Agglutination of Cercariae of *Schistosoma mansoni* by Immune Sera.* (17812)

CH'IEN LIU AND FREDERIK B. BANG.

From Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Md.

Although there are several immunologic tests which reflect helminthic infection(1), there is considerable need for an adequate and accurate test which will help one to measure the response of an infected host to the invading parasite. We here report on the presence of an agglutinin for the cercariae of *Schistosoma mansoni* which develops in the blood of monkeys. The agglutinin appears early in the course of the infection not only with the *S. mansoni* but is also found in monkeys recently infected with Trichinella. We have studied some of the characteristics of the reaction and tentatively classify it as an antigen antibody reaction.

Materials and methods. Cercariae were obtained from infected snails, *Australorbis glabratus,*† by placing them under a bright light for 2-3 hours in a beaker. A concentrated cercarial suspension was obtained by centrifuging the original suspension at about 1000 RPM for one minute. An equal volume of 1.7% sodium chloride solution was added slowly thus yielding a final suspension of cercariae in 0.85% saline. Usually a suspension of 300-400 cercariae per ml was used for the test. Sera were inactivated by heating in a water bath at 56°C for 30 minutes. Serial dilutions were made in 0.85% saline and 0.5 ml of cercarial suspension was added to 0.5 ml of the diluted serum. The test tubes (10 x 100 mm) were shaken vigorously for about 30 seconds and then left at room temperature for one hour. Agglutination usually occurred within the first half hour but the final reading was made after 4°C for 18 hours. The agglutination was read as one to 4+ positive. A 1+ agglutination was determined with the aid of a microscope. The titer was reported as the final dilution of serum in the tube with 1+ agglutination. The production of agglutinins was followed in two rhesus monkeys which were given 900 and 600 *S. mansoni* cercariae by intraperitoneal injection. Blood was drawn by cardiac puncture at weekly intervals. The direct enumeration of circulating eosinophiles (2), a total leucocyte and differential count was made at each bleeding. Serum was obtained from centrifuged clotted blood. Stool examinations by the 10% ethyl alcohol sedimentation method(3) were started near the end of the incubation period.

Results. Formation of the Agglutinate. The agglutination of cercariae was first noticed when a large number of cercariae were placed in a serum and then observed under a cover slip for the formation of a precipitate around the individual cercariae(4). This serum had been obtained from a monkey which had had *S. mansoni* for 2 years. It was then noticed that the cercariae stuck to one another whenever they came into contact so that eventually masses of entwined cercariae were formed. (Fig. 1) This did not occur in normal sera. (Fig. 2) Following this, it was decided to study the phenomenon in the test tube with varying dilutions of sera and the technique outlined above was evolved. The clumping sometimes occurred in tubes within 2 to 3 minutes but usually took place in 20 to 30 minutes at room temperature. There appeared to be no regular pattern in which one part of the

* Supported in part by a contract with the Veterans Administration.

† Courtesy of Dr. E. B. Cram, Division of Tropical Diseases. National Institutes of Health, Bethesda, Md.

