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Simultaneous free and bound radioactive vitamin B_{12} urinary excretion test

T. K. BELL, J. M. BRIDGES, AND M. G. NELSON

From the Clinical Pathology Laboratories, Royal Victoria Hospital, Belfast

SYNOPSIS The absorption of vitamin B_{12} following the simultaneous administration of ^{58}Co B_{12} and a complex of ^{57}Co B_{12} with human gastric juice was assessed by measurement of urinary excretion of radioactivity. Sixteen control subjects, 13 patients with pernicious anaemia, and four who had had total gastrectomy were studied. The method proved a reliable means of detecting those with intrinsic factor deficiency.

The urinary excretion test introduced by Schilling (1953) for assessing the absorption of vitamin B_{12} is useful in the investigation of patients suspected of having vitamin B_{12} deficiency (Van Kampen and Graafland, 1959; Adams and Seaton, 1961). In this test a small oral dose of vitamin B_{12} labelled with radioactive cobalt is given to the patient. Simultaneously a large intramuscular dose of non-radioactive B_{12} is administered to block the body stores and to flush out the labelled vitamin into the urine. The radioactivity excreted in the following 24 or 48 hours is determined and provides a measure of the amount of the oral dose which has been absorbed. When the radioactivity excreted by the patient is less than that of control subjects the test is repeated a few days later with the addition of potent intrinsic factor. If the fraction of the oral dose excreted in the urine is now increased by at least a factor of 2, the patient is presumed to be deficient in intrinsic factor.

Katz, DiMase, and Donaldson (1963) have described a diagnostic procedure which combines the two parts of the Schilling test. Vitamin B_{12} labelled with ^{58}Co is given orally to the patient together with vitamin B_{12} labelled with ^{60}Co, bound to normal human gastric juice. A large intramuscular dose of non-radioactive B_{12} is simultaneously administered and the amounts of ^{58}Co and ^{60}Co excreted in the urine are separately determined. Thus both the absorption of B_{12} and the effect of intrinsic factor may be assessed.

The present work describes a modification of this technique in which ^{57}Co B_{12} is substituted for ^{60}Co B_{12} thus reducing the dose of radiation to the patient. Normal subjects, patients with pernicious anaemia, and patients with total gastrectomy were investigated.

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PREPARATIONS OF VITAMIN B_{12} SOLUTIONS

Preparations of vitamin B_{12} labelled with ^{57}Co and with ^{58}Co of specific activity 1 microcurie per microgram B_{12} in freeze-dried form were obtained from the Radiochemical Centre, Amersham.

(1) ^{58}Co B_{12} SOLUTION An aqueous solution of ^{58}Co B_{12} containing 1·25 μg. per 100 ml. was prepared.

(2) ^{57}Co B_{12} GASTRIC JUICE COMPLEX Human gastric juice was obtained from normal subjects following maximal histamine stimulation (Kay, 1953). This was combined with ^{57}Co B_{12} to form a saturated ^{57}Co B_{12} gastric juice complex following the method of Katz *et al.* (1963).

To ensure that no unbound ^{57}Co B_{12} remained in the complex, 1 ml. volumes of the preparation were removed daily during dialysis. These aliquots were assayed in a well type scintillation counter[1] and when the radioactivity had fallen to a constant rate on two consecutive days dialysis was considered complete. Water was added to the dialysed complex to give a final concentration of 1·25 μg. B_{12} per 100 ml.

All solutions were stored at -15°C. until required.

ADMINISTRATION OF DOSES

All subjects were fasted overnight. Two oral doses were given together, one containing 0·25 μg. ^{58}Co B_{12} and the other 0·25 μg. ^{57}Co B_{12} gastric juice complex. Simultaneously and 24 hours later, 1,000 μg. of non-radioactive B_{12} was given intramuscularly. Food was allowed two hours after the oral doses were given. All urine excreted in the subsequent 48 hours was collected in two 24-hour volumes.

ASSAY OF RADIOACTIVITY IN URINE

A cell accommodating 400 ml. was used to assay the radioactivity in the urine by annular counting on the well type scintillation counter. The 0·81 Mev. gamma rays emitted by ^{58}Co can be distinguished from the 0·12

[1]Ekco Electronics type N.664 B.

Mev. gamma rays emitted by ^{57}Co by altering the discriminator bias settings on a scaler[2] which was coupled to the scintillation counter.

The amounts of ^{58}Co and ^{57}Co excreted were calculated by the method of Veall and Vetter (1958) for two component mixtures.

