Time Pattern of Vitamin B₁₂Co⁶₀ Urinary Excretion in Man After Oral Administration and Parenteral "Flushing"

By William R. Best, Wendell A. Landmann and Louis R. Limarzi

Schilling first noted that when 2.0 µg of Co⁶₀-labeled vitamin B₁₂ were given orally to normal subjects and followed in two hours by 1000 µg. nonradioactive vitamin B₁₂, significant radioactivity could be detected in the urine during the following twenty-four hours. This has been confirmed by others. It has been fairly well established that this radioactivity represents the intact, labeled vitamin. Such a response is seen in patients with pernicious anemia only if they are given a potent source of intrinsic factor along with the oral B₁₂Co⁶₀.

The present study was undertaken to more clearly define the time relationships of urinary excretion in this test.

Methods

Eight patients with pernicious anemia in clinical remissions were tested by the technic as outlined by Schilling. In seven patients twelve tests were also made using known potent doses* of intrinsic factor concentrates prepared from hog stomach. Fractional urine specimens were collected as shown in figure 1. In addition a few patients with pernicious anemia and other diseases were tested using different time intervals for urinary collections as noted in figures 2 and 3.

Urines were analyzed for radioactivity using a windowless gas-flow counter as described elsewhere. Urinary excretion was expressed in terms of µg B₁₂Co⁶₀/hr.

Results

Figure 1 presents the mean and range of excretion rates at each interval when the patients were given no intrinsic factor and when they were given known potent doses of intrinsic factor preparations.

The studies with no intrinsic factor showed a statistically significant but physiologically negligible excretion of radioactivity. After potent doses of intrinsic factor were given these patients showed little excretion of B₁₂Co⁶₀ during the first four hours. The mean excretion rate rose to a peak at 8 to 12 hours, and dropped slightly in the 12 to 24 hour interval. In most instances relatively low values were achieved by this time, but a few instances in which the peak rates occurred during this interval tended to raise the mean. Similar curves were seen in patients with other diseases given no intrinsic factor. Figures 2 and 3 demonstrate individual patterns in two selected patients. Collections were made from 24 to 28 hours in these subjects, and show that some B₁₂Co⁶₀ is excreted in this interval.

The table indicates the time at which peak excretion rates were seen in a...
Fig. 1.—Urinary excretion of radioactivity after oral vitamin B₁₂Co⁶⁰ followed by parenteral nonradioactive B₁₂ in patients with pernicious anemia.

Fig. 2.—Urinary excretion of radioactivity after oral B₁₂Co⁶⁰ together with intrinsic factor concentrate and followed by parenteral nonradioactive B₁₂ in a patient with pernicious anemia.
TIME PATTERN OF VITAMIN B\textsubscript{12}CO\textsubscript{60} URINARY EXCRETION

Fig. 3.—Urinary excretion of radioactivity after oral vitamin B\textsubscript{12}CO\textsubscript{60} followed by parenteral nonradioactive B\textsubscript{12} in a patient with hemolytic anemia.

<table>
<thead>
<tr>
<th>Hours After Oral B\textsubscript{12}CO\textsubscript{60}</th>
<th>Number of Tests</th>
<th>Number of Patients</th>
<th>Per Cent of Patients Per Hour-Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4-6</td>
<td>1</td>
<td>$\frac{1}{2}$*</td>
<td>1.6</td>
</tr>
<tr>
<td>6-8</td>
<td>8</td>
<td>$\frac{4}{3}$</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Subtotals

Pernicious Anemia Patients Given Intrinsic Factor Concentrates

| Other Patients, Control Studies                  | 24 | 10 |

Total

| 32 | 15 |

* "$\frac{1}{2}$ patient" indicates that $\frac{1}{2}$ of tests on one patient had peak at indicated time.

Larger series of patients including these. Peak excretion rates most commonly were noted between 6 and 12 hours after oral ingestion. It is likely that the actual peak rate in some instances might have occurred in the 12 to 24 hour interval, but been obscured by the length of the collection interval. Figure 3 illustrates such an instance in which more frequent collections were made. Pernicious anemia with intrinsic factor concentrates gave patterns similar to control tests in other patients.
Repeated tests on the same subject usually showed the peak excretion at a characteristic time, never differing by more than one interval. Sometimes curves would have a secondary peak for no obvious reason.

