Vitamin B_{12} 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_{12} 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_{12} and may develop megaloblastic anaemia.[1][2] These observations suggested that ileum is the site of vitamin B_{12} 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 B_{12}. Using a Geiger Müller counter during laparotomy, radioactive B_{12} was found concentrated in the ileum. Vitamin B_{12} 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 B_{12} absorption and malabsorption, and the aim of this review is to concentrate on recent developments.

Normal vitamin B_{12} absorption

Methylcobalamin, deoxyadenosylcobalamin and hydroxycobalamin are the major forms of cobalamin (vitamin B_{12}) in different food sources. The cobalamin used in clinical studies, cyanocobalamin, is an artifact of the isolation procedure. Vitamin B_{12} 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_{12}. The richest natural sources are liver and kidney, but vitamin B_{12} 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_{12} 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 B_{12} from its binding by dietary protein, and the binding of B_{12} to R-protein in the stomach; (2) degradation of the R-binder by pancreatic proteases and binding of B_{12} by intrinsic factor in the upper small bowel.

It has been shown *in vivo* that there is a rapid release of vitamin B_{12} from food in the stomach.[6] This release is facilitated by acid[5] 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 B_{12} is readily released even when the pH of the stomach is neutral.[6]

It was thought until recently that vitamin B_{12}, 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_{12}-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[7] 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 B_{12} absorption*

1 *Intragastric events*
   a   Release of B_{12} from food proteins
   b   Binding of B_{12} by R-protein
2 *Duodenal and jejunal events*
   a   Degradation of R-protein by pancreatic proteases
   b   Binding of B_{12} by intrinsic factor
3 *Ileal events*
   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 *Enterohepatic circulation*
   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 B$_{12}$ 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 ^{57}Co was administered orally to healthy volunteers. It was found that approximately two thirds of the radioactive vitamin B$_{12}$ 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 B$_{12}$, the amounts of R-protein and intrinsic factor secreted, and the gastric pH.

The vitamin B$_{12}$-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 B$_{12}$ bound to binders other than intrinsic factor nor does it take up vitamin B$_{12}$ analogues. The binding site on vitamin B$_{12}$ 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 B$_{12}$ deficiency as a result of an abnormal intrinsic factor.[8] This abnormal intrinsic factor bound vitamin B$_{12}$ normally and also reacted appropriately with antibody to intrinsic factor, but the abnormal intrinsic factor – vitamin B$_{12}$ 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 B$_{12}$-receptors may be the rate of limiting step in the absorption of vitamin B$_{12}$ 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 B$_{12}$, or it may be released at the cell surface. Vitamin B$_{12}$ is rapidly internalised, and recent experiments in pregnant mice suggest that this occurs by receptor-mediated endocytosis.[12]

From the ingestion of vitamin B$_{12}$ 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 B$_{12}$ absorption. Peters and Hoffbrand[13] in Booth's laboratory investigated the intracellular localisation of vitamin B$_{12}$ during this delay period. After feeding a physiological dose of ^{57}CoB$_{12}$ 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 B$_{12}$ 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 B$_{12}$ 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 B$_{12}$ 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 B$_{12}$, as malabsorption of the vitamin has been described in association with congenital deficiency of transcobalamin II.[15]

An enterohepatic circulation of vitamin B$_{12}$ has been suggested because the amount of vitamin B$_{12}$ excreted in the bile is much higher than that excreted in the urine and the faeces. The biliary B$_{12}$-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 B$_{12}$ analogues.[17] The bile R-protein is thought to be degraded by pancreatic enzymes with transfer of vitamin B$_{12}$ to intrinsic factor in the upper small bowel. Although the exact amount is unknown, more than half of the biliary vitamin B$_{12}$ 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 B$_{12}$; in addition the bile is the major excretory route for vitamin B$_{12}$ analogues.

It is also possible that bile itself may play a role in enhancing vitamin B$_{12}$ absorption. This was suggested from studies by Teo *et al*[18] who observed B$_{12}$-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 B$_{12}$-absorption is, however, not clear, although it has been proposed that bile acids may influence the B$_{12}$-absorption either by dissociating the B$_{12}$-intrinsic factor complex or by facilitating the uptake of B$_{12}$ by the ileal receptor.[19]

Malabsorption of vitamin B$_{12}$

DISEASES OF THE STOMACH
In patients with pernicious anaemia, and after total gastrectomy, malabsorption of vitamin B$_{12}$ is the result of intrinsic factor deficiency. In addition patients with untreated pernicious anaemia suffer

