Familial selective malabsorption of vitamin B₁₂

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Bell, M., Harries, J. T., Wolff, O. H., Dawson, A. M., and Waters, A. H. (1973). *Archives of Disease in Childhood*, 48, 896. **Familial selective malabsorption of vitamin B₁₂.** A patient with selective malabsorption of vitamin B₁₂ is described who differs in certain respects from previously published cases. Steatorrhoea was severe and was reversed by B₁₂ therapy, proteinuria was absent, and B₁₂ malabsorption was also present in one of the parents.

The differences between our patient and other reported cases probably represent a wide disease spectrum rather than a different disease entity. This being so, our patient is the first case where unequivocal vitamin B₁₂ malabsorption has been shown in one of the parents.

In 1960, Imerslund in Norway and Gräsbeck and colleagues in Finland independently described the familial occurrence of megaloblastic anaemia in children due to an isolated defect of vitamin B₁₂ absorption. Proteinuria was also a common finding in the affected children. Since then several workers in other countries have reported cases of selective B₁₂ malabsorption with proteinuria (Table I).

Both Imerslund and Gräsbeck *et al.* emphasized the familial nature of the disease and speculated that the abnormality in B₁₂ absorption was genetically determined and present from birth. Family studies of patients in published reports suggest an autosomal recessive mode of inheritance. Attempts to define a heterozygous carrier state in the parents and sibs of affected children by measuring their ability to absorb small oral doses of radioactive B₁₂ have so far been unrewarding.

The paper describes a patient with selective B₁₂ malabsorption who differs in some respects from previously reported cases. It also reports the results of B₁₂ absorption studies carried out on both parents and a brother of the patient.

Case report

Past history. The patient is the second child of healthy unrelated English parents. Pregnancy was uneventful and he was born at term weighing 4 kg. There was no relevant family history. A left cleft lip and palate were repaired at 4 months. Except for mild flexural eczema he remained in good health until the age of 5 years, when he developed severe recurrent mouth

ulcers which persisted despite treatment with many topical agents, and were responsible for much loss of schooling. At the age of 6 years both his weight and height were between the 25th and 50th centiles, but by 15

TABLE I
*Summary of published cases of selective B₁₂ malabsorption**

Authors	Year	Country	No. of cases
Najman and Brausil	1952	Yugoslavia	1
Imerslund	1960	Norway	10
Gräsbeck <i>et al.</i>	1960	Finland	2
Colle, Greenberg, and Krivit	1961	U.S.A.	1
Lamy <i>et al.</i>	1961	France	2
Wise, Lovric, and O'Gorman-Hughes	1963	Australia	1
Sievens	1964	Denmark	1
Spurling, Sacks, and Jiji	1964	U.S.A.	2
Chavelet <i>et al.</i>	1964	France	1
de Sousa	1966	Portugal	2
Mohammed, McKay, and Galloway	1966	Great Britain	2
Smrhova, Wiedermann, and Wiedermann	1966	Czechoslovakia	1
Hippe	1966	Denmark	1
Castel <i>et al.</i>	1966	France	3
Anttila and Salmi	1967	Finland	2
Gorin, Salama, and Guillien	1967	France	1
Gräsbeck and Kvist	1967	Finland	19†
Lillibridge, Brandborg, and Rubin	1967	U.S.A.	6
Furuhjelm and Kojo	1968	Finland	17
Ben-Basset, Feinstein, and Ramot	1969	Israel	18
Mackenzie <i>et al.</i>	1972	U.S.A.	3

*Modified from Visakorpi and Furuhjelm (1968).

†Including previously described cases.

Received 29 May 1973.

years they had fallen to below the 3rd centile. During this period of growth retardation there were no gastrointestinal symptoms and, except during periods of mouth ulceration, his appetite was good. Pallor was not noted.

Present admission. He was referred to The Hospital for Sick Children at the age of 14 years 9 months because of recurrent mouth ulcers and short stature. He was a depressed boy whose height (146.5 cm) and weight (35.5 kg) were both below the 3rd centile. There were no signs of puberty and, except for a few small sublingual ulcers, physical examination was normal, and no neurological abnormalities were found.

