Studies on Urinary Excretion of Vitamin $\text{B}_12\text{Co}^{60}$ in Pernicious Anemia for Determining Effective Dosage of Intrinsic Factor Concentrates

By William R. Best, Wilfrid F. White, Kenneth C. Robbins, Wendell A. Landmann and Sanford L. Steelman

The normal human intestine can absorb significant amounts of the usually ingested quantities of vitamin $\text{B}_12$ only in the presence of intrinsic factor. In recent years three different tests using orally administered, $\text{Co}^{60}$-labeled vitamin $\text{B}_12$ have been proposed for the estimation of intrinsic factor activity. One measures the unabsorbed tracer in the stools; another detects radiation emanating from the liver, where the absorbed vitamin becomes concentrated after a number of days; and the third measures urinary radioactivity following a large parenteral dose of nonradioactive vitamin $\text{B}_12$. Most of the parenteral dose is excreted in the urine and carries along a significant amount of the absorbed radioactive vitamin $\text{B}_12$. The first test requires analysis of total fecal collections for five to ten days. The second necessitates repeated body surface counts over a number of sites using a scintillation counter. Reliable quantitation requires identical measurements after a standard parenteral dose of tracer vitamin. This test does not appear to detect the minute amounts of absorption seen in patients with pernicious anemia given no intrinsic factor.

The third, or urinary excretion test is much the easiest to perform. The amount of radioactivity in the twenty-four hour urine can be directly quantitated in terms of the administered vitamin $\text{B}_12\text{Co}^{60}$. Minute amounts of radioactivity in the urine are easily and accurately measured. The theoretic disadvantage of this test lies in the unknown percentage of absorbed vitamin which is "flushed" into the urine in each individual tested. We will discuss this problem subsequently. Considering all of these factors, we have standardized our procedure using the urinary excretion test almost exclusively.

Studies reported elsewhere show a fairly good consistency of repeated urinary excretion tests in patients with pernicious anemia given known doses of intrinsic factor concentrates. Because of this it was decided to investigate the value of this test for the assay of intrinsic factor concentrates and for determination of optimal dosage schedules to be used in the oral therapy of pernicious anemia.

Methods

Urinary excretion test: clinical phase: All tests herein reported were done on established cases of pernicious anemia in partial or complete therapeutic remission at the time of testing. In the majority of instances the procedure of Schilling was followed. Usually, at 8 to

From the Department of Medicine, University of Illinois College of Medicine, Chicago, and the Biochemical Research Department, The Armour Laboratories, Chicago, Illinois. Preliminary abstracts concerning parts of this work have been published.$^1$ $^2$

Aided by a grant from Armour and Company, Chicago, Illinois. Technical assistance by Miss Diana Korbakas.

Submitted August 8, 1955; accepted for publication January 12, 1956.
9 a.m. two micrograms of B₁₂Co₆₀ (specific activity, 0.17 to 0.22 μC/μg) were taken orally in the fasting state, and the container was washed with water so that the subject drank a total of approximately 100 ml. Intrinsic factor concentrates, when given, were mixed with this fluid immediately before administration. Two hours later breakfast was allowed, and the subject was given 1000 μg non-radioactive vitamin B₁₂ intramuscularly or sub-cutaneously. A control urine specimen was always collected to establish urinary background of radioactivity. Urine was then collected as a total specimen or in selected fractions from the time of oral administration until twenty-four hours later. In most instances these studies were performed in the hospital under supervision. In a number of cases, however, studies were done on out-patients. The “flushing” dose of non-radioactive vitamin was given within fifteen minutes of the oral material in some such instances. This appears to give slightly lower recoveries. This was not done in enough cases to appreciably alter the results. In a number of instances 15 micrograms of B₁₂Co₆₀ (specific activity adjusted to approximately 0.028 μC/μg) were used in place of a two microgram dose. These are identified where used.

Urinary excretion test: radioactivity measurements: Quadruplicate two ml. samples of the twenty-four hour urine specimen were transferred to weighed aluminum planchets and dried. An infra-red lamp at a distance of eight inches provided even heat from above, which allowed drying without splattering. After two hours the samples were placed in a vacuum desiccator containing P₂O₅ for two more hours prior to counting and weighing. This drying procedure was found to give a total solids weight reproducible within a few tenths of a milligram for the four samples in a set.

