The Site of Absorption of Co$^{58}$-Labeled Vitamin B$_{12}$ in Man

An Investigation Made by Intestinal Intubation with Polyethylene Glycol as a Marker Substance

By Vagn Rønnow-Jessen and Jacob Hansen

Until recent years it was believed that the absorption of vitamin B$_{12}$ in man took place in the upper and middle portions of the small intestine. However, studies in animals, especially dogs, have revealed that considerable absorption takes place in the ileum, whereas it is negligible in the proximal small intestine unless very large doses are given.

Clinical observations in patients, after extensive resections of the ileum or intestinal short circuitings with exclusion of considerable portions of the ileum, seem to indicate that in man the absorption of B$_{12}$ also takes place almost exclusively in the distal portions of the small intestine. The methods of investigation did not show, however, whether the activity was localized to the visceral contents or the intestinal wall itself.

In this paper we report our study of the absorption of vitamin B$_{12}$ in the various divisions of the human small intestine.

Materials and Methods

The series consisted of 8 men and 2 women between 22 and 71 years of age. None of them showed signs of organic disorders in the alimentary tract, but a few of them suffered from minor dyspeptic complaints. The diagnoses are listed in Table 1.

After the small intestine had been intubated, the patients received an aqueous test meal, to which was added Co$^{58}$-labeled vitamin B$_{12}$ and a marker substance. After this, samples of intestinal juice were simultaneously collected from different levels of the small intestine.

A tri-bore polyethylene tubing was used (Portex). Each channel had a sectional area of about 3 sq. mm. The tubing of all three channels was perforated with four or five holes over a length of about 10 cm: in channel 1, 30 to 50 cm. from the distal end of the tubing; in channel 2, 80 to 100 cm. proximally to this; and in channel 3 a further 80 or 100 cm. above the most distal hole in channel 2. The lumen of every channel was occluded with a metal stopper below the most distal hole.

The tubing was introduced through the nose and drawn out at the mouth. A small rubber pouch containing about 10 Gm. of mercury was sewn to the distal end of the tubing after which the patient swallowed it. Its progress in the small intestine was controlled by x-rays with a view to placing the rubber pouch in the caecum. The bag with mercury thus showed the position of the distal end of the tubing and the metal stoppers the location of the perforations inside the small intestine. During fluoroscopy radio-opaque solution was injected into the channel leading to the distal holes. This outlined loops of the tubing and by delineating the contours of the surrounding gut, the contrast fluid sometimes helped to decide the distal position of the tubing. When this was in its place, or if after 48–60 hours it would no longer pass anally, the test meal was administered.

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The test meal consisted of 500 ml. of water with an addition of 0.5 μg. of Co⁵⁸-labeled vitamin B₁₂ with a specific activity of 1 μc. per μg. vitamin B₁₂. In order to secure satisfactory absorption, 100 mg. of hog intrinsic factor* were added. In addition, ¼ per cent of polyethylene glycol (PEG) with a molecular weight of about 4000, was added as a marker substance. Twenty to thirty minutes after the administration of the test meal each channel was connected to a water suction pump with a receptacle. Suction was intermittent from the upper and middle channels so the amount of the test meal which reached the distal end of the tubing would not be reduced too much. Suction was continued for 4–5 hours with a view to recovering at least 3–4 samples of 10–15 ml. from each level.

A radiographic survey of the abdomen at the end of the experiments showed the position of the tubing had remained unaltered in all cases.

The PEG concentration of the samples was determined by the colorimetric method described by Hydén¹ and the radioactivity was measured in a well type scintillation detector. Samples and background counting rates were counted up to 10,000 counts. The test meal was used as a standard.

The percentage activity of the collected samples as compared with that of the standard sample was determined by the following formula:

\[
\frac{\text{Activity of sample}}{\text{Activity of standard}} \times 100 \text{ per cent}
\]

The concentration of PEG in the samples was also calculated in percentages of that of the standard solution:

\[
\frac{\text{Extinction of sample}}{\text{Extinction of standard}} \times 100 \text{ per cent}
\]

The vitamin B₁₂ percentage which remained in the samples was calculated from the ratio of 1 to 2:

\[
\frac{\text{Activity of sample}}{\text{Extinction of standard}} \times \frac{\text{Extinction of sample}}{\text{Activity of standard}} \times 100 \text{ per cent}
\]

Samples with a PEG concentration below 15 per cent of the standard were rejected, as PEG in such concentrations could not be determined with sufficient accuracy. The measurement of the activity also became increasingly unreliable in these low concentrations, the background counting rates sometimes amounting to 50 per cent or more of the entire counting rates.