1. Culbertson, J. T., *Columbia University Press,* 1941.

2. Forsham, P. H., Thorn, G. W., Prunty, T. T. G., and Hills, A. G., *J. Clin. Endocrin.,* 1948, v8, 15.

3. Jahnes, W. C., and Hodges, E. P., *J. Parasitol.,* 1947, v33, 483.

4. Papirmeister, B., and Bang, F. B., *Am. J. Hyg.,* 1948, v48, 74.

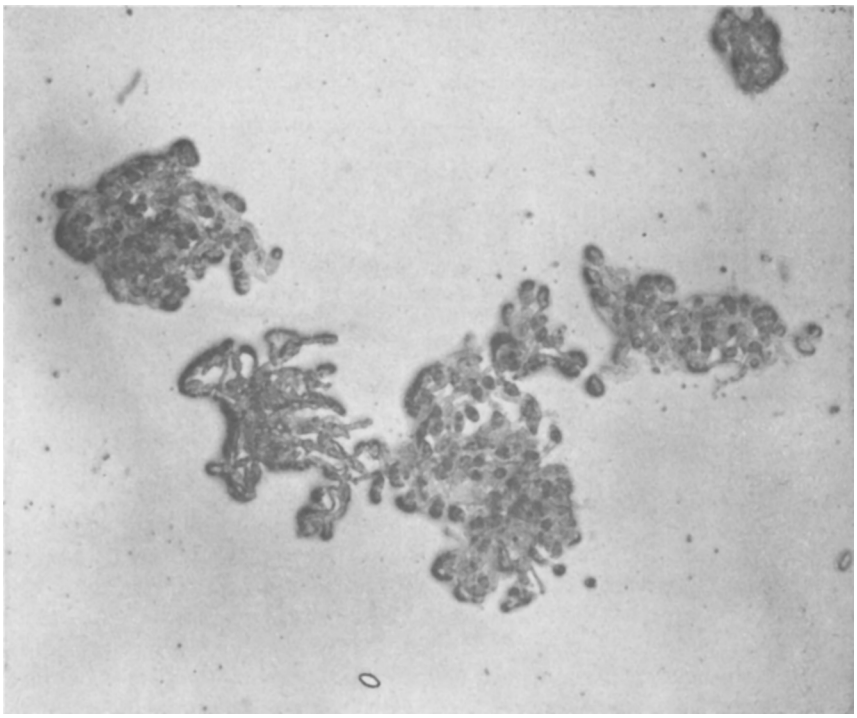

FIG. 1. Agglutinated cercariae in immune serum. ×25.

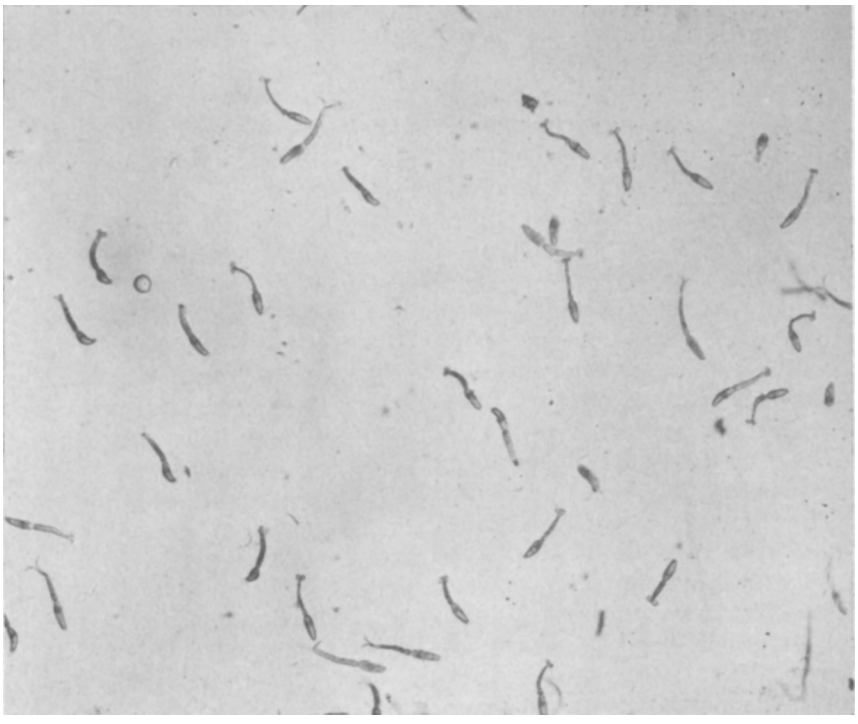

FIG. 2. Cercariae in normal serum. Not agglutinated. ×25. Serum and cercariae were mixed in a test tube for 30 minutes at room temperature and the combination was transferred to a slide for photographing.

TABLE I.
Demonstration of Prozone Phenomenon in Immune Schistosome Monkey Sera on Agglutination of Cercariae.

Monkey No.	Dilution 1/2	1/4	1/8	1/16	1/32	1/64	1/128
5	0	0	0	++++	++++	++++	+++
29	+	+	+	++	++	++	+
14	0	+	+	++++	++++	++++	+++

TABLE II.
Agglutinin Titer on Monkey Infected with *S. mansoni* March 8, '48.

