Prospective evaluation of protein bound vitamin B₁₂ (cobalamin) malabsorption in the elderly using trout flesh labelled in vivo with ^{57}Co-cobalamin


Abstract

**Background**—The frequency of dietary protein bound vitamin B₁₂ malabsorption in elderly patients remains controversial.

**Aims**—To evaluate this malabsorption in elderly hospitalised patients using a modified Schilling test.

**Patients**—Fourteen elderly patients with low B₁₂ blood levels were prospectively selected from 394 hospitalised patients.

**Methods**—The modified Schilling test was performed with trout labelled in vivo.

**Results**—The test was normal in five healthy elderly subjects, in 7/8 patients with pancreatic insufficiency, and in nine non-elderly patients with antral gastritis. The low decision limit was established at 3.3% (median 4.8%). From the 14 elderly patients with low B₁₂, prospectively selected from 394 hospitalised patients, seven had a real deficiency with anaemia and an increased homocysteine and/or methylmalonic acid serum level. The modified Schilling test showed malabsorption in five of these patients, including two in which the standard Schilling test was normal, and three in which the standard Schilling test was partially corrected by an intrinsic factor.

**Conclusions**—Protein bound vitamin B₁₂ malabsorption was detected in at least 0.5% of elderly hospitalised patients, using the labelled trout flesh absorption test.

(Gut 1997; 41: 475–479)

Keywords: cobalamin deficiency; malabsorption; gastritis; Schilling test

The frequency and aetiologies of cobalamin deficiency due to malabsorption in the elderly are still under discussion, despite the number of studies performed on this subject. Several groups have observed a low vitamin B₁₂ serum level in as many as 10% of elderly hospitalised patients. Most of these patients showed no clinical or haematological signs of B₁₂ deficiency. It has been suggested that serum B₁₂ is not sensitive enough to diagnose B₁₂ deficiency and that serum methylmalonic acid and homocysteine concentration are more sensitive.

The frequency of pernicious anaemia increases with age. A second gastric aetiology of vitamin B₁₂ deficiency corresponds to dietary cobalamin malabsorption. This can occur in the absence of intrinsic factor (IF) deficient secretion and is related to a lack of cobalamin release from food proteins, which is the consequence of decreased acid and peptic secretion. It may be assumed that such dietary cobalamin malabsorption is frequent in the elderly population as the prevalence of gastritis increases with age. The prevalence of vitamin B₁₂ deficiency due to this malabsorption has not, however, been prospectively evaluated in an elderly population. Such an evaluation requires the use of an adequate modified Schilling test, with labelled cobalamin bound to food proteins. The protein bound vitamin B₁₂ malabsorption has been studied in chronic gastritis, using modified Schilling tests performed with food proteins labelled either in vitro or in vivo. In our opinion, in vivo incorporation of labelled vitamin B₁₂ to either fish or chicken meat is a more physiological process than adding vitamin B₁₂ to chicken serum in vitro. Fish and chicken meat give an excretion percentage of the tracer in the order of 3–6% whereas chicken serum gives a percentage lower than 2% in healthy subjects. This may be explained by the different behaviour of food proteins in releasing vitamin B₁₂ at acidic pH and in being degraded by duodenal juice. In addition, the Schilling test performed with chicken serum cannot distinguish malabsorption due to chronic gastritis from that due to chronic pancreatitis.

The aim of the present study was therefore to evaluate the aetiology of vitamin B₁₂ deficiency in elderly patients with a low vitamin B₁₂ blood concentration prospectively selected from 394 elderly hospitalised patients, using a modified Schilling test performed with trout flesh.

Patients and Methods

**Patients**

**Controls**—Nine healthy, non-elderly subjects (aged 28–66 years), and five healthy, elderly subjects (aged 76–82 years) were included.
Inclusion criteria were the absence of anaemia, a normal standard Schilling test, the absence of histological gastritis, and the absence of anti-IF autoantibodies. Informed consent was obtained in accordance with the Declaration of Helsinki.

