Absorption of vitamin B₁₂ from the intestine

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In a limited space only a brief outline can be given of knowledge about the absorption of vitamin B₁₂. The size of the problem is shown by the review by Glass (1963), which covers over 300 pages and contains over 1500 references. Later developments have been summarized by Donaldson (1964) and Herbert (1965).

Historical and general aspects

Over 30 years ago Castle and his collaborators showed that patients with Addisonian pernicious anaemia, the classical disease in man due to deficiency of vitamin B₁₂, did not respond when they ate beef. If, before it was given to the patients, the beef was eaten by a normal man and was recovered from his stomach and incubated, or was incubated with normal human gastric juice, most patients showed increased formation of red blood cells (Castle, 1929; Castle & Townsend, 1929). It was suggested that an extrinsic (food) factor reacted with an intrinsic (gastric) factor in the normal stomach to form material with 'a marked hematoipoietic effect' (Castle, Townsend & Heath, 1930). The defect in pernicious anaemia was failure to secrete intrinsic factor.
In 1948 vitamin $B_{12}$ was isolated and was found to be therapeutically active when given by injection to patients with pernicious anaemia. Berk, Castle, Welch, Heinle, Anker & Epstein (1948) then showed that 5 mg vitamin $B_{12}$ given by mouth daily for 10 days to patients with pernicious anaemia was without effect; the patients responded when normal human gastric juice was given with the vitamin $B_{12}$. This confirmed the existence of intrinsic factor, but extrinsic factor and the anti-pernicious anaemia factor appeared to be the same and identical with vitamin $B_{12}$.

Until 1952 the only test for absorption of vitamin $B_{12}$ was to study the haematological response of patients with pernicious anaemia. This was tedious and uncertain because of variations in the responses of different patients. When vitamin $B_{12}$ containing radioactive cobalt became available, patients with pernicious anaemia in remission were found to excrete in the faeces most of the radioactivity from an oral dose of the labelled vitamin given alone. If normal human gastric juice or a pig stomach preparation was given with the vitamin, a smaller proportion of the dose was excreted (Heinle, Welch, Scharf, Meacham & Prusoff, 1952). Thus studies could be made on treated patients with pernicious anaemia, and the labelled vitamin could be used for investigations with normal human subjects, animals and isolated tissue preparations.

Man obtains vitamin $B_{12}$ from dietary sources which are almost all of animal origin. Liver and kidney are rich sources, muscle meats and fish contain less, while milk, eggs and dairy products are minor sources for persons on normal diets but are important for vegetarians. Animals such as the rat and the rabbit probably satisfy their requirements mainly by coprophagy. The vitamin is mainly bound to proteins in food. It is partially liberated by digestive enzymes and is then available for absorption (Reizenstein, 1959).

In almost all work with labelled vitamin $B_{12}$, cyanocobalamin, which is not the naturally occurring form of the vitamin, has been used. Conclusions from such experiments may not apply to the absorption of vitamin $B_{12}$ from food. Thus Nyberg & Reizenstein (1958) claimed that patients with pernicious anaemia absorbed as much vitamin $B_{12}$ from pig liver as did control subjects and that both patients and control subjects absorbed much less of comparable doses of cyanocobalamin. However, data on the absorption of vitamin $B_{12}$ from food are scarce and in the remainder of this discussion only results obtained with cyanocobalamin can be reviewed.

Despite many technical advances, we still do not understand the mechanism by which vitamin $B_{12}$ is absorbed. We know something about the site of absorption. Most people accept that intrinsic factor is essential for the normal absorption of the vitamin, though some deny this (Mooney & Heathcote, 1966). The nature and function of intrinsic factor remain uncertain.

The site of absorption

The lower part of the small intestine seems to be the site of maximum absorption in several species. Booth & Mollin (1957) studied patients whose ileum was intact but who had abnormalities in the intestine causing excessive growth of intestinal flora, which competed for orally administered vitamin $B_{12}$ and reduced absorption.
by the patient. Suppression of the flora with antibiotics increased absorption to normal, whereas in similar patients whose ileum had been resected or short-circuited, absorption remained low despite treatment with antibiotics. Cooke, Cox, Meynell & Gaddie (1957) and Booth & Mollin (1959) showed that the absorption of vitamin B₁₂ was subnormal and unaffected by intrinsic factor in patients whose ileum had been removed or short-circuited. Many such patients later showed evidence of vitamin B₁₂ deficiency.

In rats the site of absorption has been described as the second and third quarters of the small intestine (Reynell, Spray & Taylor, 1957), the middle segment (Booth, Chanarin, Anderson & Mollin, 1957), the middle part and just distal to it (Holdsworth & Coates, 1961), the upper and mid ileum (Moertel, Scudamore, Owen & Bollman, 1960) and the whole small intestine (Smith & Ellis, 1965). In dogs the site of maximum absorption is below the jejunum (Baker, Mackinnon & Vasudevia, 1958).

In several of these studies, in man and in animals, radioactivity was located in the wall of the small intestine after administration of labelled cyanocobalamin. The areas containing most radioactivity were assumed to be the sites of maximum absorption.

