BACTERIOLOGICAL STUDIES IN CROHN'S DISEASE

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Altered anatomy or motility of the small intestine may permit its colonisation by bacteria that interfere with the absorption of vitamin $B_{12}$ and fat (Badenoch, 1958; Donaldson, 1964; Tabaqchali and Booth, 1967) or degrade dietary protein (Jones et al., 1968). Recognition of this possibility has led to a resurgence of clinical interest in the role of the intestinal bacterial flora. Crohn's disease offers rich opportunities for bacterial overgrowth, because it may be complicated by strictures, fistulae, post-operative stagnant loops, or enteroanastomoses. It has been assumed that overgrowth often occurs in this disease and plays a part in the genesis of the malabsorption and malnutrition (van Patter et al., 1954; Bockus, 1964) that is often present, but this assumption has not been supported by any extensive bacteriological study. Drasar and Shiner (1969) cultured juice from the upper jejunum in six patients and found bacterial overgrowth in only one whose disease was complicated by jejunal obstruction.

We therefore decided to study the bacterial content of aspirates from various levels of the gastro-intestinal tract of patients with Crohn's disease in an attempt to assess the frequency and significance of any differences from a group of normal controls.

METHODS

Subjects studied. Thirteen patients with Crohn's disease of varying anatomical distribution were intubated. They were chosen partly because of their willingness to undergo intubation and in some cases because the nature of their disease suggested possible bacterial overgrowth. Their age and sex, and the location of disease, are indicated in the table. The 20 control subjects were all medical staff or students with no evidence of gastro-intestinal disorder, and all except two were male. Preliminary findings indicated the possibility of a change in the salivary bacteriology of patients with Crohn's disease. To test whether this change was common to other chronic inflammatory disorders, the saliva of ten patients with active rheumatoid arthritis and ten patients with ulcerative colitis was examined.

Intubation of subjects. After an overnight fast, the subject swallowed an open-ended polyvinyl tube with an internal bore of 1.75 mm and weighted by 1-2 ml of mercury. Samples were obtained from the stomach and upper 10 cm of the jejunum. The tube was then allowed to progress down the bowel as the subject ate normally. Samples were taken from the lower jejunum or upper ileum (mid-intestine) when the tube had progressed to 160-200 cm from the teeth and its position had been checked by X-ray screening. Samples from the terminal ileum were collected next morning after an overnight fast. The position of the tube was checked radiologically and confirmed by the introduction of air or barium after the collections had been completed. These samples were always obtained from within 30 cm of the ileo-caecal valve or ileo-colic anastomosis, although the distance from the teeth was variable.

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Samples were obtained by gentle aspiration into a 20-ml sterile syringe after a quantity equal to the capacity of the tube had been discarded. They were all delivered to the laboratory and examined within half an hour of collection. It was not technically possible to obtain samples from every site in each patient.

**Bacteriological techniques.** After the sample had been mixed in a micro-emulsifier, serial ten-fold dilutions of it were made and portions were plated on a wide variety of standard media and incubated aerobically and anaerobically as previously described (Hamilton *et al.*, 1970). Results are expressed as the log_{10} number of organisms per ml of fluid, or log_{10} number of organisms per g (wet weight) of faeces.

**Metabolic investigations.** Vitamin B₁₂ absorption was measured by the method of Schilling (1953): 0.5 μg of ^{58}Co-labelled vitamin B₁₂ was given by mouth with two capsules of intrinsic factor, each containing not less than 1 USNF unit, and an intramuscular flushing dose of 1000 μg of vitamin B₁₂. Urine was collected for 24 hr.

Three subjects were given 1 μg of labelled vitamin B₁₂ with intrinsic factor orally and the radioactivity was estimated in the stools (Heinle *et al.*, 1952).

Faecal fat was estimated by the method of van de Kamer, ten Bokkel Huinink and Weyers (1949) with the patient on a 100-g fat diet. Qualitative estimation of bile-salt deconjugation...
in the intestinal samples and measurement of urinary indican excretion were performed as described previously (Hamilton et al.).

