steadily through the years. He helped others far more than others helped him.

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Technical Papers

An Agglutinin in Normal Sera for Periodate-treated Red Cells[1]

Merwin Moskowitz[2] and Henry P. Treffers

Department of Bacteriology,
Yale University School of Medicine,
New Haven, Connecticut, **and**
The Blood Grouping Laboratory of Boston,
Boston, Massachusetts

In confirmation of the observation of Burnet (*1*), we found that periodate ions rendered red cells panagglutinable. We have extended this finding with the observation that the agglutinin for periodate-treated red cells (PTC) is distinct from the agglutinin for red cells rendered panagglutinable by the filtrates of cultures of *Vibrio comma* (VTC) (*11*). Experiments demonstrating the specificities of these agglutinins are presented and the significance of the finding is discussed.

Saline-phosphate buffer at pH 7 (*9*, p. 104) was used as a diluent and washing medium. Two percent suspensions of washed human red cells were treated with an equal volume of 0.001 M potassium periodate (in buffer) for 30 min at room temperature. The cells were centrifuged and washed three times with volumes of buffer each equal to four times the volume of the original cell suspension. The washed, treated cells were made up to a 1% suspension for testing. This procedure of treating the red cells with periodate permitted the maximum action of the periodate with minimum damage to the cells. Little or no hemolysis occurred with this treatment, whereas longer treatment or the use of more concentrated periodate solutions caused extensive hemolysis without increasing the panagglutinability of the treated red cells. *Vibrio comma* (Strain 4Z)[3] was grown in 2% Trypticase[4] broth (plus 0.5% sodium chloride) for 20 hr at 37° C and then passed through a fritted glass sterilizing filter. The red cell suspension was treated with the filtrate in the same manner as with the periodate, except that the mixture stood 1 hr at room temperature. No increase in the panagglutinability of the red cells was observed if the enzyme acted on them for a longer time at room temperature or at 37° C.

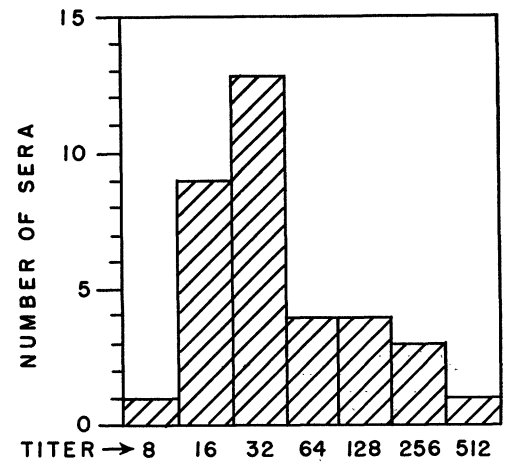

FIG. 1. Periodate agglutinin titers 35 normal human sera. sera.

PTC were agglutinated by all adult human sera tested. PTC were also agglutinated by their own serum. Two drops of the treated cell suspension were added to two drops of twofold serial dilutions of serum and, after standing at room temperature for 30 min, the mixture was centrifuged for 30 sec at 2,000 rpm on a clinical centrifuge. The tubes were gently rotated to dislodge the agglutinates and then read; the highest dilution of serum which gave macroscopic agglutination, e.g., many large clumps with few free cells, was considered the end point. Thirty-five sera from apparently normal individuals were tested with PTC and the results are presented in Fig. 1. Type O cells were used; the untreated

[1] After this article was submitted it was learned that F. S. Stewart (*10*) also noted the difference between periodate and T agglutinins. Since the article was written it has been found possible to render red cells agglutinable by filtrates of cultures of *Staphylococcus aureus, S. albus,* and *Streptococcus pyogenes.* Cells treated with *S. aureus* filtrates were agglutinated by all sera tested; those treated with *S. albus* and *S. pyogenes* filtrates were agglutinated by most, but not all sera. The agglutinin for cells treated with *S. aureus* filtrates is different than the periodate or T agglutinin; the one or more agglutin for cells treated with *S. albus* and *S. pyogenes* filtrates appear to be related to the others in a manner that is not understood at the moment.

[2] Public Health Service Postdoctoral Research Fellow of the National Heart Institute.

[3] We wish to thank Dr. B. A. Brody for this culture.

[4] Baltimore Biological Laboratories.

