Absorption of cobalamins

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Deoxyadenosylcobalamin (coenzyme $B_{12}$) is the predominant vitamin $B_{12}$ analogue present in a normal diet, although small amounts of other analogues such as methylcobalamin and hydroxocobalamin are found (Weissbach, Toohey, and Barker, 1959; Toohey and Barker, 1961; Lindstrand, 1964). Some hydroxocobalamin may arise as the result of photolysis of the coenzyme forms. The cobalamins are normally bound to protein in the cell (Cohn, Minot, Alles, and Salter, 1928; Hedbom, 1960). These vitamin $B_{12}$ analogues are absorbed largely unchanged (Okuda, Yashima, and Takamatsu, 1969), although some conversion of cyanocobalamin to deoxyadenosylcobalamin during absorption in the guinea pig has been demonstrated (Hoffbrand, Linnell, Matthews, and Peters, 1970). Further, the different vitamin $B_{12}$ analogues are absorbed equally by man (Lee and Glass, 1961; Chosy, Killander, and Schilling, 1962), although when assessed by urinary excretion methods differences in plasma and tissue binding may result in lower urinary excretion with deoxyadenosyl $B_{12}$ and hydroxocobalamin.

An average ‘low cost’ mixed diet contained 16 $\mu$g (range 1-2 to 75-6) per day (Chung, Pearson, Darby, Miller, and Goldsmith, 1961). Although a strictly vegetarian diet may be almost devoid of vitamin $B_{12}$, some vitamin $B_{12}$ may appear as the result of the increasing bacterial content of the meal following its preparation. Vegetarians may also get some vitamin $B_{12}$ from water supplies, particularly water from wells as in India. Cooking of food may serve to release vitamin $B_{12}$ from its protein link and there is little loss of the vitamin during this process. Further, almost all the vitamin in food is available for absorption. Thus vitamin $B_{12}$ in meat (lamb) was absorbed to the same extent as the equivalent dose in aqueous solution (Heyssel, Bozian, Darby, and Bell, 1966). As the daily vitamin $B_{12}$ requirement is between 2 and 5 $\mu$g the amount present in an average mixed diet is more than adequate and, indeed, the amount of vitamin $B_{12}$ available often exceeds the absorption capacity of the small gut.

The amount of vitamin $B_{12}$ that can be absorbed from a single dose or a single meal by a physiological mechanism, that is, through the agency of intrinsic factor, is about 2 $\mu$g. Thus with oral quantities of vitamin $B_{12}$ of less than 0.5 $\mu$g, over 70% is absorbed and this falls to 50% with 1 or 2 $\mu$g doses, 16% with 10 $\mu$g, and less than 5% with doses of 20 $\mu$g or more. With relatively large pharmacological doses of vitamin $B_{12}$ about 1% may diffuse across the small gut mucosa by a mechanism that does not require intrinsic factor. The limitation to the amount of vitamin $B_{12}$ absorbed may be related to saturation of specific ileal receptor sites by intrinsic factor vitamin $B_{12}$ complex. It has been suggested by Abels (1959) that the block to further vitamin $B_{12}$ uptake lasts for some three hours.

Intrinsic Factor

The field has been reviewed by Chanarin (1968a, 1968b, and 1969), Simons (1968), and Corcino, Waxman, and Herbert (1970). This glycoprotein with a molecular weight of about 55,000 is secreted by the gastric parietal cell in man. Its secretion is augmented by the polypeptide hormone gastrin and by its synthetic analogues such as pentagastrin, by histamine and histalog, and by insulin. All these substances are also potent stimulants of the other major product of the parietal cell, hydrochloric acid. Under these circumstances it is not surprising that both intrinsic factor and vitamin $B_{12}$ are tolerant to a wide range of pH and those changes in pH do not affect the capacity of intrinsic factor to unite with vitamin $B_{12}$ (McGuigan, 1967; Ashworth, Strickland, Koo, and Taylor, 1969). However, an acid pH promotes proteolytic digestion of intrinsic factor by pepsin in the gastric lumen and this results in a slow but steady loss of intrinsic factor activity.

