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The absorption of vitamin B₁₂ in the megaloblastic anaemias

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The labelling of vitamin B₁₂ with radioactive cobalt has permitted application of the tracer technique to studies of the absorption of vitamin B₁₂ and eliminated the major difficulties inherent in earlier methods. This technique enjoys the unique advantages that it can be carried out on patients who have received treatment and that it gives a moderately accurate quantitative assessment of absorption with doses of vitamin B₁₂ similar to the daily requirements of man. In Professor Witts's depart-

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ment at Oxford ^{60}Co -labelled vitamin B_{12} has been used to assess the part played by malabsorption of this vitamin in the different types of megaloblastic anaemia.

Method

The method of Heinle, Welch, Scharf, Meacham & Prusoff (1952) has been used throughout the present investigations. The radioactivity in the faeces is measured after an oral dose of $0.5 \mu\text{g}$ ^{60}Co -labelled vitamin B_{12} and it is assumed that all radioactivity not recovered has been absorbed.

Observations

Control subjects

In forty-four observations on thirty-five control subjects the amount of radioactivity recovered in the faeces varied from 9 to 47% of that present in the oral dose. The mean value was 27.3% with a standard error of the mean of $\pm 1.6\%$. There was no apparent relationship between the age of the subject and the ability to absorb an oral dose of $0.5 \mu\text{g}$ labelled vitamin B_{12} .

Pernicious anaemia

In twenty-four patients with pernicious anaemia, from 80 to 103% of the radioactivity in the oral dose of labelled vitamin B_{12} was found in the faeces. The mean value for twenty-five observations was 92.3% (S.E. 1.3%). Absorption was increased by the addition of intrinsic factor and with 25 mg of Fraction 'B' (Welch & Heinle, 1951) or 50 mg of Lederle intrinsic factor (desiccated pig's gastric mucosa) most of these results were within the limits observed in control subjects. The failure to absorb vitamin B_{12} was attributed, therefore, to loss of the secretion of intrinsic factor. This contention is supported by the findings of atrophy of the gastric mucosa and achylia in each of the nineteen patients in whom both the secretions and histology of the stomach were studied.

Previous workers have reported a haematological response in a few patients with pernicious anaemia following treatment with antibiotics (Lichtman, Ginsberg & Watson, 1950; Davis, 1951; Mogensen, 1953). The absorption of labelled vitamin B_{12} was measured in one patient with untreated pernicious anaemia following the administration of aureomycin (chlorotetracycline) for 3 weeks. During this period there had been no clinical or haematological improvement and the uptake of labelled vitamin B_{12} was not increased although cultures of the faeces showed no bacterial growth. A satisfactory remission followed treatment with vitamin B_{12} by injection.

Two patients were studied in whom the diagnosis of subacute combined degeneration had been made in the absence of anaemia. In both the absorption of labelled vitamin B_{12} was grossly impaired but was corrected by small amounts of intrinsic factor, i.e. the pattern was similar to that found in patients with pernicious anaemia.

Megaloblastic anaemia following gastric surgery

Total gastrectomy. The absorption of vitamin B_{12} after total gastrectomy is similar to that observed in patients with pernicious anaemia: little or no labelled vitamin

B₁₂ is absorbed unless intrinsic factor is given with it (Swendseid, Halsted & Libby, 1953; Callender, Turnbull & Wakisaka, 1954).

Partial gastrectomy and gastro-enterostomy. Partial gastrectomy and gastro-enterostomy should not interfere with the absorption of vitamin B₁₂ since in man intrinsic factor is secreted from the body and fundus of the stomach (Fox & Castle, 1942). However, we have seen five patients with megaloblastic anaemia after partial gastrectomy and one after gastro-enterostomy. The clinical features and special investigations have been described elsewhere (Badenoch, Evans, Richards & Witts, 1955). Five of the patients had suffered from gastric ulcers; each of these showed failure of the absorption of labelled vitamin B₁₂ which was corrected by the addition of small amounts of intrinsic factor, and biopsies from the gastric remnant revealed gastritis and moderate or severe atrophy of the gastric-body mucosa. The results in the sixth patient, who had undergone partial gastrectomy for duodenal ulcer, were anomalous. There was no evidence of a deficiency of vitamin B₁₂, only slight impairment of the absorption of labelled vitamin B₁₂ and no atrophy of the gastric-body mucosa. Steatorrhoea was not a feature of any case.

