RELATIONSHIP OF HOST ANTIBODY TO FLUCTUATIONS OF *ESCHERICHIA COLI* SEROTYPES IN THE HUMAN INTESTINE[1]

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ABSTRACT

ROBINET, HARRIETTE G. (Walter Reed Army Institute of Research, Washington, D.C.). Relationship of host antibody to fluctuations of *Escherichia coli* serotypes in the human intestine. J. Bacteriol. **84**:896-901 1962.—A study was undertaken to determine the relationship of host antibody to the changes which take place among *Escherichia coli* serotypes present in the intestine. A survey of six healthy persons examined for *E. coli* monthly for 6 months reaffirmed the periodic fluctuations of antigenic types. Serotypes were studied from each individual and their appearance and maintenance or disappearance closely followed. Representative strains were chosen each month and used to prepare a heated sodium hydroxide lipopolysaccharide extract for sensitizing human group O Rh-negative red blood cells for the hemagglutination test. These antigens were tested with the sera drawn from each of the six individuals at the time monthly cultures were made. Results showed that antibody levels for each serotype remained basically constant over the 6-month period. Titer levels tended to be characteristic of the host rather than related to the *E. coli* bacteria, as shown by tests with serotypes both from the individual's own intestinal tract and from other individuals in the study. High normal antibody levels for autologous *E. coli* serotypes did not act as a deterrent to the ability of the organisms to establish themselves in the bowel, nor did antibody titer for *E. coli* strains determine whether they would continue as residents over several months or depart as transient flora.

Several studies over the past years have revealed that *Escherichia coli* serotypes in the intestinal tract of man have periodic fluctuations. Totsuka (1902) found evidence of a shift of antigenic types of *E. coli* flora of a normal intestine; more recently Wallick and Stuart (1943) and Kauffmann and Perch (1943) made similar observations. The separation of *E. coli* into somatic, capsular, and flagellar serotypes by Kauffmann (1947) has facilitated this type of study. Sears, Brownlee, and Uchiyama (1950) characterized the *E. coli* composition of human feces in the following statement: ". . . the *E. coli* flora of the human bowel is made up of two kinds of strains, those which establish themselves firmly and continue to multiply over extended periods of time and those which are found only in a single or a few successive specimens, their total tenure being a few weeks at most. It is convenient to speak of these two kinds of strains as residents and transients respectively."

The factors which determine whether an organism will be resident or transient are elusive. Sears et al. (1950, 1956) and Sears and Brownlee (1952) have shown that a turnover of serological strains does not seem to be correlated with age of the host, diarrhea, sickness, or antibiotic treatment, nor with colicine production by the serotypes. Furthermore, experimental studies revealed that a foreign serotype of *E. coli* cannot be artificially established in an intestinal tract (Sears and Brownlee, 1952). Experiments on humans and dogs employed the feeding of massive doses of an *E. coli* serotype in enteric coated capsules for 5 days subsequent to sulfaguanidine administration. Other experiments attempted establishing a specific serotype by rectal infusion. The *E. coli* were not maintained for more than a few days and thus all such attempts were generally unsuccessful (Sears et al., 1956).

Host antibody could exert an indirect environmental influence, however, which might be a factor in determining whether an *E. coli* strain would be resident or transient. Studies on the serological behavior of nonenteropathogenic *E. coli* O1 through O25 revealed that infants with

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diarrhea developed hemagglutinating antibody to these serotypes. Of 30 children, 12 showed titer rises to these *E. coli* regardless of whether or not bacterial pathogens were present (Young et al., 1959). In adults, the relationship of antibody to *E. coli* fluctuations has not been determined. Therefore, studies were undertaken to determine whether the presence of normal antibody, or antibody response of the host to autologous *E. coli* serotypes, was correlated with the ability of specific antigenic types to establish themselves and maintain residence or to be removed from the intestinal tract.

MATERIALS AND METHODS

Bacteriological techniques. Fecal and serum samples were obtained monthly for a period of 6 months from six laboratory personnel. Fecal samples were cultured immediately. Sera were stored at −40 C until they were tested for hemagglutinating antibody.