All samples were counted in a windowless gas-flow counter, using the method of preset counts to assure a reasonable approach to a desired percentage error in the counting rate. For expediency, and to achieve practical counting times, the limits of error for weak samples were arbitrarily assigned to be no greater than 15 per cent while the stronger samples were to be no greater than 5 per cent. These limits could be realized by counting all samples for a total count (background plus sample counts) of one thousand, giving a total count for four replicates of about 4000. The sample count rates for four replicates were averaged after discarding any values falling outside the limits of one standard deviation. If more than two values fell outside these limits, new samples were prepared and counted. In all cases the results represent the average of at least three count rates, and calculated errors are within the limits imposed.

To correct for variation in counter sensitivity and self-absorption due to urinary solids, standard curves were determined for each level of specific activity of vitamin B₁₂Co₆₀ used. Equal amounts of urea and sodium chloride were used to simulate the urine solids. A family of curves was constructed using variable amounts to total solids and variable amounts of B₁₂Co₆₀. After the count rate, Rₘ (counts per minute), and total solids for each sample had been determined, the relationship W (counts per minute per mg B₁₂), of count rate to weight of vitamin B₁₂Co₆₀ could be obtained by interpolation of the appropriate curve. Then the total amount of vitamin B₁₂Co₆₀ excreted in the urine specimen could be calculated as follows:

\[
\text{mg. B₁₂ excreted} = \frac{Rₘ(\text{cts./min.})}{2(\text{ml.})} \times \frac{\text{Urine vol. (ml.)}}{W(\text{cts./min./mg.})}
\]

Results of assays carried out by this method were in good agreement with corresponding values obtained using a well-type scintillation counter.*

Hematologic assay using pernicious anemia in relapse: Data were obtained from a number

---

* Scintillation counts were determined by Dr. R. F. Schilling and by Mr. W. J. Henderson. Representative gas-flow counts and corresponding scintillation counts are: 102 and 100; 10 and 4; 50 and 8; 71 and 70; 86 and 116; and 98 and 100 μg. It is apparent that concentration of urine and measurement in a well-type scintillator or measurement of a large volume over a surface type scintillator are simpler and perhaps more precise than the method here employed.
of physicians* who conducted tests in accordance with the recommendations of the Anti-
Pernicious Anemia Board of the U. S. Pharmacopoeia. All subjects tested were anemic
either as a result of no previous therapy or were in relapse due to a lapse in treatment. Be-
fore the test was started each patient was hospitalized until the physician in charge was
satisfied that the diagnosis of pernicious anemia was correct. In all cases hospitalization
was continued throughout the entire three week period of assay. Intrinsic factor concen-
trates combined with 15 μg. vitamin B₁₂ were given orally an hour or more before breakfast.
Percentage of expected response was calculated on the basis of average red cell count for
two or three days before therapy, average count for two or three days at the twenty-one
day level, and on the U. S. Pharmacopoeia recommend tables. Reticulocyte response, and
red cell response at the end of two weeks showed good agreement with the three week red
cell response, but were not considered in these calculations. Dr. M. C. Wynes of The Armour
Laboratories Medical Department arranged for these assays, and computed the responses.

RESULTS AND DISCUSSION

The B₁₂Co⁶⁶ tests as indicators of intrinsic factor activity: It is generally agreed
that the various tests using B₁₂Co⁶⁶ in pernicious anemia patients are of value
for establishing the presence or absence of intrinsic factor activity in experi-
mental preparations made from stomach, duodenum, or gastric juice. However, there is not agreement regarding the magnitude of response characteristic
for satisfactory intrinsic factor dosage. Experimental intrinsic factor concen-
trates from various laboratories have different proportions of B₁₂-binding activity
unrelated to intrinsic factor, and may actually contain small amounts of
vitamin B₁₂. These considerations as well as minor variations in technic of
testing† may lead to dissimilar relationships between clinical potency and the
B₁₂Co⁶⁶ tests in different clinics.