Table 2 *Pathophysiological basis of vitamin B_{12}
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.[2]
3 Pancreatic insufficiency	Failure of degradation of R-protein[3]
4 Bacterial overgrowth	Bacterial uptake of B_{12}-IF[15] Dysfunction of enterocyte?[31] Conversion of B_{12} to analogues[15]
4 Ileal disease	Loss of absorptive surface
5 Imerslund-Gräsbeck disease	Lack of ileal receptor?[36]

from intestinal malabsorption. This additional cause
for malabsorption, is the result of damage of the
enterocyte caused by severe vitamin B_{12}-deficiency,
which is rapidly corrected by giving vitamin B_{12}.
Binding of intrinsic factor by intrinsic factor anti-
bodies 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 B_{12}-
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 B_{12} concentrations. About 30% of
such patients develop vitamin B_{12}-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 B_{12}. Deller, Richards
and Witts,[21] however, showed that half of those
patients with gastrectomy who had subnormal con-
centrations of vitamin B_{12}, had normal absorption of
crystalline B_{12}. This subtle form of vitamin B_{12}-
deficiency is apparently not because of intrinsic factor
deficiency, but may be caused by malabsorption of
food vitamin B_{12}. This hypothesis was investigated by
Doscherholmen and Swaim,[22] who compared the
absorption of [57]Co-labelled vitamin B_{12} incorporated
into eggs with that of crystalline [57]CoB$_{12}$. A group of
patients with gastric resections having low serum
vitamin B_{12} concentrations and normal absorption of

crystalline B_{12}, absorbed much less of [57]CoB$_{12}$
incorporated into eggs than normal subjects.
Similarly, egg vitamin B_{12} malabsorption was found
in patients with achlorhydria and severe hypo-
chlorhydria. Malabsorption of food bound vitamin
B_{12} has later been confirmed using various vitamin
B_{12} food protein preparations, including [57]CoB$_{12}$
bound *in vitro* by ovalbumin, egg yolks, and chicken
serum. Food vitamin B_{12}-malabsorption thus
appears to be an entity, and may be the result of
impaired gastric release of vitamin B_{12} in patients
with decreased secretion of acid and pepsin. Food
vitamin B_{12} malabsorption has been described
not only in patients with gastrectomy and with
achlorhydria, but also after treatment with
histamine-H_2-antagonists, and has been reported to
frequently occur in patients with unexplained low
serum vitamin B_{12} concentrations not caused by
pernicious anaemia or intestinal disease.[23] Whether
failure of liberation of vitamin B_{12} from food proteins
can always explain the vitamin B_{12} deficiency in these
patients is not clear. Most secrete intrinsic factor
poorly, and crystalline B_{12}-absorption may be inter-
mittently subnormal. Furthermore it is possible that
only some food sources may be dependent on acid
and pepsin for the release of vitamin B_{12} in the
stomach. Hence it has been shown that there was a
rapid and normal intragastric release of vitamin B_{12}
from liver in patients who had been rendered
achlorhydric by treatment with the acid inhibiting
drug omeprazole.[6] Moreover vitamin B_{12} bound by
chicken serum has been used in many of the studies
on food vitamin B_{12} absorption. Chicken serum is
not a natural food source, and the B_{12}-binding
protein in the serum belongs to the R-protein class.
The vitamin B_{12}-R-protein complex is not dependent
on peptic digestion but on pancreatic protease
secretion for the release of vitamin B_{12}, and it is not
clear why patients treated with histamine-H_2-
antagonists develop malabsorption of chicken serum
bound vitamin B_{12}.

PANCREATIC INSUFFICIENCY

In 1956 McIntyre *et al*[1] first demonstrated malabsorp-
tion of vitamin B_{12} 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 B_{12}
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 B_{12}-intrinsic factor complex on the intestinal
acceptor, and that one or more pancreatic enzymes
are essential.

How pancreatic extract stimulates vitamin B$_{12}$ absorption has been the subject of several investigations. In 1973 Toskes *et al*[25] reported that trypsin corrected vitamin B$_{12}$ 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*[26] showed that the vitamin B$_{12}$-R-binder inhibited the intestinal uptake of intrinsic factor bound vitamin B$_{12}$, and that this inhibitory effects was partially abolished by pancreatic extract; and suggested that pancreatic extract improves B$_{12}$-absorption by an effect on non-intrinsic factor vitamin B$_{12}$ binders. In 1978 Allen *et al*[7] showed that incubation of the R-protein-vitamin B$_{12}$-complex with pancreatic proteases led to rapid release and transfer of vitamin B$_{12}$ 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$_{12}$ to intrinsic factor. Marcoullis *et al*[27] provided *in vivo* evidence for this hypothesis, and showed that after the administration of ^{57}Co-labelled cyanocobalamin more than 60% of vitamin B$_{12}$ was bound to R-binders in jejunal fluid from patients with pancreatic insufficiency, whereas all vitamin B$_{12}$ was bound to intrinsic factor in healthy volunteers.

