Vitamin B₁₂ absorption and malabsorption

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(This article is one of a series linked with the *Festschrift for Christopher Booth*. See *Gut Festschrift 1989; 30.*)

When radioactively labelled vitamin B₁₂ became available after 1950, it was soon established that patients with lesions of the distal small bowel may be unable to absorb normal amounts of vitamin B₁₂ and may develop megaloblastic anaemia.^{1,2} These observations suggested that ileum is the site of vitamin B₁₂ absorption. Direct evidence was provided in 1959 by Booth and Mollin,³ who studied the distribution of radioactivity in the intestine after oral administration of labelled B₁₂. Using a Geiger Müller counter during laparotomy, radioactive B₁₂ was found concentrated in the ileum. Vitamin B₁₂ is unique in several respects; it is absorbed only in the ileum in contrast with most other substances; it requires a gastric intrinsic factor to be efficiently absorbed; and because the number of specific receptor sites in the ileum is restricted, only tiny amounts can be absorbed. There is also a delay in the transport from the lumen across the enterocyte until the vitamin appears in the plasma,⁴ which may be the result of metabolic processes within the enterocyte. In recent years there have been several studies which have enhanced our understanding of vitamin B₁₂ absorption and malabsorption, and the aim of this review is to concentrate on recent developments.

Normal vitamin B₁₂ absorption

Methylcobalamin, deoxyadenosylcobalamin and hydroxycobalamin are the major forms of cobalamin (vitamin B₁₂) in different food sources. The cobalamin used in clinical studies, cyanocobalamin, is an artifact of the isolation procedure. Vitamin B₁₂ cannot be synthesised by mammalian species, but only by microorganisms. In man bacterial synthesis takes place only in the large bowel and the caecum. From these sites absorption cannot take place, and therefore man is entirely dependent on dietary sources of vitamin B₁₂. The richest natural sources are liver and kidney, but vitamin B₁₂ is also present in meat, fish, dairy products, eggs, and shellfish. The average daily intake is about 3 nmol (4 µg), whereas the physiological needs are 0.4 to 0.8 nmol (0.5–1.0 µg).

The absorption of vitamin B₁₂ appears to result from an orderly sequence of events.⁵ These events are shown in Table 1. The events before ileal absorption include: (1) the release of vitamin B₁₂ from its binding by dietary protein, and the binding of B₁₂ to

R-protein in the stomach; (2) degradation of the R-binder by pancreatic proteases and binding of B₁₂ by intrinsic factor in the upper small bowel.

It has been shown *in vivo* that there is a rapid release of vitamin B₁₂ from food in the stomach.⁶ This release is facilitated by acid⁵ as well as pepsin.⁶ The dependence of this release on peptic digestion may, however, only apply to some food sources. Cooking and food preparation may play a role, and from some food sources, including liver, vitamin B₁₂ is readily released even when the pH of the stomach is neutral.⁶

It was thought until recently that vitamin B₁₂, after its release from food proteins, attached to gastric intrinsic factor (IF). There is now evidence that cobalamins preferentially binds in the acid stomach to another B₁₂-binding protein, R-protein or haptocorrin. R-proteins are found in many body fluids including saliva, gastric juice, bile, intestinal juice, and serum. Allen *et al* in 1978⁷ showed *in vitro* that human salivary R-protein bound cobalamin (Cbl) with affinities that were 50- and three-fold higher than those of human IF at pH 2 and pH 8 respectively. Incubation of the R-protein-cobalamin complex with pancreatic proteases led to complete and rapid transfer of cobalamin to intrinsic factor. From these studies it was suggested: (a) that in the acid milieu of the stomach Cbl is bound almost exclusively to R-protein rather than to IF; (b) that Cbl remains bound to R-protein in the small intestine until pancreatic proteases partially degrade R-

