Acute Undifferentiated Human Diarrhea in the Tropics

I. ALTERATIONS IN INTESTINAL MICROFLORA

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ABSTRACT The microflora of the small and large intestine was determined in 17 adults with acute undifferentiated diarrhea in Calcutta, India. On the basis of bacteriologic findings, the patients could be divided into two groups: those with a predominant flora of *Escherichia coli* (eight patients) and those with a mixed coliform flora (nine patients). In the former group, *E. coli* were distributed throughout the small and large bowel. Broth filtrates of these isolates contained an enterotoxin which caused fluid accumulation in the rabbit intestinal loop model. Toxigenic *E. coli* were cleared rapidly from the small bowel during the acute period; some patients only had the "hot" strains in their fecal effluent. During convalescence, the serotypes of *E. coli* changed and the new strains did not elaborate enterotoxin. Only one of the eight patients had a serotype previously associated with diarrhea. Acute undifferentiated diarrhea in the remaining cases was apparently caused by untypable *E. coli* or by typable strains not generally considered pathogenic.

Small bowel and fecal cultures from the mixed flora group revealed a heterogeneous mixture of Gram-negative enteric bacilli and a distinct pattern could not be discerned. Further study will be needed to elucidate the cause of diarrhea in these cases.

INTRODUCTION

Acute diarrheal disease is a major cause of morbidity and mortality of adults residing in the tropic. Although clinical and epidemiological data suggest an infectious etiology (1), pathogenic microorganisms cannot be iso-

lated in 50-80% of acute cases (2-5). This lack of microbiological diagnosis has led to the euphemistic terms "nonspecific gastroenteritis" and "acute undifferentiated diarrhea" (AUD).¹

Our approach to acute diarrheal disease in Calcutta, India, was to examine quantitative aspects of the intestinal microflora based on guidelines previously established in cholera diarrhea. In cholera, the invading pathogen colonizes the entire gastrointestinal tract, from the mouth to the anus (6, 7). *Vibrio cholerae* elaborates an enterotoxin which alters the physiology of the bowel mucosa causing net secretion of water and electrolytes (8). Similar alterations were looked for in patients with AUD by combining marker perfusion studies with intubation for bacteriology. Pathophysiological events were examined at the site of bacterial colonization of the small bowel during the acute and early convalescent periods; the results of the physiological studies are reported in the accompanying paper (9). Strains of bacteria designated as possible pathogens by bacteriological and marker perfusion studies were further studied in the isolated rabbit intestinal loop model. Cell-free, broth filtrates of *E. coli* isolated from diarrheal cases produced dilatation and fluid accumulation in the intestinal loop. These changes were similar to those induced by filtrates of *Vibrio cholerae*.

The purpose of these studies was to search for new pathogens in the causation of diarrheal disease in adults. Traditional emphasis on biochemical characteristics or somatic serotypes has caused microbiologists to overlook perhaps more important criteria: bacteria-induced, altered physiology in the host and reproduction of these events in animal models.

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¹ Abbreviations used in this paper: AUD, acute undifferentiated diarrhea; EEC, enteropathogenic *Escherichia coli*; TCBS, thiosulfate-citrate-bile salt-sucrose.

METHODS

During the year November 1967 to November 1968, 164 adult males with acute diarrhea were studied at the Infectious Diseases Hospital, Calcutta. Bacteriologic diagnosis was established in 112 cases and included *V. cholerae* biotype *eltor* (94 cases), noncholera *vibriosis* (16 cases), and *Shigella flexneri* (two cases). The remaining patients were considered to have acute undifferentiated diarrhea.

17 of the 52 AUD cases were selected for intubation studies on admission and comprise the material for this report. Informed consent was obtained in advance from all patients. Selection for intubation was based on choosing the more severely ill cases admitted on any particular day. Patients were intubated before the bacteriological diagnosis was known since the laboratory usually required 1 to 3 days to identify enteric pathogens. Patients with diarrhea, not selected for the intubation protocol, had bacteriological studies made of the fecal effluent as outlined below.

