

Effect of vitamin B₁₂ and folic acid deficiency on small intestinal absorption

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SYNOPSIS Three patients are described, and they provide further evidence that deficiency of folic acid and vitamin B₁₂ may sometimes affect small intestinal function. Malabsorption of both xylose and vitamin B₁₂ returned to normal in one patient after treatment of a megaloblastic anaemia due to dietary deficiency of folic acid. Impaired absorption of vitamin B₁₂ was corrected by vitamin B₁₂ therapy in the other two patients. The initial cause of the vitamin B₁₂ deficiency in one patient was not apparent, but she was taking Gynovlar 21, which may have been an aetiological factor. In the third patient the small intestinal defect was secondary to pernicious anaemia, and in a group of 98 other patients with pernicious anaemia intrinsic factor did not improve vitamin B₁₂ absorption in six, and only partially corrected absorption in 30. The significance of these observations is discussed.

The precise diagnosis of pernicious anaemia and nutritional megaloblastic anaemia depends on excluding small intestinal absorption disorders, in particular the coeliac syndrome. Recently, however, there has been some evidence that deficiency of vitamin B₁₂ or folic acid may occasionally impair the intestinal absorption of vitamin B₁₂, xylose, and carotene, in which case the measurement of the absorption of these substances could be misleading.

Impaired intestinal absorption of vitamin B₁₂, which was corrected by vitamin B₁₂ therapy, was observed in four out of 10 consecutive adult patients with pernicious anaemia (Carmel and Herbert, 1967), in a child with juvenile pernicious anaemia (Lampkin and Mauer, 1967), in a patient with nutritional vitamin B₁₂ deficiency (Schloesser and Schilling, 1963), and in one out of five patients with coeliac disease (Mollin, Booth, and Baker, 1957). Evidence of impaired absorption of carotene and xylose has also been found occasionally in patients with pernicious anaemia (Beznan, Kinnear, and Zamcheck, 1959), but steatorrhoea has not been observed (Carmel and Herbert, 1967). Treatment with folic acid corrected the malabsorption of vitamin B₁₂ and xylose in a patient with megaloblastic anaemia associated with anticonvulsant therapy (Reynolds, Hallpike, Phillips, and Matthews, 1965), and it invariably improved the small bowel function, including vitamin B₁₂ absorption, in patients with tropical sprue (O'Brien and England, 1966).

The purpose of this report is to present details of a further three patients, in whom an intestinal absorptive defect was corrected by folic acid or vitamin B₁₂. In the first patient, impaired absorption of both vitamin B₁₂ and xylose was caused by dietary deficiency of folic acid, and in the other two patients malabsorption of vitamin B₁₂ was secondary to vitamin B₁₂ deficiency. The initial cause of the vitamin B₁₂ deficiency was not apparent in one of these patients, and the other had pernicious anaemia.

METHODS

The levels of serum vitamin B₁₂ and serum folate were measured by microbiological assay using *Lactobacillus leichmanii* (Matthews, 1962) and *Lactobacillus casei* (Spray, 1964) respectively. The normal range for the vitamin B₁₂ level is 150 to 850 pg per ml, and for the serum folate level 2.1 to 10.0 ng per ml.

Vitamin B₁₂ absorption was measured by the serum counting method using ⁵⁷Co vitamin B₁₂ and without giving a parenteral dose of vitamin B₁₂ (Harwood and Forshaw, 1967). A result above 0.5% of administered radioactivity per litre of serum indicates normal absorption.

Serum intrinsic factor blocking antibody was assayed by the coated charcoal method (Gottlieb, Lau, Wasserman, and Herbert, 1965) and gastric parietal cell antibody by the immunofluorescent technique (Taylor, Roitt, Doniach, Couchman, and Shapland, 1962).

CASE REPORTS

CASE 1 A divorced woman, aged 37, complained of

increasing lassitude during the previous two years, and was admitted to Sefton General Hospital for investigation. She worked as a barmaid and for some time had been consuming an excessive amount of alcohol and taking a very poor diet. In the last three months she had lost 14 lb in weight. She had not had any previous illnesses, and she had four healthy children aged between 10 and 15 years. Physical examination revealed marked pallor and moderate enlargement of the liver.

