Aging and the alimentary tract

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

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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 methylmalonate 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.

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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.¹⁻⁹ 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.⁷⁻⁹ 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.¹⁰⁻¹³

The frequency of pernicious anaemia increases with age. A second gastric aetiology of vitamin B₁₂ deficiency corresponds to dietary cobalamin malabsorption.⁸ 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.¹⁴⁻¹⁷ It may be assumed that such dietary cobalamin malabsorption is frequent in the elderly population as the prevalence of gastritis increases with age.¹⁸⁻¹⁹ 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.¹⁴⁻²⁰⁻²⁶ 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.²⁷ 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.²⁸ 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.

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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 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 and antral biopsy samples.

Patients with *chronic gastritis* were aged 51–60 years. The diagnosis was established from endoscopic examination and the study of gastric biopsy specimens. Three 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 cyano-⁵⁸Co-

cobalamin). 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-⁵⁷Co-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.³¹ The vitamin B₁₂ serum level of this group was estimated at 83.1 (19.5) pmol/l (range 54.6–109.2 pmol/l). Fourteen of these patients were selected for further studies 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. A 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.^{32–33} 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

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

Patient	Sex	Age (y)	Serum MMA (nmol/l)	Serum HC (nmol/l)	Haemoglobin (g/100 ml)	MCV (μm ³)	Folate		Serum iron (mg/l)	Serum ferritin (ng/ml)	Serum vitamin B ₁₂ (pmol/l)
							Serum (nmol/l)	RBC (nmol/l)			
1	M	91	522	13.6	12.9	90.2	13.0	820	0.51	300	105.5
2	F	83	355	7.8	11.5	115.3	45.4	922	0.39	55	54.6
3	M	84	195	16.6	11.3	97.7	34.1	NA	0.71	NA	99.6
4	M	86	202	17.4	11.8	94.5	21.0	807	0.77	72	76.0
5	F	88	90	24.0	10.4	107.2	42.0	1312	1.25	NA	73.8
6	F	88	236	19.0	9.0	110.0	7.5	239	0.77	160	73.8
7	F	84	1029	19.2	11.1	96.6	45.4	2356	2.6	208	73.8
8	M	86	321	9.8	14.2	95.4	13.0	723	0.67	NA	108.5
9	M	81	275	15.4	13.1	92.7	5.0	945	0.57	NA	64.9
10	M	85	185	25.0	13.0	99.0	17.0	NA	0.93	220	104.1
11	F	79	298	12.4	13.2	91.3	12.0	429	0.75	540	107.7
12	F	84	257	13.8	14.1	93.7	6.0	579	0.64	100	59.0
13	F	86	228	14.4	12.4	82.5	15.0	646	0.61	102	78.2
14	M	85	185	13.0	13.1	99.8	10.6	573	0.70	64	109.2
Median		85	246.5	14.9	12.6	96.0	14.0	765.0	0.70	131	77.0
Lower quartile		86	195.0	13.0	11.3	92.7	10.6	576.0	0.61	72	73.8
Upper quartile		84	321.0	19.0	13.1	99.8	34.1	933.5	0.77	220	105.5
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.

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

Patient	Sex	Age (y)	Absorption tests				Gastritis scores		
			SST			Pepsinogen I (ng/ml)	D III	Antrum	Fundus
			Stage 1 (%)	Stage 2 (%)	MST (%)				
1	M	91	19.0	NA	1.3	55	N	1	1
2	F	83	0.6	8.4	0.0	11	N	8	5
3	M	84	16.1	NA	5.9	68	N	4	2
4	M	86	17.5	22.0	1.5	33	N	1	4
5	F	88	0.2	10.0	0.0	58	N	3	3
6	F	88	0.1	3.4	0.5	34	N	2	5
7	F	84	3.3	6.2	6.5	6	N	1	2
8	M	86	10.0	11.0	12.1	138	N	4	0
9	M	81	13.0	14.0	14.4	29	N	5	2
10	M	85	29.4	32.7	7.8	125	N	2	1
11	F	79	25.7	27.2	8.4	35	N	3	1
12	F	84	23.0	26.0	8.6	30	N	5	2
13	F	86	11.0	13.0	4.5	67	N	1	1
14	M	85	7.0	10.0	4.5	135	N	1	1
Median		85				45			
Lower quartile		84				30			
Upper quartile		86				68			
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.

