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The Complement Fixation Reaction in Toxoplasmic Infection.

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The need for a rapid and simple serological test for toxoplasmic infection prompted the present investigation of the development of complement fixing antibodies. The neutralization test described in the accompanying communications[1,2] has two chief drawbacks: (1) virulent toxoplasma must constantly be maintained by animal passage, and (2) the result of the test is not known for about a week.

Immune monkey sera possessing neutralizing antibodies were available and the first problem was to determine whether or not a toxoplasma-infected tissue could be found which would yield a suspension or extract that would fix complement specifically in the presence of a known immune serum. The data summarized in Table I reveal that the problem was not simple since the tissues which we knew to be richest in toxoplasma content, either gave no fixation (*e.g.*, mouse brain) or nonspecific fixation (*e.g.*, mouse lung), or were unsuitable for other reasons. The antigen finally found to give specific complement fixation with immune monkey sera was prepared in the following manner:

Rabbits weighing approximately 2 kg were given 0.5 cc of a 10% suspension of toxoplasma-infected mouse brain intracerebrally. When the rabbit died (4 to 8 days after inoculation) its entire brain was ground with enough physiological salt solution to make a 10% suspension which was then stored in an ordinary refrigerator for 18 hr.

TABLE I.
Search for Antigen Giving Specific Complement Fixation with Toxoplasma Immune Monkey Serum.

Toxoplasma infected animal	Tissue	Type of extract	Result
Mouse	Brain	0.85% saline, frozen, thawed 5 ×	No fixation
		Frozen with solid CO_2, pulverized, extr. with saline	" "
		Absolute methyl alcohol	Nonspecific fixation*
"	Lung	" " "	" "
		0.85% saline	" "
		Berkefeld "V" filtrate of saline extr.	" "
"	Peritoneal exudate	Whole heparinized, fresh	Anticomplementary
		Absolute methyl alcohol	"
		" ethyl "	Hemolytic
Rabbit	Spleen	Dried *in vacuo*, powder extr. with methyl alcohol	No fixation
		0.85% saline	Nonspecific fixation
"	Liver	" "	" "
"	Brain	Absolute methyl alcohol	" "
		0.85% saline, frozen, thawed 5 ×	*Specific fixation*

*Nonspecific fixation means that extracts of normal and toxoplasma-infected tissue gave same degree of fixation.

1 Sabin, A. B., and Ruchman, I., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 1.

2 Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 6.

This suspension was then alternately frozen in a mixture of solid CO_2 and alcohol and thawed 5 times, and centrifuged on an angle centrifuge at about 3,000 rpm for 30 min. The supernatant liquid constituted the antigen. It was stored without preservative both in an ordinary refrigerator and in the frozen state in an insulated box containing solid CO_2, and retained its activity for at least 3 months. The control antigen was prepared in the same manner using normal rabbit brain.

Procedure of the Complement Fixation Test. The immune sera were inactivated at 56°C for 30 min and used undiluted, 1:5, 1:10, 1:20, etc., or diluted, 1:2, 1:4, 1:8, etc. The dilutions mentioned in this paper refer to the original dilution of the serum and not that which is attained after mixture with the complement and antigen. The complement was either fresh guinea pig serum titrated immediately before use or commercially available lyophilized guinea pig serum (Sharp and Dohme). 0.2 cc of the toxoplasma immune serum was mixed with 0.2 cc of complement (containing 2 units) and 0.2 cc of the toxoplasma antigen in one series and the normal rabbit brain antigen in a duplicate series. The tubes were incubated in a water bath at 37°C for 30 min, and 0.4 cc of a mixture consisting of equal parts of a 5% suspension of washed sheep erythrocytes and hemolysin (2 units per 0.2 cc) was added to each of the tubes which were then incubated at 37°C for another 30 min. The final reading was made after an overnight incubation in the refrigerator, the degree of fixation being expressed in the conventional manner, *i.e.*, complete fixation or lack of hemolysis as ++++ and no fixation or complete hemolysis as 0. Two plus fixation or better was regarded as significant. The usual anticomplementary and hemolysis controls were included in each test.

Complement Fixing Antibodies in Toxoplasma-infected Monkeys. The sera of normal rhesus monkeys tested fresh or within 24 hr after bleeding were either completely negative with both antigens or at best reacted only when used undiluted. After storage in the refrigerator, however, both normal

TABLE II.

