serotonin creatine sulfate (20 mg/kg) or para-aminopropiophenone (32 mg or 16 mg/kg). 2. The protective effect of these agents is assumed to be due to their property of producing a temporary tissue anoxia.

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Inhibition of Bacterial (*Escherichia Coli*) Modification of Erythrocytes. (19707)

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It is an established fact that certain viral and bacterial antigens may be adsorbed on red blood cells; this may result in hemagglutination (direct microbial hemagglutination), in modification of the erythrocytes or both. These modified red blood cells are specifically agglutinated by the homologous antimicrobial antibody (indirect microbial hemagglutination). It was shown recently from this laboratory(1,2) that boiled suspensions of *E. coli* serogroups 0111 and 055 as well as sterile broth culture filtrates are capable of modifying red blood cells from a variety of animal species. These modified red cells are agglutinated and, in the presence of complement, lysed by the group-specific *E. coli* antiserum. It was demonstrated furthermore that this agglutination and hemolysis is specifically inhibited by the homologous bacterial antigen. The experiments to be recorded in this communication revealed that certain materials inhibit the modification of red blood cells by *E. coli* antigen.

Material and methods. In addition to *E. coli* serogroups 055 and 0111 used in the previous experiments several strains of *E. coli* 026 were employed, since the latter serogroup, too, has been found to be associated with epidemic diarrhea of infants(3). For the modification of red blood cells boiled suspensions from Kolle flasks were used, as described previously(2). The microorganisms were suspended in the materials to be tested for inhibitory activity, namely, serum from man and various animals, human plasma fractions, bovine albumin (Armour), egg white, egg yolk, various fractions of rat liver, and, for control purposes, physiological saline solution. The human plasma fractions were obtained from Cutter Laboratories through the courtesy of Dr. F. F. Johnson and the rat liver fractions through the kindness of Dr. Charles U. Lowe. The mixtures were kept at room temperature for 15 minutes and then used for modification of red blood cells. The treated erythrocytes were washed 3 times in physiological saline solution. The hemagglutination and hemolysis tests were carried out as described in detail previously(2).

Results. It was found that a variety of human and animal sera, including sera from healthy adults and children, human cord serum, calf, beef, horse, sheep, chicken, duck and turkey sera in dilutions of 1:10 and 1:100 either completely or partially inhibited the modification of sheep red blood cells by *E. coli* 026 antigen. Quantitative experiments revealed that the more concentrated the *E. coli* antigen the more serum is required to produce inhibition of modification of red cells. The

TABLE 1. Hemagglutination and Hemolysis by *E. coli* 026 Antiserum of Sheep Red Blood Cells Modified by Boiled *E. coli* 026 Antigen.

E. coli antigen diluted in	Titer of antiserum producing	
	Hemagglutination	Hemolysis
Saline solution	1:1600	1:12800
Human serum (1%)	—*	—
Human plasma fractions (.1%)		
Albumin	—	—
II	1:1600	1:12800
III	1:400	1:6400
II + III	1:400	1:6400
III – 0	—	1:1600
III – 1	1:800	1:3200
III – 2	1:1600	1:12800
III – 2,3	1:200	1:1600
IV – 1	—	—
IV – 6	—	1:400
IV – 7	—	—

* — = No agglutination, no hemolysis with *E. coli* antiserum in dilutions of 1:100 and higher.

same results were obtained in experiments in which *E. coli* 0111 and 055 and red blood cells of man and other animal species were employed. Furthermore, egg yolk suspensions (10% to 0.01%) in physiological saline solution had like effects. In contrast, egg white (in dilutions up to 10%) was essentially ineffective. Rat liver fractions (nucleus-free cytoplasm (1 ml = 0.2 g of liver), nuclei (1 ml = 0.5 g), mitochondria (1 ml = 0.5 g), microsomes (1 ml = 0.5 g)), also inhibited the modification of sheep cells.