**Discussion**

It has been shown that there is a prompt, marked increase in the plasma $B_{12}$ level after parenteral administration of large doses, reaching a peak level within the first hour and exhibiting an exponential decline thereafter. This is directly reflected in the rate of urinary excretion of vitamin $B_{12}$. If orally administered $B_{12}Co^{60}$ in the presence of intrinsic factor were rapidly transported across the intestinal mucosa into the circulation, one would expect the most rapid rates of $B_{12}Co^{60}$ excretion to occur during the first two hours after the large parenteral "flushing" dose. The present studies have demonstrated, on the contrary, that there is only negligible excretion during this period. The peak rate of excretion occurs at least six hours after the oral dose and four hours after the parenteral "flushing" dose, usually even later.

What does this delay represent? At the present time we can only speculate. It might represent the time necessary for transport of vitamin $B_{12}$ and intrinsic factor to that part of the bowel at which absorption takes place. Studies in dogs have shown $B_{12}$ absorption from the duodenum but not the stomach. The megaloblastic anemia of diphyllobothrium latum infestation occurs only when the worm is present in the upper intestine, presumably in the jejunum. We have not been able to demonstrate absorption from the rectum. Thus the upper intestinal tract seems to be the site of absorption, but we do not know whether a large or small segment of bowel is involved. A patient with megaloblastic anemia associated with regional enteritis failed to absorb adequate vitamin $B_{12}$, even in the presence of intrinsic factor. It is thus possible that the function of absorption is fairly localized.

A more likely explanation of the delay would be the average time necessary for cellular processes to transfer the vitamin from the intestinal lumen into the circulation.

We have examined the time-excretion rate curves for individual tests in graded dose studies of an intrinsic factor preparation in patients with pernicious anemia. Graded doses of intrinsic factor concentrates give peak excretion rates which are roughly proportional to the logarithm of the dose, but otherwise do not alter the time-pattern of excretion. The total twenty-four excretion gave more consistent values than did any aspect of the time-excretion curves. Thus, for most studies to which this test might be applied, analysis of fractional urine specimens within the first 24 hours does not appear necessary.

**Summary and Conclusions**

Serial urine collections in a number of patients with pernicious anemia given $2 \mu g B_{12}Co^{60}$ orally followed in two hours by $1000 \mu g$ nonradioactive vitamin $B_{12}$ showed little urinary radioactivity at any time. When these tests were repeated together with a potent oral dose of intrinsic factor concentrate, there was little activity during the first four hours. Peak excretion rates occurred most commonly between 6 and 12 hours after ingestion of radioactive $B_{12}$, sometimes
even later. The time of peak excretion was fairly characteristic for the individual. Secondary peaks occasionally occurred, and only slight radioactivity usually remained after 24 hours. It is postulated that the delayed peak is related to the time it takes for $\text{B}_{12}$ to be transported in the intestine to the point of absorption or to the duration of the intracellular metabolic processes of absorption. For most purposes the use of fractional urinary collections is not necessary.

**Summario in Interlingua**

Collectiones serial de urina esseva executate in un numero de patientes con anemia perniciosa qui habeva recipite 2 $\mu$g de vitamina $\text{B}_{12}$ etiquettate a Co$^{60}$ in administration oral, sequite duo horas plus tarde per 1000 $\mu$g de vitamina $\text{B}_{12}$ non-radioactive. Le specimens monstrava pauc radioactivitate a ulle tempore. Quando iste tests esseva repetite in conjunction con le administration de un potente dose oral de concentrato de factor intrinsic, pauc activitate radioactive esseva observate in le urina durante le prime quatro horas. Le excretion attingeva grados maximal communemente inter 6 e 12 horas post le ingestion de $\text{B}_{12}$ radioactive; in certe casos mesmo plus tarde. Le tempore del excretion maximal esseva satis characteristic pro le subjectos individual. Maximos secundari esseva trovate in un numero de casos. Solmente leve grados de radioactivitate persisteva post 24 horas. Nos postula que le retardation del maximo depende (1) del tempore requirite pro le transporto intraintestinal de $\text{B}_{12}$ usque al puncto de absorption o (2) del durantia del intracellular processos metabolic de absorption. Pro le majoritate del objectivos de tal tests, le uso de fractional collectiones urinari non es necessari.

**REFERENCES**

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