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Characteristics of the Toxoplasma Neutralizing Antibody.

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The development of a neutralizing antibody against toxoplasma was first reported in experimentally infected rhesus monkeys.[1] It was also pointed out that this antibody apparently does not develop in all species since none was found in rabbits recovering from infection with the same strain of toxoplasma.[1] This antibody was best demonstrated when mixtures of various dilutions of toxoplasma-infected mouse brain suspension and undiluted serum were injected intracutaneously on the back of one rabbit which received simultaneously similar mixtures prepared with Tyrode's solution or normal monkey serum. The effect of this antibody was also evident when such mixtures were injected intracerebrally or intraäbdominally in mice. This antibody was never found in very great titre since the best sera completely neutralized only 10 to 100 minimal skin lesion doses, although it modified the size and character of the lesions produced by the larger amounts of toxoplasma suspension. The occurrence of this neutralizing antibody in a proved human case of naturally acquired toxoplasmic encephalitis was first reported by one of us in 1941.[2]

In the past 2 to 3 years toxoplasma have been proved to be responsible for a variety of morbid manifestations in human beings by demonstrating their presence in certain human tissues and body fluids microscopically and by animal inoculation.[2,3,4] In order to explore the possibilities of diagnosing this infection during life as well as to ascertain its incidence and the various clinical conditions in which it may exhibit itself, it became necessary to investigate the possible usefulness of certain serological reactions. Thus, one wanted to know how soon after infection the neutralizing antibody became demonstrable, how long it persisted, and whether or not its persistence could be interpreted as indicating a continued infection with this protozoon parasite. In an attempt to answer these questions rhesus monkeys were bled before infection with toxoplasma by the intracutaneous and intraäbdominal routes and weekly thereafter. Since at least 4 sera can be tested on the back of a single rabbit, the monkey sera were tested fresh on some occasions and at other times after storage in a refrigerator at about 5°C; a number of sera

1 Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.

2 Sabin, A. B., *J. Am. Med. Assn.*, 1941, **116**, 801.

3 Wolf, A., Cowen, D., and Paige, B. H., *Am. J. Path.*, 1939, **15**, 657.

4 Pinkerton, H., and Henderson, R. G., *J. Am. Med. Assn.*, 1941, **116**, 807.

TABLE I.
Effect of Storage at 4° to 5°C on Toxoplasmic Neutralizing Antibody.

Monkey serum	Time of storage—days after bleeding: Fresh	2	5	7	9	13	14	16	21, 28, 35, 40 and 49
408-2†	10 (100)*								0 (0)
409-2	10 (100)								0 (0)
408-2		10 (500)							0 (0)
409-6		10 (100)					10 (—)		0 (0)
410-7		10 (100)							0 (0)
411-7		10 (100)							0 (0)
6-55			100 (500)			0 (0)			0 (0)
8-54			100 (500)			0 (0)			0 (0)
408-6				10 (100)			10 (—)		0 (0)
408-7				10 (—)‡				0 (0)	
409-7				10 (500)				0 (0)	
410-6					10 (—)				0 (0)
411-6					10 (100)				0 (0)
410-5								10 (—)	0 (0)
411-5								10 (100)	0 (0)