If non-intrinsic factor contaminants have a great enough B₁₂-binding activity
it is postulated that they will reduce the amount of labile B₁₂Co⁶⁶ available for
intrinsic factor-modulated absorption by the intestine, thus exhibiting an
apparent inhibitory effect. This would be especially evident with higher dosage
ratios of intrinsic factor concentrates to vitamin B₁₂. As the therapeutic use
of intrinsic factor preparations generally involves a larger amount of vitamin
B₁₂ than do the tracer studies, a more favorable ratio would be achieved in the
therapeutic situation. Thus, such a preparation might show good clinical po-
tency but rather poor absorption using the B₁₂Co⁶⁶ tests at lower dosage levels.
It would seem desirable, therefore, to test intrinsic factor preparations with the
same dosage of B₁₂Co⁶⁶ as would be incorporated with them for therapeutic
purposes. Small amounts of vitamin B₁₂ present in an intrinsic factor prepara-
tion become much less significant when a larger B₁₂Co⁶⁶ dose is used for testing.‡

* Drs. M. Block, U. of Colorado Medical Center, Denver, Colorado; G. E. Cartwright,
U. of Utah College of Medicine, Salt Lake City, Utah; Q. B. De Marsh, Seattle, Wash.;
H. L. Goodman, Wayne U. Receiving Hospital, Detroit, Mich.; L. R. Limarzi, U. of Illinois
School of Medicine, Chicago, Ill.; W. Mazzitello, Ancker Hospital, St. Paul, Minnesota;
W. G. Unglaub, Tulane U. School of Medicine, New Orleans, La.; and L. E. Young, Strong
Memorial Hospital, Rochester, New York.

† For example, some groups have administered their intrinsic factor concentrates in
capsules rather than in solution with the labeled vitamin B₁₂. These groups report
lower urinary recoveries of B₁₂Co⁶⁶ in patients with pernicious anemia given potent doses
of intrinsic factor concentrates than we have generally observed.

‡ From the technical point of view large doses of B₁₂Co⁶⁶ are less desirable because the
If the ratio of intrinsic factor to inactive $B_{12}$-binding material is reasonably constant in a series of intrinsic factor concentrates, and if they contain insignificant amounts of vitamin $B_{12}$, their relative potencies may be determined by multiple testing using smaller doses of $B_{12}Co^{60}$ in patients with pernicious anemia.

Relation of intrinsic factor dosage to urinary excretion test: A standard hog stomach intrinsic factor concentrate, Armour Laboratories Lot No. 441-254,$^*$ was tested at single or multiple dose levels in a number of patients having pernicious anemia. These tests are shown in figure 1. There were 21 tests on 11 patients using 2.0 $\mu g$ $B_{12}Co^{60}$. There were 13 tests on 7 patients using 15.0 $\mu g$. Dosages ranged from 10 to 1600 mg. of the intrinsic factor concentrate. There is a moderate scatter of the data. It appears that at each $B_{12}Co^{60}$ level the excretion of radioactivity may be linearly related to the logarithm of the intrinsic factor dosage. The data would also suggest that the curve for 15.0 $\mu g$ is approxi-

traced vitamin must be diluted to a lower specific activity, and, though the absolute $B_{12}$ absorption is increased, the percentage absorption is decreased making quantitation more difficult. The theoretic advantages, however, would appear to outweigh these technical disadvantages.

$^*$ A full response to hematologic assay (90 to 100% of U.S.P. expected) had been noted in two cases tested at 75 mg/d., and a partial response (49%) in a third tested at 50 mg/d. Similar hematologic assays were obtained for other lots prepared by the same technic. Thus, it appears that 1.0 U.S.P. unit would equal approximately 75 mg. This preparation is the same as Biopar, Armour Laboratories.
intrinsically parallel to and about 200 μg/24 hr. greater than that for 2.0 μg. One may apply the preliminary techniques used for analysis of covariance to this pooled data and arrive at the parallel regression lines which best fit the observed data. These are indicated on the figure. The standard deviation from regression, which in this instance reflects both intra- and inter-individual variation, is slightly over 100 μg/day.

The discussion which follows suggests that excessive dosage levels should not be used in these computations. Because of this, and because we have had to assume that the two slopes would be exactly parallel, a regression slope involving a single B₁₂ dosage and a smaller range of intrinsic factor levels would appear desirable.

