BACTERIA AND THE SMALL INTESTINE IN MAN

by

Alexander W. Dellipiani, M.B.,Ch.B., M.R.C.P.(Ed.),
Post-Graduate Research Scholar,
University of Edinburgh.

The extent of the colonization by bacteria of the small intestine of man in health and disease has for many years been a source of speculation and several investigations have been carried out in which sampling tubes have been passed by mouth (Kendal et al. 1927; Davidson and Gullard, 1930; Nicols and Glenn, 1940; Ollerros, 1942; Milanes et al. 1946; Girdwood, 1950; Howie et al. 1953; Duncan et al. 1954; Nadel and Gardner, 1956; Martini et al. 1957; Anderson and Langford, 1956; Goldstein et al. 1961; Wirts and Goldstein, 1963). The studies by Cregan and Hayward (1953) and Cregan, Dunlop and Hayward (1953) using the method of Blacklock and co-workers (1937) in which they sampled the small bowel contents directly at operation have helped considerably to clarify ideas on the subject. In spite of this, as French (1961) points out in a recent review of the literature, no clear picture has emerged concerning the bacteriological status of the small intestine.

We have been interested for some time in the possible relation of bacteria to small intestinal disease (Girdwood, 1950; 1955a; 1955b; Doig and Girdwood, 1960), and this paper reports the findings of the bacterial flora in four different groups of patients who were studied by an intubation technique.

As part of the investigation an attempt was made to study the uptake of labelled cyanocobalamin in vitro by the coliform type organisms isolated from the small intestine, note also being taken of the rate of uptake under these conditions.

MATERIAL

The patients studied were eight with malabsorptive disease, fifteen with pernicious anaemia and twenty-two with surgical or congenital blind or stagnant loops. There were also fifteen hospital controls who did not suffer from any
of these conditions, but whose diagnoses are given in the relevant figure and table.

**METHOD**

1. **Method of sampling and jejunal and ileal level.**

The method used was an intubation technique. The tube was similar to that suggested by Blankenhorn, Hirsch and Ahrens (1955) and was of such a nature as to withstand autoclaving, while exposing the patient to the minimum discomfort and yet allowing adequate aspiration. Six and a half foot lengths of polyvinyl tubing with radio-opaque markers attached were used. The bore of the tube was 1.5 mm. and the external diameter 2.5 mm. Aspirating ports were cut in the region of the terminal 3 to 4 inches and a mercury bag attached to the end. All materials were autoclaved prior to intubation in the morning. Owing to the duration of the study nasal intubation was thought to be most suitable. A fine plexitron tube was introduced nasally and the end drawn out of the patient's mouth. To this the **proximal** end of the polyvinyl tube was attached with a sterile metal segment. Thus the polyvinyl tube could be drawn back out of the patient's nose, this being continued until, when the **distal** end with the mercury bag attached entered the mouth, the patient was allowed to swallow this. The proximal contaminated end of the polyvinyl tube was cut off and further lengths of tube attached by sterile metal segments as and when required.

The tube was washed through with 100 mls. of sterile saline after it had reached the stomach, this being repeated when the pylorus had been passed and again on completing the jejunal aspiration. Aspiration was carried out when the aspirating ports in the tube were calculated and assessed by fluoroscopy to be at mid-jejunal and mid-ileal level, the initial aspirate being rejected in each case. In all patients there was no delay between the time at which it was calculated that the tube had reached the correct jejunal level and the time of aspiration. Owing to the fact that the tube reached the desired ileal level at varying times during the night, however, there was some delay between this time and the time of ileal aspiration on the following morning. There was always an interval of at least four hours between the time the tube was washed.
through and the time of aspiration at jejunal or ileal level.

2. **Method of sampling at gastric and jejunal level.**

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>GENUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) <strong>ENTEROBACTERIACEAE</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>ESCHERICHIA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>CITROBACTER</strong></td>
</tr>
<tr>
<td></td>
<td><strong>ALKALESCENS-DISPAR</strong></td>
</tr>
<tr>
<td></td>
<td><strong>KLEBSIELLA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>CLOACA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>HAFNIA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>PROTEUS</strong></td>
</tr>
<tr>
<td></td>
<td><strong>PROVIDENCIA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>SALMONELLA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>ARIZONA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>SHIGELLA</strong></td>
</tr>
<tr>
<td>2) <strong>LACTOBACILLACEAE</strong></td>
<td><strong>STREPTOCOCCUS FAGECALIS</strong></td>
</tr>
</tbody>
</table>

(Facing p. 3)
through and the time of aspiration at jejunal or ileal level.

2. Method of sampling at gastric and jejunial level.

The tube was similar. Since the duration of intubation in these cases was shorter the oral route was used. On intubation fasting gastric juice was aspirated, the initial aspirate being rejected. The tube was then washed through with 100 mls. normal sterile saline. With the exception of those patients with gastroenterostomies or partial gastrectomies washing was repeated when the tube had passed the pylorus. Aspiration was repeated at mid-jejunal level, the initial aspirate again being rejected. As before the position of the tube was calculated and assessed by fluoroscopy.

In all patients, passage through the pylorus was aided by turning them initially on to their right side. They were allowed a normal ward diet as soon as possible after intubation.

