Cyanocobalamin Absorption in the Elderly: Results for Healthy Subjects and for Subjects with Low Serum Cobalamin Concentration

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We examined the intestinal absorption of cyanocobalamin from a non-protein-bound test dose given to 38 subjects from a population of elderly. The subjects were 76 years old and were apparently free from conditions known to affect cyanocobalamin absorption. Their gastric mucosal function was normal, as judged from determinations of serum gastrin and pepsinogen I. The urinary excretion of radioactivity during the first 24 h was 24(7±9%), range 8.6 to 45.2%, corresponding to a health-associated reference interval of 10 to 38%. The results indicate that cyanocobalamin absorption does not decline during normal aging. Duplicate studies were performed in another 20 subjects (70–81 years old) from the same population study; these subjects had a serum cobalamin concentration <130 pmol/L. The imprecision (CV) was 23%.

Additional Keyphrases: pernicious anemia • population study of elderly • Schilling test • reproducibility • gastrin • pepsinogen I • folate • vitamin B12 deficiency • sex- and age-related effects • reference interval

Serum cobalamin (vitamin B12) concentrations in humans tend to decline with age,4 but it is not known whether this decrease reflects clinically important deficiency. It has also been a matter of dispute whether absorption of cobalamin decreases with normal aging.

The aims of this study were twofold. We wanted to investigate the absorption of free (non-protein-bound) cobalamin by apparently healthy elderly individuals selected from the population study “70-year-old people in Göteborg, Sweden.” The population samples were representative for the whole population (1–3). In addition to the clinical and laboratory features used to define absence of disease in the individuals, we measured concentrations of gastrin and pepsinogen I in serum in order to characterize the functional status of the gastric mucosa. Secondly, we examined the precision of the urinary excretion method for cobalamin absorption (4) in a group of elderly individuals with “subnormal” values for serum cobalamin concentration.

Study Population and Methods

Study Population

The population study “70-year-old people in Göteborg” is a longitudinal study, which was initiated in 1971–72 with a sampling of 30% of the total 70-year-old population (n = 1148, cohort I) (4). The participation rate was 85%. Of the participants, 41% were again studied at age 81 (72% of those still alive).

In 1976–77 another representative sample of 1281 subjects was invited, and 81% of them could be examined (cohort II). Of these, 73% (84% of those still alive) remained in the study at age 75.

In 1982–83 a third cohort of 70-year-olds (302 men and 317 women, participation rate 77%) was examined.

Comparisons between responders and nonresponders have been reported elsewhere (4). All studies included a home call with an interview on social and medical conditions as well as a thorough medical and laboratory examination at the outpatient department. The present study starts with cohort I at age 81, cohort II at age 75, and cohort III at age 70.

After determination of serum cobalamin in 486 consecutive probands from cohort II we invited probands with serum cobalamin concentration >130 pmol/L to participate in a study of cyanocobalamin absorption. Inclusion criteria were blood hemoglobin concentration >150 g/L and (or) ongoing therapy with potassium salt or anticonvulsiv drugs. Of 97 subjects invited, 38 (17 men and 21 women, age 76 years) agreed to take part in the study. The mean concentrations and distributions of blood hemoglobin, serum cobalamin, and other hematological parameters of the 38 participants did not differ from those of the 59 nonparticipants. We performed the cyanocobalamin absorption study six to 12 months after the initial survey, and we thereupon again determined blood hemoglobin, serum cobalamin, and serum...
creatinine. One proband (case no. 15) had developed iron-deficiency anemia since the initial survey. We determined serum gastrin and pepsinogen I after completing the Schilling tests. None of these results formed the basis for exclusion.

We also invited all probands from cohorts I and III with a serum cobalamin concentration <130 pmol/L to take part in a study of the precision of the Schilling test. Of the 34 subjects invited, eight men and 12 women accepted. They were examined twice at intervals ranging from three to six months. We initiated cobalamin treatment of all probands with evidence of vitamin B₁₂ malabsorption after the first test. Macrocytic anemia resolved with treatment in two 70-year-olds. There was no change in the clinical condition in the remainder. Gastrin and pepsinogen I were determined in the serum specimens collected at screening.