We have calculated such a regression for 2.0 μg B₁₂Co⁶¹ and 10 to 125 mg of the intrinsic factor concentrate:*  

\[ \text{μg in 24 hr. urine} = 290 \times \log_{10} \text{I.F.C. mg} - 215 \]

It is apparent from figure 1 that 1600 mgm. of this intrinsic factor preparation gave appreciably less urinary recovery than did 400 mg. in a patient tested using 15 μg B₁₂Co⁶⁰. An actual inhibition of intestinal B₁₂ absorption by excessive amounts of intrinsic factor preparations has been previously noted.¹⁴, ¹⁶, ¹⁷ Where an actual inhibition has not been demonstrated, a tendency to achieve a maximal absorption little effected by additional amounts of intrinsic factor preparations or human gastric juice has been seen.⁹, ¹¹, ¹₈

Whereas the present studies suggest that within the usual dosage range this tendency to form a plateau might be expressed by a log-dose curve, Baker and Mollin, using the fecal recovery method, found a linear relationship between intrinsic factor preparation dose and intestinal absorption up to the point of maximal absorption for the patient.¹¹ They noted an uptake slope characteristic for each pernicious anemia patient. Though these slopes were generally similar, one patient (Case 4) showed practically no response to intrinsic factor. It is likely that this case represents a masked malabsorption disorder. It is unfortunate that his gastric juice was not tested for intrinsic factor activity.

The lack of a pure intrinsic factor and the variation in response of individual patients make it impossible at this time to state with finality the exact relationship of intrinsic factor dose, vitamin B₁₂ dose, and intestinal absorption of vitamin B₁₂.

Daily need for absorbed vitamin B₁₂ and the urinary excretion test: One approach to assaying intrinsic factor dosage for patients with pernicious anemia is through the comparison of parenteral B₁₂ requirements with B₁₂ absorption as reflected by the urinary excretion test. In a recent review of parenteral B₁₂ requirements¹⁹ it was stated, "Tiny doses (1 μg) given daily by the intramuscular route are fully effective for maintenance treatment, but this regimen is not practical for general use. Huge single doses (1,000 μg) cannot be relied upon to give effective control for periods longer than four to six weeks.” The literature seems to amply support a contention that for optimal promotion and maintenance of remission

* Actual calculated values for the two constants respectively were 291.1 with standard error of 53.3 and -216.3 with standard error of 18.8, based on 17 determinations in eight patients with pernicious anemia. These values have been rounded out in this formula.
in the great majority of patients having pernicious anemia, an absorption of 1.0 microgram of B₁₂ daily is adequate.

The relation of B₁₂ absorption by the intestine to urinary excretion after a flushing dose of nonradioactive vitamin B₁₂ may be studied by comparison of results obtained using the three types of tests previously described. Figure 2 presents average values in separate series of studies for each of the three technics. In normal subjects one may assume that an adequate amount of intrinsic factor is present in the stomach at time of testing. Swendseid et al. studied absorption in normal subjects at various B₁₂ levels using the fecal recovery method. Glass et al. did similar studies using scintillation counts over the liver areas. The results by these two methods are in reasonably good agreement. The data suggest that the amount of B₁₂ absorbed is linearly related to the logarithm of the oral dose. An oral dose of about 3.0 to 5.0 μg appears adequate for the absorption of 1.0 μg in most cases. 15.0 μg (the maximum dose prescribed by the U.S.P. board for incorporation with intrinsic factor concentrates) gives a greater degree of absorption.

Schilling et al. have reported a series of normal subjects tested at 1.0 μg using the urinary excretion test. We have two series of pernicious anemia patients given known adequate amounts of intrinsic factor concentrates together with either 2.0 or 15.0 μg B₁₂. Comparison of these values with absorption values in normals by the other two technics would suggest that 25 to 50 per cent of
the $B_{12}Co^{60}$ absorbed appears in the urine during the first twenty-four hours after the usual flushing dose.

This is further substantiated by use of two techniques simultaneously in a single patient. Callender and Evans using simultaneous fecal recovery and urinary excretion tests found 22 to 48 per cent of the absorbed vitamin flushed into the urine in all but 10 of 83 tests showing greater than 20 per cent absorption. The mean value was 34 per cent, and the extreme range was 13 to 70 per cent. The percentage did not appear related to the absolute absorption.