Naish & Capper (1953) suggested that megaloblastic anaemia after partial gastrectomy might be related to sepsis in the cul-de-sac of duodenum created by the Polya type of operation. In one of our patients who developed megaloblastic anaemia owing to a deficiency of vitamin B₁₂ after a Polya partial gastrectomy, the absorption of labelled vitamin B₁₂ was measured after sterilization of the intestine with aureomycin but no improvement was noted.

Removal of the distal half of the stomach does not *per se* interfere with the absorption of vitamin B₁₂ (Evans, 1955). This has been shown by studies with labelled vitamin B₁₂ before and after partial gastrectomy in nine patients without megaloblastic anaemia. Impaired absorption was detected 3 months after operation in two patients but at a later examination both results were within normal limits.

Megaloblastic anaemia with idiopathic steatorrhoea

Of seven patients with idiopathic steatorrhoea and megaloblastic anaemia, the absorption of labelled vitamin B₁₂ was normal in one young patient, slightly impaired in another and negligible in the remaining five all of whom had suffered from severe steatorrhoea for many years. Increasing doses of intrinsic factor were given with 0.5 µg ⁶⁰Co-labelled vitamin B₁₂ to those with defective absorption, and a variable response was observed (Fig. 1).

The quantity of intrinsic factor effective in patients with pernicious anaemia (50 mg of either preparation) improved absorption in only one of the patients, but with progressively larger doses in three others a proportionate increase in absorption was observed. In these four patients there was evidence of impaired gastric secretion, i.e. achlorhydria and reduced excretion of uropepsinogen in the urine. In the remaining two patients, both of whom had free acid in the gastric juice and normal excretion of uropepsinogen, the defective absorption of vitamin B₁₂ was not improved by the giving of as much as 1000 mg intrinsic factor. Absorption was reassessed in one patient after treatment with aureomycin but no change was noted.

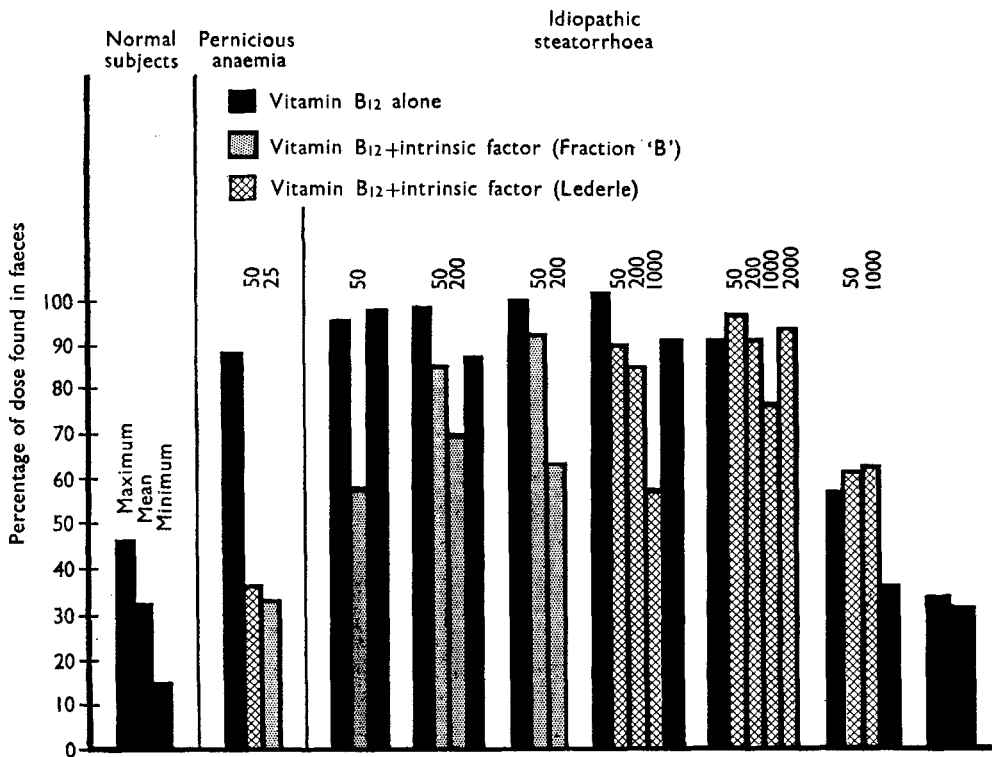


Fig. 1. Unabsorbed radioactivity in the faeces after an oral dose of $0.5 \mu\text{g}$ ^{60}Co -labelled vitamin B₁₂ in seven patients with idiopathic steatorrhoea and megaloblastic anaemia. In six patients the effect of increasing amounts of intrinsic factor is illustrated. The results in control subjects, and the effect of 25 mg of Fraction 'B' or 50 mg of Lederle intrinsic factor (see p. 127) on the absorption of labelled vitamin B₁₂ in patients with pernicious anaemia, are shown for comparison. Figures above the columns are the weights in mg of the preparations given.