Figure 1: Schilling tests performed with trout flesh labelled in vivo with ^{57}Co -vitamin B_{12} .

Discussion

The group of elderly subjects with a low vitamin B_{12} 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 B_{12} blood level, only seven had a vitamin B_{12} 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 B_{12} deficiency rather than to a folate deficiency as they all had low B_{12} and normal red blood cell folate levels. Methylmalonate and homocysteine blood levels have been reported to be sensitive markers for detecting vitamin B_{12} deficiency.¹⁰⁻¹² In a study by Stabler *et al*³⁴ on patients with a vitamin B_{12} 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,³⁵ in the absence of haematological or clinical abnormality. The specificity of these parameters for diagnosing a B_{12} 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 B_{12} 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 B_{12} serum level.³⁷ 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 B_{12} deficiency (fig 1).

Our study is the first to evaluate prospectively the frequency of vitamin B_{12} deficiency due to protein bound vitamin B_{12} malabsorption in elderly hospitalised patients, using a modified Schilling test with trout flesh labelled in vivo with ^{57}Co -vitamin B_{12} . This modified Schilling test was previously described by Dorscherholmen *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 B_{12} release due to deficient gastric acid secretion, and a lack of haptocorrin degradation due to pancreatic deficiency.³⁸ An abnormal modified Schilling test was found in seven patients (2% of elderly hospitalised patients).

Vitamin B_{12} 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 B_{12} 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 B_{12} 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 B_{12} release from food protein

is the only factor responsible for malabsorption.²⁶ This was the 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.^{14 20 26} It should be noted that in certain cases, malabsorption is due to both deficient food vitamin B₁₂ release and to bacterial overgrowth related to deficient gastric acid secretion.^{41 42} In such cases normal vitamin B₁₂ absorption can be restored by oral antibiotic therapy.^{41 42} 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.⁴³

