are required to define the importance of the polysaccharide moiety of the endotoxin molecule for the histamine-releasing activity.

1. Thomas, L., *Physiol. Rev.*, 1954, v16, 467.
2. Landy, M., Johnson, A. G., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 57.
3. Rothschild, A. M., Rocha e Silva, M., *Brit. J. Exp. Path.*, 1954, v35, 507.
4. Webster, M. E., Sagin, J. E., Landy, M., Johnson, A. G., *J. Immunol.*, 1955, v74, 455.
5. Carey, F. J., Braude, A. I., Zalesky, M., *J. Clin. Invest.*, 1958, v37, 441.
6. Noyes, H. E., McInturf, C. R., Blahuta, G. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 65.
7. Westphal, O., Presented at Nat. Inst. Health under sponsorship of Lab. Chem. Pharm., Nat. Cancer Inst., Nov. 20, 1959.
8. Kuida, H., Hinshaw, L. B., Gilbert, R. P., Visscher, M. B., *Am. J. Physiol.*, 1958, v192, 335.
9. Gilbert, R. P., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 346.
10. Weil, M. H., Spink, W. W., *J. Lab. and Clin. Med.*, 1957, v50, 501.
11. Novy, F. G., DeKruif, P. H., *J. Infect. Dis.*, 1917, v20, 776.
12. Rocha e Silva, M., *The Mechanism of Inflammation*, Pub. Acta, Inc., Montreal, 1953.

Received January 18, 1960. P.S.E.B.M., 1960, v103.

Bactericidal Activity of Normal Serum Against Bacterial Cultures. II. Activity Against *Escherichia coli* Strains. (25619)

LOUIS H. MUSCHEL (Introduced by G. Edsall)
Division of Immunology, Walter Reed Army Inst. of Research, Washington, D.C.

The preceding paper of this series(1) on bactericidal action of normal serum against strains of *Salmonella typhosa* established that a strain's resistance to serum bactericidal action was associated with its O-inagglutinability or Vi content(2). The most resistant of the strains tested were a strain of maximum mouse virulence and of proven virulence for chimpanzees as well as another strain isolated during a severe human outbreak. From the point of view of the host, moreover, it was shown that natural bactericidal antibody of marked specificity played the major role in serum bactericidal action. The present report represents a study of bactericidal action of normal human serum against strains of *Escherichia coli*. Though usually non-pathogenic, this species was chosen for study because it may cause acute or chronic disease, and recognition of this disease is increasing. In addition, there is evidence that certain serological types of *E. coli* are responsible for acute enteritis in newborn infants and calves(3). For a given host, therefore, wide variations in virulence of different strains of the same species may exist. Distinguishing characteristics have been ascribed to potentially virulent strains of *E. coli*. These have included necrosis of the skin of rabbits and of cells in culture, hemolysis of horse erythrocytes, ability to elicit the Shwartzman phenomenon, greater mouse virulence, and O-inagglutinability(4,5). These properties have not led to well-defined distinctions between virulent and non-virulent strains. To these may now be added resistance of strains to bactericidal action of normal human serum. It would seem likely, therefore, that virulence results from an association of various properties, rather than from a single characteristic.

Materials and methods. E. coli strains. Most of the cultures and the information concerning their source were received through the generosity of Dr. W. H. Ewing, Enteric Bacteriology Unit, Communicable Disease Center, U.S.P.H.S., Chamblee, Ga., except for 0114 serotypes which were kindly provided by Dr. Joan Taylor, Central Public Health Lab., London. Most of the cultures were isolates from normal healthy children or from infants with diarrhea. *Sera.* Several pools of sera from normal humans were tested. Each pool was composed of equal volumes of sera of 4 individuals whose sera lacked detectable *E. coli* agglutinins against the test organisms. Tests with the different pools invariably gave

TABLE I. Bactericidal Action of Pooled Normal Human Serum Against *E. coli* Isolates from Normal Individuals.

A. Isolates from normal children

Serotype	ED_{50}, ml serum
02	.40
02	.35
03	.35
07	.34
07	.025
07	.017
021	.12
036	.14
037	.24
04	.17
0127a, 0127b:B10	.014
086a, 086b:B9	.012

B. 3 Isolates from one individual

Type	ED_{50}, ml serum
Anaerogenic 011	>.45
01	.028
091	.24

C. Isolates from healthy hospital personnel serving on the ward when an outbreak of diarrhea among newborn occurred. These isolates were not of the same serotype as that found in the cases.

