ALTERATIONS IN SMALL-BOWEL MICROFLORA IN ACUTE INTESTINAL OBSTRUCTION

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THE RESIDENT bacterial flora in the lumen of the small bowel is complex, and the relationship with its host in health and disease has received increased attention in recent years (Donaldson, 1970; Tabaqchali and Booth, 1970; Gorbach, 1971). The increased knowledge of it in normal subjects and in patients with chronic intestinal disorders has developed from studies of small-bowel content obtained by per-oral methods (Kalser *et al.*, 1966; Drasar and Shiner, 1969; Drasar, Shiner and McLeod, 1969; Vince *et al.*, 1972; Challacombe, Richardson and Anderson, 1974). These methods of sampling are not suited to the investigation of the acutely disordered bowel, so there is little knowledge of alterations in small-bowel microflora that may occur in acute intestinal obstruction. Needle aspiration at laparotomy is an alternative method of collecting samples that is more applicable to acute disorders. We have used this method to obtain specimens to study the microflora in a control group and in patients with acute small-bowel obstruction and acute large-bowel obstruction.

We present the results of an investigation into some of the difficulties encountered in this study, namely the problem of qualitative and quantitative bacterial analysis of small-volume samples after varying periods of storage in transport medium, in addition to the changes in microflora observed in acute intestinal obstruction.

MATERIALS AND METHODS

Samples of small-bowel content were obtained either from the outflow from an ileostomy or by needle aspiration of the small bowel at operation. The volume of the specimen was measured and each sample was placed in 10 ml of Stuart's Transport Medium (Modified) (Oxoid) and kept at room temperature until investigated by qualitative and quantitative bacteriological methods.

Quantitative bacteriological methods. From the original dilution in transport medium, serial 10-fold dilutions to 1 in 10^{10} were made with NaCl 0.85% (w/v); a fresh pipette was used for each dilution and the work was done on an open bench. From each dilution the culture media listed in the table were inoculated.

For MacConkey's and Columbia agars, pour-plates were made from 1 ml of each

Received 9 Sept. 1974; revised version accepted 6 May 1975.

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TABLE
*Culture media used for quantitative and qualitative bacteriological analysis
of specimens. All incubation was at 37°C.*

Medium	Organisms favoured	Incubation time (h)	Atmosphere
Columbia Agar No. 2 (Oxoid)	Aerobes	48	Aerobic
MacConkey Agar (Oxoid)	Coliform bacilli	48	Aerobic
Sabouraud's Agar (Oxoid)	Yeasts	72	Aerobic
Rogosa Agar with Nystatin (Difco)	Aerobic lactobacilli	72	Aerobic
Rogosa Agar with Nystatin (Difco)	Anaerobic lactobacilli	72	Anaerobic
6.5% Salt Agar (Oxoid)	Staphylococci	96	Aerobic
Azide Blood Agar (Oxoid)	Streptococci	96	Aerobic
10% horse-blood agar with nalidixic acid (30 µg per ml)	<i>Bacteroides</i> spp.	120	Anaerobic
10% horse-blood agar with neomycin (200 µg per ml)	Clostridia	48	Anaerobic

dilution. For all the other media, inoculation and counting were by the method of Miles, Misra and Irwin (1938).

Anaerobic methods. Freshly prepared plates were inoculated on the open bench. For anaerobic incubation of plates a Baird and Tatlock (BTL) jar was used with the cold catalyst supplied by BTL and the GasPak (Baltimore Biological Laboratories) system of hydrogen and carbon-dioxide production. A methylene-blue reduction indicator was included in each jar.

Identification of organisms was by colonial appearance on selective media, Gram's stain and morphology, and other tests as detailed below.

Gram-positive cocci, grown on salt agar and shown to be catalase-positive, were termed "staphylococci". Gram-negative bacilli, grown anaerobically in the presence of nalidixic acid and intolerant of aerobic conditions, were termed *Bacteroides*.

The effect of storage in Stuart's medium. Many operations for the relief of acute intestinal obstruction are performed at night and, in these cases, it is not easy to examine the small-bowel content fresh; it is therefore desirable to ascertain whether a transport medium can hold organisms in a state of "suspended animation", until the next morning.