Investigations The haemoglobin was 3.8 g per 100 ml, and the red blood cells were macrocytic and normochromic. The bone marrow showed megaloblastic erythropoiesis. Secretion of free acid in the gastric juice after maximal histamine stimulation was normal. The level of serum vitamin B₁₂ was 305 pg, and of serum folate 0.6 per ml. In the vitamin B₁₂ absorption tests there was 0.33% of the administered radioactivity per litre of serum when histamine was given and 0.31% when 60 mg of an intrinsic factor preparation (Armour) was given. (The first of these tests was carried out before, and the second two weeks after starting vitamin B₁₂ therapy.) After an oral dose of 25 g xylose, 2.9 g was excreted in the urine in five hours (normal range 4.0 to 8.0 g). The stool fat excretion during a period of three days was 0.7 g per day. The level of serum albumin was 3.4 g, of globulin 2.5 g, and of alkaline phosphatase 14 K-A units per 100 ml. Radiographs of the gastrointestinal tract showed hypertrophic rugae in the stomach, but the small bowel appeared normal. Jejunal mucosal biopsy was normal.

Treatment and progress She was given one intramuscular dose of 500 µg vitamin B₁₂, followed by six daily doses of 250 µg, which produced a reticulocyte response of 28%, and a rise in the haemoglobin level to 5.2 g per 100 ml within seven days. In view of the normal serum vitamin B₁₂ and low serum folate levels, treatment was then changed to folic acid 5 mg three times daily, but this did not produce a further reticulocyte response. Six weeks later the haemoglobin had risen to 11.8 g per 100 ml and the red cells were hypochromic. The vitamin B₁₂ absorption test with histamine then showed 0.5% of the administered radioactivity per litre of serum, and in the xylose excretion test there was 4.5 g xylose in a collection of urine over five hours.

CASE 2 A married woman, aged 32, attended Sefton General Hospital with a history of lassitude, anorexia, and loss of 7 lb in weight during the previous two months. Before the onset of these symptoms her appetite was good and her diet normal. She had not had diarrhoea and she could not recall any previous illnesses. There was no family history of anaemia and she had two healthy children, aged 6 and 10 years. She had been taking the contraceptive drug Gynovlar 21 during the previous nine months but had not received any other drugs. On physical examination, apart from pallor there were no abnormal signs.

Investigations The haemoglobin was 6.1 g per 100 ml, and the red blood cells were macrocytic and normochromic. The bone marrow showed megaloblastic erythropoiesis. Secretion of free acid in the gastric juice after maximal histamine stimulation was normal. The level of serum vitamin B₁₂ was 60 pg, and of serum folate 2.8 ng

per ml. In the vitamin B₁₂ absorption tests there was 0.3% of the administered radioactivity per litre of serum when histamine was given, and 0.2% when 60 mg intrinsic factor preparation was given. Antibodies to intrinsic factor and gastric parietal cells were not detected in the serum. In the xylose excretion test 4.6 g xylose was present in urine collected over five hours. The stool fat excretion during three days was 0.5 g per day. The serum albumin level was 4.4 g, globulin 2.0 g, and alkaline phosphatase 6 K-A units per 100 ml. Radiographs of the stomach and small intestine were normal.

Treatment and progress Intramuscular vitamin B₁₂ was given as follows: one dose of 500 µg, then 250 µg daily for five days, weekly for six weeks, and monthly thereafter. There was a reticulocyte response of 20% followed by a steady rise in the haemoglobin level to 14.6 g per 100 ml. The blood film then appeared normal. Her symptoms disappeared completely and she gained 20 lb in weight. She has continued to take Gynovlar.

Six weeks after commencing vitamin B₁₂ therapy the serum folic acid level was 2.3 ng per 100 ml. After five months' treatment the vitamin B₁₂ absorption test with intrinsic factor was repeated and 0.76% of the administered radioactivity was present in 1 litre of serum. Seven weeks later the test was performed with histamine and there was 0.77% of the administered radioactivity per litre of serum.

CASE 3 A married woman, aged 34, was admitted to Sefton General Hospital on account of increasing lassitude for 18 months, and nausea, anorexia, and loss of 16 lb in weight during the past three months. Her brother had a bleeding duodenal ulcer, but there was no other family history of anaemia. She had two healthy children, 8 and 10 years old. Her only previous illness was an abortion two years previously when the haemoglobin level was 9.9 g per 100 ml and the red blood cells hypochromic; the anaemia was treated by transfusion with 2 pints of blood. She had taken Valium for a few weeks three months previously but had not received any other drugs. On physical examination she was pale and slightly jaundiced, and the tip of the spleen was palpable.

