is not known. To find the correct explanation would be an important contribution to a better understanding of the virus-cell relationship.

The possibility that the ring zones are the result of mesodermal cellular infiltration or that they are caused by external nonspecific stimulations or by antigen-antibody reactions could be excluded. On the other hand, it is not unlikely that the formation of ring zones is influenced to some extent by the alternating physiological condition of the eggs. This hypothesis is supported by the observation that pox viruses settle better in viable embryos than they do in fertilized eggs of low vitality. In the latter, the secondary foci do not show the same degree of development as in more viable eggs after the same period of incubation.

A. MAYR
G. WITTMANN
Federal Research Institute for Animal Virus Diseases, Tübingen, Germany

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Hemagglutination Test for Toxoplasmosis

The *in vitro* dye test of Sabin and Feldman (*1*) is at present the most useful diagnostic procedure for toxoplasmosis. However, it has difficulties in its performance which make it unsuitable for use in most routine diagnostic laboratories. It requires live parasites, which are dangerous to handle, and it requires microscopic reading of the tests, large quantities of an accessory factor that is found in normal human serums and that is sometimes difficult to obtain, and considerable care in most of the manipulations involved. The reports of Middlebrook and Dubos (*2*), Boyden (*3*), Keogh *et al.* (*4*), Alexander *et al.* (*5*), Stavitsky (*6*), and others have demonstrated the applicability of the hemagglutination procedure to a variety of antigens, both polysaccharide and protein in nature. The technique has already been tested in parasitic infections, such as schistosomiasis and trichinosis, by Kagan (*7*). In an effort to devise a practicable test for the diagnosis of toxoplasmosis, we have explored the applicability of this procedure.

A number of *Toxoplasma* antigens have been prepared and tested in the hemagglutination test, using the following procedure. Toxoplasmas are harvested from the peritoneal exudates of intraperitoneally infected mice into a tared tube containing saline, buffered at *p*H 7.2. The harvested organisms are centrifuged and the supernatant discarded. A volume of sterile distilled water 10 times the wet weight of the sediment is added, the sediment is resuspended, and the mixture is allowed to stand at 5°C for at least 18 hours, with occasional shaking. The solid particles are then removed by centrifugation, and an equal volume of 1.7-percent saline is added. The antigen now constitutes approximately a 1/20 (weight/volume) dilution of the original parasite harvest but contains only the water-soluble components of the parasites. It is stored in the deepfreeze until it is used.

Sensitization of red cells with these antigens requires treatment of the red cells with 1/20,000 tannic acid. Sheep red cells have been used, and the techniques are like those used by Stavitsky (*6*). The red-cell concentration used was close to that recommended by Stavitsky. The density of the red-cell suspension was measured in each case by lysing 1 ml of the diluted cells with 5 ml of distilled water and measuring the optical density at 520 mμ in a Beckman spectrophotometer. It was found that the most usable concentrations gave readings varying from 0.4 to 0.6. A diffuse settling of cells is regarded as a 4+ reaction, whether or not any crinkling of the outer edges of the settled film occurs. The 2+ reaction of Stavitsky is the same as our 2+ reaction, and is taken as the endpoint, at which the titer of the test serum is measured.

Block tests have been performed to determine more closely the optimal concentrations of reagents to use in the hemagglutination test. In general, the results indicate that an antigen concentration of 1/200 and a red-cell suspension giving an optical density reading of 0.4, as described, gave the highest titers with the test serums, with adequate negative controls. A reading of 0.4 is obtained with a suspension of 2.0 to 2.5 percent red cells, depending on length of storage. It appears also that antigens vary in their initial potency, and each one must be adjusted for optimal concentration prior to use.

Table 1. Agreement between dye-test titers and hemagglutination titers in tests with different lots of erythrocyte-sensitizing antigens.

Dye-test titer	No. of tests	Hemagglutination tests on same serums agreeing within	
		2-fold difference	4-fold difference
1/16	11	7	11
1/64	12	9	12
1/256	9	7	9
1/1024	10*	4	6

* All tests agreed within an eightfold difference.

About 15 *Toxoplasma* antigens have been tested by this procedure, using a variety of human serums, either pooled or individual specimens, with different dye-test titers. Not all antigens have been used with the same serums. Good agreement has been found between dye-test titers and hemagglutinating titers, as seen in Table 1. Fourfold differences in titer can frequently be found in different dye tests on the same serums. Therefore, the hemagglutination test agrees as well with the dye test as can be expected. The hemagglutination reaction, in tests on serums from one proved human case of lymphadenopathic toxoplasmosis, became positive slightly later than the dye test, but much earlier than the complement-fixation test.

Results thus far obtained in more than 100 tests similar to those described in Table 1, but using pooled lots of antigen, continue to show excellent agreement between the hemagglutination and dye tests. All except one serum found positive with the dye test have been positive also by hemagglutination. Agreement between the tests within a four-fold difference in titer has been obtained with 93 percent of the specimens. With only 4 percent of the serums was the disagreement in titer greater than 16-fold. All of this work has been done with human serums. While additional problems may be encountered with animal serums, it seems justifiable to conclude that the hemagglutination reaction offers promise of providing a practicable serological procedure for the diagnosis of toxoplasmosis.

LEON JACOBS
MILFORD N. LUNDE
Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

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