The effect of *E. coli* on the absorption of vitamin B₁₂

C. C. Booth and Janet Heath

*From the Department of Medicine, Postgraduate Medical School of London*

SYNOPSIS  Cultures of *E. coli* are capable of inhibiting the absorption of vitamin B₁₂ in the rat. Their inhibitory effect is totally abolished when the organisms are killed by heat and partially abolished when test doses of labelled B₁₂ are previously bound by intrinsic factor.

Several observations suggest that the absorption of vitamin B₁₂ may be inhibited by bacteria. Both in experimental animals and in patients with blind loops, strictures, or fistulae involving the small intestine, heavy growths of bacteria have been found in areas of the bowel which are normally sterile (Seyderhelm, Lehmann, and Wichels, 1924; Doig and Girdwood, 1960). In patients with such lesions the absorption of radio-active vitamin B₁₂ is usually subnormal, and these patients may absorb B₁₂ normally if they are treated with a suitable intestinal antibiotic (Badenoch, Bedford, and Evans, 1955; Mollin and Baker, 1955; Halsted, Lewis, and Gasster, 1956; McIntyre, Sachs, Krevans, and Conley, 1956; Mollin, Booth, and Baker, 1957).

The precise way in which bacteria are able to block B₁₂ absorption is uncertain. Since bacteria, particularly *E. coli*, take up vitamin B₁₂ *in vitro*, it is usually supposed that bacteria utilize B₁₂ in competition with their host (Doig and Girdwood, 1960). Under physiological conditions, however, vitamin B₁₂ is present in the intestine bound to intrinsic factor, and *in vitro* such bound B₁₂ is not available to microorganisms (Ternberg and Eakin, 1949; Hoff-Jørgensen, 1952). It therefore seems unlikely that bacteria interfere with B₁₂ absorption simply by competition.

In order to determine the effect of bacteria on the absorption of free and bound B₁₂, we have studied in the rat the absorption of ⁵⁸Co-labelled B₁₂ given either alone or with varying concentrations of *E. coli*, an organism often isolated from the small intestine of patients with blind loops or strictures who fail to absorb B₁₂ (Doig and Girdwood, 1960); we have also studied the effect of *E. coli* on the absorption of vitamin B₁₂ previously incubated with rat gastric juice.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS  Black and white laboratory rats weighing between 200 and 250 g. were used throughout the experiments.

RADIOACTIVE VITAMIN B₁₂  Vitamin B₁₂ labelled with ⁵⁸Co was obtained from the Radiochemical Centre, Amersham. The material initially had a specific activity of 10 µc per µg. By suitable dilution, test doses of 0.01 µg. (0.1 µc) in 1-ml volumes were prepared.

RAT GASTRIC JUICE  A saline extract of the gastric mucosa of 10 freshly killed rats was prepared. The mucosa were rapidly sliced into 20 ml. of iced saline and extracted by rapid stirring for half an hour. The resulting material was filtered and stored at −20°C. until required.

CULTURES OF *E. coli*  *E. coli* was first cultured on standard agar slopes and the resulting growth was suspended in saline. These suspensions were then added to a protein-free culture medium half an inch deep in a flat-bottomed five-litre flask. The culture medium contained only K₂HPO₄, KH₂PO₄, Na₂ citrate SH₄O, Mg SO₄, 7 H₂O, (NH₄)₂SO₄, and glucose and was prepared according to the formula described by Lederberg (1950). Cultures were incubated at 37°C for 48 hours until growth was satisfactory. The organisms were then concentrated by repeated centrifuging and suitable concentrations were prepared by dilution. An estimate of the number of organisms in the bacterial suspensions was obtained by comparison with a series of Wellcome opacity tubes. The concentrations used were either 1.9 or 3.8 × 10⁹ organisms per ml.

EXPERIMENTAL PROCEDURE

EFFECT OF *E. coli* ON ABSORPTION OF VITAMIN B₁₂  Two groups of six rats were given successive test doses of 0.01 µg. of ⁵⁸Co-labelled B₁₂ either alone or after incubation for half an hour with different concentrations of *E. coli*. The first group also received test doses of labelled B₁₂ together with *E. coli* killed by heating to 56°C for four hours. The second group received further test doses given...
with 1 ml. of rat gastric juice, first alone and then after subsequent incubation with *E. coli*.

**MEASUREMENT OF B₁₂ ABSORPTION** The test doses were usually given in 1 ml. volume through a fine polythene tube passed into the stomach. The animals were placed in separate metabolism cages and the faeces were collected in waxed cardboard containers for three days. The radioactivity in the faeces was then measured by positioning each carton between two scintillation counters according to the method described by Lewis and Porter (1960). This counting arrangement made it possible to count faecal radioactivity without homogenization. Absorption was then calculated by assuming that the radioactivity not recovered had been absorbed.