The Appearance and Persistence of Complement Fixing Antibodies in the Serum of Monkeys with Experimental Toxoplasmosis.

Wks after inoculation	Monkey No. 281	392	393	408	409	410	411
Before inoculation	0 U*	0 (0)	0 (0)	*10 (10)*	*30 (20)*	*U (U)*	*Anti-complementary*
1	U (0)	16 (0)	0 (0)				
2	U (0)	16 (0)	U (0)	*20 (10)*	*40 (20)*	*30 (20)*	*60 (10)*
3	U (0)	8 (0)	0 (0)	*30 (10)*	*50 (20)*	*30 (10)*	*70 (10)*
4	2 (0)	8 (0)	8 (0)	*20 (5)*	*80 (40)*	*30 (10)*	*80 (10)*
5	4 (0)	8 (0)	8 (0)	*20 (5)*	*100 (70)*	*60 (5)*	*70 (10)*
6				20 (U)	*90 (50)*	50 (0)	60 (5)
7				10 (U)	90 (5)	10 (0)	30 (5)
8				10 (0)	90 (5)	10 (0)	60 (5)
9				0 (0)	80 (5)	0 (0)	
10				U (0)	90 (10)	10 (0)	60 (10)
12						U (0)	60 (10)
13				0 (0)	90 (0)	U (0)	
14		16 (U)	4 (U)		60 (5)		
17					30 (2)	U (0)	
28		2 (U)	4 (2)				
34						8 (2)	
45					4 (2)	U (U)	

Figures not in parentheses indicate titre with Toxoplasma-infected rabbit brain, while those in parentheses refer to the titre with normal rabbit brains.

The data in italics were obtained with sera which had been stored in the refrigerator for varying periods of time before the test, and show the degree of nonspecific fixation which develops in normal and immune monkey sera on storage (this does not occur when the sera are frozen). The tests on all the other sera were carried out within 24 hr after bleeding.

*U—undiluted.

and immune monkey sera have been found to develop the capacity to fix complement in varying degrees with both normal and toxoplasma rabbit brain antigens. With the exception of a certain number of sera (indicated in Table II) which were tested after various periods of storage in the refrigerator, all the other tests were, therefore, carried out within 24 hr after bleeding.

The sera of 7 rhesus monkeys which were infected by the intracutaneous and intra-abdominal routes and used in the neutralizing antibody investigation,[1] were studied at weekly intervals early after inoculation and at longer intervals subsequently. Monkeys 281, 392, 393, 408 and 409, received their toxoplasma in mouse brain suspension and monkeys 410 and 411 in rabbit brain suspension. Monkey 392, whose serum was entirely negative with both antigens before infection, developed specific complement fixing antibodies for the toxoplasma antigen as early as one week after inoculation, its serum giving a positive reaction in a dilution of 1:16. Definite evidence of specific complement-fixing antibodies appeared later in the other monkeys, the interval being as long as 4 weeks in some (Table II). While the maximum specific complement fixing titres varied from 1:4 in monkey 281 to 1:90 in monkey 409, it is not yet clear how much this variation depended on the individual monkeys and how much on the possible differing potencies of the antigens.

Unlike the neutralizing antibody the complement fixing antibody either completely disappeared (monkeys 408 and 410) or was diminished to very low titre after periods of 2 months or longer after infection (the antigen involved in some of these negative results gave high titres with other sera in simultaneous tests). It cannot be said, however, that persistent complement fixing antibody necessarily indicates a persistent infection since monkey 411 had a high titre of complement fixing antibody at a time when the toxoplasma had already disappeared from the body.[1]

Absence of Complement Fixing Antibodies in Toxoplasma-infected Rabbits, Dogs, and Cats. No complement fixing antibodies were found in the sera of 4 rabbits which were proved to be immune to toxoplasma. Four dogs, 2 adult and two 3 months old, and 3 cats were tested before and at intervals after experimental infection with toxoplasma, but no complement fixation was demonstrable.

