HI tests for 2 or more bacterial strains isolated from an infant with diarrhea usually yield findings at considerable variance from one another. These would be difficult to explain except on the assumption that the HI test is specific. For example, the acute phase serum from a 5-months-old infant had no demonstrable antibodies or inhibitors for enteropathogenic *E. coli* 0127 isolated from his feces, but had a 3 tube inhibition against *E. coli* 015 which he also carried in his intestine. Similarly, the acute phase serum obtained from a 7-months-old child had a hemagglutinin titer of 128 for *E. coli* 018 and a 3 tube inhibition for *E. coli* 015 though both serotypes were isolated from his feces at the same time.

The HI test presented here has been successfully used to provide evidence for the presence in sera of patients with diarrhea of substances which are apparently related antigenically to bacteria present in their intestine. Results of such tests are presented elsewhere(13). The nature of the circulating inhibitor is unknown. It might be a complex substance or a simple sugar(14). Studies are continuing to further its characterization.

Summary. A simple hemagglutination-inhibition (HI) technic is presented which enables detection of inhibitors in patients' sera during diarrhea. These inhibitors thus far appear to be antigenically related to bacteria isolated from the patients' intestine at the same time and may well be substances derived from them.

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Infectious Agents in Infant Diarrhea: II. Serological Reactions with *Escherichia coli* 01 through 025. (26202)

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A number of *Escherichia coli* serotypes have been established as being associated with diarrhea in infants and young children, *i.e., E. coli* 0111:B_4, 086:B_7, 026:B_6, 0.55:B_5, etc.(1). Some of the *E. coli* serotypes 01 through 025 have also been described as possessing pathogenic properties(2,3). Grönroos(4), in an attempt to relate *E. coli* serotypes 01 through 025 to infant diarrhea, studied the feces of healthy and diarrheal infants and was unable to demonstrate significant differences in the two groups. Ørskov (5) carried out a similar study with 130 *E. coli* 0 immune sera and found that strains representing specific serotypes were found in about the same frequency in both groups of

infants. The response of the host by antibody formation against "normal" *E. coli* serotypes 01 through 025 during attacks of diarrhea, however, has apparently not been determined. This study presents the results of an investigation of serological reactions of these serotypes both for antibody formation and for substances in the circulation which appear to be antigenically related to the *E. coli* serotypes isolated from the intestinal tract of infants.

Materials and methods. 1. Bacteriologic procedure. Rectal swabs were obtained from 118 infants and young children with diarrhea from Children's Hospital, Washington, D.C., and from 29 others from the Municipal Hospital, San Juan, Puerto Rico. These samples were frozen immediately in dry ice, then thawed within one week and cultured on eosin-methylene-blue, MacConkey and blood agar plates. All organisms were isolated, identified biochemically, and the serotypes determined using methods described by Edwards and Ewing(6). The first serum was drawn on admission during the acute place of the disease and a second serum, whenever possible, was obtained from 7 to 21 days later during convalescence.

2. Serologic procedures. a. Hemagglutination (HA) test: Neter *et al.*(7) have shown that the HA test affords a sensitive method of determining antibody response to *E. coli* infections. Our modification of this technic(8) was used for this study.

b. Hemagglutination-inhibition test: All sera negative for hemagglutinins at a final dilution of 1:8 were further tested for the presence of "inhibitors"(8,9) which were antigenically related to the homologous serotype of *E. coli* isolated from the infant's intestinal tract. Procedure for the HI test is published in the preceding paper of this series(8).

Results. Fifty-eight strains of *E. coli* 01 through 025 representing 17 O serotypes were isolated from the feces of 147 diarrheal infants and young children studied. Table I shows the number of strains studied and whether hemagglutinins and/or inhibitors were detected in the patients' sera. *E. coli* 015 was the most frequently encountered of the 17 serotypes found; 19% of the 58 *E. coli* strains were of this antigenic type. The serotypes not listed in Table I were isolated from only 1 or 2 patients. Of the total of 30 infants who had *E. coli* serotypes 01 through 025 present in their feces, and from whom acute and convalescent sera were available, 12 or 40% responded with a rise in antibody titer. Furthermore, of a total of 21 infants who had no hemagglutinins present in their acute phase sera, 14 or two-thirds had inhibitors present which were antigenically related to the *E. coli* serotype isolated from their fecal flora. Eleven convalescent sera were obtained from these same infants 1 to 3 weeks after the acute sera were drawn. Seven of the sera had inhibitors present, one showed a disappearance of inhibitor, and in 3 instances hemagglutinins had developed.

TABLE I. Distribution and Serologic Reactions of the Most Common O Groups of *E. coli* 01 through 025 in Infants with Diarrhea.

