ACTIVITY OF ANTICEREBRAL ANTIBODIES (FROM THE SERUM OF PATIENTS WITH NEUROPSYCHIC DISORDERS) WITH RESPECT TO WATER-SALT BRAIN EXTRACTS SUBJECTED TO PHYSICOCHEMICAL TREATMENT

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TABLE 1. Reaction of Serum (from Patients with Neuropsychic Disorders) with Water-Salt Extracts from the Brains and Livers of Rats Treated by Various Methods

Patient	Dilution of serum	Water-salt extracts from brain I	II	III	IV*	V	VI	VII	from liver
B-a	1:10	++++	++++	++++	+-+-+	—	—	—	—
	1:20	++++	++++	+-+++	+++	—	—	—	—
	1:40	+++(+)	+++(+)	+++(+)	+-+	—	—	—	—
	1:80	++(+)	+++	++(+)	+	—	—	—	—
M-k	1:10	+++	++	+++(+)	++	—	—	—	—
	1:20	++(+)	+(+)	+++	+-+	—	—	—	—
	1:40	+(+)	+(+)	++(+)	—	—	—	—	—
	1:80	+	+	++	—	—	—	—	—
G-a	1:10	++(+)	++++	+++-+	+	—	—	—	—
	1:20	++	++(+)	+++	±	—	—	—	—
	1:40	+	++	++	—	—	—	—	—
	1:80	±	±	±	—	—	—	—	—
I-v	1:10	++++	++++	++++	—	∅	±	∅	—
	1:20	+++(+)	+-+++	++++	—		±		—
	1:40	+	++	++(+)	—		—		—
	1:80	—	—	—	—		—		—
M-v	1:10	++++	++++	++++	+(+)	—	—	—	—
	1:20	++++	+++	+-+	+(+)	—	—	—	—
	1:40	++(+)	++(+)	++	±	—	—	—	—
	1:80	±	++(+)	±	—	—	—	—	—
Z-a	1:10	++++	++++	+++		±	+	++	+/+
	1:20	+++(+)	+++(+)	++	∅	—	—	—	—
	1:40	++	+	+(+)		—	—	—	—
	1:80	—	—	—		—	—	—	—

TABLE 1 (continued)

Patient	Dilution of serum	Water-salt extracts from brain I	II	III	IV *	V	VI	VII	from liver
L-a	1:10	++++(+)	++++(+)	++++					—
	1:20	+++	+++	+++	∅	∅	∅	∅	—
	1:40	++	+++	++(+)					—
	1:80	+(+)	++	±					—
G-v	1:10	++++		++++		—			±
	1:20	++++	∅	++++	∅	—	∅	∅	—
	1:40	+++		++++		—			—
	1:80	++		++++		—			—
Z-v	1:10	++	++(+)						—
	1:20	++	++(+)	∅	∅	∅	∅	∅	—
	1:40	++	++						—
	1:80	+	+(+)						—
V-v	1:10	++++	++++	++++	+++	—	—	++	—
	1:20	+++	++++	+++	++	—	—	±	—
	1:40	++	++	+(+)	±	—	—	—	—
	1:80	+	+	+	—	—	—	—	—
R-v	1:10	++++			++++				—
	1:20	+++	∅	∅	±	∅	∅	∅	—
	1:40	+			—				—
	1:80	—			—				—
S-a	1:10	++++			+	—	—		—
	1:20	+++(+)	∅	∅	+	—	—	∅	—
	1:40	++(+)			+	—	—		—
	1:80	±			—	—	—		—
L-v	1:10	++++					—		—
	1:20	++	∅	∅	∅	∅	—	∅	—
	1:40	—					—		—
	1:80	—					—		—
M-n	1:10	++++				—			—
	1:20	++	∅	∅	∅	—	∅	∅	—
	1:40	+				—			—
	1:80					—			—
R-n	1:10	++(+)				—			—
	1:20	++	∅	∅	∅	—	∅	∅	—
	1:40	+				—			—
	1:80	±				—			—

Notations: [++++, +++(+), +++, ++(+), ++, +(+), +] various degrees of positive reaction; ± doubtful reaction; —negative reaction; ϕ not investigated. I) native water-salt extract; II) water-salt extract dried by a lyophilic method; III) water-salt extract heated at 50° and preserved with carbolic acid; IV) water-salt extract heated at 100°; VII) water-salt extracts treated with ether, chloroform, and carbon tetrachloride, respectively.