Bled	Dilution 1/4	1/8	1/16	1/32	1/64	1/128
4/20/48	+	+++	++++	++++	0	0
6/14/48	0	0	+	++++	++++	±
7/26/48	not done		+++	+	+	0
10/15/49	+	+	++++	++++	+	0

cercariae stuck to another, and the motility of the cercariae was still present after 24-48 hours at 4°C. Up to date, only living cercariae have given the agglutination. Cercariae killed either by heat, formalin, ultraviolet light or chemicals have not given agglutination. The age of the cercariae has had very little effect on the agglutinin titer. Positive agglutination had been obtained even in a 24-hour-old living cercarial suspension, and washing of living cercariae with 0.85% saline did not affect the agglutination. Finally a prozone phenomenon was consistently present in several sera as shown in Table I.

Development of agglutinins in experimentally infected monkeys. The time of appearance of agglutinins was studied by infecting 2 monkeys with 900 and 600 cercariae of *S. mansoni*. The appearance of agglutinins which was roughly concomitant with the appearance of eggs in the stools is shown in Fig. 3 and 4. That the antigen stimulating this antibody response is not unique to the schistosome worms is indicated by a study of the serum of a monkey infected with trichinella. This monkey also developed agglutinins for schistosome cercariae on the 34th day after having been fed some 620 trichina larvae. The titer increased to 1/64 on the 50th day at which time the monkey was challenged with 50-60 *S. mansoni* cercariae cutaneously. The antibody titer rose to 1/128 on the 58th day and stayed approximately at this level thereafter. There was a marked but temporary eosinophilia up to 2200/cmm accompanying the initial rise of the agglutinin. This monkey died on the 43rd day of schistosome infection due to rupture of the heart from cardiac puncture.

Autopsy showed 6 pairs of matured schistosomes in the mesenteric veins leading from the large intestines. Many yellowish-white nodules were found on the surface of the liver and in the mucosa of the large intestines. No ulcerations were present. These nodules contained living schistosome eggs when examined by pressing between two slides. Examination of the diaphragmatic muscle by a similar compression method(5) showed a moderate number of encapsulated trichina larvae. Thus, there is no evidence of protection produced by one infection against the other. The agglutinins apparently remain present in high titer for at least a year and a half as shown by a study of 2 monkeys (*M. irus*) which had been previously infected. In one the serum drawn 6 weeks after infection and stored at about −16°C was found to have a titer of 1/32. This titer later went to 1/64 and was maintained throughout as shown in Table II.