It is not entirely clear, however, how pancreatic insufficiency leads to vitamin B$_{12}$ malabsorption. Thus no correlation has been found between pancreatic exocrine function (including faecal fat excretion, trypsin and chymotrypsin secretion) and B$_{12}$ absorption.[28 29] The absorption of vitamin B$_{12}$ 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$_{12}$ becomes bound to intrinsic factor. Moreover although more than 60% of vitamin B$_{12}$ 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 B$_{12}$-R-complex in the ileum where vitamin B$_{12}$ is absorbed.

Although vitamin B$_{12}$ malabsorption occurs in up to 40–50% of patients with pancreatic insufficiency, vitamin B$_{12}$ 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*[30] observed that patients who failed to absorb radioactive B$_{12}$ 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 B$_{12}$ 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$_{12}$, as the patients may absorb B$_{12}$ normally when they are given metronidazole, lincomycin or tetracycline. Booth and Heath[31] reported that oral administration of B$_{12}$ bound to cultures of viable *E coli* inhibited the absorption of vitamin B$_{12}$ in the rat. When B$_{12}$ 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,[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 B$_{12}$ by small intestinal brush borders,[34] but so far it is unknown whether there are any disturbances in B$_{12}$-uptake by the ileal mucosa of patients with bacterial overgrowth.

The most important pathophysiological event appears to be the competive uptake of IF-bound vitamin B$_{12}$ by bacteria which deprive the host of vitamin B$_{12}$.

The hypothesis that intestinal bacteria take up intrinsic factor bound vitamin B$_{12}$ in the intestinal lumen, was investigated by obtaining aspirates from the upper ileum at timed intervals after an oral test meal containing radioactive B$_{12}$ 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$_{12}$ bound by bacteria. Treatment with antibiotics resulted in improved B$_{12}$ 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 B$_{12}$ is not clear. Presumably vitamin B$_{12}$ is bound to intrinsic factor in the lumen of the small intestine. Although most intestinal bacteria avidly take up unbound vitamin B$_{12}$, the uptake is much reduced if B$_{12}$ is bound to intrinsic factor *in vitro*. The inhibitory effect is partial when B$_{12}$ 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 B_{12} 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 B_{12} occurs only when they are present in very high concentrations. In addition, some of the ingested vitamin B_{12} may be converted by the bacterial flora to vitamin B_{12} analogues, making less vitamin B_{12} available to the host.[37]

Alternatively intestinal bacteria may, as seems to be the case with the fish tapeworm,[38] release vitamin B_{12} from its bond to intrinsic factor. So far, however, no intestinal bacteria have been found capable of splitting vitamin B_{12} from gastric intrinsic factor.

As intrinsic factor and bacteria have similar affinities for vitamin B_{12}, it has been proposed that the malabsorption is the result of competition for vitamin B_{12} between bacterial binding sites and those of IF-binders and non-IF-binders.[39] In the upper small bowel vitamin B_{12} 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 B_{12} malabsorption and deficiency. These diseases include coeliac disease, tropical sprue and Crohn's disease of the ileum. In tropical sprue vitamin B_{12} 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 competive uptake of vitamin B_{12}.[40]

In coeliac disease vitamin B_{12} 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 B_{12}.[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, B_{12} malabsorption is almost invariably present. Because the B_{12}-receptors are restricted to the ileum, there appears to be no adaptation of B_{12} 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 B_{12}-absorption has been reported.[42]

In Imerslund-Gräsbeck's disease familial selective malabsorption of vitamin B_{12} 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 B_{12}-uptake by homogenates of ileal biopsies from a single patient; and suggested that the absorptive defect appears after IF-B_{12} 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 B_{12}. 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

1 McIntyre PA, Sachs MV, Krevans JR, Conley CL. Pathogenesis and treatment of macrocytic anemia. *Arch Intern Med* 1956; **98:** 541–9.

2 Booth CC, Mollin DL. Importance of the ileum in the absorption of vitamin B_{12}. *Lancet* 1957; ii; 1007.

3 Booth CC, Mollin DL. The site of absorption of vitamin B_{12} in man. *Lancet* 1959; i: 18–21.

4 Booth CC, Mollin DL. Plasma, tissue and urinary radioactivity after oral administration of ⁵⁸Co-labelled vitamin B_{12}. *Br J Haematol* 1956; **2:** 223–36.