The radioimmunoassay for intrinsic factor introduced by Ardeman and Chanarin in 1963 has been widely employed in the diagnosis of pernicious anaemia and in the study of gastric secretion. A unit of intrinsic factor has been defined as the quantity binding 1 nanogram of vitamin $B_{12}$, and 400 to 500 such units are required to promote optimum absorp-
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Absorption of vitamin B_{12} in pernicious anaemia (Ardeman and Chanarin, 1965a). On the other hand, the normal daily output of intrinsic factor is in excess of 70,000 units with an average concentration of 30 units per ml in the basal secretion and a concentration of about 55 units per ml after a stimulus to secretion.

Interaction between Intrinsic Factor and Vitamin B_{12}

The stoichiometric reaction between intrinsic factor and vitamin B_{12} which occurs with great rapidity in vitro is largely complete in the stomach but can also occur in the small gut. Digestion in the stomach serves to release vitamin B_{12} in food, a process that may remain incomplete after partial gastrectomy. There may be some exchange between intrinsic factor-bound vitamin B_{12} and free vitamin B_{12} (Donaldson and Katz, 1963). A further important aspect of the link between vitamin B_{12} and intrinsic factor is that each becomes considerably more stable (Abels and Schilling, 1964). Thus, intrinsic factor when linked to vitamin B_{12} is much more resistant to proteolytic digestion as well as to heat, and adenosylcobalamin becomes resistant to photolysis (Okuda et al., 1969). It has also been suggested that the vitamin B_{12}-intrinsic factor complex is less easily taken up by bacteria than is free vitamin B_{12} (Booth and Heath, 1962). The complex passes down the small gut to be taken up by specific receptor sites on the brush border of the epithelial cells of the villi in the ileum.

Events in the Ileum

Normal intestinal uptake of the intrinsic factor-vitamin B_{12} complex depends not only on the presence of the normal epithelial cells but on a normal milieu in the small gut lumen. Hyperacidity, as in the Zollinger-Ellison syndrome (Shimoda, Saunders, and Rubin, 1968), loss of pancreatic secretion (Toskes and Deren, 1970), or interference with the integrity of the cell during colchicine therapy (Race, Paes, and Faloof, 1970) or by alcohol (Lindemann, Rybak, Gerson, Rubin, and Leibler, 1970) all interfere with the ileal phase of vitamin B_{12} absorption.

The site of vitamin B_{12} absorption in man is the distal ileum and the evidence has been reviewed by Chanarin (1969). Following an oral dose of vitamin B_{12} detectable amounts of the vitamin appear in the blood some four hours later and reach a peak in eight to 12 hours (Booth and Mollin, 1956). This long lag is due to delay in passage of vitamin B_{12} from the surface of the epithelial cell to the portal blood but the precise events that occur are uncertain.

Specific receptors for the uptake of the vitamin B_{12}-intrinsic factor complex appear to be present on the microvillous membranes of the ileal epithelial cells. Thus, a homogenate of human ileum (but not jejunum) showed an enhanced uptake of intrinsic factor-vitamin B_{12} that was inhibited by the addition of intrinsic factor antibody (Carmel, Rosenberg, Lau, Streiff, and Herbert, 1969). Donaldson, Mackenzie, and Trier (1967) localized this activity to the microvillous fraction. Carmel et al. (1969) found that calcium or magnesium ions were required for the attachment of the vitamin B_{12}-intrinsic factor complex to human ileal homogenate and that the optimal pH was 6.6. Attempts to isolate an ileal receptor were reported by Donaldson et al. (1967) and by Rothenberg (1968).

It is believed that intrinsic factor separates from vitamin B_{12} during the passage of vitamin B_{12} to the portal blood. This belief is based on repeated failure to demonstrate intrinsic factor in blood and the fact that some patients may have intrinsic factor antibody in plasma while still having normal vitamin B_{12} absorption (Ardeman, Chanarin, Krafchik, and Singer, 1965). Administration of hog intrinsic factor labelled with $^{51}$Cr and vitamin B_{12} labelled with $^{52}$Co showed that vitamin B_{12} entered the blood but that the chromium label was recovered from the faeces (Yamaguchi, Rosenthal, and Glass, 1970). Peters and Hoffbrand (1970a) labelled human intrinsic factor with $^{125}$I and fed this with $^{57}$Co-labelled vitamin B_{12} to four guinea pigs. Fractionation of the ileal cells appeared to show that the $^{125}$I label remained attached to the brush border while the $^{57}$Co label entered the cell and was attached to mitochondria. If confirmed, this would indicate that separation of intrinsic factor from vitamin B_{12} takes place on the cell surface and that intrinsic factor does not enter the epithelial cell. Further, since this separation preceded the lag in vitamin B_{12} transport to portal blood such separation may not be the cause for this lag. Vitamin B_{12} entering the epithelial cell is found in the mitochondrial fraction (Peters and Hoffbrand, 1970b). On the other hand if the mode of entry of vitamin B_{12} to the ileal epithelial cell was by pinocytosis (Wilson, 1963) localization to lysosomes would be anticipated, since pinocytic vesicles fuse with lysosomes. Although the evidence is against entry of vitamin B_{12} by pinocytosis, could not secondary localization of vitamin B_{12} to mitochondria occur?

