

Determination of vitamin B₁₂ absorption by a simple whole body counter

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SYNOPSIS This paper reports the results of estimating vitamin B₁₂ absorption by whole body counting in patients without known gastrointestinal disorder, and in patients with pernicious anaemia, idiopathic achlorhydria, achlorhydria following gastric operations, and various forms of small intestinal disease. Patients with pernicious anaemia absorbed less than 30% of the test dose; they could be distinguished from patients without gastrointestinal abnormality and from most achlorhydric patients who secreted more than 300 mg units of intrinsic factor in the post-histamine hour. Nevertheless, the wide range of normal absorption and the variable absorption from the normal gastrointestinal tract is emphasized and discussed. There is no relation between histamine-stimulated intrinsic factor production and vitamin B₁₂ absorption in patients with small intestinal disease.

There are at present a number of different methods for measuring vitamin B₁₂ absorption, of which the urinary excretion method of Schilling is the most widely used. However, none of these methods is wholly satisfactory, either because of the difficulty of ensuring complete urine or stool collections from patients, or because of difficulties in quantitating absorption. The whole body counter offers the possibility of estimating absorption quantitatively without incurring the sources of error implicit in making urine and stool collections. Previous workers (Warner and Oliver, 1966; Callender, Witts, Warner, and Oliver, 1966) have illustrated a whole body counter for determination of vitamin B₁₂ absorption.

The present paper reports the results obtained from a particularly simple whole body counting machine, eminently suitable for routine hospital use in estimating vitamin B₁₂ absorption. Patients with and without gastrointestinal disease were studied, the reproducibility of the method was investigated, and intrinsic factor production was correlated with vitamin B₁₂ absorption.

METHODS

The whole body counter used in the present study has already been described (Simpson and Shearman, 1968). Before the present study, patients were given vitamin B₁₂ as described below, and by counting the radioactivity

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of the stool excreted subsequently it was found that there was no activity at seven days.

In preparation for the test, the patient was fasted for 12 hours overnight and then counted to get a background reading. He was then given 1 μC ⁵⁸Co contained in 0.5 μg of cyanocobalamin with 100 ml water, and counts were repeated to obtain the increase in count rate due to administered radioactivity, mainly in the stomach. One gram of Sennokot was given three days later to ensure adequate elimination of unabsorbed radioactivity, a final count being carried out on the seventh day to determine the amount of radioactivity retained, now mainly in the liver. On each occasion a standard was counted to allow for drift in the apparatus and for radioactive decay. The reproducibility of the method was tested by carrying out two tests of vitamin B₁₂ absorption in successive weeks.

Where vitamin B₁₂ absorption was related to ability to secrete intrinsic factor, the latter was measured in the hour following augmented histamine stimulation after the method of Kay (1953). These tests were carried out under carefully controlled conditions as described previously (Shearman, Finlayson, and Wilson, 1967). Intrinsic factor was measured by the method of Ardeman and Chanarin (1963).

PATIENTS

Eleven patients without a history or clinical evidence of gastrointestinal disease were studied. Seven had had uncomplicated myocardial infarcts, two suffered from cerebral vascular disease, one had chronic bronchitis, and one an ovarian cyst. One patient with a benign gastric ulcer, who had a normal acid output on aug-

mented histamine stimulation and no other gut pathology, was also studied.

Seventeen patients with pernicious anaemia were diagnosed on the basis of a serum vitamin B₁₂ level of less than 100 µg/ml, and achlorhydria to augmented histamine stimulation with a secretion of less than 300 ng units of intrinsic factor in the post-histamine hour.

Twelve patients with achlorhydria were studied. This followed partial gastrectomy in five cases, pyloroplasty and vagotomy in one case, and in six cases it was of unknown cause.

Eighteen patients studied were shown to have small intestinal malabsorption. Of these, 14 had idiopathic steatorrhoea as judged by flat intestinal biopsy without definable cause, faecal fat excretion of more than 7 g/day on a normal ward diet, and folic acid malabsorption, one patient had proven fibrocystic disease of the pancreas, one had biopsy-proven amyloidosis of the small intestine, one had biopsy-proven Crohn's disease which involved the whole small intestine, and one had radiological terminal ileal disease.

RESULTS

These are shown in Figures 1, 2, and 3. In general, normal patients absorbed more than 30% of the test dose, while those with pernicious anaemia absorbed less than 30%. In patients with achlor-

hydria from various causes, absorption was correlated with intrinsic factor output, while in those with small intestinal disease there was no such correlation.