Investigations The haemoglobin was 5.1 g per 100 ml and the red blood cells were macrocytic and normochromic. The bone marrow showed megaloblastic erythropoiesis. There was no free acid in the gastric juice after maximal histamine stimulation. The level of serum vitamin B₁₂ was 15 pg, and the serum folate 12.3 ng per ml. In the vitamin B₁₂ absorption tests, with histamine there was 0.1% of the administered radioactivity per litre of serum, and with 60 mg intrinsic factor preparation 0.2%. Antibodies to intrinsic factor were not detected in the serum, but the test for gastric parietal cell antibodies was strongly positive. In the xylose excretion test, 4.4 g xylose was present in urine collected over five hours. Stool fat excretion could not be measured because of severe constipation. The serum albumin was 4.9 g, globulin 2.0 g, and alkaline phosphatase 7 K-A units per 100 ml. Radiographs of the stomach and small intestine were normal.

Treatment and progress She was given vitamin B₁₂ intramuscularly in a dose of 250 µg daily for 10 days and

then monthly. There was a reticulocyte response of 33% followed by a steady rise in the haemoglobin level to 12.4 g per 100 ml. The appearance of the blood film became normal.

Two months after beginning vitamin B₁₂ therapy the vitamin B₁₂ absorption test with intrinsic factor was repeated and 0.77% of administered radioactivity was present in 1 litre of serum. Four months later the absorption test was carried out with histamine and no radioactivity appeared in the serum.

DISCUSSION

The three cases reported here provide further evidence that folic acid and vitamin B₁₂ deficiency may sometimes cause a defect in small intestinal absorption of xylose and vitamin B₁₂. A small intestinal mucosal biopsy will often help to distinguish such cases from those of the coeliac syndrome, but may not always clarify the diagnosis, as there is some evidence that both vitamin B₁₂ and folic acid deficiency can also produce structural changes in the small intestine. Foroozan and Trier (1967) reported that both the duodenal and jejunal mucosa of patients with pernicious anaemia showed slight histological abnormalities, which were corrected by vitamin B₁₂ therapy, and O'Brien and England (1966) observed that folic acid corrected the partial villous atrophy of the jejunal mucosa in patients with tropical sprue. There is also indirect evidence that partial villous atrophy may occasionally be caused by dietary folic acid deficiency (Forshaw, 1968). If there remains any doubt, therefore, about the diagnosis the absorption tests should be repeated after correcting the folic acid or vitamin B₁₂ deficiency.

The initial cause of the vitamin B₁₂ deficiency in our second patient is obscure, as there was no evidence of pernicious anaemia or coeliac disease and her diet was normal. It is pertinent to speculate whether Gynovlar, which belongs to the progesterone group of oral contraceptives, could have been an aetiological factor. It has been shown that the long-term administration of progesterone to animals impairs cellular metabolism, in particular nucleic acid production and protein biosynthesis (Beaconsfield and Ginsburg, 1968), and it is possible that intestinal mucosa with its high rate of turnover of surface epithelial cells could be affected. However, even if Gynovlar did affect the function of the ileal mucosa it was not taken for long enough to have produced vitamin B₁₂ deficiency by malabsorption alone.

Our third patient had pernicious anaemia, and Carmel and Herbert (1967) observed an intestinal

defect of vitamin B₁₂ absorption in four out of 10 patients with pernicious anaemia. Our experience, however, suggests that the incidence of severe malabsorption of vitamin B₁₂ caused by vitamin B₁₂ deficiency is lower than this. We studied the effect of 60 mg intrinsic factor preparation in a further 116 patients with impaired absorption of vitamin B₁₂. Absorption was not improved in 16 patients, six of whom probably had pernicious anaemia, one had a partial gastrectomy, and nine had small intestinal disorders (seven cases of coeliac disease, one of Crohn's disease, and one in which several feet of ileum had been resected). Absorption was only partly corrected in 30 of the other 92 patients with pernicious anaemia, and in one of the six patients with a partial gastrectomy. These observations suggest that an ileal defect causing impaired vitamin B₁₂ absorption may occur in about 36% of cases of pernicious anaemia, but that the defect is severe in less than 10% of cases. However, we did not have the opportunity to observe the effect of vitamin B₁₂ therapy on intestinal absorption and it is possible that in some of these cases intrinsic factor activity was affected by the presence of antibodies in the gastric juice (Schade, Feick, Muckerheide, and Schilling, 1966).

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