Type	ED_{50}, ml serum
011	.45
014	.0048
06	>.45

results in close agreement. Sera were stored at about –30°C; tests were performed within 3 weeks after blood collection. For absorption of serum, 14 ml of an aliquot of pool of serum was added to the washed, heat killed (56°C for 30 minutes) organisms obtained after 16 hours growth from the surface of meat extract agar in 4 Kolle flasks. The organisms were suspended in the serum, and suspension was kept at 4°C for 1 hour. Then the serum was separated from the organisms by high speed centrifugation in the cold. *Bactericidal tests.* Tests for measuring bactericidal activity of normal serum were performed by the quantitative photometric assay previously described(1). Results are expressed in terms of estimated volume (ml) of serum required to inhibit growth of 50% (ED_{50}) of test culture. *O-inagglutinability.* The O-inagglutinable status of a strain was determined by comparison of the titer of a homologous anti-O rabbit serum against organisms killed by heating for 1 hour at 100°C, and living cells. Reactions were read after 20 hours incubation in a water bath at 50°C with the killed cultures and after 2 hours at 37°C followed by 18 hours at room temperature with the living cultures. Inhibition of O-agglutination is believed to result from presence of K antigens. These are somatic antigens which act as masking antigens in a manner similar to the Vi antigens in *S. typhosa* strains (4). The K antigens are divisible into at least 3 varieties, L, A, and B based on their physical behavior.

Results. Bactericidal tests. ED_{50} of the serum pool against the isolates from normal, healthy individuals are given in Table I. The results indicated an almost 100-fold variation in susceptibility of different strains to serum bactericidal action ranging from the most resistant strains of serotype 06 and anaerogenic 011 to the most sensitive strain of serotype 014. Three of the strains tested were isolated from a healthy graduate student working with enteric bacteria (Table I,B). The serum of that individual collected about 8 months after isolation of the strains showed no greater bactericidal activity than that of other normal sera against the 3 strains. Thus, presence of non-pathogenic *E. coli* did not lead to a detectable serological response probably because the organisms were not invasive. Similar tests were performed with isolates from cases of infantile diarrhea. Results are given in Table II. Several points are of interest: relatively high level of resistance of most of these cultures, extremely high resistance of the 5 strains of serotypes 018 and 044, and marked variation in resistance of strains of serotypes 0111:B4, 0112a, 112c:B11 and 0124:B17.

O-inagglutinability. The O-inagglutinability of several *E. coli* strains and resistance of these strains to serum were compared; a significant association was observed (Table III). The highly O-inagglutinable strains such as those of serotype 02 and 0111:B4 were the most resistant to bactericidal action, although slight O-inagglutinability given by the strain of serotype 0127a, 0127b was not associated with increased resistance to normal serum bactericidal action. On the other hand, strains of serotype 07 and 086a, 086b, almost completely O-agglutinable, were extremely sensitive to normal serum bactericidal action. If the strains are ranked according to consecu-

TABLE II. Bactericidal Action of Normal Human Serum Against *E. coli* Isolates from Cases of Infantile Diarrhea.

Serotype	ED_{50}, ml serum
026:B6:H–	.31
Idem	.32 (approx.)
026:B6:H11	>.40
" : " :H32	.28
" : " :H11	.25
055:B5:H11	.22
" : " :H2	.34
" : " :H4	.30
" : " :H7	.15
" : " :H10	.29
086a, B7:H–	.19
" " :H7	.12
" " :H10	>.40
" " :H34	.23
" " :H11	>>.40
0111:B4:H–	.082
" : " :H–	.16
" : " :H2	.080
" : " :H4	.038
" : " :H6	.13
" : " :H12	.078
0112a, 112c:B11:H–	.24
Idem	.14
"	.10
"	.03
"	.11
0114:H2	.17
" :H10	.11
" : unknown	.30
0119:B14:H–	.13
" : " :H4	.11
" : " :H6	.13
" : " :H18	.23
0124:B17:H–	>>.40
" : " :H30	.13
" : " :H32	.090
" : " :H32	.092
" : " :H19	.27
0125a, 125b:B15:H21	.13
" 125c: " :H6	.12
0126:B16:H27	.10
0127:B8:H–	.058
Idem	.069
"	.092
"	.14
"	.086
0128a, 128b:B12:H8	.20
" " : " :H12	.23
" 128c: " : "	.12
018a, 18b:B20:H14	>.40
" 18c:B21:H7	>.40
044:K?:H34	>.40
" :K74:H34	>.40
" : " :H12	>.40
025:K?:H1	.13
" : " :H–	>>.40
06	>>.40
Related to 086; but not 086:B7 (K undeter.)	.29
020a, 20c:B7:H–	.19
" 20b:new K unnumbered:H26	.40

tive order in degree of O-inagglutinability and resistance to serum, a simple ordering test(6) indicates a significant degree of association ($p = <0.05$).

