Vitamin B<sub>12</sub> absorption and malabsorption

H SCHJØNSBY

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

When radioactively labelled vitamin B<sub>12</sub> 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 B<sub>12</sub> and may develop megaloblastic anaemia. These observations suggested that ileum is the site of vitamin B<sub>12</sub> absorption. Direct evidence was provided in 1959 by Booth and Mollin, who studied the distribution of radioactivity in the intestine after oral administration of labelled B<sub>12</sub>. Using a Geiger Müller counter during laparotomy, radioactive B<sub>12</sub> was found concentrated in the ileum. Vitamin B<sub>12</sub> 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, 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 B<sub>12</sub> absorption and malabsorption, and the aim of this review is to concentrate on recent developments.

Normal vitamin B<sub>12</sub> absorption

Methylcobalamin, deoxyadenosylcobalamin and hydroxycobalamin are the major forms of cobalamin (vitamin B<sub>12</sub>) in different food sources. The cobalamin used in clinical studies, cyanocobalamin, is an artifact of the isolation procedure. Vitamin B<sub>12</sub> 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 B<sub>12</sub>. The richest natural sources are liver and kidney, but vitamin B<sub>12</sub> 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 B<sub>12</sub> appears to result from an orderly sequence of events. These events are shown in Table 1. The events before ileal absorption include: (1) the release of vitamin B<sub>12</sub> from its binding by dietary protein, and the binding of B<sub>12</sub> to R-protein in the stomach; (2) degradation of the R-binder by pancreatic proteases and binding of B<sub>12</sub> by intrinsic factor in the upper small bowel.

It has been shown in vivo that there is a rapid release of vitamin B<sub>12</sub> from food in the stomach. This release is facilitated by acid as well as pepsin. 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 B<sub>12</sub> is readily released even when the pH of the stomach is neutral.

It was thought until recently that vitamin B<sub>12</sub>, 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 B<sub>12</sub>-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 1978 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-

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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. Liver homogenate labelled in vivo with 14C was administered orally to healthy volunteers. It was found that approximately two thirds of the radiolabelled vitamin B12 in splanchnic blood 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. This is supported by the report of a child who developed vitamin B12 deficiency as a result of an abnormal intrinsic factor. 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, and has been shown in the entire distal three fifths of the human small intestine.

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.

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.

From the ingestion of vitamin B12 until its appearance in the blood there is a delay for several hours. This delay appears to be related to the intracellular phase of vitamin B12 absorption. Peters and Hoffbrand in Booth’s laboratory investigated the intracellular localisation of vitamin B12 during this delay period. After feeding a physiological dose of 3H-B12 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 Chananin et al 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.

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. 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 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.

**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
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, as in the patient reported by Katz et al. 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, 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 Swain, who compared the absorption of 15Co-labelled vitamin B12 incorporated into eggs with that of crystalline 15CoB12. A group of patients with gastric resections having low serum vitamin B12 concentrations and normal absorption of crystalline B12, absorbed much less of 15CoB12 incorporated into eggs than normal subjects. Similarly, egg vitamin B12 malabsorption was found in patients with achlorhydria and severe hypo- chlorhydria. Malabsorption of food bound vitamin B12 has later been confirmed using various vitamin B12 food protein preparations, including 15CoB12 bound in vitro to 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.

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. 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. 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. 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 B\textsubscript{12} absorption has been the subject of several investigations. In 1973 Toskes et al\textsuperscript{25} reported that trypsin corrected vitamin B\textsubscript{12} 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\textsuperscript{26} showed that the vitamin B\textsubscript{12}-R-binder inhibited the intestinal uptake of intrinsic factor bound vitamin B\textsubscript{12}, and that this inhibitory effect was partially abolished by pancreatic extract; and suggested that pancreatic extract improves B\textsubscript{12}-absorption by an effect on non-intrinsic factor vitamin B\textsubscript{12} binders. In 1978 Allen et al\textsuperscript{27} showed that incubation of the R-protein-vitamin B\textsubscript{12} complex with pancreatic proteases led to rapid release and transfer of vitamin B\textsubscript{12} 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 B\textsubscript{12} to intrinsic factor. Marcoullis et al\textsuperscript{27} provided in vivo evidence for this hypothesis, and showed that after the administration of \textsuperscript{57}Co-labelled cyanocobalamin more than 60\% of vitamin B\textsubscript{12} was bound to R-binders in jejunal fluid from patients with pancreatic insufficiency, whereas all vitamin B\textsubscript{12} was bound to intrinsic factor in healthy volunteers.

