to warrant any conclusions at this time. This investigation will include cultures of material from the oral pharynx before, during, and after treatment with paraffin-sulfadiazine. The observations made to date are given so that other groups may carry out investigations relative to the therapeutic value.

Summary. When 0.325 g of sulfadiazine is incorporated into approximately 1.5 g of paraffin, and the paraffin-sulfadiazine mixture is chewed by human subjects, high concentrations of free sulfadiazine were attained in the saliva. Such a procedure may favorably affect the course of acute hemolytic streptococcic infections such as pharyngitis and tonsillitis without recourse to larger systemic doses.

14023

Immunologic Studies in Experimental *Trypanosoma cruzi* Infections: 2. Slide Agglutination and Intradermal Tests.*

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Whereas the antigenic composition of bacteria is fairly well known, little work has as yet been done on the antigenic composition of protozoa pathogenic to man. Flagellar 'H' and somatic 'O' agglutinogens have been demonstrated by the writer[1] for *Leishmania tropica,* and positive skin tests have been obtained by the writer[1] with the polysaccharide and protein fractions of *L. tropica* in cutaneous leishmaniasis.

The Machado[2] complement fixation test, using as antigen glycerine and aqueous extracts of the heart and spleen of infected puppies, has been used for the diagnosis of "Chagas" disease in man, and more recently[3] cultures of *T. cruzi* have been utilized as antigen. Precipitin[4] and agglutination[5] tests have also been used in experimental *T. cruzi* infections. Rabbits were immunized with killed and living cultures of *T. cruzi,* and their agglutinin titers ranged from 2,000 to 130,000, with an average of 2,000 to 4,000. Intravenous method of immunization gave rise to higher titers. Rabbits and guinea pigs which were given living cultures showed a lower titer (256 to 1,024). Sera of animals infected with *T. brucei* or *T. hippicum* agglutinated *T. cruzi* in very low titers (1:32). Lysins[6] for *T. cruzi* have also been observed.

Preparation of antigens for immunization. Five strains of *T. cruzi,* 2 arthropod strains from Texas (Packchanian, Davis), one armadillo strain from Brazil (Tatu) and 2 human strains, one from Brazil (1114) and another from Panama, were each cultured on modified Senekjie's medium[7,8] in Roux bottles or Erlenmeyer flasks for 7 to 10 days. The growths were emulsified in physiologic salt solution and killed in 0.3 % formalin. After 24 hours the trypanosomes were collected by centrifugation, and were suspended in 0.5 % phenol in saline. The suspension was standardized to contain 30 million trypanosomes per cc (by making counts in the blood-counting chamber).

Rabbits were immunized with respective cultures by intravenous injections. The dosage

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was gradually increased from 0.5 cc until 1 cc doses were reached. Twelve such injections were given twice a week. Ten days after the last injection, a trial bleeding was carried out by cutting the ear vein, and the titer of agglutinins was determined. Two weeks after the last injection of killed trypanosomes, immunization was started with living trypanosomes. The injections were given intravenously once a week for a period of 2 months. Skin tests were performed by injecting these animals intradermally with 0.2 cc of living and dead cultures of trypanosomes. The rabbits were then bled and the homologous agglutinin and lysin titers determined. At no time could living trypanosomes be found in the peripheral blood. Two of the rabbits died and sections of the various organs did not reveal any leishmania stages of the trypanosome.

Preparation of antigens for tube agglutination. 1. *Flagellar 'H' antigen.* The trypanosomes were grown on Senekjie's medium[7,8] at room temperature for 10 days. The colonies were emulsified in physiological salt solution containing 0.3% formalin, and the suspension left at room temperature for 24 hours. The trypanosomes were then collected by centrifugation, resuspended in saline and kept in the ice chest. The suspensions were standardized to contain 30 million trypanosomes

2. *Somatic 'O' antigens.* The trypanosomes were grown as before. The colonies were emulsified in saline, the trypanosomes collected by centrifugation, and resuspended in 90 % alcohol, kept at room temperature for 24 hours, then washed three times in saline, suspended in saline to contain 30 million trypanosomes per cc and stored in the ice chest.

Preparation of antigen for slide agglutination. The Tatu (*i.e.,* armadillo) strain was very smooth, and formed a very homogeneous suspension. The antigen was prepared from the Tatu strain. It was grown on Senekjie's medium at room temperature for 10 days. The colonies were emulsified in physiological salt solution containing 0.5 % phenol, and standardized to contain 100 million trypanosomes per cc. When kept in the ice box, the trypanosomes remained alive for about a week.

