Simultaneous free and bound radioactive vitamin B₁₂ urinary excretion test

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SYNOPSIS The absorption of vitamin B₁₂ following the simultaneous administration of ⁵⁸Co B₁₂ and a complex of ⁵⁷Co B₁₂ 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 B₁₂ is useful in the investigation of patients suspected of having vitamin B₁₂ deficiency (Van Kampen and Graafland, 1959; Adams and Seaton, 1961). In this test a small oral dose of vitamin B₁₂ labelled with radioactive cobalt is given to the patient. Simultaneously a large intramuscular dose of non-radioactive B₁₂ 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 B₁₂ labelled with ⁶⁰Co is given orally to the patient together with vitamin B₁₂ labelled with ⁶⁰Co, bound to normal human gastric juice. A large intramuscular dose of non-radioactive B₁₂ is simultaneously administered and the amounts of ⁶⁰Co and ⁶⁰Co excreted in the urine are separately determined. Thus both the absorption of B₁₂ and the effect of intrinsic factor may be assessed.

The present work describes a modification of this technique in which ⁵⁷Co B₁₂ is substituted for ⁶⁰Co B₁₂ thus reducing the dose of radiation to the patient. Normal subjects, patients with pernicious anaemia, and patients with total gastrectomy were investigated.

PREPARATIONS OF VITAMIN B₁₂ SOLUTIONS

Preparations of vitamin B₁₂ labelled with ⁵⁷Co and with ⁶⁰Co of specific activity 1 microcurie per microgram B₁₂ in freeze-dried form were obtained from the Radiochemical Centre, Amersham.

1) ⁵⁷Co B₁₂ SOLUTION An aqueous solution of ⁵⁷Co B₁₂ containing 1-25 μg per 100 ml. was prepared.

2) ⁵⁷Co B₁₂ GASTRIC JUICE COMPLEX Human gastric juice was obtained from normal subjects following maximal histamine stimulation (Kay, 1953). This was combined with ⁵⁷Co B₁₂ to form a saturated ⁵⁷Co B₁₂ gastric juice complex following the method of Katz et al. (1963).

To ensure that no unbound ⁵⁷Co B₁₂ 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 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 B₁₂ 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. ⁵⁸Co B₁₂ and the other 0-25 μg. ⁵⁷Co B₁₂ gastric juice complex. Simultaneously and 24 hours later, 1,000 μg. of non-radioactive B₁₂ 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 ⁵⁸Co can be distinguished from the 0-12

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Mev. gamma rays emitted by $^{57}$Co by altering the
discriminator bias settings on a scaler$^3$ which was coupled
to the scintillation counter.

The amounts of $^{54}$Co and $^{57}$Co excreted were calculated
by the method of Veall and Vetter (1958) for two com-
ponent 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. Thir-
ten 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 achlorhydia
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>
<thead>
<tr>
<th>Subjects</th>
<th>% of Dose Excreted</th>
<th>Ratio $^{57}$Co/$^{54}$Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (16)</td>
<td>$8.1 \pm 2.1$</td>
<td>$10.0 \pm 3.0$</td>
</tr>
<tr>
<td>Addisionian pernicious (13)</td>
<td>$8.0 \pm 2.0$</td>
<td>$18.5 \pm 6.3$</td>
</tr>
<tr>
<td>Total gastrectomy (4)</td>
<td>$0.4 \pm 2.2$</td>
<td>$2.8 \pm 10.6$</td>
</tr>
</tbody>
</table>

In the 16 control subjects the excretion of B$_{12}$
bound to gastric juice was similar to that of the un-
bound, 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 dis-
crimination between controls and subjects deficient
in intrinsic factor was obtained by the analysis of
the urine collected over the longer period.

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 radio-
active 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 absorp-
tion. 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

$^{*}$Ekco Electronics type N.610 A.
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by a factor of 9. As $^{57}\text{Co}$ is easily discriminated from $^{56}\text{Co}$ it would seem preferable to use $^{57}\text{Co}$ instead of $^{60}\text{Co}$ in the bound B\textsubscript{12} complex.

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