It is not entirely clear, however, how pancreatic insufficiency leads to vitamin B\textsubscript{12} malabsorption. Thus no correlation has been found between pancreatic exocrine function (including faecal fat excretion, trypsin and chymotrypsin secretion) and B\textsubscript{12} absorption.\textsuperscript{28,29} The absorption of vitamin B\textsubscript{12} 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 B\textsubscript{12} becomes bound to intrinsic factor. Moreover although more than 60\% of vitamin B\textsubscript{12} was bound by R-protein in the jejunum, in patients with pancreatic insufficiency,\textsuperscript{27} it is still unknown whether there is an increase in the vitamin B\textsubscript{12}-R-complex in the ileum where vitamin B\textsubscript{12} is absorbed.

Although vitamin B\textsubscript{12} malabsorption occurs in up to 40–50\% of patients with pancreatic insufficiency, vitamin B\textsubscript{12} 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\textsuperscript{30} observed that patients who failed to absorb radioactive B\textsubscript{12} in the fasting state, had normal absorption when the vitamin was administered together with food. Furthermore the malabsorption may be present only intermittently,\textsuperscript{30} which might prevent or postpone the development of deficiency.

**Bacterial overgrowth**

In patients with the bacterial overgrowth syndrome or stagnant loop syndrome vitamin B\textsubscript{12} 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 B\textsubscript{12}, as the patients may absorb B\textsubscript{12} normally when they are given metronidazole, lincomycin or tetracycline. Booth and Heath\textsuperscript{31} reported that oral administration of B\textsubscript{12} bound to cultures of viable E coli inhibited the absorption of vitamin B\textsubscript{12} in the rat. When B\textsubscript{12} 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,\textsuperscript{32} but Ament et al\textsuperscript{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 B\textsubscript{12} by small intestinal brush borders,\textsuperscript{34} but so far it is unknown whether there are any disturbances in B\textsubscript{12}-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 B\textsubscript{12} by bacteria which deprive the host of vitamin B\textsubscript{12}.

The hypothesis that intestinal bacteria take up intrinsic factor bound vitamin B\textsubscript{12} in the intestinal lumen, was investigated by obtaining aspirates from the upper ileum at timed intervals after an oral test meal containing radioactive B\textsubscript{12} 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 B\textsubscript{12} bound by bacteria. Treatment with antibiotics resulted in improved B\textsubscript{12} absorption, decreased amount of radioactivity in the ileal deposits, and markedly decreased concentrations of bacteroides.\textsuperscript{35}

Nevertheless the way in which bacteria interact with vitamin B\textsubscript{12} is not clear. Presumably vitamin B\textsubscript{12} is bound to intrinsic factor in the lumen of the small intestine. Although most intestinal bacteria avidly take up unbound vitamin B\textsubscript{12}, the uptake is much reduced if B\textsubscript{12} is bound to intrinsic factor in vitro. The inhibitory effect is partial when B\textsubscript{12} is bound by rat intrinsic factor,\textsuperscript{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.  
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.  

Alternatively intestinal bacteria may, as seems to be the case with the fish tapeworm, 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. 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.  

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. 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.  

In Imerslund-Gräsbeck’s disease familial selective malabsorption of vitamin B12 is associated with proteinuria. 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 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 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.

Medical Department,  
Aker University Hospital,  
Oslo, Norway

References

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