The aspirates were immediately made up into serial dilutions using nutrient broth. Viable counts were done on each specimen using the Miles and Misra technique (1938). The method will demonstrate the presence of organisms in as low a concentration as 250/ml. All estimations were performed in duplicate. In those patients undergoing jejunal and ileal aspiration it was hoped to isolate as many organisms as possible by using selective as well as ordinary media. Thus blood agar and MacConkey plates were cultured aerobically whilst blood agar, Willis and Hobbs' medium (1959) (for Clostridia), Rogosa agar (1961) (for Lactobacillus), Neomycin blood agar (for Bacteroides) and thallus acetate agar plates (Barnes 1956) (for anaerobic Streptococci) were cultured anaerobically. In those patients undergoing gastric and jejunal aspiration, organisms of the family Enterobacteriaceae were searched for, together with Streptococcus faecalis on MacConkey agar (Table I). Enterobacteriaceae were subdivided by their biochemical reactions to conform closely with the groups proposed by the Enterobacteriaceae subcommittee of the International Committee on Bacterial Nomenclature and Taxonomy.

The ability of organisms to assimilate cyanocobalamin in vitro was
<table>
<thead>
<tr>
<th>No.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>2x10^3</td>
</tr>
<tr>
<td>8</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>5x10^2</td>
</tr>
<tr>
<td>14</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>5x10^3</td>
</tr>
<tr>
<td>15</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td></td>
</tr>
</tbody>
</table>

* incl. Providencia
G - Gastric Aspirate
J - Jejunal Aspirate

(Facing p. 4)
BACTERIOLOGICAL FINDINGS IN THE JEJUNUM AND ILEUM OF SIX CONTROL PATIENTS (Nos. 1-6)

JEJUNUM

- Strep. viridans
- Staph. albus
- Diphtheroid bacilli
- Lactobacilli
- Strep. faecalis
- Coliform bacilli
- C. welchii
- Yeasts

Diagnosis
1. Normal
2. Hysterical Neurosis
3. Rheumatoid Arthritis
4. Thyrotoxicosis
5. Hypochondriasis
6. Obesity

ILEUM

- Yeasts

Figure I

(Facing p. 4)
estimated by preparing 10 ml. aliquots of a mixture of 150 mls. of Difco microinoculum broth to which 0.5 ug of $^{58}$Cobalt-labelled cyanocobalamin (Radiochemical Centre, Amersham) had been added. The solution was sterilised at 15 lbs. pressure for 10 minutes. One ml. of a fresh overnight culture of an organism was added to an aliquot. Two standards of 10 mls. of broth with added $^{58}$Cobalt-labelled cyanocobalamin, but inoculated with 1 ml. sterile saline, were used as controls. After overnight incubation at $37^\circ$C. the material was centrifuged at 3000 revs per minute for 30 minutes. The extent of radioactivity in the supernatant was calculated using a well type scintillation counter and compared with that in the uninoculated control samples. Measurement of radioactivity in the organisms confirmed that they had removed it from the culture medium. The radioactivity could not be washed off the organisms.

The rate of uptake of cyanocobalamin by the organisms was investigated by preparing 6x10 ml. of broth containing $^{58}$Cobalt-labelled cyanocobalamin as above and adding to this 1 ml. of a fresh overnight culture of the organism under study. Centrifugation was done as above after incubation at zero time, one hour, two hours, five hours, twelve hours and twenty-four hours, and the activity of the supernatant calculated at each time.

Vitamin B$_{12}$ absorption was calculated by the Schilling test (1953), modified in that carbachol, 0.25 mg., was given intramuscularly 15 minutes before the oral dose of labelled cyanocobalamin. Where required the test was repeated with intrinsic factor or after a five day course of tetracycline in a dosage of 250 mg. four times daily.

**RESULTS**

Figure I shows the findings obtained on culturing the jejunal and ileal aspirates for the organisms previously mentioned in six hospital control patients. The thyrotoxic patient was mildly toxic. The striking feature is the absence of faecal type organisms in the upper small intestine. Two type patients, i.e. 1 and 6, had faecal/organisms in the ileum.

Table 2 shows the findings in a series of similar patients who had their...
TABLE 3

FAECAL TYPE FLORA IN STOMACH AND INTESTINE OF
FIFTEEN PATIENTS WITH PERNICIOUS ANAEMIA

<table>
<thead>
<tr>
<th>No.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
</tr>
<tr>
<td>24</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>$1 \times 10^8$</td>
<td>$1 \times 10^8$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>$7 \times 10^6$</td>
<td>$3 \times 10^6$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>-</td>
<td>$4 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>$4 \times 10^6$</td>
<td>$2 \times 10^7$</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* incl. Providencia

N.D. - NOT DONE
G - GASTRIC ASPIRATE
J - JEJUNAL ASPIRATE

(Facing p. 5)
BACTERIOLOGICAL FINDINGS IN THE JEJUNUM AND ILEUM OF EIGHT PATIENTS (Nos. 16-23) WITH MALABSORPTIVE DISEASE.

JEJUNUM.

Strep viridans
Stapp Neisseria
Lactobacilli
Strep faecalis
Caliform bacilli
C. welchii
Yeast
Anaerobic streps.