**Elderly patients**—A total of 394 elderly patients (aged 70–91 years), hospitalised in the Department of Internal Medicine of the University Hospital Centre, Nancy, were included in the study. None were receiving any treatment or were known to suffer from any intestinal disease impairing cobalamin absorption. Biological parameters measured included serum vitamin B₁₂, and folates, erythrocyte mean corpuscular volume, haemoglobin and ferritin blood levels, and serum anti-IF autoantibodies. Patients who had low vitamin B₁₂ in serum and at least one of the other parameters out of the normal range were investigated for vitamin B₁₂ deficiency and malabsorption, by means of determination of homocysteine and methylmalonic acid in serum, modified and standard Schilling tests, and gastric endoscopic examination of fundic biopsy specimens. Three patients had fundic gastritis and nine had antral gastritis.

Patients with **chronic gastritis** were aged 51–60 years. The diagnosis was established from endoscopic examination and the study of gastric biopsy specimens. Nine patients had fundic gastritis and nine had antral gastritis.

Patients with **chronic pancreatitis** (n=8) were aged 37–45 years. The diagnosis was established from clinical signs, NBT-PABA test, ultrasonography, and x ray computed tomography.

**SERUM ASSAYS**

Plasma pepsinogen I was measured by radioimmunoassay (ORIS, Gif sur Yvette, France). Serum vitamin B₁₂ and folates were measured using a radioisotopic dilution assay (Becton Dickinson Immuno Diagnostics Company, New York, USA). Serum methylmalonate and homocysteine were assayed in serum using capillary gas chromatography mass spectrometry, as recently described.²⁹–³¹

**HISTOLOGICAL STUDY OF GASTRIC BIOPSY SPECIMENS**

The gastric biopsy specimens were examined histologically by a single pathologist, who was unaware of the endoscopic findings. Biopsy tissue, fixed in 10% formalin and embedded in paraffin wax, was sectioned at 5 µm and stained with haematoxylin and eosin. Gastritis was graded according to the Sydney system.³² This grades the severity of inflammation, activity (the degree of polymorphonuclear neutrophil infiltration), atrophy, and intestinal metaplasia on a scale from 0 to 3. A subsequent “gastritis score” for each biopsy site was obtained by combining the scores of the four individual characteristics (maximum possible score 12).

**SCHILLING TESTS**

The standard Schilling test was performed with the Dicopac test (Amersham, UK). The second stage (ingestion of cobalt-57 labelled cyanocobalamin-IF) was started four days after the first stage (ingestion of cyanocobalamin). Urine was collected for 48 hours at each stage. The limit of normal values was established at 10% and 11% of urinary excretion of the tracer for the first and second stages, respectively. The modified Schilling test was performed using trout meat labelled with cyano-³⁷Co-cobalamin. Labelled vitamin B₁₂ (0.02 µCi) was injected into five week old trout after anaesthesia with 0.03% (vol/vol) ethylene glycol monophenyl ester (Merck, FRG). The injection was repeated one week later and the trout were sacrificed two weeks later, cooked, wrapped in aluminium foil for 10 minutes at 200°C, then dissected. The meat was liquidised, divided into fractions (one fraction of 54 (10 g per test) corresponding to a radioactivity of 177 000 (5500) cpm and stored at –18°C. The protocol of the test was similar to that of the classic test and included the determination of the meal fraction radioactivity before its ingestion, the intramuscular injection of 1000 µg of non-labelled vitamin B₁₂, and the collection of urine for 48 hours. The determination of excreted radioactivity was estimated by 10 minute γ counting of five 10 ml urine samples. The modified Schilling test was performed at least eight days after the standard test. Correlation of the urinary excretion rate of the tracer with either the Sydney score for the gastric biopsy samples or the pepsinogen blood level was studied using the Spearman rank correlation coefficient.

**Results**

A low vitamin B₁₂ serum concentration was observed in 40/394 subjects (10.2%); the lowest limit of serum vitamin B₁₂ was 110 pmol/l in our reference population.³³ The vitamin B₁₂ serum level of this group was estimated at 83.1 (19.3) pmol/l (range 54.6–109.2 pmol/l). Fourteen of these patients were selected for further study of vitamin B₁₂ assimilation as they were suspected of having either vitamin B₁₂ deficiency or chronic gastritis, using the criteria defined in the methods section. Nine had a low haemoglobin level and/or an increased erythrocyte mean corpuscular volume, two had a low serum folate level, and three underwent a gastric endoscopic examination for epigastric pain and presented histological signs of chronic gastritis (tables 1 and 2). Anti-IF autoantibodies were detected in the serum of only one patient. The red blood cell folate and the ferritin and iron serum levels were normal in all 14 patients (table 1). None had any neurological symptoms or renal failure.