We cannot, however, conclude that the

5. Gould, E. S., *Trichinosis*, Springfield, The Charles C. Thomas Co., 1945, 1st Edition, 170-175.

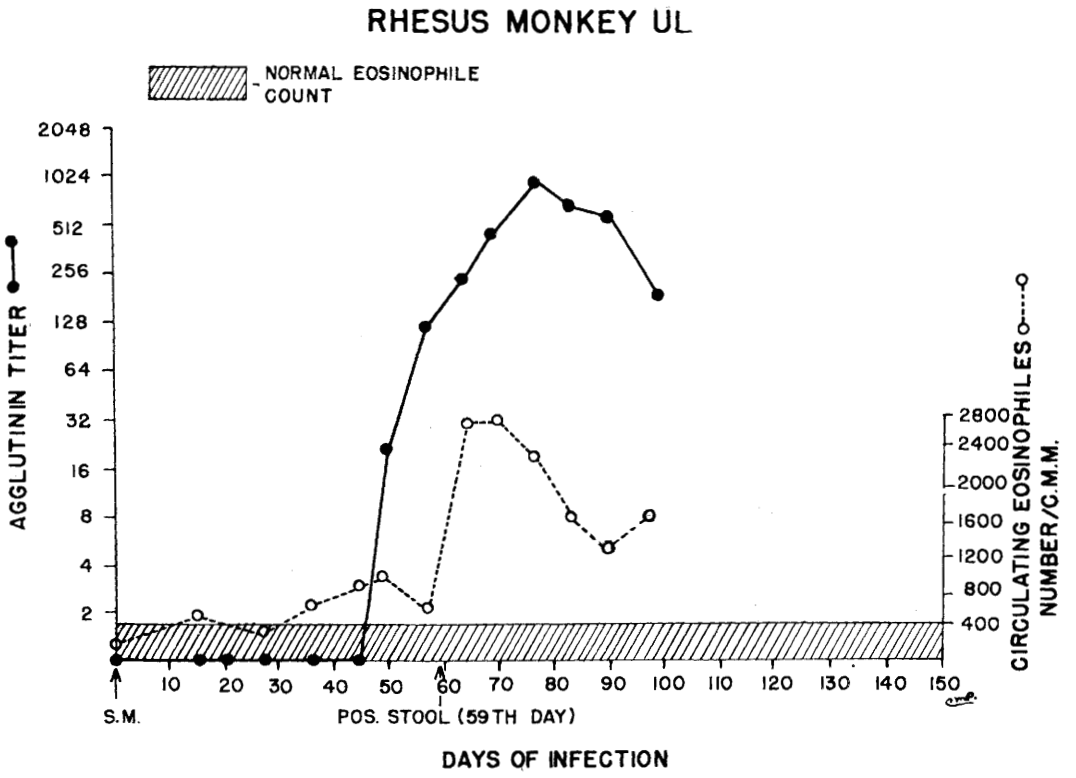
RHESUS MONKEY UL
NORMAL EOSINOPHILE COUNT
AGGLUTININ TITER
2048
1024
512
256
128
64
32
16
8
4
2
CIRCULATING EOSINOPHILES NUMBER/C.M.M.
2800
2400
2000
1600
1200
800
400
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
S.M.
POS. STOOL (59TH DAY)
DAYS OF INFECTION

FIG. 3.

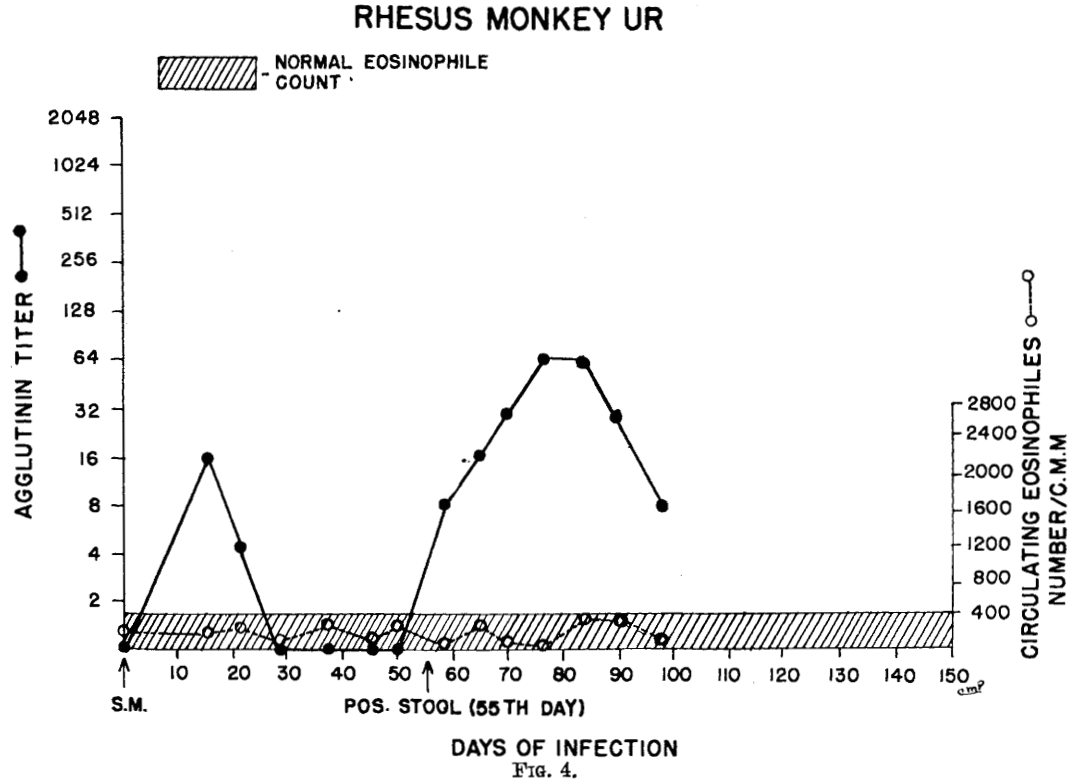
RHESUS MONKEY UR
NORMAL EOSINOPHILE COUNT
AGGLUTININ TITER
2048
1024
512
256
128
64
32
16
8
4
2
CIRCULATING EOSINOPHILES NUMBER/C.M.M
2800
2400
2000
1600
1200
800
400
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
S.M.
POS. STOOL (55TH DAY)
DAYS OF INFECTION

