# EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON ORGAN-SPECIFIC ANTIGENS OF BRAIN TISSUE

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It is known that brain tissue possesses immunological specificity and under pathological conditions may act as an antigen for the organism proper and may promote the appearance of specific antibodies [7]. Such antibodies appear in the serum of patients with neuropsychiatric illnesses along with different brain antigens, including antigens normally contained in an aqueous—salt extract of brain tissue [1, 7-9]. The latter antigens are used as unpreserved preparations,and the conditions which determine the preservation or inactivation of their antigenic properties have not been studied. It is important that special investigation in this area be carried out.

Brain tissue has a complex antigenic structure. Water—saline extracts of brain contain, in addition to antigens common to all tissues, a number of antigens specific for brain tissue. The organ-specific antigens of the brain may reflect a species specificity of brain tissue or may be present in brain tissue of various unrelated species of animals [6, 13, 14, 16, 18, 19] and even characterize the peculiarities of the morphologic structure of the brain [3, 5, 10, 11, 17].

In the present work we studied the action of some physicochemical factors (heat, treatment with ether and chloroform) on the antigenic properties of the human brain, possessing immunologic similarity to the antigenic properties of brain tissue of two other species (mouse and rat) and on antigenic properties specific for each of these species. Water—saline extracts of brain tissue as well as suspensions of tissue subjected to such treatment were studied.

## EXPERIMENTAL

Water—saline extracts (20%) of human and animal brain, obtained by the method of triple freezing and thawing, were heated in a water bath at temperatures of 45, 50, 56, 60, and 100°C for 30 min, with subsequent centrifugation for 20 min in the angular centrifuge type MOM 6 × 25 (Hungary) at 1000 rpm. Ethyl ether and chloroform were added to the water—saline extracts in ratios of 1:1, and the mixture agitated for five minutes and centrifuged for 20 min at 1000 rpm.

The protein content was determined for all antigens by the method of Lowry and coworkers [15]. Prior to the experiment the antigens were diluted so that their protein contents were equal (about 0.4 mg/ml). The studies were performed by the method of prolonged complement fixation in the cold with doses of complement comprising 170% of its titer, determined by the method of warm fixation. The results of the experiment were calculated after hemolysis occurred in the control.

Immune antibrain sera containing antibody to the thermolabile components of the brain antigen but reacting weakly or not at all with boiled water—saline extracts were used.

To expose the absorptive properties of the tissues subjected to various actions, the precipitate of tissue washed many times was mixed with a double volume of antibrain serum diluted 1:10, and left for an hour at four °, after which it was centrifuged for 20 min at 1000 rpm. The supernatant liquid was heated for 30 min at 56° and again centrifuged, then a complement fixation test performed.

TABLE 1. Effects of Temperature, Ether, and Chloroform on the Immunologic Activity of Organ-specific Antigens of Brain Tissue

| Antigen used to obtain serum | Serum series | Serum dilution | Water saline extract of brain tissue: human, untreated | human, treated, heated to 45° | human, treated, heated to 50° | human, treated, heated to 56° | human, treated, heated to 60° | human, treated, ether | human, treated, chloroform | animal (rat, mouse), untreated | animal, treated, heated to 45° | animal, treated, heated to 50° | animal, treated, heated to 56° | animal, treated, heated to 60° | animal, treated, ether | animal, treated, chloroform |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human brain | 7 | 1:80 | ++++ | ++++ | ++++ | ++++ | +++ | ∅ | ∅ | ++++ | ++++ | ++++ | ± | ± | — | ± |
| | | 1:160 | ++++ | ++++ | ++++ | ++++ | +(+) | | | + | + | +(+) | — | — | — | — |
| | | 1:320 | + | ± | + | + | — | | | — | — | — | — | — | — | — |
| | 11 | 1:80 | ++++ | ++++ | ++++ | ++++ | +(+) | — | — | +++ | +++(+) | ++(+) | ± | ± | — | ± |
| | | 1:160 | ++++ | +++(+) | ++++ | ++++ | + | — | — | +(+) | + | + | ± | — | — | — |
| | | 1:320 | — | — | ± | ± | — | — | — | + | — | — | — | — | — | — |
| | 10 | 1:40 | +++ | +++ | +++ | +++ | + | — | — | ++++ | ∅ | ∅ | ∅ | ∅ | — | — |
| | | 1:80 | ++(+) | +++ | ++ | ++(+) | ± | — | — | + | | | | | — | — |
| | | 1:160 | — | — | — | — | — | — | — | — | | | | | — | — |
| Rat brain | 178 | 1:80 | +++(+) | ++(+) | +(+) | — | — | ∅ | ∅[1] | ++++ | ++++ | ++++ | ++++ | + | ∅ | ∅ |
| | | 1:160 | ++(+) | ++ | — | — | — | | | ++++ | ++++ | ++++ | +++ | — | | |
| | | 1:320 | +(+) | + | — | — | — | | | +++ | ++ | ++ | + | — | | |
| | 69 | 1:20 | ++(+) | ++ | ++ | — | — | — | — | +++(+) | +++(+) | +++(+) | +(+) | + | ∅ | ∅ |
| | | 1:40 | ++ | +(+) | +(+) | — | — | — | — | ++(+) | ++(+) | ++(+) | + | ± | | |
| | | 1:80 | + | + | + | — | — | — | — | +(+) | +(+) | + | ± | — | | |
| | 88 | 1:20 | ++++ | ∅ | ∅ | ∅ | ∅ | — | — | ++++ | ++++ | ++++ | ++++ | ++(+) | — | — |
| | | 1:40 | ++++ | | | | | — | — | ++++ | ++++ | ++++ | ++ | ± | — | — |
| | | 1:80 | ++++ | | | | | — | — | ++(+) | ++(+) | +(+) | — | — | — | — |

Note: For all three tables: ++++, +++(+), +++, (+), ++, +(+), +) various degrees of positive reactions; ±) questionable reaction; −) negative reaction; ∅ not studied.

