Gut, 1970, **11**, 1-6

Alterations in human intestinal microflora during experimental diarrhoea

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SUMMARY Large quantities of fluid administered through an intestinal tube caused alterations in small bowel bacteriology in four of seven subjects. In two normal individuals, colonic bacteria were found after fluid infusion in areas of the small bowel which previously had none. The faecal microflora was also altered by the fluid purge: anaerobes were reduced in some subjects and large numbers of *Enterobacter* species emerged in others.

In a patient with pancreatic insufficiency and diarrhoea, the concentration of *Enterobacter* in the faeces was directly related to the number of bowel motions per day. Treatment with pancreatic enzymes curtailed the diarrhoea and markedly reduced these organisms. Intubation showed that *Enterobacter* were harboured in the small intestine and suppressed in the large bowel during periods of normal bowel action. Diarrhoea caused by pancreatic enzyme withdrawal or fluid purgation removed the colonic inhibition and allowed these organisms to appear in the faeces.

Acidic diarrhoea induced by lactose feeding to three hypolactasic patients caused reductions in the numbers of *E. coli* in the stool but increases in *Enterobacter* species. Bacteroides also declined in one subject.

Certain alterations in small and large bowel bacteriology observed in these forms of experimental diarrhoea have also been described in naturally occurring diarrhoea of diverse aetiologies.

Diarrhoeal diseases of diverse aetiologies are known to alter the intestinal microflora. Abnormalities of faecal bacteriology, especially coliforms, have been noted in 'summer' diarrhoea (Topley and Wilson, 1936), traveller's diarrhoea (Kean, 1963), amoebic dysentery (Albert, Ricossé, and Picq, 1968), and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (Gorbach, Nahas, Plaut, Weinstein, Patterson, and Levitan, 1968). Furthermore, there are reports of bacterial contamination of the small intestine by salmonellae and enteropathogenic *E. coli* in children (Thomson, 1955) and by 'non-

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specific' enteric microorganisms in adults (Cohen, Kalser, Arteaga, Yawn, Frazier, Leite, Ahearn, and Roth, 1967) during acute gastroenteritis.

When the resident microflora is eclipsed by pathogenic bacteria (salmonellae, shigellae, or enteropathogenic *E. coli*), there is a direct causal relationship to the clinical disease. However, in 'non-specific' diarrhoea, no apparent explanation exists for changes in intestinal microbial populations. These changes may precede and be directly responsible for the diarrhoea or the bacterial flora may be secondarily disturbed by altered gastrointestinal motility and physiology.

In this paper we describe the bacteriology of the intestine in diarrhoea induced by fluid infusion or by feeding lactose to patients with hypolactasia. We show that experimental diarrhoea may cause changes in intestinal microflora similar to those reported in the naturally occurring disease.

Methods

INFUSION OF FLUID INTO THE SMALL INTESTINE

Fluid was infused in eight normal subjects and two post-gastrectomy patients (one post-gastrectomy patient also had chronic pancreatic insufficiency). A double-, or triple-lumen, polyvinyl tube was passed perorally under fluoroscopic control into the small intestine. Approximately 1,500 to 2,400 ml of sterile, isotonic electrolyte solution was administered via an infusion pump into the proximal tube at a steady rate over a two-hour period. All patients were fasting during the infusion and sampling period and had taken no food for at least eight hours before the study.

Sampling of the small intestine was done in six of the normal subjects and one of the post-gastrectomy patients by aspirating from the distal tube orifice 30 minutes after the infusion had ceased. Details of the sampling procedure and criteria for localization of the tube have already been described (Gorbach *et al*, 1968).

Liquid stool specimens were collected approximately 30 minutes after the infusion from four normal subjects and the post-gastrectomy patient with pancreatitis. (In two of the normal individuals both stool and small bowel specimens were obtained.)

STUDIES ON PATIENTS WITH HYPOLACTASIA

Three patients were studied who gave a history of lactose intolerance and in whom malabsorption of lactose was demonstrated radiographically (Laws, Spencer, and Neale, 1967). In two of the patients the deficiency of lactase was confirmed by estimating the enzyme activity in jejunal biopsies (G.N. 2·2 Units; E.M. 2·2 Units; normal values, greater than 2·5 Units). Diarrhoea was induced by giving 50 g of lactose by mouth.

