METHODS

A NEW METHOD OF INDICATING THE PRESENCE OF Toxoplasma gondii IN TISSUE CULTURES

G. T. Akinshina and G. D. Zasukhina

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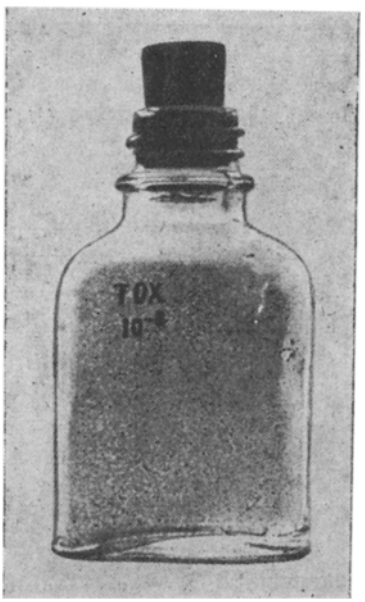
Several methods exist for detecting the presence of Toxoplasma gondii in tissue cultures. Among the more important of these are the method of direct counting of the parasites in the culture fluid and of estimating their number in the tissue culture by their cytopathogenic action.

However, the use of the plaque method, widely used in virology, for indicating the presence of T. gondii has shown that it is more effective than these two methods. The plaque method can be used for the study of many problems in theoretical and practical protozoology (production of pure lines, investigation of processes of interaction between parasite and cell, the study of certain problems in the genetics of the Protozoa, and so on). Yet this method has not been used for these purposes. The plaque method has been applied to the study of these parasites only for determining their viability and for their direct counting [4].

The authors have used the plaque method to study certain genetic characteristics of T. gondii, to isolate clones of this organism and investigate them from the point of view of variability, to detect the possible correlation between signs of pathogenicity and signs which can be studied in experiments in vitro, and to examine certain other problems.

To obtain plaques of T. gondii, various virological methods were used, the conditions of plaque formation were studied in relation to the method, and some possible genetic characteristics were examined: the size of the plaques formed by T. gondii; the effect of the type of the serum and agar inhibitors on the size of the plaques; the ability of T. gondii to propagate at a raised temperature of cultivation (42°); the pathogenicity of clones of T. gondii isolated from individual plaques toward mice, and so on.



Plaques formed by T. gondii on the 6th day after inoculation of the culture.

Monolayer cultures of chick fibroblasts were obtained by trypsinization of 10-day chick embryos. The nutrient medium was a 0.5% solution of lactalbumin hydrolyzate with 10% bovine serum. A cell suspension was prepared to contain 1,000,000 cells/ml. The cells were grown in 50-ml flasks. After formation of the monolayer the nutrient medium was removed, and 0.5 ml of tenfold dilutions of T. gondii prepared in a 0.5% solution of lactalbumin hydrolyzate was added to each flask with its monolayer. Peritoneal exudate of infected albino mice was used for inoculation. After contact for 1 h between the parasites and cells, 5 ml of covering medium was added to each flask.

Three modifications were used when preparing the covering medium [1, 2, 5]. In the experiments with the covering medium of Hsiung and Melnick [5], bovine or calf serum was used. The plaques were counted from 4 to 12-14 days after inoculation, for the number of plaques increased depending on the time of observation. In the modifications of L. K. Karpovich and G. D. Zasukhina, one component of the covering medium was ecmolin,* preventing the action of inhibitors of sulfopolysaccharide type in the agar, as reflected by an increase in the size of the plaques in the inhibitor-sensitive variant of different viruses [6]. In contrast to the modification of Hsiung

*A liver extract with bacteriostatic properties.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR O. V. Baroyan). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 4, pp. 119-121, April, 1967. Original article submitted August 23, 1965.

TABLE 1. Relationship between Diameter (in mm) of Plaques of T. gondii and Addition of Ecmolin to the Covering Medium

Covering medium	Time after inoculation (in days)			
	4	7	9	11
With ecmolin	0,2—0,3	0,5—0,8	0,9—1,1	1,0—1,1
Without ecmolin	0,2—0,3	0,5—0,8	0,9—1,1	1,0—1,1

TABLE 2. Comparative Characteristics of Ability of T. gondii to Propagate at Different Temperatures (intensity of plaque formation)

Temp. of cultivation (in degrees)	Dilution of original material	Time after inoculation (in days)	
		6	11
37	10^{-1}	Confluent	Confluent
	10^{-2}	>100	»
	10^{-3}	>100	>100
	10^{-4}	20	>100
42	10^{-1}	>100	Confluent
	10^{-2}	>100	>100
	10^{-3}	51	>100
	10^{-4}	0	12

TABLE 3. Pathogenicity of Clones of T. gondii Isolated from Individual Plaques for Mice

Clone No.	Size of plaques (in mm)	Pathogenicity for mice	No. of T. gondii cells injected	Time of death of mice (in days)
21	1,0	5/5*	500	10—11
42	1,0	5/5	500	9—10
67	1,1	0/5	50 000	—
71	1,05	0/5	50 000	—
115	0,95	0/5	10 000	—
126	1,0	5/5	500	9—10
128	1,0	0/5	10 000	—
136	1,15	0/5	10 000	—
145	1,0	5/5	1 000	11—12
146	0,95	5/5	1 000	10—11

*Numerator – number of mice dying; denominator – number of mice inoculated.

and Melnick [5], these methods could be used to determine a genetic characteristic of the toxoplasmas: their sensitivity to the inhibitors in the covering agar.