Table 1 *Phases of vitamin B₁₂ absorption*

1	<i>Intragastric events</i>
a	Release of B ₁₂ from food proteins
b	Binding of B ₁₂ by R-protein
2	<i>Duodenal and jejunal events</i>
a	Degradation of R-protein by pancreatic proteases
b	Binding of B ₁₂ by intrinsic factor
3	<i>Ileal events</i>
a	Attachment of B ₁₂ -IF to receptor
b	Transport of B ₁₂ across enterocyte
c	Binding of B ₁₂ by transcobalamin II in portal blood
4	<i>Enterohepatic circulation</i>
a	Biliary excretion of B ₁₂ and B ₁₂ -analogues bound by R-protein
b	Degradation of R-protein by pancreatic proteases and formation B ₁₂ -IF
c	Ileal reabsorption of B ₁₂ and faecal excretion of B ₁₂ -analogues

protein and enable Cbl to become bound exclusively to IF. Results of a recent *in vivo* study support that B₁₂ preferentially binds to R-protein in the stomach and is transferred to IF in the upper small bowel.⁶ Liver homogenate labelled *in vivo* with ⁵⁷Co was administered orally to healthy volunteers. It was found that approximately two thirds of the radioactive vitamin B₁₂ in the gastric aspirate was bound to R-protein and one third to intrinsic factor, whereas in jejunal aspirates only 25% was bound to R-protein. The degree of binding to R-protein and intrinsic factor in the stomach, however, may be dependent on the oral dose of vitamin B₁₂, the amounts of R-protein and intrinsic factor secreted, and the gastric pH.

The vitamin B₁₂-intrinsic factor complex is carried to its site of absorption in the ileum, where it attaches to specific membrane receptors. The attachment is highly specific, and the receptor does not take up vitamin B₁₂ bound to binders other than intrinsic factor nor does it take up vitamin B₁₂ analogues. The binding site on vitamin B₁₂ for intrinsic factor is separate from that which attaches to the receptor.¹⁻⁵ This is supported by the report of a child who developed vitamin B₁₂ deficiency as a result of an abnormal intrinsic factor.⁸ This abnormal intrinsic factor bound vitamin B₁₂ normally and also reacted appropriately with antibody to intrinsic factor, but the abnormal intrinsic factor - vitamin B₁₂ complex failed to attach to the ileal receptor. The attachment to the receptor is non-energy dependent and requires the presence of calcium-ions and a neutral pH. The receptor which has not been fully characterised, has been located at the bottom of the pits between the microvilli,⁹ and has been shown in the entire distal three fifths of the human small intestine.¹⁰

The number of B₁₂-receptors may be the rate of limiting step in the absorption of vitamin B₁₂ and as shown in Booth's laboratory may show an adaptive increase in response to jejunal pathology.¹¹

How cobalamins are transported across the enterocyte is not fully understood, and the fate of intrinsic factor is also unclear; intrinsic factor is not absorbed into the blood, and may either be transported into the cell along with vitamin B₁₂, or it may be released at the cell surface. Vitamin B₁₂ is rapidly internalised, and recent experiments in pregnant mice suggest that this occurs by receptor-mediated endocytosis.¹²

From the ingestion of vitamin B₁₂ until its appearance in the blood there is a delay for several hours.⁴ This delay appears to be related to the intracellular phase of vitamin B₁₂ absorption. Peters and Hoffbrand¹³ in Booth's laboratory investigated the intracellular localisation of vitamin B₁₂ during this delay period. After feeding a physiological dose of ⁵⁷CoB₁₂ the ileal mucosa of the guinea pig showed a

highly significant rise in mitochondrial radioactivity during two hours postfeeding. This activity subsequently decreased coincidentally with a rise in hepatic specific activity. Although this suggests that vitamin B₁₂ is delayed in the mitochondria before the vitamin enters the portal plasma, the metabolic events within the mitochondria during the delay remains to be elucidated.

