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PRELIMINARY NOTE

Determination of Absorption of Vitamin B_{12} by a Double Isotope Tracer Technique

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The conventional methods for the quantitative estimation of the absorption of labeled vitamin B_{12} require either a prolonged collection of faeces (1) or a complete 24-hour urinary collection, as in the Schilling's test (2). It is usually necessary to admit the patient to a hospital for this investigation; even then, a reliable total collection of urine or faeces is impossible without intelligent cooperation from the patient and the nursing staff. Furthermore, the Schilling's test, which is by far the most commonly employed procedure, does not give a true quantitative measure of vitamin B_{12} absorption, as it measures only that per cent of the administered dose which is flushed out into the urine after a large parenteral dose of stable vitamin B_{12}. This flushing dose also hinders subsequent hematological studies.

The new technique described in this paper gives an accurate estimate of vitamin B_{12} absorption by counting radioactivity in an aliquot of a random sample of faeces. An unabsorbable marker in the form of ^{51}Cr labeled chromic oxide is administered orally with ^{58}Co labeled vitamin B_{12}. The ratio of the two radioisotopes in the administered dose is compared with the ratio of these isotopes in a sample of faeces collected after 24 or 48 hours. Any change in the ratio would be due to the absorption of labeled vitamin B_{12} and the proportion of ^{58}Co labeled vitamin B_{12} absorbed is calculated by reference to the ^{51}Cr excreted.

METHOD

A mixture of 5 μc of ^{51}Cr labeled chromic oxide and 1 μc of ^{58}Co labeled vitamin B_{12} was administered orally to fasting patients. The specific activities of chromic oxide and labeled vitamin B_{12} were 12 mc/mg and 1 $\mu c/\mu g$, respectively. Two samples of faeces were collected from each patient, the first after 24 hours and the second after 48 hours, directly in a small wide-mouth plastic bottle (250 ml capacity). The standard was mixed with 250 ml of water and counted in bottles identical to those used for faecal collection.

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The counting of radioactivity in the sample was done by placing the bottle in front of a 2 inch sodium iodide crystal probe which was connected to a gamma ray spectometer. Figure 1 shows the gamma ray spectrum of a mixture of ^{51}Cr and ^{58}Co. ^{51}Cr has only one gamma emission at 0.32 mev, while ^{58}Co has a principal gamma emission of 0.81 mev and an additional small annihilation peak of positron emission at 0.51 mev. The gamma energies of ^{51}Cr and ^{58}Co are widely separated and counting of the individual radioactivity of the two isotopes can be easily accomplished by differential counting with appropriate window settings. With the small amount of radioactivity administered to our patients, the count rates obtained with the stool samples were too low to permit statistically satisfactory computations unless prolonged counting of each sample was undertaken. We overcame this difficulty by adopting a method of 'surplus integral counting'. This was done by putting a base level cut off at the point shown in Fig. 1. Counts obtained at this base level setting were due to ^{58}Co only; counts in the ^{51}Cr region were obtained by subtracting these counts from the total integral counts obtained without any base level cut off. However, counts in the ^{51}Cr region were due to both the ^{51}Cr counts and the Compton scatter contribution from ^{58}Co. To obtain true counts due to ^{51}Cr only, the scatter contribution of ^{58}Co had to be subtracted from the counts in the ^{51}Cr region. To do this, a sample of ^{58}Co alone was counted in a similar bottle, with and without the base level cut off, and a ratio of ^{58}Co counts in the cobalt region and the chromium region was obtained. This ratio was applied to the observed counts of ^{58}Co in a sample containing a mixture of ^{51}Cr and ^{58}Co, and an appropriate deduction made from the ^{51}Cr region for the scatter contribution of ^{58}Co.

