A PHYSICIST’S INTERPRETATION OF SOME ASPECTS OF VITAMIN B\textsubscript{12} METABOLISM AND ITS USE TO ROUTINELY ESTIMATE TOTAL-BODY B\textsubscript{12}

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It is with great deference that a physicist offers an interpretation of material derived from clinical studies. In mitigation, a serious attempt has been made to understand some of the clinical problems of our medical colleagues to improve the effectiveness of collaboration. As would be expected, a physicist lacks a detailed knowledge of vitamin B\textsubscript{12} metabolism and of the pertinent literature, but this detachment may avoid preconceptions. Although the interpretation will consequently be oversimplified, it might be adequate for practical purposes, particularly for other nonspecialists.

Many studies of vitamin B\textsubscript{12} metabolism are based on the simple hypothesis of effective “equilibrium” in the body between administered doses of tracer B\textsubscript{12} and native B\textsubscript{12}, which are then assumed to behave identically. When this assumption was disputed (1–4), it was necessary to consider its basis more closely and to demonstrate whether it was valid or not. Despite an extensive literature, no comprehensive examination of available data appears to have been undertaken in this particular respect. A preliminary report (5) concluded that there was clinical evidence in favor of the simple hypothesis of “equilibrium” and that it was an adequate approximation for most clinical purposes. A more detailed consideration of this hypothesis is presented here from which an interpretation of some aspects of vitamin B\textsubscript{12} metabolism has been developed.

METABOLIC “EQUILIBRIUM” OF VITAMIN B\textsubscript{12} AND ITS IMPLICATIONS

If metabolic “equilibrium” is established between tracer and native B\textsubscript{12}, then its implications can be related to the available experimental data. This infers the following five points:

1. Vitamin B\textsubscript{12} in the body is effectively in a single compartment. A kinetic analysis by Reizenstein and colleagues (3,4) chose initially three compartments but, paradoxically, showed that 99.3\% of B\textsubscript{12} was in a single compartment while a further 0.3\% had been allocated to a second compartment before the analysis was carried out. It was not demonstrated whether 99.3 or 99.6\% was significantly different from 100\%, presumably because an analog computer was used. For most practical purposes, it seems reasonable to assume that the difference is not significant and that to a first approximation, all of the body B\textsubscript{12} is effectively in a single compartment.

2. The specific activity of significant tissues should be the same. When “equilibrium” is established after administration of B\textsubscript{12} labeled with a radionuclide, the specific activity (m\textsubscript{Cl}/gm vitamin) should be the same in all relevant tissues. A significant correlation (r = 0.84) was obtained between the concentration of labeled and native B\textsubscript{12} in the tissues of a rooster, pig, and calf which were slaughtered only 5, 7, and 16 days, respectively, after the final injection of tracer (6). Similar findings have been reported in dogs (7) and in rats (8,9).

3. The excretion rate should be adequately described by a single exponential term. Using whole-body monitors, it has been possible to measure the body turnover of labeled vitamin B\textsubscript{12} over periods of up to 2 years or more. The excretion rate could be adequately described by a single exponential term (10–14), and in our studies (15,16) there was a standard deviation of about 10\%. We further showed (16) that the excretion rate did not change significantly after about 1 week postadministration of a tracer dose (0.1 \mu g i.v.) of B\textsubscript{12}.

4. Excretion rates should correspond with B\textsubscript{12} turnover rates in other tissues. Whole-body excretion rates (0.1–0.2\%/day) agree well with hepatic clearance rates (0.08–0.46\%/day) (17–19) and with long-term plasma turnover rates (0.1\%/day) (20). In a recent study (21), long-term whole-body excretion rates were compared with plasma turnover.
in the same patients following massive intravenous doses of vitamin B₁₂. Good agreement was obtained.

The rate of loss of nonlabeled B₁₂ in feces was estimated indirectly by microbiological assay of bile (22). A rate of only 0.03%/day was obtained. However, this value is probably an underestimate of loss from the body since it ignores both urinary losses and the possibility that much of the vitamin may have been in a microbiologically inactive form (20).