When vitamin B₁₂ leaves the enterocyte and enters the portal plasma, it is bound to transcobalamin II, but whether binding to transcobalamin II occurs within the enterocyte or in the plasma is not clear. Studies in volunteers by Chanarin *et al*¹⁴ suggest indirectly that vitamin B₁₂ enters blood bound to transcobalamin II, which may be derived from the ileal enterocyte. Transcobalamin II may also be essential for normal absorption of vitamin B₁₂, as malabsorption of the vitamin has been described in association with congenital deficiency of transcobalamin II.¹⁵

An enterohepatic circulation of vitamin B₁₂ has been suggested because the amount of vitamin B₁₂ excreted in the bile is much higher than that excreted in the urine and the faeces. The biliary B₁₂-secretion involves about 3 nmol (4 µg) all bound to bile R-binder. Of the total amount of corrinoids in the bile more than 50% may represent vitamin B₁₂ analogues.¹⁷ The bile R-protein is thought to be degraded by pancreatic enzymes with transfer of vitamin B₁₂ to intrinsic factor in the upper small bowel. Although the exact amount is unknown, more than half of the biliary vitamin B₁₂ may be reabsorbed, whereas the analogues that do not bind intrinsic factor, are excreted. The enterohepatic circulation therefore seems to play a major role in conserving vitamin B₁₂; in addition the bile is the major excretory route for vitamin B₁₂ analogues.

It is also possible that bile itself may play a role in enhancing vitamin B₁₂ absorption. This was suggested from studies by Teo *et al*¹⁸ who observed B₁₂-malabsorption in five patients with T-tube bile duct drainage, which improved after the T-tubes had been removed. Whether bile or bile acids play a role in physiological B₁₂-absorption is, however, not clear, although it has been proposed that bile acids may influence the B₁₂-absorption either by dissociating the B₁₂-intrinsic factor complex or by facilitating the uptake of B₁₂ by the ileal receptor.¹⁹

Malabsorption of vitamin B₁₂

DISEASES OF THE STOMACH

In patients with pernicious anaemia, and after total gastrectomy, malabsorption of vitamin B₁₂ is the result of intrinsic factor deficiency. In addition patients with untreated pernicious anaemia suffer

Table 2 Pathophysiological basis of vitamin B₁₂ malabsorption

Disease	Pathophysiology
1 Pernicious anaemia, gastrectomy	Intrinsic factor deficiency
2 Achlorhydria, vagotomy, H ₂ -receptor antagonists, partial gastrectomy	Decreased release of vitamin B ₁₂ from food protein. ²²
3 Pancreatic insufficiency	Failure of degradation of R-protein ⁷
4 Bacterial overgrowth	Bacterial uptake of B ₁₂ -IF ¹⁸ Dysfunction of enterocyte? ²¹ Conversion of B ₁₂ to analogues ¹⁷
4 Ileal disease	Loss of absorptive surface
5 Imerslund-Gräsbeck disease	Lack of ileal receptor? ²⁶

from intestinal malabsorption. This additional cause for malabsorption, is the result of damage of the enterocyte caused by severe vitamin B₁₂-deficiency, which is rapidly corrected by giving vitamin B₁₂. Binding of intrinsic factor by intrinsic factor antibodies secreted into the gastric juice or bacterial overgrowth of the small bowel may contribute to the malabsorption in some patients.

In pernicious anaemia there is a permanent failure of intrinsic factor and acid secretion. An exception is congenital pernicious anaemia which develops before the age of two. These patients are unable to secrete intrinsic factor, but otherwise the structure and the function of the gastric mucosa are normal. It has been suggested that some, if not all, of these patients may actually represent examples of B₁₂-malabsorption because of structurally abnormal intrinsic factor,²⁰ as in the patient reported by Katz *et al.*^{8,20}