MICROBIOLOGICAL METHODS

Small bowel and faecal specimens were delivered promptly to the laboratory for qualitative and quantitative bacteriological analysis. One gram of solid faeces or 1 ml of liquid sample was comminuted in 9 ml sterile saline and 10-fold tube dilutions were carried out. Aliquots (0·1 ml) of each dilution were applied to the surface of bacteriological media for aerobic cultivation (MacConkey, blood agar with and without 25 μg/ml neomycin, Mitis-Salivarus agar and mannitol salt agar) and anaerobic cultivation (blood agar with neomycin and Nagler-neomycin agar). Pour plates were made from Rogosa SL agar (aerobic and anaerobic) and Sabouraud's agar with chloramphenicol.

Representative colonies were subcultured from MacConkey plates for identification by biochemical characters (IMViC, carbohydrate utilization, O-F test in glucose, oxidase, motility, and amino acid decarboxylation). *Enterobacteriaceae* were classified according to the criteria of Cowan and Steel (1965).

The results are expressed as the $\log_{10}$ of the mean viable count per gram or millilitre of intestinal specimen. For example, 5,000 organisms /ml $= 5 \times 10^3$/ml $= 3{\cdot}7$ $\log_{10}$/ml. Previous studies with these techniques have shown that differences greater than 1·0 to 1·5 logs are generally significant.

Results

EFFECTS OF INFUSING FLUID INTO THE SMALL INTESTINE

Changes in the microflora of the small intestine

Small bowel sampling revealed that four of seven subjects developed significant changes in the microflora during the fluid purge. These included three normal subjects and a patient with a Polya gastrectomy. The remaining three normal subjects showed no significant alterations in small bowel bacteriology.

As shown in Table I, two normal subjects (nos. 1 and 2) had no coliforms or bacteroides in the mid jejunum and upper ileum respectively before infusion. After fluid administration, these organisms could be cultured from these sites in concentrations of 10^4 to 10^5/ml. The other normal subject (no. 3) had small numbers of coliforms, mostly *E. coli*, in the mid ileum before infusion. The tube remained in place for three days, and infusions were performed on the first and second days. Although there was an initial reduction in coliforms following administration of fluid, the viable count rose on each subsequent day (3·5/ml to 4·6/ml to 7·3/ml). Furthermore,

Subject Age/Sex	*Site of Tube*	*Time of Aspiration*	*Before (B) or After (A) Infusion*	*No. of Microorganisms (mean $\log_{10}$/ml)*			
				Coliforms	*Streptococci*	*Bacteroides*	*Anaerobic Lactobacilli*
1	Mid-jejunum	Day 1	B	0	2·6	0	0
38/F			A	4·0 Enterobacter	4·6	4·7	0
2			B	0	4·9	0	0
18/M	Upper ileum	Day 1	A	5·0 *E. coli*	2·0	5·4	0
3				5% Enterobacter			
24/M	Mid-ileum	Day 1	B	3·5 95% *E. coli*	2·3	7·0	0
			A	3·0 50% Enterobacter 50% *E. coli*	0	5·8	0
			B	4·6 50% Enterobacter 50% *E. coli*	0	6·5	3·7
		Day 2					
			A	3·0 95% Enterobacter 5% *E. coli*	0	4·8	2·4
		Day 3	—	7·3 95% Enterobacter 5% *E. coli*	0	7·6	4·0
4[2]		Day 1	B	8·7 50% Enterobacter 50% *E. coli*	0	0	2·3
45/M	Mid-ileum	(1st study)					
			A	9·7 50% Enterobacter 50% *E. coli*	6·0	10·6	5·7
		Day 1 (2nd study	B	7·8 1% Enterobacter 99% *E. coli*	2·6	0	1·6
		7 days later)	A	8·8 50% Enterobacter 50% *E. coli*	5·0	5·5	4·7

Table I *Small bowel microflora before and after fluid infusion in three normal subjects and one patient with a Polya gastrectomy*[1]

[1]Three other normal subjects (not shown in the table) had no changes in microflora following infusion (see text)
[2]Case 4 had a Polya gastrectomy

there was a transformation in coliform biotypes from a predominant *E. coli* population to *Enterobacter* species. This latter microorganism was also cultured in large numbers from faecal specimens at this time. Bacteroides were reduced immediately after the infusions but the concentration of this organism returned to control levels by day 3 (7·6/ml vs 7·0/ml). On days 2 and 3, anaerobic lactobacilli could be found in the small bowel specimens. Previous studies have shown that intestinal intubation as such for 48 to 72 hours is not generally associated with changes in the microflora (Gorbach, Plaut, Nahas, Weinstein, Spanknebel, and Levitan, 1967).