Fecal samples were cultured on blood agar and eosin methylene blue plates and ten or more *E. coli* strains were selected. Biochemical determinations included lactose, sucrose, maltose, and mannitol fermentations and indol, methyl red, Voges-Proskauer, and Simmons citrate reactions (the IMViC test). Only those strains which fermented lactose, and were IMViC ++−−, were used in the study.

E. coli strains conforming to the above pattern were typed according to methods of Edwards and Ewing (1955). The typing included O somatic antigens for enteropathogenic *E. coli* serotypes O26, O55, O86, O111, O112ab, O112ac, O124, O125ab, O125ac, O126, O127, O128ab, and O128ac, and for *E. coli* O1 through O25. *E. coli* strains, which belonged to none of the serotypes mentioned above, were used to immunize rabbits, employing techniques of Ewing (1956) for O antisera. These antisera in turn were used to distinguish the members of that particular serotype. Each *E. coli* strain was agglutinated to titer with specific antiserum, and reciprocal adsorptions were done to establish further the identity of some representative strains.

Hemagglutination technique. Lipopolysaccharide antigens were prepared each month from smooth *E. coli* strains which were representative of resident and transient flora for the six individuals in the study. Preparation of lipopolysaccharide extracts, for sensitizing the erythrocytes, and the hemagglutination test have been described elsewhere (Young, Sochard, and Hemphill, 1958). Briefly, cells harvested in physiological saline from Trypticase Soy Agar (BBL) enriched with 5% glycerine were mixed with an equal quantity of 0.1 N sodium hydroxide, heated to 100 C for 1 hr, neutralized with 1.0 N hydrochloric acid, and, after dialysis in distilled water for 24 hr, Seitz filtered to obtain water-clear extracts. These extracts, diluted 1:5, were used to sensitize an equal volume of a 10% suspension of washed human group O Rh-negative erythrocytes. The sensitized, and subsequently washed, erythrocytes were diluted to a 1% suspension by volume for use. The monthly serum samples of the six laboratory personnel were thawed, inactivated, and tested in final dilutions ranging from 1:10 to 1:5,120. Titers were read as the reciprocal of the highest dilution showing complete agglutination of the sensitized blood cells. Controls included tests for spontaneous agglutination of sensitized and nonsensitized erythrocytes in saline, and nonspecific agglutination of nonsensitized erythrocytes in the test sera.

RESULTS AND DISCUSSION

Bacteriological findings. The six persons in the study afforded a wide range of antigenic types of *E. coli* which were followed as the bacteria appeared and subsequently established themselves or disappeared; these data form the foundation for the investigation of the host's serological influence on the flora. Table 1 presents a series of tabulations on the *E. coli* flora of each individual. In some instances, a total of ten *E. coli* colonies did not grow on the media employed for culturing fecal samples, as from subjects A, B, and F. No *E. coli* strains grew from several stool cultures taken in August, October, and November from individual B. These and other aberrant findings have been reported elsewhere (Young et al., 1960).

Only two enteropathogenic serotypes, *E. coli* O126 and O112ab, were encountered; these were in individual C. The isolation of *E. coli* O112ab was made within 1 week after a mild diarrhea occurred in the children of individual C.

The number of *E. coli* serotypes harbored during the 6-month period varied from only two in individual A to as many as nine different antigenic types harbored by subject C. The persistence of *E. coli* serotypes in some persons, in contrast with the transience of a multiplicity of serotypes in other persons, is being currently studied in relation to colicine production (Branche et al., 1960) and phage activity of the bacteria.

TABLE 1. *Escherichia coli serotypes isolated in six persons*

Individual	Serotypes* isolated	Month of isolation					
		June	July	Aug.	Sept.	Oct.	Nov.
A	z		10	6	10	10	8
	O22			1			
	Rough†	4					2
	Total no. strains	4	10	7	10	10	10
B	O20	4					
	p		10		3		
	q				7		
	Total no. strains	4	10	0	10	0	0
C	O1	1	3		2		10
	s	9		3			
	t		7				
	g			2			
	O14			1			
	O126			1			
	e			3			
	O112ab				8		
	k					10	
	Total no. strains	10	10	10	10	10	10
D	O2	10					
	x		10	10	10	10	8
	y						2
	Total no. strains	10	10	10	10	10	10
E	a	6					
	O22		10		1		10
	b			3			
	c				3		
	O18				3		
	O6					10	
	Rough	4		7	3		
	Total no. strains	10	10	10	10	10	10
F	d	3	5	1			
	O2	3		2	10		
	h	4		4			
	n						4
	Rough		4	3		10	
	Total no. strains	10	9	10	10	10	4

* Small letters = *E. coli* serotypes which were not included in the *E. coli* O1 through O25 or enteropathogenic serotypes and which were identified by antisera prepared during the study.

