SEROLOGICAL STUDIES OF O ANTIGENS OF *ESCHERICHIA COLI* BY MEANS OF THE HEMAGGLUTINATION TEST

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ABSTRACT

KUNIN, CALVIN M. (University of Virginia, Charlottesville) AND MARY V. BEARD. Serological studies of O antigens of *Escherichia coli* by means of the hemagglutination test. J. Bacteriol. **85**:541–548. 1963.—The serological interrelationships among most of the known O antigens of *Escherichia coli* were studied by the hemagglutination test. Good agreement with the bacterial agglutination test was found with the major antigens, but cross reactions among groups were much more limited with the hemagglutination test. Heterogenetic cross reactions with many *Enterobacteriaceae* species were observed with rabbit antisera to *E. coli* O14, O56, O124, and O144. This effect tended to elevate antibody titers observed in human serum against almost all O antigen groups, but could not by itself account for the wide diversity of antibodies to them. *E. coli* antibodies were localized in human γ-globulin, but were present in the β-globulin fractions of a number of domestic animals. Rabbits differed from other animals studied in that antibodies to *E. coli* were rarely found in their sera; this was attributed in part to the low density of *E. coli* populations in this animal. The hemagglutination test may be used in identification of unknown O antigens, but does not have special merit over the bacterial agglutination test; it does not further characterize rough or untypable strains other than by demonstrating that they also possess heterogenetic antigens.

Three in vitro methods are commonly employed to characterize the antibody response to the O antigens of *Escherichia coli*: the bacterial agglutination test (Edwards and Ewing, 1962), serum bactericidal tests (Muschel, 1960; Landy, Michael, and Whitby, 1962), and the hemagglutination test (Neter et al., 1952). The bacterial agglutination test, although fairly specific, particularly when absorption is used to eliminate cross reactions between groups, suffers principally from lack of sensitivity with sera from nonhyperimmunized animals such as man. The bactericidal test has the merit of measuring biological activity. Although excellent quantitative methods have been devised, it is relatively complex to perform, requires optimal concentrations of complement from an antibody-free source, and results occasionally have not been well quantitated. The hemagglutination test does not require complement and has the advantage of great specificity, relatively good quantitative sensitivity, and ease of performance.

In a previous study (Kunin, 1962), the hemagglutination test was employed to assess the distribution of antibodies against 11 nonenteropathic *E. coli* groups in human serum according to age, sex, and past history of urinary tract infections in the donors, and to measure transplacental transfer and excretion of antibody into human milk and urine. Cross absorption, antigen inhibition studies, and comparison with standard *E. coli* group antisera revealed the test to be highly specific and sensitive. The present study was undertaken to characterize fully the antigenic cross relationships that exist among the almost 145 established *E. coli* antigens, as revealed by the hemagglutination test, to determine the presence of antibody to these groups in human serum and in serum fractions from various animals, and to ascertain the significance of heterogenetic antibody to *Enterobacteriaceae* (Kunin, Beard, and Halmagyi, 1962) in interpretation of these tests. In addition, an effort was made to characterize clinical isolates of *E. coli*, including rough strains, by the hemagglutination test.

