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Demonstration of *Escherichia coli* 055 and 0111 Antigens by Means of Hemagglutination Test.* (19343)

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It has been shown by Keogh, North and Warburton(1,2) that certain bacterial antigens may be adsorbed onto red blood cells, thus rendering the erythrocytes agglutinable by the anti-bacterial antibodies. Subsequently this indirect hemagglutination test was used for the demonstration of antibodies against tubercle bacilli (Middlebrook and Dubos)(3), *H. influenzae* type b (Warburton, Keogh and Williams)(4), streptococci (Kirby)(5) and *Pasteurella pestis* (Amies)(6). Furthermore, Hayes and Stanley(7) showed that the somatic antigen (polysaccharide) isolated from an untyped strain of *Escherichia coli* was operative in producing hemagglutination by the homologous bacterial antiserum. The experiments to be described in this communication revealed that the hemagglutination test can be used for the identification of the 0 antigens of two groups of *E. coli*, namely, serogroups 055 and 0111. These antigenic groups of *E. coli* are of particular interest, since they are closely associated with, and, indeed, may be considered as a cause of, epidemic and sporadic diarrheal disease of infants. Furthermore, the observations reported here are of interest in connection with the problem of the nature of the phenomenon of bacterial inagglutinability.

Material and methods. Several strains of *E. coli* serogroups 055 and 0111, isolated from infants suffering from diarrheal disease, were used; in addition, one strain each was kindly supplied by Dr. Joyce Wright, London, England. The organisms were maintained on brain veal agar and transferred weekly. For the hemagglutination test brain heart infusion cultures were used, after the pH had been adjusted to 7.0. Group-specific antisera were prepared in rabbits by injection of suspensions of the organisms; and, for control purposes, *E. coli* 055 B5 and 0111 B4 antisera, made available through the courtesy of Dr. Wm. H. Ewing, Communicable Disease Center, were employed. For the hemagglutination test both human and rabbit red blood cells were washed in physiological saline solution. To the sediment of such a suspension were added *E. coli* infusion broth cultures or filtrates thereof to make a 2.5% red blood suspension. The mixture was kept for 2 hrs at 37°C and stirred repeatedly. The cells were then washed three times with physiological saline solution. The 2.5% cell suspension (vol. 0.25 ml) was added to 0.25 ml of various *E. coli* antisera in serial dilutions and to normal human and rabbit sera, serving as controls. The mixtures were incubated in the waterbath at 37°C and the resulting agglutination was read grossly at various intervals up to 2 hrs.

Results. In the first experiments human red cells were treated with broth cultures of several strains of *E. coli* 0111. Agglutination of these treated cells occurred only irregularly and with relatively concentrated amounts of group-specific antiserum. It was then decided to study the activity of *E. coli* cultures and filtrates which had been heated at 100°C for 2 hrs. The data obtained in one representative experiment, read after 2 hrs. at 37°C, are summarized in Table I.

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TABLE I. Demonstration of *E. coli* 0111 Antigen by Means of Hemagglutination Test. Agglutination by *E. coli* antisera of human red cells treated with heated *E. coli* 0111.*

E. coli antisera	Broth culture	Broth culture filtrate	Washed agar suspension sediment	Agar suspension supernate
		0111 antiserum		
1/100	4	4	4	4
1/200	4	4	3	4
1/400	4	4	1	4
1/800	4	4	—	3
1/1600	—	2	—	2
1/3200	—	—	—	—
0	—	—	—	—
		055 antiserum		
1/100	—	—	—	—

— = No agglutination. 1 to 4 = Various degrees of agglutination.

* Human red blood cells treated with the 4 unheated preparations failed to agglutinate in the above dilutions of antisera. The 055 antiserum strongly agglutinated red cells treated with *E. coli* 055.

It is evident from the data presented in this table that the boiled broth culture, broth culture filtrate, suspension of the organisms and the agar suspension supernate, in contrast to the unheated materials, rendered the red blood cells agglutinable by the *E. coli* 0111 antiserum. That this agglutination is specific for the group-specific antigen 0111† is evident from the fact that several *E. coli* 055 antisera as well as normal sera in like dilutions failed to produce agglutination. Identical results were obtained with red blood cells of both man (blood groups O, A, B, as well as Rh positive and Rh negative) and rabbit.

The specificity of this hemagglutination with *E. coli* 0111 antiserum is substantiated further by the observations that all 4 strains of *E. coli* 0111 rendered the blood cells agglutinable by the 0111 group-specific antisera and that 2 strains of *E. coli* 055 were operative in producing hemagglutination with *E. coli* 055 but not with *E. coli* 0111 antisera. Furthermore, treatment of blood cells with a strain of *E. coli* of a group other than 0111 and 055 (No. 7402) failed to induce hemagglutination by either 055 or 0111 antisera. By means of this indirect hemagglutination test group-specific 0 antigen was demonstrated in 1 to 11 days old infusion broth cultures.