Complement Fixing Antibodies in Human Beings. 63 specimens of serum from 56 individuals were tested for complement fixing antibodies. At no time did any of the human sera react with the normal rabbit brain antigen, while 10 individuals yielded sera which fixed complement to varying degrees with the toxoplasma rabbit brain antigen. These 10 positive reactions were associated with the following conditions: One infant (J.F.) who at one month of age presented hydrocephalus, cerebral calcification, and bilateral chorioretinitis and from whose cerebrospinal fluid Wolf, Cowen, and Paige isolated toxoplasma by animal inoculation, gave a positive reaction with only the undiluted serum at about 3 months of age and again at 4 months of age (we are indebted to Dr. Rustin McIntosh of New York, for these sera and the history); one 8-year-old boy (W.V.) exhibiting cerebral calcification and convulsions since 11 months of age had a serum with a titre of 1:4 (Dr. Bronson Crothers of Boston, kindly supplied this specimen and history); 3 mothers of infants in whom a diagnosis of congenital toxoplasmosis was considered on clinical grounds, and one mother who gave birth to an anencephalic monster, had titres varying from undiluted to 1:8; one was a man without any significant history other than rabbit hunting who was tested because his infant daughter to whom he gave blood for a transfusion was also positive. The sera of the 8 individuals just listed also had neutralizing antibodies against toxoplasma. The remaining two individuals (a patient and his mother) gave specific complement fixation reactions in repeated tests, but the neutralization tests with their sera were regularly negative. The patient was a 14-month-old infant who was suspected of having congenital toxoplasmosis by Drs. P. M. Levin and H. Moore of Dallas, Texas, since he presented microcephaly, cerebral calcification, chorioretinitis, convulsions and psychomotor

retardation. Two specimens of this infant's serum gave titres of 1:8, while two different specimens of the mother's serum had titres of 1:2. Among the sera of 43 selected individuals which were tested both for neutralizing and complement fixing antibodies, 8 had both, 20 had neutralizing but not complement fixing antibodies, 2 had complement fixing but not neutralizing antibodies, and 13 had neither antibody.

Discussion. Rhesus monkeys, but not cats, dogs, or rabbits, have been found to develop specific complement fixing antibodies during the course of experimental toxoplasmic infection. Unlike the neutralizing antibody, that responsible for complement fixation disappeared in many monkeys during convalescence. The complement fixing antibody, furthermore, differs from the neutralizing antibody in that it is destroyed neither by heating at 56°C for 30 min nor by storage in the refrigerator. Positive complement fixation reactions were also obtained with sera of human beings who were known or could be suspected of having experienced a toxoplasmic infection. The fact that as many as 20 individuals whose sera yielded positive neutralization tests gave negative complement fixation reactions may perhaps be dependent on the following factors: (1) the complement fixing antibody unlike the neutralizing antibody, did not persist for a very long time (as in most of the experimentally infected monkeys), (2) in none of these 20 individuals was the suspected toxoplasmic infection either active or recent, and (3) the toxoplasma antigen used in these tests may have been of low potency.

Further experiments are necessary to elucidate the conditions which will yield the most potent complement fixing antigen. We know of no reason, for example, why the extract of toxoplasma-infected rabbit brain should be an effective antigen, while similar extracts of mouse brain or other tissues containing even larger numbers of toxoplasma, should be devoid of any detectable complement fixing antigen. The only other investigation of this problem of which we are aware is that by Nicolau and Ravelo,[3] who reported positive complement fixation reactions with the sera and extracts of various tissues of experimentally infected rabbits (a smaller number of dogs, cats, guinea pigs, pigeons and monkeys were also tested) as the source of antibody and a methyl alcohol extract of powdered, dry, parasitized rabbit spleen as the antigen. This antigen gave no fixation in our hands and the sera of rabbits, dogs, and cats were found to be devoid of both complement fixing and neutralizing antibodies.

Summary. A frozen and thawed extract of toxoplasma-infected rabbit brain (but not similar extracts of certain other heavily parasitized tissues) yielded an antigen which fixed complement specifically in the presence of immune sera from experimentally infected rhesus monkeys but not rabbits, dogs, and cats. This complement fixing antibody appeared within 1 to 4 weeks after inoculation, but unlike the neutralizing antibody disappeared again in most monkeys, sometimes as early as 2 months. A certain number of human beings who were known or suspected to have experienced toxoplasmic infection also gave positive complement fixation reactions. However, twenty selected individuals whose sera had neutralizing antibodies against toxoplasma gave negative complement fixation reactions, but in none of these was there evidence of recent or active infection. While a positive complement fixation reaction does not in all cases indicate active or even recent infection, it is believed that this test may have its greatest usefulness in the rapid diagnosis of active toxoplasmosis.

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[3] Nicolau, S., and Ravelo, A., *Bull. Soc. path. exot.*, 1937, **30**, 855.