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MATERIALS AND METHODS

Hemagglutination test. Cultures of the 145 prototype *E. coli* O antigen strains, with the exception of O 29, 31, 47, 67, 68, 72, 87, 99, and 107, together with unabsorbed hyperimmune rabbit serum prepared against all of the O groups with the exception of O 8, 31, 47, 57, 67, 68, 72, 87, 88, 94, 107, 122, and 141 were obtained from the Communicable Disease Center, U.S. Public Health Service. (We are indebted to John Winn for supplying these cultures and antisera. The rabbit antisera were preserved with 50% glycerol; all dilutions are expressed in terms of rabbit serum prior to addition of glycerol.) Thus, 136 antigens and 131 antisera were available for study; these are referred to as standard CDC O antigens and antisera. [O group 31, 47, 67, 72, 94, and 122 are no longer considered to belong in the *E. coli* schema, so that only 139 O groups are now recognized (Edwards and Ewing, 1962).] Other organisms were obtained from the collection of the Department of Microbiology, University of Virginia, or isolated from clinical material. Saline (0.9% NaCl) suspensions of the cultures were inoculated on the surface of Heart Infusion Agar (Difco) in petri dishes and incubated overnight at 37 C. Cultures were harvested in 10 ml of saline per plate and boiled for 2 hr, followed by centrifugation at 5,000 $\times$ *g* for 15 min at 0 C, and final addition of 1 ml of 95% alcohol to supernatants. Supernatants were stored at 4 C until used as antigen. Bacterial lipopolysaccharides obtained commercially (Difco) included *E. coli* O55:B5, O127:B8, *Salmonella abortivo-equina*, *S. typhosa* O901, *S. typhimurium*, *S. enteriditis*, *Serratia marcescens*, *Shigella flexneri*, and *Brucella abortus*.

A dilution (1:10) of crude antigen or 100 μg per ml of the lipopolysaccharides was incubated in a water-bath shaker with a 2.5% suspension of human O cells (obtained in heparin) and washed three times in Hank's balanced salt solution (BSS; employed as diluent in all experiments). The treated erythrocytes were washed three times in BSS and adjusted to a final 2.5% suspension. Antigen-coated cells were employed in the hemagglutination test immediately after preparation.

Hemagglutination tests were conducted in white plastic trays (Limbro Manufacturing Co., New Haven, Conn.) containing 96 cups (8 rows, 12 to a row). By use of multiple trays, 0.25 ml of both 1:100 and 1:400 dilutions of each of the 131 rabbit *E. coli* antisera was distributed into rows 180 cups long. In this manner, 245 trays were prepared for each of the two antiserum dilutions, sealed with paraffin sheets, and stored at -20 C until used. Similar replicate trays were prepared and stored after addition of serial dilutions of serum obtained from three healthy young human adults and human, bovine, equine, porcine, canine, and rabbit β- and γ-globulins. (Human γ-globulin was obtained from Merck, Sharp and Dohme, West Point, Pa., as poliomyelitis immune globulin; the other serum fractions were obtained as powders from Pentex Inc., Kankakee, Ill., and prepared as 2% solutions in BSS.) Additional trays were prepared as required for supplementary studies to be presented below.

On the test day, sets of trays containing 12 rows of replicate dilutions of each rabbit antiserum and preparations of other reagents were warmed to room temperature. Samples of erythrocytes were coated with 12 different antigens, and 0.25 ml of each was added to rows of antisera. Mixtures were incubated for 1 hr at 37 C and read by pattern, and, after gentle shaking, for gross agglutination over a horizontally placed X-ray view box. Pattern reading was somewhat more sensitive than observation of clumping after shaking.

Hemagglutination-inhibition tests were performed by adding 0.1 or 0.25 ml of antigen to cups containing various serum dilutions, followed by incubation for 1 hr at 37 C prior to addition of sensitized erythrocytes.

Bacterial agglutination test. Bacterial strains were grown for 6 hr at 37 C in Brain Heart Infusion broth. They were then boiled for 1 hr, permitted to cool, and 0.9 ml of a 1:2 dilution in formalinized saline was added to 0.1 ml of pooled or individual rabbit antisera to make a final serum dilution of 1:500. Pools were prepared according to the system developed by Ewing et al. (1956). Mixtures of antigen and serum were incubated overnight in a water bath at 50 C and read for gross agglutination. Rough strains were defined as those producing agglutination in all pools.

Serum bactericidal test. These tests were conducted essentially as described by Landy et al. (1962), with the following modifications: human complement was prepared from a single donor,

previously shown to have normal complement level, and stored in 5-ml portions at −70 C until used; washed bacterial cells employed to absorb antibody from human serum were boiled for 1 hr, and petri dishes were employed rather than cups in plastic trays.

