SOME CONDITIONS FOR SPECTROPHOTOMETRIC DETERMINATION OF SUCCINATE DEHYDROGENASE AND CYTOCHROME OXIDASE ACTIVITY IN THE MITOCHONDRIA OF THE BRAIN

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The methods most widely used for determining the activity of the oxidative enzymes succinate dehydrogenase (succinate: cytochrome c-oxidoreductase, I.C.E. 1.3.99.1) and cytochrome oxidase (cytochrome c : O_2–oxidoreductase, I.C.E. 1.9.3.1) are the methods of Potter and Schneider [11] and Hess and Pope [7], which were developed for tissue homogenates.

Results of determination of the activity of these enzymes in the mitochondria of the cat's brain are described in this paper. The determinations were based on the methods indicated above. The only modifications concerned the concentrations of the components used in the enzyme reaction. The effect of different method of treatment of the mitochondria on the level of activity of the oxidative enzymes was also studied.

EXPERIMENTAL METHOD

Mitochondria isolated from the cerebral cortex of the cat's brain in 0.25 M sucrose, made up in 0.01 M tris buffer as described previously [3], were investigated. The purity of the suspensions of the mitochondria was verified in the phase–contrast microscope and by staining with acid fuchsin by Altmann's method [12]. It has been verified by the use of the electron microscope that the mitochondria of the cat's cerebral cortex, isolated as described above, are adequately pure [13].

Protein in the mitochondria was determined by Lowry's method [8], using Folin's reagent [1].

Determination of Succinate Dehydrogenase Activity. The succinate dehydrogenase activity was determined on the SF-4 spectrophotometer at 550 mμ.

The following reagents were used; 2.5 M sodium succinate, 0.15 M KCN solution, and the "mother" solution consisting of a 1.9×10^{-3} M solution of cytochrome in 0.15 M phosphate buffer (pH 7.4). The "working" solution of cytochrome (4.4×10^{-5} M) was prepared from the "mother" solution in 0.04 M phosphate buffer on the day it was to be used. Aqueous solutions of $AlCl_3$ and $CaCl_2$ of equal molarity (4×10^{-3} M) were mixed in equal volumes before the experiment.

The mitochondria, to the amount of about 0.14 mg protein, were added to 0.1 ml sodium succinate and the mixture was carefully stirred and incubated for 2 min at 22-23°. Next, 0.1 ml of KCN was added, followed after 1 min by 0.3 ml of the mixture of $AlCl_3$ and $CaCl_2$ and 2.5 ml of cytochrome c solution. The change in extinction taking place during reduction of the cytochrome c was recorded every 30 sec for 3 min. At the end of the determination, sodium hydrosulfite was added to the sample until the cytochrome c was completely reduced, as shown by the termination of the increase in optical density of the incubated mixture. The difference between the optical density of the sample before and after enzymic reduction of the cytochrome c ($\triangle E$), corresponding to 1 mg protein of the mitochondria, was a measure of the succinate dehydrogenase activity.

Remembering that potassium cyanide may react with cytochrome c, in a control sample all the components were present except the mitochondria. However, no significant change was found in the optical density of the control during a period of 3 min, in agreement with results obtained by other authors [5].

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TABLE 1. Succinate Dehydrogenase Activity in Mitochondria with Different Concentrations of Sodium Succinate and Potassium Cyanide

Final concentration of potassium cyanide (mole)	0,005			0,01	0,0025	0,0025
Final concentration of sodium succinate (mole)	0,04	0,08	0,16	0,08		0,04
Enzyme activity (M ± m)	12,1±1,34	14,7±0,87	10,5±0,96	9,3±1,90	12,4±1,94	9,5±0,91
No. of measurements	12	11	10	4	4	9

Note. Activity of enzyme $M = \frac{\Delta E\ 550 \cdot 60\ \text{min}}{\text{mg protein}}$.

TABLE 2. Effect of Hypotonic Treatment of Mitochondria on Succinate Dehydrogenase Activity

Index	Intact mitochondria	Mitochondria preincubated in 0.02 M tris buffer
Enzyme activity (M ± m)	12.2±0.564	14.7±0.478
Number of measurements	19	11

Determination of Cytochrome Oxidase Activity. Because the cytochrome oxidase is localized on the inner membrane of the mitochondria and inaccessible to the oxidation substrate—exogenous cytochrome c, the mitochondria were first treated in various ways.