RESULTS

One intubation of the small intestine was carried out in each patient. In 4 cases (numbers 2, 4, 7 and 8) the pouch with mercury was successfully placed in the caecum. When the distance from the nostrils to the pylorus was estimated at 60 cm., the distances from the pylorus to the caecum were 320, 240, 250 and 330 cm., respectively, in these cases. In 1 case (number 9) the tubing passed down to the middle of the transverse colon and when it was withdrawn a "creasing" or "folding" of the gut took place so it only measured 210 cm. from the pylorus to the caecum. In these 5 cases the position of the perforations inside the intestine could be easily determined since their places on the tubing were known.

*Intrinsic factor was prepared from fresh hog pyloric mucosa which was desiccated in vacuum and defatted. One hundred mg. of the preparation corresponds with 600 mg. fresh pyloric mucosa. The preparation was standardized by its effect on the Schilling test in patients with pernicious anemia.
In the last 5 cases in which the tubing did not reach the caecum, only the distance of the perforations from the pylorus was known. According to Blankenhorn et al. and our observations in the first four experiments, the length of the small intestine was estimated at 300 cm. Holes at a distance of less than 100 cm. from the pylorus were considered to be situated in the proximal third of the gut, holes at distances between 100 cm. and 200 cm. (from the pylorus) in the middle third. When the distance exceeded 200 cm., they were considered to be in the distal third of the gut. This occurred in experiments number 6 and 10.

As a rule, the sampling periods varied between 5 and 20 minutes although they were sometimes of longer duration because the suction was initially unsuccessful. Very often the suction started suddenly and then the “effective” period was in reality only of few minutes duration.

In 9 subjects a total of 42 samples were collected from the proximal third of the small intestine, 36 samples from the middle third in 8 cases and 26 samples from the anal third of the small intestine in 7 cases. The results are given in table 1. The activities actually found in the samples are indicated in per cent of the activities expected from the concentration of PEG. Figure 1 gives a graphical representation of the average values found in the various experiments.

In 6 of the experiments (1, 2, 5, 6, 8 and 10) samples from the oral third of the small intestine showed values around 100 per cent, whereas in the 3 other experiments (3, 4 and 7) they grouped themselves just under 90 per cent. The difference is significant whether it is calculated on the basis of single samples (12.1 per cent, S.E. ± 2.23 per cent (p < 0.001)) or from the average values (12.9 per cent ± 1.69 per cent (p < 0.001)).

In 7 experiments samples were collected from both the upper and middle third of the small intestine. The difference between the values is significant, whether it is calculated on the basis of the single samples (6.2 per cent, S.E. ± 2.41 per cent (p < 0.02)) or by comparison of the average values of each experiment (5.9 per cent ± 2.14 per cent (p < 0.05)).

In 6 experiments there were samples from both the middle and anal third. The difference between the results of the two groups is highly significant, both when calculated on the basis of the single sample (24.3 per cent, S.E. ± 4.31 per cent (p < 0.001)) and when the average values are compared (27.4 per cent ± 5.85 per cent (p < 0.01)).

**Discussion**

As far as we have been able to ascertain, previous investigations of the absorption of vitamin B₁₂ in the human small intestine have not been done along the lines described above. The properties of PEG as a marker substance—water solubility, no absorption in the small intestine or to the mucous membrane, protein or the intestinal juice, no destruction in the alimentary tract, and an exact method for quantitative determination—have been prerequisites for obtaining reliable results.⁶,¹²,¹³,¹⁶,¹⁷
Table 1.—Absorption of Vitamin B₁₂ in Various Parts of the Human Intestine