To evaluate viability in Stuart's medium, 8 ml of an ileostomy effluent were divided into four equal parts. One of these was examined whilst fresh and the other three were examined after periods of 24, 48, and 72 h in Stuart's medium. This investigation was performed on specimens from nine patients who had recently undergone pan-proctocolectomy.

Alterations in acute intestinal obstruction. Bacteriological studies were made of the small-bowel content of 13 patients undergoing operation for acute small-bowel obstruction and five patients undergoing operation for acute large-bowel obstruction. Any patient who had received antibiotics during the 4 weeks before operation was excluded from the study. Eight patients undergoing operation for gall stones confined to the gall bladder, without other evidence of gastro-intestinal-tract disease, were studied as controls.

Whenever possible, 2-ml specimens were obtained by needle aspiration of undiluted small-bowel content at operation from three sites—the jejunum, the mid-ileum, and the distal ileum.

In the control group it was not always possible to obtain 2 ml even after "milking" intraluminal content to the aspiration site, and in some cases, 0.3-ml specimens were used for analysis. It proved impossible to obtain specimens from the collapsed small bowel

FIG. 1.—Effect of storage of organisms in Stuart's transport medium for periods up to 72 h. Each continuous line represents one specimen.

distal to the site of a small-bowel obstruction and in these patients only specimens proximal to the obstruction were analysed.

The effect of the volume of the specimen. From a group of six patients who had recently undergone pan-proctocolectomy, 0.3-ml, 1-ml, and 2-ml specimens of ileostomy effluent were examined by the methods described above.

RESULTS

Effect of storage in Stuart's medium

The difference observed, on quantitative analysis, between cultures of fresh specimens and those cultured after storage for 24 hours was less than one log count for coliform bacilli, streptococci, aerobic and anaerobic lactobacilli, clostridia, and fungi, and was less than 1.5 log counts for staphylococci and *Bacteroides*. The results (fig. 1) show a good correlation for individual organisms between the colony counts from fresh specimens and from specimens after storage in Stuart's medium.

Alterations in acute intestinal obstruction

Control group. A gradient of aerobic organisms was demonstrated in the small bowel, the bacterial counts rising from 10^4 or 10^5 per ml in the jejunum to

FIG. 2.—Control group; ● = numbers of aerobes and ▲ = anaerobes in aspirated contents.

FIG. 3.—Control group; numbers of aerobic organisms; ● = gram-positive cocci, = ▲ gram-negative rods.

FIG. 4.—All aerobic organisms; ● = small-bowel obstruction, ▲ = controls.

10^7 or 10^8 per ml in the distal ileum (fig. 2). Gram-positive and gram-negative organisms were present in about equal numbers (fig. 3).

Anaerobic organisms were isolated in only eight of 15 specimens but, when organisms were isolated, a gradient down the small bowel existed, although the counts were generally lower than for aerobes, 10^2 – 10^5 per ml in the jejunum rising to 10^5 – 10^7 in the ileum (fig. 2). *Bacteroides* were found in six of 15 specimens, most often in the mid and distal ileum. Fungi were isolated from seven of 15 specimens.

Small-bowel obstruction. All specimens were obtained from the distended small-bowel loops proximal to the site of obstruction.

FIG. 5.—Small-bowel obstruction. Numbers of aerobic organisms; ● = gram-positive cocci, ▲ = gram-negative rods.

FIG. 6.—All anaerobic organisms; ● = small-bowel obstruction, ▲ = controls.

There was a general increase in the numbers of organisms and a loss of the normal gradient of aerobic organisms from jejunum to distal ileum, the absolute level being 10^9 – 10^{11} per ml throughout the small bowel (fig. 4). This increase is significant in comparison with the control group ($P < 0.01$). The increase in aerobic organisms included gram-negative organisms and gram-positive organisms but the counts of gram-negative organisms were generally higher, 10^9 – 10^{11} per ml, than of gram-positive organisms, 10^6 – 10^8 per ml (fig. 5). The counts of gram-negative bacilli found in the obstructed small bowel were higher than those found in the distal ileum of the control group, in contrast to the counts of gram-positive organisms which were of the same order in both groups.