The absorption of labelled vitamin B₁₂ was also measured in four patients suffering from idiopathic steatorrhoea but without megaloblastic anaemia. It was normal in three; in the fourth, a mild defect was detected which was not improved by 50 mg intrinsic factor. All four patients were young and had free acid in the gastric juice. In no case was there a long history of steatorrhoea.

For comparison four patients with severe symptomatic steatorrhoea were investigated. In these the absorption of labelled vitamin B₁₂ was normal even in the presence of marked intestinal hurry.

Megaloblastic anaemia with structural abnormalities of the intestine

Three patients were encountered in whom megaloblastic anaemia was associated with structural abnormalities of the small intestine. Steatorrhoea was present in

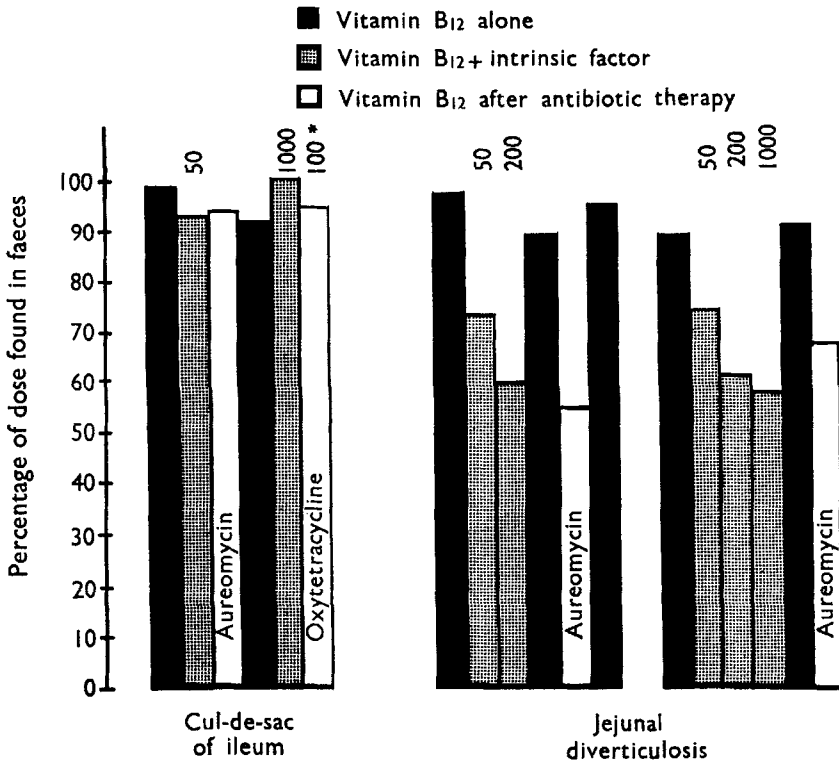


Fig. 2. Effect of increasing amounts of intrinsic factor, and of sterilization of the intestine with antibiotics, on the absorption of an oral dose of $0.5 \mu\text{g}$ ^{60}Co -labelled vitamin B₁₂ in three patients with megaloblastic anaemia and structural abnormalities of the small intestine. Figures above the columns are the weights in mg of the intrinsic-factor preparation given.

*Given while patient was receiving oxytetracycline.

each. The absorption was measured of labelled vitamin B₁₂ alone, with varying amounts of intrinsic factor, and after the administration of aureomycin or oxytetracycline (terramycin) for a period of at least 1 week (Fig. 2).

One of these patients had developed megaloblastic anaemia 11 years after hemicolectomy for carcinoma of the hepatic flexure. Radiological examination revealed a cul-de-sac of ileum and changes in the remainder of the small intestine such as are seen in severe idiopathic steatorrhoea. The patient did not absorb labelled vitamin B₁₂ when it was given alone or with as much as 1000 mg intrinsic factor. Separate courses of aureomycin and oxytetracycline did not improve absorption even when 100 mg intrinsic factor were given together with the labelled vitamin B₁₂. In spite of this, a haematological response occurred while the patient was receiving aureomycin.

The other two patients had numerous diverticula of the small intestine, the effect of which is similar to a series of blind loops of bowel (Badenoch, Bedford & Evans, 1955). Both failed to absorb the oral dose of labelled vitamin B₁₂. When intrinsic factor was added absorption was increased but the amount required was much greater than that necessary to produce a similar effect in patients with pernicious anaemia. A course of aureomycin improved the absorption of labelled vitamin B₁₂ in both patients without the addition of intrinsic factor.