Another hypothesis is that intrinsic factor is separated by hydrolase enzymes on the brush border (Mackenzie and Donaldson, 1969). This is supported by the preliminary observations of Peters and Hoffbrand (1970a) that labelled intrinsic factor remains on the cell surface. Vitamin B_{12} entering the portal blood does so attached to a specific carrier.
protein, transcobalamin II (Hall and Finkler, 1965). The transcobalamin II-vitamin B₁₂ complex has a very short half-life so that absorbed vitamin B₁₂ is rapidly transferred to tissues such as the liver (Hall, 1969; Hom, 1967). Transcobalamin II may be synthesized in the ileal epithelial cell and the complex with vitamin B₁₂ transferred to the blood. Lag in production of the carrier protein is another possible explanation of the long lag in delivery of absorbed vitamin B₁₂ to blood.

Finally absorbed vitamin B₁₂ reappears in the gut via excretion in bile and this vitamin B₁₂ is re-absorbed.

Vitamin B₁₂ Malabsorption

This falls into three categories: (1) inadequate intrinsic factor which may be congenital, due to gastric atrophy, gastric resection, or neutralization by antibody; (2) luminal factors, such as abnormal small gut flora, fish tape worm, pancreatitis, Zollinger-Ellison syndrome, laxatives (cascara), or drugs, eg, slow-K, EDTA; (3) ileal factors which can be congenital (Imerslund-Gräsbeck), due to resection, gluten-sensitive enteropathy, tropical sprue, drugs, eg, ethanol, colchicine, para-aminosalicylic acid, or neomycin, or folate and vitamin B₁₂ deficiency states.

Inadequate Intrinsic Factor

Failure of intrinsic factor production may be inherited as a recessive characteristic and present as a familial form of megaloblastic anaemia in the first two years of life. This has been reviewed by Chanarin (1969).

It has been recognized for some time that the malabsorption of vitamin B₁₂ in pernicious anaemia is not fully corrected by additional intrinsic factor. There are a number of possible explanations for this. Free antibody to intrinsic factor in the gastrointestinal tract has been found in a significant proportion of patients (Fisher, Rees, and Taylor, 1965; Schade, Feick, Muckerheide, and Schilling, 1966; Herbert, Carmel, and Li, 1967; Rose and Chanarin, 1969; Goldberg and Bluestone, 1970). More recently Rose and Chanarin (1971) have found a significant correlation between intrinsic factor antibody, particularly in gastric secretion, and the absorption of vitamin B₁₂ in pernicious anaemia. In the absence of demonstrable antibody the mean absorption of vitamin B₁₂ with intrinsic factor was the same as that in healthy subjects. Absorption in the presence of excess intrinsic factor was most depressed in those with antibody in both serum and gastric juice. The corollary is the restoration of vitamin B₁₂ absorption in pernicious anaemia by steroid therapy and this is accompanied by a steady decline in titre of intrinsic factor antibody (Ardeman and Chanarin, 1965).

Long-term vitamin B₁₂ deficiency of itself depresses the absorption of vitamin B₁₂. This is corrected slowly over many months following the start of vitamin B₁₂ therapy and is a further factor contributing to impaired vitamin B₁₂ absorption when given with intrinsic factor in pernicious anaemia (Haurani, Sherwood, and Goldstein, 1964; Brody, Estren, and Herbert, 1966; Carmel and Herbert, 1967; Forshaw, 1969). A third contributory factor to intestinal malabsorption in pernicious anaemia may lie in the very high serum gastrin level in pernicious anaemia which of itself may produce intestinal malabsorption (McGuigan and Trudeau, 1970; Wright, Hersh, Floch, and Weinstein, 1970).