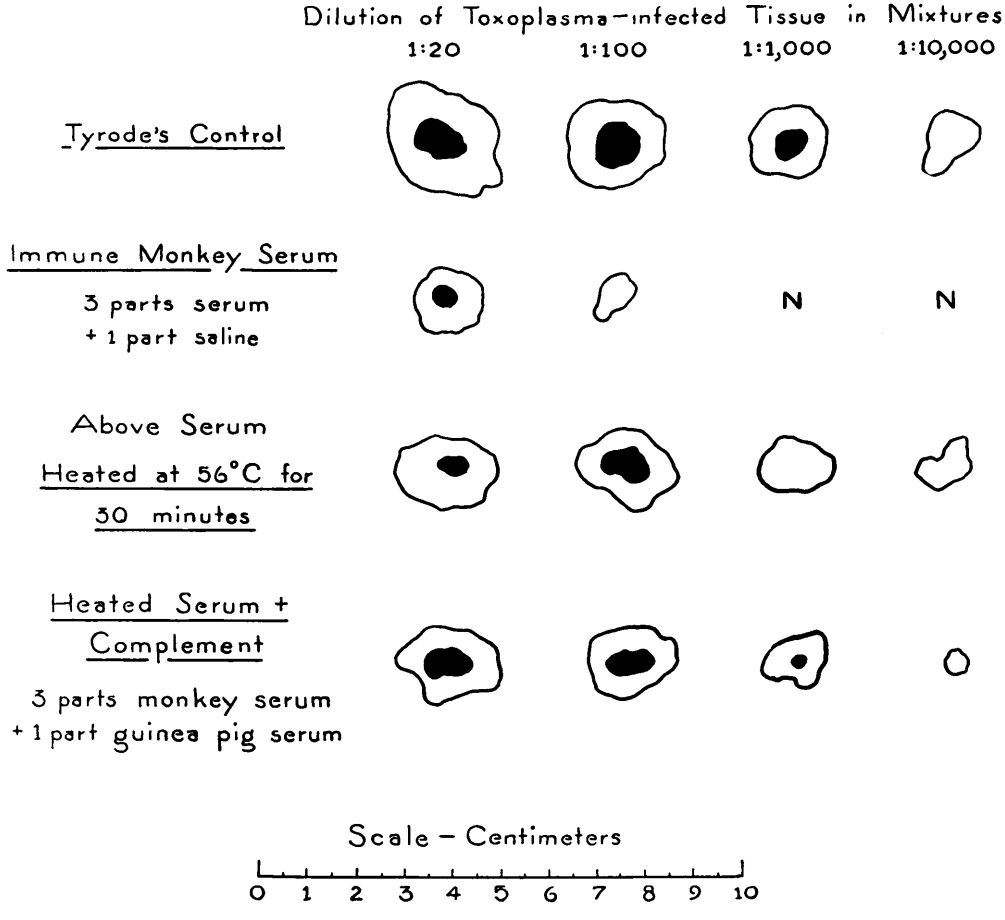
†408-2 Serum of monkey 408, 2 weeks after inoculation with toxoplasma.
*10 (100) Serum neutralized 10 minimal infective skin doses completely and 100 m.i.s.d. partially.
‡10 (—) Serum neutralized 10 minimal infective skin doses completely and had no effect on the other doses.

obtained at different intervals from the same monkey were tested simultaneously on the back of a single rabbit. This method of study at first led us to the erroneous conclusion that this antibody may disappear as early as 6 weeks after inoculation[2] and later it seemed as if the results obtained in this neutralization test were not reproducible. It appeared, however, that when several different sera obtained at weekly intervals from one monkey were tested at the same time the most recent sera regardless of the time after infection, invariably neutralized while those which had been stored in the refrigerator for 2 weeks or longer were usually negative. Furthermore, when a previously positive serum was retested after more than 2 weeks' storage it was almost always negative. When the data on 15 different positive monkey sera which had been tested more than once after different times of storage in the refrigerator were tabulated (Table I), a significant property of the toxoplasmic neutralizing antibody emerged, namely that the antibody, or some part of it, is very unstable even at refrigerator temperature. All the 15 sera which originally had the neutralizing antibody were completely negative after storage in the refrigerator for 21 days or longer; 2 sera were negative on the 13th day and 2 on the 16th day. When it was then found that the neutralizing antibody in a fresh monkey serum was destroyed by exposure to a temperature of 56°C for a half hour, it became necessary to determine whether or not complement was a factor in the reaction and whether or not the neutralizing effect could be restored by the addition of complement to an inactivated serum. The results of such a test shown in Chart I indicate that complement is not a factor (the guinea pig serum used in the test produced complete lysis of sensitized sheep erythrocytes in a dilution of 1:18) and that the toxoplasmic neutralizing antibody is itself so unstable at 56°C. It has proved possible to preserve the antibody by freezing the serum with solid CO_2 and maintaining it in the frozen state in a CO_2 refrigerator or by drying it from the frozen state.