Biochemical Methods

Serum cobalamin concentration was determined with *Euglena gracilis* (strain Z) according to Hutner et al. (5), as modified by Andersson (6). The reference interval was 130–740 pmol/L, calculated from analysis of samples from 54-year-old men. We performed the cyanocobalamin absorption test without addition of intrinsic factor (4), using 0.5 μCi (18.5 kBq) of cyano[57Co]cobalamin (specific activity 0.5 Ci/g; The Radiochemical Centre, Amersham, England), given orally after an overnight fast. Cykobemin* (1 mg, nonradioactive vitamin B₁₂; Kabi, Sweden) was administered by intramuscular injection after 1.5 and 24 h. Urine was collected for the following two days. The results were recorded as 24-h urinary excretion of radioactivity in per cent of the dose given. Radioactivity was determined with an LKB Wallac 1260 Multigamma counter.

We determined gastrin in serum specimens collected during the initial survey and stored at −20 °C, using a nonselective double antibody RIA (7). The upper limit of the reference interval for elderly has been assessed (7) to be within the range 100–150 ng/L (50–75 pmol/L). Serum pepsinogen I was determined by a double-antibody RIA (8). The central 0.95 fractile interval, from determination of an unselected population of 256 women ages 54–60 years, was 21–183 μg/L.

Statistical Methods

Standard methods were used for calculation of mean and standard deviation, and for linear regression analysis. Student's *t*-test and the Pitman test (9) were used for statistical analysis and differences were considered significant at a level of *p* < 0.05.

Results

Reference Values

*Cyanocobalamin absorption*. During the first 24-h period the excretion ranged from 8.6 to 45% (mean 24%, SD 7.1%), giving a reference interval of 10–38% (mean ± 2 SD). The distributions were similar for men and women (mean values 24/SD 6.2)% and 26/SD 8.0, respectively. During the second 24-h period the excretion ranged from 2.0 to 12% with a mean of 7.8(± 2.8)%. We found no correlation between values for the first and the second 24-h period.

The urine-specimen volumes for the first and second 24-h periods were of similar magnitude in 34 cases. In four cases (nos. 1, 10, 18, and 23) the urinary volume was low during the first period but considerably higher during the second. Of these, cases nos. 1 and 18 had the lowest results for urinary excretion during the first 24-h period. There was a significant correlation (*r* = 0.53, *p* < 0.01) between urinary volume and excretion of radioactivity during the first 24 h in the women; but not in the men (*r* = 0.39). Neither urinary volume nor amount of urinary radioactivity was correlated with serum creatinine concentration. Furthermore, the excretion of radioactivity was not correlated with the concentrations with serum cobalamin, blood hemoglobin, serum gastrin, or serum pepsinogen.

*Serum gastrin* concentrations ranged from 23 to 155 ng/L for 37 of the 38 subjects, without sex-related difference. Case no. 19 had a concentration of 253 ng/L. This woman excreted 12% of the radioactivity during the first 24-h period and had a serum pepsinogen concentration of 31 μg/L.

*Serum pepsinogen I* concentrations ranged from 20 to 143 μg/L in 35 of the 38 subjects. The other three subjects had concentrations exceeding 190 μg/L, possibly indicating superficial gastritis (10). Those individuals with the lowest pepsinogen concentrations did not differ from the rest with respect to urinary excretion of radioactivity.

Precision

*Cyanocobalamin absorption*. Figure 1 shows the results from duplicate studies of cyano-cobalamin absorption (first 24-h period), performed on 10 subjects from cohort I and 10 subjects from cohort III, all having serum cobalamin concentration <130 pmol/L. A CV of 23% was obtained, calculated from the differences between the results for day 1 in the two tests. Using figures for excretion during both the first and second 24-h periods, we obtained a CV of 26%. Of the six patients with abnormally low values in both tests, one had previously been subjected to gastrectomy and had a serum gastrin concentration close to the detection limit; five had

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6 These data are available upon request from the authors or from the Editorial Office of this journal.