After an initial period of rehydration (generally 1 to 3 hr), a triple-lumen, polyvinyl tube was passed perorally under fluoroscopic control. All samples were obtained while the patients were fasting or at least 2 hr after eating. During convalescence, generally 7 to 10 days after admission, intubation for bacteriology and perfusion was repeated. The details of sampling in the small bowel and collection of fecal specimens have been outlined in previous publications (6, 7, 10, 11).

Quantitative and qualitative studies of intestinal samples were performed in eight media under aerobic and anaerobic conditions (6). To search for enteric pathogens, additional selective media were employed: SS agar, bismuth sulfate agar, MacConkey agar, bile salt agar and TCBS agar (thio-sulfate-citrate-bile salt-sucrose, Difco Laboratories, Inc., Detroit, Mich.). All specimens were enriched in selenite broth and alkaline peptone water (pH 9.2) and replated on SS agar and TCBS agar respectively.

With the aid of a hand lens and a dissecting microscope, multiple colonies were picked off MacConkey agar in an attempt to identify as many species and biotypes as possible. Suspicious colonies from the other selective media were also examined by biochemical and serological methods. In addition, 5-10 colonies were subcultured from the highest dilution MacConkey plates and preserved for serotyping. These procedures were carried out on all small bowel and fecal specimens.

Biotyping by biochemical and physiological characteristics was performed according to Edwards and Ewing (12). Specific antisera were available for salmonellae, shigellae, *V. cholerae*, and enteropathogenic *E. coli*. Oxidase-positive bacteria were classified according to Eddy and Carpenter (13). *E. coli* serotyping was accomplished at the National Communicable Disease Center, Atlanta, Georgia, under the supervision of Dr. W. Martin and Miss Betty Davis.

Viral isolation was attempted in 14 of the 17 patients selected for intubation. Small bowel samples collected during the acute illness were inoculated in Hep-2 and monkey kidney tissue cultures and in suckling mice. (This work was performed by Dr. William Hillis).

Rabbit intestinal loop test. Strains of *E. coli* were studied for enteropathogenicity in the rabbit model originally described by De and Chatterjee (14) and revised by Burrows and Musteikis (15). The test strain was grown in syncase broth (16) for 18 hr in a shaking water bath at 37°C. Following centrifugation, the supernatant was filtered through a 0.45 μ Millipore filter (Millipore Corp., Bedford, Mass.).

Leunberg rabbits (Scientific Small Animal Laboratories), weighing approximately 2 kg with an average age of 10 wk, were used throughout these experiments. These are the same animals obtained by Dr. W. Burrows and our techniques were standardized by cooperation and advice from his laboratory. After 48 hr of fasting, the animals were anesthetized and six intestinal loops of 10-12 cm length were constructed starting at the terminal ileum and proceeding proximally. Four of these loops were injected with 2 ml of sterile broth filtrate from different test strains; the remaining two loops were used for a negative control (un-inoculated broth) and a positive control ("whole cell lysate" from *V. cholerae* 569B supplied by Dr. Burrows). The order of loop inoculation was randomized among the six test materials and changed in each animal. The animals were sacrificed at 18 hr and the loops were examined for dilatation and fluid accumulation. A positive loop had a volume: length ratio of greater than 1.0.

Approximately five strains of each *E. coli* serotype were examined in this rabbit model. Sterile broth filtrates of these strains were prepared on two separate occasions; each filtrate was injected into four animals. Hence, the five strains of a particular serotype were each tested in eight intestinal loops in as many animals.

RESULTS

The 17 AUD cases with complete bacteriological studies of their small and large intestine could be divided into 2 groups.

TABLE I
Rank Order of Prevalence of Microorganisms in Stool of Patients with Diarrhea (Mean Log₁₀/ml or gm)