The questions dealing with the time of appearance, persistence, and significance of the neutralizing antibody were then reinvestigated in new rhesus monkeys which were experimentally infected with toxoplasma by the intracutaneous and intraäbdominal routes. It was found (Table II) that, (1) fresh normal monkey sera were entirely devoid of any neutralizing effect, (2) a small amount of the antibody was already detectable one week after inoculation or 5 days after the first fever

Chart I

Effect of Complement on Inactivated Toxoplasmic Neutralizing Antibody



in the monkey, (3) the amount of neutralizing antibody present at 2 weeks after inoculation persisted without apparent increase or decrease for more than a year (the limit of observation thus far), and (4) the amount of this antibody is so small that it is no longer detectable by this method with serum diluted 1:10. In order to determine whether this persistence of the neutralizing antibody indicated a persistent infection with toxoplasma,

TABLE II.
Time of Appearance and Persistence of Neutralizing Antibodies in Monkeys with Experimental Toxoplasmosis

Wks. after inoc.	Monkey No. 281	392	393	408	409	410	411	6	8
Before inoculation	0(0)	0(0)	0(0)						
1	1(10)	1(—)	1(10)						
2	10(100)	10(500)	10(100)	10(500)	10(100)				
3									10(100)
4								10(100)	
5							10(100)		
6				10(100)	10(100)		10(100)		
7					10(500)	10(100)	10(100)		
9				10(—)	10(100)				
12						10(100)	10(100)		
13				10(100)	10(100)				
15				1(100)					
17						10(100)			
20					10(100)				
29		10(100)	100(500)						
45-46					10(100)	100(500)			
54-55								100(500)	100(500)
60					100(500)	100(500)		100(500)	

