Reproducibility of the hepatic uptake test of vitamin B₁₂ absorption

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The absorption of vitamin B₁₂ can be determined by several methods based on the measurement of the radioisotope-labelled vitamin in serum, faeces, and urine, and by external counting over the liver. In general these test procedures distinguish clearly between normal values and those obtained in individuals with pernicious anaemia, but it has been claimed that serial tests by the Schilling urinary excretion method failed to give results which were consistently normal or abnormal in patients with primary malabsorption syndromes or who had had a partial gastrectomy (Adams and Cartwright, 1963). These discrepancies could be due in part to difficulty in ensuring complete urinary collections in those with diarrhoea, and perhaps to variation in flushing of the dose absorbed. Another, more direct technique might therefore prove more satisfactory. Results obtained with the hepatic uptake test have been shown to agree well with those given by the faecal excretion method when both were performed simultaneously (Fone, Cooke, Meynell, and Harris, 1961) and the hepatic uptake test has the advantage that it avoids the problems of complete urinary or faecal collection, which can be considerable in patients with malabsorption syndromes. We have therefore studied the reproducibility of the hepatic uptake test (Glass, Boyd, Gellin, and Stephenson, 1954; Glass and Boyd, 1957) by performing the examination on two occasions in individuals with malabsorption syndromes and in those who had had a partial gastrectomy. The results have been compared with those obtained in patients with pernicious anaemia and in individuals without evidence of gastrointestinal disease.

MATERIALS AND METHODS

The hepatic uptake of radioactively labelled cyanocobalamin was determined on two occasions one week to one month apart in patients with pernicious anaemia or malabsorption syndromes, after partial gastrectomy, and in a group without gastrointestinal disease. ⁵⁷Co labelled cyanocobalamin of specific activity 1μCi per microgram was used in these investigations. At each test 0.5 μg of the labelled vitamin was given in the morning by mouth in 100 to 200 ml of water. Each patient ate a normal breakfast beforehand, for there is evidence that, at least after partial gastrectomy, vitamin B₁₂ absorption is reduced by fasting (Deller, Germar, and Witts, 1961). After one week hepatic radioactivity was determined using a scintillation counter having a 1½ in. diameter × 1 in. diameter sodium iodide crystal and fitted with a wide angle collimator. Counts were made for five minutes each at the centre of liver dullness in the midclavicular and midaxillary lines, over the anterior or posterior aspect of the thigh, and over the lower abdomen at a point midway between the umbilicus and the symphysis pubis. The liver counts were corrected for background radiation by deduction of the thigh count and results were expressed in counts per minute per microcurie of radioactive dose ingested (counts/min/μCi). The abdominal count was used as an estimate of residual intracolonic radioactivity and if it was more than 50% greater than the thigh count then the liver values obtained at that time were rejected as unreliable and the measurement was repeated a few days later. When the test was the second of a pair a correction had to be applied to allow for the effect of the radioactivity absorbed during the first test. The liver count rate at the first test was deducted from the value obtained in the second test after allowance for radioactive decay since the first test had been made. In a small series of preliminary studies it was established that when counts were performed twice within a few minutes of each other the values showed good agreement. It was also found that the proportion of the dose of radioactive vitamin B₁₂ taken up by the seventh day was retained for the period necessary to complete the second test. However, at the end of the investigation it was found that test results tended to be higher at the second than the first measurements, suggesting that there was a small continued uptake of radioisotope by the liver more than seven days after a dose.

The individuals studied were inpatients or outpatients of the Central Middlesex Hospital. Criteria for inclusion in the various clinical categories were as follows.
PERNICIOUS ANAEMIA. All 10 patients had previously had an anaemia which was megaloblastic in type or was associated with a serum vitamin B₁₂ level below 140 μg/ml. In eight of the patients bone marrow studies and serum vitamin B₁₂ estimation were both performed. All the patients had complete achlorhydria on augmented histamine or histalog testing.

PARTIAL GASTRECTOMY. Billroth I or Polya partial gastrectomy had been performed from one to 17 years previously, one being combined with vagotomy. At the time of the investigation the patients were under routine observation, but clinical evidence of anaemia or malabsorption was not necessarily present. Two of the 15 patients in the group had steatorrhoea, faecal fat excretion rates being in excess of 6g daily, four had blood haemoglobin concentrations below 11-0g%, and one had been treated elsewhere for megaloblastic anaemia some years before, but the cause of anaemia had not been established and treatment had later been discontinued.

MALABSORPTION. All the 10 patients had a faecal fat excretion in excess of 6g daily when measured over a three-day period, or a urinary d-xylose excretion of less than 5g within five hours after a 25g loading dose. The clinical diagnoses in the patients studied were idiopathic steatorrhoea (4), Crohn’s disease (2), and tropical sprue, amyloidosis, abdominal lymphoma, and Zollinger Ellison syndrome (1 each).

CONTROL SUBJECTS. These were 10 inpatients who had been admitted to the wards with cardiorespiratory or neurological diseases: none had symptoms referable to the gastrointestinal tract and none was anaemic.

RESULTS

Table I gives the means and ranges found at the first and second estimates of vitamin B₁₂ uptake in individuals in each clinical category. The results for anterior and lateral counts were similar within each group except that one control subject had a lateral count which was within the pernicious anaemia range at the first estimate. In this individual the second lateral count and also both anterior uptake measurements were, however, clearly within the normal range, and the discrepancy was probably due to difficulty in ensuring that the patient lay on his side under the vertically suspended scintillation counter without moving. Lateral counts had to be repeated on several occasions because of this problem and since anterior measurements gave at least equally good results comparisons of reproducibility have been based upon them. Although preliminary studies had suggested that uptake of radioactivity by the liver ceased after seven days it was found that the uptake measured by the second test was higher than that found at the first in 23 of the 35 individuals without pernicious anaemia.