Rank order	E. coli flora (8 patients)		Mixed flora (9 patients)	
	Acute	Convalescent	Acute	Convalescent
1	<i>E. coli</i> (8.6)	<i>Bacteroides</i> * (8.6)	Coliforms (mixed) (7.4)	<i>Bacteroides</i> * (8.7)
2	<i>Bacteroides</i> (5.8)	Coliforms* (7.6)	Streptococci (6.5)	Streptococci* (7.8)
3	Streptococci (5.3)	Streptococci* (7.5)	<i>Bacteroides</i> (5.8)	Coliforms‡ (7.2)
4	Anaerobic lactobacilli (4.0)	Anaerobic lactobacilli* (7.4)	Clostridia (3.9)	Anaerobic lactobacilli* (7.1)
5	Aerobic lactobacilli (3.8)	Aerobic lactobacilli* (5.2)	Anaerobic lactobacilli (3.0)	Aerobic lactobacilli* (5.9)
6	Fungi (2.1)	Clostridia* (4.0)	Aerobic lactobacilli (2.2)	Clostridia* (5.1)
7	Staphylococci (2.0)	Fungi* (3.0)	Staphylococci (1.8)	Fungi‡ (2.3)
8	Clostridia (1.0)	Staphylococci‡ (1.7)	Fungi (1.6)	Staphylococci‡ (2.2)

* Differences between acute and convalescent values are significant ($P < 0.02$).

‡ Differences between acute and convalescent values are not significant ($P > 0.05$).

E. coli group (eight patients)

Acute studies. The microflora of the intestinal tract was composed largely of *Escherichia coli*. The concentration of *E. coli* in the fecal effluent was generally 1-3 logs higher than other aerobic or anaerobic bacteria (Table I). In seven of the eight patients, these organisms were also found in the upper small bowel. The most proximal site was the stomach in five cases, duo-

denum in one case, and midjejunum in one case (Table II).

Serotyping of *E. coli* isolates showed that five of the eight cases had a single serotype in the small and large bowel (Table II). Patient 931 had two serotypes (078 and 0126) in approximately equal concentrations. Two patients had different serotypes in the small bowel and stool. In patient 79, nine isolates from the fecal effluent were 06:H16, but multiple colonies cultured

TABLE II
Concentrations and Serotypes of E. coli in the Gastrointestinal Tract of Eight Patients with Diarrhea and a Predominant E. coli Flora

Patient	Concentrations and serotypes of <i>E. coli</i>				
	Stomach and Small Bowel			Stool	
	Location	<i>E. coli</i> /ml	Serotypes	<i>E. coli</i> /ml	Serotypes
924	Stomach	10 ⁵	015:H11	10 ⁷	015:H11
	Duodenum	10 ⁵	015:H11		
	Mid jejunum	10 ⁴	015:H11		
928	Mid jejunum	10 ³	078:H12	10 ⁹	078:H12
	Upper ileum	10 ⁶	078:H12		
	Terminal ileum	10 ⁸	078:H12		
931	Stomach	10 ⁶	078:H12* 0126:H12:K?*	10 ⁸	078:H12* 0126:H12:K?*
	Duodenum	10 ⁵	078:H12* 0126:H12:K?*		
	Upper jejunum	10 ⁶	078:H12* 0126:H12:K?*		
936	Stomach	10 ⁶	025:H42	10 ⁸	025:H42
	Duodenum	10 ⁷	025:H42 026:H32‡ 0115:H42‡		
	Mid jejunum	10 ⁶	025:H42		
969	Stomach	10 ³	015:H11	10 ⁹	015:H11
	Lower jejunum	10 ³	015:H11		
79	Stomach	10 ²	0115:H27	10 ⁸	06:H16
	Duodenum	10 ⁵	0115:H27		
	Upper jejunum	10 ⁶	0115:H27		
	Mid jejunum	10 ⁷	0115:H27		
	Lower jejunum	10 ⁶	0115:H27		
89	Duodenum	10 ³	0126:H12:B16	10 ⁹	0126:H12:B16
	Mid jejunum	10 ⁶	0126:H12:B16		
966	Mid ileum§	10 ⁸	012:untypable H untypable 0:H27‡	10 ⁸	Untypable 0:H27

* Two serotypes present in approximately the same concentration.

‡ One colony.

§ No *E. coli* in the upper small bowel.

FIGURE 1 Intestinal microflora in a patient (No. 924) with AUD.

from the small bowel were 0115:H27. Similarly, six colonies from stool of patient 966 were untypable 0:H27; however, of six colonies from the midileum, five were 012:untypable H and only one was untypable 0:H27.

Type 0126 containing K antigen were isolated from patients 931 and 89. The K antigen from the latter was B16, indicating a recognized enteropathogenic serotype (0126:B16). The K antigen from patient 931 was not B16, but it could not be further identified.