Severe atrophic gastritis in the absence of pernicious anaemia may lead to impaired intrinsic factor production (Ardeman and Chanarin, 1966) and half the patients without pernicious anaemia have malabsorption of vitamin B₁₂ (Chanarin, 1969). In a significant number of these patients the absorption of vitamin B₁₂ is improved by an injection of carbachol (Whiteside, Mollin, Coghill, Williams, and Anderson, 1964). However, carbachol does not stimulate intrinsic-factor secretion (Ardeman, Chanarin, and Doyle, 1964) but may act by producing intestinal hurry and hence propelling small amounts of intrinsic factor-vitamin B₁₂ complex to the ileum.

Megaloblastic anaemia due to vitamin B₁₂ deficiency develops in about 5% of patients undergoing partial gastrectomy, although malabsorption of vitamin B₁₂ is present in some 30% of patients when the test is carried out in the fasting state (Lous and Schwartz, 1959). On the other hand absorption of labelled vitamin B₁₂ is improved when the dose is given with a meal (Deller, Germar, and Witts, 1961) or with an injection of histamine (Turnbull, 1967). This implies that a stimulus to intrinsic factor production may be necessary after partial gastrectomy. A further complication after partial gastrectomy is interference with vitamin B₁₂ absorption by an abnormal gut flora so that there is impaired absorption of the vitamin B₁₂-intrinsic factor complex. This is the case in 12% of patients who have impaired vitamin B₁₂ absorption after partial gastrectomy (Chanarin, 1969).

Luminal Factors

Both infestation with the fish tape worm and an abnormal small-intestinal bacterial flora, by taking up dietary vitamin B₁₂, make it unavailable to the host. The disorders leading to an abnormal bacterial gut flora (reviewed by Chanarin, 1969) are associated with small gut stasis and the malabsorption may be corrected temporarily after administration of wide-spectrum antibiotics. These disorders are blind
intestinal loops surgically produced or otherwise, strictures, entero-entero or entero-colic Anastomosis, fistulae, small-intestinal diverticulosis, interference of gut motility as in scleroderma, Whipple's disease, post-vagotomy, and after administration of ganglion-blocking agents.

Pancreatic insufficiency may be associated with impaired vitamin B₁₂ absorption (MacIntyre, Sachs, Krevans, and Conley, 1956; Perman, Gullberg, Reizenstein, Snellman, and Allgen, 1960; Nieweg, Abels, Veeger, and Hillemans, 1962; Toskes and Deren, 1970). This was the case in nine out of 22 consecutive patients with pancreatic insufficiency reported by Toskes and Deren (1970) and in five out of nine patients studied by Perman et al. (1960). In these cases the absorption of vitamin B₁₂ is improved by carrying out the absorption test with the addition of a single dose of pancreatic enzyme and also by the addition of bicarbonate. Factors leading to impaired vitamin B₁₂ absorption in pancreatitis may be an acid intestinal pH due to loss of bicarbonate secretion from the pancreas, and reduction in the amount of ionic calcium. However, Toskes and Deren (1970) have not found any difference in ileal pH and in total calcium concentration in those patients who absorbed vitamin B₁₂ normally and those who did not. They suggest that pancreatic extract contains a nondialyzable, thermolabile factor needed for vitamin B₁₂ absorption. Pancreatic enzymes conceivably could be required for digestion of intrinsic factor-vitamin B₁₂ complex as part of the process of the passage of vitamin B₁₂ into the ileal epithelial cell (LeBauer, Smith, and Greenberger, 1968).

Impaired absorption of vitamin B₁₂ in the Zollinger-Ellison syndrome was noted by Shimoda et al. (1968) and was ascribed to the acid pH in the small gut. When the pH at the duodeno-jejunal junction was maintained at 7 normal vitamin B₁₂ absorption was restored, the patient's own gastric secretion serving as the source of intrinsic factor.

Administration of cascara was associated with increased faecal excretion of vitamin B₁₂ although the urinary excretion remained within normal limits (Webb, Chodos, Mahar, and Faloon, 1968). If confirmed, this would be a very unusual pattern but could be explained by a failure to reabsorb vitamin B₁₂ excreted via the bile.