ILEUM.

Figure II

Table I shows the findings in patients 16, 17, 18, 19, and 20. The other findings are to be found in patients 21, 22, and 23. The table also includes the findings of other patients with similar conditions.

(Facing p. 5)
gastric and jejunal aspirates cultured for faecal type organisms. Those with a testicular seminoma had been treated surgically and given radiotherapy at least three years previously and had not relapsed. The thyrotoxic patient was euthyroid and on potassium iodide prior to operation, at the time of intubation. The absence of a faecal type flora in the stomach and upper small intestine is clearly seen in this table. As far back as 1919 Bessau and Bossert stated that they regarded as abnormal the presence of even a few coliform bacilli in the duodenal juice. I would agree with this and would classify a concentration of faecal type organisms, as represented by the family Enterobacteriaceae and Streptococcus faecalis, of \(10^4/\text{ml.}\) as abnormal in the upper small intestine.

Figure II shows the findings of full bacteriological investigation in the jejunum and ileum of eight patients with malabsorptive disease. The diagnosis in these patients was made on the basis of examination of the stool for fat and nitrogen, the xylose absorption test, the folic acid absorption test of Girdwood (1953), radiological examination of the small intestine and histological examination of the small intestinal mucosa as obtained by the Crosby-Kugler capsule. Except for patients 16 and 22, who were on a gluten free diet, and patient 18 who was on steroid therapy, the patients were untreated. All patients except 22 and 23 showed impaired absorption of cyanocobalamin. As is seen faecal type organisms in the upper small intestine were found in significant numbers in patients 18 and 19, in the latter the concentration of Streptococcus faecalis only being significant. Yeast type organisms and Lactobacilli were generally found only in small concentrations in the upper small bowel.

The abnormal coliform concentration in patient 18 and the abnormal concentration of Streptococcus faecalis in patients 18 and 19 were reflected in quite high concentrations of similar organisms in the ileum. In addition patient 19 had now quite a high concentration of coliform organisms in the ileum and similar findings are to be found in patients 16, 17 and 20. Lactobacilli and yeasts were found, usually in low concentrations, in the ileum of most patients.

In table 3 there are illustrated the findings in fifteen patients with pernicious anaemia in whom the upper small bowel was investigated for faecal type organisms. In all these patients the diagnosis was made on the basis of
TABLE 4

FAECAL TYPE FLORA IN STOMACH AND INTESTINE OF THREE PATIENTS WITH MULTIPLE INTESTINAL DIVERTICULA

<table>
<thead>
<tr>
<th>NO.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
<th>Vit. B₁₂ Absorpt.</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANAEMIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IRON DEFICIENT</td>
</tr>
<tr>
<td>40</td>
<td>N.D.</td>
<td>5x10⁸</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G.I. SYMPTOMS</td>
</tr>
<tr>
<td>41</td>
<td>4x10³</td>
<td>5x10²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8x10³</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G.I. SYMPTOMS</td>
</tr>
</tbody>
</table>

* incl. Providencia

N.D. - NOT DONE
A - ABNORMAL
N - NORMAL
G - GASTRIC ASPIRATE
J - JEJUNAL ASPIRATE

(Facing p. 6)
TABLE 5

FAECAL TYPE FLORA IN STOMACH AND INTESTINE OF FOUR PATIENTS
WITH LOOPS IN LOWER SMALL BOWEL AND IMPAIRED ABSORPTION OF CYANOCOBALAMIN

<table>
<thead>
<tr>
<th>No.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
<th>B12 Absorption after Tetracycline</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>G 3x10^4</td>
<td>J - - - -</td>
<td>G - - - -</td>
<td>G - - - -</td>
<td>J - - - -</td>
<td>G - - - -</td>
<td>2x10^3 1x10^3</td>
<td>IMPROVED</td>
</tr>
<tr>
<td>43</td>
<td>- 3x10^3</td>
<td>- - - -</td>
<td>- - - -</td>
<td>1x10^3</td>
<td>- - - -</td>
<td>- 2x10^4</td>
<td>CORRECTED</td>
<td>ILEO-TRANSVERSE COLOSTOMY FOR APPENDICITIS</td>
</tr>
<tr>
<td>44</td>
<td>- 2x10^7</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>CORRECTED</td>
<td>ILEO-TRANSVERSE COLOSTOMY FOR APPENDICITIS</td>
</tr>
</tbody>
</table>

* incl. Providencia

N.D. - NOT DONE
G - GASTRIC ASPIRATE
J - JEJUNAL ASPIRATE

(Facing p. 6)
TABLE 6

FAECAL TYPE FLORA IN STOMACH AND INTESTINE OF SIX PATIENTS
WITH LOOPS IN LOWER SMALL BOWEL AND IMPAIRED ABSORPTION OF CYANOCOBALAMIN