From patients with acute small-bowel obstruction, more specimens yielded anaerobic organisms than from the control group. Growth was obtained in 23 of 25 specimens, compared with eight of 15 control specimens, and *Bacteroides* were cultured from 20 of 25 specimens, compared with six of 15 control specimens. There was an increase to 10^5 – 10^7 anaerobic organisms per ml in the jejunum and mid-ileum, which eliminated the normal gradient from jejunum to distal ileum. The numbers in the obstructed small bowel, 10^5 – 10^7 , were similar to those in the distal ileum of control patients from whom growth was obtained (fig. 6). This increase of anaerobic organisms in small-bowel obstruction as compared with the control group is statistically significant ($P < 0.01$).

Fungi were isolated from eight of 25 specimens; this does not differ significantly from the control group.

Large-bowel obstruction. Specimens of small-bowel content were obtained from five patients with acute large-bowel obstruction.

A general increase in the numbers of aerobic organisms throughout the small bowel and a loss of the normal gradient from jejunum to ileum was found,

FIG. 7.—All aerobic organisms; ● = large-bowel obstruction, ▲ = controls.

FIG. 8.—All anaerobic organisms; ● = large-bowel obstruction, ▲ = controls.

similar to that demonstrated for patients with acute small-bowel obstruction; the absolute numbers were 10^{10} – 10^{11} per ml in all areas (fig. 7). This increase of aerobic organisms included gram-positive and gram-negative organisms and is highly significant in comparison with the control group ($P < 0.001$). In the distal ileum, the gram-negative organisms were present in greater numbers than in the control group but the number of gram-positive organisms was of the same order as in the control group.

Anaerobic organisms were cultured from all 10 specimens, in comparison with eight of 15 in the control specimens. A loss of the normal gradient from jejunum to distal ileum was demonstrated (fig. 8) and the absolute numbers, 10^8 – 10^{11} per ml, were considerably higher than in the distal ileum of controls, 10^5 – 10^7 per ml, or proximal to acute small-bowel obstruction, 10^5 – 10^7 per ml. The majority of these organisms were *Bacteroides*, which were present in all but one specimen.

The increased count of anaerobic organisms in acute large-bowel obstruction, in comparison with the control group, is highly significant ($P < 0.001$).

Fungi were present in three of 11 specimens; this does not differ significantly from the control group.

Effect of the volume of the specimen

There was a good correlation of counts from 0.3-ml, 1-ml, and 2-ml specimens for coliform bacilli, staphylococci, streptococci, lactobacilli and clostridia. The growth of *Bacteroides* correlated well in five of six specimens, but in one specimen 10^6 *Bacteroides* per ml were cultured from the 2-ml sample, whereas there had been no growth from the small-volume samples. The results of qualitative and quantitative analysis of 0.3-ml, 1-ml, and 2-ml portions of ileostomy effluent are shown in fig. 9.

FIG. 9.—Effect of the volume of the specimen on viable counts for various organisms. Each continuous line represents one specimen.

DISCUSSION

Gorbach *et al.* (1967*b*) have shown that bacteriological results obtained after needle aspiration from the small bowel of starved and anaesthetised patients were comparable with those obtained by intubation in the conscious, non-fasting subject. One of the problems of direct needle aspiration is that only a small volume may be obtained for analysis. Bishop and Anderson (1960), in an attempt to overcome this difficulty, injected a known volume of isotonic saline into the lumen of the bowel and then aspirated a mixture of small-bowel content and saline, but this method introduces an unknown dilution effect which Gorbach *et al.* (1967*b*) showed to invalidate the method for quantitative analysis. We found 0.3 ml to be enough for quantitative analysis.

For quantitative studies, the storage method must maintain a viable count that is either constant or predictably altered after different periods. Cohn (1965) attempted to store intestinal specimens by freezing but Kalser *et al.* (1966) showed that this reduced the counts in an unpredictable fashion. Drasar *et al.* (1969) froze small-bowel contents in 10% glycerol which Crowther (1971) found to be satisfactory for quantitative analysis.