RESULTS
General bacteriological findings

The bacterial flora at different levels of the gut in healthy subjects and in patients with Crohn’s disease is indicated in figs. 1–4. The numbers of organisms recovered from the stomach of patients generally fell within the normal range, the only notable exception being in a patient (M. L.) who had a duodeno-colic fistula. In the upper jejunum, counts of enterobacteria were raised in four patients, increases in other organisms again being restricted to the patient with the duodeno-colic fistula. In the mid-gut, raised counts of several organisms were present in several patients. Organisms whose numbers were most often increased were enterobacteria, enterococci, yeasts, and lactobacilli. In the terminal ileum, many aerobes and anaerobes were found in healthy subjects in
whom counts of enterobacteria, enterococci, lactobacilli, streptococci, yeasts, neisseria, and diphtheroids often equalled or exceeded those found in patients with Crohn’s disease. Consequently it was difficult to define an abnormal flora in this region, though raised counts of anaerobes were found in some patients. The bacterial flora at any level was defined as abnormal if counts of

AEROBES
- Enterobacteria
- Lactobacilli
- Enterococci
- Staphylococci
- Streptococci
- Yeasts
- Neisseria
- Diphtheroids
- Bacillus spp.
- Strep. viridans

ANAEROBES
- Clostridia
- Bacteroides
- Bifidobacteria
- Veillonellae

Fig. 3.—Bacteriology of the mid-intestine in 11 patients with Crohn’s disease compared with 11 normal controls. ▲ = Patient with an ileal resection of more than 50 cm. ○ = Patient with an ileal resection of less than 50 cm. Other symbols as in fig. 1.

one or more of enterobacteria, enterococci, clostridia, or bacteroides exceeded those found in normal subjects by a factor of ten or more (table). On this basis, eight patients were judged to have some degree of overgrowth. In three (P. S., R. S., L. A.) the abnormality was confined to a small increase in a single species, so the finding was of doubtful significance. In the remaining five patients (M. H., H. P., M. L., J. U., S. F.) substantial overgrowth involved a wide variety of species, so that in these patients the findings were similar to those described in the stagnant-loop syndrome.
In one patient (M. L.) overgrowth was clearly due to the presence of a fistula connecting sparsely and densely populated areas of the gut. The other four with overgrowth had extensive disease. Two (J. U., S. F.) had had much of the ileum resected, but still had segments with recurrent disease and radiological evidence of stasis. The other two (M. H., H. P.) had diffuse small-gut disease involving the jejunum and the ileum. In neither would the tube progress beyond the mid-gut, probably because of impaired peristalsis rather than stricture.

**Significance of the ileo-caecal valve**

In two of three patients in whom the ileo-caecal valve had been resected (L. A., S. A. S.), the small-gut flora resembled that found in normal subjects. In the third (J. U.) there was gross bacterial overgrowth, but this was no greater than that found in S. F. who had undergone ileal resection with preservation of the ileo-caecal valve.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Site of disease</th>
<th>Bacterial flora: species and log&lt;sub&gt;10&lt;/sub&gt; organisms per ml in</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; absorption tests: per cent. of oral dose</th>
<th>Fat absorption tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>stomach</td>
<td>upper jejunum</td>
<td>mid-bowel</td>
</tr>
<tr>
<td>J. A.</td>
<td>F</td>
<td>47</td>
<td>Terminal ileum</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>L. G.</td>
<td>M</td>
<td>38</td>
<td>Terminal ileum</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>M. H.</td>
<td>M</td>
<td>16</td>
<td>Jejunum, proximal ileum*</td>
<td>St 7.4</td>
<td>Bi 6.7</td>
<td>Eb 8-2</td>
</tr>
<tr>
<td>F. F.</td>
<td>M</td>
<td>58</td>
<td>Jejunum, ileum</td>
<td>...</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>H. P.</td>
<td>M</td>
<td>71</td>
<td>Jejunum, ileum*</td>
<td>N</td>
<td>N</td>
<td>Eb 8-2</td>
</tr>
<tr>
<td>S. L. S.</td>
<td>M</td>
<td>31</td>
<td>Colon</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P. S.</td>
<td>M</td>
<td>24</td>
<td>Colon</td>
<td>N</td>
<td>Eb 4-1</td>
<td>N</td>
</tr>
<tr>
<td>R. S.</td>
<td>M</td>
<td>33</td>
<td>Terminal ileum Sigmoid fistula</td>
<td>N</td>
<td>N</td>
<td>Eb 3-5</td>
</tr>
<tr>
<td>M. L.</td>
<td>F</td>
<td>25</td>
<td>Ileo-colic* Duodenal fistula</td>
<td>Bt 8-5</td>
<td>Eb 5-8</td>
<td>Es 4-9</td>
</tr>
<tr>
<td>S. A. S.</td>
<td>F</td>
<td>37</td>
<td>Resection 50 cm ileum, ileo-caecal valve and ¼ colon</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>L. A.</td>
<td>F</td>
<td>26</td>
<td>Resection 30 cm ileum, ileo-caecal valve and all colon</td>
<td>N</td>
<td>N</td>
<td>Es 5-8</td>
</tr>
<tr>
<td>J. U.</td>
<td>F</td>
<td>25</td>
<td>Reselection 130 cm ileum, ileo-caecal valve and ¼ colon*</td>
<td>N</td>
<td>N</td>
<td>Eb 8-6</td>
</tr>
<tr>
<td>S. F.</td>
<td>F</td>
<td>54</td>
<td>Resection 70 cm ileum*</td>
<td>N</td>
<td>Eb 4-8</td>
<td>Ye 6-6</td>
</tr>
</tbody>
</table>