Components of the normal microflora were also present during the acute episode. In patient 924, (Fig. 1), *Streptococci* and *Bacteroides* were recovered from all areas of the bowel; in addition, *Klebsiella* and *Pseudomonas* were found in the ileum and stool. (The *Bacteroides* strains in the small bowel failed to grow in 10% bile suggesting that they originated in the oral cavity [17]).

FIGURE 2 Intestinal microflora in a patient (No. 966) with AUD. Coliforms were not present in the jejunum.

E. coli were not found in the upper small bowel of patient 966, but rather large concentrations (10^8 /ml) were present in the ileum (Fig. 2). Smaller numbers of *Streptococci*, *Klebsiella*, and *Pseudomonas* were isolated from the small bowel, but *Bacteroides* were only in the feces.

Convalescent studies. When restudied, 7–10 days after admission, three patients had *E. coli* in the upper small bowel (Fig. 3). The serotypes changed in two (Nos. 924 and 931) and remained the same in the third (No. 79) (Table III). In the last patient, the original serotype (0115) isolated from the small bowel persisted during convalescence and also colonized the large bowel; however, the serotype (006) formerly present in the feces was eliminated. Patient 924 was restudied 2 months after the acute episode and coliforms were still present in the jejunum (Fig. 3). No coliforms were found in the jejunum of the other four patients who previously had organisms in this region.

Fecal microbial populations also changed in the convalescent studies (Table I). For example, coliforms were significantly reduced. *E. coli* remained the most prevalent species, but there were two to four different biotypes represented. Furthermore, serotypes found in the acute period were not present in the follow-up specimens.

The most striking change in the convalescent fecal specimens was the increased concentration of anaerobic bacteria. Viable counts of *Bacteroides*, anaerobic lactobacilli, and clostridia rose in all patients by 1–4 logs.

Mixed coliform group (nine patients)

Acute studies. The fecal microflora contained several serotypes and biotypes of enteric bacteria, and it was

FIGURE 3 Concentrations of coliforms in jejunal fluid of patients with AUD. Studies were performed in the acute period and in the early and late convalescent periods.

difficult to select a predominant strain (Table IV). *E. coli* was the most prevalent species in five of the nine patients, but several biotypes were isolated as well as other Gram-negative bacteria. The small bowel also contained two to three different species, and a distinctive pattern was not obvious.

Viable coliform counts were not increased in the acute phase (Table I). The change in rank order of prevalence in the follow-up studies was due to increased numbers (1-4 logs) of anaerobic and microaerophilic bacteria.

E. coli isolated from the small bowel or stool during the acute stage were screened with the enteropathogenic *E. coli* typing sera.^a There were no positive agglutinations among the 25 strains from these nine patients.

Convalescent studies. Small numbers of coliforms (*E. coli*, *Klebsiella* and *Enterobacter*) persisted in the jejunum of three of five patients during convalescence (Fig. 3). As in the *E. coli* group, patients with initial coliform concentrations of less than 3.5 log/ml in the jejunum appeared to clear these organisms after the acute diarrheal episode. One patient (No. 912) was available for restudy 4 months later and coliforms were no longer found in the upper small bowel.

Rabbit loop studies. Broth filtrates obtained from *E. coli* strains in the five patients who had a single serotype in their small bowel and feces produced positive reactions in the intestinal loop (Table V). Patient 931 had two serotypes (078 and 0126) in the GI tract in approximately equal concentrations; broth filtrates of both strains were reactive in the rabbit intestinal loop.

In patient 966, two serotypes were isolated from the mid ileum, 012:untypable H (10⁸/ml) and untypable 0:H27 (10⁷/ml); in the stool, however, only the untypable 0:H27 could be identified. The latter serotype from either the small bowel or stool was found to be toxigenic whereas the 012:untypable H was unreactive in the rabbit loop. Similarly, patient 79 had different serotypes in the small bowel and feces. The strains from the feces (06:H16) were toxigenic but the small bowel isolates (0115:H27) were unreactive. The toxigenic strain (06:H16) may have been cleared from the small bowel at the time of the initial study. It was replaced by 0115:H27 which persisted in convalescence and by that time had colonized the entire GI tract.