**BINDING OF B₁₂ BY E. COLI OR RAT GASTRIC JUICE** The B₁₂ binding capacity of live *E. coli* or *E. coli* which had been killed by heat was determined by ultrafiltration. ⁵⁸Co-labelled B₁₂ 0.01 µg, was incubated for half an hour with suspensions of *E. coli* at similar concentrations to those used in the absorption tests, and the radioactivity of an ultrafiltrate was compared with that of the unfiltered solution. The binding capacity of 1 ml. of gastric mucosal extract was determined similarly.

**RESULTS**

**EFFECT OF E. COLI ON ABSORPTION OF FREE VITAMIN B₁₂**

Six rats (nos. 1 to 6) were given test doses of 0.01 µg of ⁵⁸Co-labelled B₁₂ first alone, then after incubation for half an hour with *E. coli* at concentrations of 1.9 or 3.8 × 10⁹ organisms per ml. They also received a further test dose after incubation with the larger concentration of *E. coli* but on this occasion the organisms had been previously killed by heating at 56°C. for four hours. The results of these absorption tests are given in Table I and illustrated in Fig. 1.

The animals absorbed between 44.8 and 70.6% (mean 60.2%) when the dose was given alone (Fig. 1). When the dose was incubated with the two different concentrations of *E. coli* absorption was reduced, the degree of inhibition being directly proportional to the number of organisms given. The rats given the dose of labelled B₁₂ incubated with *E. coli* at a concentration of 1.9 × 10⁹ organisms per millilitre absorbed from 28.9 to 42.7% (mean 34.2%). When the concentration of *E. coli* was 3.8 × 10⁹ organisms per millilitre only between 0.0 and 30.0% (mean 16.7%) was absorbed.

When the bacteria were previously killed by heat at 56°C. for four hours, this inhibitory effect on the absorption of B₁₂ was abolished (Fig. 1).

**EFFECT OF GASTRIC JUICE ON INHIBITION OF B₁₂ ABSORPTION BY E. COLI** The second group of six rats (nos. 7 to 12) received four consecutive test doses of 0.01 µg of ⁵⁸Co-labelled B₁₂. The dose was given first alone, then after incubation with 1 ml. of the gastric mucosal extract. For the third absorption test, the doses were previously incubated for half an hour with live *E. coli* at a concentration of 3.8 × 10⁹ organisms per millilitre. In the final test, the labelled B₁₂ was first incubated for half an hour with 1 ml. of the gastric mucosal extract, and then with live *E. coli* at a concentration of 3.8 × 10⁹ organisms per millilitre. The results of these tests are given in Table II and illustrated in Fig. 2.

When the dose of B₁₂ was given alone, the rats absorbed from 27.2 to 63.8% (mean 51.4%) and similar amounts were absorbed when the dose was given with 1 ml. of gastric mucosal extract (Fig. 2, Table II). As in the first group of animals, *E. coli* at

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**TABLE I**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Dose Given Alone</th>
<th>Dose Plus E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose 10⁶</td>
<td>3.8¹</td>
</tr>
<tr>
<td>1</td>
<td>70.6</td>
<td>42.7</td>
</tr>
<tr>
<td>2</td>
<td>65.5</td>
<td>31.3</td>
</tr>
<tr>
<td>3</td>
<td>44.8</td>
<td>29.9</td>
</tr>
<tr>
<td>4</td>
<td>66.1</td>
<td>40.4</td>
</tr>
<tr>
<td>5</td>
<td>57.0</td>
<td>32.0</td>
</tr>
<tr>
<td>6</td>
<td>57.3</td>
<td>28.9</td>
</tr>
</tbody>
</table>

Mean = 60.2

1 Concentration of *E. coli* as number of organisms per ml. × 10⁹.

2 *E. coli* heated at 56°C. for four hours before incubation with B₁₂.
TABLE II
PERCENTAGE ABSORPTION OF 0-01 μg. 58Co-LABELLED B12 GIVEN ALONE, WITH GASTRIC JUICE AND WITH E. COLI ALONE OR AFTER PREVIOUS INCUBATION WITH GASTRIC JUICE