5 Cooper BA, Castle WB. Sequential mechanisms in the enhanced absorption of vitamin B_{12} by intrinsic factor in the rat. *J Clin Invest* 1960; **39:** 199–214.

6 Kittang E, Schjønsby H. Effect of gastric anacidity on the release of cobalamins from food and their subsequent binding to R-protein. *Scand J Gastroenterol* 1987; **22:** 1031–7.

7 Allen RH, Seetharam B, Podell E, Alpers DH. Effect of proteolytic enzymes on the binding of cobalamin to R protein and intrinsic factor. In vitro evidence that a failure to partially degrade R protein is responsible for cobalamin malabsorption in pancreatic insufficiency. *J Clin Invest* 1978; **61:** 47–54.

8 Katz M, Lee SK, Cooper BA. Vitamin B_{12} malabsorption due to a biologically inert intrinsic factor. *N Engl J Med* 1972; **287:** 425–9.

9 Levine JS, Nakane PK, Allen RH. Immunocytochemical localization of intrinsic factor-cobalamin bound to the guinea pig ileum in vivo. *Gastroenterology* 1982; **82:** 284–90.

10 Hagedorn CH, Alpers DH. Distribution of intrinsic factor-vitamin B_{12} receptors in human intestine. *Gastroenterology* 1977; **73:** 1019–22.

11 Mackinnon AM, Short MD, Elias E, Dowling RH. Adaptive changes in vitamin B_{12} absorption in coeliac disease and after proximal small bowel resection in man. *Am J Dig Dis* 1975; **20:** 835–40.

12 Robertson JA, Gallagher ND. In vivo evidence that cobalamin is absorbed by receptor-mediated endocytosis in the mouse. *Gastroenterology* 1985; **88:** 908–12.

13 Peters TJ, Hoffbrand AV. Absorption of vitamin B_{12} by the guinea pig. 1. Subcellular localization of vitamin B_{12} in the ileal enterocyte during absorption. *Br J Haematol* 1970; **19:** 369–82.

14 Chanarin I, Muir M, Hughes A, Hoffbrand AV. Evidence for intestinal origin of transcobalamin II during vitamin B_{12} absorption. *Br Med J* 1978; i: 1453–5.

15 Hakami N, Neiman PE, Canellos GP, Lazerson J. Neonatal megaloblastic anemia due to inherited transcobalamin II deficiency in two siblings. *N Engl J Med* 1971; **285:** 1163–70.

16 Reizenstein PG. Excretion of non-labeled vitamin B_{12} in man. *Acta Med Scand* 1959; **165:** 313–9.

17 Kanazawa S, Herbert V, Herzlich V, Drivas G, Manusselis C. Removal of cobalamin analogue in bile by the enterohepatic circulation of vitamin B_{12}. *Lancet* 1983; i: 707–8.

18 Teo NH, Scott JM, Neale G, Weir DG. Effect of bile on vitamin B_{12} absorption. *Br Med J* 1980; ii: 831-3.

19 Seetharam B, Jimenez M, Alpers DH. Effect of bile and bile acids on binding of intrinsic factor to cobalamin and intrinsic factor-cobalamin complex to ileal receptor. *Am J Physiol* 1983; **245:** G72–7.

20 Katz M, Mehlman CS, Allen RH. Isolation and characterization of an abnormal intrinsic factor. *J Clin Invest* 1974; **53:** 1274–83.

21 Deller DJ, Richards WCD, Witts LJ. Changes in the blood after partial gastrectomy with special reference to vitamin B_{12}. *Q J Med* 1962; **31:** 89–102.

22 Doscherholmen A, Swaim WR. Impaired assimilation of egg Co^{57} vitamin B_{12} in patients with hypochlorhydria and achlorhydria and after gastric resection. *Gastroenterology* 1973; **64:** 913–9.

23 Carmel R, Sinow RM, Siegel ME, Samloff M. Food cobalamin malabsorption occurs frequently in patients with unexplained low serum cobalamin levels. *Arch Intern Med* 1988; **148:** 1715–9.

24 Veeger W, Abels J, Helleman N, Nieweg HO. Effect of sodium bicarbonate and pancreatin on the absorption of vitamin B_{12} and fat in pancreatic insufficiency. *N Engl J Med* 1962; **267:** 1341–4.

25 Toskes PP, Deren JJ, Conrad ME. Trypsin-like nature of the pancreatic factor that corrects vitamin B_{12} malabsorption associated with pancreatic dysfunction. *J Clin Invest* 1973; **52:** 1660–4.