E. coli serotype	Total No. of patients	No. of patients with HA antibody titer rise	No. of patients with Acute serum inhibitors
015	11	4/6*	5/5†
02	6	0/3	3/4
04	6	1/4	NS
06	5	0/2	2/2
07	5	1/4	1/3
017	4	1/3	1/3
08	3	1/2	NS
011	3	1/1	NS
016	3	1/2	1/2
019	3	2/3	1/2
Totals	49	12/30	14/21

* No. of patients with HA titer rise/No. of paired sera available for testing.

† No. of patients with serum inhibitors/No. of acute phase sera available for testing which contained no hemagglutinins.

NS = No serum available.

Note: Less common serotypes isolated: *E. coli* 013 and 025, 2 each; *E. coli* 01, 03, 012, 018 and 021, 1 each. (Only one of these strains, *E. coli* 012, had any significant serologic reaction, *i.e.*, inhibitors present in patient's serum.)

The serologic reactions with these *E. coli* groups can best be observed by examination of the results obtained with the most frequently encountered serotype, *E. coli* 015. These serologic findings are presented in Table II, together with the reactions of associated organisms and ages of the patients. Table II is arranged in sequence with those reactions listed first in which inhibitors were

TABLE II. Serologic Reactions with *E. coli* 015 and Associated Organisms.

Patient No.	Patient's age	*E. coli* 015 and associated organisms	Serologic findings: Acute serum titers HI*	Acute serum titers HA†	Convalescent serum titers HI*	Convalescent serum titers HA†
1	2 mo	015	4	0	4	0
		017	1	0	1	0
2	4 "	015	3	0	3	0
3	5 "	015	3	0	NS	NS
		0127	0	0	NS	NS
4	7 "	015	3	0	—	16
		018	—	128	—	128
5	8 "	015	3	0	—	32
6	10 "	015	—	256	NS	NS
7	1 yr	015	—	32	NS	NS
8	2 "	015	—	32	—	128
		Sh. flexneri 2	—	16	—	20480
9	3 "	015	—	32	—	128

* HI titer = No. of 2-fold dilutions of specific antiserum inhibited in HI reaction: Measurement of bacterial substance present in patient's serum.

† HA titer = Reciprocal of highest dilution yielding complete hemagglutination: Measurement of antibody.

NS = No serum available. — = Hemagglutinins present; test not performed.

present in both acute and convalescent phase sera; second are shown reactions in which inhibitors in acute phase sera were followed by antibody in the convalescent phase sera; and lastly are shown those reactions with antibodies present in acute phase sera which may or may not have shown an increase in the convalescent phase sera. When the data, arranged in sequence as in this table, are compared to the patients' ages, it can be seen that some association exists. Table II shows that in general the highest antibody titers were found in children over 7 months of age (Patients No. 4 through 9) and inhibitors were prevalent in the younger infants. Inhibitor persisted in the convalescent serum of one 8-months-old child harboring *E. coli* 06; otherwise it appeared only in the convalescent sera of infants 4 months of age or younger. On the other hand, hemagglutinin titer rises were not confined to older age groups, though they were much more frequently observed in children over 6 months of age as shown in Table III, which presents *E. coli* serotypes 01 through 025 which stimulated an antibody response and ages of the children from whom each was isolated. Patients No. 1, 2 and 3, 7 weeks old, 3 months old and 4 months old respectively, had *E. coli* serotypes 011, 016 and 08 isolated from their fecal samples during an attack of diarrhea and responded with a rise in hemagglutinating antibodies. Nine other children over 6 months of age experienced similar rises.

Discussion. An investigation of the serological reactions with *E. coli* 01 through 025 clearly revealed that a hemagglutinin titer rise for these organisms is a common finding when these serotypes are present in an infant's intestine during an attack of diarrhea. Utilizing the bacterial agglutination test Grönroos(4) was able to demonstrate ho-

TABLE III. *E. coli* Serotypes 01 through 025 Stimulating an Antibody Response and Ages of Patients from Whom Each Was Isolated.

Patient No.	Patient's Age	*E. coli* serotype	Hemagglutination titers: Acute sera	Hemagglutination titers: Convalescent sera
1	7 wk	011	8	128
2	3 mo	016	0	8
3	4 "	08	8	64
4	7 "	015	0	16
5	8 "	04	8	64
6	8 "	015	0	32
7	15½ "	019	0	16
8	20 "	019	32	256
9	22 "	07	128	1024
10	2 yr	015	32	128
11	3 "	015	32	128
12	7 "	017	16	256

0 = No hemagglutinins demonstrable in a serum dilution of 1:8.

mologous antibodies in the sera of only 7 of 115 patients positive for enteropathogenic *E. coli*. McNaught(10) found HA and agglutination tests to be of much the same sensitivity for detecting presence of serum antibodies to homologous strains of *E. coli*, but Neter *et al.*(11) in a study of the serological aspects of feeding experiments with human volunteers, found the hemagglutinin titers 5 to 20 times higher than O-agglutinin titers. In later studies with a variety of enteric pathogens Neter *et al.*(7) found hemagglutinin titer levels 2 to 64-fold higher in every case. Our experience with the HA test(9) corroborates that of Neter.