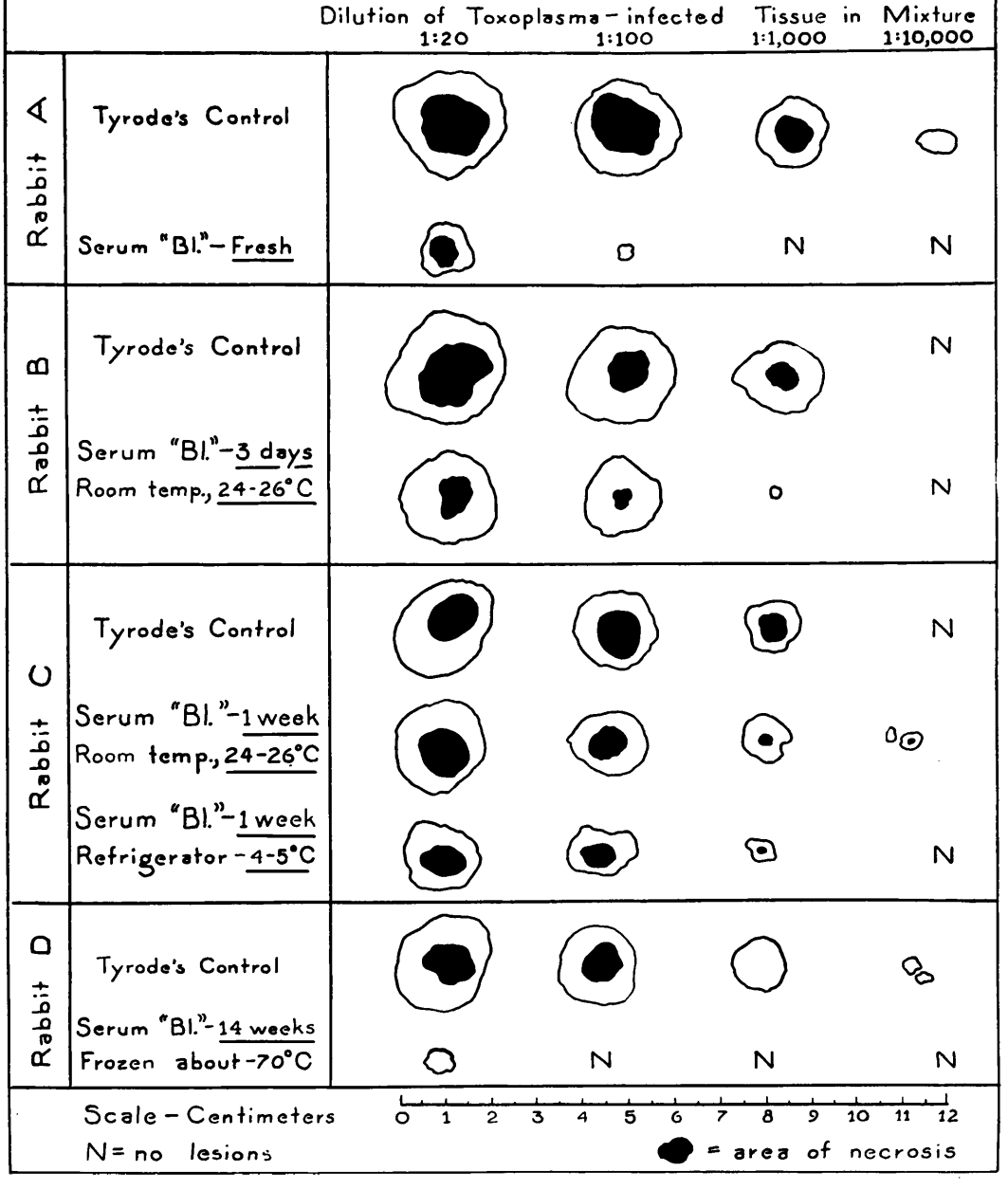
For legends see Table I.

a thorough search was made for the parasite in numerous tissues of 3 convalescent monkeys at various intervals after infection. Rhesus monkeys inoculated with toxoplasma intracutaneously and intraäbdominally usually develop skin lesions and fever which disappear within 10 to 14 days and the animals appear entirely normal thereafter. Rhesus 411 was sacrificed at 14 weeks and Rhesus 408 at 17 weeks after such an inoculation and the following tissues were examined histologically and tested for toxoplasma by intracerebral and intraäbdominal inoculation of mice (each tissue suspension into 4 mice): (1) the axillary and inguinal lymph nodes (corresponding to the inoculated skin sites), (2) spleen, (3) liver, (4) kidneys, (5) adrenals, (6) lungs, (7) brain, and (8) spinal cord. No evidence of persistent infection was found either histologically or by animal inoculation. None of the inoculated mice showed any signs of illness and at the end of one month 2 of each group of 4 mice were sacrificed and the brain and viscera subinoculated into a new group of 4 mice which in turn remained well. This is the most rigorous method that can be used to detect toxoplasma. This procedure was also followed and negative results obtained with the same tissues of Rhesus 392 which died 7 months after inoculation of an adhesive pericarditis, apparently the result of repeated cardiac punctures. *It would appear, therefore, that the persistence of the toxoplasmic neutralizing antibody is not the result of persistent infection with this protozoon parasite.*

Paradoxical behavior of one lot of hyperimmune monkey serum. One of us (A.B.S.) prepared a pool of "hyperimmune" monkey serum in 1935 by bleeding out 4 rhesus monkeys which had received repeated intraabdominal injections of toxoplasma-containing tissues. At first this "hyperimmune" serum neutralized no better than any other convalescent monkey serum. When it was retested in 1939, however, it regularly completely neutralized even the largest concentration of toxoplasma with which it was mixed both upon injection into the rabbit's skin and intracerebrally in mice. This serum has been stored in an ordinary refrigerator for the past 7 years and, contrary to the experience with all the other sera containing toxoplasmic neutralizing antibody, is still as potent as ever. Also contrary to the effect which heating at 56°C for 30 minutes has on all the other toxoplasma-immune sera, this "hyperimmune" serum lost none of its antitoxoplasmic effect after exposure to this temperature. Because of its history and prop-