Agar gel diffusion test. Gel diffusion tests were conducted with undiluted rabbit antiserum and antigen. Plates, containing 0.6% ionagar no. 2 (Oxoid) in saline with sodium azide as preservative, were held for 3 weeks at 4 C in a moist chamber before discarding.

RESULTS

Cross reactions observed with standard CDC antigens and antisera. All but 6 of the 129 available antigen-antiserum pairs reacted strongly in the hemagglutination test at rabbit serum dilutions of 1:100 and 1:400. Pairs failing to give a homotypic reaction were O 12, 40, 90, 92, 99, and 117, and these were not considered further. Cross reactions observed with the hemagglutination test are summarized in Table 1. Antisera against *E. coli* O 14, 56, 124, and 144 were termed heterogenetic because they gave cross reactions with almost all of the *E. coli* antigens and with antigens prepared from other *Enterobacteriaceae* as well (Kunin et al., 1962). The O14 antiserum was by far the most active in this regard. Further studies with these antisera are described below.

Comparison of the cross reactions among *E.*

TABLE 1. *Cross reactions among Escherichia coli O groups observed with the hemagglutination test*

Antiserum	Antigen	Antiserum	Antigen	Antiserum	Antigen	Antiserum	Antigen
1	*2**, *50*, 4	35		73	*44*	113	
2	*50*, 23, 38	36	43	74	*2*, *50*	114	5
3	*23*, 6, 53	37		75	105	115	*105*
4		38	*23*	76	*22*	116	34, 59
5	*70*, *114*, 71, 74	39		77	*17*, *44*, *66*	118	18 ab
6		41		78	*137*, 89	119	
7	*5*, *71*	42		79		120	*105*, 69, 79
9	104	43		80		121	
10		44	*73*, *106*	81	*21*, *32*, 33, 39	123	62
11		45		82		124	Heterogenetic
13	*19*, *53*, *129*, *133*, *135*	46	130, 134	83		125	71
14	Heterogenetic	48	*19*, *37*, *59*	84		126	
15	98, 99	49		85		127	*90*
16	62, 98, 135	50	*2*, 74, 105	86		128	
17	*73*, *77*, 44, 66	51	69, 71	89	101	129	*135*
18 ab		52		91		130	45
18 ac	18 ab	53	2, 50, 105	93		131	
19	*48*, 69, 70, 133	54	19, 40	96		132	
20		55	22	97		133	19, 79
21	*32*, *83*, 30, 33	56	Heterogenetic	98		134	46, 48, 130
22	*76*, *83*	58		100		135	*13*, *129*, *133*
23	*38*	59		101	*76*, 89		19, 59, 79
24		60		102		136	
25		61		103		137	
26		62	*16*, 57	104		138	
27		63		105	*115*	139	
28	42	64		106	*44*	140	
29	*28*	65		108		142	101
30		66		109		143	
32	33	69	*19*, 71	110	45, 51	144	Heterogenetic
33		70	*5*, *71*, *74*, 53	111		145	
34	39	71	*5*, *7*, *70*	112			

* Italics denote groups positive on both hemagglutination and bacterial agglutination tests.

coli O antigens determined by the hemagglutination test with the results of the bacterial agglutination test reported by Ewing et al. (1956) revealed agreement between the two tests in 59 instances, positive hemagglutination and negative bacterial agglutination in 66, and negative hemagglutination but positive bacterial agglutination in 363. In most instances, hemagglutination results best fitted those reported to be strong crosses (e.g., reciprocal and at high titer) by the bacterial agglutination test, but this was not always true. It must be pointed out that the rabbit antisera employed in this study and that reported by Ewing et al. (1956) were not identical, although prepared against the same antigens in the same institution.