TABLE 1

RESPONSE OF RABBITS TO INJECTIONS WITH PERIODATE-TREATED CELLS (PTC), CELLS TREATED WITH *Vibrio comma* ENZYME (VTC), AND NORMAL CELLS (NC)

Rabbits	Sera			Titer with :		
				PTC	VTC	NC
I and II	Before injection :		I	32	128	16
			II	32	128	16
	After injection with PTC :	unabsorbed	I	7,500	500	500
			II	10,000	500	500
		absorbed	I	5,000	< 100	< 100
		with VTC	II	10,000	< 100	< 100
		absorbed	I	7,500	200	< 100
		with NC	II	10,000	200	< 100
III and IV	Before injection :		III	32	256	16
			IV	64	512	64
	After injection with VTC :	unabsorbed	III	2,500	15,000	2,500
			IV	2,500	10,000	2,500
		absorbed	III	< 100	10,000	< 100
		with PTC	IV	< 100	10,000	< 100
		absorbed	III	< 100	10,000	< 100
		with NC	IV	100	10,000	< 100
V and VI	Before injection :		V	64	256	16
			VI	16	128	8
	After injection with NC :	unabsorbed	V	2,500	7,500	7,500
			VI	2,500	10,000	10,000
		absorbed	V	< 100	500	1,000
		with PTC	VI	< 100	500	5,000
		absorbed	V	100	< 100	2,500
		with VTC	VI	100	< 100	5,000
		absorbed	V	100	200	< 100
		with NC	VI	100	200	< 100

cells were not agglutinated by the sera at room temperature. Periodate treatment had no effect on the ability of Type A or Type B red cells to agglutinate with their specific agglutinins but it apparently destroyed the Rh (D) factor, as Rh-positive PTC did not agglutinate with agglutinating Rh antiserum in saline or with incomplete Rh antiserum in albumin. Before testing for the specific agglutinins, the agglutinin for PTC was absorbed from the sera mentioned. It has previously been shown that VTC agglutinate with A, B (*4*), and Rh (*11*) antisera.

Human sera having a titer of 8 to 32 for PTC and VTC were absorbed with an equal volume of packed PTC for 15 min at room temperature and the sera still agglutinated VTC to the same extent, but no longer agglutinated PTC. After absorption with VTC the sera still agglutinated PTC to the same extent but no longer agglutintaed VTC. The specific absorption of the agglutinins for PTC and VTC was also demonstrated with sera from sensitized rabbits (Table 1).

Heating dissociated the agglutinins from sensitized cells as it does in the Rh (*5*) and other systems (*7*). Six percent suspensions of VTC and PTC were mixed with two volumes of serum and, after standing at room temperature for 15 min, the cells were centrifuged and washed three times at 0° C. The packed cells were mixed with an equal volume of buffer, heated at 56° C for 5 min, and centrifuged. The supernatant fluid from sensitized VTC agglutinated only VTC, and the supernatant fluid from sensitized PTC agglutinated only PTC. Neither supernatant fluid agglutinated normal cells.

Rabbits were injected with normal cells, PTC and VTC. One ml of a 25% cell suspension was injected into the ear veins on two successive days. After a lapse of 10 days the rabbits were injected intraperitoneally and then intravenously the next day. Seven days after the last injection they were bled. The sera were absorbed as described except that 100-fold dilutions of the sera were used. The results are tabulated in Table 1. It is observed that the agglutinin titers for PTC and VTC were increased as a result of the injections and the increase was specific for each type of treated cell. These experiments indicate that the agglutinin for PTC is not the same as the one acting on VTC and that it has the characteristics of an antibody, e.g., the agglutinin titer of the sera could be increased with PTC. This will be referred to as the periodate agglutinin.

Thomsen (*12*) observed that the red cells in some contaminated bloods were agglutinated by all sera, and in an extensive study of this panagglutination phenomenon Friedenreich (*4*) showed that it was an enzyme produced by the bacteria that rendered the red cells panagglutinable. He found that only a few organisms, among them several strains of *Vibrio comma,* produced this effect. The agglutinin for the treated cells, called the T agglutinin, could be absorbed from the serum by red cells treated with the filtrates from cultures of a number of bacteria which he tested in this respect. Burnet and Anderson (*2*) showed that rabbits could be specifically sensitized to VTC, and conjectured that the T agglutinins may be involved in the pathogenesis of various diseases such as blackwater fever. Some viruses also render red cells panagglutinable (*11*), and studies

(*8*) were made of the T agglutinin titer of patients with various virus diseases. A significant rise in T agglutinin titer was observed only in patients with primary atypical pneumonia. These workers were observing only the T agglutinin; but, as has been shown here, there is more than one panagglutinin in sera and the panagglutinin titer for cells such as PTC may be increased without being detected if only the T agglutinin is observed.