Investigations. He had a megaloblastic anaemia due to B₁₂ deficiency. The haematological findings are summarized in Table II.

TABLE II
Haematological findings

Blood count	Hb 10.8 g/100 ml Leucocytes 4700/mm ³ Differential—neutrophils 57%, lymphocytes 43%, monocytes 1%, eosinophils 5% Platelets—normal on film
Blood film	Anisocytosis with macrocytosis
Bone marrow	Gross megaloblastic changes
Serum B ₁₂	10 pg/ml (normal 160–960 pg/ml)
Serum folate	11 ng/ml (normal 4–18 ng/ml)
Serum iron	77 µg/100 ml
TIBC	257 µg/100 ml

TIBC, total iron binding capacity.

There were no antibodies in the serum to thyroid, gastric parietal cells, or intrinsic factor. Serum B₁₂ concentration was 10 pg/ml and unsaturated B₁₂ binding capacity 2200 pg/ml. The proportion of serum binding due to the β-fraction, which contains transcobalamin II, was 66%, and that due to the α-fraction containing transcobalamin I, was 34%. These results are similar to those found in patients with severe B₁₂ deficiency due to causes other than pernicious anaemia (Herbert, 1968).

Gastric juice was acid (pH 2.8) with normal intrinsic factor activity by the method of Ardeman and Chanarin (1963)—before and after stimulation with pentagastrin 44 and 96 units/ml, respectively. A Schilling test showed severe B₁₂ malabsorption (2.7% of a 1 µg oral dose of ⁵⁷Co-B₁₂ excreted in 24 hours), which was not improved by the addition of hog intrinsic factor (2.6% excreted in 24 hours). Quantitative cultures of duodenal juice for aerobic and anaerobic bacteria showed a normal flora, and urinary excretion of indoles was not increased.

Faecal fat excretion was grossly abnormal (76 g fat excreted over 5 days). Xylose excretion was normal (34% of 5 g loading dose excreted in urine in 24 hours). Barium meal and follow-through showed slight dilatation of duodenum and proximal jejunum with normal appearance of distal small intestine. Peroral biopsy (2

weeks after starting B₁₂ therapy) showed the duodenal mucosa to be normal under light microscopy. Liver function tests (serum bilirubin, transaminases, and serum albumin), and pancreatic function tests (sweat sodium and chloride concentration; fasting trypsin, lipase, and amylase activities in duodenal juice after pancreozymin-secretin stimulation) were normal. Plasma vitamins A and E levels, plasma calcium, inorganic phosphorus, and alkaline phosphatase were all within the normal range.

Sensitive tests of renal tubular and glomerular function, namely urinary lysozyme excretion by the method of Barratt and Crawford (1970), and urinary albumin measured by an immunochemical technique on several occasions, were normal. In addition, blood urea, creatinine clearance, and intravenous pyelography were also normal.

Management. He was given 1000 µg B₁₂ weekly for 3 weeks by intramuscular injection. This produced a complete haematological remission. His mood improved and he became less depressed and truculent, and more co-operative. The mouth ulcers disappeared. He was discharged on a maintenance dose of 250 µg B₁₂ monthly.

Follow-up. 6 months later he was admitted for reassessment. The improvement in mood had been maintained, the mouth ulcers had not recurred, and growth velocity had increased from below the 3rd centile to between the 10th and 25th centiles. Faecal fat excretion had returned to normal (11.3 g fat excreted over 5 days), and the radiological abnormalities in the small intestine had disappeared. Despite this improvement there was no change in B₁₂ absorption. The patient excreted 2.9% of a 1 µg dose of ⁵⁷Co-B₁₂ given with hog intrinsic factor, and when the test was repeated with intrinsic factor and 50 ml normal human duodenal juice there was no change, 2.5% of the dose being excreted in 24 hours.