26 von der Lippe G, Andersen KJ, Schjønsby H. Pancreatic extract and the intestinal uptake of vitamin B_{12} binder. *Scand J Gastroenterol* 1977; **12:** 183–7.

27 Marcoullis G, Parmentier Y, Nicolas JP, Jimenez M, Gerard P. Cobalamin malabsorption due to non-degradation of R proteins in the human intestine. *J Clin Invest* 1980; **66:** 430–40.

28 Toskes PP, Hansell J, Cerda J, Deren JJ. Vitamin B_{12} malabsorption in chronic pancreatic insufficiency. *N Engl J Med* 1971; **284:** 627–32.

29 Matuchansky C, Rambaud JC, Modigliani R, Bernier JJ. Vitamin B_{12} malabsorption in chronic pancreatitis. *Gastroenterology* 1974; **67:** 406–7.

30 Henderson JT, Simpson JD, Warvick RRG, Shearman DJC. Does malabsorption of vitamin B_{12} occur in chronic pancreatitis? *Lancet* 1972; ii: 241–3.

31 Booth CC, Heath J. The effect of *E coli* on the absorption of vitamin B_{12}. *Gut* 1962; **3:** 70–3.

32 Schjønsby H, Andersen KJ, Nordgård K, Skagen DW. Enzymatic activities in jejunal biopsy specimens from patients with the stagnant loop syndrome. *Scand J Gastroenterol* 1983; **18:** 599–602.

33 Ament ME, Shimoda SS, Saunders DR, Rubin CE. Pathogenesis of steatorrhea in three cases of the stagnant loop syndrome. *Gastroenterology* 1972; **63:** 728–47.

34 Schjønsby H, Tabaqchali S. Uptake of rat gastric-juice-bound vitamin B_{12} by intestinal brush borders isolated from blind-loop rats. *Scand J Gastroenterol* 1971; **6:** 515–21.

35 Schjønsby H, Drasar BS, Tabaqchali S, Booth CC. Uptake of vitamin B_{12} by intestinal bacteria in the stagnant loop syndrome. *Scand J Gastroenterol* 1973; **8:** 41–7.

36 Welkos SL, Toskes PP, Baer H. Importance of anaerobic bacteria in the cobalamin malabsorption of the experimental rat blind loop syndrome. *Gastroenterology* 1981; **80:** 313–20.

37 Brandt LJ, Bernstein LH, Wagle A. Production of vitamin B_{12} analogues in patients with small-bowel bacterial overgrowth. *Ann Intern Med* 1977; **87:** 546–51.

38 Nyberg W. The influence of Diphyllobotrium latum on the vitamin B_{12}-intrinsic factor complex. II. In vitro studies. *Acta Med Scand* 1960; **167:** 189–92.

39 Giannella RA, Broitman SA, Zamcheck N. Competition between bacteria and intrinsic factor for vitamin B_{12}: Implications for vitamin B_{12} malabsorption in intestinal bacterial overgrowth. *Gastroenterology* 1972; **62:** 255–60.

40 Kapadia CR, Bhat P, Jacob E, Baker SJ. Vitamin B_{12} absorption – a study of intraluminal events in control subjects and patients with tropical sprue. *Gut* 1975; **16:** 988–93.

41 Stewart JS, Pollock DJ, Hoffbrand AV, Mollin DL, Booth CC. A study of proximal and distal intestinal structure and absorptive function in idiopathic steatorrhoea. *Q J Med* 1967; **36:** 425–44.

42 Nygaard K, Helsingen N, Rootwelt K. Adaptation of vitamin B_{12} absorption after ileal bypass. *Scand J Gastroenterol* 1970; **5:** 349–51.

43 Imerslund O. Idiopathic chronic megaloblastic anemia in children. *Acta Paediatr* 1960; **49** [suppl 119]: 1–65.

44 Gräsbeck R, Gordin R, Kantero I, Kuhlbäck B. Selective vitamin B_{12} malabsorption and proteinuria in young people. A syndrome. *Acta Med Scand* 1960; **167:** 289–96.

45 Mackenzie IL, Donaldson RM, Trier JS, Mathan VI. Ileal mucosa in familial selective vitamin B_{12} malabsorption. *N Engl J Med* 1972; **286:** 1021–5.

46 Burman JF, Jenkins WJ, Walker-Smith JA *et al*. Absent ileal uptake of IF-bound vitamin B_{12} *in vivo* in the Imerslund-Gräsbeck syndrome (familial vitamin B_{12} malabsorption with proteinuria). *Gut* 1985; **26:** 311–4.