<table>
<thead>
<tr>
<th>NO.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
<th>Correction of impaired absorption of Vit. B12 by Tetracycline</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D. 5x10^6</td>
<td>N.D.</td>
<td>N.D.</td>
<td>None</td>
<td>CROHNS ILEO-TRANSVERSE COLOSTOMY</td>
</tr>
<tr>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>CROHNS ILEO-TRANSVERSE COLOSTOMY</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>ILEO-TRANSVERSE COLOSTOMY FOR APPENDICITIS</td>
</tr>
<tr>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>ILEO-TRANSVERSE COLOSTOMY FOR ADHESIONS</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>ILEAL RESECTION P.A.</td>
</tr>
<tr>
<td>51</td>
<td>2x10^3</td>
<td>2x10^3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>NOT done CROHNS ILEO-TRANSVERSE COLOSTOMY GASTRO-ENTEROSTOMY</td>
</tr>
</tbody>
</table>

* incl. Providencia
G - GASTRIC ASPIRATE
J - JEJUNAL ASPIRATE
N.D. - NOT DONE

(Facing p. 6)
BACTERIOLOGICAL FINDINGS IN THE JEJUNUM AND ILEUM OF FIVE PATIENTS (Nos. 24-28) WITH PERNICIOUS ANAEMIA.

**JEJUNUM**
- Strep. viridans
- Strep. albus
- Lactobacilli
- Strep. faecalis
- Coliform bacilli
- C. welchii
- Yeasts

**ILEUM**

*Figure III*

(Facing p. 6)
finding a megaloblastic anaemia, a low serum vitamin B\textsubscript{12} level, achlorhydria on maximal histamine stimulation, and an abnormal Schilling test corrected by intrinsic factor. All patients responded to therapy with cyanocobalamin. Most patients had been treated at the time of intubation. Patient 34 also had an intestinal diverticulum. Patients 30, 34 and 38 had abnormal coliform counts in the stomach and upper small intestine. Patients 35 and 37 had an abnormal concentration of Streptococcus faecalis in the upper small intestine. In the others there was no significant difference from the findings in the control group. Figure III shows the results of the more extensive bacteriological studies at jejunal and ileal levels in five of these patients (cases 24 to 28). In addition to the absence of faecal type organisms it will be seen that Clostridium welchii were also absent from the upper small intestine. In two patients Clostridium welchii were found in the ileum and patient 28 had coliforms in the same region.

Table 4 shows the bacteriological findings of faecal type flora in three patients with small intestinal diverticula. Patients 39 and 40 exhibited impaired absorption of cyanocobalamin. This was corrected by antibiotics in these patients. In patient 41 who had normal absorption of cyanocobalamin there is no increase of the number of faecal organisms in the small intestine. The abnormal findings in patients 39 and 40 are not unexpected in that there was impaired absorption of vitamin B\textsubscript{12} correctable by antibiotics.

Table 5 illustrates the small intestinal findings in patients with surgical blind loops (ileo-transverse colostomies) involving the lower small bowel. All these patients had impaired absorption of vitamin B\textsubscript{12} correctable by antibiotics. None had steatorrhoea. An abnormal flora was present in patients 43, 44 and 45. Though the small intestinal findings in case 42 were normal the gastric coliform count may be significant.

Table 6 presents a similar group of patients but in these the impaired not absorption of cyanocobalamin was/corrected by antibiotics and only in case 50, who had had an unknown length of ileum resected with an end to side anastomosis, was it corrected by intrinsic factor. This suggests that the impaired vitamin B\textsubscript{12} absorption in cases 46, 47, 48 and 49 was due to disease or bypassing of the
### TABLE 7

**FAECAL TYPE FLORA IN STOMACH AND INTESTINE OF TEN PATIENTS WITH GASTRIC OPERATIONS**

<table>
<thead>
<tr>
<th>No.</th>
<th>E. Coli</th>
<th>Citrobacter</th>
<th>Alk. Dispar</th>
<th>Klebsiella</th>
<th>Proteus*</th>
<th>Strep. faecalis</th>
<th>Vit. B12 Absorpt.</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>G</td>
<td>J</td>
<td>-</td>
<td>Not done</td>
</tr>
<tr>
<td>52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>G.E.</td>
</tr>
<tr>
<td>53</td>
<td>-</td>
<td>9x10⁵</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>54</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P.G.</td>
</tr>
<tr>
<td>56</td>
<td>1x10⁷</td>
<td>6x10⁶</td>
<td>5x10²</td>
<td>8x10⁵</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>57</td>
<td>2x10⁴</td>
<td>3x10³</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>58</td>
<td>6x10⁴</td>
<td>3x10³</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3x10³</td>
<td>A</td>
</tr>
<tr>
<td>59</td>
<td>2x10⁵</td>
<td>1x10⁶</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P.G.</td>
</tr>
<tr>
<td>60</td>
<td>8x10⁷</td>
<td>8x10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>G.E.</td>
</tr>
</tbody>
</table>

* incl. Providencia

A - ABNORMAL  
N - NORMAL  
P.G. - PARTIAL GASTRECTOMY  
G.E. - GASTROENTEROSTOMY  
G - GASTRIC ASPIRATE  
J - JEJUNAL ASPIRATE