Chart II

Tests on Single Specimen of Human Toxoplasma Immune Serum Stored under Different Conditions



erties the antitoxoplasmic effect of this "hyperimmune" serum cannot be regarded as being due to the regular neutralizing antibody. At one time before 1939 this serum became overgrown with molds during the course of its storage in the refrigerator and it was sterilized by Seitz filtration and has remained sterile ever since (pH 7.2). Suspecting that the particular mold may have produced an antitoxoplasmic substance, we filtered 5

old, moldy, normal monkey sera and tested them against toxoplasma, but with completely negative results.

Neutralizing antibodies in other animals. Fresh sera of normal rabbits, mice, cats, and dogs contained no antibody nor was any found in these animals after recovery from experimental infection with toxoplasma (the studies on the sera of cats and dogs were carried out in association with Dr. Joel Warren).

Lability of toxoplasmic neutralizing antibody in human sera. Although a number of scattered tests had indicated that the toxoplasmic neutralizing antibody was probably as labile in human as in monkey sera, it was desirable for practical and other reasons to determine some of the limits of this lability. Serum, known to contain neutralizing antibody, was obtained from an 18-month-old infant with congenital toxoplasmosis[5] and divided into several portions. Part was immediately frozen and stored in a solid CO_2 refrigerator, part was tested fresh, and other portions were tested after storage at room temperature (about 24° to 26°C) or in an ordinary refrigerator (about 4° to 5°C). The data illustrated in Chart II reveal that the same serum which yielded strongly positive results when tested fresh or after being frozen at about 70°C for 14 weeks gave an equivocal result after 3 days at room temperature or 1 week in the refrigerator and a distinctly negative result after 1 week at room temperature. The neutralizing antibody in the human serum was also inactivated by heating at 56°C for 30 minutes.

Summary. The toxoplasma neutralizing antibody was found to be so labile even at temperatures of about 5°C, that it could disappear after one to 2 weeks of storage in an ordinary refrigerator. The antibody effect was destroyed by heating at 56°C for 30 minutes and could not be restored by the addition of fresh complement. The antibody could be preserved for months at the low temperature provided by solid CO_2 in an insulated box. Antibody was absent in the fresh sera of rhesus monkeys before infection with toxoplasma but appeared within one to 2 weeks after infection and persisted for at least 14 months which is the limit of observation thus far. This persistence of neutralizing antibody was not associated with persistence of toxoplasma in the monkeys, since none could be found in tests on a large number of tissues from 3 monkeys, 14, 17, and 26 weeks after infection. No neutralizing antibody was found in the fresh sera of normal rabbits, mice, cats, or dogs, nor did these animals develop any appreciable antibody after recovery from experimental infection with toxoplasma.

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

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Toxoplasma Neutralizing Antibody in Human Beings and Morbid Conditions Associated With It.

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By actual demonstration of the organisms in the tissues, by microscopic and animal inoculation methods, toxoplasma have already been proved to be responsible for the following morbid conditions in human beings: (a) congenital encephalomyelitis becoming apparent in the newborn period or *in utero*,[1,2] (b) acute encephalitis in childhood,[3] and (c) a spotted-fever-like syndrome associated with pneumonitis.[4] The fact that mothers who had given birth to infants with proved

1 Wolf, A., Cowen, D., and Paige, B. H., *Am. J. Path.*, 1939, **15**, 657; *Science*, 1941, **93**, 548.

2 Paige, B. H., Cowen, D., and Wolf, A., *Am. J. Dis. Child.*, 1942, **63**, 474.

3 Sabin, A. B., *J. A. M. A.*, 1941, **116**, 801.