

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

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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 micro-organisms (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 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°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, Na₃ citrate SH₂O, 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 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 × 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

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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,

TABLE I

PERCENTAGE ABSORPTION OF 0.01 µg. ⁵⁸CO-LABELLED B₁₂ GIVEN ALONE OR AFTER INCUBATION WITH VARYING CONCENTRATIONS OF *E. COLI* AND WITH *E. COLI* PREVIOUSLY HEATED AT 56°C. FOR FOUR HOURS

| Rat No. | Dose Given Alone | Dose Plus <i>E. coli</i> | | |
|---------|------------------|--------------------------|------------------|------------------|
| | | 1.9 ¹ | 3.8 ¹ | 3.8 ² |
| 1 | 70.6 | 42.7 | 19.0 | 54.4 |
| 2 | 65.5 | 31.3 | 15.8 | 69.2 |
| 3 | 44.8 | 29.9 | 25.5 | 70.5 |
| 4 | 66.1 | 40.4 | 30.0 | 38.5 |
| 5 | 57.0 | 32.0 | 0.0 | 75.0 |
| 6 | 57.3 | 28.9 | 9.6 | 74.7 |
| Mean = | 60.2 | 34.2 | 16.7 | 63.7 |

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

²*E. coli* heated at 56°C. for four hours before incubation with B₁₂.

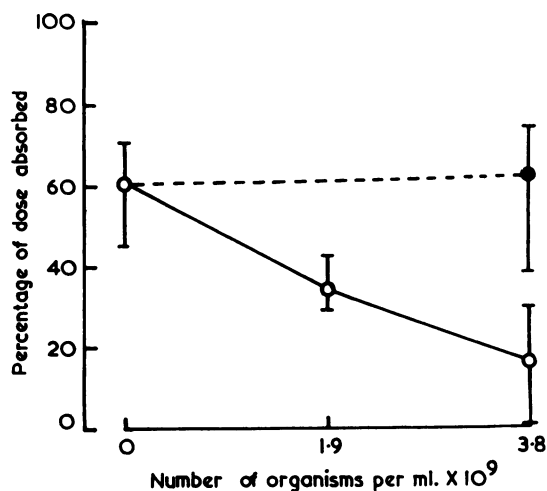


FIG. 1. Mean and range of absorption of 0.01 µg. ⁵⁸Co-labelled B₁₂ given alone or after incubation with varying concentrations of live *E. coli* (○—○), and with *E. coli* killed by heating at 56°C. for four hours (●).

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

TABLE II

PERCENTAGE ABSORPTION OF 0.01 μg . ^{58}CO -LABELLED B_{12} GIVEN ALONE, WITH GASTRIC JUICE AND WITH *E. COLI* ALONE OR AFTER PREVIOUS INCUBATION WITH GASTRIC JUICE

| Rat No. | Dose Alone | Dose Plus Rat Gastric Juice | Dose Plus <i>E. coli</i> ¹ | Dose Plus Gastric Juice then Incubated with <i>E. coli</i> ¹ |
|---------|------------|-----------------------------|---------------------------------------|---|
| 7 | 63.8 | 47.0 | 0.0 | 24.5 |
| 8 | 59.7 | 56.4 | 3.5 | 33.3 |
| 9 | 40.5 | 28.2 | 17.0 | 28.3 |
| 10 | 54.6 | 54.4 | 18.5 | 32.9 |
| 11 | 27.2 | 59.3 | 10.5 | 50.5 |
| 12 | 61.2 | 65.4 | 25.0 | 29.2 |
| Mean = | 51.4 | 51.8 | 12.4 | 33.1 |

¹Concentration of *E. coli* was 3.9×10^9 organisms per millilitre.

a concentration of 3.8×10^9 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 B_{12} 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 B_{12} BY *E. COLI* OR BY RAT GASTRIC JUICE
The capacity of 1 ml. of *E. coli* at a concentration of 3.8×10^9 organisms per millilitre to bind 0.01 μg . of ^{58}Co -labelled B_{12} 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 B_{12} binding capa-

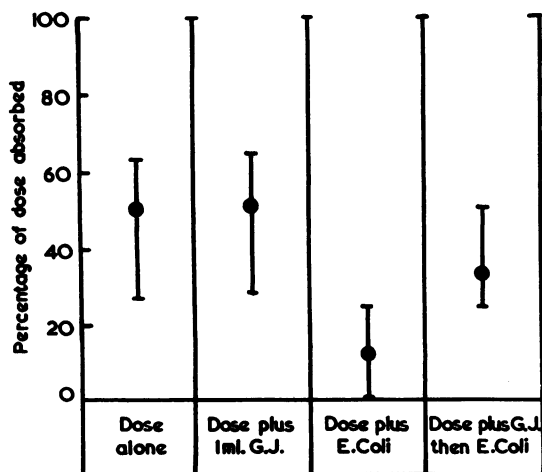


FIG. 2. Mean and range of absorption of 0.01 μg . ^{58}Co -labelled B_{12} given alone, with gastric juice, and with *E. coli* alone or after previous incubation with gastric juice.

city of 1 ml. of the gastric mucosal extract is also shown in Table III.

TABLE III

BINDING OF 0.01 μg . ^{58}CO -LABELLED B_{12} BY *E. COLI* BEFORE AND AFTER HEATING OR BY RAT GASTRIC JUICE

| Material Added to B_{12} | Counts in Sample | Counts in Ultra-filtrate | % Bound |
|---|------------------|--------------------------|---------|
| 1 ml. unheated <i>E. coli</i> ¹ | 3,170 | 1,466 | 54 |
| 1 ml. <i>E. coli</i> ¹ heated at 56°C. for four hours | 4,411 | 1,557 | 65 |
| 1 ml. <i>E. coli</i> ¹ heated at 100°C. for half an hour | 3,345 | 2,696 | 20 |
| 1 ml. gastric juice | 2,620 | 1,520 | 42 |

¹Concentration of *E. coli* was 3.9×10^9 organisms per millilitre.

The live organisms bound 54% of the labelled vitamin B_{12} 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 B_{12} (Table III).

DISCUSSION

The results given in this paper indicate that cultures of *E. coli* are capable of inhibiting the absorption of ^{58}Co -labelled vitamin B_{12} 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 B_{12} 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 B_{12} (Fig. 1, Table III), as do preparations of intrinsic factor similarly treated (Spray, 1952).

The results also show that when test doses of labelled B_{12} are previously incubated with gastric mucosal extracts, cultures of *E. coli* lose much of their capacity to inhibit B_{12} 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 B_{12} absorption in patients with the 'blind loop' syndrome therefore remains uncertain, but it is

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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