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

- Elsborg L, Lund V, Bastrup-Madsen P. Serum vitamin B12 levels in the aged. *Acta Med Scand* 1976; **200**: 309-14.
- Bailey LB, Wagner PA, Christakis GJ, Araujo PE, Appledorf H, Davis CG, et al. Vitamin B12 status of elderly persons from urban low income households. *J Am Geriatr Soc* 1980; **28**: 276-8.
- McEvoy AW, Fenwick JD, Boddy K, James OFW. Vitamin B12 absorption from the gut does not decline with age in normal elderly humans. *Age Ageing* 1982; **11**: 180-3.
- Magnus EM, Bache-Wig JE, Anderson TRA, Melbostad E. Folate and vitamin B12 (cobalamin) blood levels in elderly persons in geriatric homes. *Scand J Haematol* 1982; **28**: 300-66.
- Garry PJ, Goodwin JS, Hunt WC. Folate and vitamin B12 status in a healthy elderly population. *J Am Geriatr Soc* 1984; **32**: 719-26.
- Hsu JM, Smith JC. B-vitamins and ascorbic acid in the aging process. In: Ordy JM, Harman D, Alfin-Slater R, eds. *Nutrition in gerontology*. New York: Raven Press, 1984: 87-118.
- Blundell EL, Matthews JH, Allen SM, Middleton AM, Morris JE, Wickramasinghe SN. Importance of low serum vitamin B12 and red cell folate concentrations in elderly hospital in-patients. *J Clin Pathol* 1985; **38**: 1179-84.
- Nilsson-Ehle H, Jagenburg R, Landahl S, Lindstedt G, Swolin B, Westin J. Cyanocobalamin absorption in the elderly: results for healthy subjects and for subjects with low serum cobalamin concentration. *Clin Chem* 1986; **32**: 1368-71.
- Marcus DL, Shadick N, Crantz J, Gray M, Hernandez F, Freedman ML. Low serum B12 levels in a hematologically normal elderly sub-population. *J Am Geriatr Soc* 1987; **35**: 635-8.
- Stabler SP, Marcell PD, Podell ER, Allen RH, Savage DG, Lindenbaum J. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. *J Clin Invest* 1988; **81**: 466-74.
- Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid and total homocysteine concentrations. *Am J Hematol* 1990; **34**: 99-107.
- Moelby L, Rasmussen K, Jensen MK, Petersen KO. The relationship between clinically confirmed cobalamin deficiency and serum methylmalonic acid. *J Intern Med* 1990; **228**: 373-8.
- Joosten NE, Van den Berg A, Riezler R, Naurath HJ, Lindenbaum J, Stabler SP, Allen RH. Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr* 1993; **58**: 468-76.
- Cattan D, Belaiche J, Zittoun J, Yvart J, Chagom JP, Murit Y. Rôle de la carence en facteur intrinsèque dans les malabsorptions de la vitamine B12 dans les achlorhydries. *Gastroenterol Clin Biol* 1982; **6**: 570-5.
- Nicolas JP. Le protocole classique du double test de Schilling est-il toujours bien adapté au diagnostic des malabsorptions intestinales de la vitamine B12? Importance de la sécrétion chlorhydro-peptique et de la forme d'administration de la vitamine. *Gastroenterol Clin Biol* 1982; **6**: 554-6.
- Kittang E, Schjonsby H. Effect of gastric anacidity on the release of cobalamins from food and their subsequent binding to R-protein. *Scand J Gastroenterol* 1987; **22**: 1031-7.
- Scarlett JD, Read H, O'Dea K. Protein-bound cobalamin absorption declines in the elderly. *Am J Haematol* 1992; **39**: 79-83.
- Bird T, Hall MRP, Schade ROK. Gastric histology and its relation to anaemia in the elderly. *Gerontology* 1977; **23**: 309-21.
- Kekki W, Sipponen P, Siruala M, Lasiewicz W. Peptic ulcer and chronic gastritis: their relation to age and sex and to location of ulcer and gastritis. *Gastroenterol Clin Biol* 1990; **14**: 217-23.
- Dorsherholmen A, Swain WR. Impaired assimilation of egg Co 57 vitamin B12 in patients with hypochlorhydria and achlorhydria and after gastric resection. *Gastroenterology* 1973; **64**: 913-9.
- King CE, Leibach J, Toskes PP. Clinically significant vitamin B12 deficiency secondary to malabsorption of protein-bound vitamin B12. *Dig Dis Sci* 1979; **24**: 387-402.