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Dose Alone</th>
<th>Dose Plus Rat Gastric Juice</th>
<th>Dose Plus E. coli</th>
<th>Dose Plus Gastric Juice then Incubated with E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>63.8</td>
<td>47.0</td>
<td>0.0</td>
<td>24.5</td>
</tr>
<tr>
<td>8</td>
<td>59.7</td>
<td>56.4</td>
<td>3.5</td>
<td>33.3</td>
</tr>
<tr>
<td>9</td>
<td>40.5</td>
<td>28.2</td>
<td>17.0</td>
<td>28.3</td>
</tr>
<tr>
<td>10</td>
<td>54.6</td>
<td>54.4</td>
<td>18.5</td>
<td>32.9</td>
</tr>
<tr>
<td>11</td>
<td>27.2</td>
<td>59.3</td>
<td>10.5</td>
<td>50.5</td>
</tr>
<tr>
<td>12</td>
<td>61.2</td>
<td>65.4</td>
<td>25.0</td>
<td>29.2</td>
</tr>
<tr>
<td>Mean</td>
<td>51.4</td>
<td>51.8</td>
<td>12.4</td>
<td>33.1</td>
</tr>
</tbody>
</table>

1Concentration of E. coli was 3.9 x 10⁹ organisms per millilitre.

a concentration of 3.8 x 10⁹ organisms per millilitre inhibited absorption, the range of absorption under these conditions being from 0-0 to 25-0 % (mean 12.4%). However, this inhibitory effect was greatly reduced when the B₁₂ was incubated with gastric mucosal extract before the organisms were added, the animals now absorbing from 24.5 to 50.5% (mean 33.1%) (Fig. 2, Table II).

BINDING OF B₁₂ BY E. COLI OR BY RAT GASTRIC JUICE
The capacity of 1 ml. of E. coli at a concentration of 3.8 x 10⁹ organisms per millilitre to bind 0-01 μg. of 58Co-labelled B₁₂ is given in Table III, together with the binding capacity of similar concentrations of E. coli killed by heating at 56°C. for four hours, or at 100°C. for half an hour. The B₁₂ binding capacity of 1 ml. of the gastric mucosal extract is also shown in Table III.

TABLE III
BINDING OF 0-01 μg. 58Co-LABELLED B₁₂ BY E. COLI BEFORE AND AFTER HEATING OR BY RAT GASTRIC JUICE

<table>
<thead>
<tr>
<th>Material Added to B₁₂</th>
<th>Counts in Sample</th>
<th>Counts in Ultra-filtrate</th>
<th>% Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml. unheated E. coli</td>
<td>3,170</td>
<td>1,466</td>
<td>54</td>
</tr>
<tr>
<td>1 ml. E. coli heated at 56°C. for four hours</td>
<td>4,411</td>
<td>1,557</td>
<td>65</td>
</tr>
<tr>
<td>1 ml. E. coli heated at 100°C. for half an hour</td>
<td>3,345</td>
<td>2,696</td>
<td>20</td>
</tr>
<tr>
<td>1 ml. gastric juice</td>
<td>2,620</td>
<td>1,520</td>
<td>42</td>
</tr>
</tbody>
</table>

1Concentration of E. coli was 3.9 x 10⁹ organisms per millilitre.

The live organisms bound 54% of the labelled vitamin B₁₂ and a similar amount was bound by the E. coli when killed by heating at 56°C. for four hours (Table III). After heating at 100°C. for half an hour, however, the binding capacity of E. coli was much reduced, being only 20%.

One millilitre of the gastric mucosal extract bound 42% of the labelled B₁₂ (Table III).

DISCUSSION
The results given in this paper indicate that cultures of E. coli are capable of inhibiting the absorption of 58Co-labelled vitamin B₁₂ in the rat (Fig. 1, Table I). This inhibitory effect was apparently a vital function of living organisms and was not due merely to binding of the B₁₂ by E. coli, for when killed by heating at 56°C. for four hours the organisms were no longer capable of inhibiting absorption although they retained their capacity to bind B₁₂ (Fig. 1, Table III), as do preparations of intrinsic factor similarly treated (Spray, 1952).

The results also show that when test doses of labelled B₁₂ are previously incubated with gastric mucosal extracts, cultures of E. coli lose much of their capacity to inhibit B₁₂ absorption. As shown in Fig. 2 and Table II, this protective action of gastric juice was not complete, for the absorption of the test doses incubated with gastric juice before the addition of E. coli was not entirely normal. Table III shows that the rat gastric mucosal extract which was used only bound 42% of the test dose and the E. coli were presumably able to take up the proportion of the oral doses which was not bound. The rat gastric juice was only likely to protect that fraction of the dose which was bound.

The precise way in which bacteria are capable of influencing B₁₂ absorption in patients with the 'blind loop' syndrome therefore remains uncertain, but it is...
The effect of E. coli on the absorption of vitamin B₁₂ may be suggested that E. coli may interfere in some way with the transport mechanism in the mucosa of the distal small intestine.

We wish to thank Dr. D. L. Mollin for his encouragement, criticism, and advice. We are also grateful to Dr. Mary Barber for supplying cultures of E. coli.

REFERENCES


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*Gut* 1962 3: 70-73
doi: 10.1136/gut.3.1.70

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