The effect of *E. coli* on the absorption of vitamin B$_{12}$

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SYNOPSIS Cultures of *E. coli* are capable of inhibiting the absorption of vitamin B$_{12}$ in the rat. Their inhibitory effect is totally abolished when the organisms are killed by heat and partially abolished when test doses of labelled B$_{12}$ are previously bound by intrinsic factor.

Several observations suggest that the absorption of vitamin B$_{12}$ 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, Lehmman, and Wielches, 1924; Doig and Girdwood, 1960). In patients with such lesions the absorption of radio-active vitamin B$_{12}$ is usually subnormal, and these patients may absorb B$_{12}$ 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 B$_{12}$ absorption is uncertain. Since bacteria, particularly *E. coli*, take up vitamin B$_{12}$ in *vitro*, it is usually supposed that bacteria utilize B$_{12}$ in competition with their host (Doig and Girdwood, 1960). Under physiological conditions, however, vitamin B$_{12}$ is present in the intestine bound to intrinsic factor, and *in vitro* such bound B$_{12}$ is not available to microorganisms (Ternberg and Eakin, 1949; Hoff-Jørgensen, 1952). It therefore seems unlikely that bacteria interfere with B$_{12}$ absorption simply by competition.

In order to determine the effect of bacteria on the absorption of free and bound B$_{12}$, we have studied in the rat the absorption of $^{58}$Co-labelled B$_{12}$ 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 B$_{12}$ (Doig and Girdwood, 1960); we have also studied the effect of *E. coli* on the absorption of vitamin B$_{12}$ previously incubated with rat gastric juice.

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MATERIALS AND METHODS

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

RADIOACTIVE VITAMIN B$_{12}$ Vitamin B$_{12}$ labelled with $^{58}$Co was obtained from the Radiochemical Centre, Amersham. The material initially had a specific activity of 10 $\mu$C per $\mu$g. By suitable dilution, test doses of 0.01 $\mu$g. (0.1 $\mu$C) in 1-ml. volumes were prepared.

RAT GASTRIC JUICE A saline extract of the gastric mucosa of 10 freshly killed rats was prepared. The mucosae 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 $-20^\circ$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 K$_2$HPO$_4$, KH$_2$PO$_4$, Na$_4$ citrate SH$_2$O$_4$, MgSO$_4$, 7 H$_2$O, (NH$_4$)$_2$SO$_4$, and glucose and was prepared according to the formula described by Lederberg (1950). Cultures were incubated at 37$^\circ$C. for 48 hours or 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 $\times$ 10$^8$ organisms per ml.

EXPERIMENTAL PROCEDURE

EFFECT OF E. COLI ON ABSORPTION OF VITAMIN B$_{12}$ Two groups of six rats were given successive test doses of 0·01 $\mu$g. of $^{58}$Co-labelled B$_{12}$ either alone or after incubation for half an hour with different concentrations of *E. coli*. The first group also received test doses of labelled B$_{12}$ together with *E. coli* killed by heating to 56$^\circ$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 $B_{12}$ 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 position-
ing 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 $B_{12}$ BY *E. coli* OR RAT GASTRIC JUICE** The $B_{12}$
binds with capacity of live *E. coli* or *E. coli* which had been
killed by heat was determined by ultrafiltration. $^{58}$Co-
labelled $B_{12}$, 0-01 $\mu$g, 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 $B_{12}$**
Six rats (nos. 1 to 6) were given test doses of 0-01 $\mu$g.
of $^{58}$Co-labelled $B_{12}$ first alone, then after incubation
for half an hour with *E. coli* at concentrations of
1-9 or 3-8 $\times 10^9$ 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 by
$56^\circ C$. 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,

**TABLE I**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Dose Given Alone</th>
<th>Dose Plus E. coli</th>
<th>1-9$^a$</th>
<th>3-8$^a$</th>
<th>3-8$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70-6</td>
<td>42-7</td>
<td>19-0</td>
<td>54-4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65-5</td>
<td>31-3</td>
<td>15-8</td>
<td>69-2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44-8</td>
<td>29-9</td>
<td>25-5</td>
<td>70-5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>66-1</td>
<td>40-4</td>
<td>30-0</td>
<td>38-5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>57-0</td>
<td>32-0</td>
<td>0-0</td>
<td>75-0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>57-3</td>
<td>28-9</td>
<td>9-6</td>
<td>74-7</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>60-2</td>
<td>34-2</td>
<td>16-7</td>
<td>63-7</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Concentration of *E. coli* as number of organisms per ml. $\times 10^9$.

the degree of inhibition being directly proportional
to the number of organisms given. The rats given
the dose of labelled $B_{12}$ incubated with *E. coli* at a
concentration of 1-9 $\times 10^9$ organisms per millilitre
absorbed from 28-9 to 42-7% (mean 34-2%). When
the concentration of *E. coli* was 3-8 $\times 10^9$ organisms
per millilitre only between 0-0 and 30-0% (mean 16-7%)
was absorbed.

When the bacteria were previously killed by heat
at $56^\circ C$. for four hours, this inhibitory effect on the
absorption of $B_{12}$ was abolished (Fig. 1).

**EFFECT OF GASTRIC JUICE ON INHIBITION OF $B_{12}$
ABSORPTION BY *E. coli*** The second group of six
rats (nos. 7 to 12) received four consecutive test doses
of 0-01 $\mu$g. of $^{58}$Co-labelled $B_{12}$. 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 $\times 10^9$
organisms per millilitre. In the final test, the labelled
$B_{12}$ 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 $\times 10^9$ organisms per
millilitre. The results of these tests are given in
Table II and illustrated in Fig. 2.

When the dose of $B_{12}$ 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
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. coli1</th>
<th>Dose Plus Gastric Juice then Incubated with E. coli1</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 × 10⁹ organisms per millilitre.

...a concentration of 3.8 × 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).

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. coli² 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. coli² 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>

²Concentration of E. coli was 3.9 × 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...
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possible that they do not inhibit absorption simply by competitive uptake. As an alternative hypothesis, it may be suggested that E. coli may interfere in some way with the transport mechanism in the mucosa of the distal small intestine.

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REFERENCES