RESULTS

Sixteen hospital patients, 13 males and three females, aged between 22 and 64 years in whom there was no clinical or haematological evidence of deficiency of vitamin B_{12}, were selected as control subjects. Thirteen patients with Addisonian pernicious anaemia were studied. They showed a clinical picture consistent with the diagnosis: a macrocytic peripheral blood picture; a megaloblastic bone marrow; a histamine-fast achlorhydria and made a satisfactory response to vitamin B_{12} therapy. Many had low serum levels of B_{12} before therapy and also showed the typical pattern of pernicious anaemia with the standard Schilling test. Four patients who had had total gastrectomy carried out between two and five years previously were also studied.

All 16 control subjects excreted at least 8·1% of the oral unbound dose in the first 24 hours with a range of 8·1 to 32·1%, whereas of the 13 pernicious anaemia and four total gastrectomy patients the maximum excretion was 3·6% of the oral unbound dose with a range of 0·4 to 3·6% (Table).

TABLE

THE URINARY EXCRETION OF FREE AND BOUND B_{12} DOSES IN 24 HOURS

Subjects	*% of Dose Excreted*		*Ratio $^{57}Co/^{58}Co$*
	^{58}Co B_{12}	*^{57}Co B_{12} G.J.*	
Controls (16)	8·1 to 32·1 (16·2 ± 7·8)	10·0 to 30·6 (18·5 ± 6·3)	0·85 to 1·55
Pernicious anaemia (13)	0·8 to 3·6 (1·6 ± 0·8)	1·8 to 10·8 (5·2 ± 2·3)	2·0 to 6·0
Total gastrectomy (4)	0·4 to 2·2	2·8 to 10·6	2·13 to 7·0

In the 16 control subjects the excretion of B_{12} bound to gastric juice was similar to that of the unbound, being 10·0 to 30·6% of the oral dose. However, in the 13 pernicious anaemia patients and four total gastrectomy patients the excretion of bound B_{12} was increased and lay in the range of 1·8 to 10·8% of the oral dose. Thus the ratio of bound to unbound B_{12} in the control group was 0·85 to 1·55 but in those subjects with pernicious anaemia or with total gastrectomy it was much higher, being respectively 2·0 to 6·0 and 2·13 to 7·0.

The ratio of bound to unbound B_{12} was similar in the 24- and 48-hour samples and no better discrimination between controls and subjects deficient in intrinsic factor was obtained by the analysis of the urine collected over the longer period.

[2]Ekco Electronics type N.610 A.

DISCUSSION

Our experience has been similar to that of Katz *et al.* (1963) in that we have found the combined test to be a reliable method for the differentiation of normal subjects from those with intrinsic factor deficiency.

The combined test is more acceptable for patient, clinician, and laboratory staff than the standard Schilling test. It is more convenient for the patient, especially the out-patient, in that it requires only one urine collection rather than two. The clinician benefits since the result is available more rapidly. Extra work is involved in collection of the gastric juice and preparation of the bound B_{12} complex. However, some 40 tests can be performed with the gastric juice obtained from one subject and the labour involved in the laboratory is balanced by the fact that fewer specimens need to be assayed for radioactivity.

The test is dependent on the use of gastric juice containing an adequate amount of potent intrinsic factor. For this reason we have obtained gastric juice from a number of subjects with normal acidity and pooled the samples. However, it would be more satisfactory if the actual content of intrinsic factor in the gastric juice sample was determined before the preparation of the complex.

We have attempted in a small number of cases to substitute commercially available hog stomach preparations for human gastric juice as a source of intrinsic factor in this test, but, as might be expected, the results were not satisfactory. Adams and Seaton (1961) found it was necessary to use at least 60 mg. of hog stomach intrinsic factor with 0·5 μg. of radioactive vitamin B_{12} in order to achieve satisfactory absorption. In terms of the quantities used this represents a gross excess of intrinsic factor which appears to be necessary to promote adequate absorption. Such an excess of intrinsic factor is clearly impracticable in the combined test, as the intrinsic factor must be fully bound to one of the radioactive labelled vitamin B_{12} components and not be available to bind the other free radioactive vitamin B_{12}.

Our technique has differed from that of Katz *et al.* (1963) in that we have used ^{57}Co B_{12} instead of ^{60}Co B_{12} in the B_{12} gastric juice complex. Seltzer, Kereiakes, and Saenger (1964) have calculated the radiation dose to subjects associated with B_{12} labelled with various radioactive isotopes of cobalt. Using their data it is calculated that by substituting ^{57}Co for ^{60}Co the total radiation given to a standard man from the bound and unbound B_{12} doses is reduced

by a factor of 9. As ^{57}Co is easily discriminated from ^{58}Co it would seem preferable to use ^{57}Co instead of ^{60}Co in the bound B_{12} complex.

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