The mitochondria were broken up with 0.37, 0.75, and 2.5% sodium desoxycholate during incubation for 10–15 min with this substance (0°) or by freezing and thawing, and they were also incubated for 30 min in a hypotonic medium of sucrose (0.06 M) in 0.02M tris buffer, pH 7.4, 0°, leading to an increase in the permeability of their outer membrane.

Solutions of cytochrome c were prepared in the same concentrations as for determination of the succinate dehydrogenase activity. The solution of 0.5M sodium hydrosulfite in 0.04M phosphate buffer was made up before each measurement from previously weighed out samples.

The maximal value of the optical density of cytochrome c, reduced by hydrosulfite, was determined beforehand. For determination of the activity of the enzyme, 0.02–0.04 ml of sodium hydrosulfite solution was added to 2.5 ml of the "working" solution of cytochrome c. The mixture was carefully stirred for 5–10 min to obtain complete oxidation of the sodium hydrosulfite, for even a slight excess of this salt disturbs the course of the reaction, the suspension of mitochondria was then added, and the optical density was recorded every 30 sec for 3 min. The activity was calculated just as when determining the succinate dehydrogenase activity.

EXPERIMENTAL RESULTS

The results of experiments to determine the succinate dehydrogenase activity when using different concentrations of oxidation substrate and potassium cyanide are given in Table 1.

Maximal succinate dehydrogenase activity was determined within a wide range of concentrations of sodium succinate and potassium cyanide. The differences between the activities of the enzyme during the measurements indicated above were not statistically significant. In most determinations, however, the enzyme activity was greatest with sodium succinate in a concentration of 0.08 M and potassium cyanide in a concentration of 0.005 M. For this reason, in later experiments the succinate dehydrogenase activity was determined with these concentrations of ingredients.

TABLE 3. Effect of Various Ways of Treatment of Mitochondria on Cytochrome Oxidase Activity

Index	Method of treatment of mitochondria				
	hypotonic medium	sodium desoxycholate			freezing and thawing
		2.5%	0.75%	0.37%	
Enzyme activity (M±m)	35.4±0.88	23.1±0.47	29.6±0.4	25.6±1.02	24.5±0.6
Number of measurements	23	8	18	5	8

A marked increase in the concentrations of sodium succinate (0.16M) or potassium cyanide (0.01M) and a simultaneous decrease in the concentrations of both these substances, led to a statistically significant increase in the succinate dehydrogenase activity.

To detect succinate dehydrogenase activity, intact mitochondria and mitochondria preincubated in isotonic medium were used. The results of these experiments (Table 2) showed that in mitochondria treated with isotonic medium, the activity of the enzyme was on the average 20% higher than its activity in intact mitochondria.

The results of experiments to determine the cytochrome oxidase activity in the mitochondria treated by various methods are given in Table 3.

As Table 3 shows, maximal cytochrome oxidase activity was characteristic of the mitochondria preincubated in hypotonic conditions. After treatment with sodium desoxycholate, the highest results were obtained when this substance was used in a concentration of 0.75%. The activity of the enzyme in mitochondria frozen and thawed three times was almost the same as in mitochondria treated with sodium desoxycholate in a concentration of 2.5%. The results obtained were statistically significant.

Preincubation of the mitochondria in a hypotonic medium, as used in this investigation, increased the permeability of their membranes for sodium succinate and cytochrome and enabled maximal activity of the corresponding enzymes to be obtained. The lower activity of the cytochrome oxidase in mitochondria destroyed by sodium desoxycholate or by freezing and thawing was evidently attributable both to partial disturbance of the chain of electron transport and to the inhibitory action of sodium desoxycholate on the activity of this enzyme [6, 9].

The increase in succinate dehydrogenase activity following preincubation of the mitochondria in hypotonic conditions evidently took place on account of that part of the enzyme located in the cristae and not detectable in the intact mitochondria because of the insufficient permeability of their membranes for the oxidation substrate and cytochrome c.

The results obtained may clearly be interpreted as confirming the view that a large proportion of the succinate dehydrogenase is located in the inner membranes of the mitochondria [4]. They are also in agreement with results obtained by Pearce [10], who considers that higher succinate dehydrogenase activity can be detected histochemically in swollen mitochondria than in intact mitochondria.

An increase in succinate dehydrogenase activity was also found during hypotonic treatment of mitochondria from other parts of the cat's and rabbit's brain possessing a less marked ability to swell in a hypotonic medium.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