**ILEAL FACTORS**

A specific failure of transport of vitamin B₁₂ across the ileal cell (congenital vitamin B₁₂ malabsorption) was described by Imerslund (1960) and by Gräsbeck, Gordin, Kantero, and Kuhlbläck (1960). This is the commonest cause of vitamin B₁₂ malabsorption in children. It has a recessive mode of inheritance and these children present with a severe megaloblastic anæmia in the first or second year of life. The stomach and gastric secretion is normal, that is, has normal content of acid and of intrinsic factor. Other than malabsorption of the intrinsic factor-vitamin B₁₂ complex all other intestinal function tests are normal. Proteinuria is a constant finding.

Ileal resection, for example for regional enteritis or following a volvulus, may lead to vitamin B₁₂ malabsorption. Involvement of the ileum in patients with gluten-sensitive enteropathy results in impaired vitamin B₁₂ absorption in about one third of patients. The majority of patients with tropical sprue have impaired vitamin B₁₂ absorption and this may be the only manifestation of the disease in the chronic state. There is improvement in the manifestations of tropical sprue with long-term antibiotic therapy (Klipstein, 1968), and although this evidence has been cited as support for the view that an abnormal intestinal flora is the principal aetiological agent (Banwell and Gorbach, 1969) it is more probable that an abnormal mucosa is responsible.

A number of drugs or chemicals may lead to vitamin B₁₂ malabsorption. These include ethanol, slow-K, neomycin, colchicine, and ethylenediaminetetraacetate (EDTA).

Depression of vitamin B₁₂ absorption was found in all five volunteers taking large amounts of alcohol (46 to 66% of total caloric intake) although none were inebriated (Lindenbaum et al., 1970). Abnormal vitamin B₁₂ absorption was present in eight out of 17 chronic alcoholics admitted to an Alcoholic Unit (Roggin, Iber, Kater, and Tabon, 1969). Ethanol probably exerts a direct toxic effect on intestinal cells which, if an analogy can be drawn from its effect on the marrow, is transient and reversible on ethanol withdrawal.

Administration of colchicine also produces a reversible malabsorption state, one of the manifestations of which is malabsorption of vitamin B₁₂. Thus 12 out of 13 subjects showed impaired absorption reaching lowest values on the second to the seventh day after the start of oral colchicine (Webb et al., 1968). Normal vitamin B₁₂ absorption was restored three to five days after withdrawal of the drug. It is assumed that the effect is due to interference with cell renewal on the ileal surface.

Neomycin therapy produces intestinal malabsorption, one of the manifestations of which is interference with vitamin B₁₂ absorption (Jacobson, Chodos, and Faloon, 1960).

Since the original report by Heinivaara and Palva (1964) that para-aminosalicylic acid produced vitamin B₁₂ malabsorption there have been contradictory reports in the literature (Paaby and Norvin, 1966) but other evidence of malabsorption in such patients, including that of fat, was reported by Coltart (1969).
and Hess, Gregory, Thompson, and Welsh (1970). The effects were reversed on drug withdrawal and were partly overcome by oral folic acid (Palva, Heinivaara, and Mattela, 1966; Hess et al., 1970).

Salokannel, Palva, and Takkunen (1970) reported that regular administration of potassium chloride (500 mg) in a slow release tablet led to impaired vitamin B₁₂ absorption in 11 out of 30 patients in hospital. A fall in pH in the intestinal lumen was suggested as a possible factor.

Gräsbeck and Nyberg (1958) found that administration of EDTA when given orally with vitamin B₁₂ interfered with its absorption possibly by binding calcium ions.

Prolonged deficiency of either folate or vitamin B₁₂ leads to impairment of the capacity of the ileum to absorb the vitamin B₁₂-intrinsic factor complex. This is reversible after specific therapy but in many cases improvement becomes evident only after six to 12 months of therapy. Lees (1961) noted impaired vitamin B₁₂ absorption in epileptics which improved after folate therapy, and further examples were recorded by Scott, Kammer, Burger, and Middleton (1968) in megaloblastic anaemia due to alcoholism and pregnancy and by Forshaw (1969) in nutritional folate deficiency.

Impaired uptake of the vitamin B₁₂-intrinsic factor complex by the ileum may result from vitamin B₁₂ deficiency due to fish tape worm infestation (Nyberg, Gräsbeck, and Sippola, 1958), in pernicious anaemia (Brody et al., 1966; Lawrence, 1966; Forshaw, 1969), and in infants with congenital absence of intrinsic factor (Lampkin and Maurer, 1967).
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guinea pig during fasting and absorption of cyanocobalamin. J. Physiol. (Lond.), 208, 669-673.


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