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CHANGES IN MITOCHONDRIAL CYTOCHROME COMPOSITION AND FUNCTION IN THE ISCHEMIC HEART

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A decrease in the level of cytochromes $a + a_3$ and c (or $c + c_1$) has been observed in mitochondria isolated from the ischemic heart [9] and liver [8]. However, no change in cytochrome oxidase activity was found during ischemia [1, 8, 10]. The contradictory nature of functional and quantitative data may be attributable to differences in the experimental conditions, due mainly to the variety of methods of oxidation and reduction of cytochromes and incomplete separation of the peaks of individual cytochromes at room temperature.

In this investigation the effect of ischemia on the quantitative composition of cytochromes in isolated cardiac mitochondria was studied by differential spectrophotometry at the temperature of liquid nitrogen; derivative spectra of the fourth degree also were recorded, which give much better separation of the cytochrome peaks with overlapping absorption bands [5]. Dependence of oxidation of succinate on exogenous cytochrome c was studied in a parallel investigation.

EXPERIMENTAL METHODS

Rabbits weighing 2.5-3.5 kg were used. The composition of the cytochromes and activity of succinate oxidation were studied in mitochondria isolated from the heart. Ischemia was produced by autolysis of heart tissue. The heart was washed in ice-cold (0°C) 0.9% NaCl solution and divided into two parts: One part acted as the control (mitochondria were isolated immediately after washing), whereas the other part, wahsed in warm (37°C) 0.9% NaCl solution, was subjected to autolysis for 2 h at 37°C in a humid chamber [4]. Homogenization was carried out in a glass-Teflon homogenizer. Mitochondria were isolated by differential centrifugation in medium containing 0.18 M KCl, 2 mM EDTA, pH 7.0, at 0-4°C [7] and suspended in the same medium. Differential spectra of the cytochromes were recorded at the temperature of

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TABLE 1. Effect of Ischemia on Quantitative Composition of Cytochromes of Isolated Cardiac Mitochondria (n = 6)

Duration of ischemia, h	ΔA of amplitudes of fourth degree derivative spectra					ΔA $a \pm a_3$
	$c_{(544-546)}$	$c_{(548-550)}$	$c_{1\,(553-555)}$	$b_{557\,(557-559)}$	$b_{561\,(561-564)}$	
0	0,268±0,02	0,440±0,04	0,111±0,013	0,076±0,007	0,430±0,035	0,05±0,003
2	0,132±0,01	0,214±0,03	0,104±0,014	0,084±0,010	0,444±0,035	0,05±0,001
	—51%	—52%				
P	<0,001	<0,002				

Legend. ΔA values given for mitochondrial concentration of 20 mg protein/ml. ΔA of amplitudes calculated from fourth degree derivative spectra (1 and 2); first number in parentheses denotes maximum, second number minimum of amplitude (in nm). ΔA for cytochromes $a + a_3$ calculated from spectra 1 and 2 between waves 596-630 nm.

TABLE 2. Effect of Myocardial Ischemia and Cytochrome c on Succinate Oxidation in Rabbit Heart Mitochondria (n = 6)

Presence of cytochrome c in medium	Duration of ischemia, h	V_2	V_3	V_{BTB}
—	0	104±10	287±23	20,0±1,7
	2	86±7	93±9	41,4±4,8
			—67,6%	+100%
P			<0,001	<0,01
+	0	324±32	563±47	111±11
	2	520±59	527±56	184±19
		+60%	—6,4%	+65%
P		<0,001	<0,05	<0,01

Legend. V_2 and V_3) Rate of respiration with succinate before and after addition of ADP respectively; V_{BTB}) Rate of respiration with succinate in medium containing BTB, without ADP.

liquid nitrogen in isolation medium which also contained 1 mM KCN, 10 μM rotenone, 10 μM carbonylcyanide-m-chlorophenylhydrazone, and mitochondria in a concentration of 20 mg protein/ml. The base line was recorded with mitochondria only. Cytochromes were oxidized with H_2O_2 [6] (1 μl 10% H_2O_2 in 0.2 ml of medium) and reduced by the addition of a few grains of dithionite. Cooling of the mitochondrial suspension in a chamber with liquid nitrogen was complete within about 20 min, after which the readings were stabilized. Spectra were recorded on a dual-beam two-wave model 557 spectrophotometer (Hitachi, Japan). The scanning speed was 12 nm/min, the slit was 1 nm wide, and the optical path of the cuvette was 1 mm. A high concentration of mitochondrial protein (20 mg/ml) was used when recording the spectra in order to reduce the sensitivity of the apparatus to noise when recording the derivative spectra of the fourth degree. The rate of oxidation of succinate (20 mM) was measured polarographically at 37°C by an electrode of Clark type, in medium containing 150 mM KCl, 5 mM KH_2PO_4, 10 mM Tris, 5 mM $MgCl_2$, pH 7.5, and 2 μM rotenone. The concentration of mitochondrial protein was 0.5 mg/ml, of ADP 1 mM, and of cytochrome c from horse heart 30 μM. In special experiments 80-100 μM of the dicarboxylate transport inhibitor bromthymol blue (BTB) [3] was added, and the concentration of mitochondrial protein in these experiments was 1 mg/ml. The significance of changes was estimated by the method of paired comparisons. Protein was determined by the biuret method with extraction by diethyl ether [2].