Specificity of normal serum bactericidal action. An aliquot of a test serum pool was absorbed with a strain of serotype 0111:B4 and another aliquot with a strain of 127a, 127b:B10. Tests were performed with the untreated serum and the 2 absorbed aliquots against 8 strains of 7 different serotypes (Table IV). Absorption of the serum pool with the 0111:B4 strain caused no reduction in its bactericidal activity against heterologous strains although activity against homologous strain was very considerably diminished. Similarly, absorption with strain 0127a, 0127b:B10 caused relatively slight reduction (less than 2-fold) in activity against heterologous strains compared to a disproportionately greater (at least 7-fold) loss against 0127 strains.

Effect of growth of cultures at different temperatures. The finding that *S. typhosa* was more susceptible to serum bactericidal action when cultivated at temperatures above or below 37°C(1) prompted an examination of this effect on *E. coli* cultures. Cultures were grown at 14°C, 37°C, 42°C and 45°C, then tested at 37°C. The results (Table V) indicate that cultures grown at 14°C and 45°C were considerably more sensitive to normal serum than those grown at 37°C, but that growth at 42°C did not result in enhanced sensitivity.

Discussion. Previous work has indicated that resistance to serum may be an important determinant of mouse virulence in *E. coli* as tested by intraperitoneal challenge(7). In this study, the data in Tables I and II did not disclose significant evidence of a relationship between resistance of a strain to serum bactericidal action and its virulence for man despite the fact that certain strains of *E. coli* isolated from normal individuals were quite sensitive to serum bactericidal action. Moreover, many *E. coli* strains isolated from normal material possessed greater resistance to normal human serum than did highly virulent strains of *S. typhosa*(1). Thus, these results indicate that resistance to normal serum cannot itself be correlated with an organism's

TABLE III. Comparison of Resistance of Strains of *E. coli* to Serum and Their O-inagglutinability.

	Strain serotype							
	02	0111:B4	0127:B8	055:B5	0128:B12	07	086a, 086b	0127a, 0127b:B10
ED_{50} of normal human serum, ml	.40	.17	.16	.12	.12	.017	.014	.012
Homologous anti-O titer* with living organisms	<20	<20	40	160	40	10,240	2560	320
Homologous anti-O titer* with heat-killed organisms	5120	640	5120	10,240	640	10,240	5120	5120

* Reciprocal of anti-O agglutinin titer.

pathogenicity. Nevertheless, a relatively high level of serum resistance may represent one of the requisites for human pathogenicity since *E. coli* isolates from cases of infantile diarrhea were not among the most sensitive to serum bactericidal action.

On the other hand, an association was found between O-inagglutinability of *E. coli* strains and their resistance to normal sera (Table III). The highly O-inagglutinable strains of serotypes 02, 0111:B4 and 0127:B8 were the most resistant to bactericidal action. Slight O-inagglutinability manifested by the strain of serotype 0127a, 0127b:B10, however, provided no protection against normal serum components. This result was comparable to that observed with *S. typhosa* strains (1). Since the K antigens of *E. coli* strains are responsible for their O-inagglutinability, the results suggest that natural bactericidal antibodies are directed against O antigens of smooth strains and that antibodies against K antigen are either not present, their concentration is too low, or they are ineffective bactericidally. The increased susceptibility to serum bactericidal action of strains cultivated at temperatures other than 37°C is also probably related to loss of O-inagglutinability. A discussion of the significance of these observations in relation to the role of fever in resistance to infections with Gram-negative organisms must await studies to determine if antigenic changes *in vitro* occur in the animal host.

The absorption experiments were done to determine whether the bactericidal activities of normal serum could be attributed to one non-specific substance, to a relatively few such substances, or to many specific substances. The findings with *E. coli* (Table IV) were similar to those with other Enterobacteriaceae (1,8) and indicated a marked specificity of

TABLE IV. Bactericidal Activity of Pooled Human Serum (ED_{50}, ml) and Absorbed Aliquots of the Serum.