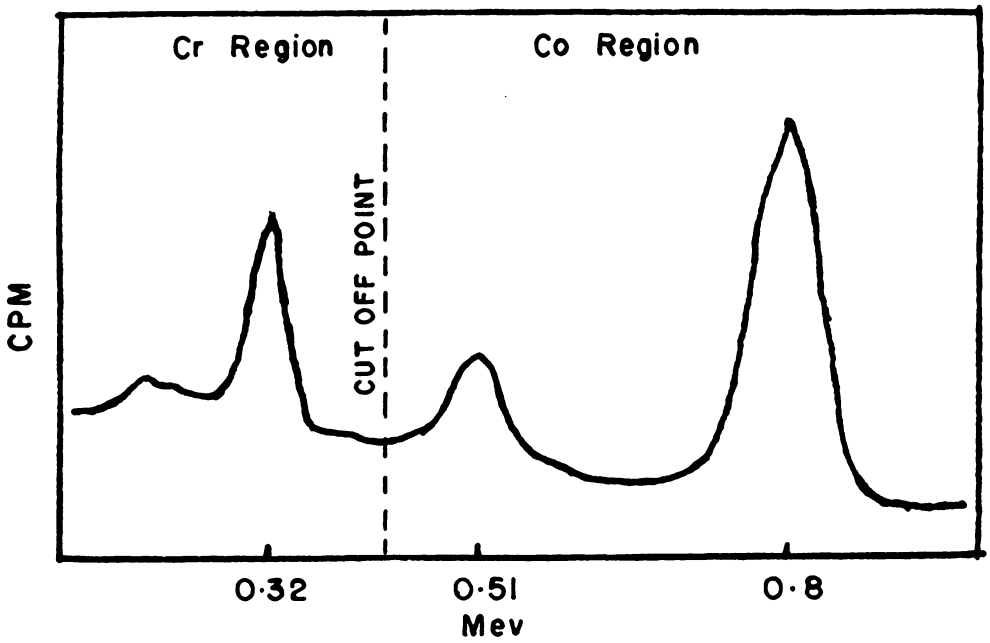

Fig. 1. A gamma ray spectrum of a mixture of ^{51}Cr and ^{58}Co, showing the base level cut-off point used in surplus integral counting method of separating ^{51}Cr and ^{58}Co.

A typical set of calculations would run as follows:

1. Net counts in the Cr region
   = Counts without cut off—Counts with base level cut off—Scatter counts due to Co in the Cr region.
2. Net counts in the Co region
   = Counts obtained with base level cut off.
3. Net counts due to Compton scatter in Cr region
   = Observed counts in (2) $\times \frac{\text{Counts in Cr region}}{\text{Counts in Co region}}$ of a standard of ^{58}Co alone.

A series of linear simultaneous equations comparing the net counts due to ^{51}Cr and ^{58}Co in the standard and the stool sample lead to the following:

4. % excretion of ^{58}Co–vitamin B_{12} in the stools
$$= \frac{^{51}Cr \text{ in Std.} \times ^{58}Co \text{ in stool} \times 100}{^{51}Cr \text{ in stool} \times ^{58}Co \text{ in Std.}}.$$
5. % absorption of ^{58}Co–vitamin B_{12}
   = 100 − % excretion of ^{58}Co–vitamin B_{12} in stools.

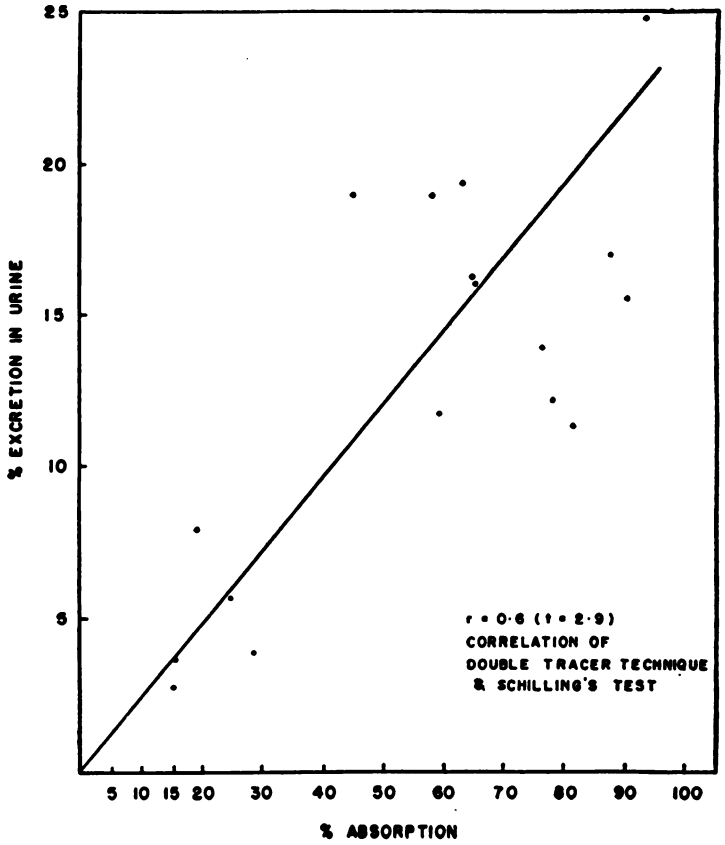

Fig. 2. Correlation between double tracer technique and the Schilling test. X axis shows % absorption as determined by the double tracer technique, and the Y axis shows % excretion in urine as determined by the Schilling test.

The new technique was compared with the Schillings's test in 17 patients, and with the method of total collection of faeces in two patients. The collected faeces were counted in an annular G.M. Counter. In 3 normal subjects, whole body counting was carried out together with the double tracer technique. The whole body counting was done with a 9 inch solid sodium iodide crystal placed in a low background steel room.

RESULTS

Figure 2 shows good correlation between the double tracer technique and the Schilling's test. Figure 3 emphasizes the fact that the ratio of ^{51}Cr and ^{58}Co does not differ significantly in the 24-hour and 48-hour sample, and that almost identical results are obtained with either of the samples. Table I shows the comparison of results of the new technique and those of the total faecal collection and whole body counting. The number of patients in each group is too small to permit any statistical tests. However, agreement among the results obtained with different methods is striking.

