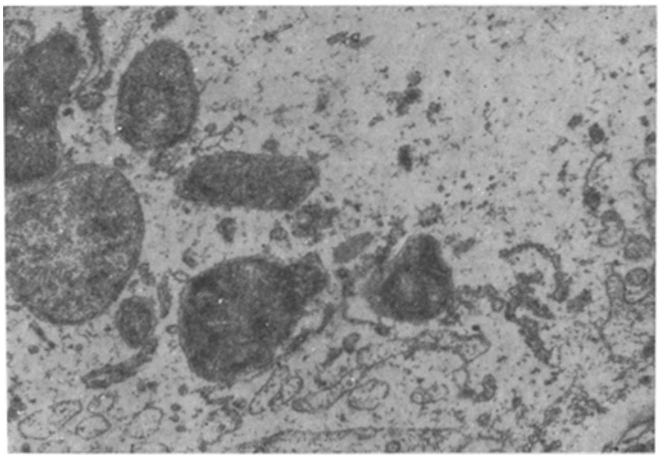

Fig. 3. Rat liver tissue fixed and treated in the same way as isolated mitochondria resembles isolated mitochondria with condensed configuration. Only solitary mitochondria are of the orthodox type.

processes can predominate in the tissues. Kopteva and Biryuzova [2, 3] had described two types of mitochondria which they isolated from heart tissue. Mitochondria with an ultrastructure similar to that of the condensed mitochondria of the liver contained more than twice as much RNA as mitochondria of the orthodox type. It must evidently be concluded that these so-called unproductive mitochondria use a considerable proportion of their energy in satisfying their own needs [7, 8].

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