After partial gastrectomy there is a progressive fall in serum vitamin B₁₂ concentrations. About 30% of such patients develop vitamin B₁₂-deficiency, but this is frequently mild with no overt haematological or neurological abnormalities. The main cause of the nutritional deficiency is thought to be lack of intrinsic factor as intrinsic factor enhances the absorption in those patients who have impaired absorption of crystalline radioactive vitamin B₁₂. Deller, Richards and Witts,²¹ however, showed that half of those patients with gastrectomy who had subnormal concentrations of vitamin B₁₂, had normal absorption of crystalline B₁₂. This subtle form of vitamin B₁₂-deficiency is apparently not because of intrinsic factor deficiency, but may be caused by malabsorption of food vitamin B₁₂. This hypothesis was investigated by Doscherholmen and Swaim,²² who compared the absorption of ⁵⁷Co-labelled vitamin B₁₂ incorporated into eggs with that of crystalline ⁵⁷CoB₁₂. A group of patients with gastric resections having low serum vitamin B₁₂ concentrations and normal absorption of

crystalline B₁₂, absorbed much less of ⁵⁷CoB₁₂ incorporated into eggs than normal subjects. Similarly, egg vitamin B₁₂ malabsorption was found in patients with achlorhydria and severe hypochlorhydria. Malabsorption of food bound vitamin B₁₂ has later been confirmed using various vitamin B₁₂ food protein preparations, including ⁵⁷CoB₁₂ bound *in vitro* by ovalbumin, egg yolks, and chicken serum. Food vitamin B₁₂-malabsorption thus appears to be an entity, and may be the result of impaired gastric release of vitamin B₁₂ in patients with decreased secretion of acid and pepsin. Food vitamin B₁₂ malabsorption has been described not only in patients with gastrectomy and with achlorhydria, but also after treatment with histamine-H₂-antagonists, and has been reported to frequently occur in patients with unexplained low serum vitamin B₁₂ concentrations not caused by pernicious anaemia or intestinal disease.²³ Whether failure of liberation of vitamin B₁₂ from food proteins can always explain the vitamin B₁₂ deficiency in these patients is not clear. Most secrete intrinsic factor poorly, and crystalline B₁₂-absorption may be intermittently subnormal. Furthermore it is possible that only some food sources may be dependent on acid and pepsin for the release of vitamin B₁₂ in the stomach. Hence it has been shown that there was a rapid and normal intragastric release of vitamin B₁₂ from liver in patients who had been rendered achlorhydric by treatment with the acid inhibiting drug omeprazole.⁶ Moreover vitamin B₁₂ bound by chicken serum has been used in many of the studies on food vitamin B₁₂ absorption. Chicken serum is not a natural food source, and the B₁₂-binding protein in the serum belongs to the R-protein class. The vitamin B₁₂-R-protein complex is not dependent on peptic digestion but on pancreatic protease secretion for the release of vitamin B₁₂, and it is not clear why patients treated with histamine-H₂-antagonists develop malabsorption of chicken serum bound vitamin B₁₂.

PANCREATIC INSUFFICIENCY

In 1956 McIntyre *et al.*¹ first demonstrated malabsorption of vitamin B₁₂ in five patients with pancreatic insufficiency. In 1962 an important role for the exocrine pancreatic secretion was suggested by Veeger *et al.*²⁴ In three patients with vitamin B₁₂ malabsorption and pancreatic insufficiency the absorption improved partially with pancreatic extract or sodium bicarbonate or optimally by both. They concluded that the secretion of sodium bicarbonate is required for a suitable pH for the absorption of the vitamin B₁₂-intrinsic factor complex on the intestinal acceptor, and that one or more pancreatic enzymes are essential.

How pancreatic extract stimulates vitamin B₁₂ absorption has been the subject of several investigations. In 1973 Toskes *et al*²⁵ reported that trypsin corrected vitamin B₁₂ malabsorption in patients with pancreatic insufficiency, and suggested that 'the pancreatic intrinsic factor' was of a trypsin like nature. In 1977 von der Lippe *et al*²⁶ showed that the vitamin B₁₂-R-binder inhibited the intestinal uptake of intrinsic factor bound vitamin B₁₂, and that this inhibitory effects was partially abolished by pancreatic extract; and suggested that pancreatic extract improves B₁₂-absorption by an effect on non-intrinsic factor vitamin B₁₂ binders. In 1978 Allen *et al*¹⁷ showed that incubation of the R-protein-vitamin B₁₂-complex with pancreatic proteases led to rapid release and transfer of vitamin B₁₂ to intrinsic factor; and proposed that the primary defect is lack of pancreatic proteases and a failure to alter R-protein and effect transfer of vitamin B₁₂ to intrinsic factor. Marcoullis *et al*²⁷ provided *in vivo* evidence for this hypothesis, and showed that after the administration of ⁵⁷Co-labelled cyanocobalamin more than 60% of vitamin B₁₂ was bound to R-binders in jejunal fluid from patients with pancreatic insufficiency, whereas all vitamin B₁₂ was bound to intrinsic factor in healthy volunteers.