Subject no. 4 (Table I) had undergone a Polya gastrectomy five years previously. Two ileal infusions were performed in this subject, seven days apart. After the first infusion, there were significant increases in coliforms, streptococci, bacteroides, and anaerobic lactobacilli. The second study also showed increases in these same microorganisms although bacteroides did not rise to previous levels. However, in the second study, there was a change in the coliform biotypes. The control sample contained mostly *E. coli* but following infusion equal numbers of *Enterobacter* and *E. coli* were found. (Equal concentrations of these coliforms were also noted in cultures from the previous week.)

Fluid infused failed to alter the bacteriology of the small bowel in three normal subjects. The tube was positioned in the mid jejunum in two of these subjects: samples taken before and after infusion contained only small numbers of streptococci. An ileal infusion was performed in the third subject. Coliforms (10^4/ml) and streptococci (10^8/ml) were found in the pre-infusion samples and the concentration of these organisms was unchanged by the fluid purge.

Changes in faecal microflora

The faecal bacteriology of three normal subjects and one patient with Polya gastrectomy and associated pancreatic insufficiency was examined following fluid infusion. The three normal individuals had reductions in coliforms 30 minutes after perfusion: two decreased by 0·5 to 1·0 log (probably not significant) and the third by 6 logs (Figure 1). Case 6 also had an alteration in coliform biotypes: *E. coli* was most prelavent before infusion but *Enterobacter* became most prevalent during and after infusion. The initial decline in total coliforms in this subject was due to reduced numbers of *E. coli* and the subsequent increase was caused by a higher concentration of *Enterobacter*. Anaerobes (bacteroides, anaerobic lactobacilli, and clostridia) fell in two of the three subjects. In case 5, the decrease in anaerobic flora was considerably greater than the aerobic flora, ie, bacteroides declined by 2 logs, anaerobic lactobacilli and clostridia by 4 logs, whereas coliforms were lowered by 0·7 log. This appeared to be a selective decrease in anaerobes rather than simple dilution by infusion fluid.

The patient with gastrectomy and pancreatitis had five to 10 loose stools per day and marked steatorrhoea (faecal fat 50 g/day). These symptoms were relieved by oral ingestion of commercial