A method of assay using the urinary excretion test: In keeping with these considerations the simplest method by which a preparation at a particular dose could be tested for adequate potency is through use of the urinary excretion test at $15^* \mu g \ B_{12}Co^{60}$ in a number of characteristic pernicious anemia patients. An average 24 hour excretion for all patients tested in excess of 340 mg would indicate the likelihood of an average absorption in excess of 1.0 $\mu g$, or an apparently adequate daily dose for the average patient with pernicious anemia. A standard preparation should be adequate for practically all cases, not just the average. As a margin of safety any accrediting agency might require that the average exceed some higher figure such as 400 or 500 mg. The exact figure might be determined by further comparisons with hematologic assays as outlined subsequently.

As a further refinement two or more dosages might be tested in each patient in an effort to bracket the prescribed mean excretion by the observed means at the different levels. Arithmetic or logarithmic interpolation could be used to determine the dosage equivalent of a standard unit. Our studies would suggest the use of logarithmic interpolation, but arithmetic computation is simpler and gives a greater and, therefore, safer estimate of the unit dosage.

Assay by hematologic response compared with urinary excretion test: Thirteen patients with pernicious anemia in relapse (red blood cell counts = 55 – 2.75 million/cm$^3$) were given various dose levels of several intrinsic factor preparations for U.S.P. hematologic assay. After completion of the clinical assay they were given a urinary excretion test using the same dose of the same preparation. In six instances this was tested using 2.0 $\mu g \ B_{12}Co^{60}$; in seven, using 15.0 $\mu g$. The two tests showed a correlation, but individual values were at times quite divergent, as noted in figure 3. Full hematologic response was seen with as little as 190 mg twenty-four hour excretion after 2.0 $\mu g$, and with as little as 385 mg after 15.0 $\mu g$. Less than a full response, on the other hand, was seen with as much as 210 mg after 2.0 $\mu g$, and with as much as 565 mg after 15.0 $\mu g$.

Variable and seemingly inconsistent clinical responses have also been noted when patients in relapse were treated parenterally with preparations having various $B_{12}$ activities. In fact, the U.S.P. Board has referred to, "...the long recognized and considerable variability of the responses of different patients even to identical preparations of liver injection U.S.P...." This represents part of the biologic variability seen in the disease. Because of this variability, the results from a single study, be it $B_{12}Co^{60}$ test or clinical response, can never be extrapolated to the entire population of patients with pernicious anemia.

* This dose is chosen because most commercial preparations will be standardized at this level.
WILLIAM R. BEST ET AL.

1.0

\[ 15 \mu g \, B_{12}Co^{60} \times \]
\[ 2 \mu g \, B_{12}Co^{60} \]

Regression through origin of urinary excretion on RBC response at 15 \( \mu g \) 
\( B_{12}Co^{60} \)

*Intrinsic factor concentrate plus 15 \( \mu g \) \( B_{12} \) daily by mouth to patients with pernicious anemia in relapse

Fig. 3.—Red cell response to therapy compared to urinary excretion test with same intrinsic factor dosage in same patients.

The greater the number of observations, the sounder is such extrapolation. The supply of patients with pernicious anemia under treatment is much greater than that of patients in relapse. For this reason, the \( B_{12}Co^{60} \) test would appear a more practical method of assay.

The amount of data in figure 3 is meager when one is trying to set a positive boundary to separate adequate from inadequate therapeutic dose levels. The difficulties of obtaining such data are well known. From the data on the figure it can be calculated that the average excretion of the three patients given 15 \( \mu g \, B_{12}Co^{60} \) who showed a full hematologic response was .57 \( \mu g \). The three patients showing a 50 to 90\% hematologic response, on the other hand, had a mean excretion of .41 \( \mu g \). On this basis it would seem that the mean excretion signifying adequate dosage should be somewhere around .50 to .55 \( \mu g \) using a 15 \( \mu g \, B_{12}Co^{60} \) urinary excretion test.