* Comparable to stagnant-loop syndrome.
† Normal is >12 per cent.
‡ Normal is >50 per cent.
§ Normal is <6 g per 24 hr.
--+ None detected.
+++ <50 per cent.
+++ >50 per cent.

**Note:** The species outside the normal range are indicated in the table at each site.
Specific bacteriological findings

There was a virtual absence of aerobic diphtheroids in patients with Crohn's disease (figs. 1-4). They were recovered from only one of 44 samples of gastro-enteric juice from the patients, compared with 39 of 85 samples from healthy controls. This depression was evident even in saliva, from which diphtheroids were recovered less often and in smaller numbers from the patients than from healthy controls (fig. 5). The concentration of diphtheroids appeared to be similarly reduced in the saliva of patients with ulcerative colitis, but not of those with rheumatoid arthritis.

**Fig. 5.**—Diphtheroids and yeasts recovered from (a) the saliva of 24 normal subjects, 24 patients with Crohn's disease, ten patients with ulcerative colitis, and ten patients with rheumatoid arthritis, (b) the mid-bowel of 11 normal subjects and 11 patients with Crohn's disease, and (c) the faeces of 14 normal subjects and eight patients with Crohn's disease.
In contrast to this depression of diphtheroids, higher counts of yeasts were noted in all parts of the gut sampled from patients with Crohn's disease. This increase was present but less marked in some patients with ulcerative colitis and rheumatoid arthritis.

**Vitamin-B_{12} absorption**

Results from individual subjects are given in the table. Excretion of less than 12 per cent. of the dose of labelled vitamin B_{12} (the lower limit of normal excretion) was found in eight patients. Four of these had undergone ileal resection and the other four had ileal disease, so in no case was it necessary to invoke bacterial overgrowth as the cause of malabsorption. Nevertheless, substantial overgrowth, including organisms that are thought to impair vitamin B_{12} absorption, was present in four of the eight subjects (H. P., M. L., J. U., S. P.). Another patient (M. H.) with substantial overgrowth and disease of the jejunum and proximal ileum (but not of the distal ileum—the site of vitamin B_{12} absorption) absorbed vitamin B_{12} normally.

**Faecal-fat excretion**

Results for individual subjects are given in the table. Only five of the 13 patients had steatorrhoea; four of these had undergone extensive ileal resection or had combined disease of the jejunum, where fat is absorbed, and of the ileum, where bile-salts are reabsorbed, which was sufficient to account for the finding. Increased counts of bacteroides, enterococci, and enterobacteria were found in four of these patients while in only two was there more than 50 per cent. splitting of conjugated bile salts. One patient (M. H.), who had gross contamination of the mid-bowel but no bile-salt deconjugation, did not have steatorrhoea.

**Antibiotic therapy**

Two patients (M. L., H. P.) were given tetracycline (500 mg q.d.s.) after organisms present in the upper gut had been shown to be sensitive to the agent *in vitro*. There was a reduction or elimination of some members of the anaerobic flora, but in both patients representation of aerobic organisms increased quantitatively and qualitatively. Both had impaired absorption of vitamin B_{12}, which was not improved by tetracycline, possibly because both also had ileal disease.