DISCUSSION

It is a well recognized fact that the addition of a nonabsorbable marker to a test meal facilitates the estimation of the absorption of a test substance (3-8). Chromic oxide has been a favourite choice in the past as an unabsorbable marker (6), but labeled chromic oxide has been seldom used for this purpose. The advantages of using labeled marker together with the labeled test substance are obvious. Availability of reliable gamma scintillation spectrometers has simplified the counting of a mixture of several radioisotopes, and, in many cases, *i.e.* the present study, it is possible to count the radioactivity of each isotope in a mixture separately, without any chemical processing. While attempting to estimate the absorption of ^{58}Co labeled vitamin B_{12}, ^{51}Cr labeled chromic oxide becomes a natural choice, as it is easy to separate the gamma energies of these radioisotopes with a spectrometer.

TABLE I

COMPARISON OF DIFFERENT METHODS OF ESTIMATING VITAMIN B_{12} ABSORPTION

No. of Patients	*Double Tracer Technique % Absorption*	*Total Faecal Collection % Absorption*	*Whole Body Counting % Absorption*
1	93.1%	97.3%	—
2	63.5%	70 %	—
3	68	—	61.2%
4	81	—	69 %
5	77	—	63 %

The results show that by counting of any random sample of faeces it was possible to obtain a satisfactory measure of vitamin B_{12} absorption. Collection of faeces directly in the wide-mouth counting bottles avoids any handling of faecal material. The similarity of the results obtained by counting the 24-hour and the 48-hour faecal samples suggests that a stool sample collected anytime in the first 48 hours after the administration of the dose, can be a substitute for the total collection of faeces. It also indicates that the unabsorbed chromic oxide is not handled any differently from the unabsorbed vitamin B_{12} in the intestinal tract. Both the unabsorbed isotopes appear to be thoroughly mixed in the intestine, so that any faecal sample would give the same relative proportions of the two isotopes.

^{51}Cr and ^{58}Co are administered in the ratio of 5:1 to compensate for the different counting efficiency of the scintillation crystal for the two radioisotopes. Certain error is involved in the fact that the standard is counted in an aqueous form, while the stool sample is either in semi-solid or solid form. We are continuing our experiments to minimise this error. One way to achieve this would be the use of a larger scintillation crystal.

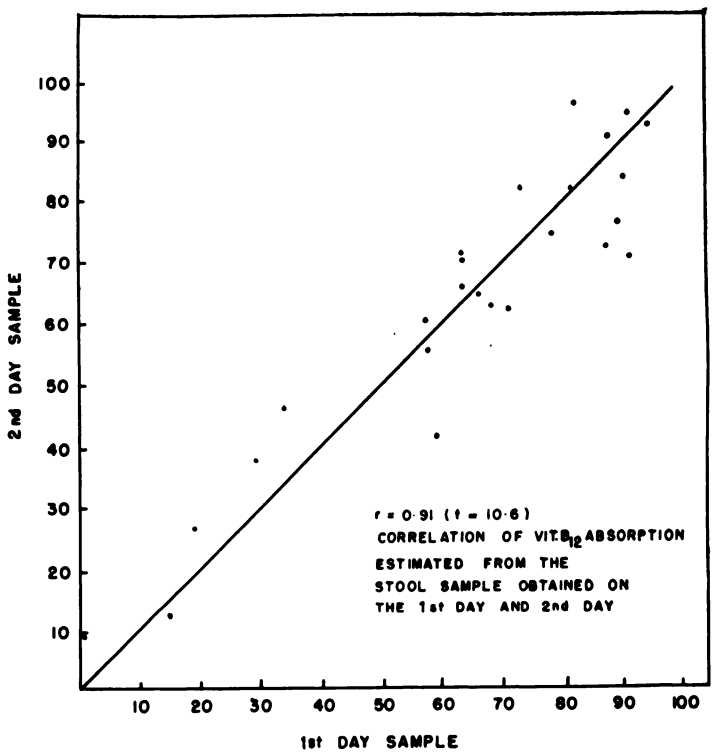

Fig. 3. X axis shows the % absorption of labeled vitamin B_{12} as estimated from a stool sample collected 24 hours after the administration of a mixture of labeled chromic oxide and labeled vitamin B_{12}. Y axis shows the absorption value obtained from the stool sample collected after 48 hours.

The present method showed good correlation with Schilling's test, total faecal collection and whole body counting. The former two require accurate collection of excreta and the latter requires prohibitively expensive equipment. As the new method does not entail cumbersome 24-hour collection of urine and prolonged collection of faeces, it is eminently suitable as an out-patient procedure in a busy general hospital.

SUMMARY

A new simple method for the study of vitamin B_{12} absorption involving the use of two tracers is described. Along with ^{58}Co labeled vitamin B_{12} an unabsorbable marker in the form of ^{51}Cr labeled chromic oxide is administered to the patients. The ratio of the activities of the two isotopes in the standard is compared with the ratio of the activities of these isotopes in an aliquot of a stool sample collected after 24 or 48 hours. The absorption of labeled vitamin B_{12} is estimated from the alteration in the ratio of absorbable and unabsorbable isotopes in the stool sample. The method compares well with other methods of estimating vitamin B_{12} absorption such as Schilling's test, total faecal collection and whole body counting.

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