(Facing p. 7)
TABLE 8

UPTAKE OF LABELLED CYANOCOBALAMIN
BY ORGANISMS ISOLATED FROM THE G.I. TRACT

<table>
<thead>
<tr>
<th>ORGANISM GENUS</th>
<th>NO. OF STRAINS</th>
<th>MEAN % UPTAKE OF RADIOACTIVITY</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ESCHERICHIA</td>
<td>13</td>
<td>88.5%</td>
<td>78 - 98%</td>
</tr>
<tr>
<td>2. KLEBSIELLA</td>
<td>5</td>
<td>87%</td>
<td>80 - 93%</td>
</tr>
<tr>
<td>3. ALKALESCENS DISPAR</td>
<td>3</td>
<td>87%</td>
<td>80 - 91%</td>
</tr>
<tr>
<td>4. CITROBACTER</td>
<td>1</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>5. CLOACA</td>
<td>1</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>6. PROTEUS*</td>
<td>3</td>
<td>70%</td>
<td>56 - 83%</td>
</tr>
<tr>
<td>7. STREPTOCOCUS FAECALIS</td>
<td>9</td>
<td>6%</td>
<td>0 - 20%</td>
</tr>
</tbody>
</table>

* incl. Providencia
UPTAKE OF \textsuperscript{58}Co-B\textsubscript{12} BY BACTERIA.

- E. coli (13)
- E. coli (40)
- E. coli (45)
- E. coli (60)
- E. coli (69)

Figure IV

UPTAKE OF LABELLED CYANOCOBALAMIN BY INTESTINAL BACTERIA.

- Cloaca (10)
- Citrobacter (30)
- Klebsiella (12)
- Alkalescens-Disper (52)
- Proteus mirabilis (14)
- Alkalescens-Disper (53)

Figure V

(Facing p. 7)
UPTAKE OF LABELLED CYANOCOBALAMIN BY THE INTESTINAL BACTERIA OF PATIENTS WITH BLIND OR STAGNANT LOOPS.

% UPTAKE OF $^{58}$Co-$\text{B}_{12}$ BY BACTERIA

HOURS AFTER INCUBATION.

Figure VI

UPTAKE OF LABELLED CYANOCOBALAMIN BY CONTROL SERIES OF PROTEUS ORGANISMS

% UPTAKE OF $^{58}$Co-$\text{B}_{12}$ BY BACTERIA

HOURS AFTER INCUBATION

Figure VII

(Facing p. 7)
vitamin B₁₂ absorbing area in the ileum. In case 50 there must have been sufficient ileum remaining for vitamin B₁₂ absorption to occur. Except in case 46 the small intestinal flora in this group was normal in terms of faecal type organisms. This patient had subacute intestinal obstruction due to a tumour of the colon.

The results of examining the upper bowel content for faecal type organisms in patients with gastric operations are found in Table 7. It was not unusual to be able to isolate coliform organisms from the gastric and jejunal contents of this group and in some cases the number of organisms was in the abnormal range.

Studies of the uptake of labelled cyanocobalamin were carried out with thirty-five strains of organisms, and the results are shown in Table 8. These results confirm previous findings (Girdwood, 1955b; Doig and Girdwood 1960). It will be seen that the Proteus group of organisms are less active in their ability to remove vitamin B₁₂ from the culture medium than are other organisms of the family Enterobacteriaceae. Strains of Streptococcus faecalis took up very little labelled cyanocobalamin.

It is seen from Figures IV, V and VI that, when the rates of uptake of the vitamin were measured, on an average an organism required about five hours to attain its maximum uptake. The pattern of uptake by organisms isolated from patients with blind or stagnant loops was similar to that of organisms isolated from other patients. The number of Proteus strains isolated was only three and six organisms obtained from the National Collection of Type Cultures (Collindale Avenue, London N.W.9) were therefore examined (Figure VII). The curves confirm that these organisms are less active in their uptake of cyanocobalamin.

DISCUSSION

Method of Sampling

As French (1961) points out, the inaccessibility of the small intestine and the rapid alteration of its content during digestion make it very difficult to characterise what is taking place so far as gut flora is concerned, and it is not surprising that no clear picture has yet emerged. There is considerable difficulty about any method of study. The direct sampling technique (Blacklock
et al. 1937; Cregan and Hayward, 1953) is attractive, but only a semi-quantitative estimation of organisms is possible with it and the state of the bowel in the fasting anaesthetised patients is not necessarily the same as that in the ambulant patient. In the experimental work to date most studies have dealt with the patient in the fasting state (French, 1961) but in the present studies the patients have been on a normal ward diet except when gastric juice has been aspirated, this being done with the patient fasting.

One difficulty peculiar to an intubation technique is that little weight can be attached to the finding of oropharangeal type flora in the small intestine as much of this has almost certainly been carried down by the tube. In an attempt to get round this various ingenious methods have been employed for occluding the aspirating ports on intubation. These probably do little to exclude the error introduced by the carrying down of organisms, but it is not claimed that the attempt employed here to solve this problem, by washing the tube through with saline, is entirely satisfactory. For this reason little comment is made about the finding of such flora.

It was because of these difficulties that the emphasis was turned to the study of a faecal type flora. The finding of these is of some significance in that they are unlikely to have been carried down by the tube. Considerable simplicity is also introduced since one can with reasonable justification classify a faecal flora by the family Enterobacteriaceae and the genus Streptococcus faecalis and the isolation and identification of these is reasonably easy.