In Fig. 1, the results of two successive absorption tests on the same patients are shown. All patients studied are included. It will be seen that one normal patient had a 32% absorption on one occasion, whilst one patient with pernicious anaemia absorbed 31% of the oral dose. The correlation coefficients between the two tests for the various subgroups are as follows: achlorhydria 0.30, achlorhydric partial gastrectomy 0.93, intestinal disease 0.86, pernicious anaemia 0.64, normals 0.0029.

Figure 2 compares absorption of vitamin B₁₂ with intrinsic factor output in all achlorhydric patients. With a single exception, all patients who produced less than 300 ng units in the post-histamine hour absorbed less than 30% of the vitamin B₁₂ test dose. The sole exception was a patient with pernicious anaemia who absorbed 31% on one occasion and 26% on the other. Three patients who secreted more than 300 ng units of intrinsic factor in the post-histamine hour absorbed less than 30% of the test dose. One patient had a partial gastrectomy and absorbed 21% on one occasion and 46% on the other. The second patient had idiopathic achlor-

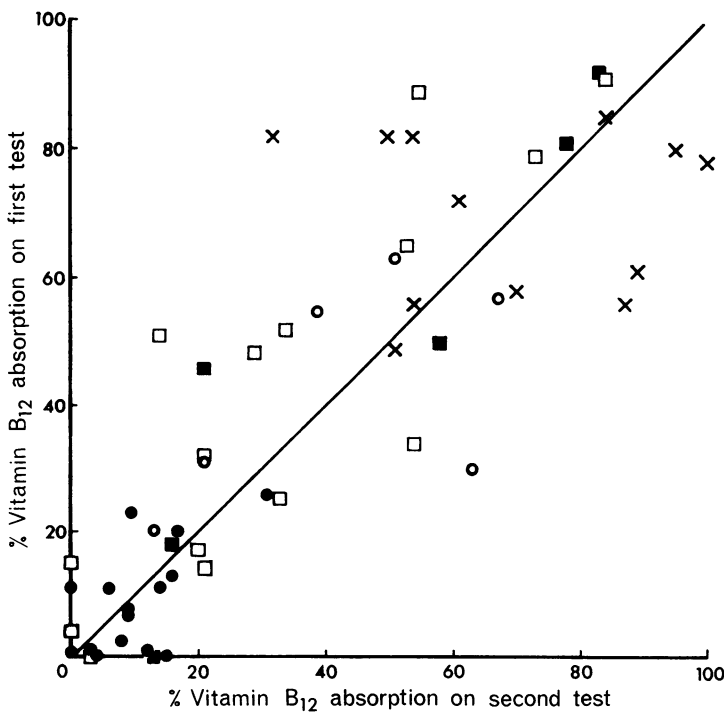


FIG. 1. Results of two successive tests of vitamin B₁₂ absorption plotted together. ● Pernicious anaemia; ○ achlorhydria; □ intestinal disease; ■ achlorhydric partial gastrectomy; X normal.

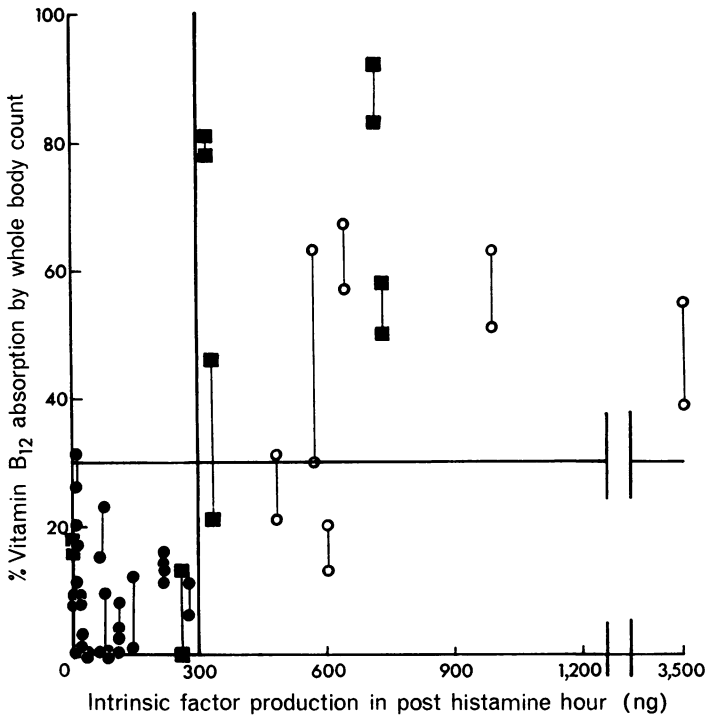


FIG. 2. Comparison of vitamin B₁₂ absorption by whole body counting and intrinsic factor production during augmented histamine stimulation in achlorhydria. ● Pernicious anaemia; ○ achlorhydria; ■ achlorhydric partial gastrectomy.