*Sera of patients I-v, R-v, and S-a were investigated with extracts produced from tissue heated at 100°.

Clinical observations of patients whose blood contains anticerebral complement-fixing antibodies, indicate the promising nature of further research pertaining to the practical utilization of these data for the prognosis and characterization of the severity of neuropsychic disorders [1, 6, 7]. The purpose of this work was to determine the possibility of preserving antigens from the brain tissue, as well as to determine their stability to various methods of treatment, which may be used to isolate the antigens in pure form. Previous investigations have shown that water-salt extracts from rat brain tissue exhibit high antigenic activity and can be used for detection of anticerebral antibodies in the serum of patients with neuropsychic disorders [4].

EXPERIMENTAL

Water-salt extracts (20%) from the rat brain, produced by the method three freezings and thawings, using dry ice, were subjected to lyophilic drying, heating at 50°C, followed by preserving with carbolic acid, boiling, treatment with ether, chloroform, and carbon tetrachloride. Extracts preliminarily diluted with an equal amount of a stabilizing medium, containing 1% gelatin and 10% sucrose, were preliminarily subjected to lyophilic drying. * The extracts were heated at 50° for 30 min, then centrifuged in a circular centrifuge (MOM 6 x 25, Hungary) at 5,000 rpm for 45 min. An equal amount of phosphate buffer (pH 8.0) was added to the supernatant liquid, along with a 5% solution of carboxylic acid dropwise, in an amount such that its concentration in the antigen would comprise 0.1%. Another antigen was produced from a 20% brain homogenate, heated at 100°, which was thoroughly pulverized, centrifuged, reheated at 100° for 10 min, and then centrifuged. The treatment of the antigens with ether and chloroform is described in another work [5]. The extracts were treated analogously with carbon tetrachloride. The protein content was determined in the antigens according to the method of Lowry et al. [8], and before the experiment the antigens were diluted in an amount such that the protein concentration would comprise 0.332 mg/ml. The investigation was conducted by the reaction of complement fixation in the cold. The complement was taken in a dose comprising 170% of its titer, determined by a thermal method.

RESULTS

Table 1 presents the results of our investigation of serum (from patients with various neuropsychic disorders) with water-salt extracts from the brains of rats subjected to the indicated treatment. An investigation of the serum with a water-salt extract from rat liver was the control for the specificity of the reaction with the brain.

As can be seen from Table 1 extracts subjected to lyophilic drying and heated at 50°, followed by treatment with carbolic acid, retained their activity. In the first case the antigens possessed good activity, in spite of storage for more than two years (at 4°). The second antigen was active in testing after 9 months of storage at 4°. Such a method of treating antigens from brain tissue is both simple and readily available and can be recommended for the preparation of active antigens, capable of withstanding prolonged storage. Moreover, the treatment of tissue antigens by heating at 50° followed by centrifuging, makes it possible to free them from part of the protein substances, to which the type antigens (nonspecific for a given tissue) detected in the reaction of complement fixation, are bound [5].

As can be seen from Table 1, the water-salt extracts from brain tissue, after heating at 100%, reacted with the serum of the patients to a lesser extent than did the native antigens and antigens subjected to lyophilic drying, as well as to heating at 50°. However, the extracts from the serum of certain patients (B-a, V-v, R-v), heated to 100°, reacted with relatively high intensity. This permits us to assume the presence of complement fixing antibodies for the thermally stable antigens of the brain tissue in the sera of the patients. Such antigens, characterizing the immunological specificity of the brain, have been detected earlier [9], and they have been assigned to the class of lipids, which, however, cannot be considered as finally proven.

Treatment of water-salt extracts from brain tissue with ether, chloroform, and carbon tetrachloride causes an inactivation of the antigens in them. Such antigens are inactive both with respect to specific anticerebral antibodies, and in immune sera [5]. This is evidence that the specific antigens of the brain, interacting with the anticerebral antibodies in the reaction of complement fixation, are rather unstable to such a relatively "mild" method of treatment as the action of organic solvents, which are used in the practice of preparation of viral and microbial antigen preparations [2]. However, the mechanism of the inactivating effect of organic solvents with respect to the antigenic properties of water-salt extracts of the brain, the clarification of which is closely involved in the solution of the question of the chemical nature of the brain antigens and their intracellular distribution, is unknown.

*Performed at the L. A. Tarashevich State Control Institute of Medical and Biological Preparations.

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