It is not entirely clear, however, how pancreatic insufficiency leads to vitamin B₁₂ malabsorption. Thus no correlation has been found between pancreatic exocrine function (including faecal fat excretion, trypsin and chymotrypsin secretion) and B₁₂ absorption.^{28,29} The absorption of vitamin B₁₂ may also be normal in severe pancreatic insufficiency and after total pancreatectomy. Other factors, such as the secretory output of intrinsic factor and R-binders as well as the pH of the stomach might influence whether or not B₁₂ becomes bound to intrinsic factor. Moreover although more than 60% of vitamin B₁₂ was bound by R-protein in the jejunum, in patients with pancreatic insufficiency,²⁷ it is still unknown whether there is an increase in the vitamin B₁₂-R-complex in the ileum where vitamin B₁₂ is absorbed.

Although vitamin B₁₂ malabsorption occurs in up to 40–50% of patients with pancreatic insufficiency, vitamin B₁₂ deficiency appears to be rare. This may be related to the unphysiological nature of tests of absorption in the fasting state. Thus Henderson *et al*³⁰ observed that patients who failed to absorb radioactive B₁₂ in the fasting state, had normal absorption when the vitamin was administered together with food. Furthermore the malabsorption may be present only intermittently,²⁸ which might prevent or postpone the development of deficiency.

BACTERIAL OVERGROWTH

In patients with the bacterial overgrowth syndrome

or stagnant loop syndrome vitamin B₁₂ malabsorption is associated with a profuse growth of microorganisms in the small intestine; and bacteroides and coliform bacteria are frequently isolated in the highest concentrations. The bacterial flora probably plays an important role for the malabsorption of vitamin B₁₂, as the patients may absorb B₁₂ normally when they are given metronidazole, lincomycin or tetracycline. Booth and Heath³¹ reported that oral administration of B₁₂ bound to cultures of viable *E coli* inhibited the absorption of vitamin B₁₂ in the rat. When B₁₂ was bound to organisms killed by heat, the inhibitory effect was abolished; and they proposed that microorganisms in some way interfere with the transport mechanism in the distal small intestine. It is still not clear, however, whether dysfunction of the ileal epithelium plays a role in the malabsorption. The structure of the mucosa is usually normal, and the mucosal enzyme activities have been shown to be similar to those of control patients,³² but Ament *et al*³³ reported a mucosal defect in the absorption of fat in three patients. In the blind loop rat it has been shown that there is unimpaired uptake of vitamin B₁₂ by small intestinal brush borders,³⁴ but so far it is unknown whether there are any disturbances in B₁₂-uptake by the ileal mucosa of patients with bacterial overgrowth.

The most important pathophysiological event appears to be the competitive uptake of IF-bound vitamin B₁₂ by bacteria which deprive the host of vitamin B₁₂.

The hypothesis that intestinal bacteria take up intrinsic factor bound vitamin B₁₂ in the intestinal lumen, was investigated by obtaining aspirates from the upper ileum at timed intervals after an oral test meal containing radioactive B₁₂ bound to human intrinsic factor. The proportion of radioactivity in the centrifuged deposits of the ileal aspirates was much higher in patients with the stagnant loop syndrome (43–72%) than in control subjects (0 to 11%). Presumably the radioactivity in the deposits represented B₁₂ bound by bacteria. Treatment with antibiotics resulted in improved B₁₂ absorption, decreased amount of radioactivity in the ileal deposits, and markedly decreased concentrations of bacteroides.³⁵