It is possible that agglutinins for altered red cells are implicated in the pathogenesis of various diseases and, for instance, may sometimes be the cause of intravascular agglutination ("sludged blood" [*6*]). However, as there may be many different agglutinins acting on cells altered by different agents, these agglutinins would not be observed if the sera were tested with cells altered by a single agent. As an example, the tubercle bacillus may form an enzyme which alters some of the infected host's red cells and these altered red cells could then serve as antigens, having become "foreign" to the body. The antibodies produced against these altered cells may then act on other altered cells, causing intravascular agglutination. The agglutinins produced in such cases need not necessarily be panagglutinins, since they may agglutinate only red cells altered by the specific agent. In this hypothetical case, the specific agent is an enzyme produced by the tubercle bacillus, and thus the agglutinin for red cells altered by this agent may be found only in individuals suffering from tuberculosis. If a specific altering agent was produced by an organism such as the staphylococcus, the agglutinin acting on red cells altered by this agent may be found in all sera, due to the staphylococci normally present in the body, but it may increase in titer in individuals with staphylococcal infections. It is conceivable that an endogenous abnormal enzyme system may also produce the general picture described here.

Friedenreich (*4*) tested most of the common bacteria for the ability of their culture filtrates to render red cells panagglutinable, but found that only a few were able to do so. However, Chu (*3*) recently found that filtrates of the cultures of many common bacteria did render red cells panagglutinable. Chu did not describe the manner in which he grew his organisms, and differences in the media and conditions of growth may explain the differences in their results. He also did not report any cross-absorption studies to see if different agglutinins were responsible for the agglutination in the various cases. The organism with which Friedenreich did most of his work produced active filtrates only if it was grown at 22° C; the filtrates were inactive if the organisms were grown at 37° C. In this laboratory and in other laboratories (*11*) active filtrates are routinely obtained when the organisms are grown at 37° C. Thus the conditions necessary for the production of active filtrates probably vary with different organisms. Studies are under way in this laboratory on the ability of filtrates of cultures of pathogenic bacteria grown under various conditions to render red cells agglutinable by normal sera and by sera from patients with infections caused by the specific bacteria.

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Reduction of Mortality from X-Radiation by Treatment with Antibiotics[1]

C. Phillip Miller, Carolyn W. Hammond, and Marianne Tompkins

Department of Medicine and the Institute of Radiobiology and Biophysics, University of Chicago, Chicago

A preceding communication (*1*) presented results of blood and spleen cultures on mice subjected to a single exposure of 600 or 450 roentgen units total body x-radiation. The results showed an incidence of bacteremia which rose and fell during the second postirradiation week roughly parallel with the daily death rate. This finding suggested that infection might be a significant factor in death from radiation injury. An attempt, therefore, was made to reduce the mortality from x-radiation by controlling the bacteremia by the administration of antibiotics. As the bacteremia was found to be caused by microorganisms (mostly Gram-negative bacilli) normally inhabiting the lower intestinal tract of these mice, it was realized that to be effective an antibiotic must be active against a wide variety of bacterial species.

Methods. Male Swiss mice were exposed to a single dose of 450 r x-radiation delivered at 20 kv, 15 ma, at a distance of 27 in., using ½-mm copper and 3-mm Bakelite filter.[2] The dose rate was approximately 20 r per min. Their LD_{50} (30 days) was about 400 r.

After irradiation, they were divided into control and treated groups, so that each therapeutic trial contained a group of control mice that had received the same dose of irradiation on the same day. From the 4th to the 28th day after irradiation, the treated mice were injected

[1] This investigation was initiated as part of the U. S. Army Contract No. W39-007-MD-425 and has been continued under Contract No. At(11-1)-46 between the U. S. Atomic Energy Commission and the University of Chicago.

[2] Most of the mice were irradiated at the Argonne National Laboratory with the assistance of Mr. Joseph Trier and Mr. Emil Johnson. Some were irradiated by Dr. James W. J. Carpender of the Section of Roentgenology, Department of Medicine, University of Chicago.