*Serum was studied with mouse brain antigens; results obtained with serum in dilutions of 1:10, 1:20, 1:40.

TABLE 2. Effect of Temperature on the Absorptive Properties of Human and Rat Brain Tissue

| Series of serum | Antigen used for obtaining sera | Dilution | Antigen for complement fixation test | Tissue used for absorption | Result of complement fixation test with serum absorbed by brain tissue | | | | | | Serum prior to absorption |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | untreated | heated to 45° | heated to 50° | heated to 56° | heated to 60° | heated to 100° | |
| 88 | Mouse brain | 1:10 | Human brain | Human brain | — | — | +(+) | +(+) | ++++ | ++++ | ++++ |
| | | 1:20 | | | — | — | ± | + | ++(+) | ++++ | ++++ |
| | | 1:40 | | | — | — | — | — | + | ++(+) | ++(+) |
| 1440 | Rat brain | 1:20 | Rat brain | Rat brain | — | — | — | +(+) | ++++ | ++++ | ++++ |
| | | 1:40 | | | — | — | — | ± | +++ | ++++ | ++++ |
| | | 1:80 | | | — | — | — | — | — | ++ | +++ |

TABLE 3. Results of Absorption of Sera by Tissues Kept for a Month in Ether and Chloroform

| Serum 67 against mouse brain | dilution of serum | Results of complement fixation test with water saline antigens from brain tissue | |
|---|---|---|---|
| | | human | rat |
| Prior to absorption | 1:10 | ++++ | ++++ |
| | 1:20 | +++ | ++++ |
| | 1:40 | + | +++(+) |
| Absorbed in brain tissue | | | |
| Human* | 1:10 | — | +++ |
| | 1:20 | — | ++ |
| | 1:40 | — | ± |
| Rat* | 1:10 | + | + |
| | 1:20 | ± | ± |
| | 1:40 | — | — |
| Rat† | 1:10 | | +++(+) |
| | 1:20 | | — |
| | 1:40 | | — |

*Kept in ether.

†Kept in chloroform.

## RESULTS

In Table 1 are presented the results of investigation into the action of temperature, ether and chloroform on the organ-specific antigens of brain tissue. All sera were absorbed beforehand by liver tissue and in the indicated dilutions did not react with extracts of liver. As seen from Table 1, heating the brain extracts at 45 and 50° essentially did not affect their activity. Extracts heated to 56° reacted with serum against brain suspensions of the corresponding species, but did not react with sera against brain tissue of other species. The data obtained did not depend on the titer of the immune sera. Heating water–saline extracts to 60° either destroyed their activity or decreased it sharply even when these antigens were studied with the corresponding immune serum. Extracts subjected to heating at 100°, as mentioned earlier, as a rule did not react with the given sera.

Extracts treated with ether and chloroform did not display antigenic activity. It remains unclear if such inactivation is the result of denaturation of brain proteins by the organic solvents or if it is caused by a disruption of the bonds in the protein–lipoid complex which, possibly, are the determinants of antigenic specificity in the brain. The antigenic properties of 20% extracts of tissue preserved in ether and chloroform were also studied. Such extracts either do not interact with the corresponding immune sera or possess anticomplementary activity.

Because it is known that after treatment with various substances tissues are capable of fixing organ-specific antigens in the cells and resisting the extraction of the substances from the cells [4], we studied the absorption capacity of brain tissue subjected to the effect of various temperatures and of ether and chloroform (Tables 2 and 3). The absorption was performed with sera which were freed from nonspecific antibodies by preliminary treatment with liver tissue.

As seen from Table 2, brain tissue heated to 45° fully retains its absorptive properties in relation to homologous antibody as does brain tissue heated to 50°. Heating to 56° produces some weakening of the absorptive properties of the brain; they decrease sharply after heating to 60° and completely disappear when the tissue is boiled. This confirms the hypothesis that some brain antigens are related to thermolabile protein substances [19].

Brain tissue placed in ether and chloroform retains the capacity to absorb specific antibody (see Table 3). Evidently ether or chloroform treatment is sufficient to inactivate the water-soluble organ-specific antigens, but

this treatment does not appear to have a similar effect on specific antigens fixed in the brain tissue cells. The organ-specific antigens which characterize the interspecies immunologic similarity of the brain are less resistant to the effect of high temperature. These properties of brain antigens should receive attention in the preparation and use of brain preparations for scientific and practical purposes. In this connection it should be stressed that the use of brain extracts prepared by the method of Il'enko [2], which includes treatment with ether or chloroform [12] as antigens to produce antibrain autoantibodies in the serum of patients, evidently does not permit formation of antibodies to those components of the brain which are inactivated as the result of treatment with the above-mentioned substances.

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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 this issue.**

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