5. The amount of B₁₂ lost daily from the body as estimated by the simple model should correspond with the daily intake. At "equilibrium," the daily loss of B₁₂ (μg) is obtained simply by multiplying the excretion rate (0.1–2%/day) by the total-body content of B₁₂ (1,000–3,500 μg). The daily loss is then about 1–7 μg/day which is in agreement with an estimated dietary absorption of 2–9 μg B₁₂/day (12).

The simple assumption of "equilibrium" between tracer and native B₁₂ and its implications seem to be in reasonable accordance with available clinical and experimental evidence. On the other hand, the final exponential term by kinetic analysis (0.036%/day) (3,4) agrees only with the estimated loss of nonlabeled B₁₂ (22) which is itself evidently an underestimate for the reasons discussed above.

EXTENSION OF THE HYPOTHESIS OF "EQUILIBRIUM"

If the hypothesis of "equilibrium" is accepted, then an interpretation, which unifies and is supported by previously isolated findings from several disciplines, can be developed for other aspects of vitamin B₁₂ metabolism as is shown diagrammatically in Fig. 1. The hypothesis is that vitamin B₁₂ is taken into the body primarily as hydroxocobalamine, with possibly some unconverted or unhydrolyzed coenzyme B₁₂ and cyanocobalamine and converted to, stored, utilized, and transferred to the excretory system in a common form, possibly as coenzyme B₁₂. This simple concept obviously requires justification and supporting evidence although the specialist may consider that certain aspects have been well established already in his particular field of expertise.

There are several analog forms of vitamin B₁₂, the most important being hydroxocobalamine, cyanocobalamine, methylcobalamine, and coenzyme B₁₂ (α-(5′:6-diamethylbenzimidazolyl) 5′-deoxyadenosyl cobamide). The bulk of vitamin B₁₂ in the body is evidently in the coenzyme form (23,24) and will be primarily in this form in raw meats from animals, which constitute the major dietary source of the vitamin. However, coenzyme B₁₂ is readily hydrolyzed to hydroxocobalamine and will generally be in the latter form in food since few meats are eaten uncooked. The conversion or reconversion in vivo of hydroxocobalamine to coenzyme B₁₂ has been demonstrated (25,26). As indicated above, there is evidence that the vitamin is stored and used in the body as coenzyme B₁₂. It would then follow that whatever form of the vitamin was administered, following "equilibrium" the excretion rate should be the same since only a common form, possibly coenzyme B₁₂, reaches the excretory system and is subsequently lost to the body. It would not necessarily follow that the vitamin B₁₂ will be in this form in excreta since there is evidence (27) that conversion can take place in urine after excretion by the kidney and an analogous situation may pertain in losses, via bile, in feces. In this case, the microbiological activity of coenzyme B₁₂ in vitro would not contradict the present hypothesis and the suggestion (20,22) that only a fraction of excreted vitamin B₁₂ is microbiologically active.

On the basis of the simple "equilibrium" model some quantitative confirmation of the present hypothesis can be derived:

1. The daily absorption (μg) should equal the conversion rate of hydroxocobalamine: coenzyme B₁₂ and the daily loss. As discussed earlier, the daily loss estimated from the model agreed with the daily absorption, amounting to 1–10 μg/day.

Unfortunately, few data are available describing the amount of hydroxocobalamine or cyanocobalamine converted daily to coenzyme B₁₂ (25,26). Uchino and colleagues (26) studied the in vivo conversion in rats of the analogs to coenzyme B₁₂ up to 24 hr after parenteral administration of 0.1 μg of B₁₂ dose. The ranges obtained were 6.3–11.4 μg hydroxo-
cobalamin per day and 2.1–3.0 μg cyanocobalamin per day. To permit a crude extrapolation to man and to facilitate comparison with the findings of Pawelkiewicz and colleagues (25), these ranges can be expressed from the data given as 32–76 μg/kg body weight/day and 11–20 μg/kg body weight/day, respectively. The equivalent conversion rate of hydroxocobalamin in man would be about 2–5 μg/day which is similar to the daily absorption and excretion.