While many of the antibody elevations for *E. coli* 01 through 025 during diarrhea are of a low order, as revealed even by the sensitive HA test, many of the infants were quite young and some had no detectable antibodies prior to the diarrheal attack. The antibody rise is not due to a non-specific anamnestic reaction, as shown by Neter, *et al.*(7) who found no such rise to enteric pathogens other than the causative agent in cases of diarrhea, and also indicated in our results by the frequent antibody titer rises to only one of the enteric bacteria isolated from the intestine. A rise in antibody titer cannot be interpreted as proof that the corresponding organism was responsible for the disease state, but does clearly show that the *E. coli* serotype present in the infant's intestine, or some fraction thereof which is capable of stimulating antibody production, is passing the mucosal barrier. Such episodes provide one mechanism by which the child develops antibodies against so-called "normal" *E. coli* intestinal flora.

The second finding revealed by the serological investigation of *E. coli* 01 through 025 in infant diarrhea, would tend to corroborate the presumption that bacterial substances pass from the infants' intestine into the circulation. Substances were found to be circulating in the blood stream of the infant patient which were closely enough related to the organisms isolated from the intestinal contents to combine with specific antisera and inhibit the HA test. Such substances derived from bacteria might theoretically be any one of a number. Protein (12) and lipids(13) are capable of such inhibition by interfering with the sensitization of red blood cells by antigen. Since previously sensitized cells are added to the patient serum-rabbit antiserum in the HI test, lipids and proteins should not be capable of inhibiting the test through interference with red cell sensitization. However, material as simple as single sugars has been shown to neutralize agglutinins(14). Davies *et al.* (15) demonstrated that abequose inhibited HA tests performed with red blood cells sensitized with polysaccharide derived from *E. coli* 055 and 0111. The possibility that such simple sugars would be adsorbed in sufficient quantities to be inhibitory without being metabolized or eliminated, is remote. Bacterial polysaccharides, on the other hand, are known to be capable of inhibiting the HA test(16) and a relatively large amount circulating in the blood is known to temporarily depress the reticuloendothelial system thereby interfering with polysaccharide removal(17). Circulating polysaccharide has been detected in diseases such as pneumococcal pneumonia in sufficient quantity to give a positive precipitin test(18). Buxton (19) in experiments with *Salmonella gallinarum* in young turkeys found that, at the peak of infection, polysaccharide was present in the circulation in sufficient quantity to adsorb onto the turkeys' red blood cells. It might be speculated that in young infants the large numbers of *E. coli* found as high up as the duodenum(20) would provide optimum conditions for adsorption of inhibitory substances.

The adsorbed polysaccharide in the experiments of Buxton(19) was demonstrated by use of the antiglobulin hemagglutination test, *i.e.*, incomplete antibodies were demonstrated to have adsorbed onto the sensitized cells *in vivo*. Neter *et al.*(21) have shown such incomplete antibodies to be present in maternal and cord blood. It is to be noted that the most strongly inhibitory sera in the present series were from the youngest infants in whom incomplete antibodies would be expected to be highest. Theoretically such incomplete antibodies should be able to in-

hibit the homologous rabbit antiserum HA titer. However, experiments designed to further investigate this possibility(8) proved negative in a number of strongly inhibitory sera. Red blood cells from the acutely ill cases presented here were not obtained, so tests for sensitization of the cells *in vivo* could not be made. Preliminary studies with cases of colienteritis lead us to believe that it could occasionally occur(22).

The disease-producing mechanisms of established enteropathogenic *E. coli* serotypes are little understood. An antibody response to *E. coli* serotypes 01 through 025 during diarrheal attacks and presence in the circulation of substances related to these serotypes has been demonstrated. These findings may provide a clue to some of the dynamic interrelationships between infant host and organisms present in the intestine.

Summary. An investigation was undertaken to determine the immunologic sequence of events during attacks of diarrhea associated with the presence of "normal" fecal flora, *E. coli* serotype 01 through 025, in the intestinal tract. It revealed that 12 of 30 or almost half of those infants from whom acute and convalescent phase sera were available exhibited a rise in hemagglutinin titer. At some time during their illness a greater number, two-thirds, had inhibitors present in their sera which were antigenically related to the *E. coli* serotype isolated from their fecal flora.

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