- Steinberg GW, King CE, Toskes PP. Malabsorption of protein-bound cobalamin but not unbound cobalamin during cimetidine administration. *Dig Dis Sci* 1980; **25**: 188-92.
- Doscherholmen A, Mc Mahon J, Economon P. Vitamin B12 absorption from fish. *Proc Soc Exp Biol Med* 1981; **167**: 480-4.
- Doscherholmen A, Silvius S, McMahon J. Dual isotope Schilling test from measuring absorption of food-bound and free vitamin B12 simultaneously. *Am J Clin Pathol* 1983; **80**: 490-5.
- Dawson DW, Sawers AH, Sharma RH. Malabsorption of protein bound vitamin B12. *BMJ* 1984; **288**: 675-8.
- Dawson DW. Diagnosis of vitamin B12 deficiency. *BMJ* 1984; **289**: 938-9.
- Belaiche J. Conséquences de la réduction de la sécrétion acide gastrique sur la sécrétion de facteur intrinsèque et l'absorption des cobalamines. *Gastroenterol Clin Biol* 1991; **15**: 109C-15C.
- Belaiche J, Zittoun J, Marquet J, Yvart J, Cattan D. In vitro effect of duodenal juice on R binders cobalamin complexes in subjects with pancreatic insufficiency: correlation with cobalamin absorption. *Eur J Clin Invest* 1987; **17**: 70-4.
- Straczek J, Felden F, Dousset B, Guéant JL, Belleville F. Quantification of methylmalonic acid in serum measured by capillary gas chromatography-mass spectrometry as t-butylidimethylsilyl derivatives. *J Chromatogr* 1993; **620**: 1-7.
- Brattström B, Israelsson B, Lindgarde F, Hultberg B. Higher total plasma homocysteine in vitamin B12 deficiency than in heterozygosity for homocysteinuria due to cystathionine β-synthase deficiency. *Metabolism* 1988; **37**: 175-8.
- Guéant JL, Djalali M, Nicolas JP. Cobalamines. In: Siest G, Henny J, Schiele F, eds. *Références en biologie clinique*. Paris: Elsevier, 1990: 225-32.
- Misiewicz JJ, Tytgat GNJ, Goodwin CS, Price AB, Sipponen P, Strickland RG, Cheli R. The Sydney System: a new classification of gastritis. *J Gastroenterol Hepatol* 1990; **144**: 53-7.
- Samloff IM. Pepsinogens I and II. Purification from gastric mucosa and radioimmunoassay in serum. *Gastroenterology* 1982; **82**: 26-33.
- Stabler SP, Marcell PD, Podell ER, Allen RH, Lindenbaum J. Assay of methylmalonic acid in the serum of patients with cobalamin deficiency using capillary gas chromatography-mass spectrometry. *J Clin Invest* 1986; **77**: 1606-12.
- Guéant JL, Gastin I, Vidailhet M. Méthodes biologiques de diagnostic positif et étiologique des carences vitaminiques. *Nutr Clin Metabol* 1995; **9**: 29-42.
- Carmel R. In vitro studies of gastric juice in patients with food-cobalamin malabsorption. *Dig Dis Sci* 1994; **39**: 2516-22.
- Joosten E, Pelemans W, Devos P, Lesaffre E, Gossens W, Griel A, Verhaeghe R. Cobalamin absorption and serum homocysteine and methylmalonic acid in elderly subjects with low serum cobalamin. *Eur J Haematol* 1993; **51**: 25-30.
- Guéant JL, Djalali M, Gaucher P, Champigneulle B, Bigard MA, Hassouni A. In vitro test of haptocorrin degradation for biological diagnosis of exocrine pancreatic dysfunction using duodenal juice collected during endoscopy. *Lancet* 1986; **ii**: 709-12.
- Bellou A, Aimone-Gastin I, De Korwin JD, Bronowicki JP, Moneret-Vautrin A, Nicolas JP, et al. Cobalamin deficiency with megaloblastic anaemia in one patient under long-term omeprazole therapy. *J Int Med* 1996; **240**: 161-4.
- Kittang E, Aaland E, Schjonsby H. The effect of omeprazole on gastric acidity and the absorption of liver cobalamins. *Scand J Gastroenterol* 1987; **22**: 156-60.
- Guéant JL, Djalali M, Duc MA, Nicolas JP. Déséquilibre de la flore intestinale: facteur aggravant la carence en vitamine B12 dans un cas de gastrite chronique. *Gastroenterol Clin Biol* 1982; **6**: 225-7.
- Suter PM, Goluer BB, Goldin BR, Morrow FD, Russel RM. Reversal of protein-bound vitamin B12 malabsorption with antibiotics in atrophic gastritis. *Gastroenterology* 1991; **101**: 1039-45.
- Carmel R. *Helicobacter pylori* infection and food-cobalamin malabsorption. *Dig Dis Sci* 1994; **39**: 309-14.