In view of the inhibitory effects of human serum it was deemed of interest to determine the activity of various human plasma fractions. In these experiments boiled *E. coli* 026 antigen (diluted 1:50) was used to yield approximately 2 minimal hemagglutinating units; the antigen was diluted in the available plasma fractions (0.1%), human and chicken sera (1%) and in physiological saline solution. Blood cells modified by this antigen were specifically agglutinated—and in the presence of complement lysed—by *E. coli* 026 B6 antiserum but not by *E. coli* 055 and 0111 antisera. The results are summarized in Table I. It can be seen that certain fractions prevented hemagglutination and hemolysis completely, whereas others were minimally effective or completely ineffective. Since, as shown previously(2), *E. coli* antiserum produces hemolysis of sheep cells in far higher titers than hemagglutination, it is not surprising to find that some of the fractions inhibited hemagglutination to a greater extent than hemolysis. Commercially available human gamma globulin (up to 10%) was found to be ineffective.

This inhibitory effect takes place rapidly, since the inhibitor is in contact with the bacterial antigen for a total of less than one hour; the dissolved inhibitor is removed by repeated washing of the red cells. Continued contact of the inhibiting plasma fraction IV-1 for 18 hours at 4°C did not result in substantially greater inhibition than contact for less than one hour.

Regarding the heat stability of the inhibitory materials the following observations were made. Heating of the inhibitors in the concentrations used for one hour at 56°C did not reduce their activity. Boiling of human and animal sera (1%) for 10 minutes impaired their inhibitory activity but slightly and boiling for one hour only moderately. A substantial or complete loss of inhibitory activity occurred following boiling for 10 minutes of human plasma fractions IV-1, IV-4, IV-7, and albumin as well as bovine albumin and egg yolk (all in 0.1% concentration).

Since, as shown in repeated experiments, treatment of sheep cells with the above mentioned inhibitors (human serum (1%), human plasma fraction IV-4 (0.1%), sheep serum (0.1% to 10%), bovine albumin (0.3%), and egg yolk (0.1%)) did not interfere with their modification by subsequent treatment with *E. coli* antigen, the inhibitors must act either on the bacterial antigen itself or on the process of its adsorption on red blood cells. To determine which of the two possible modes of action actually takes place, the following experiments were carried out.

A boiled suspension of *E. coli* 026 was diluted 10 fold (vol. 10 ml) in 1) bovine albumin (3%), 2) human plasma fraction IV-1 (1%), 3) normal human serum (20%), and 4) physiological saline solution. The suspensions were kept at room temperature for 15 minutes. The bacteria were then washed 3 times with physiological saline solution;

TABLE II. Effect of Heat on Inhibitory Effect of Egg Yolk on *E. coli* Modification of Red Blood Cells.

E. coli antigen in	Titer of *E. coli* antiserum producing	
	Hemagglutination	Hemolysis
Saline solution	1:1600	1:25600
Egg yolk (.1%)	—	1:200
Boiled egg yolk (.1%)	1:200	1:3200
Egg yolk (.1%) (mixture boiled)	—	1:100

— = No agglutination, no hemolysis with *E. coli* antiserum in dilution of 1:100 and higher.

separation of bacterial cells from the surrounding fluid was accomplished by means of a refrigerated centrifuge (International, model P R-1: r.c.f. at tip of tube calibrated to be 24,000; centrifugalization for 20 minutes). The sediment was suspended in 5 ml of physiological saline solution and then used for modification of sheep red blood cells. The experiment revealed that the bacteria treated with the 3 inhibitors—in contrast to the untreated bacteria—failed to modify the red cells, as indicated by absent hemagglutination and hemolysis by *E. coli* antiserum. These results suggest that the inhibitors act directly on the bacterial antigen.* This conclusion is substantiated further by the results of the following experiment. *E. coli* 026 antigen was diluted in 1) physiological saline solution, 2) egg yolk (0.1%), 3) egg yolk (0.1%) which had been boiled for one hour and 4) egg yolk (0.1%). All mixtures were kept at room temperature for 15 minutes. The latter mixture was then boiled for one hour to inactivate the inhibitor. The materials were used for modification of sheep red blood cells and the modified red cells were tested with *E. coli* 026 antiserum. The results are summarized in Table II. It is evident that boiled egg yolk is less effective as an inhibitory agent than the unboiled material and that egg yolk which had been allowed to act on the bacterial antigen and was subsequently boiled and thus partially inactivated had produced greater inhibition than was effected by boiled egg yolk.