EXPERIMENTAL RESULTS

The results showed that the quantity of cytochromes $a + a_3$ and cytochrome oxidase activity [1, 10] were unchanged during ischemia (Table 1; Fig. 1). Analysis of the fourth degree derivative spectra showed (Table 1; Fig. 1) that only the characteristic amplitudes for cytochrome c with maxima at 548 and 544 nm decrease in ischemia, whereas amplitudes with maxima of 553, 557, and 561 nm, characteristic of cytochromes c_1, b_{557}, and b_{561} [5], remain unchanged.

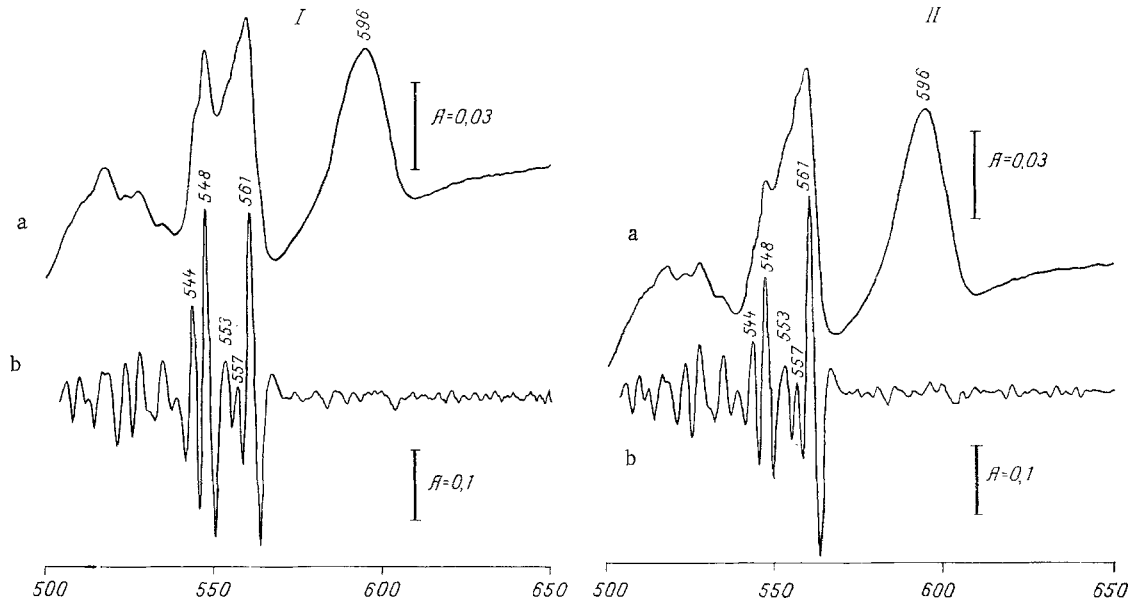

Fig. 1. Differential spectra of mitochondrial cytochromes (a) and their fourth degree derivative spectra (b) in control (I) and ischemia (II). Abscissa, wavelength (in nm); ordinate, optical density.

During ischemia, therefore, only the content of cytochrome c, weakly bound with the internal mitochondrial membrane, falls in isolated mitochondria. This is in agreement with the results of polarographic studies of mitochondrial function. It will be clear from Table 2 that during ischemia the rate of respiration in state 3 in medium without cytochrome c is reduced by 67%, but in medium with cytochrome c, only by 6.4%. The reduction in cytochrome c content in the mitochondria in ischemia leads also to an increase in the degree of activation of respiration in state 3 by exogenous cytochrome c, i.e., the ratio of respiration in state 3 in medium with cytochrome c to the same rate in medium without it. In the control this ratio is 1.96, but during ischemia it is 5.7 (calculated for data in Table 2). These changes are evidently connected with damage to the outer membrane of the mitochondria and to an increase in its permeability for protein molecules, as a result of which the release of endogenous cytochrome c from the mitochondria is increased.

It will also be clear from Table 2 that in ischemia, in medium without cytochrome c, the rate of mitochondrial respiration before addition of ADP (V_2) was unchanged, but in medium with cytochrome c it was considerably increased, and almost reached the respiration rate in state 3. Oxidation of succinate also was increased under these circumstances and was not reduced by BTB. These findings indicate that damage to the inner mitochondrial membrane in ischemia is accompanied not only by increased proton and potassium permeability [11], but also by an increase in nonspecific permeability for succinate and also, perhaps, for other low-molecular-weight compounds.

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