duced synthesis of transport proteins, as was shown indirectly by a decrease in the number of attached ribosomes.

Starting from 24 h of recirculation, the dominant processes in the hepatic parenchyma were thus those of restoration of hepatocyte ultrastructure, whereas synthetic processes, aimed at export, were evidently depressed both during ischemia and throughout the period of recirculation until the 14th day. This was shown indirectly by a decrease in the number of attached ribosomes and some delay in lipid transport.

Some workers consider that 30 min of ischemia is a safe period of exclusion of the liver from the circulation in man [1] and in animals [2, 4, 5]. The results of the present investigation indicate that hepatic ischemia for 30 min leads to serious changes in the hepatocytes at the subcellular level, and that complete recovery of the ultrastructure of the hepatic parenchyma has not taken place even by the 14th day of recirculation. This state of affairs raises the question of the need to search for ways of correcting this situation in order to prevent possible complications in the postischemic period or after other states associated with disturbance of the blood flow in the liver.

LITERATURE CITED

1. G. I. Veronskii, Anatomo-Physiological Aspects of Resection of the Liver [in Russian], Novosibirsk (1983).
2. A. Bernelli-Zazzera and G. Gaja, Exp. Mol. Pathol., 3, 351 (1964).
3. I. D. Bowen and R. A. Lockshin, Cell Death in Biology and Pathology, London (1981).
4. K. R. Chien, J. Abrams, R. G.Pfau, et al., Am. J. Pathol., 88, 539 (1977).
5. J. J. Frederiks, K. S. Bosch, M. J. R. Schröder, et al., Exp. Pathol., 22, 245 (1982).
6. E. R. Weibel, Int. Rev. Cytol., 26, 235 (1969).

A POSSIBLE MORPHOLOGIC APPROACH TO THE ASSESSMENT OF MITOCHONDRIAL ENERGY POTENTIAL

V. T. Vasilenko

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Modern cytopathology has many facilities for obtaining objective morphometric data on the state of structural and chemical components of the cell. Nevertheless, not even this level of cytomolecular research can always provide exhaustive information on the state of function of the cell and, in particular, of its mitochondria. Technical papers dealing with the assessment of energy efficiency of the mitochondria have been published [1]. In the writer's view, for a more complete assessment of the state of mitochondrial function it is preferable to use a conventional parameter (coefficient) which would characterize the potential energy capacity or level of power of the mitochondria, having in mind not the physical definition, but the translated meaning of the latin word "potentia" — meaning power. In the writer's view, analysis of a set of enzyme-histochemical and ultrastructural data relating to the ability of mitochondria to engage in oxidation-reduction reactions with electron transfer along the respiratory chain, could provide an objective idea of such a parameter, for it is these reactions that are components of respiratory assemblages, and directly precede energy production. It is now possible to obtain exact information about components of respiratory assemblages such as electron transfer enzymes — NADH-dehydrogenase (NADH-DH), succinate dehydrogenase (SDH), and cytochrome oxidase, as well as additional enzymes, such as D-β-hydroxybutyrate dehydrogenase and α-glycerophosphate dehydrogenase, which are firmly bound with respiratory fragments. These enzymes of energy production perform vector processes

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TABLE 1. Dynamics of Changes in Structural and Functional Parameters of Cardiomyocyte Mitochondria at Stages of Deep Hypothermia

Stage of experiment	Area of mi mitochondria (S_{mch}, M ± m), μ	P	Mean extinction (ME)		CEPM				ICEPM	
					for SDH		for NADH-DH			
			SDH	NADH-DH	conventional units	%	conventional units	%	conventional units	%
1. Initial biopsy (conventional norm)	5,70±0,58	—	0,62	0,75	0,1	100	0,13	100	0,23	100
2. Period of cooling before circulatory arrest	2,90±0,21	<0,05	0,19	0,13	0,07	70	0,04	30,7	0,11	47,8
3. End of period of circulatory arrest for 40 min	3,70±0,50	<0,05	0,66	0,48	0,17	170	0,13	100	0,30	130,4
4. End of reheating period	2,85±0,29	<0,05	0,27	0,15	0,09	90	0,05	38,4	0,14	60,8

that take place in the mitochondria, whose volume changes depending on their functional state. It is therefore logical to assume that the quantitiative characteristics of activity of the enzyme mentioned above always correlate with the area of these organelles. By comparing the coefficient of energy potential of the mitochondria (CEPM) with its original values, or with a conventional norm, it is therefore possible to obtain information on quantitative changes in the energy potential and the trend of the change in CEPM. It will also be possible both to assess the contribution of individual oxidation substrates — succinic acid, NADH, and so on, for example — to the energy metabolism of the cell, and also to determine their integral CEPM (ICEPM).