This difference could be explained if small amounts of radioactive B₁₂ had in fact continued to accumulate in the liver after the estimate of uptake had been made on the seventh day following administration of the first dose. Such a tendency would cause some degree of spurious correlation between first and second test results in a single individual, but should not invalidate comparisons between the clinical groups for it would be expected to affect equally the consistency of the results obtained in them.

Figure 1 shows the two measurements of hepatic radioactivity in the individual patients in each clinical group, except pernicious anaemia, plotted against each other. For comparison with these values the upper limits of radioactivity recorded in first and in second tests in patients with pernicious anaemia have been shown as dotted lines. The highest correlation coefficient (r = 0·86) was obtained in patients with malabsorption whilst those who had had a partial gastrectomy had slightly more consistent results than the control individuals (r = 0·61 and 0·49 respectively). However, the differences between the three correlation coefficients, which were compared by Fisher’s Z test, could well have been due to chance. The lowest correlation coefficient (r = 0·20) was obtained for patients with pernicious anaemia, but this finding must largely be due to the small uptake of vitamin B₁₂ in these individuals compared with the level of background radiation.

Although the results in patients with malabsorption syndromes were in general the most reproducible, two of the measurements which were initially normal were definitely within the abnormal range.
Reproducibility of the hepatic uptake test of vitamin $B_{12}$ absorption

FIG. 1. Results of duplicate hepatic vitamin $B_{12}$ uptake tests in individuals in different clinical categories, comparing the results of first and second tests. Correlation coefficient $r = 0.49$ in control patients, $0.61$ in patients treated by partial gastrectomy, and $0.86$ in those with malabsorption syndromes (for the differences between these figures, $p < 0.1$ by Fisher's $Z$ test). $O$ Controls; $X$ partial gastrectomy; $\bullet$ malabsorption.

at the second test. Fourteen pairs of measurements in patients treated by partial gastrectomy were consistently within the control range; the one remaining individual who had two subnormal results was known to have had a megaloblastic anaemia in the past.

Comparison of the results at the first and second tests in all patients with normal uptakes at both measurements showed considerable variation between the two estimates. The mean difference between pairs was 352 counts/min/$\mu$Ci or 47% of the original estimate, though is should be noted that this figure is due in part to the tendency for test results to be higher at the second measurements.

DISCUSSION

Tests of vitamin $B_{12}$ absorption should, ideally, distinguish clearly between results which are normal and those which fall within the pernicious anaemia range. They should also allow comparisons between measurements on the same individual, to determine whether the capacity to absorb vitamin $B_{12}$ has altered, for instance, with time after partial gastrectomy, following treatment with antibiotics or with the administration of intrinsic factor.

Our results have confirmed the finding of Adams and Cartwright (1963) by the Schilling test technique that measures of vitamin $B_{12}$ uptake may not discriminate reliably between normal and subnormal absorption in patients with malabsorption syndromes. In another study Lamar, McCracken, Miller, and Goldsmith obtained consistent results in repeated tests on patients with malabsorption syndromes; this difference from our findings and those of Adams and Cartwright may, however, be due to the method of selection of patients used by Lamar, McCracken, Miller, and Goldsmith (1965). All subjects studied by them had previously been shown to have anaemia, and Schilling tests with and without intrinsic factor had indicated that vitamin $B_{12}$ absorption was defective. These patients, therefore, were likely to have a true and fixed defect of vitamin $B_{12}$ so that no single test could be expected to give a high result.

Although Adams and Cartwright found tests of vitamin $B_{12}$ absorption to be unreliable in patients treated by partial gastrectomy we have obtained reasonably consistent results. One possible explanation of this difference is that we gave a normal breakfast to our patients before the radioactively labelled vitamin $B_{12}$ was administered. It has previously been shown that the absorption of vitamin $B_{12}$ in patients after partial gastrectomy is increased if the individual is not fasted but takes a normal meal beforehand (Deller et al., 1961).
Although the anterior hepatic uptake measurements distinguished consistently between normal and abnormal results, except in patients with malabsorption syndromes, the two figures often differed greatly when pairs of estimates were within the normal range. This variation was such that no reliable conclusion could probably be drawn from individual comparisons of results in such patients to determine whether the capacity to absorb vitamin B\textsubscript{12} had improved or deteriorated.

The particular value of the hepatic uptake test is probably in distinguishing normal from subnormal vitamin B\textsubscript{12} absorptive capacity, provided that the patient does not suffer from malabsorption.

**SUMMARY**

The reproducibility of results obtained by the hepatic uptake test of vitamin B\textsubscript{12} absorption has been studied by performing the test twice within a month in each of 45 patients. The ranges of results obtained in 10 control patients and in 10 with pernicious anaemia could be distinguished clearly on each occasion. Pairs of measurements made in 14 patients after partial gastrectomy were consistently normal whilst both results were subnormal in one remaining individual, who had previously had megaloblastic anaemia. In two of 10 patients with malabsorption syndromes, however, results were initially within the normal range, but on repetition were subnormal.

The interpretation of repeated tests within the same individual should, however, be made with caution even when there is no evidence of malabsorption, for comparisons of pairs of results obtained when both were within the normal range showed marked fluctuation.

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