Megaloblastic anaemia of pregnancy or the puerperium

Nine patients with megaloblastic anaemia of pregnancy or the puerperium were studied. Four were seen before term and the remaining five after delivery. The clinical features and results of special investigations have been reported in detail previously (Badenoch, Callender, Evans, Turnbull & Witts, 1955). In all cases the absorption of labelled vitamin B₁₂ was within normal limits indicating adequate secretion of intrinsic factor.

Megaloblastic anaemia associated with epilepsy

During the past 2 years thirteen cases of megaloblastic anaemia have been described in epileptic patients. We have seen three such patients and in each the absorption of labelled vitamin B₁₂ was normal.

Discussion

The present observations on the pathogenesis of different types of megaloblastic anaemia serve for the most part to confirm the classical experiments performed by Castle and his colleagues a quarter of a century ago.

The essential defect in pernicious anaemia and in subacute combined degeneration is failure to secrete intrinsic factor. Without this substance insufficient vitamin B₁₂ is absorbed from the diet to meet the requirements of the body. Apart from exceptional cases the loss of intrinsic factor is due to atrophy of the gastric-body mucosa. The significance of intestinal sepsis in pernicious anaemia remains obscure, but the present observations after sterilization of the bowel indicate that the abnormal bacterial flora is not directly responsible for the failure to absorb vitamin B₁₂.

After total gastrectomy a deficiency of vitamin B₁₂ would be expected since, in man, the production of intrinsic factor is limited to the stomach. Often there are no manifestations of this deficiency for several years. Experience has shown, however, that if the patient survives the operation long enough the body's stores of vitamin B₁₂ are exhausted and megaloblastic anaemia develops (Tomoda, 1954; Paulson & Harvey, 1954).

In contrast, megaloblastic anaemia is rare after partial gastrectomy and gastroenterostomy. The pathogenesis of the anaemia was obscure in one of our patients but in the remaining five there was evidence of a deficiency of vitamin B₁₂ due primarily to lack of intrinsic factor. Since partial gastrectomy *per se* does not interfere with the absorption of vitamin B₁₂, failure to secrete intrinsic factor in these cases must be attributed to atrophy of the mucosa of the gastric remnant. It is unlike-

ly that these were patients with pernicious anaemia in whom the operation was only incidental since in each case there was a history of proven peptic ulcer and the histology of the stomach at partial gastrectomy was in no way similar to that seen in pernicious anaemia. The atrophic changes which subsequently developed in the remaining portion of stomach were probably the result of prolonged gastritis originally present with the gastric ulcer and persisting owing to the conditions after operation.

When megaloblastic anaemia occurs in patients with idiopathic steatorrhoea it is usually attributed to lack of folic acid. A deficiency of vitamin B₁₂, however, is not uncommon (Mollin & Ross, 1954) and in some cases subacute combined degeneration may develop (Woltman & Heck, 1937). The absorption of labelled vitamin B₁₂ in this disease may be normal, grossly impaired or between these extremes. Impaired absorption is not due solely to lack of intrinsic factor although decreased secretion of intrinsic factor may play a major part in a few cases. More often the interference seems to be at the site of absorption in the small bowel. The nature of the disturbance is uncertain but it may be due to inactivation of intrinsic factor, to atrophic changes in the intestinal mucosa or to excessive mucus acting as a barrier to absorption. Previous workers have suggested that when impaired absorption occurs in steatorrhoea it cannot be corrected by the addition of intrinsic factor. In the present study, however, an increase in absorption was demonstrated in certain patients with evidence of reduced gastric secretion but, in general, the amount of intrinsic factor required was much greater than that necessary to produce a comparable effect in patients with pernicious anaemia. In other patients with severe or long-standing idiopathic steatorrhoea the failure of absorption was not improved by intrinsic factor irrespective of the quantity given. It was assumed that in such cases the power to absorb vitamin B₁₂ had been lost owing to atrophic and inflammatory changes in the wall of the intestine as described by Paulley (1954). Failure to absorb fat *per se* is not of primary importance since the absorption of labelled vitamin B₁₂ may be normal in patients with severe symptomatic steatorrhoea.