Methods for comparing the activity of similar preparations: In searching for a pure intrinsic factor, and in checking the adequacy of production methods for various concentrates, one would like to compare the potency of preparations which do not differ greatly in their content of inactive \( B_{12} \)-binding substances. Tests for this purpose could be made using 0.5 to 2.0 \( \mu g \, B_{12}Co^{60} \). The best method would be to test the “standard” intrinsic factor preparation at several dosages in each of a number of patients. The “unknown” preparation would be tested at one or more levels, and the corresponding doses of “standard” would be obtained by interpolation. The relative potencies could then be averaged.

We have evolved a method which required fewer tests on each patient and which appears adequate for our purposes. Although, as previously stated, our data seem to support a log-dose curve over the whole range of dosages studied,
in a narrower range of low dosages (about 1 U.S.P. unit and lower) a reasonable case may be made for a linear relationship. The findings of Baker and Mollin strongly support a linear relationship in this low range extending back to practically zero absorption at zero dosage. If we assume that this is generally the case, then the percent of "standard" potency of an "unknown" preparation can be estimated as follows when relatively low dosages are used:

\[
\text{Per cent of "standard" potency} = \frac{E_u \times W_s}{E_u \times W_u} \times 100
\]

Where \( W_s \) is weight of "standard" preparation tested, \( E_u \) is excretion of \( B_{12}Co^{60} \) in the urine after test with this "standard"; and \( W_u \) and \( E_u \) are corresponding values for the "unknown" preparation.

In the table this method has been applied to two lots of intrinsic factor concentrate in which all conditions of manufacture were identical except the method of drying to a powder. The activity of preparation U is calculated to be 60 to 77 per cent of the standard with a mean value of 65 per cent. This reveals rather conclusively that some of the intrinsic factor activity is destroyed by the alternate method of drying. In this instance each patient was tested with identical quantities of the two concentrates. This would not actually be necessary as long as excessive doses are avoided.

**How should the urinary excretion test be standardized?** When Schilling first evolved this test it is apparent that he gave this question serious consideration. Various authors have contributed additional information since that time, however, which suggest that further refinements might improve the quantitative value of the test:

1. **Fasting state:** Swendsen et al. noted a greater absorption of \( B_{12}Co^{60} \) in normal subjects after a test meal than after an overnight fast. Presumably this is due to post-prandial increase of intrinsic factor secretion. Though this would

<table>
<thead>
<tr>
<th>Pernicious Anemia Patient</th>
<th>Intrinsic Factor Preparation*</th>
<th>( \mu g B_{12}Co^{60} )</th>
<th>mg ( B_{12}Co^{60} ) in 24 hr. urine</th>
<th>Prep. B Activity as % of Prep. A Activity Using Equation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S</td>
<td>62.5</td>
<td>2</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>U</td>
<td>62.5</td>
<td>2</td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>40</td>
<td>2</td>
<td>244</td>
</tr>
<tr>
<td>4</td>
<td>U</td>
<td>40</td>
<td>2</td>
<td>154</td>
</tr>
<tr>
<td>4</td>
<td>S</td>
<td>40</td>
<td>2</td>
<td>140</td>
</tr>
<tr>
<td>4</td>
<td>U</td>
<td>40</td>
<td>2</td>
<td>108</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>

* A batch of intrinsic factor concentrate was prepared in a manner identical to that used in preparing the standard preparation, 441-254, except that it was divided into two parts before the final drying. Preparation S was dried by the standard technic, preparation U, by an alternate technic under investigation.
be of little or no influence in patients with pernicious anemia, dietary vitamin B₁₂ and the possibility of binding substances in the food might interfere with the test. We, therefore, feel it is best to keep the subject in the fasting state until at least two hours after the oral administration of B₁₂Co⁶⁰ and intrinsic factor concentrates.

(2) Method of giving B₁₂Co⁶⁰ and intrinsic factor concentrates: Schilling recommended that the oral material be arbitrarily standardized at a volume of 100 ml. We know of no systematic study regarding effects of different oral volumes, but for the sake of uniformity recommend that this be followed. Bishop et al. noted that preliminary incubation of B₁₂Co⁶⁰ and intrinsic factor does not enhance absorption. However, it would seem advantageous to mix them in solution before administration rather than to give the intrinsic factor separately as a capsule. Uniform contact of intrinsic factor with the B₁₂ is thus insured.