Since then he has remained in excellent health, and at the age of 17½ years signs of puberty appeared, and height and weight were between the 3rd and 10th centiles. B₁₂ malabsorption persists and maintenance therapy remains necessary. The mouth ulcers have not recurred.

Family studies. Vitamin B₁₂ absorption was measured in both parents and a brother. A double isotope modification of the Schilling test was used (Bayly, Bell, and Waters, 1971) and the results are summarized in Table III. The severe malabsorption of B₁₂ in the patient was confirmed by this method. The father also had unequivocal malabsorption of B₁₂ (though less severe than the patient), which was not corrected by intrinsic factor. B₁₂ absorption was normal in the brother, and results in the mother were at the lower end of the normal range.

Serum B₁₂ concentrations measured by *Lactobacillus leichmannii* assay were normal in the father (315 pg/ml), mother (355 pg/ml), and brother (330 pg/ml).

TABLE III
*Family B₁₂ absorption studies**

Subjects	Age (yr)	Date of test	⁵⁸ Co-B ₁₂ alone (% excretion in 24 hr)	⁵⁷ Co-B ₁₂ IF bound (% excretion in 24 hr)
Patient	15½	4 Sept 1969	1.2	1.5
		5 Jan 1970	1.3	1.5
Father	50	18 Mar 1970	6.6	6.3
		6 June 1970	6.8	5.6
Mother	55	18 Mar 1970	13.2	15.7
Brother	12½	18 Mar 1970	20.1	18.8

*Two capsules containing, respectively, 0.25 µg ⁵⁸Co-B₁₂ and 0.25 µg ⁵⁷Co-B₁₂ bound to normal human gastric juice were taken simultaneously and followed immediately by intra-muscular injection of 1 mg nonradioactive B₁₂. Urine was collected for 24 hr. Control subjects excrete equal amounts of the two isotopes in the range 13–36% of the administered dose.
IF, intrinsic factor.

Discussion

The present case has several features which are similar to those described in other patients with the syndrome of selective B₁₂ malabsorption. He had severe B₁₂ deficiency, megaloblastic anaemia, and B₁₂ malabsorption that could not be corrected with either hog or human intrinsic factor. Free acid was present in the gastric juice and intrinsic factor secretion was normal by *in vitro* assay. Tests for serum antibodies against intrinsic factor, gastric parietal cells, and thyroid were all negative.

Certain features, however, distinguish our patient from previously reported cases. The absence of generalized malabsorption is one of the diagnostic criteria of this syndrome. Though slight abnormalities of small bowel structure and function may occur (Visakorpi and Furuhielm, 1968), we are not aware of such severe steatorrhoea having been previously reported. This was reversible, however, and faecal fat excretion had returned to normal when measured after 6 months of B₁₂ therapy. The initial dilatation of the proximal small bowel had also disappeared. These changes are probably due to the effect of severe B₁₂ deficiency on the small intestine. Similar reversible changes in the absorption of vitamin B₁₂ itself have been reported in patients with severe B₁₂ deficiency (Schloesser and Schilling, 1963; Haurani, Sherwood, and Goldstein, 1964; Brody, Estren, and Herbert, 1966; Mollin and Waters, 1968).

Proteinuria, sometimes associated with renal tract abnormalities, is almost invariably an integral part of the syndrome. Several examinations of urine by sensitive techniques failed to reveal increased amounts of albumin in our patient.

The late diagnosis of megaloblastic anaemia

requires some explanation. Most cases have presented with megaloblastic anaemia in the first 2 years of life, presumably at a time when maternally derived stores of B₁₂ are depleted. Though the exact diagnosis has often been delayed for several years (Gräsbeck *et al.*, 1960; Ben-Basset *et al.*, 1969), such patients have usually received B₁₂ or folic acid therapy. As far as is known, our patient received no B₁₂ or folate therapy until the diagnosis was first made at the age of 14 years 9 months. The early onset of growth failure and mouth ulcers, however, suggested that B₁₂ deficiency had been present for several years, and it is possible that bone marrow examination at an earlier age would have revealed megaloblastosis even though there were no signs of anaemia. A possible explanation is that the dietary folate intake was enough to prevent the onset of anaemia in spite of the associated B₁₂ deficiency. It is also possible that the slow rate of growth may have spared the available B₁₂ for haemopoiesis, and thereby delayed the onset of anaemia; a similar explanation has been suggested for the absence of overt megaloblastic anaemia in coeliac children with folate deficiency (Dormandy, Waters, and Mollin, 1963).

