ably complicated by bacteremia, terminate with death in about 2 weeks. Chronic cases and one spontaneous recovery have also been observed. Carrier cases, with the passing of cysts in the stools, apparently do not develop in dogs. 4. The cecum is the primary seat of infection with *E. histolytica* in the dog. 5. The dog apparently receives its amebic infection from man but due to the failure to produce cysts the infection apparently cannot be transferred in nature from dog to dog or from dog to man.

5035

Observations on the Significance of the Agglutinins and Lysins Produced by Typhoid Inoculation.

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

From the Department of Pathology and Bacteriology of Tulane University of Louisiana.

In a previous paper the results of agglutination reactions performed upon the sera of students inoculated with typhoid vaccine were reported. Two sets of tests were carried out, one with serum taken before inoculation, the other with serum taken 10 days after inoculation.

This paper deals with a third set of agglutination reactions, together with parallel complement fixation reactions and bacteriolytic tests, performed upon sera from the same group of 90 students 4 months after inoculation. The antigen used in the complement fixation reactions was a saline suspension of typhoid bacilli from which lipoids had been extracted with alcohol and ether. The bacteriolytic tests were carried out by diluting one capillary drop (about 0.025 cc.) of serum to 0.5 cc. with saline, and adding to this about 500 to 1000 typhoid bacilli. This mixture was incubated for one hour and then plated in plain agar. In negative control sera there was practically always a marked multiplication of the bacilli.

Because of the marked difference in the agglutinin titer between students who had received previous typhoid vaccine and those who had not, a second inoculation was given to a number of volunteers from the latter group. Each of the tests mentioned above were performed upon sera drawn from these students 10 days after inoculation.

Analysis of the data collected in this study reveals the following observations: 1. Agglutinins are demonstrable more universally 4

months after inoculation in sera of those who have had typhoid (100%), or previous typhoid vaccine (81%), than in sera of those who have had neither (47%).

2. Agglutinins are maintained in higher titer for 4 months by those who have had typhoid (1/50), or previous vaccine (1/54), than by those who have had neither (1/28).

3. The percent of students showing agglutinins in titer strong enough for diagnosis (Widal Reaction, 1/40 dilution) 4 months after inoculation was higher among those who have had typhoid (75%), or previous typhoid vaccine (60%), than among those who have had neither (18%).

4. The percent of students showing positive complement fixation tests was slightly higher among those who have had typhoid (75%), and previous typhoid vaccine (75%), than among those who have had neither (65%).

5. The average complement fixing titer was higher among those who have had typhoid (1.6—almost++), than among those who have had previous typhoid vaccine (1.2), or neither (1.0).

6. The percent of students showing marked bacteriolytic power (over 90% of typhoid bacilli killed) was higher among those who have had typhoid (63%), and previous typhoid vaccine (50%), than among those who have had neither (31%).

7. The average bacteriolytic titer was somewhat higher among those who have had typhoid (87%), than among those who have had previous typhoid vaccine (77%), or neither (77%).

8. There is some correlation between the strength of the agglutination and complement fixation reactions. (Correlation coefficient $+0.212 \pm 0.067$.)

9. There is practically no correlation between the complement fixing antibodies and the bacteriolysins produced. (Correlation coefficient +0.068.)

10. There is practically no correlation between the agglutinins and bacteriolysins produced. (Correlation coefficient +0.099.)

11. The immunity resulting from a second inoculation some months after the first is much greater than that produced by the original inoculation, as shown by the agglutination reactions of 12 students given a second inoculation. (Before first inoculation no agglutinins, 10 days after 1/69, 4 months after 1/36; 10 days after second inoculation 1/322.) There was a similar exaltation in the complement fixation and bacteriolytic reactions.

Conclusions. 1. The immunity produced by a single course of 3 doses of typhoid vaccine may not be as great as usually believed.

A second inoculation probably ought to be given some weeks or months after the first. 2. The Widal Reaction is much less reliable as a test for typhoid fever among those who have had more than one inoculation with vaccine than among those who have had only a single inoculation.

5036

Effects of Sodium Citrate on Bile Salt Hemolysis.

JOHN W. WILLIAMS. (Introduced by W. H. Harris.)

From the Department of Pathology, College of Medicine, Tulane University.

In a previous report[1] we observed an increase in toxicity of bile salts* injected intraperitoneally into the white mouse when sodium citrate was added. In the present study the effects of varying dilutions of bile salts and sodium citrate on human blood cells *in vitro* have been observed.

When bile salts are added to red blood cells of different individuals and allowed to stand at room temperature for 18 hours, hemolysis occurs through dilutions of 1:200 to 1:6400. The bloods of 100 undiagnosed individuals were examined in order to determine their susceptibility to bile salt hemolysis. The large variation in ability of bile salts to hemolyse different bloods is independent of changes due to age of cells, and independent of the temperature and humidity under which the cells are kept.

When sodium citrate is added to bile salts, hemolysis of red cells takes place in higher dilutions of the bile salts than when the bile salts *per se* are employed and the dilution at which hemolysis takes place depends upon the concentration of the sodium citrate added. If concentrations of less than 0.032% (1:3200) of sodium citrate are added there is no increase in the hemolytic power of bile salts; however, as the concentration of sodium citrate added is increased above 0.032% the hemolytic power of the bile salts is likewise increased. For example, if 1% (1:100) sodium citrate is added to the bile salts dilutions, hemolysis takes place in the 1:400 dilution, while if 4% (1:25) sodium citrate is added hemolysis takes place in the 1:1200 dilution of bile salts. We see by these figures the gradual increase in hemolytic power of bile salts produced by addi-

[1] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 637.

* Merck & Co., about 47% sodium taurocholate.