

## Case report

# Absent ileal uptake of IF-bound vitamin B<sub>12</sub> *in vivo* in the Imerslund-Grasbeck syndrome (familial vitamin B<sub>12</sub> malabsorption with proteinuria)

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**SUMMARY** A Syrian family is described with three children who had inherited selective vitamin B<sub>12</sub> malabsorption associated with proteinuria. (Imerslund-Grasbeck syndrome). Although inherited the defect was apparently not present at birth. A third child had less severe vitamin B<sub>12</sub> malabsorption, was not vitamin B<sub>12</sub> deficient and had no proteinuria. Studies on two of the affected children with subcellular fractionation of the uptake of radioactive vitamin B<sub>12</sub> by ileal tissue *in vivo* indicate a defect in the ileal receptors for IF-bound vitamin B<sub>12</sub>. These findings are different from the single *in vitro* experiment on a patient with this condition that has been previously reported.<sup>1</sup>

Familial selective malabsorption of vitamin B<sub>12</sub> associated with proteinuria was first reported by Imerslund.<sup>2</sup> Her observations were confirmed by Grasbeck *et al.*<sup>3</sup> Some of these children had anatomical defects of the urinary tract. The inheritance of the condition is recessive.<sup>4</sup>

The ileum is normal histologically,<sup>1 5-7</sup> and by electron microscopy.<sup>1</sup> The underlying defect in this condition has been assumed to be a loss of receptors in the ileum for intrinsic factor bound vitamin B<sub>12</sub>. Binding of vitamin B<sub>12</sub> by ileal tissue, however, has been studied in only one patient with this condition, and was found to be normal.<sup>1</sup> We report for the first time a detailed study of ileal structure and function which shows the site of the defect in the absorption of vitamin B<sub>12</sub> in a family with the Imerslund-Grasbeck syndrome.

### Case history

An Arab boy born in 1973 of Syrian parents who were first cousins, presented to St Bartholomew's

Hospital in 1980 at the age of 7 years. He became unwell at the age of 3 years. Anaemia was noted at the age of 4, and at 5 years of age megaloblastic anaemia was diagnosed in Saudia Arabia. He was treated with large doses of vitamin B<sub>12</sub> by injection and responded well clinically and haematologically. From the age of 5-7 years he was treated with regular injections of 1000 µg of vitamin B<sub>12</sub> every month. On examination he was healthy, of normal height and weight, and was physically normal.

### FAMILY HISTORY

His parents were healthy. They had had seven other children of whom two had died in infancy from unidentified illnesses which were probably infective. Five other children survived. There were three boys aged 18, 11, and 1½ years, and two girls aged 14 and 8 years. None of the children had symptoms attributable to anaemia or vitamin B<sub>12</sub> deficiency.

### Methods

Haematology was performed by standard methods.<sup>8</sup> Assays of serum vitamin B<sub>12</sub> were performed using *Euglena gracilis*,<sup>9</sup> assay of serum and red cell folate were performed using *L casei*.<sup>10</sup> Analysis of serum

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vitamin B<sub>12</sub> binding proteins was carried using silica precipitation.<sup>11</sup> The deoxyuridine suppression test was performed using a modification of the method of Wickramasinghe and Longland.<sup>12</sup> Measurement of intrinsic factor in gastric juice used cobinamide to differentiate R binders from Intrinsic Factor.<sup>13</sup> Vitamin B<sub>12</sub> absorption was studied using cyanocobalamin labelled with <sup>58</sup>Co and doses of 1 μg (containing 1 microcurie <sup>58</sup>Co vitamin B<sub>12</sub>) were given. Absorption was measured using a Nuclear Enterprises whole body counter.

In addition to, and on a separate occasion from the measurement of vitamin B<sub>12</sub> absorption, vitamin B<sub>12</sub> uptake in the ileum *in vivo* was examined by subcellular fractionation of ileal biopsy specimens.<sup>14</sup> For these studies ileal tissue was obtained by colonoscopy two hours after a dose of radioactive vitamin B<sub>12</sub> by mouth (20 microcuries of <sup>57</sup>Co vitamin B<sub>12</sub> containing 1 μg vitamin B<sub>12</sub>). The uptake of radioactive vitamin B<sub>12</sub> *in vivo* by ileal tissue was studied in specimens from the patient, from an affected but untreated brother, and from an unaffected normal brother as control. After washing, samples of ileal tissue were gently homogenised and the subcellular organelles were separated by centrifugation on a Percoll density gradient. The subcellular organelles were identified by marker enzyme assay and the distribution of <sup>57</sup>CoB<sub>12</sub> determined by counting the radioactivity of each fraction.<sup>14</sup>