The findings regarding the presence of these organisms in the small intestine at jejunal level agree with those of Blacklock et al. (1937) and Cregan and Hayward (1953). Such organisms are very unusual at this site. It is more difficult to interpret the finding of these organisms at ileal level. By intubation Nicols and Glenn (1940) found coliforms to be unusual in the ileum of normal controls. Blacklock and his co-workers found coliform organisms in the lower ileum of one third of his young patients, though the concentrations are not stated. Cregan and Hayward found them in three of fourteen patients, in two of these in significant concentrations. Unlike the present study, all the above were done in fasting subjects. One of the controls in this study (No. 6, Figure
I) had coliform organisms present in the ileum and another some Clostridium welchii. The finding of a faecal type flora in this site is therefore difficult to interpret, and it would seem that the demonstration of this type of flora is likely to be of more significance, the higher up the small bowel that it is found.

These were the considerations which led to the curtailing of the earlier more extensive bacteriological studies to the study of a faecal type flora and, in particular, in most of the patients to the study of samples obtained from the jejunum.

It is felt that the tube chosen for the present work has some advantages. In particular it causes little discomfort and is extremely well tolerated by patients who are able to partake of a normal diet with no difficulty. Previous studies have involved the use of larger tubes, including Miller Abbott tubes. The tube used in the present investigation is perhaps less likely to disturb small bowel physiology than a larger tube in that its size and marked pliability at body temperature interfere to a lesser degree with small intestinal function. Also, the "creeping" of the small bowel up the larger tube, which adds to the difficulty of estimating the level at which one is aspirating is probably less marked with this tube.

Patients with Malabsorptive Disease

Bearing in mind the difficulty in interpreting the findings of oropharangeal type flora it is seen that in these patients the upper small intestinal flora differs little from those in the control group of patients. As in the controls a faecal type flora is absent from the jejunum in most of the patients. Streptococcus faecalis appears in abnormal concentrations in the jejunum of patients 18 and 19, and we would also classify the coliform count in patient 18 as just abnormal. This last patient was on steroid therapy and other patients on these drugs are at present being studied to see if such therapy is associated with an abnormal small bowel flora. It is difficult to explain the concentration of Streptococcus faecalis in case 19.

With regard to the ileal findings in these patients it is seen that patients 18 and 19, who had jejunal abnormalities, also had coliform organisms in the ileum. However, three other patients (16, 17 and 20) were found to have these
as well. In spite of the previous remarks regarding the difficulty of interpreting faecal flora at this site, it may be that the incidence of these organisms in this group of patients is greater than might have been expected.

Several of these patients had impaired absorption of vitamin B₁₂ but it was impossible to draw any relationship between this and the presence of Enterobacteriaceae in the small bowel. Certainly in patients 17 and 18 broad spectrum antibiotics did not improve absorption of the vitamin.

In the search for specific organisms as the cause of malabsorptive disease it has been suggested that Lactobacilli or yeasts are responsible. It will be noticed that in the group of patients investigated here these organisms were not infrequently isolated from the small bowel. It is not suggested that this indicates that they are responsible for the disease and, in fact, in the low concentrations found they are very likely to be a secondary phenomenon, as previously suggested (French, 1961).

**Patients with Pernicious Anaemia**

Many observers have emphasised the existence of an abnormal flora in the small intestine of patients with pernicious anaemia and much of the literature prior to 1930 is reviewed by Moench and his colleagues (1925) and Davidson and Gulland (1930). However the descriptions of the abnormal flora in patients with pernicious anaemia have been based on the findings in the gastro-duodenal juice and faeces, and not on the findings in the small intestine itself. Although the view that the features of the disease are attributable to the absorption of a toxin from the alimentary tract is not now accepted, it is still a hypothesis that an abnormal small intestinal flora exists, particularly as regards faecal type organisms. The high pH of the gastric and therefore the intestinal contents is invoked as being responsible for this (Davidson and Gulland, 1930). The findings of Cregan, Dunlop and Hayward (1953) in patients with low gastric acidity indicated that the flora of the small intestine was independent of acid secretion by the stomach, and this was an additional reason for investigating the matter further in patients with pernicious anaemia.

The results obtained in these patients indicate that as far as faecal flora is concerned there is no demonstrable difference between the findings in the
majority of patients with pernicious anaemia and those in the control patients. In patient 34 (Table 3) the increase in coliform organisms may have been due to the presence of a diverticulum in the upper small intestine. Radiological examination of the small intestine in cases 30 and 38, both of whom had an abnormal faecal flora in the jejunum, revealed no local abnormality which might explain the findings. In the light of the findings in the control patients the concentration of Streptococcus faecalis in patients 35 and 37 is also abnormal. It appears therefore that in some patients with pernicious anaemia there is an increase of faecal type organisms in the jejunum.

Patients with pernicious anaemia have been reported as having a markedly increased concentration of Clostridium welchii in the faeces (Koench et al. 1925). The reason for this is obscure, but Davidson and Gulland (1930) thought that it must be due to an increase of spores in the faeces of these patients as the vegetative forms of the organisms which were present were found to be dead. They suggested that the "altered biochemical reaction of the contents of the small intestine" in pernicious anaemia might be largely responsible for the findings in the faeces. Koench and his co-workers (1925) supported the opinion of previous observers in suggesting that this finding indicated an increase in the concentration of these organisms in the small intestine of patients with pernicious anaemia. Davidson and Gulland were unable to demonstrate any significant increase in the number of Clostridia in the gastroduodenal contents of their patients with this disease and the present work has shown that these organisms were absent from the upper small intestine of the patients studied (Figure III).