(3) Dose of B₁₂Co⁶⁰: Schilling initially used 2.0 µg, but later changed to 1.0 µg. Most other groups have followed his original recommendation of 2.0 µg. Other groups have used dosages of approximately 0.5 µg, 0.7 µg, 2.3 µg, and 3.0 µg. We have primarily used 2.0 µg but have also had experience with 1.0 and 15.0 µg. Some of those who have used intermediate dosages have standardized the amount of radioactivity rather than the amount of vitamin B₁₂ present. As is seen from figure 2 such a policy would call for a new set of standard values for each change in specific activity of the radioactive vitamin. It appears more logical to maintain a uniform vitamin B₁₂ dosage as long as the specific activity is within a convenient range. We have studied the data of the above cited groups carefully in attempting to arrive at the optimal dose for testing. It appears that on the average a greater percentage of the tracer vitamin is recovered in control tests on patients with pernicious anemia with low dosages of B₁₂Co⁶⁰ than with larger amounts. It is rare to find typical cases of pernicious anemia who show greater than 9 per cent excretion after 0.5 µg; greater than 4 per cent after 1.0 µg; greater than 3 per cent after 2.0 µg; and greater than 1.5 per cent after 15 µg. The lower limit of secretion in normal individuals appears to be about twice this upper limit for pernicious anemia at each level, though selected cases of achlorhydria may show intermediate values. From the point of view of measuring radioactivity, smaller doses of B₁₂ would thus appear desirable. However, for the purpose of testing experimental intrinsic factor preparations small doses may lead to misleading results due to non-specific binding as previously mentioned. There is considerable discrepancy in the ranges of excretions noted between experimental groups, each group using its own known potent, “standard” intrinsic factor preparation.

For any given study it seems most important that a uniform dosage of B₁₂Co⁶⁰ in terms of B₁₂ weight be used throughout. 2.0 µg has the benefit of a greater accumulated experience, but 1.0 µg has the recommendations of a greater percentage excretion for more accurate measurement. Percentage is readily converted into absolute terms with this dose. We have now adopted the policy of clinical diagnostic testing and comparison of similar intrinsic factor concentrates using 1.0 µg doses. For minimization of binding artefacts in assessing the clinical potency of dissimilar intrinsic factor preparations we prefer 15.0 µg doses despite the relatively small recovery of radioactivity in the urine.
Intrinsic Factor and Excretion of Vitamin B₁₂Co₆₀

(4) **Time and amount of first parenteral dose:** We have shown that there is minimal urinary excretion during the first four hours of the test. The flushing dose of B₁₂ itself has an inhibitory effect on intestinal absorption. When this flushing dose is given two hours before the oral material there appears to be slightly greater inhibition of uptake. Larger doses or frequent repetition of non-radioactive vitamin B₁₂ within six hours after the oral tracer do not appear to enhance urinary recovery. We have not seen any studies using smaller flushing doses of vitamin B₁₂. It would seem that Schilling’s original schedule of 1000 µg s.c. two hours after the oral material should be followed. For clinical screening we have found satisfactory results when this injection is given simultaneously with the oral material.

(5) **Collection of urine and subsequent B₁₂ injections:** We have studied the pattern of excretion during the first 24 hours, and find that for quantitative purposes fractionation has no advantage over a single 24 hour collection. In some patients, however, the greatest rate of excretion occurred in the final collection period; and in some patients small but significant degrees of excretion were seen on the following morning. Ellenbogen et al. have collected successive 24 hour specimens with a 1000 µg flushing dose of nonradioactive vitamin B₁₂ repeated each day. They observed a second day’s excretion equal on the average to 1/3 of that on the first; and a third day’s excretion equal to 1/3 of the second. Individual patients may deviate significantly for this pattern. One of their patients demonstrated 3.1 per cent recovery on the first day, 4.3 per cent on the second, and 5.7 per cent on the third! It would appear then, that for best quantitative results, at least two and preferably three successive days with parenteral B₁₂ and 24 hour collection would be desirable for each intrinsic factor test.

(6) **Value to be reported:** There is convincing evidence that urinary radioactivity represents excretion of the intact vitamin. There is no right or wrong in the choice between “percentage,” “micrograms,” or “millimicograms” for reporting. It is important, however, that each author clearly state the oral dose in micrograms of vitamin B₁₂. Toporek et al. in assessing the effects of intrinsic factor preparations, have considered as most important the difference between excretion following no intrinsic factor and that following the intrinsic factor preparation under consideration, rather than the latter value alone. Such a correction would generally not be large, but appears to be a logical method for considering the data.