E. coli antibodies in human serum and pooled serum fractions. In a previous study employing the hemagglutination test (Kunin, 1962), it was shown that virtually all humans studied possessed antibody to *E. coli* O 1, 2, 4, 7, 20, 39, 58, 75, and 110 at a dilution of 1:10 or greater by 2 years of age. Sera from three additional young adults (two females and one male) and pooled human γ-globulin were tested in the present study against all of the available *E. coli* antigens. Positive tests were observed with all of these dilutions at 1:10 or greater against all antigens except O 10, 11, 18, and 25. Antigens O 12 and 92, which had not given homotypic crosses with standard rabbit sera, also failed to react with human sera. The remaining antibody titers against *E. coli* O antigens ranged from 1:10 to 1:640, with a geometric mean titer of 1:23, 1:40, and 1:53 for the three serums, respectively, and 1:46 for the human pooled γ-globulin.

To assess the possible influence of heterogenetic antibody in human serum (which would give nonspecific cross reactions with all *E. coli* antigens in the hemagglutination test), 0.25 ml of dilutions of serum from a single adult female was incubated with undiluted *E. coli* O14 crude antigen for 1 hr at 37 C prior to addition of erythrocytes sensitized with each of the 136 available antigens. Titers observed in these tests were compared with those obtained after simultaneous incubation of the same serum dilutions with BSS. Preincubation with O14 antigen clearly eliminated reactivity of the human serum with erythrocytes coated with O14 antigen, but reduced heterologous antibody titers usually only by onefold and at most by twofold; the mean was a onefold reduction. Geometric mean titer against all antigens was 1:53 prior to and 1:26 after incubation with O14 antigen. Only in a few instances in which antibody titers were in the range of 1:10 did the O14 antigen appear to block hemagglutination completely. Thus, at best, only part of the very broad distribution of *E. coli* O antibodies in human serum could be attributed to heterogenetic antibody.

Human pooled β-globulin (shown on paper electrophoresis to contain small amounts of γ-globulin), prepared at a dilution of 4%, gave positive hemagglutination tests with the same 136 antigens, but a 20% solution of this fraction was only slightly active.

E. coli antibodies in serum fractions from various animals. All available antigens were tested against 4% and 20% solutions in BSS of β- and γ-globulins from bovine, canine, and equine pools and porcine γ-globulin. Only slight or no hemagglutination was observed with 4% equine, canine, and porcine γ-globulins against various *E. coli* antigens, and 20% solutions were completely inactive. Bovine γ-globulin was active against almost all antigens at a dilution of 4%, but was virtually inactive at 20%.

β-Globulin fractions of these animal sera, in contrast, gave strongly positive tests at 20% concentrations against 130 of the 136 *E. coli* antigens (the negative were O 10, 11, 12, 18ac, 25, and 92), behaving in this respect very much like human γ-globulin. Inhibition studies in which O14 antigen was added to β-globulins resulted in marked reductions in antibody titer; for example, low titers at about 1:10 observed against many *E. coli* O antigens with bovine β-globulin were reduced to <1:10 when O14

TABLE 2. *Reduction of antibody titers against Escherichia coli O antigens after preincubation of pooled animal β-globulins with O14 crude antigen*

Coated RBC*	Pooled β-globulins			
	Canine		Equine	
	Control	O14	Control	O14
O55	1:80	—†	1:80	—†
O127	1:80	—†	1:80	1:10

* Red blood cells.

† Undiluted 2% solution in BSS.

antigen was added. Other studies employing O55 and O127 coated erythrocytes are shown in Table 2. These studies suggest that a considerable proportion of reactivity of these fractions with *E. coli* is due to heterogenetic antibody.