Tube agglutination test. The sera of immunized rabbits were diluted in series in physiological salt solution and equal amounts of homologous 'H' and 'O' antigens were added. The tubes were incubated at 37°C overnight and 2 readings were taken, one immediately after and the second 5 hours after the racks were placed at room temperature. No difference was noted between the two readings. Tube agglutination was performed using the antigen prepared for the slide agglutination but diluted to contain 30 million of organisms per cc.

Slide agglutination test. One and one-half-inch squares were marked off on a glass plate. To various dilutions of each serum in the different squares an equal amount of homologous antigen was added. The mixtures were stirred with a match stick and the plate was gently shaken so that there was a uniform mixing of the serum and antigen. Agglutination became apparent in one to two minutes and was complete in 3 to 5 minutes. The agglutination was very marked in the presence of low dilutions of sera but became progressively less and less marked as the dilution was increased until the end titer was reached. Negative controls were included in these tests.

Lysis. Living trypanosomes were suspended in various dilutions of the immune sera and kept at room temperature, and at intervals were examined. Controls were included, using normal serum and physiological salt solution.

Results. Table I shows the agglutinin titers of the sera of rabbits immunized with killed cultures. The 'O' titers were lower than the 'H' titers. Those for the slide agglutination were roughly half those for the 'O.'

Table II shows the titers of the sera of the rabbits which were given living cultures of trypanosomes following the killed trypano-

TABLE I.

Agglutination Titers of Rabbits Following Immunization with Killed Cultures of *T. cruzi.*

Serum	Tube agglutination Homologous 'H' antigen	Tube agglutination Homologous 'O' antigen	Slide agglutination
Packchanian	1:1,600	1:400	1:100
Davis	1:400	1:200	1:100
Tatu	1:400	1:200	1:100
1114	1:400	1:200	1:50
Panamá	1:400	1:200	1:200

TABLE II.

Agglutination Titers of Rabbits Following Immunization with Killed and Living Cultures of *T. cruzi*.

Serum	Tube agglutination Homologous 'H' antigen	Tube agglutination Homologous 'O' antigen	Slide agglutination
Packchanian	1:3,200	1:1,600	1:1,600
Davis	1:1,600	1:800	1:800
Tatu	1:6,400	1:1,600	1:1,600
1114	1:3,200	1:800	1:400
Panamá	1:6,400	1:800	1:800

somes. These titers were higher and ranged from 1:1,600 to 1:6,400 in the case of the 'H' agglutinins, and 1:800 to 1:1,600 in the case of 'O' agglutinins. There was more correlation between the slide agglutination and the 'O' agglutination.

Table III shows the relation between the slide agglutination and the tube agglutination, using the Tatu antigen for the tube and slide agglutination. The titers for the tube agglutination ranged from 1:1,600 to 1:6,400, while the slide agglutination ranged from 1:800 to 1:1,600 with a ratio of about 2:1 to 4:1.

TABLE III.

Comparison of the Tube and Slide Agglutination Titers Using Sera of Rabbits Immunized with *T. cruzi* (Tatu antigen used)

Serum	Tube agglutination	Slide agglutination
Packchanian	1:1,600	1:400
Davis	1:1,600	1:800
Tatu	1:6,400	1:1,600
1114	1:6,400	1:1,600
Panamá	1:6,400	1:1,600

The flagellar 'H' agglutination was in large flakes, and settled to the bottom of the tube, leaving a clear supernate. On shaking the tube the large flakes were broken up and a fine homogeneous suspension with very fine clumps was produced. The somatic 'O' agglutination was in small flakes, and there was a general turbidity of the fluid in the tube due to small clumps. Some of the clumps settled to the bottom of the tube but never broke up into finer clumps on shaking the tube. In the case of the slide tests the agglutination clumps were rather large and resembled those of the large flaking type. They appeared first on the margin of the drop and progressively the whole drop showed the agglutination.

Skin tests. Following the intradermal injection of the cultures of *T. cruzi* into the skin of immunized rabbits no reaction was noticed for a period of 12 hours, but later a red papule was formed which increased in size to a 5-cent piece and reached its height in 24 hours, fading away in 3 days.

Lysis. The immune sera lysed the trypanosomes in 1:100 dilution in 2 hours at room temperature. Saline and normal serum controls showed no change. At first the motility ceased and progressively the trypanosomes disintegrated.