Methylmalonate and homocysteine serum levels were determined in these 14 patients. In most of the anaemic patients, elevation of both parameters was dissociated. Methylmalonate and homocysteine were the only increased parameters in three and five patients, respectively. At least one of these parameters was increased in all patients with macrocytic anaemia (table 1). Plasma pepsinogen I was measured as an index of gastric atrophy.³²–³⁵ The lowest normal blood pepsinogen I level limit was established at 20 ng/ml. Pepsinogen I was lower than this limit in only one woman.
with pernicious anaemia (patient 2, table 1) and one patient with moderate fundic gastritis (patient 7). It was normal in another patient with severe fundic gastritis (patient 6).

The standard Schilling test was performed on the 14 patients (table 2). Stage 1 (without IF) was abnormal (lower than 10%) in four patients with macrocytic anaemia. Stage 2 (with IF) showed a partial correction of the malabsorption in all four patients. One patient (patient 2) had pernicious anaemia with serum anti-IF autoantibodies. All patients with an abnormal haemoglobin blood level had a normal standard Schilling test, except one (patient 14) whose urinary excretion index of free vitamin B₁₂ was slightly decreased. This patient had macrocytosis.

The normal value of the modified Schilling test was established as the lowest value obtained in the two control groups (n=14). It was estimated to be 3.3%. Values were higher than this limit in the five elderly, healthy subjects, in 7/8 cases of chronic pancreatitis, and in 8/9 non-elderly patients with antral gastritis. In contrast, it was abnormal in all three non-elderly patients with fundic gastritis. The modified Schilling test was performed in the 14 patients and was found to be abnormal in five of them (table 2, fig 1). Two of these patients showed malabsorption of food vitamin B₁₂ as they had both a normal standard Schilling test and an abnormal modified Schilling test (table 2). Only one of these two cases presented histological signs of atrophy of the fundus (table 2). A significant negative correlation was found between the modified Schilling test and the Sydney score for fundic mucosa (r=0.62, p<0.05).

### Table 1
Analytical data from 14 elderly patients with a low serum vitamin B₁₂ concentration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Serum MMA (nmol/l)</th>
<th>Serum HC (mmol/l)</th>
<th>Haemoglobin (g/100 ml)</th>
<th>MCV (µm³)</th>
<th>Serum folate (nmol/l)</th>
<th>Serum iron (mg/l)</th>
<th>Serum ferritin (ng/ml)</th>
<th>Serum vitamin B₁₂ (pmol/l)</th>
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Median: 85; Lower quartile: 84; Upper quartile: 86; Normal values: F 50–300, 7.0–15.0, 11.5–15.0, 80–94, 9.3–24.0, 340–1600, 0.50–1.6, 15–250, 111–500; M 50–300, 7.0–15.0, 13.0–16.0, 81–99, 9.3–24.0, 340–1600, 0.55–1.7, 20–350, 111–500.

MMA, methylmalonate; HC, homocysteine; MCV, mean corpuscular volume; RBC, red blood cell.

### Table 2
Schilling tests and gastric analytical and histological data of 14 elderly patients with a low serum vitamin B₁₂ concentration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Serum MMA (nmol/l)</th>
<th>Serum HC (mmol/l)</th>
<th>Haemoglobin (g/100 ml)</th>
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<th>Serum folate (nmol/l)</th>
<th>Serum iron (mg/l)</th>
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<td>1.3</td>
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Median: 85; Lower quartile: 84; Upper quartile: 86; Normal values: F 10.0, 10.0, 3.3 >20; M 10.0, 10.0, 3.3 >20.

Gastritis was graded according to the Sydney system using a score from 0 to 12.