**DISCUSSION**

In healthy subjects the numbers and variety of organisms recovered from all levels of the bowel were greater than those reported by some previous workers (Shiner, Waters and Gray, 1963; Tabaqchali *et al.*, 1966) but similar to those reported by others (Gorbach *et al.*, 1967; Gorbach *et al.*, 1969). Much less information is available about the microflora of the mid-gut and terminal ileum than of the upper small bowel. In agreement with Drasar, Shiner and McLeod (1969), we found that the numbers of organisms recovered increased as the small bowel was traversed.
Mid-intestinal contents were aspirated at various levels, so conclusions must be tentative, but this region yielded more information about bacterial overgrowth than did terminal ileal intubation. In four of the patients studied, the flora would have been passed as normal if aspirates had been restricted to the stomach and upper jejunum, because overgrowth was apparent only in more distal parts of the small bowel. On the other hand, aspirates from the terminal ileum of normal subjects usually contained large numbers of organisms that were seldom exceeded in samples from similar areas in patients with Crohn's disease, making it almost impossible to define bacterial overgrowth. Study of the terminal ileum is therefore considered to be of limited value in the demonstration of small-gut overgrowth.

Crohn's disease is a difficult condition to study because disturbances of metabolism and bacteriology vary with the site of the disease. The high proportion of patients found to have some abnormality of the small-gut flora was to be expected in a disease characterised by inflamed or stenosed bowel or fistulae, but motility is undoubtedly important in maintaining the low bacterial population normal in the jejunum (O'Grady and Vince, 1971). Colonisation cannot occur at any site unless the rate at which organisms multiply or are supplied to the area exceeds the rate at which they are removed. This was confirmed by the finding that only patients with obvious stasis or a duodeno-colic fistula exhibited considerable overgrowth in the small gut. Those with comparatively normal gut-motility, or disease restricted to the terminal small bowel, did not show increased bacterial colonisation of the upper small gut.

The deficiency of diphtheroids and relative abundance of yeasts in this heterogeneous collection of patients is difficult to explain and could not be accounted for by recent antimicrobial or steroid therapy. The change is probably not a non-specific response to chronic inflammation or ill-health, as the same trends were not apparent in patients with rheumatoid arthritis. Reduced recovery of diphtheroids from the saliva of patients with ulcerative colitis suggests that there may be an association with inflammatory gut disease, but with so few specimens studied this speculation must remain tentative.

It proved impossible to demonstrate a coherent relationship between the absorptive and metabolic defects of patients with Crohn's disease and the presence of an abnormal bacterial flora. This study has shown that malabsorption of vitamin B\textsubscript{12} usually depends more on disease or resection of the terminal ileum than on the presence of an abnormal flora. Disease or excision of the ileum in patients with Crohn's disease may explain why they differ from other patients with the blind-loop syndrome in whom the ileal absorptive areas are normal in failing to improve their vitamin B\textsubscript{12} absorption after antibiotic therapy (Cameron, Watson and Witts, 1949; Mollin, Booth and Baker, 1957). Furthermore, patient M. H. confirms previous findings that gross contamination of the small gut by faecal-type organisms is compatible with normal vitamin B\textsubscript{12} absorption (Paulk and Farrar, 1964; Hamilton \textit{et al.}, 1970). Three patients who showed significant deconjugation of bile salts—which seems to be the governing factor in the production of steatorrhoea in the blind-loop syndrome (Tabaqchali, Hatzioannou and Booth, 1968)—had steatorrhoea but bacterial
过生长，包括能够分解胆盐的物种，不总是与脂肪的增加分泌相关。

**Summary**

细菌群落从胃、上回肠、中肠和末端回肠的13例克罗恩病患者的抽吸物与健康人的相似部位的细菌群落进行了比较。菌群与病变部位的关系以及维他命B₁₂和脂肪的吸收进行了研究。

增加了上消化道的殖民化只在由于上消化道疾病导致明显滞留的患者中或当上消化道与下消化道通过瘘管相连时才被看到。在具有相对正常运动性或病变仅限于末端小肠的患者中，菌群与健康人的相似。需氧型假单胞菌几乎不存在，而酵母的计数在所有肠道区域的克罗恩病患者的菌群中都较高。

没有一致的联系能够证明吸收缺陷与异常菌群的存在。维生素B₁₂的吸收异常通常更依赖于末端回肠的丢失或疾病，而不是小肠内细菌数量的增加。三名患者显示出显着的胆盐分解，但因细菌过生长，包括能够分解胆盐的物种，不总是与脂肪的增加分泌相关。

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**References**


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