(7) **Frequency of tests:** A number of authors have noted that heavy loading of the system with vitamin B₁₂ will inhibit the intestinal absorption of B₁₂Co₆₀. This being the case it would be advantageous to allow a reasonable interval in which no large doses of vitamin B₁₂ are given before subsequent tests are performed. Blood levels of vitamin B₁₂ are elevated as long as 48 hours after a large parenteral dose, but levels are not excessively high. It would be our recommendation that an interval of at least two days elapse after the last large injection of vitamin B₁₂ (e.g., 200 µg or more) before a test is initiated. The studies we have reported herein were not performed according to all the above recommendations because many of them are based on very recent reports.
Studies were made in pernicious anemia patients on the urinary excretion of $\text{B}_{12}\text{Co}^{\text{60}}$ after a small oral dose followed by a large parenteral injection of non-radioactive vitamin $\text{B}_{12}$.

1. Increasing doses of intrinsic factor concentrates give increasing excretions of radioactivity at low doses; little additional increase at moderate doses; and at times a subsequent diminution at excessive doses. Data on 34 tests of a particular intrinsic factor concentrate in 18 pernicious anemia patients tend to support an excretion proportional to the logarithm of intrinsic factor dosage at low to moderate levels, but do not exclude the possibility of a linear approach to a plateau.

2. Assay by hematologic response was compared with the urinary excretion tests in 13 pernicious anemia patients. This data shows a relation between the two tests though the correlation is far from complete.

3. Methods are outlined for testing all intrinsic factor preparations with the same amount of tracer vitamin $\text{B}_{12}$ as will be incorporated commercially. More sensitive comparative tests of similar intrinsic factor preparations may be made using smaller amounts of $\text{B}_{12}\text{Co}^{\text{60}}$.

4. The literature is reviewed to determine which variations in technic might lead to the most reliable quantitation of intrinsic factor activity.

**SUMMARIO IN INTERLINGUA**

In patientes de anemia perniciosa, le excretion urinari de vitamina $\text{B}_{12}$ etiquettate per $\text{Co}^{\text{60}}$ esseva studiate post le administratio de un parve dose oral de iste substantia, sequite per un grande injection parenteral de non-radioactive vitamina $\text{B}_{12}$.

1. Crescente doses de concentratos de factor intrinsec resulta in crescente excretiones de radioactivitate a basse doses, in parve crescentias additional a doses moderate, e a vices in subsequente decrescentias a doses excessive. Datos ab 34 tests de un specific concentrato de factor intrinsec, executate in 18 patientes de anemia perniciosa, tende a justificar le these que le excretion es proportional al logarithmo del dosage de factor intrinsec a basse e moderate nivellos sed non exclude le possibilitate de un curva linear attingente un plateau.

2. Studios del responsa hematologic esseva comparate con le tests del excretion urinari in 13 patientes de anemia perniciosa. Iste investigation monstrava un relation inter le duo methodos, sed le correlation certemente non es complete.

3. Es delineate methodos pro tests de omne preparatos de factor intrinsec per medio de dosages de $\text{B}_{12}$ a $\text{Co}^{\text{60}}$ identic con le quantitates del vitamina incorporate in le preparatos in uso therapeutic. Plus delicate essayos comparative de simile preparatos de factor intrinsec pote esser executate per medio de plus parve quantitates de $\text{B}_{12}$ a $\text{Co}^{\text{60}}$.

4. Es presentate un revista del litteratura pro determinar qual variationes del technica resultarea in le plus precise quantitation del activitate de factor intrinsec.
350  

intrinsinc factor and excretion of vitamin B12Co60

REFERENCES


Studies on Urinary Excretion of Vitamin B$_{12}$Co$^{60}$ in Pernicious Anemia for Determining Effective Dosage of Intrinsic Factor Concentrates

WILLIAM R. BEST, WILFRID F. WHITE, KENNETH C. ROBBINS, WENDELL A. LANDMANN and SANFORD L. STEELMAN