CHANGES IN CYTOCHROME OXIDASE AND SUCCINATE DEHYDROGENASE ACTIVITY OF THE MITOCHONDRIA OF THE RAT'S BRAIN UNDER THE INFLUENCE OF ANTI-BRAIN ANTIBODIES IN IMMUNE SERA AND SERA OF PATIENTS WITH NEUROPSYCHIC DISEASES

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It has been shown that specific antibodies (against the mitochondria of the liver and kidney of a rat) are capable of modifying the activity of certain mitochondrial enzymes of the rat's liver and kidney [6]. In this connection it was interesting to investigate the action of anti-brain antibodies on the enzyme activity of the mitochondria of the brain, which possess high antigenic activity. It was considered that investigations of this type would help to shed light on the biological significance of anti-brain autoantibodies in neuropsychic diseases.

In the present investigation the action of anti-brain antibodies was studied on the enzymes of the mitochondria of the rat's cerebral cortex—cytochrome oxidase and succinate dehydrogenase.

EXPERIMENTAL METHOD

Mitochondria were obtained by the method of Fonyo and Somogy [10], slightly modified in the laboratory of biohistochemistry of the Institute of the Brain, Academy of Medical Sciences of the USSR. The purity of the isolated preparations was checked under the phase-contrast microscope. The protein content of the mitochondria was determined by the method of Lowry and co-workers [7], the cytochrome oxidase activity spectrophotometrically by the method of Hess and Pope [8], and the succinate dehydrogenase activity by the method of Potter and Schneider [9]. Both methods were adapted for the mitochondrial fraction in the laboratory of biohistochemistry of the Institute of the Brain. The enzyme activity was expressed as the difference between the initial (at 550 mμ) and final (after 3 min) extinction values after incubation of a suspension of mitochondria with a substrate, related to 1 mg mitochondrial protein, and adjusted for an exposure of 1 h:

$$\Delta E \frac{60 \text{ min}}{1 \text{ mg protein}}.$$

During investigation of the action of antibodies on the enzyme activity of the mitochondria, the latter were preincubated with the test serum (0.2 ml in a dilution of 1:2) in the presence of complement (0.1 ml),* which was diluted with phosphate buffer solution (pH 7.4) 1:10 before the experiment. One batch of complement was used.

To determine the cytochrome oxidase activity, the above mixture was introduced into an identification medium—cytochrome c ($4.4 \cdot 10^{-5}$ M solution in 0.04 M phosphate buffer) reduced with sodium hydrosulfite. To determine the succinate dehydrogenase activity, all the remaining ingredients were added to the mixture of mitochondria, serum, and complement (0.1 ml of 2.5 M sodium succinate, 0.1 ml of 0.15 M KCN, 0.3 ml of a mixture of $4 \cdot 10^{-3}$ M solutions of $AlCl_3$ and $CaCl_2$, and 2.5 ml of a $4.4 \cdot 10^{-5}$ M solution of cytochrome c). In the control series the test serum and complement were replaced by an equivalent volume of buffer solution. The sera obtained from rabbits were diluted 1:4.

* The complement used was a dried preparation supplied by the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera.

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TABLE 1. Changes in Cytochrome Oxidase and Succinate Dehydrogenase Activity under the Influence of Sera with Different Characteristics of Results of the CFR

Persons from whom serum was obtained	No. of persons	Results of CFR		Activity of enzyme (in % of control)										
				Cytochrome oxidase						Succinate dehydrogenase				
		assessment	no. of persons	100 and below	101—125	126—150	151—175	176—200	Over 200	100 and below	99—85	84—70	69—50	49—25
Patients with neuropsychic diseases*	25	+	12	—	—	2	3	1	6	—	—	5	4	3
		±	1	1	—	—	—	—	—	1	—	—	—	—
		−	12	6	5	1	—	—	—	4	4	2	2	—
Patients with other diseases†	4	+	0	—	—	—	—	—	—	—	—	—	—	—
		±	0	—	—	—	—	—	—	—	—	—	—	—
		−	4	1	3	—	—	—	—	3	1	—	—	—
Donors	7	+	0	—	—	—	—	—	—	—	—	—	—	—
		±	2	2	—	—	—	—	—	1	—	1	—	—
		−	5	2	1	1	1	—	—	—	3	2	—	—
Total	36	+	12	—	—	2	3	1	6	—	—	5	4	3
		±	3	3	—	—	—	—	—	2	—	1	—	—
		−	21	9	9	2	1	—	—	7	8	4	2	—

Legend: + positive result, ± doubtful, - negative.

* Schizophrenia—in 10 patients, epilepsy—4, prolonged reactive state—2, disturbance of cerebral circulation—2, traumatic brain lesion—1, cyclothymia—1, involutional psychosis—1, psychopathia—1, paranoid syndrome and Kandinskii's syndrome—2, no mental disease was found in one subject.