<table>
<thead>
<tr>
<th>Sex, Age</th>
<th>Oral third of Intestine</th>
<th>Middle third of Intestine</th>
<th>Anal third of Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1. d' 54</td>
<td>92-101-109</td>
<td>97-98</td>
<td>- - -</td>
</tr>
<tr>
<td>Psychoneurosis</td>
<td>Coronary sclerosis ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. d' 43</td>
<td>106-105-106</td>
<td>95-97-102-96</td>
<td>82-49</td>
</tr>
<tr>
<td>Psychoneurosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. d' 54</td>
<td>91-93-92-93-</td>
<td>79-89-86-86-</td>
<td>78-77-84-90</td>
</tr>
<tr>
<td>Remote myocardial infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroduodenitis ?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. d' 35</td>
<td>90-81-80-97</td>
<td>- - -</td>
<td>106-73-68-61-</td>
</tr>
<tr>
<td>Psychoneurosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. d' 49</td>
<td>112-100-102-91-</td>
<td>101-99-97-88</td>
<td>- - -</td>
</tr>
<tr>
<td>Cerebral arteriosclerotic disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychoneurosis</td>
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<td></td>
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</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. d' 71</td>
<td>84-85-96-94</td>
<td>83-85</td>
<td>85-79</td>
</tr>
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<td>Aerophagia</td>
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<td></td>
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</tr>
<tr>
<td>Psychoneurosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. d' 51</td>
<td>- - -</td>
<td>90-88-99</td>
<td>68-63-68-70</td>
</tr>
<tr>
<td>Psychoneurosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerophagia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. d' 50</td>
<td>102-104-101</td>
<td>104-96-92</td>
<td>82-78-49</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ten persons were given a test meal containing Co³⁵-B₁₂ (500 ml. specific activity 0.5 μg. per 0.5 μc. of Co³⁵). PEG was used as marker substance. The activities actually found in samples from upper, middle and lower third of the small bowel are indicated in per cent of the values expected from the concentration of PEG.

As previously mentioned, it is generally accepted that the absorption of vitamin B₁₂ in the human alimentary tract takes place almost exclusively in the ileum, whereas it is negligible in the duodenum and jejunum. The results of this study corroborate this in that we have found a considerable absorption of vitamin B₁₂ taking place during the passage of the test meal from the middle to the anal third of the small intestine, whereas only a modest, though significant, absorption takes place from the oral to the middle third. The results of our investigations of the absorption in the oral portion of the small intestine can only be explained by the assumption that in certain cases an absorption also takes place in the proximal portion of the jejunum. As mentioned above, in 3 cases out of 9, the content of B₁₂ was reduced in samples from the oral third of the small intestine, collected 95, 50 and 50 cm. distally to the pylorus. If this reduction is due to erroneous determinations, there is every probability, in view of the grouping of the samples just under 90 per cent, that the error had been made in the measurements of the standard samples. This would imply that the contents of PEG had been
Fig. 1.—Content of Co$^{58}$-vitamin B$_{12}$ in various parts of the human intestine after a test meal containing Co$^{58}$-B$_{12}$ and PEG as marker substance. The content is indicated in per cent of the values expected from the concentration of PEG.

underestimated or the radioactivity overestimated, or both. If so, these experiments (cf. formula 3) would also be expected to reveal a low content of B$_{12}$ in the middle and anal third of the small intestine. As shown in figure 1, this was not the case.

The findings must, therefore, be presumed to indicate that removal of vitamin B$_{12}$ has taken place from the test meal. This removal is likely indicative of an absorption, but the possibility cannot be excluded that it has only been reversibly adsorbed to the mucous membranes of the stomach and the proximal portion of the small intestine.

Clinical symptoms distinguishing these 3 patients (3, 4, 7) from the other 6 could not be demonstrated. The absorption of B$_{12}$ in the proximal portions of the small intestine might be due to concentration of the test meal during its passage down the gut. This was not the case, however, since it was found that the maximum concentration of PEG in proportion to the test meal in these 3 cases was 60, 120 and 110 per cent, whereas in the others it ranges from 40 to 160 per cent.

Our observations are supported in part by the results obtained by Doscherholm et al. who administered Co$^{58}$-labeled vitamin B$_{12}$ to eleven very sick patients. After death, 2 hours to 113 days later, it was found that in 7 cases the radioactivity of the small intestine was localized to the ileum, and in 2, evenly distributed in the whole gut. In 2 cases it was almost exclusively localized to the jejunum which, according to these authors, might suggest an absorption of B$_{12}$ in the jejunum in certain cases.

The limited number of patients in our study does not permit an evaluation
of how frequently the absorption of B₁₂ begins in the jejunum. Comparing our results and those of Doscherholmen et al., it can be concluded that absorption in the jejunum takes place not infrequently.

**SUMMARY AND CONCLUSION**

Intestinal intubation was performed with a tri-bore tubing in ten subjects without signs of gastrointestinal disease. After administration of a test meal containing Co⁵⁸-labeled vitamin B₁₂ and with polyethylene glycol as a marker substance, samples of intestinal juice were collected from the upper, middle and anal thirds of the small intestine.