E. coli strains which were obtained during convalescence from the small bowel and feces were studied in four patients, (Nos. 924, 931, 966, and 79). Broth filtrates of convalescent strains were uniformly negative in the rabbit intestinal loop.

Viral studies. No enteroviruses were isolated from

^a 026:B6, 055:B5, 0111:B4, 0127:B8, 086:B7, 0119:B14, 0124:B17, 0125:B15, 0126:B16, 0128:B12. (Difco Laboratories, Inc., Detroit, Mich.).

TABLE III
*Coliforms in the Jejunum of Patients Convalescing from Diarrhea Who Had a Predominant E. coli Flora during the Acute Episode**

Patient	Microorganisms in jejunal fluid	
	Biotypes	<i>E. coli</i> serotypes
924	<i>E. coli</i> (3 types) <i>Alcaligenes faecalis</i>	Changed to untypable 0 (Rough:H19)†
931	<i>E. coli</i> (3 types)	Changed—not typed§
966	<i>Klebsiella</i> species	-----
79	<i>E. coli</i>	0115:H27‡—Same as previous small bowel strains; previous stool strains (06:H16) were not found

* Patients 928, 936, 969, and 89 had no coliforms in the jejunum when studied during convalescence.

† Same serotype present in stool.

§ These strains did not agglutinate with 015 serum (previous serotype); however, definitive typing was not done.

small bowel specimens of 14 patients either by direct tissue culture inoculation or by two subsequent blind passages. There was a single isolation of a reovirus-type 1 from patient 85.

DISCUSSION

Certain serotypes of *Escherichia coli* are known to cause acute gastroenteritis in humans. Such strains, designated enteropathogenic *E. coli* (EEC), have generally been associated with outbreaks of diarrhea in infant nurseries (18, 19). The disease can be reproduced by feeding EEC to healthy adult volunteers (20, 21).

While the search for enteropathogenic serotypes has proven fruitful in infantile diarrhea, sporadic cases of gastroenteritis in children or in adults are not usually associated with these type-specific strains. This was our experience in Calcutta, since only 1 of 17 cases of AUD had a recognized enteropathogenic serotype (0126:B16).

The location of coliform organisms within the GI tract is altered during acute diarrhea. In healthy individuals, the stomach, duodenum, and jejunum are free of coliform organisms (10, 11). Colonization of this area occurs in AUD and in diarrhea caused by *Vibrio cholerae* (7) and EEC (22). Coliforms are generally cleared from the upper small bowel within 7-10 days of the acute episode but some patients may have persistence of this abnormality for several weeks (7).

The rabbit intestinal loop model was originally designed to study the toxigenicity of cholera vibrios (14). Taylor et al. subsequently showed that pathogenic

strains of *E. coli* from cases of infantile diarrhea also caused dilatation of the rabbit intestinal loop (23, 24). However, the same serotypes of *E. coli* isolated from the feces of healthy carriers or from extra-intestinal sources such as the urinary tract were unreactive in this model.

The rabbit is not the only animal susceptible to the enteropathogenic effects of *E. coli*. In our laboratory

we have observed net secretion and fluid accumulation in the GI tract of dogs receiving broth filtrates of *E. coli* isolated from our cases of AUD. Furthermore, in collaboration with Dr. Sam Formal of the Walter Reed Army Institute of Research, diarrhea has been produced in monkeys challenged with viable cultures of these strains.

There are other enteropathogenic serotypes of *E. coli*,

TABLE IV
Concentrations and Biotypes of Coliforms in the Gastrointestinal Tract of Nine Patients with Diarrhea and a Mixed Coliform Flora