With regard to the ileal contents in these patients, one had some coliforms present in this region and two had some Clostridium welchii. In view of the findings in the control group (Figure 1) this should not be interpreted as being necessarily of any significance.

In general it may be said that the present findings do not support the contention of previous authors that, in patients with pernicious anaemia, the small intestinal contents of faecal type organisms consistently differ from that of controls and this supports the observations of Cregan, Dunlop and Hayward.
that the manner in which the small intestine is kept relatively free of these organisms is independent of gastric acidity.

Patients with Blind or Stagnant Loops

Patients with a blind or stagnant loop not uncommonly exhibit evidence of impaired absorption of cyanocobalamin and it has been suggested that this is due to intestinal organisms which compete with the host for the vitamin (Doig and Girdwood, 1960). It has been demonstrated that organisms of the family Enterobacteriaceae, the predominant organism in these loops, are active as regards their uptake of vitamin B₁₂ (Table 8). The view that this might occur in vivo has been challenged recently by Booth and Heath (1962).

It has been shown in the past that various substances including intrinsic factor will bind cyanocobalamin, rendering it unavailable to micro-organisms that would otherwise take it up in vitro (Ternberg and Bakin, 1949; Bird and Hoevet, 1951; Burkholder, 1952; Hoff-Jørgensen, 1952; Drexler, 1958). Since, under physiological conditions, vitamin B₁₂ is present in the intestine bound to intrinsic factor it is suggested that the vitamin is not available to the organisms in vivo. Booth and Heath (1962) have shown that, though Escherichia coli are capable of inhibiting absorption of vitamin B₁₂ in the rat, the inhibitory effect of the organism was partially abolished when the vitamin had previously been bound by intrinsic factor (in this case rat gastric extract). Girdwood (1955b), working with gastric juice, was however unable to confirm that in the presence of this, vitamin B₁₂ was not available to Escherichia coli and more recent work here again suggests that human gastric juice does not necessarily render cyanocobalamin unavailable to this organism.

C. welchii has not been found in the increased faecal flora which the present study has demonstrated in the jejunum of patients with blind or stagnant loops giving rise to impaired vitamin B₁₂ absorption. In vitro studies with these organisms show that human A strains, as well as C and D strains, take the vitamin up poorly both after 24 and 48 hours of anaerobic incubation.

The in vitro studies on the rates of uptake by organisms of the family Enterobacteriaceae have demonstrated that an organism requires about five hours to attain its maximum uptake of the vitamin. Since food takes about four hours
UPTAKE OF LABELLED CYANOCOBALAMIN. VARIATION OF CONCENTRATION OF INOCULUM.

Figure VIII

(Facing p. 13)
from the time of intake to reach the large intestine (Starling and Lovatt Evans, 1962) it is not difficult to imagine how blind or stagnant loops in the proximal small bowel are able to flood this region with organisms which might bind the vitamin rendering it unavailable for absorption at the terminal ileum. Indeed in the two patients with jejunal diverticula and impaired absorption of cyanocobalamin, which was correctable by antibiotics, the coliform concentration in the jejunal juice was high (Table 4, Nos. 39, 40).

Though in vitro findings must be applied with caution to the human, the five hour lapse before maximum uptake of the vitamin makes it more difficult to understand how a blind or stagnant loop in the distal reaches of the small bowel allows its inhabiting organisms sufficient time to bind the vitamin rendering it unavailable for absorption. Force of numbers of bacteria in the distal small bowel might diminish the importance of the delay factor. Thus in Figure VIII it is seen that in vitro, after two hours a quadruple strength inoculum of organisms has taken up as much vitamin B₁₂ as a single strength inoculum does in five hours. It is particularly difficult to apply such studies to man in whom not only the numbers of bacteria but the amount of nutrient as well as the amount of available vitamin B₁₂ will vary, and in whom it is possible that gastrointestinal secretions affect the interaction between micro-organisms and the vitamin.

In this context the findings expressed in Tables 5 and 6 are of interest. All these patients had undergone similar bowel operations and with one exception, who was not available for absorption studies (Table 6, Case 51), all exhibited impaired absorption of cyanocobalamin. Antibiotic therapy corrected or markedly improved absorption in the patients in Table 5 and certainly in three of these coliforms were found in higher than normal concentrations in the upper small intestine. The gastric coliform count in patient 42 may be significant.

Antibiotics did not affect the absorption of cyanocobalamin in any of the patients in Table 6, and only in case 50 was absorption corrected by intrinsic factor. Except for case 50, therefore, the impaired absorption of cyanocobalamin was due to disease or bypassing of the terminal ileum. In these cases coliform organisms were, with one exception, not in the abnormal range. The one exception,
case 46, had subacute intestinal obstruction due to a malignant tumour of the ascending colon. Bishop and Allcock (1960) found that patients with intestinal obstruction can exhibit a very abnormal flora in the small intestine and this probably accounts for the abnormal findings in this case.