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grosely increased serum gastrin concentrations, indicating achlorhydria (7). Of the 13 patients with normal absorption of free cyanocobalamin in both tests, three had evidence of achlorhydria. Another patient had a normal gastrin concentration but low concentration of pepsinogen I (16 μg/L), a finding typical for atrophic gastritis type B (7, 8).

We found one case with discordant results when using a decision limit of 10% excretion of ingested 57Co. This 70-year-old man excreted 10.4% in the first test, 4.3% in the second. Clinical evaluation revealed a duodenal diverticulum and intestinal bacterial overgrowth as possible causes of his low serum cobalamin concentration and of the unusual variability in Schilling test results.

**Discussion**

Vitamin B12 deficiency in humans is usually caused by defective absorption as a result of deficient production of intrinsic factor from the gastric mucosa or disease of the lower parts of the small intestine, but other causes have been recognized (11–16). Investigation of cobalamin absorption is therefore considered to be a major diagnostic tool in the evaluation of cases with suspected vitamin B12 deficiency. Conventional techniques for the study of vitamin B12 absorption are based on the use of an oral tracer dose of radioactive cyanocobalamin. Widely used is the urinary excretion method, first described by Schilling (4); other, technically less simple, methods have also been used (17, 18). The precision of the urinary excretion method reportedly ranges from good to fair in healthy controls and in patients with pernicious anemia, to less satisfactory in cases of intestinal malabsorption and after partial gastrectomy (19–21). We obtained a CV of 23% in the present study of 70- and 81-year-old outpatients. Although the difference in urine volumes between the two 24-h periods exceeded 25% for 12 of the 38 healthy probands there were only two individuals (nos. 1 and 15) for whom a concomitant low excretion of radioactivity suggested an incomplete collection of urine. Not unexpectedly, our results indicate correct urine collection to be a greater problem for women than for men in old age. However, the results presented in Figure 1 indicate that the precision of the test was at least as satisfactory in women as in men. Instruction of the patient in order to ensure correct urine collection is a prerequisite to the application of this test, both in hospital and in outpatient service. Decreased glomerular filtration rate, a frequent finding in old age (22), is another source of error, which by definition was excluded in our material; it may influence the result, especially if only urine from the first 24-h period is used for the measurements.

Reports on reference intervals for the Schilling test are summarized in Table 1. There are several other reports, either from study of smaller numbers of reference subjects or which suffer from lack of information regarding age and (or) health status of the subjects, in particular the studies of elderly. As indicated by Table 1, however, subjects with abnormally low cobalamin absorption have been included. We now went into considerable detail in order to obtain an apparently healthy study population of elderly (representative population sample, rigorous exclusion criteria for relevant abnormality). Gastric mucosal function assessed from assays of serum gastrin and pepsinogen I was normal in all, with one possible exception (7). The reference interval of 10 to 38% of the ingested radioactivity excreted during the first 24-h period may therefore be regarded as a reliable one for healthy elderly subjects and accords well with those for younger age groups.

In the precision study, done on subjects with low serum cobalamin concentration, we found several subjects with Schilling test results within the reference interval. Failure to absorb dietary or protein-bound vitamin B12 (11–14) may have been due to gastric achlorhydria, partial gastrectomy, or bacterial overgrowth in some of these subjects, and such conditions may possibly lead to high variability in cyanocobalamin absorption. The high prevalence of achlorhydria reported in the elderly (23) and the alleged importance of gastric acidity and peptidase activity for the liberation of dietary vitamin B12 (14, 24–26) call for studies of the reproducibility of tests for absorption of protein-bound cobalamin in groups of elderly with different degrees of abnormality in gastric mucosal function as well as in patients with intestinal malabsorption. Determination of the absorption of protein-bound cobalamin is not generally available, either for lack of food-bound labeled cyanocobalamin or because of hesitation to administer native animal serum to patients (14). Absence of adequate gastric proteolytic activity may, however, be inferred from findings of high serum gastrin concentration and (or) low pepsinogen I concentration (the nosological sensitivity of the latter test is, however, controversial, 8) if direct determination of gastric capacity for hydrogen-ion secretion is not available.

We conclude that cobalamin absorption as measured by the urinary excretion test does not decline during normal aging. The urinary excretion method shows acceptable reproducibility also in the elderly.

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**References**