	Test strain							
Test serum	0111	127a, 127b	02	07	055	0127	0128	086a, 086b
Unabsorbed	.14	.014	>.4	.034	.19	.06	.35	.010
Abs. c̄ 127a, 127b:B10	.17	.110	"	.045	.37	.43	.60	.022
" c̄ 0111:B4	>.40	.013	"	.035	.24	.07	.40	.011

TABLE V. Resistance to Bactericidal Action of Normal Human Serum of *E. coli* Strains Cultivated at Different Temperatures.

Test culture	ED_{50} of human serum (ml) against cultures grown at different temperatures			
	14°C	37°C	42°C	45°C
055, CDC #2559 - 54	.048	>.3	>.3	.17
" " #1565 - 56	.076	.13	.11	.065
0111, " #380 - "	.066	.15	.15	Not done
02, " #5086 - "	Not done	>.4	Not done	.018
0127, " #1039	"	.12	"	.0086

action. Absorption of serum with one organism resulted generally in complete inactivation against the homologous organism but little or no loss of activity against antigenically unrelated organisms.

The relationship of these findings to the nonspecific properdin system is of interest. Bactericidal activity of normal serums which were assumed to be devoid of antibody against certain bacteria has been attributed to the properdin system(9). Absorption experiments with *E. coli, S. typhosa*(1), other Gram-negative organisms(8) and also bacteriophage (10) have indicated that substances selectively removed by absorption and which may be regarded as antibodies are invariably present, however, in normal mammalian serums. Because of their low concentration, these antibodies may be unable to effect agglutination reactions, but may be detected by the bactericidal reaction, one of the most sensitive methods for antibody determination(11). In absence of antibody, normal human serum is devoid of bactericidal activity notwithstanding the presence of the components of the properdin system(8). Antibodies of marked specificity appear to play the major part, therefore, in bactericidal action of serum. Possibly, the greater susceptibility of infants to *E. coli* disease may be related to the lower concentration of these antibodies in infant's fluids, but this thesis remains to be tested.

Summary. Resistance to bactericidal action of normal human serum of *E. coli* isolates from normal children and from those with diarrhea was determined. Results indicated that serum resistance did not provide a basis for differentiation of virulent and non-virulent strains. Serum resistance was associated with the O-inagglutinability of the organisms. In addition, bactericidal action of normal human serum against *E. coli* was shown to be dependent upon natural antibodies of marked specificity.

1. Muschel, L. H., Chamberlin, R. H., Osawa, E., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 376.
2. Felix, A., Pitt, R. M., *J. Hyg.*, 1951, v49, 92.
3. Edwards, P. R., Ewing, W. H., *Identification of Enterobacteriaceae,* Burgess Publishing Co., Minneapolis, 1955, p140.
4. Kauffman, F., *Enterobacteriaceae,* 2nd Ed., Ejner Munksgaard, Copenhagen, 1954, p186.
5. Lindberg, R. B., Young, V. M., *Ann. N. Y. Acad. Sci.*, 1956, v66, 100.
6. Quenouille, M. H., *Associated Measurements,* Academic Press, N. Y., 1952.
7. Rowley, D., *Brit. J. Exp. Path.*, 1954, v35, 1.
8. Osawa, E., Muschel, L. H., *J. Immunol.*, 1960, v84, 203.
9. Wardlaw, A. C., Pillemer, L., *J. Exp. Med.*, 1956, v103, 553.
10. Toussaint, A. J., Muschel, L. H., *Nature*, 1959, v183, 1825.
11. Treffers, H. P., in Dubos, R. J., ed., *Bacterial and Mycotic Infections of Man,* 3rd ed., Lippincott Co., Philadelphia, 1958, ch. 5.

Received January 19, 1960. P.S.E.B.M., 1960, v103.

Desoxyribonucleic Acid (DNA)-Bentonite Flocculation Test for Lupus Erythematosus. (25620)

JOHN BOZICEVICH, JOHN P. NASOU AND DONALD E. KAYHOE
(Introduced by J. Freund)
(With technical assistance of Stanley B. Ward)
Laboratories of Immunology and Clinical Investigation, Nat. Inst. of Allergy and Infect. Dis., N.I.H., PHS, DHEW, Bethesda, Md.

It has been found that serum from patients with active systemic lupus erythematosus may flocculate through a wide range of titers with a suspension of a volcanic ash, bentonite, and purified desoxyribonucleic acid. Results of the test agree generally with those of lupus cell preparations. However, significant differences appear to exist especially in some patients with rheumatoid arthritis between the flocculation test and the L. E. test. In such instances, the DNA-bentonite test is negative. The present report describes the procedure for