Megaloblastic anaemia associated with structural abnormalities of the small intestine may be cured by restoration of the continuity of the intestine or relieved temporarily by the oral administration of antibiotics (Siurala & Kaipainen, 1953). The antibiotics may exert their effect by increasing the concentration of citrovorum factor within the intestine (Waisman, Green, Munoz, Ramenchik & Richmond, 1951) but the present observations indicate that they also improve the absorption of vitamin B₁₂. In both patients with jejunal diverticulosis failure to absorb labelled vitamin B₁₂ was corrected by sterilization of the bowel, and it is logical to assume that interference with the uptake of vitamin B₁₂ was due to the abnormal bacterial flora present high in the small intestine. The fact that very large quantities of intrinsic factor also increased absorption suggests that intrinsic factor may be capable of preserving vitamin B₁₂ from the action of bacteria but that the usual doses of intrinsic factor are destroyed by the micro-organisms. If bacteria can deprive the host of vitamin B₁₂ from the diet the situation is similar to that which occurs in patients with megaloblastic anaemia due to infestation with the fish-tapeworm. Nyberg & von Bonsdorff (1955) have shown that when labelled vitamin B₁₂ is given to such

patients from 85 to 100% of the administered radioactivity is taken up by the parasite and little or none is absorbed.

The patient with a cul-de-sac of ileum following hemicolecotomy had evidence of severe damage to the remaining intestinal mucosa and it is not surprising that the absorption of labelled vitamin B₁₂ was improved neither by antibiotics nor by large amounts of intrinsic factor. The haematological remission during aureomycin therapy was probably mediated by the absorption of some haematinic principle other than vitamin B₁₂.

When megaloblastic anaemia develops during pregnancy and the puerperium or when it occurs in epileptic patients there is seldom evidence of an absolute deficiency of vitamin B₁₂. Although some cases respond to treatment with large doses of vitamin B₁₂ the absorption of the labelled vitamin is not impaired and the serum level of vitamin B₁₂ is usually within normal limits. A deficiency of folic acid has been suspected but it seems more likely that these two forms of megaloblastic anaemia are due to metabolic interference with the utilization of haematopoietic factors.

SUMMARY

Studies with labelled vitamin B₁₂ reveal distinctive patterns of absorption which may be useful in classifying the different forms of megaloblastic anaemia according to their pathogenesis. When impaired absorption is due to lack of intrinsic factor the defect may be corrected by giving small amounts of intrinsic factor together with the oral dose of labelled vitamin B₁₂. Where anaemia is associated with intestinal disease the absorption of labelled vitamin B₁₂ is rarely improved by the usual doses of intrinsic factor but may be increased by very large doses. In some patients with structural abnormalities of the intestine failure to absorb labelled vitamin B₁₂ is corrected by sterilization of the bowel suggesting that bacterial contamination of the small intestine is responsible for the interference with absorption. In other forms of megaloblastic anaemia there appears to be no defect in the absorption of vitamin B₁₂.

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Anaemia and vitamin B₁₂ dietary deficiency

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Dietary deficiency of vitamin B₁₂ with low serum levels occurred (Wokes, Badenoch & Sinclair, 1955*a,b*) in persons termed 'vegans' (Hill, 1952) after several years on a diet containing no animal food, and therefore probably deficient in this vitamin. This deficiency did not lead to the characteristic blood picture of pernicious anaemia, or indeed to any pronounced macrocytosis, but it was accompanied by marked changes in the nervous system, extending in some instances as far as subacute combined degeneration of the cord (J. Badenoch, 1954; A. G. Badenoch, 1956), and there were two deaths attributed to these changes. However, about half of the vegans escaped serious illness even after many years on the diet, and in some of them normal serum vitamin B₁₂ levels indicated a normal vitamin B₁₂ status. The wide variation in response to the dietary deficiency suggested significant variations either in dietary intake of the vitamin or in dietary conditions concerned with intestinal synthesis of the vitamin and its absorption from the intestine. These variations could have wide and far-reaching implications in many parts of the world where economic conditions necessitate adherence to diets very low in animal protein. Hence, although the vegans constitute a minute fraction of our population, data on their diets may help in tackling world food problems.

Previous findings with subnormal intakes by man of animal food, particularly animal protein, have been summarized in recent reviews (see Dean, 1953). McCance & Widdowson (1946*b*) at the beginning of World War II carried out on British adults an experimental study of rationing in which the intake of animal protein was gradually reduced, falling below 2% on the calorie basis in two of the subjects, and below 4% in another two subjects, during the last 2 weeks of the 3-month experimental period. These intakes supported severe muscular exertion in persons who normally consume much more animal protein. The diet was adequate in vitamins A, B₁ and C, nicotinic acid, calcium, phosphorus, iron and other minerals.

In an investigation by Widdowson & McCance (1954), 160 German children aged 4-15 years were fed for a year on diets in which the animal protein, half from