FIG. 4.

agglutinin remains present for the duration of infection. Of 7 monkeys (*M. philippinensis*) infected with *S. japonicum* 4½ years previously, which still had active infections as demonstrated by positive stool examinations, only 3 showed agglutinins in a titer of 1:128-1:160. The others were negative. It is, of course, possible that the different species of parasites (*S. japonicum* instead of *S. mansoni*) is responsible for these negative tests, but in view of the overlap of the reaction with the trichina infection this is unlikely as an explanation. Six normal monkeys have given negative results.

Discussion. The agglutinin reaction here reported differs from the formation of a precipitate around the cercariae of *Schistosoma mansoni* in that a heat labile factor, possibly complement is necessary for the precipitate reaction(4). It may, however, have some basic similarity to the reaction reported by Vogel and Minning(6) in which a sharply defined homogeneous membrane is formed around the individual cercariae when put in either fresh or inactivated sera. We, however, have not observed any membrane formation during our tests for agglutination. The cross reaction between trichinosis and schistosomiasis was not unexpected for we had previously found that fresh sera from monkeys infected with trichina would form a precipitate around the cercariae of *S. mansoni*(7).

The application of this test to studies in man remains to be explored. Of some seven sera from old but active infections in man with *S. mansoni*‡ or *S. japonicum*, only one *S. mansoni* serum was positive in a dilution of 1/8. We have not tested any sera from humans known to be recently infected.

Summary. The agglutination of living cercariae of *Schistosoma mansoni* by immune sera in high dilutions is reported. This reaction which occurs in heat inactivated as well as in fresh serum also appears in the serum of monkeys recently infected with trichina. The agglutinin develops early in the acute infections and was absent in four of seven chronic schistosome infections.

6. Vogel, H., and Minning, W., *Zbl. Bakter. usw.*, Abt. 1 Orig. 153, 91.

7. Bang, F. B., and Papirmeister, B., unpublished data.

‡ Courtesy of Dr. H. Most, New York University Medical College, N. Y. City.

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Practical Application of a Hemagglutination Reaction in Tuberculosis.* (17813)

SIDNEY ROTHBARD, A. S. DOONEIEF, AND K. E. HITE. (Introduced by R. J. Dubos.)
From the Division of Pulmonary Diseases, Montefiore Hospital, New York City.

Serological methods in the past have been unsatisfactory in the diagnosis of tuberculosis. Although a variety of immunological techniques had been employed, consistent results from different laboratories were not obtained. Sera from many patients with active tuberculosis failed to react, and in turn, the sera from many normal individuals showed positive tests by these techniques. Difficulties may have arisen from the lack of a suitable antigen of known chemical composition which was capable of reacting with its corresponding antibody. These difficulties were for the most part obviated in 1948 when Middlebrook and Dubos(1) described a specific hemagglutination reaction between sheep erythrocytes treated with a water soluble fraction (rich in polysaccharide) of mammalian tubercle bacilli and the sera of tuber-

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1. Middlebrook, G., and Dubos, R. J., *J. Exp. Med.*, 1948, v88, 521.