## Results

### CLINICAL INVESTIGATIONS

The patient, who had received regular monthly injections of B<sub>12</sub>, was haematologically normal (No 4 in the Table). In particular there was no evidence of vitamin B<sub>12</sub> deficiency (normal serum vitamin B<sub>12</sub>, serum and red folate concentrations and normal morphology of the peripheral blood and bone marrow and normal results of the deoxyuridine suppression test). Transcobalamin 1 and 11 were normal. He had proteinuria but renal function was normal. Investigation showed selective malabsorption of intrinsic factor-bound vitamin B<sub>12</sub> and this persisted after a course of broad spectrum antibiotics by mouth (cotrimoxazole and metronidazole) for seven days. Gastrointestinal investigations were normal (jejunal biopsy, gastric acid production in response to pentagastrin, radiologically normal stomach and small bowel, normal xylose and fat absorption). He had no antibodies in serum to gastric parietal cells or to intrinsic factor. His ileum was normal by light and electron microscopy.

An elder sister and brother (No 1 and 2 in the

Table Haematological investigations

Age (yr)	1	2	3	4	5
	F 13	M 11	F 9	M 7	M 1.25
Haemoglobin g/dl	11.6	12.9	12.5	11.6	12.1
Red cells × 10 <sup>12</sup> /l	3.86	4.18	4.71	4.22	5.16
MCV fl	90	94	78	80	72
White cells × 10 <sup>9</sup> /l	5.9	5.3	4.7	6.0	7.1
Platelets × 10 <sup>9</sup> /l	331	232	271	299	310
Serum B12 ng/l	85	25	430	750*	370
Serum folate microg/l	7.0	>18	>18	14.5	>18
Red cell folate microg/l	202	298	416	516	501
Serum B12 binding ng/l					
UBBC	1567	1846	2000	1178	1426
TC 1	498	662	700	373	605
TC 11	1069	1184	1300	805	821
B12 absorption % dose					
Dose alone	<1	4	24	1	44
Dose + IF	<1	2	25	1	—
Dose after antibiotics	—	—	—	1	—
Deoxyuridine suppression test (normal <5%) (uptake of tritiated thymidine as percentage of control tubes)					
dU alone	25.6	23.5		3.1	
dU + B12	13.7	7.9		2.9	
dU + folic acid	9.7	11.6		2.8	

\* treated with B12. RBC = × 10<sup>6</sup>/mm<sup>3</sup>; WBC = × 10<sup>3</sup>/mm<sup>3</sup>; platelets = × 10<sup>3</sup>/mm<sup>3</sup>.

Table) were found to have megaloblastic anaemia due to vitamin B<sub>12</sub> deficiency: changes in the peripheral blood and bone marrow of megaloblastic anaemia, subnormal serum vitamin B<sub>12</sub> concentrations, and abnormal results of the deoxyuridine suppression test which were typical of severe vitamin B<sub>12</sub> deficiency (see Table). They also had selective malabsorption of intrinsic factor-bound vitamin B<sub>12</sub> (radiologically normal stomach and small bowel, jejunal and ileal biopsies were normal, and normal ileal mucosa by electron microscopy). They also had proteinuria. The parents and two other sons, aged 18 years and 1 years 4 months, were haematologically normal, had normal absorption of radioactive B<sub>12</sub> and did not have proteinuria. A 9 year old sister (No 3 in the Table) was also haematologically normal but despite a normal serum vitamin B<sub>12</sub> concentration she had malabsorption of radioactive vitamin B<sub>12</sub>. She absorbed 24% of the dose of radioactive vitamin B<sub>12</sub>. She did not have proteinuria.