E. coli antibodies in rabbits. Rabbit serum fractions were remarkable, in that solutions of both β- and γ-globulin as low as 2% did not give hemagglutination reactions with any of the *E. coli* antigens. In view of this finding and the extensive use of serum from immunized rabbits for serological work with *E. coli*, strains of *E. coli* were isolated directly from the caecae of seven healthy rabbits and identified by the bacterial agglutination test. *E. coli* were isolated from three of the animals; 15 isolates from one animal failed to react with any of the *E. coli* pools; of 14 isolates from a second animal, 4 were identified as O18, the remainder being nonreactive; and 3 strains from a third animal were members of group O1. Serum obtained from all seven animals failed to react at a 1:10 dilution with the *E. coli* O1 isolate; serum from the animal which yielded an unidentifiable strain reacted with it at a titer of 1:20, but all other rabbit sera were unreactive at a 1:10 dilution. Serum from the animal which yielded an O18 strain gave a positive hemagglutination test at a titer in excess of 1:80, but all other rabbit sera were unreactive at a dilution of 1:10. Thus, it appeared that, although *E. coli* could be isolated from rabbit feces, rabbit sera did not possess the broad *E. coli* antibody pattern observed in other animals and in man. These results are in accord with previous observations that rabbit serum at a dilution of 1:10 was virtually unreactive with 11 different *E. coli* antigens in the hemagglutination test (Kunin, 1962).

Further studies on properties of heterogenetic Enterobacteriaceae antibody. Four rabbit antisera pools prepared against *E. coli* O14, O56, O124, and O144 agglutinated erythrocytes sensitized with crude O antigens and Boivin-type lipopolysaccharides from many species of *Enterobacteriaceae*, including *Salmonella*, *Shigella*, *Proteus*, and *Aerobacter*, but did not react with similar fractions from other gram-negative or -positive bacterial species. Of these, O14 antiserum was by far the most potent, followed by O 144, 124, and 56. Studies with O14 antiserum have been reported in detail elsewhere (Kunin et al., 1962) and confirmed by Whang and Neter (1962). The properties of the other antisera were indistinguishable from those of O14; e.g., they did not agglutinate heterologous bacteria, did not form precipitins with heterologous soluble antigens, and cross reactions could be inhibited by preincubation with any one of many *Enterobacteriaceae* antigens.

Serum bactericidal tests employing O14 rabbit antisera (Table 3) indicated that this serum was weakly bactericidal only against the homologous strain of *E. coli*, but not against other antigenic groups, nor against *Salmonella* or *Shigella* strains.

Identification of clinical isolates of E. coli by the hemagglutination test. Crude O antigens were prepared from 32 clinical isolates of *E. coli* obtained from school children with urinary tract infections (Kunin and Halmagyi, 1962). Of these strains, 20 were smooth and could be grouped by the bacterial agglutination test; 12 were rough strains and could not be identified according to the O group. Human erythrocytes were sensitized with each of these antigens and tested for reactivity with all of the available rabbit O antisera. The results with the smooth strains are summarized in Table 4. In 15 of 20 instances (75%), at least one of the cross reactions corresponded with the grouping previously established. Cross reactions were largely restricted to those previously summarized in Table 1, although a few were in accord with the observations of Ewing et al. (1956) and some

TABLE 3. *Bactericidal activity of Escherichia coli O14 rabbit antiserum and human serum expressed as approximate 50% end point*

Organism	O14 Antiserum*		Human serum
	Pre	Post	
E. coli O14....	<1:5	1:20	1:20
E. coli O1.....	—	<Undil.†	<Undil.
Salmonella enteritidis.....	—	Undil.	>1:5; <1:10
Shigella dysenteriae.....	—	<Undil.	1:5

* Prepared by immunization of rabbit with broth culture heated for 2 hr at 100 C and treated with 0.5% formalin; hemagglutination titer vs. O14 antigen: pre, <1:10; post, 1:640.

† Undiluted.

TABLE 4. *Cross reactions observed between O antigens of Escherichia coli isolated from urinary tract infections and standard rabbit antiserum*

