Localization and Persistence of Toxoplasma in Tissues of Experimentally Infected White Rats.* (18635)

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Jacobs and Jones(1) recently reported the occurrence of parasitemia in various animals inoculated with toxoplasma. In rats the observed parasitemia was sporadic though parasites persisted in the tissues long after the blood was free. In our experiments we wish to report the finding of toxoplasma regularly in the blood stream of experimentally infected rats and of the localization of the parasites in other tissues of the body, especially, their persistence in the brain for as long as two years.

Materials and methods. The R.H. strain of toxoplasma(2) was used throughout. The brains of mice that succumbed after intracerebral inoculation were removed on the fourth day and ground in saline solution to make a 10% suspension. After the larger particles had settled out, the supernatant fluid was used to infect the rats. Intracerebral inoculation into mice of decimal dilutions of this suspension revealed titers of $10^{-5.3}$ to $10^{-5.5}$. Albino rats weighing between 200 and 350 g were inoculated intraperitoneally with one cc of the 10% suspension and they developed inapparent infection. They were sacrificed at various intervals and blood was removed by cardiac puncture. One part of heparin (1:500) was added to 9 parts of blood to inhibit clotting, and 10-fold dilutions of the blood were prepared in saline solution. The animal was then opened and tissues were removed aseptically. Separate instruments were used for each tissue. At first only a portion of each tissue was used but in later experiments the entire organ was removed and made into a 10% suspension either by grinding with mortar and pestle or by homogenizing in a Waring blendor. The presence of toxoplasma was established by subinoculating one cc of the blood or tissue suspensions intraperitoneally into groups of mice and recording the animals that died. Tissue suspensions and dilutions were prepared in saline solution. Frequent checks were made for the presence of toxoplasma by the microscopic examination of Wright stained smears of mouse brains. Surviving mice were tested for immunity by intracerebral inoculation of a 10% suspension of infected mouse brain but all of them died.

Occurrence in the blood stream. After a preliminary experiment had indicated that organisms could be demonstrated in the blood stream within several hours after intraperitoneal inoculation, later experiments were designed to test for the presence of toxoplasma at 4 hours and at daily intervals thereafter (Table I). Organisms were found in the blood at 4, 24, 48 and 72 hours. They were also found in some instances in dilutions of at least 1:10. It was at the fourth and fifth days, however, that the maximal number of organisms was found in the circulating blood. Dilutions of 1:100 and in one instance 1:1000 caused toxoplasmic infection in mice. After the sixth day organisms were present sporadically in the blood stream up to the sixteenth day. The blood was not examined beyond the fourth week.

Occurrence in the viscera. At 4 hours subinoculation of the liver, spleen and lung showed that parasites were present. Toxoplasma were found regularly for nearly a week and more or less regularly for a few more days in liver and spleen 24 hours after inoculation and in lung 48 hours after inoculation. Up to the tenth week they were discovered occasionally in the liver, up to the fourteenth day in the spleen and up to the tenth week in the lungs. The viscera were not examined beyond the twelfth week.

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1. Jacobs, L., and Jones, F. E., *J. Infect. Dis.*, 1950, v87, 78.

2. Sabin, A. B., *J.A.M.A.*, 1941, v116, 801.

TABLE I. Distribution of Toxoplasma in Tissues of Rats Following Intraperitoneal Inoculation. Composite of several tests.

Rat tissue subinoculated into mice	Result of subinoculation of rat tissues into mice at*																												
	Hours					Days																Weeks							
	4	24	48	72	96	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	3	4	5	6	7	8	10	12
Blood																													
Undiluted	6	4	10	3	13	11	6	4	0	2	1	4	1	0	0	0	1	0	0	0	0	0	0						
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	11	10	11	9	13	11	10	11	6	8	8	8	8	8	8	3	5	3	3	3	3	3	3						
1:10	0	2	5	3	8	6	2	0	0																				
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	9	9	9	6	6	6	3																				
1:100	0	0	1	0	4	5	0	0																					
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	6	6	6	6	6	6																					
1:1000					1	0	0	0																					
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
					6	6	6	6																					
10% suspensions																													
Liver	3	8	9	8	9	9	9	9	0	2	1	3	1	1	0							0	0	0	3	0	0	1	0
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	9	9	9	9	9	9	3	3	3	3	3	3	6							6	6	6	6	6	3	3	3
Spleen	2	7	9	5	9	8	6	7	2	3	1	3	0	0	1							0	0	0	0	0	0	0	0
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	9	9	9	9	9	9	3	3	3	3	3	3	6							6	5	6	6	6	3	3	3
Lung	2	1	6	3	9	9	6	6	3	3	3	3	2	1	5							3	1	0	0	1	0	1	0
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	9	9	9	9	9	9	3	3	3	3	3	3	6							6	6	6	6	6	3	3	3
Brain	0	0	2	0	7	7	6	7	3	3	3	3	3	3	3							6	6	6	6	6	3	3	3
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	9	9	9	9	9	9	9	9	3	3	3	3	3	3	3							6	6	6	6	6	3	3	3

* Numerator denotes number of mice that died; denominator denotes number of mice inoculated.

TABLE II. Persistence of Toxoplasma in Brains of Rats.