Patient	Concentrations and biotypes of coliforms				
	Stomach and Small Bowel			Stool	
	Location*	Organisms/ml	Biotypes	Organisms/ml	Biotypes
912	Upper jejunum	10 ⁵	<i>Klebsiella</i> species	10 ⁸	<i>Escherichia coli</i>
		10 ³	<i>Pseudomonas</i> species	10 ⁸	<i>Escherichia coli</i> -A-D†
		10 ²	<i>Escherichia coli</i>	10 ⁶	<i>Pseudomonas</i> species
				10 ⁵	<i>Proteus morganii</i>
				10 ⁴	<i>Vibrio</i> species (non-cholera)
916	Upper ileum	10 ⁵	<i>Aeromonas formicans</i>	10 ⁷	<i>Escherichia coli</i> (3 serotypes)
		10 ⁵	<i>Escherichia coli</i>	10 ⁵	<i>Proteus morganii</i>
927	Lower jejunum	10 ³	<i>Enterobacter cloacae</i>	10 ⁵	<i>Enterobacter cloacae</i>
		10 ²	<i>Escherichia coli</i>	10 ³	<i>Escherichia coli</i> (2 biotypes)
934	Upper jejunum	10 ⁷	<i>Pseudomonas</i> species	10 ⁷	<i>Escherichia coli</i> (3 biotypes)
		10 ⁵	<i>Klebsiella</i> species		
		10 ⁴	<i>Escherichia coli</i>		
941	Terminal ileum	10 ⁵	<i>Escherichia coli</i>	10 ⁶	<i>Escherichia coli</i>
		10 ⁴	<i>Klebsiella</i> species	10 ⁶	<i>Escherichia coli</i> -slf§
				10 ⁵	<i>Klebsiella</i> species
				10 ⁵	<i>Enterobacter cloacae</i>
				10 ²	<i>Pseudomonas</i> species
945	Mid ileum			10 ²	<i>Alcaligenes faecalis</i>
		10 ⁵	<i>Enterobacter cloacae</i>	10 ⁷	<i>Escherichia coli</i> (2 biotypes)
		10 ⁴	<i>Klebsiella</i> species	10 ⁶	<i>Klebsiella</i> species
		10 ³	<i>Pseudomonas</i> species	10 ⁶	<i>Enterobacter cloacae</i>
				10 ⁴	<i>Alcaligenes faecalis</i>
85	Stomach	10 ⁵	<i>Escherichia coli</i>	10 ⁷	<i>Escherichia coli</i>
		10 ⁵	<i>Klebsiella</i> species	10 ⁷	<i>Klebsiella</i> species
		10 ⁵	<i>Aeromonas formicans</i>	10 ⁷	<i>Aeromonas formicans</i>
86	Upper ileum	10 ⁷	<i>Escherichia coli</i>	10 ⁷	<i>Escherichia coli</i>
		10 ⁶	<i>Alcaligenes faecalis</i>	10 ⁷	<i>Escherichia coli</i> -slf§
				10 ⁷	<i>Klebsiella</i> species
914	Upper jejunum			10 ⁶	<i>Plesiomonas shigelloides</i>
		10 ³	<i>Escherichia coli</i>	10 ⁷	<i>Aeromonas formicans</i>
		10 ²	<i>Klebsiella</i> species	10 ⁶	<i>Escherichia coli</i>
				10 ⁶	<i>Vibrio</i> species (non-cholera)
				10 ⁵	<i>Klebsiella</i> species

* Most proximal site of positive culture.

† Alkalescens-Dispar Group (12).

§ slf-slow lactose fermenter.

different from the human strains, associated with diarrhea in hogs and calves. Extensive studies with the animal strains have defined a toxic material which causes fluid accumulation in the isolated intestinal loop model (25-27). Our preliminary experiments show that this material is similar to the enterotoxin from strains of *E. coli* isolated in human cases of diarrhea.⁸

The bacteriological and physiological techniques used in the present study of AUD have been previously applied to acute cholera (6-8). Several points of similarity can be seen between cholera and AUD associated with a predominant *E. coli* flora: (a) In both situations, the entire gastrointestinal tract may be heavily colonized by a specific pathogenic microorganism. (b) The production of diarrheal fluid is apparently due to net secretion in the small bowel at the site of bacterial colonization (9). However, mucosal architecture remains intact and there is no tissue penetration by vibrios (28) or coliforms (9). (c) Filterable, toxic material can be obtained from in vitro cultivation of the microorganism. Instillation of this product in an appropriate animal reproduces the pathophysiological events.