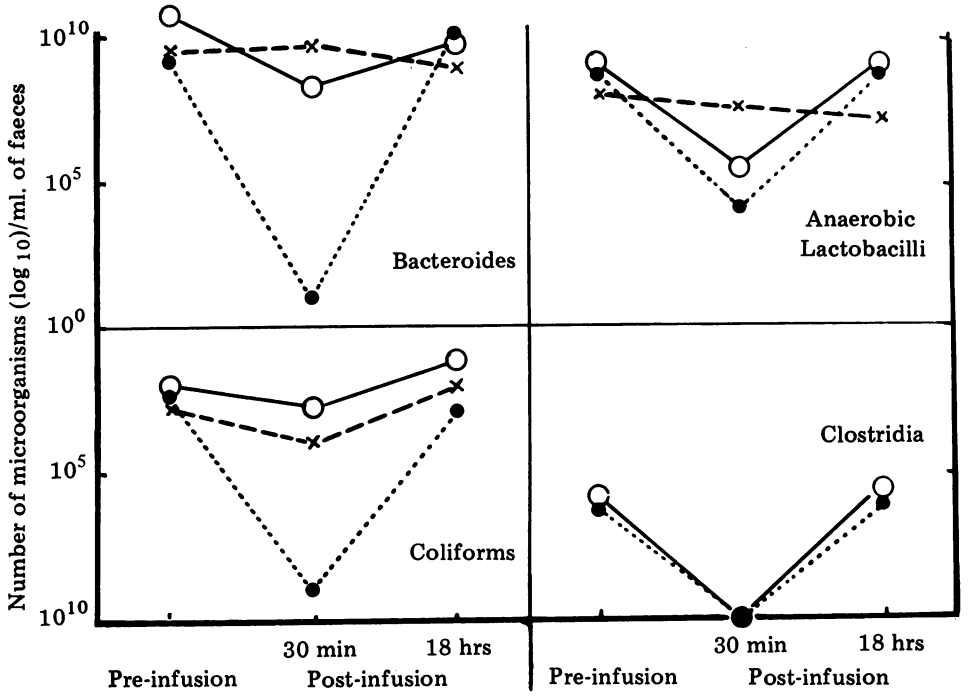

Fig. 1 *Faecal microflora before and after saline purgation in three normal subjects.*

pancreatic enzyme (Cotazym); the number of motions declined to two to three per day and stool fat to 20 g per day.

Examination of faecal coliforms before and after Cotazym showed a correlation between the concentration of *Enterobacter* species and the degree of diarrhoea (Table II). During the initial study when the patient received no therapy, *Enterobacter* were abundant (8·3/g). A low dose of Cotazym (2 capsules with each meal) reduced the number of motions to three to five/day and there was a 200-fold decrease in the number of *Enterobacter* (8·3 to 6·0/g). Two weeks later, Cotazym was again discontinued and the *Enterobacter* count rose as the patient developed loose motions. A larger dose of this preparation (4

Drug	*No. of Bowel Motions/Day*	*Stool pH*	*No. of Microorganisms (log_{10}/g)*	
			E. coli	*Enterobacter*
Off Cotazym	5-10	5·2	7·5	8·3
On Cotazym (low dose)[1]	3-5	6·4	7·5	6·0
Two weeks later				
Off Cotazym	5-10	5·2	8·3	8·0
On Cotazym (high dose)[2]	1-2	7·0	8·4	

Table II *Relation of faecal coliforms and oral administration of pancreatic enzyme (Cotazym) in a subject with chronic pancreatitis*

[1]Two capsules with each meal
[2]Four capsules with each meal

Site of Sample	*No. of Coliforms (log_{10})/ml or g*	
	E. coli	*Enterobacter*
Upper jejunum	6·6	7·4
Mid-jejunum	6·8	7·0
Upper ileum	7·7	7·9
Terminal ileum	8·4	8·9
Stool (formed)	8·4	10/g[2]
After 1,000 ml fluid infusion		
Terminal ileum	7·7	7·8
Stool (liquid)	8·7	8·0

Table III *Small bowel and faecal microflora during fluid infusion in a subject with chronic pancreatitis*[1]

[1]Patient receiving pancreatic enzymes (Cotazym)

capsules with each meal) relieved the diarrhoea and caused a striking reduction of *Enterobacter* species. The stool *p*H also varied with the administration of Cotazym: 5·2 (no enzymes), 6·4 (low dose), and 7·0 (high dose).

Intubation during the period when Cotazym was given in high dosage demonstrated the presence of *Enterobacter* and *E. coli* throughout the small intestine (Table III). Despite the predominance of *Enterobacter* in the small intestine, samples of solid stool taken at the same time contained very few of these microorganisms.

This situation could be altered by fluid purgation. A tube was positioned in the terminal ileum and 1,000 ml of sterile saline was infused over a 30-minute period. Samples of the watery stool taken 30 minutes later now contained large numbers of *Enterobacter* (8·0/g). The viable coliform counts after saline purging were similar to those observed during diarrhoeal episodes induced by withdrawal of Cotazym.

DIARRHOEA INDUCED BY LACTOSE

After obtaining control stool specimens, a single oral dose of 50 g of lactose was administered to three hypolactasic subjects, causing abdominal fullness, colicky pain, and acidic diarrhoea (*p*H 5·9-6·4 in all subjects). Viable bacterial counts of the diarrhoea stool showed a reduction in total coliforms, especially *E. coli*, during lactose administration (Table IV). However, in two subjects there was an increase in the number of *Enterobacter* species. These bacteria were 'too few to count', ie, below 10^5/ml, in the pre-lactose stools but rose to 10^6 to 10^7/g during the diarrhoeal episode, and persisted for at least two days after the last loose motion. A repeat specimen was available in one subject (G.N.) 14 days later and these microorganisms could no longer be detected.