Source	Antigen group	Cross-reacting antiserum	Reaction with O14 antiserum
J.C.	O1	*1**, 53	+
C.P.	O2	None	−
M.M.	O2	None	+
N.H.	O4	None	−
R.M.	O4	*4*, 7, 108	+
L.H.	O6	*3*, *6*	+
T.M.	O6	*6*, 15, 16	+
M.W.	O6	*6*	−
B.F.(1)	O7	1, 5, *7*, 25, 36, 63, *71*, 73	+
B.F.(2)	O7	*7*, *71*	+
M.G.	O7	*7*, *71*	+
L.L.	O7	None	−
M.L.	O7	*7*, *71*	+
V.W.	O25	*25*	−
B.P.	O30	*30*	+
H.R.	O40	13, 18, 19, 25, *40*, *54*, 102	+
P.M.	O52	*52*, 116	+
M.M.	O75	*75*	+
S.M.	O75	None	−
D.M.	O86	*86*	+

* Italics denote cross reactions for this antigen group also observed in the hemagglutination test summarized in Table 1. Antigen groups determined by bacterial agglutination; cross-reacting antiserum by hemagglutination test.

had not been previously observed. Considerable variation was encountered, even among isolates of the same group. Five isolates (25%) failed to react with any of the antisera, and of these only one reacted with the heterogenic O14 antiserum; they presumably produced poor antigens.

The 12 rough strains failed to react with any of the antisera except those with heterogenetic properties; 11 (92%) reacted with O14 and with O144, 5 (42%) with O124, but none with O56 antiserum. Thus, the rough strains were shown to possess common *Enterobacteriaceae* hapten, but not specific O group antigens.

DISCUSSION

The current study was devised to assess the value of the hemagglutination test in serological characterization of *E. coli* O antigens and for measurement of antibodies in serum. Homologous *E. coli* O antigen groups and rabbit antisera representing 129 pairs obtained from the Communicable Disease Center reacted quite well in all but a few instances in the hemagglutination system. Cross reactions among groups were much less frequent than reported with the bacterial agglutination test; only a small number of previously unrecognized cross reactions were observed. The most striking observation was the capacity of certain rabbit antisera to hemagglutinate cells coated with O antigen preparations from many different *Enterobacteriaceae* species, including rough strains of *E. coli* isolated from clinical material. These heterogenetic reactions, particularly prominent with O14 antiserum, have been described in detail elsewhere (Kunin, et al., 1962) and recently confirmed by Whang and Neter (1962). The same, although much less marked, heterogenetic activity was observed in the current study with rabbit antisera prepared against *E. coli* groups O56, O124, and O144. Investigations now in progress are concerned with separation of heterogenetic material from O antigen preparations and with study of the relation of these factors to the rough antigen.

The role of heterogenetic antibody in resistance to infection, if any, is probably minor, since O14 antisera were found to be only weakly bactericidal against homotypic strains and inactive against other *E. coli* or *Enterobacteriaceae* species. More important is the possibility that heterogenetic antibody may be confused with specific O group antibody in serological studies employing the hemagglutination test. Antibodies to almost all of the known *E. coli* groups were detected in individual samples of human serum, pooled γ-globulin, and serum fractions from various animals. Antigen inhibition studies, however, indicated that, on the average, one-half of the titer obtained against the various antigens in serum from a single human was probably due to heterogenetic antibody, and that this heterogenetic activity was even more marked in canine and equine β-globulins. It must be emphasized, however, that although preincubation of serum with crude O14 antigen reduced titers against heterologous antigens, the wide spectrum of antibody against many groups persisted. The antigenic stimulus which accounts for such a wide reactivity of human and other sera against many *E. coli* O groups is not entirely clear, but presumably much of it is due to the fecal micro-

flora. The limited number of cross reactions detected by the hemagglutination test suggests that the stimulus is provided by many different organisms, rather than by a few with broad cross reactivity. In view of the presence of heterogenetic *Enterobacteriaceae* antibody in serum, it is suggested that serological studies employing the hemagglutination test be controlled by either absorbing this component or blocking it by addition of O14 or other antigens. Almost every *Enterobacteriaceae* O antigen studied has the capacity to block this reaction.