### UPTAKE OF RADIOACTIVE VITAMIN B<sub>12</sub> IN VIVO

The ileal tissue from the two affected children showed virtually no uptake of radioactive vitamin B<sub>12</sub> *in vivo*. In contrast ileal tissue from the normal control brother took up radioactive vitamin B<sub>12</sub>, and two hours after ingestion, the radioactive vitamin B<sub>12</sub> was located in the brush border, cytosol, and

denser organelles, probably the lysosomes (see Figure). The uptake of radioactive vitamin B<sub>12</sub> into subcellular fractions of the ileum *in vivo* has not been studied previously in man but the distribution of radioactive vitamin B<sub>12</sub> in the normal control was the same as previously found in the guinea pig ileum *in vivo*.<sup>14</sup>

The small amount of vitamin B<sub>12</sub> recovered in the density gradient fractions of ileal tissue from the affected children remained in the sample layer and probably represents contamination from luminal vitamin B<sub>12</sub> not removed by washing. There was no evidence of vitamin B<sub>12</sub> binding in the brush border fraction of ileal tissue from the affected children. This indicates absence or dysfunction of ileal receptors for intrinsic factor-bound vitamin B<sub>12</sub>.

### Discussion

Our findings clearly differ from the *in vitro*

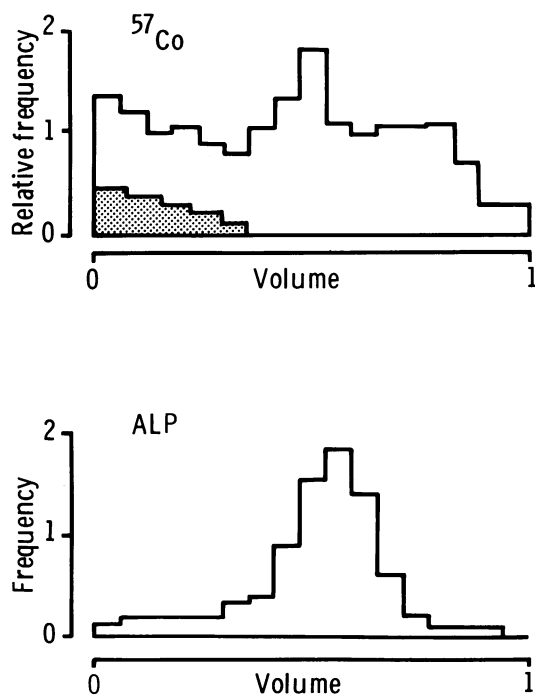


Figure Subcellular fractionation of postnuclear supernatants from ileal homogenates of two siblings with Imerslund-Grasbeck syndrome (shaded area) and a normal control sibling (unshaded area). Graphs show frequency volume-volume histograms for (<sup>57</sup>Co)B<sub>12</sub> and alkaline phosphatase (ALP), a marker for the brush border. Frequency is defined as the fraction of total recovered activity present in the individual fractions divided by the fractional volume.

experiment of Mackenzie *et al.*<sup>1</sup> The design of their experiments, however, was relatively crude and subcellular fractionation allows a more precise analysis of the defect. We found no uptake of vitamin B<sub>12</sub> by the ileum in the children in this family with the Imerslund-Grasbeck syndrome. It seems unlikely therefore that the condition is caused by lysosomal defect.

The most likely explanation for the failure of uptake of intrinsic factor bound vitamin B<sub>12</sub> is a defect in the ileal receptors. The failure of uptake of radioactive B<sub>12</sub> by the ileal cells was not because of the vitamin B<sub>12</sub> deficiency itself as it was found in the child who had been treated with vitamin B<sub>12</sub> and was in haematological remission, as well as the untreated child.

It is hard to explain why in this inherited condition it is so long before vitamin B<sub>12</sub> deficiency develops. In the children in this family indications of vitamin B<sub>12</sub> deficiency were not seen until the ages of 4, 11, and 13 years. If vitamin B<sub>12</sub> malabsorption had been present from birth, however, vitamin B<sub>12</sub> deficiency would have developed much earlier.<sup>15</sup>

It seems likely therefore that the ileal receptors for intrinsic factor-bound vitamin B<sub>12</sub> in the Imerslund-Grasbeck syndrome may be normal at birth. For some reason they decline either in number or functional quality as time passes. The basic defect in familial selective vitamin B<sub>12</sub> malabsorption may be a defect in the ileal receptors which renders them unstable. The exact nature of this defect awaits structural analysis. Perhaps such a study may indicate whether a common defect exists in the ileum and the kidney and so explain the association of proteinuria and vitamin B<sub>12</sub> malabsorption.

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