Study *in vitro* of the *E. coli* and *Enterobacter* strains failed to demonstrate any difference in their resistance to acid: both organisms could multiply at *p*H 5·5 to 6·5.

Two individuals with no history of lactose intolerance were also given 50 g of lactose. They developed no symptoms or changes in their bowel motions. Faecal microbial populations were unaltered by lactose in these subjects.

Patient	*Study Period*	*Faecal Microorganisms (mean $\log_{10}$/g or ml)*		
		E. coli	*Enterobacter*	*Bacteroides*
G.M.	Control	8·8	Too few to count	9·5
	Lactose	5·5	Too few to count	7·7
	Off lactose (14 days)	8·0	Too few to count	9·9
G.N.	Control	7·7	Too few to count	10·1
	Lactose	6·9	7·3	10·0
	Off lactose (2 days)	6·7	7·1	10·4
	Off lactose (14 days)	8·3	Too few to count	10·2
E.M.	Control	7·3	Too few to count	10·0
	Lactose	6·7	6·3	10·2
	Off lactose (2 days)	7·3	6·0	10·6

Table IV *Faecal microflora in three subjects with intestinal lactase deficiency given a single oral dose of 50 g of lactose*

Discussion

Diarrhoea induced by fluid infusion or the administration of lactose to hypolactasic subjects was capable of producing alterations in the resident intestinal microflora. In two normal subjects, the mid-small bowel became contaminated with coliforms during fluid purgation. Since these microorganisms are normally confined to the distal ileum and colon, we must assume that bacterial spread occurred in a retrograde fashion, apparently against the flow of infusion fluid and natural peristalsis. (Contamination from above was excluded by pre-infusion sampling and by withholding food during the study period.)

Microorganisms in the stomach and small bowel have been noted in acute infectious diarrhoea due to salmonellae, *Vibrio cholerae*, and enteropathogenic *E. coli*. However, as suggested in the present study, the presence of 'non-specific' colonic bacteria in these aberrant sites does not necessarily imply pathogenicity. Small bowel bacterial contamination may also be the result of a diarrhoeal episode rather than its cause.

Reduction in faecal anaerobes (bacteroides, anaerobic lactobacilli, and clostridia) was noted in some subjects during the induced diarrhoeal episode. Similar findings are currently being observed in a bacteriological study of diarrhoea in the tropics (Gorbach, unpublished data). The natural habitat of anaerobes in the gastrointestinal tract appears to be the large bowel (Gorbach *et al*, 1967). In normal circumstances, they are not encountered in the upper small intestine unless stagnant or 'blind' loops are created by strictures, diverticula, or surgical procedures (Drasar, Hill, and Shiner, 1966). Thus, the apparent prerequisite for the growth of these oxygen-labile microorganisms within the intestine is stasis of luminal contents. The reduction in faecal anaerobes during diarrhoea may be due to rapid intestinal transit through the natural area of stasis, the colon.

Alterations in the anaerobic flora may have important implications for other microbial populations. For example, suppression of salmonellae and coliforms in the mouse intestine has been related to the production of short-chain fatty acids by bacteroides species in an environment of low oxidation-reduction potential (Meynell, 1963; Bohnhoff, Miller, and Martin, 1964). Meynell and Subbaiah (1963) showed that this antibacterial activity occurred in the mouse caecum and colon and that contents remained in the caecum for approximately two hours. In experimental diarrhoea in man, the period of

residence in the large bowel was considerably reduced; it is possible that normal control mechanisms were disturbed by intestinal hurry and alterations in anaerobic bacteria. This proposition is supported by the finding of *Enterobacter* species in diarrhoeal stools. In one patient, suppression of this organism was demonstrated during normal passage through the colon. Induction of diarrhoea removed the inhibitory effect of the large bowel and *Enterobacter* now appeared in the faecal effluent.

The present study suggests that alterations in intestinal bacteriology associated with diarrhoeal diseases should be interpreted with caution: isolations of unusual coliforms from the small bowel or faeces does not necessarily infer pathogenicity.

These investigations were sponsored in part by training grant 5TIAI-276, National Institute of Allergy and Infectious Disease, and research grants AM-09332 and AM-13387, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

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