There are, however, important differences between *E. coli* diarrhea and acute cholera. The most obvious is the reduced morbidity of *E. coli* gastroenteritis as reflected by smaller volume of diarrheal fluid and shorter duration of symptoms (9). This may be related to a more rapid clearance of toxigenic *E. coli* from the small bowel; in some patients the toxigenic strain was apparently cleared or greatly reduced in the small bowel within the time required for intubation, i.e., 24-36 hr after onset of symptoms. By contrast, similar studies in cholera patients have shown that vibrios were not cleared from the small bowel until 4-8 days after admission (7).

The patients with a mixed coliform flora still represent a diagnostic enigma. Although alterations in coliform biotypes have been observed in a variety of naturally occurring and experimental diarrheal diseases (29-34), it is still possible that certain microorganisms from the feces of our patients with a mixed flora might be pathogenic. For example, the experiments of Koya, Kosakai, and Fukasawa (35) with implantation of EEC in healthy volunteers showed that subjects who developed only mild diarrhea had a mixed intestinal microflora consisting of the pathogenic *E. coli* as well as other elements of the coliform flora. Those with severe diarrhea had a flora predominantly of the implanted EEC strain. Since the patients with a "mixed flora" in our series had relatively mild disease, pathogenic bacteria may be included among the several strains which were

TABLE V
The Effect of Broth Filtrates of *E. coli** on the Rabbit Intestinal Loop Model.

Patient	Acute period		Convalescent period	
	<i>E. coli</i> strain	Rabbit loop test	<i>E. coli</i> strain	Rabbit loop test
924	015:H11	+	?‡	0
931	078:H12	+	?‡	0
	0126:H12:K?	+		
79	06:H16	+	0115:H27	0
	0115:H27	0		
966	Untypable 0:H27	+	?‡	0
	012:untypable H	0		
928	078:H12	+	—	—
936	025:H42	+	—	—
	026:H32	0		
	0115:H42	0		
969	015:H11	+	—	—
89	0126:H12:B16	+	—	—

* *E. coli* strains were isolated from patients with diarrhea during the acute and convalescent periods.

‡ Convalescent strains were different from the serotypes of the acute period; however, definitive typing was not done.

isolated. As noted above, clearance of pathogenic strains from the gastrointestinal tract occurs very rapidly, further compounding the difficulty in recognizing them in patients with mild disease. Experiments are currently underway to examine each of the strains from the mixed flora group in a variety of animal models.

In addition to the changes in coliform flora, marked reductions in fecal anaerobes were noted in diarrheal stool. We have previously reported similar findings in cholera "rice-water" stool (6), and in diarrheal stool produced experimentally in volunteers by fluid purgation or by feeding lactose to hypolactasic subjects (34). Moore, Cato, and Holdeman (36) have also found reduced concentrations of anaerobes in the feces of patients with gastroenteritis. These oxygen-sensitive strains require an environment of low oxidation-reduction potential and stasis; rapid passage through the colon, such as occurs in diarrhea, appears to adversely alter the natural physiochemical environment which supports the growth of these fastidious microorganisms.

The main emphasis of this study of AUD in Calcutta is to show that strains of *E. coli* may cause diarrheal disease without necessarily being recognized as "enteropathogenic" by standard serological and biochemical reactions. In order to produce clinical diarrhea, it would

⁸ Sack, R. B., S. Gorbach, J. G. Banwell, B. Jacobs, and B. D. Chatterjee. 1970. Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. *J. Infect. Dis.* In press.

appear that three conditions must be fulfilled: The strain must be "toxigenic" as demonstrated in animal models; the concentration of these organisms must be of sufficient quantity— 10^6 /ml appears to be too few, but 10^6 – 10^8 /ml may be adequate; and the toxigenic strains of *E. coli* must be in contact with "sensitive" mucosa. This implies colonization of the upper small bowel since this area appears to be sensitive to *E. coli* toxin whereas the colon is probably unaffected (25).

Rowe, Taylor, and Bettelheim (37) have suggested that a previously unrecognized *E. coli* serotype, now designated 0148:K?:H28, was the cause of "Traveller's diarrhea" in English soldiers recently transferred to Aden. This is consistent with our findings in India that untypable *E. coli* or typable strains generally considered nonpathogenic may in fact be responsible for cases of acute undifferentiated diarrhea.

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