In the investigation described below the informative value of the above criteria was tested in relation to calculations for SDH and NADH-DH.

EXPERIMENTAL METHOD

A morphometric study was made of the area of mitochondria on electron micrographs of heart muscle cells of puppies exposed to general hypothermia. Material from an initial biopsy (conventional norm), taken immediately after thoracotomy, and also material taken during the period of hypothermia before stopping the circulation, at the end of circulatory arrest for 40 min, and at the end of the period of reheating of the animal, were investigated. A semiautomatic electronic computer and memory system (MOP-AM 03, from Reichert, Austria) was used for the morphometric investigation. Measurements were made on only those electron micrographs in which the myofibrils were oriented longitudinally. The relative data were converted into absolute units (microns). The results were subjected to statistical analysis by the Fisher—Student test. SDH and NADH-DH activity was determined in parallel tests on freshly frozen sections. Activity of these enzymes was estimated cytospectrophotometrically on an SMP-0.1 scanning cytospectrophotometer (Opton, West Germany). The equation used to calculate CEPM, suggested bythe writer, was:

$$CEPM = \frac{ME}{S_{mch}},$$

where ME denotes the mean extinction obtained when determining activity of a particular enzyme, and S_{mch} the mean area of a mitochondrion. The value of ICEPM was obtained by summation of the separate values of CEPM, expressing the contribution of the various components of energy production to the total:

$$ICEPM = CEPM_{SDH} + CEPM_{NADH\text{-}DH}$$

and so on.

EXPERIMENTAL RESULTS

Stereometric analysis showed a statistically significant decrease in the absolute area of mitochondria of the cardiomyocytes during deep hypothermia (Table 1). Meanwhile differences in the areas of the mitochondria at each consecutive stage of the experiment compared with

the previous stage were not statistically significant. The greatest decrease in area of the mitochondria was observed when the animal was cooled to a rectal temperature of 19-20°C. Greatly reduced values of the area of the mitochondria were obtained before final reheating of animal. SDH and NADH-DH activity fluctuated: a decrease was observed at the 2nd and 4th stages, with a sharp increase up to the original values in the third stage of the experiment. Hence it follows that it is difficult to judge the functional state of the mitochondria purely on the basis of their area or activity of their enzymes. Undoubtedly mitochondria undergo certain conformational changes, but to what degree these are still compatible with the vital activity of the cell is difficult to determine from the results of this kind of analysis. Meanwhile CEPM, calculated from the cytospectrophotometric characteristics of succinate oxidation, whose value was 0.07, indicated a reduction of 30% in the energy potential of the mitochondria during cooling. The fraction of electrons supplied to the respiratory chain from NADH under these circumstances was reduced even more — by almost 70% compared with initially (CEPM = 0.04). Despite low values of the area of the mitochondria and of extinction during the reheating period, CEPM for SDH approached the original values, whereas for reduced NAD it was only 38.4% of the original level (Table 1). The total energy potential of the mitochondria at the end of the experiment, on restoration of cardiac activity and of the circulation, was only 60.8% of the original level (ICEPM = 0.14), evidence of their functional insufficiency. Meanwhile, it follows from the results that mitochondria of cardiomyocytes are subjected to the greatest energy strain at the end of the period of circulatory arrest ($CEPM_{SDH}$ = 0.17, i.e., 70% higher than the initial level; ICEPM = 0.30, i.e., 30.4% higher than the initial value), and this evidently reflects an intracellular stress situation at this stage of the experiment.

By the use of the tests suggested above it was thus possible to show that changes undergone by mitochondria (a decrease in their area, fluctuations of SDH and NADH-DH activity) reflect mechanisms of self-regulation of their energy (maintenance of CEPM and ICEPM at their optimal levels).

The coefficients of energy potential of the mitochondria suggested in this paper, in the writer's opinion, permit a more objective and complete evaluation of the structural and functional state of the mitochondria and of the cell as a whole, and also of the contribution of individual oxidation substrates and mechanisms in the maintenance of intracellular homeostasis and they thus widen the field of possible application of morphologic and cytologic analysis in diagnosis and prognosis.

LITERATURE CITED

1. V. S. Paukov, T. A. Kazanskaya, and V. A. Frolov, Byull. Éksp. Biol. Med., No. 4, 122 (1971).