From these findings it would seem that patients with blind or stagnant loops in the distal small bowel may be divided into two groups. There are those with an abnormal content of faecal type organisms in the upper small intestine and those with a normal faecal flora at the same site. The former group exhibit features of a blind loop syndrome in relation to vitamin B\textsubscript{12} absorption. The latter group do not, and hence it can be concluded in these patients, provided intrinsic factor is unable to correct absorption, that a diseased or bypassed ileum is responsible for the impaired absorption of the vitamin. It must be said, however, that from the results of tests available to us at present it is difficult to exclude the possibility of a blind loop syndrome being present in these patients also.

A hypothesis that is suggested by the bacteriological findings in these two groups of patients is that where a blind or stagnant loop in the distal small bowel gives rise to impaired absorption of cyanocobalamin, the blind loop is found to be associated with an abnormal content of coliform organisms in the upper small intestine. Obviously this brings the organisms into contact with the vitamin at quite an early stage during its passage down the small bowel. In view of the in vitro five hour delay demonstrated between availability and maximum uptake of the vitamin by the organisms this early access to vitamin B\textsubscript{12} might be one way of enabling organisms in these distal loops to bind the vitamin before it reaches its site of absorption at the ileum.

General Points

It would be of great interest to try and establish the source of the abnormal faecal flora in the upper small intestine which occurs in some patients. One should resist the temptation to put it down to growth from below and indeed the observations of Bishop and Allcock (1960) would seem to indicate that the abnormal flora is obtained from ingestion, at least in patients with intestinal obstruction. What exactly prevents these organisms from establishing a hold in
the upper reaches of the small intestine in normal circumstances it is impossible to say. Peristaltic activity is probably of importance though unlikely to be the only, or even the most important factor. Thus it is worth recalling the observations of Howie and his colleagues on the findings of Clostridia in the stomachs of patients recently exposed to gastric operations (Howie et al. 1953; Duncan et al. 1954). It is possible that we are constantly ingesting faecal type organisms in low concentrations and that these remain undetected until some change in the pattern of bowel function allows their multiplication. In this context previous authors have used the terms "transient" and "permanent" flora — the latter description being given to organisms which are present in such concentration that they can be said to be capable of multiplication in the part of the intestine sampled (Cregan and Hayward, 1953). Using the present technique the finding of faecal type organisms in a concentration of \(10^4/\text{ml}\) has been defined as abnormal but one is reluctant to use the word "permanent". Thus the finding of faecal type organisms in the upper small bowel in patients with stagnant or blind loops in the proximal bowel following gastric operations is not unusual, and in a group of ten patients studied here, four had abnormal counts in the upper small intestine (Table 7, Nos. 53, 56, 59 and 60). Using the Schilling test it is difficult to establish the part played by coliform organisms in impaired absorption of vitamin B\(_{12}\) when there is another cause for this present. Thus in patients 56, 59 and 60 the Schilling test was completely corrected by intrinsic factor and so it is unlikely that the organisms were playing any significant part. However, in case 53, vitamin B\(_{12}\) absorption was normal in spite of the abnormal concentration of Escherichia coli in the upper small intestine. It may be that the bacteriological findings merely reflect evidence of a transient phenomenon such as the discharge of proximal loop content into the small intestine just prior to sampling. This may also explain the abnormal counts in the patients with pernicious anaemia and an intestinal diverticulum (Table 3, case 34).

It is impossible to compare the present findings in these patients with the findings of Goldstein, Wirts and Kramers (1961) and Wirts and Goldstein (1963) who investigated the flora in the afferent loops (or failing this, at the gastrojejunal stoma) of patients with partial gastrectomies. They related the presence
of steatorrhoea to the finding of high coliform counts in these patients though they also describe one patient with, in addition to steatorrhoea, impaired vitamin B₁₂ absorption which was corrected by antibiotics. Only patients 53, 56 and 59 have counts approximating to the abnormal counts they found in their patients. None of these patients had steatorrhoea and neither did the patient with a gastro-enterostomy and high coliform counts in the upper bowel (case 60).

It is felt that the presence of faecal type flora in the upper small intestine must be interpreted with caution and it would seem that a high intestinal aspirate containing an abnormally high concentration of coliform organisms, may be evidence of either a transient or permanent invasion of the upper small intestine and can only be interpreted in the light of knowledge of intestinal anatomy and retrospectively in the light of the clinical and investigative findings. Thus, for example, the absence of faecal type organisms in the upper small intestine of a patient with a blind or stagnant loop which was giving rise to impaired absorption of cyanocobalamin, regardless of the site of this loop in the small intestine, would be surprising. However, the presence of such a flora even in obviously high concentrations is not diagnostic of a blind loop syndrome.

**SUMMARY**

By means of an intubation technique the bacteriological flora of the small intestine has been examined in a group of control patients, a group of patients with malabsorptive disease, a group with pernicious anaemia, and a group of patients with blind or stagnant loops involving the small bowel.

The findings are presented and discussed and where possible an attempt has been made to correlate these with studies in vitro of the uptake of vitamin B₁₂ by organisms.
REFERENCES


DAVIDSON, L.S.P. and GULLAND, G.L. "Fernicious Anaemia" (1930), Henry Kimpton, Lond., Chap. IV.