In rabbits (25) the in vivo conversion of cyanocobalamin amounted to 600 μg/kg body weight/day, and a similar value could be deduced from parallel in vitro studies (25) using slices of human liver and kidneys. This value is higher than that found by Uchino et al (26), possibly because a comparatively large dose of 10 μg cyanocobalamin/kg body weight was injected into the rabbits and in vitro 6 μg per 5–10 gm liver or kidney was used. Although this explanation is speculative, if it were correct, it might imply that the rate of conversion can be increased in response to a large dose of B₁₂ in excess of the normal daily intake.

Despite the obvious uncertainties involved in the extrapolation of animal data to man, it is encouraging that the values obtained tend to support the hypothesis and that the lower estimates of the conversion rate would be compatible with the estimated daily balance of vitamin B₁₂ in man.

2. The metabolic pattern of large intramuscular doses of hydroxocobalamin and cyanocobalamin should be qualitatively similar, but the quantity retained will be roughly proportional to the conversion rates of these analogs to coenzyme B₁₂. The metabolic pattern of a similar dose of coenzyme B₁₂ will differ from that of the other analogs. The retention of 1,000 μg intramuscular doses of hydroxocobalamin, cyanocobalamin, and coenzyme B₁₂ has been studied by whole-body monitoring (28). The mean percentage retentions at 3 and 28 days are summarized in Table 1. The ratio of hydroxocobalamin: cyanocobalamin retained at 3 days is about 3:1 and is a similar value at 28 days. Their rates of conversion (μg/day) to coenzyme B₁₂ are also in the ratio of about 3:1 (26). This suggests that the metabolism of these analogs is similar. Differences in the absolute amount retained may be explained in simple terms if the conversion rates are proportional to the respective number of reaction sites available for conversion to be effected; viz., preferential conversion of hydroxocobalamin will be associated with proportionately greater retention.

The ratio of coenzyme B₁₂: cyanocobalamin retained at 3 days is about 2.7:1, but at 28 days the ratio is significantly less, 1.4:1. Evidently the metabolism of coenzyme B₁₂ is different from that of the other analogs until "equilibrium" is reached. This would be expected if hydroxocobalamin and cyanocobalamin must undergo conversion, while coenzyme B₁₂ is already in the form in which the vitamin is used, stored, and passed to the excretory system.

Differences in the metabolism of coenzyme B₁₂ and cyanocobalamin in rats (29,30) and in man (31) have been noted which seems to support this interpretation. The transport and tissue distribution were different as shown by preferential uptake of coenzyme B₁₂, especially by liver and kidneys (29), and by the dissimilar clearance rates from blood (29,31) possibly due to the greater binding capacity of plasma for coenzyme B₁₂ than for cyanocobalamin.

3. The calculated period for conversion to coenzyme B₁₂ of the hydroxocobalamin and cyanocobalamin retained following massive intramuscular doses should correspond roughly to the period at which "equilibrium" is established and a steady rate of loss is established. Following intramuscular injection of 5,000 μg vitamin B₁₂, there is a very rapid loss over a period of a few days, then less rapid loss during a few months, and thereafter a slow rate of loss (15). This is shown diagrammatically in Fig. 1. About 10% (500 μg) of hydroxocobalamin and about 5% (250 μg) of cyanocobalamin are retained. From the respective conversion rate of about 2–5 μg/day and 0.7–1.4 μg/day, the retained analogs will have been largely converted by about 150 days, or less if the conversion rate is higher (25). As predicted, it is also about this time that a steady rate of loss is established following intravenous doses of 5,000 μg (15).

4. When "equilibrium" has been reached following parenteral doses of hydroxocobalamin, cyanocobalamin, and coenzyme B₁₂, the steady rate of loss should be the same. If administered hydroxocobalamin and cyanocobalamin have been converted

| Table 1. Metabolism of 1,000 μg Intramuscular Doses of Vitamin B₁₂ Analogs |
|-----------------------------|-------------------|-------------------|
| Analog                      | At 3 days         | At 28 days        |
| Hydroxocobalamin            | 42.1              | 32.5              |
| Cyanocobalamin              | 14.3              | 11.5              |
| Coenzyme B₁₂                | 40.9              | 18.5              |
to coenzyme B₁₂, then a common form will reach the excretory system.

Consequently, the excretion rates following "equilibrium" should be independent of the form of the administered analog. Excretion rates measured in man up to 488 days postadministration of 5,000-μg vitamin B₁₂ showed no significant difference among the analogs (15), and similar findings have been reported in rats (30).