Effect of size of dose on absorption

Patients with pernicious anaemia absorb vitamin B₁₂ given by mouth if the dose is sufficiently large. For example, haematological responses occurred after single doses of between 3000 and 10 000 µg (Conley & Krevans, 1955) and after daily doses of between 20 and 500 µg (Chalmers & Shinton, 1958). This absorption is probably due to passive diffusion through the intestinal wall. The doses are much higher than the amount of vitamin B₁₂ likely to be provided in the diet, except perhaps in the 120–240 g liver daily which was the first effective treatment for pernicious anaemia (Minot & Murphy, 1926).

In normal men and normal rats the amount of vitamin B₁₂ absorbed is proportional to the dose over a limited range, further increases in dose resulting in little further absorption (Glass, 1955; Taylor, Mallett, Witts & Taylor, 1958). Rabbits apparently absorb a fairly constant proportion of the dose when between 0.055 and 150 µg is given by mouth (Simnett & Spray, 1965). Doscherholmen & Hagen (1957) studied the rate of appearance of radioactivity in the blood of human subjects who were given various doses of labelled vitamin B₁₂ by mouth. They distinguished three types of curve, depending on the size of the dose, and concluded that large doses were absorbed partly through a mechanism involving intrinsic factor and partly by a mechanism independent of intrinsic factor. Smaller doses are absorbed by the first mechanism only.

Source, nature and mode of action of intrinsic factor

Intrinsic factor is secreted by the stomach in all species which have been studied. The site of production is the fundus and body of the stomach in man, the pyloric mucosa in the pig, and the glandular portion of the stomach in the rat (Glass, 1963).
Intrinsic factor has not been isolated in an unequivocally pure state, though preparations have been described of which less than 1 mg promoted the absorption of vitamin B₁₂ in man. It is heat-labile and non-dialysable and is mucoprotein or mucopolysaccharide in nature. Molecular weights between 5000 and 200,000 have been assigned to materials of pig and human origin. There may be two or more substances with intrinsic factor activity from both these species. These aspects have been summarized in another review by Glass (1964).

Intrinsic factor is antigenic, and about 40% of patients with pernicious anaemia have autoantibodies in intrinsic factor in their blood (Taylor, 1963). It has been alleged that intrinsic factor is species-specific, but there is little basis for this claim. For example, pig intrinsic factor is active in man, though resistance may develop after prolonged use (Blackburn, Spray, Swan, Tudhope & Wilson, 1959). Rat gastric juice promoted the absorption of vitamin B₁₂ in man (Abels, Woldring, Vegter & Nieweg, 1957) and human intrinsic factor is active in the rat (Taylor et al. 1958).

Intrinsic factor combines with or binds vitamin B₁₂, but this property is shared by other proteins which are not active as intrinsic factor (Bird & Hoevet, 1951; Abels, Nieweg & Schipperijn, 1964).

The mechanism by which intrinsic factor promotes the absorption of vitamin B₁₂ is unknown. It seems anomalous that vitamin B₁₂, with a molecular weight of about 1400, should have to interact with a substance with a molecular weight of several thousand before it can be absorbed. The process seems to be dependent on the presence of calcium ions or of closely related divalent cations (Okuda & Sasayama, 1965). The results described in the section on the site of absorption show that in the first stage of absorption the vitamin becomes fixed to the intestinal wall, and it remains at the surface of, or within, the wall for several hours. It has been suggested that intrinsic factor attaches itself to receptor sites and then facilitates the passage of vitamin B₁₂ through the intestinal wall. The vitamin may be split from intrinsic factor and absorbed alone into the mucosal cells and thence into the blood stream, or the vitamin B₁₂–intrinsic factor complex may be absorbed as a whole into the cells, subsequently being split to release its vitamin B₁₂ into the blood. There is little evidence to confirm or deny these possibilities, but once it has reached the blood the vitamin is no longer bound to intrinsic factor (Herbert, 1965).

Some light may be shed on this problem by a recent report from France (Wolff, Nabet, Jamaigne & Bertheau, 1966). Pig gastric mucosal extract was treated with trypsin and dialysed under pressure and the dialysate contained material with intrinsic factor activity both in vitro and in patients with pernicious anaemia. No studies were reported of the behaviour of the intrinsic factor–vitamin B₁₂ complex under such conditions, but the observations suggest that intestinal enzymes may split the complex to render the vitamin B₁₂ absorbable and that small dialysable molecules can have intrinsic factor activity.

Conclusions

Until intrinsic factor is completely purified and characterized neither its role in the absorption of vitamin B₁₂ nor the more general aspects of this problem are...
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likely to be fully understood. The unanswered questions are of fundamental biochemical and physiological interest and it is hoped that their solution will not be long delayed.

REFERENCES


Iron absorption

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We do not yet know how iron traverses the mucosal cell of the intestine. Granick’s ingenious theory of the mechanism of control of iron absorption through the ferritin–apoferritin system of the mucosal cell was founded on very few experiments and there has since accumulated abundant evidence that ‘mucosal’ block if it does occur is a relatively inefficient factor in regulating iron absorption.