A guinea pig which was inoculated with trypanosomes recovered from the rectum of an assassin bug developed infection, and its blood showed an agglutinin titer of 1:400. A second guinea pig infected with *T. equiperdum* agglutinated *T. cruzi* in 1:20 dilution.

A hundred Wassermann sera were tested by the slide agglutination method, and all were found to be negative. Two acute tertian malaria cases and 5 paretics who had induced quartan malaria also gave negative slide agglutination. One patient suffering from an undiagnosed fever gave a titer of 1:10.

Discussion. The same types of antibody are present in *Trypanosoma cruzi* infections as are present in bacterial infections, *i.e.*, complement fixing antibodies,[2,3] precipitins,[4] agglutinins[5] and lysins.[6] The writer confirms the findings of Packchanian but shows that the agglutinin consists of 'H' and 'O' types. He also confirms the findings of Denison on the presence of lysins, and provides evidence on the presence of a skin-sensitizing antibody which gives rise to positive skin tests.

In bacterial infections it is claimed that the 'O' agglutinin is the one which protects against infections. The importance of 'H' and 'O' agglutinins is not yet known in *Trypanosoma cruzi* infections. It is very probable that the 'H' antigen is located somewhere in the flagellum or in the undulating membrane, and the 'O' antigen in the body of the trypanosome. These two antigens may be of great importance in the diagnosis and the protective vaccine in Chagas' disease in man.

The slide agglutination test is very easy to perform and requires very little equipment. It is of particular value when large numbers of

sera are to be examined to find suspected cases. Xenodiagnosis needs laboratory-bred assassin bugs, making it a complicated test to perform. The same thing holds true of biopsy material. A simple, easy serological test would be of great value in diagnosis of Chagas' infection in man, such as the slide agglutination test.

Summary and conclusions. 'H' and 'O' agglutinins, skin sensitizing antibodies and lysins are present in the blood of animals infected or immunized with *Trypanosoma cruzi.* A simple, easy slide agglutination test is described for the diagnosis of experimental *Trypanosoma cruzi* infection in animals. Out of 108 human sera obtained from a hospital laboratory, only one agglutinated *T. cruzi* in 1:10 dilution.

14024 P

Effect of Hematin Upon Stability of Thiochrome in Solutions of Alkaline Ferricyanide.*

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The usual technic for determining thiamin by the thiochrome reaction consists in converting thiamin to thiochrome by alkaline oxidation with ferricyanide, extracting the thiochrome with iso-butyl alcohol and measuring its fluorescence with a photo-electric fluorometer.[1] In the course of a study of the thiamin inactivating enzyme of carp,[2] it has been observed that this procedure of analysis gives erratic results in the presence of hemolyzed red blood cells. Closer investigation has indicated that traces of hematin markedly increase the rate of destruction of thiochrome in solutions of alkaline ferricyande. In the presence of this pigment before the thiochrome formed by alkaline oxidation of thiamin can be extracted with iso-butyl alcohol, a considerable portion may be destroyed and falsely low values of thiamin analysis obtained.

Table I illustrates the disappearance of thiochrome fluorescence which follows addition of 1 ml of a dilute solution of hematin† to 9 ml of an aqueous solution of 0.82 μg of thiochrome containing alkali and ferricyanide in amounts comparable to those used in the thiochrome technic of thiamin estimation. The hematin effect is dependent upon the presence of both alkali and ferricyanide in the solution and may be observed with high dilutions of hemolyzed red blood cells as well as with dilutions of hematin.

TABLE I.

Effect of Hematin upon the Rate of Destruction of Thiochrome in Solutions of Alkaline Ferricyanide.

Solution	Time in minutes 0	1	3	5	10	20	30
Control	47.0*	47.0	46.5	46.5	45	44.5	43.0
Hematin (1:1,000,000)	46.0	6.0	1.5	1.5	0.5	0	0
Hematin (1:10,000,000)	46.5	37.5	28.5	22.0	10.0	3.0	2.0

*Fluorescence in galvanometer units, Coleman Electronic Photofluorometer—Model 12.

Boiling, peptic digestion, carbon monoxide, or preliminary treatment with strong acid or alkali does not diminish the activity of the

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2 Owen, Philip S., and Ferrebee, Joseph W., in press.

† Crystalline preparation of chlor-hemin obtained from Dr. Otto Schales, Peter Bent Brigham Hospital, Boston, Massachusetts.