Mouth ulcers have been reported in two other children with selective B₁₂ malabsorption (Colle *et al.*, 1961; Mackenzie *et al.*, 1972). This was the presenting feature in our patient who suffered from severe recurrent mouth ulcers over a period of 10 years. This caused considerable loss of schooling. The ulcers disappeared with B₁₂ therapy and have not recurred.

Familial occurrence is a striking feature of the syndrome of selective B₁₂ malabsorption, and family studies of patients in published reports suggest an autosomal recessive mode of inheritance (Gräsbeck and Kvist, 1967; Visakorpi and Furuhielm, 1968). Attempts have been made to define a heterozygous carrier state by measuring B₁₂ absorption in the parents and sibs of affected children, but the results have so far been unrewarding. Normal B₁₂ absorption (assessed by Schilling tests) has been reported in the parents of several cases (Gräsbeck *et al.*, 1960; Chavelet *et al.*, 1964; Spurling *et al.*, 1964; Ben-Basset *et al.*, 1969; Mackenzie *et al.*, 1972). Mohammed *et al.* (1966) found abnormal B₁₂ absorption in the father of one of their patients, but when subsequently reassessed, the Schilling test was normal. However, in the father of our patient, B₁₂ absorption was abnormal on two separate occasions, and in the mother the results were at the lower end of the normal range. The test was normal in a brother. These findings are consistent with an autosomal recessive mode of inheritance.

The nature of the absorptive defect in selective B₁₂ malabsorption has not been defined, though evidence points to the ileal mucosa as the most likely site of the lesion. Patients with this condition secrete normal amounts of biologically active intrinsic factor (Gräsbeck *et al.*, 1960; Imerslund and Bjornstad, 1963; Mackenzie *et al.*, 1972), there are no circulating antibodies to intrinsic factor or the B₁₂-IF complex (Gräsbeck and Kvist, 1967; Mackenzie *et al.*, 1972), and the B₁₂ carrier proteins in plasma are not deficient (Gräsbeck and Kvist, 1967; Mackenzie *et al.*, 1972). The absorption of small doses of radioactive-B₁₂ is not improved by the addition of normal jejunal juice (Bjornstad and Imerslund, 1963; Spurling *et al.*, 1964; Gräsbeck and Kvist, 1967) or by administration of broad spectrum antibiotics (Imerslund and Bjornstad, 1963). All these observations point towards the ileal mucosa as the site of the defect in B₁₂ absorption. Examination of ileal biopsies by light or electron microscopy has revealed no morphological abnormality (Gräsbeck and Kvist, 1967; Visakorpi and Furuhielm, 1968; Mackenzie *et al.*, 1972). It has been suggested that there may be a lack of specific ileal receptors for the B₁₂-IF complex (Herbert, 1960; Gräsbeck and Kvist, 1967). Mackenzie *et al.* (1972), however, showed that uptake of B₁₂-IF by a homogenized ileal biopsy from a patient with selective B₁₂ malabsorption was normal, which argues strongly against the lack of ileal receptors. Furthermore, serum from three of their patients with selective B₁₂ malabsorption did not impair the uptake of B₁₂-IF by guinea-pig ileum brush-border preparations, suggesting that there were no antibodies against ileal receptors. These observations led Mackenzie *et al.* (1972) to suggest that the defect in B₁₂ absorption occurs after attachment of B₁₂-IF to the ileal mucosa, but before the absorbed B₁₂ binds to its carrier protein (transcobalamin II).

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