A COMPARATIVE ESTIMATE OF COMPLEMENT-FIXATION METHODS IN THE COLD, OF DOUBLE DIFFUSION IN AGAR, AND OF PASSIVE HEMAGGLUTINATION FOR THE DEMONSTRATION OF ANTICEREBRAL ANTIBODIES IN THE SERUM OF MENTALLY DISTURBED PATIENTS

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It has been shown that complement-fixing antibodies reacting with aqueous saline extracts of cerebral tissue are present in the serum of patients suffering from psychiatric disorders [2, 5, 10, 12]. Also there is evidence that in the serum of patients with cerebral disorders antibodies of the precipitation type may be present, as may be shown by precipitation in agar, or by agglutination of tanned erythrocytes or of latex particles treated with tissue extracts [8, 9, 11].

The object of the present investigation has been to determine whether sera containing anticerebral complement-fixation antibodies are active in precipitating aqueous-saline extracts of cerebral tissue. The theoretical and practical significance of investigations of this kind is that they give an indication of the sensitivity of one or other methods with respect to the various immunological systems to be estimated, and they also indicate the part played by various types of antibodies in the pathogenesis of autoimmune diseases.

METHOD

Serum from patients with various nervous disorders were investigated by the complement-fixation reaction in the cold; in order to reveal precipitating antibodies we used the method of double diffusion in agar and Boyden's passive hemagglutination. The antigens were aqueous-saline extracts from human brain tissue, or from rat or mouse brains. As has been shown in previous investigations [2, 4, 5], extracts of cerebral tissue of these animals in many cases give a selective complement-fixation reaction in the presence of sera from patients with mental disorders. As a control we used liver extracts.

The method of precipitation of antigens has been described previously [3]. For the complement-fixation reaction the amount of antigen used was equal to 0.4 mg of protein per ml. The amount of complement was 170% of the titer determined at body temperature. The hemolytic system was added after incubation for 18 h at 4°, and 1 h at 37°. The count was made after hemolysis had occurred in the control.

The experiment of diffusion precipitation was carried out in agar plates about 3 mm thick, and the diameter of the central and peripheral holes was 5 mm; the concentration of the agar was 1-1.5% in physiological saline with phosphate buffer at pH 7.4 (7.2). The central hole was filled with the serum under test, and the peripheral holes were filled with the antigens. In most cases the sera were investigated in the undiluted state. The amount of protein in the antigens was about 2-4 mg per ml. As a preservative we used merthiolate at a concentration of 1:100,000. The measurements were made over a period of one week during incubation at 37°, and in most cases it was continued for 3 weeks at 4°. The experiment of passive hemagglutination was carried out with pig erythrocytes, with human erythrocytes of group 0 (1), and in most cases with rabbit erythrocytes. The latter were found to be most suitable, because they gave no non-specific agglutination in the control. Thrice-washed erythrocytes as a 4% suspension in physiological saline with phosphate buffer at pH 7.4 (7.2) were added to 1:30,000 tannin in

TABLE 1. Results of a Comparative Study of Sera in the Complement-Fixation Reaction in the Cold, and in the Method of Double Diffusion in Agar

No. of observations	Surname of patient	Diagnosis	Complement-fixation reaction				Method of double diffusion in agar
			Antigen from tissue				
			Brain		Liver		
			Human	Rat	Human	Rat	
1	Z-va	Schizophrenia	+++	++	–	–	–
2	K-v	"	++	+++	–	–	–
3	B-va	"	+	+	–	–	–
4	Z-a	"	+++	+++	–	–	–
5	K-va	"	++	+++	–	–	–
6	S-na	"	++	+	–	–	–
7	M-v	Organic damage of the brain, alcoholism	++	++	–	–	–
8	B-a	Schizophrenia	–	+++	–	–	–
9	Ch-va	"	–	+	–	–	–
10	O-v	"	–	+++	–	–	–
11	M-n	Organic damage to the brain	–	+++	–	–	–
12	G-v	Alcoholism	–	++++	–	–	–
13	I-n	Organic damage to brain	–	+	–	–	–
14	S-a	The same	±	++	–	–	±
15	R-n	" "	++++	±	–	–	–
16	A-v	Progressive paralysis	±	+	–	–	–
17	Ch-v	" "	++++	++++	+	++++	–
18	L-v	" "	++	+++	++	+++	–

Indications for Table 1 and 2: ++++ reactions strongly positive (delay of hemolysis ++++ in dilutions 1:2-1:20, and ++++ or ++ in dilutions 1:40-1:80); +++ reaction positive (delay of hemolysis ++++ or +++ in dilutions 1:10 and 1:20, and not less than ++ in dilutions 1:40 and 1:80); ++ reaction positive (delay of hemolysis ++++ or +++ in dilutions 1:10 and 1:20, and not less than ++ at a dilution of 1:40); + reaction weakly positive (delay of hemolysis in dilutions 1:10-1:40, but less intense than the reaction ++); ± reaction doubtful (delay of hemolysis in dilutions 1:10-1:20, but not more than +++); – reaction negative (hemolysis in all dilutions of serum or reaction not more than ++ in dilutions 1:10).

phosphate buffer at pH 7.4 (7.2); after 30 min they were washed three times in the same buffer, and again suspended in a phosphate buffer at pH 6.4, and twice the volume of antigen diluted in the same buffer to a protein content of about 0.2-0.4 mg per ml was added. After 30 min the erythrocytes were washed three times in buffer solution at pH 7.4 containing 1% rabbit serum. Next this medium was used to suspend the washed erythrocytes and for dilution of the serum under test. The experiment was carried out in test tubes 5 mm in diameter; one drop of a 1.5% suspension of erythrocytes sensitized with antigen was added to 2 drops of serum previously inactivated and absorbed by erythrocytes; the test tubes were left for 18 h at 4°, and measurements were made in terms of the configuration of the deposit.