† Rough = *E. coli* strains which underwent spontaneous agglutination in broth, physiological saline, or nonspecific sera, and therefore could not be typed.

The serotypes isolated in the study were classified in the defined categories of residents and transients.

Resident serotypes tended to be the "dominant" flora, i.e., they frequently composed all of the *E. coli* strains isolated from a fecal sample. Examples of dominant residents are "z" of individual A and "x" of individual D, both of which were the only serotypes isolated during four of the six months and were predominant during a fifth month. These are prototypes of ". . . residents which establish themselves firmly . . ." (Sears et al., 1950). However, resident *E. coli* were not always present as dominant flora. Some resident serotypes appeared to increase in cell population over several months. *E. coli* O1 of subject C constituted one of ten colonies in June, three of ten in July, two in September, and all ten colonies in November. *E. coli* O2 of individual F was a similar example. This probably represented slow "establishment" of resident serotypes. Apparently some concurrent attributes of host habitat and *E. coli* serotype resulted in bacterial ascendency.

Transient serotypes, on the other hand, generally constituted a small proportion of the total *E. coli* strains isolated, in contrast with the tendency of residents to be numerically dominant. Transients were represented by antigenic types such as "b" of individual E and "g", "e", O14, and O126 of individual C. Each occurred only once and in small numbers approximately midway in the 6-month survey. Some serotypes could not be defined as resident or transient due to isolation at the beginning or the end of the study. An example is *E. coli* O2 of individual D, which may or may not have been a transient strain occurring only in June.

Several patterns of *E. coli*-host relationships emerge from the bacteriological data. These are the persistence of dominant resident strains, the slow ascension to dominance of other residents, and the disappearance of numerically sparse transients.

Serological findings. The host's antibody stimulation, if any, and the general serological relationship to the fluctuating *E. coli* flora were then investigated. Table 2 shows results of hemagglutination tests using autologous *E. coli* serotypes against all of the monthly serum samples from each individual. The study revealed that serotypes were lost and acquired in the bowel of hosts irrespective of high or low antibody levels

TABLE 2. *Hemagglutinating antibody titers* for autologous Escherichia coli serotypes*

Individual	Serotype and month of isolation	Month of serum sample					
		June	July	Aug.	Sept.	Oct.	Nov.
A	z..... July	20	20	20	160	20	20
	z..... Aug.	40	40	20	80	20	40
	z..... Sept.	40	80	80	80	40	40
	z..... Oct.	40	40	40	80	40	40
	z..... Nov.	320	160	320	640	320	320
B	O20..... June	640	320	320	320	640	640
	p..... July	160	160	160	160	160	160
	q..... Sept.	320	320	320	320	320	320
C	s..... June	40	40	40	40	40	20
	O1..... June	160	160	160	160	160	160
	t..... July	10	10	20	10	20	10
	g..... Aug.	80	80	80	80	80	80
	O112ab.... Sept.	20	10	10	10	20	10
	k..... Oct.	40	40	40	40	40	80
	O1..... Nov.	80	80	80	80	80	80
D	O2..... June	640	320	320	320	320	320
	x..... July	80	80	80	80	80	80
	x..... Aug.	80	80	80	80	80	80
	x..... Sept.	80	80	80	80	80	80
	x..... Oct.	10	20	10	10	10	10
	y..... Nov.	160	80	80	80	80	80
E	a..... June	10	20	10	10	10	10
	O22..... July	160	160	160	160	160	160
	b..... Aug.	160	160	160	160	160	160
	O18..... Sept.	160	160	160	160	160	160
	O6..... Oct.	20	20	10	20	20	40
	O22..... Nov.	20	10	20	10	20	20
F	O2..... June	640	640	640	640	640	1280
	Rough..... July	160	160	160	80	80	160
	h..... Aug.	640	640	640	640	640	640
	O2..... Sept.	640	640	640	640	640	640
	Rough..... Oct.	320	320	320	320	320	320
	n..... Nov.	320	320	320	320	320	320