During the passage of the test dose from the upper to the middle third of the gut a modest, but significant, absorption of about 6 per cent was discovered. During the passage from the middle to the anal third of the intestine a considerably larger absorption took place, on an average of about 25 per cent of the oral dose.

These results agree entirely with those obtained from experiments performed in animals and with clinical observations in man after operations on the small intestine. The most outstanding feature was that, contrary to the generally accepted view, our study seems to indicate that in certain cases the absorption of B₁₂ begins far up in the jejunum. This seemed to be the case in 3 out of 9 experiments. The study throws no light on the cause of this.

**SUMMARIO IN INTERLINGUA**

Intubation intestinal con tubos tri-luminate eseva executate in dece subjectos sin signos de morbo gastrointestinal. Post le administration de un repasto experimental, contiente vitamina B₁₂ marcate con Co⁵⁸, con glycol polyethylenic como substantia de marcare, specimenes de succo intestinal eseva colligite ab le tertio superior, le tertio intermedie, e le tertio anal del intestino tenue.

Durante le passage del dose experimental ab le tertio superior al tertio intermedie del intestino, un modeste (sed significative) absorption de circa 6 pro cento esseva discoperite. Durante le passage ab tertio intermedie ad le tertio anal del intestino, un considerabilemente plus marcate absorption occurreva, amontante al media a circa 25 pro cento del dose oral.

Iste resultatos se trova completemente de accordo con illos obtenite in experimentos animal e con le observationes in patientes human post operationes in le intestino tenue. Le plus notable caracteristica eseva que—per contrasto con le generalmente acceptate notion—nostre studio pare indicar que in certe casos le absorption de vitamina B₁₂ comencia multo in alto in le jejuno. Isto pareva esser le caso in 3 de 9 experimentos. Le studio non provide datos que poterea explicar iste constatation.

**REFERENCES**

4. von Bonsdorff, B., and Gordin, R.: Anti-
anemic activity of dried fish tape-
worm. Diphyllobothrium latum and 
pernicious anemia. Acta med. Scandi-
nav. (suppl.) 266:283, 1952.
5. Booth, C. C., and Mollin, D. L.: The 
site of absorption of vitamin B\textsubscript{12} in 
6. Borgström, B., Dahlqvist, A., Lundh, 
G., and Sjövall, J.: Studies of in-
testinal digestion and absorption in 
the human. J. Clin. Invest. 36:1521, 
1957.
7. Castle, W. B.: Medical progress: De-
velopment of knowledge concerning 
the gastric intrinsic factor and its re-
lation to pernicious anemia. New 
8. Citrin, Y., DeRosa, C., and Halsted, J. 
A.: Sites of absorption of vitamin B\textsubscript{12} 
9. Dorscherholmen, A., Finley, P. R., and 
Hagen, P. S.: Distribution of radio-
activity in man after the oral inges-
tion of small test doses of radio-
10. Drapanas, T., Williams, J. S., McDon-
ald, J. C., Heyden, W., Bow, T., and 
Spencer, R. P.: Role of the ileum in 
the absorption of vitamin B\textsubscript{12} and 
intrinsic factor (NF). J.A.M.A. 184: 
site of absorption of orally adminis-
tered Co\textsuperscript{60}-labeled vitamin B\textsubscript{12} in 
dogs: The effect of dose. Gastroenter-
the determinations of higher poly-
ethylene glycols in biological mate-
139, 1955.
13. Jacobson, E. D., Bondy, D. C., Broit-
man, S. A., and Fordtran, J. S.: Valid-
ity of polyethylene glycol in estimating 
intestinal water volume. Gastroentero-
14. McIntyre, P. A., Sachs, M. V., Krevans, 
J. R., and Conley, C. L.: Pathogenesis 
and treatment of macrocytic anemia. 
15. Rosenthal, H. L., and Hampton, J. K.: 
The absorption of cyanocobalamin 
(vitamin B\textsubscript{12}) from the gastrointesti-
nal tract of dogs. J. Nutrition 56:67, 
1955.
16. Shaffer, C. B., and Critchfield, F. H.: 
The absorption and excretion of the 
solid polyethylene glycols ("Carbo-
17. Sperber, I., Hydén, S., and Ekman, J.: 
The use of polyethylene glycol as a 
reference substance in the study of 
(Sweden) 20:337, 1953.

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