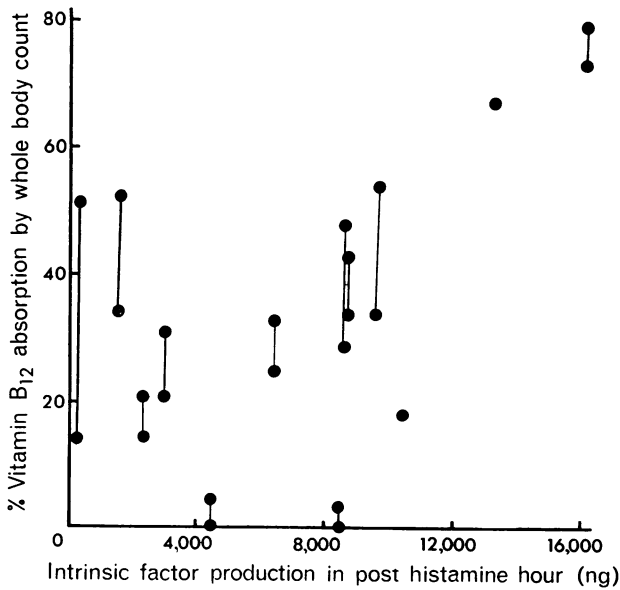


FIG. 3. Comparison of vitamin B₁₂ absorption by whole body counting and intrinsic factor production during augmented histamine stimulation in various malabsorptive states.

hydria and on the two tests absorbed 21% and 31%. The third was a lady of 89 years who presented with a severe megaloblastic anaemia due to folic acid deficiency (serum folate 1.6 m μ g/ml, serum vitamin B₁₂ 143 μ g/ml) who was also achlorhydric. Unfortunately she died of an unrelated cause before small bowel studies could be done, but her vitamin B₁₂ absorption on two occasions was 13% and 20%.

Figure 3 illustrates that for intestinal disease there is no correlation between the production of intrinsic factor and absorption of labelled cyanocobalamin.

DISCUSSION

The whole body counter gives an accurate measure of the percentage absorption of labelled cyanocobalamin from a test dose on a given occasion, provided that unabsorbed radioactivity has been eliminated in the stool. In view of the fact that we have found unabsorbed activity to be excreted in less than seven days, it was surprising that there was such a large variation in absorption in patients without gastrointestinal disease. Not only was this variation large from one patient to another, but it was also considerable in the same patient on two successive tests so that the correlation between the two tests was poor ($r = 0.0029$). As the machine used for counting has been shown to count reproducibly to within $\pm 8\%$ and as the position of the patient during the counting procedure was standardized, it appears that the variability must result from variable degrees of absorption on different occasions. The explanation for this may lie in the nature of the test used, for it seems most likely that the percentage absorption of vitamin B₁₂ on any occasion would be most closely related to the amount of intrinsic factor which happened to be free in the stomach at the time of the test, and which was secreted in response to the drink of water given with the vitamin B₁₂ test dose. It is probably incorrect to compare the percentage vitamin B₁₂ absorption in this test with intrinsic factor output measured under conditions of augmented stimulation of the stomach. In addition, variability in gut motility and in absorption from the terminal ileum could affect the results. It is interesting to note that the reproducibility of the Schilling method of measuring vitamin B₁₂ absorption is not good, at least in partial gastrectomy and primary malabsorption (Adams and Cartwright,

1963). While this may be due to incomplete urine collections, it may also result from variable absorption of vitamin B₁₂.

It would be valuable to study vitamin B₁₂ absorption under more physiological conditions in which the test dose would be given with a vitamin B₁₂-free meal, especially as there is now evidence that maximal gastric secretion occurs when a meal is taken (Rune, 1966). Such studies are in progress.

From the viewpoint of diagnosis, it was found that patients with pernicious anaemia could be separated from normals, the former absorbing less than 30% of the test dose. Nonetheless, on a single occasion one patient without gastrointestinal disorder absorbed as little as 32%, and while the next lowest figure for such a patient was 49%, it seems likely that there may occasionally be overlapping absorption values in these two groups. On the evidence presented it seems unlikely that patients unable to secrete more than 300 ng units of intrinsic factor in the first histamine hour will absorb more than 30% of the test dose. However, it does appear that some achlorhydric patients who can secrete more than 300 ng units will absorb less than 30% in spite of there being no evidence of small bowel disease, as was the case in two of the three patients described above. Hence values between 20 and 30% may represent an equivocal range.

Vitamin B₁₂ absorption was also compared with intrinsic factor production in a group of patients with small intestinal malabsorption, and, as would be expected, there was no correlation between these two groups of observations.

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