In the experiments carried out by the three methods, we investigated not only the patients' serum but also immune sera containing antibodies to brain tissue; the results of the experiments were considered significant if the immune sera gave a positive reaction with the corresponding antigens.

RESULTS

Altogether 33 specimens of serum obtained from 24 patients with various mental disorders were used for comparative experiments utilizing complement-fixation in the cold and double diffusion in agar. Of this number the

TABLE 2. Results of a Comparative Investigation of Sera in the Complement-Fixation Reaction in the Cold, and in Passive Hemagglutination

No. of observations	Surname of patients	Diagnosis	Results of complement-fixation reaction				Passive hemagglutination
			Antigens from tissues				
			Brain		Liver		
			Human	Rat (mouse)	Human	Rat (mouse)	
1	Ch-v	Schizophrenia	+	+	–	–	–
2	Sh-ko	Organic brain damage	+++	+++	–	±	–
3	R-ov	The same	+	++	–	–	–
4	S-v	Schizophrenia	–	+++	–	–	–
5	Sh-v	"	±	++	–	–	–
6	K-v	"	–	+	–	–	–
7	R-v	"	–	++	–	–	–
8	L-v	"	–	+	–	–	–
9	N-ov	"	±	+++	–	–	–
10	Yu-n	Organic brain damage	–	++	–	–	–
11	Zh-v	The same	–	+++	–	–	–
12	G-ov	" "	–	+++	–	–	–
13	P-v	Psychopathic state	±	++	–	–	–
14	S-a	Organic brain damage	–	++++	–	–	–
15	G-v	Alcoholism	–	++	–	–	–
16	R-k	Organic brain damage; syphilis; phychopathy		++		±	–
17	L-v	Progressive paralysis	+		++	–	–
18	O-v	Schizophrenia	–	+		–	–
19	V-v	"	±	++	–	–	–

sera of 18 persons contained anti-tissue complement-fixating antibodies (Table 1): in the serum of 16 patients we found antibodies to human and to rat (or mouse) cerebral tissue, or only to heterologous brain; in the serum of 2 persons (observations Nos. 17 and 18, see Table 1) the complement-fixation reaction occurred also in the presence of liver extracts (patients with progressive paralysis). The occurrence of precipitation with tissue antigens was established in only one case (observation No. 14, see Table 1). In this case the serum reacted weakly with antigens from the brain in the complement-fixation reaction, but gave a precipitation reaction which however could have been interpreted as non-specific with respect to the brain, because antigens from human and mouse brain were also precipitated. The precipitation bands in the antigenic zones from brain and liver could not be distinguished, and appeared as isolated continuous lines. Evidently their appearance must be interpreted either as the result of the interaction of sera with antigens which are identical but non specific only for brain tissue, or as a result of a precipitation reaction of a non-immune nature, as described by several authors [6, 7, 14].

The sera of patients in which the complement-fixation reaction was negative gave a negative result also in the precipitation in agar test.

By the passive hemagglutination method of tanned erythrocytes we investigated 62 samples of sera from 41 patients. Of this number the sera from 19 patients (Table 2) reacted in the complement-fixation reaction with antigens from brain tissue, in most cases heterologous brain tissue. As can be seen from Table 2, not one of these sera reacted in the passive hemagglutination tests. A negative result was also obtained in studies of sera from 22 patients in whom the complement-fixation reaction was negative. The results of these investigations showed that

the method of complement-fixation in the cold was able to reveal anticerebral antibodies, i.e., antibodies which cannot be shown up by means of tests on precipitating antibodies. However, because of the small number of experiments we cannot in general deny the possibility of the appearance of anti-tissue auto-antibodies of a precipitation type in sera of patients suffering from mental disorders. In this respect the results of other authors must be considered encouraging [8, 9, 11, 12].

It is known that the complement-fixation reaction, passive hemagglutination, and double diffusion in agar are often used as a means of searching for anti-tissue auto-antibodies [16]. Also the lack of agreement in results obtained by different methods has been pointed out [13, 15], and the disagreement has been attributed to the fact that each of the methods used is sensitive to particular immunological systems. In damage to tissues possessing a complex antigenic structure auto-antibodies related to various antigens of one and the same tissue or to various components of one and the same antigen may arise. As a consequence, in order to demonstrate such auto-antibodies a number of different methods, or a set of methods must be used. Because the antibodies to the various tissue antigens appear not all to be of the same pathogenic significance, such a complex investigation allows us to determine what immunological reactions correspond to the degree of clinical severity of the disease, and possibly also to find out which reactions indicate merely the degree of severity, being (in the terminology of A. D. Ado [1]) merely "witnesses" of a pathological process.

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