Discussion. Bacterial hemagglutination and hemolysis are of considerable interest from several points of view. In the first place, direct bacterial hemagglutination may be used for the differentiation of closely related microorganisms, for example, *H. aegyptius* and *H. influenzae*(4). Secondly, indirect bacterial hemagglutination and hemolysis are useful tools for the serologist, as exemplified by the Middlebrook-Dubos test for the demonstration of antibodies against products of the tubercle bacillus(5). Thirdly, the possibility exists that these phenomena may take place *in vivo* and conceivably could be responsible for hemolytic anemia associated with bacterial infections, similar to the hemolytic process described to develop concomitant with certain viral diseases(6). The above reported experiments revealed that sera of man and various animals as well as certain, but not all, human plasma fractions inhibit the modification of red blood cells by *E. coli* antigen. Due to this inhibition these erythrocytes are not agglutinated or lysed by the homologous group-specific *E. coli* antiserum. Inhibition was also demonstrated with egg yolk and certain fractions obtained from rat liver. It has been shown furthermore that these inhibitors act mainly on the bacterial antigen and not on the red cells. This inhibition, then, differs from that due to receptor destroying enzyme interfering with viral hemagglutination.

This inhibition of *E. coli* modification of red cells is of interest also in connection with the previously reported(2) fact that unheated, in contrast to boiled, *E. coli* suspensions fail to modify red blood cells. It is conceivable that inhibitor is present in the unheated bacterial suspension which is destroyed by heating. Since the B antigen of *E. coli* interferes with bacterial O agglutination, it is possible that it is this antigen which also interferes with the modification of red blood cells. When purified B antigen becomes available, it will be of interest to determine its effects on *E. coli* modification of erythrocytes. The presence of inhibitors in various bacterial species may account for their inability to modify red blood cells. Further studies on the presence of red blood cell modifying antigens and their inhibitors in microorganisms are clearly indicated.

*The inhibitors do not interfere with bacterial agglutination.

It is of interest to point out that certain human plasma fractions (III-0, IV-1 and albumin) which inhibit bacterial modification of red cells also bring forth agglutination of red blood cells in the presence of incomplete Rh antibodies(7). Whether these 2 phenomena have an underlying mechanism in common remains to be determined.

Summary. 1. The modification of red blood cells by boiled suspensions of *E. coli* serogroups 026, 055 and 0111 is inhibited by human and animal sera as well as by egg yolk and various fractions of rat liver. Certain human plasma fractions in concentration of 0.1% (particularly fractions IV-1, IV-7 and albumin) are likewise effective, whereas others are not. 2. This inhibition is due largely to the action of the inhibitor on the bacterial antigen rather than on the red blood cell. 3. The significance of the results is discussed.

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XYZ Effect in Strain of Origin: EO771 Carcinoma in C57 BL/6 Mice. (19708)

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The xyz factors(1-11) are specific agents present in long-frozen or lyophilized tissue or fresh supernatant fluid from certain malignant tumors which, when injected, render the animal hosts more susceptible to the subsequent transplant of the same tumor. If the xyz factors are to affect the malignancy of spontaneous tumors in nature then it must be shown that each factor in transplanted tumors will influence the course of the same neoplasm in the inbred strain of origin. Prior experiments have shown that the EO771 factor would enhance the course of malignant disease in the C57 black strain(7,8) as well as in foreign strains of mice(5,6,11). It did not affect the course of transplanted tumor growth in C57 blacks for 3 other mammary cancers which arose in C57 black mice (755, 241-5, 241-16) nor did extracts of the other 3 tumors affect the course of EO771 in C57 blacks (7,8). This seemed significant, particularly since the EO771 xyz factor has been demonstrated in some concentration in the normal liver and kidney of the Jackson Laboratory strain of C57 blacks but not in normal spleen (10,11). Mammary cancers 755, 241-5, 241-16 had been carried in the National Cancer Institute strain of C57 blacks, and although the EO771 tumors used came from the Jackson Laboratory the experiments were carried out with the Carworth Farms strain of C57 black mice. The possibility arose that the diversity of C57 black strains used might have influenced the results. The present paper reports experiments on the effect of the EO771 xyz factor obtained from tumors inoculated in the Jackson Memorial Laboratory and tested on C57 BL/6 mice obtained from the same source.

Materials and methods. Fifty-two C57 BL/6 mice, 3-4 months of age, were obtained in one batch from the Jackson Memorial Laboratory in Bar Harbor, and were caged in 8 boxes of 6 or 7 mice in each.

Twenty-six mice (7 males and 19 females) were injected subcutaneously into the left