To determine the ability of the toxoplasmas to propagate at 42°, half the infected flasks lined with covering medium were kept for 24 h at 42°, and then transferred to the incubator at 37°. The other half of the infected flasks were immediately incubated at 37° [3].

To study the pathogenicity of the clones of T. gondii for mice, the parasites were isolated from individual plaques situated not less than 1-2 cm from each other, and introduced into a separate preparative test tube with nutrient medium (0.5% lactalbumin hydrolyzate with 10% bovine serum). By direct counting in a Goryaev's chamber, the number of parasites was then determined, after which the fluid obtained was injected intraperitoneally into albino mice weighing 16-18 g.

Altogether 20 experiments were carried out with T. gondii by the method of Hsiung and Melnick, in 12 of which bovine serum was used and in the other 8—calf serum. Six experiments were performed by the other two modifications. Plaque formation by T. Gondii was observed when all three methods were used, but the method of Hsiung and Melnick was rather less sensitive than the other two.

The plaques were round in shape, with clearly defined borders, and they appeared on the 4th-5th day after inoculation, when their diameter was 0.2-0.3 mm (see the figure). With an increase in the time of incubation the diameter of the plaques increased to 1.0-1.1 mm (Table 1).

The type of serum is known to have a significant effect on the size of the plaques formed by certain viruses. For instance, a population of the virus of Western equine encephalomyelitis, when calf serum was used, contained a higher percentage of large plaques than when bovine serum was used [7].

In the present experiments, however, the type of serum used had no effect on the size of the plaques formed by T. gondii, and in all the experiments, whether with bovine or with calf serum, the diameter of the plaques on the 4th-7th day after inoculation was 0.3-0.8 mm. Addition of ecmolin, abolishing the action of inhibitors in the agar, to the covering medium likewise had no effect on the size of the plaques (Table 1).

A differential sign for many viruses, distinguishing pathogenic from apathogenic variants, is their ability to propagate at different temperatures of cultivation [3].

In the course of three experiments in which T. gondii was cultivated at 42°, comparison of the results of plaque formation with those at 37° showed that the difference in titers (PFU/ml) was approximately 1.0 (Table 1). Evidently the population of T. gondii was nonhomogeneous by this criterion, and some of the cells propagated less intensively or, possibly, did not propagate at all at 42°.

The results of the separate experiments to study the pathogenicity of clones of T. gondii isolated from the plaques for albino mice are given in Table 3. In five cases, the mice died from acute toxoplasmosis on the 9th-11th day after receiving injections of between 500 and 1000 parasites, while in the other five cases, receiving 10 times the number of parasites, the mice did not die but were still alive when observed after one month. Consequently, the population of T. gondii was nonhomogeneous by the criterion of pathogenicity for albino mice when injected intraperitoneally. No correlation likewise was found between the criteria of pathogenicity and five of plaques formed by T. gondii under agar.

Hence, the plaque method, widely used nowadays only in virology, proved to be very valuable both for diagnosis of toxoplasmosis and for studying the actual causative agent T. gondii. In particular, this method may be used to obtain genetically pure lines, to study the correlation between the criterion of pathogenicity with criteria that can be studied in experiments in vitro, making it possible to investigate different strains of T. gondii, and to find apathogenic variants with respect to one sign or to all signs collectively in vitro, which are essential for containing diagnostic sera and vaccines and for other purposes.

LITERATURE CITED

1. G. D. Zasukhina, Vopr. Virusol., No. 5 (1966), p. 617.
2. L. G. Karpovich, in the book: Tick-Borne Encephalitis, Kemerova Tick Fever, Hemorrhagic Fevers, and Other Arbovirus Infections [in Russian], Moscow (1944), p. 44.
3. R. Carp and H. Koprowski, Virology, 16 (1962), p. 71.
4. S. Chaparas and R. Schlesinger, Proc. Soc. Exp. Biol., 102, New York (1959), p. 431.
5. G. Hsiung and J. Melnick, Virology, 1 (1955), p. 533.
6. H. Liebhaber and K. Takemoto, Virology, 14 (1961), p. 502.
7. L. Quersin-Thiry, Brit. J.Exp. Path., 42 (1961), p. 511.