Nevertheless the way in which bacteria interact with vitamin B₁₂ is not clear. Presumably vitamin B₁₂ is bound to intrinsic factor in the lumen of the small intestine. Although most intestinal bacteria avidly take up unbound vitamin B₁₂, the uptake is much reduced if B₁₂ is bound to intrinsic factor *in vitro*. The inhibitory effect is partial when B₁₂ is bound by rat intrinsic factor,³⁶ whereas pure cultures of bacteroides and coliforms from patients with the stagnant loop syndrome are able to take up only small

amounts of B₁₂ bound by human intrinsic factor.³⁵ Quantitatively these bacteria may be among the most important in the stagnant loop syndrome, and it is probable that significant malabsorption of vitamin B₁₂ occurs only when they are present in very high concentrations. In addition, some of the ingested vitamin B₁₂ may be converted by the bacterial flora to vitamin B₁₂ analogues, making less vitamin B₁₂ available to the host.³⁷

Alternatively intestinal bacteria may, as seems to be the case with the fish tapeworm,³⁸ release vitamin B₁₂ from its bond to intrinsic factor. So far, however, no intestinal bacteria have been found capable of splitting vitamin B₁₂ from gastric intrinsic factor.

As intrinsic factor and bacteria have similar affinities for vitamin B₁₂, it has been proposed that the malabsorption is the result of competition for vitamin B₁₂ between bacterial binding sites and those of IF-binders and non-IF-binders.³⁹ In the upper small bowel vitamin B₁₂ is thought to be released from its binding to R-protein, and then transferred to intrinsic factor. One might theorise that bacterial growth in the proximal small bowel could interfere with this transfer by taking up the released vitamin in competition with intrinsic factor.

ILEAL DISEASE

Any condition associated with ileal dysfunction may lead to vitamin B₁₂ malabsorption and deficiency. These diseases include coeliac disease, tropical sprue and Crohn's disease of the ileum. In tropical sprue vitamin B₁₂ malabsorption is thought to be the result of an ileal mucosal lesion. In these patients there is also a persistent overgrowth of enterobacteria in the proximal small bowel, but in patients with longstanding disease the bacteria apparently do not cause malabsorption by their competitive uptake of vitamin B₁₂.⁴⁰

In coeliac disease vitamin B₁₂ deficiency is relatively uncommon, and only occurs when the mucosal lesion extends to involve the ileum. Thus a significant correlation was found between epithelial cell height in the ileum and the absorption of vitamin B₁₂.⁴¹ In Crohn's disease the absorption is dependent on the extent of the mucosal lesion and whether ileal resection has been carried out. When the resection is more than 100 cm, B₁₂ malabsorption is almost invariably present. Because the B₁₂-receptors are restricted to the ileum, there appears to be no adaptation of B₁₂ absorption in those patients who have extensive ileal disease. But in patients with partial loss of the ileum such as partial ileal bypass for hypercholesterolaemia, adaptation of B₁₂-absorption has been reported.⁴²

In Imerslund-Gräsbeck's disease familial selective malabsorption of vitamin B₁₂ is associated with proteinuria.^{43,44} The inheritance is recessive.

Although the morphology of the ileal mucosa is normal, the absorption defect has been localised to the ileal enterocyte, but the specific defect has still not been defined. Mackenzie *et al*⁴⁵ showed that intrinsic factor stimulated vitamin B₁₂-uptake by homogenates of ileal biopsies from a single patient; and suggested that the absorptive defect appears after IF-B₁₂ attaches to the surface of the enterocyte and before the absorbed vitamin binds to transcobalamin II. Burman *et al*⁴⁶ recently described different findings in a Syrian family with three affected children. Studies of the ileal mucosal by subcellular fractionation in two of the children was carried out two hours after the oral administration of radioactive vitamin B₁₂. There was no uptake of radioactivity by the brush border fraction. This clearly indicates that the malabsorption is the result of a receptor defect. Whether there is reduced number of receptors or a decline in functional quality requires further study.

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