THE ROUTINE ESTIMATION OF TOTAL-BODY B₁₂

Serum levels of vitamin B₁₂ are a factor considered in the diagnosis of pernicious anemia. It is implied that the fraction of total-body B₁₂ in serum is the same among individuals. In some circumstances it might be more meaningful to obtain an estimate of the total-body vitamin B₁₂.

It was shown (16) that following a tracer dose of labeled vitamin B₁₂ the excretion rate was effectively constant from about 8–10 days postadministration when about 95% of the dose was retained. If "equilibrium" between the tracer B₁₂ and native B₁₂ was established by this time, then the specific activity (μCi/gm) of tissues and fluids would be the same. Thus if a random urine sample is obtained and assayed:

\[
\frac{0.95 \times \text{administered dose (μCi)}}{\text{Total-body B₁₂ (μg)}} = \frac{\text{Activity/ml urine (μCi/ml)}}{\text{B₁₂ content/ml urine (μg/ml)}}
\]

Hence:

\[
\text{Total-body B₁₂ (μg)} = 0.95 \times \text{administered dose (μCi)} \times \frac{\text{B₁₂ content/ml urine (μg/ml)}}{\text{Activity/ml urine (μCi/ml)}}
\]

This is simply a statement of isotope dilution with a factor of 0.95 to correct approximately for loss of the tracer dose. If a dose identical to that administered or a known fraction of that administered is counted in the same geometry as the urine sample, then the ratio of the counting rates represents the activity content (μCi).

The test would involve administering the tracer dose to the patient intravenously and preparing an identical standard or known fraction. About 1–2 weeks later, the patient provides a urine sample. A known volume of urine is counted in the same volume and geometry as the standard and a further known volume of urine is assayed to give the amount of vitamin B₁₂ in each volume (μg B₁₂/ml). The total-body B₁₂ can then be estimated from the relationship.

In practice, the test would be simple, avoiding the necessity for complete urine collection; in addition it uses routinely available facilities and permits examination on an outpatient basis. It can be incorporated with measurements of vitamin B₁₂ absorption by whole-body monitoring, which is being used increasingly. An obvious potential problem is the fact that only a fraction of the vitamin B₁₂ excreted in urine may be assayable by conventional methods such as microbiological assay (20,22). Investigations are in progress to assess the test experimentally.

In principle, the test avoids the assumption implied in the measurements of serum B₁₂ that the fraction of total-body B₁₂ in the serum is the same among individuals. However, it substitutes the assumption of "equilibrium," for which the justification has been submitted here.

CONCLUSIONS

Following administration of a tracer dose of vitamin B₁₂, the simple and common assumption of "equilibrium" and the assumption that the tracer and native B₁₂ behave identically from about 1 week postadministration seem to agree reasonably well with available clinical and experimental data. The alternative of kinetic analysis appears to offer no advantage in interpreting B₁₂ metabolism and evidently leads to the conclusion that effectively all of the native B₁₂ is in a single compartment as implied by the simple assumption of equilibrium. Because computing facilities and mathematical skills are not required in its interpretation, the simple model is not inferior per se and some might even consider this an advantage. Although the model may be an oversimplification, it appears to be an adequate approximation for most practical clinical purposes.

The assumption of "equilibrium" can be extended to the metabolism of B₁₂ analogs. A simple interpretation that hydroxocobalamin and, to a lesser extent, unhydrolysed coenzyme B₁₂, and cyanocobalamin are the principal dietary forms and that these analogs are converted to, stored, utilized, and, following "equilibrium," pass to the excretory system in a common form possibly as coenzyme B₁₂ seems to explain much of the relevant published data. The present hypothesis does not consider the absorption mechanism of vitamin B₁₂, but it may be capable of extension to this complicated aspect.

A method of estimating total-body B₁₂ is proposed, based on the assumption of "equilibrium," which involves only the measurement of the specific activity of a casual urine sample about 1–2 weeks postadministration of a labeled tracer dose. Complete urine collection is not required nor is sophisticated equipment needed. The test can be made on an outpatient basis using routinely available techniques. It is currently being evaluated experimentally.
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