Another approach is to use a storage medium which Gästrin, Kallings and Marcetic (1968) state should hold organisms in a viable state but prevent

multiplication, so that the organisms are preserved in the same numbers and proportions as at the moment of sampling. To fulfil these requirements, the medium should have a low nutrient content, no inhibiting factors, a low oxidation-reduction potential, and a physiological pH. Stuart (1959) had based his transport medium on these premises. Sodium thioglycollate was used as a reducing agent, agar was added to preserve anaerobic conditions, and a reduction indicator, methylene blue, was included. Although Stuart originally introduced his medium for transport of the gonococcus it has become widely used as a general storage medium for other organisms. Gästrin *et al.* (1968) showed that staphylococci, streptococci, clostridia, and *Escherichia coli* are held "in status quo" for 48 h, whilst the loss of *Salmonella*, *Shigella*, gonococci, and meningococci is only one log count in 48 h. We found that mixed intestinal microflora can be stored for 24 h in a modified Stuart's medium without unacceptable quantitative changes.

The present study confirms the existence of a resident microflora in the upper and lower small bowel similar to that demonstrated by Tabaqchali and Booth (1970) in studies of normal subjects. The principal difference between the microflora of our control group and that described by other workers from normal subjects is that previously coliform organisms have been isolated only occasionally from the normal jejunum (Donaldson, 1970), whereas in this study a low count of these organisms was present in the majority of "control" jejunal samples. This may be related to the choice of cholecystectomy patients as a control group, because coliform bacilli have been found in the bile of such patients (Elkeles and Mirizzi, 1942; Anderson and Priestley, 1951; Edlund, Mollstedt and Ouchterlony, 1959).

Many factors are thought to affect the ecology of the small bowel, including the antibacterial effect of the gastric acid and the availability of nutrients (Donaldson, 1970), but the most important factor is probably the cleansing effect of intestinal movement (Drasar *et al.*, 1969). Certainly, in conditions of intestinal stasis, bacterial overgrowth is commonly present (Gorbach, 1971) and has been shown in multiple jejunal diverticulosis (Polter *et al.*, 1968), in ileal blind loops (Drasar and Shiner, 1969), and in conditions of abnormal motility, e.g., scleroderma (Kahn, Jeffries and Sleisenger, 1966; Salen, Goldstein and Wirts, 1966). It would, therefore, be expected that bacterial overgrowth in the small bowel would occur proximal to intestinal obstruction. The only previous quantitative studies of the microflora in intestinal obstruction were not accurate because of the introduction of an unknown dilution factor (Bishop and Allcock, 1960; Bishop and Anderson, 1960).

The present study provides evidence of an increase in aerobic and anaerobic organisms in the small bowel in intestinal obstruction. Because very high anaerobic counts are common in the normal colon (Gorbach *et al.*, 1967a), the high counts in the small bowel in large-bowel obstruction may reflect incompetence of the ileo-caecal valve. The increase in aerobic and anaerobic organisms proximal to small-bowel obstruction cannot be due to colonic contamination and may be due either to proliferation of the existing small-bowel microflora or to colonisation from above.

The higher small-bowel anaerobic count in acute large-bowel obstruction, in comparison with acute small-bowel obstruction, is particularly interesting in view of the much higher morbidity and mortality associated with the former condition. Asymptomatic bacteraemia has been shown to occur commonly during intestinal operations (Burton, 1971) and it might occur during intestinal handling during operation for large-bowel obstruction. In several reports of bacteroides septicaemia, most patients have had antecedent colonic disease or operation (Public Health Laboratory Service, 1973; Mackenzie and Litton, 1974). In the present study, no correlation emerged between alterations in microflora and the prognosis in any individual patient.

SUMMARY

Small-bowel content was examined bacteriologically whilst fresh and after storage for 24, 48, and 72 h in a modified Stuart's medium. There was little alteration in the viable count of individual intestinal organisms. For accurate quantitative analysis, 0.3 ml of intestinal content was enough.

Specimens of small-bowel content were obtained by needle aspiration, and a qualitative and quantitative study was made of the microflora of patients with acute intestinal obstruction and of a control group of patients. Results in the control group confirmed the findings of the results of intubation studies by other workers, that a quantitative gradient of aerobic and anaerobic organisms exists from jejunum to distal ileum. In acute small-bowel obstruction and acute large-bowel obstruction there was a loss of the normal gradient and an increase in the absolute numbers of organisms present; this was particularly marked for anaerobic organisms in large-bowel obstruction.

We thank Dr H. Jackson for his generosity with laboratory facilities, advice and assistance, Professor P. Collard and Dr F. Tuxford for encouragement and helpful criticism, and Dr Wendy Schofield for critical and secretarial help.

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