† Patients from surgical clinic.

TABLE 2. Action of Rabbits Sera on Enzyme Activity of Mitochondria of the Rat's Brain

Serum no.	Tissue against which serum obtained	Serological characteristics (titer of antibodies against corresponding antigen)	Act. of enzyme (in % of control)	
			cytochrome oxidase	succinate dehydrogenase
1440 1417 1294	Homogenate of rat's brain	1 : 640 (++)*	166 180 241	58 59 63
1424 1279	Homogenate of human brain	1 : 320(++)*	138 160	52 69
668	Rat's serum	1 : 40 000†	82	102
1415	Human serum	1 : 15 000†	107	87
Normal rabbit serum	—	1 : 40*	118	96

* Investigated by the complement fixation method in the cold.
† Investigated by the passive hemagglutination method.

The degree of the change in enzyme activity following the action of the serum was expressed as a percentage of the activity of the enzyme in the control series, taken as 100%. In each experiment simultaneous tests were made of sera with and without anti-brain antibodies. These antibodies were determined by the complement fixation method in the cold [5]. The antigens were saline extracts and mitochondria from the rat's brain. The brain of the rat and certain other rodents (mouse, hamster) contains antigenic substances against which the serum of patients with neuropsychic diseases may contain antibodies with higher activity than is shown against antigens from the brain tissue of other species of animals and man [2]. The results of the complement fixation reaction were assessed as positive, negative, and doubtful, using for guidance the criteria of intensity of the reaction previously established [3]. In some experiments the sera (immune sera against serum proteins) were investigated by Boyden's passive hemagglutination method [4]. Some experiments were carried out with sera preliminarily absorbed by homogenates of untreated brain tissue and liver tissue of the rat [5].

The present paper describes the results of the investigation of serum from 36 human subjects and 8 rabbits Twenty-five specimens of serum were obtained from patients admitted to hospital for various neuropsychic disorders, and 11 specimens from donors and patients admitted to the surgical clinic.

The serum of 12 persons gave a positive complement-fixation reaction with antigenic preparations from rats' brain tissue. The remaining 24 samples gave doubtful (3 sera) or negative results of the complement fixation reaction.

EXPERIMENTAL RESULTS

The results of the investigation of the effect of the serum from human patients and healthy donors on the cytochrome oxidase and succinate dehydrogenase activity of the mitochondria from the rat's cerebral cortex are given in Table 1. Under the influence of the "positive" sera, the cytochrome oxidase activity rose to 150-200% of the control level; conversely, the succinate dehydrogenase activity fell by 25-75% of the initial activity. In the serum of persons with doubtful and negative results of the complement fixation reaction, an increase in cytochrome oxidase activity within the limits mentioned above was found in only 3 of 24 cases; 7 sera had an inhibitory action on the succinate dehydrogenase activity within the limits corresponding to the inhibitory activity of the serum with anti-brain antibodies.

The differences between the intensities of action of sera differing in their serological characteristics on the enzymes are statistically significant [1].

TABLE 3. Changes in Activity of Cytochrome Oxidase and Succinate Dehydrogenase of Mitochondria of the Brain under the Influence of Sera Absorbed by Homogenate of Rat's Brain and Liver Tissues

Serum	Activity of enzyme (in % of control)					
	cytochrome oxidase			succinate dehydrogenase		
	original serum	absorbed		original serum	absorbed	
		by brain	by liver		by brain	by liver
Immune						
No. 1440	177	107	153	72	100	71
No. 1417	180	122	140	59	93	81
Taken from patient						
K.	180	120	160	13	93	44
L.	179	106	158	75	85	80

It was thus shown that the action of sera on the enzymes of the mitochondria depends on whether or not they contained antibodies. To confirm the above-mentioned results, the activity of these enzymes was investigated under the influence of immune rabbit sera obtained against homogenate of human and rat's brain tissue. For control purposes immune rabbit sera against human and rat's serum proteins and also the serum of an unimmunized rabbit were used. The results of these investigations are given in Table 2. This table shows that the sera of rabbits immunized with brain homogenates caused considerable activation of cytochrome oxidase and inhibition of succinate dehydrogenase; the control sera had no such action.

Absorption of the sera by homogenates of brain tissue greatly diminished their action on the activity of these enzymes. Absorption of the sera by liver tissue also slightly reduced the action of the serum, although not to the same extent as by treatment of the serum with brain tissue (Table 3).

The results obtained thus show that the serum of man and animals, containing anti-brain antibodies, is capable of modifying the activity of the enzyme present in the mitochondria of the brain—stimulating the cytochrome oxidase activity and inhibiting succinate dehydrogenase activity. Further investigations will define this phenomenon in more detail and also explain its biological significance.

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