Boiling of *E. coli* 0111 and 055 broth culture filtrates for two hours resulted in greater activity than boiling for one hour only; heating at 100°C for 3 or 4 hours did not substantially increase further the activity of the filtrate. It was found that autoclaving of the filtrates at 15 lbs pressure for 5 and 15 minutes produced active antigenic material, although autoclaved filtrates were somewhat less effective than those boiled for 2 hrs.

Hemagglutination appears within a few minutes after the test is set up and increases in strength during 2 hrs of incubation at 37°C. Centrifugalization at 2,000 r.p.m. for one minute enhances the degree of agglutination. Since it was shown by Wheeler, Luhby and Scholl(8) that trypsinized red blood cells may be agglutinated by incomplete Rh antibodies in saline solution, it was decided to determine whether such cells differ from untreated cells in the above described hemagglutination test. In repeated experiments it was found that trypsinized cells are agglutinated somewhat more strongly than untreated cells and that one-half to one-fourth of the minimal amount of group-specific antiserum causing agglutination of non-trypsinized red blood cells produced specific agglutination of trypsin-treated cells.

Discussion. The above described experiments revealed that both human and rabbit red blood cells may adsorb the somatic antigen of *Escherichia coli* serogroups 055 and 0111 from broth cultures as well as from sterile Seitz filtrates, thus rendering these erythrocytes agglutinable by the respective group-specific antiserum. Since certain bacteria produce agglutination of red blood cells, referred to as bacteriogenic hemagglutination,

† Both Dr. F. Kauffmann, Copenhagen, and Dr. P. R. Edwards, Chamblee, Georgia, who have seen the manuscript, raised the question as to whether or not the antigen demonstrated in the hemagglutination test is the O or the thermolabile B antigen. That the above described test demonstrates the O antigen is evident from the facts that, as shown by Dr. Edwards (personal communication) and in this laboratory, O antiserum gives agglutination to the same degree as serum containing O and B antibodies and that red cells treated with heated broth culture filtrates or agar suspensions engender specific *E. coli* antibodies in rabbits (unpublished data).

the term "indirect bacterial hemagglutination" is suggested for the specific agglutination of red blood cells produced by antibacterial antibodies acting on the antigen adsorbed onto the erythrocytes.

The observation that *E. coli* cultures and their filtrates become highly effective in this indirect hemagglutination test after being boiled for one or two hours in comparison to the unheated specimens is of interest in connection with the phenomenon of bacterial inagglutinability. It has been known for many years that certain strains of enteric bacilli are not agglutinated by the specific O antisera, unless the bacteria are first heated or the agglutination test is carried out at 50°C instead of 37°C. Kauffmann(9) made the important discovery that the heat-labile L or B antigens of *E. coli* are the cause of O inagglutinability. These antigens are not capsular antigens, since, with the exception of a single strain, capsular swelling is not produced by L or B antibodies. Kauffmann calls these antigens surface or envelope antigens, which, because of their location on the surface of the bacterial cell, make it impossible for the O antibody to reach the O antigen. Inactivation of the L or B antigens by boiling of the bacterial suspension exposes the O antigen to its homologous antibody. The observation that boiling of the sterile *E. coli* broth filtrate also renders the filtrate active in the above described indirect bacterial hemagglutination test for the demonstration of the O antigen suggests that the interference of the L or B antigens with O agglutination or hemagglutination is not necessarily dependent on the topographic arrangement of these antigens inside the bacterial cell. Rather, one may consider the possibility that L or B and O antigens may be present in the intact bacterial cell as well as in culture filtrates as a complex and that the L or B antigens block the reactive group or groups of the O antigen.

The question arises as to whether or not adsorption of bacterial antigens onto red blood cells occurs also *in vivo*. If this be so, one may be able to demonstrate in various infections red blood cells which are specifically agglutinable by the homologous antibacterial antiserum. Furthermore, it is conceivable that in such patients hemolysis and anemia may follow the development of bacterial antibodies. Such an occurrence of events would represent indirect bacterial isohemolysis, since the patient's red blood cells are being agglutinated and/or hemolyzed by his own antibodies and the antibodies do not react with red blood cell antigens but rather with bacterial antigens. Studies are now in progress to elucidate these moot questions.

Summary. (1) Boiled broth cultures and sterile Seitz filtrates thereof of *Escherichia coli* serogroups 055 and 0111 render red blood cells of man and rabbit specifically agglutinable by the homologous group-specific antisera. Unheated cultures and filtrates are only slightly effective. Trypsinized red blood cells are somewhat better agglutinated than non-trypsinized cells. (2) The significance of the findings regarding the indirect bacterial hemagglutination test is discussed with particular reference to the phenomenon of bacterial inagglutinability.

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