* Titers are expressed as the reciprocal of the highest dilution yielding complete hemagglutination.

specific for the antigenic types. Loss of serotype O2 in D for the month of June was combined with a relatively high normal antibody titer of 1:320 which was higher than the titer of 1:80 for the new *E. coli* serotype "x" of July. Individual E, on the other hand, had an antibody titer of 1:10 for his departing June flora and acquired a new serotype in July for which he had a constant titer of 1:160. Persistence of residents did not seem to be correlated with antibody level. The resident strains "x" of individual D, "z" of subject A, *E. coli* O1 of individual C, and O2 of individual F established themselves and remained in the colon of their hosts regardless of whether the homologous antibody level was as low as 1:10 or as high as 1:640. The same lack of correlation was true for the evanescence of transients. Transients such as "b" of individual E, and "g" and O112ab of subject C, disappeared from the intestinal tract of individuals with homologous antibody levels of 1:160, 1:80, and 1:10 to 1:20, respectively.

Although the earlier study, which revealed that hosts develop antibody to autologous *E. coli* flora, was carried out on infants during attacks of diarrhea (Young et al., 1959), it was presumed that a similar antibody response might be demonstrable in adults as well. In the present study, three of the six persons (D, E, and F) had diarrhea of unknown etiology one or more times during the study, but no rise of serum antibody titer for their *E. coli* flora occurred. Each person tended to have similar antibody levels for both resident and transient flora, and the titers remained basically constant throughout the study. The antibody-producing mechanism of the host apparently was not stimulated during either long- or short-term residence. A possible exception to this was subject A. During late summer desensitization to hay fever pollens, individual A had higher antibody titer for "z" strain of July, August, and November. He developed greater serum avidity which was visible for all the hemagglutination readings of July, August, and September sera, but October and November serum readings were equivalent to those of June. This may have been due to immunization with pollen antigens similar to the bacterial antigens, or to a nonspecific anamnestic reaction whereby any immunization will cause a boost in normal antibody titers.

In several instances, polysaccharide preparations of different isolations of a single serotype were apparently variable in antigenic potency for the hemagglutination test (Table 2). The dominant resident "x" of subject D reacted with the host's sera to a titer of 1:80 for three successive months but, during the fourth month, the strain isolated reacted to a titer of only 1:10. Other examples of similar strain variations are *E. coli* O22 of subject E and "z" serotype of individual

A. The latter is an example of polysaccharide antigens of an *E. coli* serotype which exhibited higher titers of 1:160 to 1:640 with the November isolate in contrast to a previous titer range of 1:20 to 1:160. These changes may be explained as either a quantitative loss or gain of specific polysaccharide available for extraction (Tawil and El Kholy, 1959) or a minor qualitative difference in the antigenic composition of the strain. A loss of polysaccharide content which occurs when *E. coli* strains become rough (Wardlaw, 1960) would result in a sensitizing antigen of lowered potency and therefore lowered ability of the sensitized red blood cells to combine with specific antibody. The organism may also change its antigenic composition by acquiring minor antigens for which the host may or may not have antibody. The resulting altered bacterial polysaccharide, containing newly acquired fractions, would have higher or lower antibody titers in the hemagglutination test.

Further studies revealed that normal hemagglutinating antibody levels were more characteristic of the host than of his *E. coli* flora. For example, subject F maintained high antibody titers of 1:320 to 1:640 for *E. coli* isolated from other persons as well as for those inhabiting his intestinal tract. Likewise, individual A showed relatively low antibody titers (1:80) for heterologous as well as autologous flora. Normal antibodies in man may or may not be produced in response to specific bacterial antigens, and a wide variety of natural substances are probably responsible for their stimulation (Wilson and Miles, 1955). Such antibody levels therefore understandably vary with the individual host. Furthermore, the effect of normal antibody on bacteria not in direct contact with the circulation, i.e., dwelling in an intestinal habitat, is still unknown.

Apparently the persistence of an *E. coli* flora in these six individuals did not serve to stimulate the antibody-producing mechanism of the host as measured by hemagglutination tests, nor did the hosts' normal antibody levels in any way seem to influence the course of the numerous *E. coli* serotypes which were either resident or transient during the study.

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