Interval tested, mo.	Result of subinoculation into mice Brain suspension			
	1:10	1:100	1:1000	1:10000
4	6			
	–	–	–	–
	6			
5	6			
	–	–	–	–
	6			
6	6			
	–	–	–	–
	6			
11	5			
	–	–	–	–
	5			
12	2	2	1	
	–	–	–	–
	2	2	2	
18		4	2	0
	–	–	–	–
		4	4	4
24	4	4	3	
	–	–	–	–
	4	4	4	

Occurrence in the brain. For the first few days practically no organisms were detected in the brain (Table I), but on the fourth day and up to a period of 2 years, the longest interval of time studied, the parasites were present with great regularity. In order to obtain a rough idea of their concentration in the brains at the end of one, 1½ and 2 years, 10-fold dilutions of brain were prepared in saline and subinoculated into groups of mice. As seen in Table II organisms were present not only in the 10% suspension but also in dilutions of 1:100 and 1:1000, indicating that although the animals were apparently well they still harbored large numbers of organisms in the brain.

Rat to rat passage by peripheral inoculation. Having decided that organisms were harbored indefinitely in the brains of rats it next became of practical interest to determine if toxoplasma could be maintained by rat to rat passage especially when the interval between passages was long. A rat injected intraperitoneally (Passage I) with infected mouse brain material was sacrificed at the end of one year (Table III). A suspension of its brain was transferred to other rats by the peritoneal route (Passage II). Simultaneously, inoculation of this suspension into mice revealed a titer of $10^{-3.0}$. The rats of passage II were sacrificed at 6 months and 18 months and parasites were demonstrated in the brains. Inoculation of the brain of the animal sacrificed at 6 months was made into 2 rats (Passage III) and into mice which yielded a titer of $10^{-3.0}$. One of the rats of passage III was killed at the end of one year and as soon as it became apparent that parasites were still demonstrable in the brain, the other was also sacrificed. A suspension of its brain was transferred to rats (Passage IV). Again organisms were present in the brain as shown by subinoculation into mice. Passage IV rat was sacrificed at the end of 2 months and titration of its brain in mice showed a titer of $10^{-2.7}$. Thus after 4 consecutive passages in rats which consumed a total of 32 months, the number of organisms present in the rat brain remained undiminished. This indicated that the parasites had not only survived but that they had multiplied. Further rat passages were not undertaken.

Discussion. Although individual variations occur among infected rats nevertheless a general pattern is detectable. Organisms begin to circulate in the blood stream as early as 4 hours after inoculation and continue to do so for about one week, the peak concentration occurring between the fourth and fifth days. After the first week relatively few organisms are found. This period coincides with the first appearance of antibodies in the blood stream. It has been found that experimentally infected white rats develop complement-fixing antibodies towards the end of the first week (3), cytoplasm-modifying antibodies at the end of one week(4) and neutralizing antibodies at the end of 2 weeks(3). As the antibodies appear, the organisms disappear from the blood stream but they may remain longer in some of the tissues of the viscera. Owing to their "protected" environment in the brain toxoplasma may survive in this tissue indefin-

3. MacFarlane, J. O., and Ruchman, I., unpublished data.

4. Sabin, A. B., and Feldman, H. A., *Science*, 1948, v108, 660.

TABLE III. Multiplication and Survival of Toxoplasma in Brains of Rats Following Rat to Rat Passage.

Passage No.	Suspension inoculated*	Date		Time interval, mo.	Results with rat brain suspension	
		Inoculated	Sacrificed		Intraperitoneal titer in mice†	Passed to rats
I	10% mouse brain	4- 9-48	3-17-49	11	$10^{-3.0}$	Yes
II	10% rat brain	3-17-49	9-22-49	6	$10^{-3.0}$	Yes
			12- 7-50	18	$10^{-1.5}$‡	No
III	" " "	9-22-49	9-22-50	12	$10^{-1.5}$*	No
			10- 6-50	12	$10^{-1.5}$*	Yes
IV	" " "	10- 6-50	12- 7-50	2	$10^{-2.7}$	No

* 3 cc intraper.
† 1 cc intraper. into groups of mice.

itely. The occurrence of chronic toxoplasmosis in wild rats has already been reported(5). The long residence of organisms in the brain and the known tendency of rodents to devour brain tissue suggests one method of maintaining a reservoir of infection in nature. The fact that toxoplasma can be found in the brains of experimentally infected white rats for periods of at least 2 years becomes of practical importance to investigators working with the organism. For many years it has been the usual procedure to maintain toxoplasma by alternate storage in the refrigerator for 2 weeks and then passage into mice especially when uniformly fatal strains are used. In this laboratory it is now more convenient to inoculate rats. Whenever the organisms are required, a rat is sacrificed and its brain passed to mice. After one or two passages the organism is ready for use. Additional rats may then be inoculated intraperitoneally with this material. Thus the white rat may be used to demonstrate a parasitemia or an antibody response, which can be determined by several serological methods. It also serves as an excellent means of maintaining the organisms conveniently and for long periods of time, and finally it can be used to study the carrier state.

Summary. Intraperitoneal inoculation of toxoplasma into rats yielded the following data. Organisms were shown to be present in the blood 4 hours after inoculation and daily thereafter for the next week. The maximal concentration was reached between the fourth and fifth days. Organisms were found sporadically during the next few weeks. Parasites were present in small numbers in the liver, spleen and lungs at 4 hours but were more regularly found after that for 7 days in the liver, 9 days in the spleen and 11 days in the lungs. They were less regularly found after these periods of time for 10 weeks in the liver and lungs and for 2 weeks in the spleen. Toxoplasma appeared in the brain at 4 days and were uniformly present thereafter for a period of 2 years, which was the longest interval of time that the tissue was examined. Intraperitoneal inoculation of rats is a convenient method to maintain the parasites indefinitely.

5. Perrin, T. L., Brigham, G. D., and Pickens, E. G., *J. Infect. Dis.*, 1943, v72, 91.

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