SST, standard Schilling test after oral intake of free labelled vitamin B₁₂ (stage 1) or bound to intrinsic factor (stage 2); MST, modified Schilling test after oral intake of labelled vitamin B₁₂ bound to trout flesh; D III, duodenal biopsy specimens.
The group of elderly subjects with a low vitamin B12 blood level represented 10.1% of the 394 subjects included in our study. This percentage is close to that observed by other authors.1–9 Of the 40 patients with a low vitamin B12 blood level, only seven had a vitamin B12 deficiency with macrocytic anaemia and an elevated blood level of methylmalonate and/or homocysteine. Another patient (patient 10) had a haemoglobin blood level and an erythrocyte mean corpuscular volume at the normal value limit and an increased level of homocysteine (table 1). The increase in homocysteine corresponded to a vitamin B12 deficiency rather than to a folic acid deficiency as they all had low B12 and normal red blood cell folate levels. Methylmalonate and homocysteine blood levels have been reported to be sensitive markers for detecting vitamin B12 deficiency.10–12 In a study by Stabler et al14 on patients with a vitamin B12 deficiency, 92% of the patients had macrocytosis, 95% an elevated methylmalonate blood level, and 99% an elevated homocysteine level. More recently, Joosten et al showed that 23% and 30% of elderly ambulatory subjects had abnormal methylmalonate and homocysteine blood levels, respectively.13 Our group also observed that methylmalonate and homocysteine blood levels increase in elderly healthy subjects,15 in the absence of haematological or clinical abnormality. The specificity of these parameters for diagnosing a B12 deficiency in the elderly therefore remains to be established.

The present study was the first to use labelled trout meat for performing modified Schilling tests on elderly patients, concurrently with younger adult patients and healthy controls. The value of modified Schilling tests for detecting dietary protein bound vitamin B12 has been discussed in recent literature.17 18 22 24 36 Joosten et al failed to find an advantage of the protein bound Schilling test performed with chicken serum over the standard Schilling test, in the diagnosis of cobalamin malabsorption in 41 elderly patients with a low vitamin B12 serum level.18 In addition, Scarlett et al recently showed that the diagnostic value of this protein bound Schilling test was limited by the frequent finding of reduced absorption in the healthy elderly.17 This was not the case with our test as no abnormal modified Schilling test was observed in elderly patients without vitamin B12 deficiency (fig 1).

Our study is the first to evaluate prospectively the frequency of vitamin B12 deficiency due to protein bound vitamin B12 malabsorption in elderly hospitalised patients, using a modified Schilling test with trout flesh labelled in vivo with 57Co-vitamin B12. This modified Schilling test was previously described by Dorsherholmen et al.23 It was normal in 7/8 (87%) patients with chronic pancreatitis and therefore enabled the distinction to be made between a lack of dietary vitamin B12 release due to deficient gastric acid secretion, and a lack of haptocorrin degradation due to pancreatic deficiency.17 An abnormal modified Schilling test was found in seven patients (2% of elderly hospitalised patients).

Vitamin B12 malabsorption in chronic gastritis involves two aetiological factors: deficient intrinsic factor, which occurs in pernicious anaemia, and deficient gastric acid and pepsin secretion, which may lead to protein bound malabsorption despite normal or subnormal intrinsic factor secretion.14 20–22 27 We found a significant negative correlation of the modified Schilling test with the Sydney score for fundic mucosa but not with the score for antral mucosa nor with pepsinogen blood levels. It may therefore be suggested that deficient gastric acid secretion was the predominant factor responsible for protein bound vitamin B12 malabsorption, assuming that blood pepsinogen reflected pepsin secretion.33 This hypothesis is in agreement with a recent case report from our group which described vitamin B12 deficiency with protein bound vitamin malabsorption in a patient receiving long term omeprazole treatment.39 This drug is known selectively to inhibit gastric acid secretion and has no effect on pepsin and intrinsic factor secretion.40

The modified Schilling test is the only Schilling test to be disturbed when the deficient vitamin B12 release from food protein...
is the only factor responsible for malabsorption. It was this case in two patients in our study, representing 0.5% of the elderly hospitalised patients and 28% of the patients with vitamin B12 malabsorption. The trout flesh modified Schilling test may provide a functional test for diagnosing protein bound vitamin B12 malabsorption. It is important to establish this diagnosis, as these patients can be treated by oral administration of vitamin B12.

More recently it has also been shown that Helicobacter pylori infection of the stomach and food vitamin B12 are intimately associated, H pylori predisposing to a more severe form of malabsorption.

In conclusion, our study indicated that 2/398 (0.5%) hospitalised elderly patients had vitamin B12 deficiency related exclusively to protein bound vitamin B2 malabsorption. The Schilling test with trout flesh seems to be an efficient and specific method for investigating this type of malabsorption in elderly patients.

41 Carmel R. Helicobacter pylori infection and food-