E. coli antibody appears predominantly in the γ-globulin fraction of human serum but in dogs, pigs, cows, and horses is primarily in the β-fraction. Typhoid O antibodies have been found exclusively in the 19 S fraction of human serum (Fink et al., 1962). *E. coli* antibodies may be in this same fraction, since they are also poorly transmitted to the fetus across human placenta (Vahlquist, 1958; Kunin, 1962), which appears to be quite permeable to 7 S but not to 19 S globulins. The failure to detect antibodies to *E. coli* in pooled rabbit β- and γ-fractions and in most of the individual rabbit sera studied at a dilution of 1:10 may be a reflection of the generally low population densities of *E. coli* found in rabbit feces. Smith and Crabb (1961) failed to find *E. coli* in four of ten rabbit fecal specimens studied; the mean concentration per g of feces was only $10^{2.7}$ in their study, much lower than observed in other domestic animals. *E. coli* were found in only three of seven caecal samples in this study, and only two of these animals possessed antibodies to strains isolated from rabbits. This observation is of significance, since rabbits are extensively used to prepare *E. coli* antisera and for the study of experimental infections with these organisms.

It is possible to identify *E. coli* strains according to their O antigens by the hemagglutination test. The necessity of preparing crude O antigens, coating erythrocytes, prediluting sera, and the problem of heterogenetic reactions indicate that this approach has no great advantage over the well-established bacterial agglutination method. The hemagglutination test failed to characterize further rough or untypeable strains other than to demonstrate heterogenetic antigen.

It appears from these and other studies that the hemagglutination reaction is a sensitive and fairly specific test for antibodies directed against *E. coli* O antigens, provided that both the limited cross reactions found among O groups and the broad heterogenetic cross reactions noted for *Enterobacteriaceae* species be taken into account or controlled.

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LITERATURE CITED

EDWARDS, P. R., AND W. H. EWING. 1962. Identification of *Enterobacteriaceae*, 2nd ed. Burgess Publishing Co., Minneapolis.

EWING, W. H., H. W. TATUM, B. R. DAVIS, AND R. W. REAVIS. 1956. Studies on the serology of the *Escherichia coli* group. U.S. Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center, Atlanta.

FINK, C. W., W. E. MILLER, JR., B. DORWARD, AND J. LOSPALLUTO. 1962. The formation of macroglobulin antibodies. II. Studies on neonatal infants and older children. J. Clin. Invest. **41**:1422–1428.

KUNIN, C. M. 1962. Distribution of antibodies against various non-enteropathic *E. coli* groups: Relation to age, sex and breast feeding. Arch. Internal Med. **110**:676–686.

KUNIN, C. M., M. V. BEARD, AND N. E. HALMAGYI. 1962. Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other *Enterobacteriaceae*. Proc. Soc. Exptl. Biol. Med. **111**:160–166.

KUNIN, C. M., AND N. E. HALMAGYI. 1962. Urinary tract infections in school-children. II. Characterization of the invading organisms. New Engl. J. Med. **266**:1297–1301.

LANDY, M., J. G. MICHAEL, AND J. L. WHITBY. 1962. Bactericidal method for the measurement in normal serum of antibody to gram-negative bacteria. J. Bacteriol. **83**:631–640.

MUSCHEL, L. H. 1960. Bactericidal activity of normal serum against bacterial cultures. II. Activity against *Escherichia coli* strains. Proc. Soc. Exptl. Biol. Med. **103**:632–636.

NETER, E., L. F. BERTRAM, D. A. ZAK, M. R. MURDOCK, AND C. E. ARBESMAN. 1952. Studies

on hemagglutination and hemolysis by *Escherichia coli* antisera. J. Exptl. Med. **96**: 1–15.

SMITH, H. W., AND W. E. CRABB. 1961. The faecal bacterial flora of animals and man: Its development in the young. J. Pathol. Bacteriol. **82**:53–66.

VAHLQUIST, B. 1958. The transfer of antibodies from mother to offspring. Advan. Pediat. **10**:305–338.

WHANG, H. Y., AND E. NETER. 1962. Immunological studies of a heterogenetic enterobacterial antigen (Kunin). J. Bacteriol. **84**:1245–1250.