A Modified Urinary Excretion Test for Measuring Oral Cobalt^{60} Labeled Vitamin B_{12} Absorption and its Application in Certain Disease States

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ABSORPTION of orally administered radioactive vitamin B_{12} can be measured by three methods: fecal excretion,[1] urinary excretion following a "flushing dose" of non-radioactive vitamin B_{12}[2] and external monitoring over the liver.[3] The first method presents practical difficulties which limit its clinical usefulness, and the third method, although the simplest to perform, is difficult to quantitate. The urinary excretion method is simple to perform, requires less total time than the other two methods and for a given dose exposes the patient to the least amount of radioactivity. It was the purpose of this study to: (a) investigate factors influencing the urinary excretion test, (b) apply certain modifications to the test in order to obtain a better separation between normal and pernicious anemia, and (c) determine the effect of various disease states on the oral absorption of vitamin B_{12} as measured by this test.

Methods

Clinical Material. The control group consisted of hospitalized patients who had no evidence of renal, liver, gastroenterologic, or hematologic disease. Some of these patients were convalescing from cerebral vascular accidents. Patients listed as having pernicious anemia had typical features of the disease plus a significant reticulocyte response to vitamin B_{12}. Several patients were throught to have combined system disease with little or no anemia and are listed separately. The remainder of the patients were hospitalized with the disorders designated.

Experimental Procedures. Cobalt 60 labeled vitamin B_{12} of specific activity 1.08 $\mu c/\mu g$. was used throughout the study; 0.5 ml. of the stock solution was made up to a volume of 25 ml. with distilled water; 1 ml. of this dilution was placed directly in a counting tube, made up to 75 ml. with distilled water and served as a standard. The remaining 24 ml., containing 0.48 μg. of vitamin B_{12}, was administered orally to fasting patients. Patients usually ate 15–30 minutes later. "Flushing" doses of 1 mg. of nonradioactive vitamin B_{12} were given intramuscularly at various times thereafter. Urine was collected for 24 hour periods. In one normal subject and one patient with chronic renal insufficiency, renal excretion and plasma levels were determined concomitantly by injecting intravenously 1 mg. of vitamin B_{12} containing 3 μc of radioactivity. Timed urine collections and serial plasma samples were obtained. Patients with acute infections were tested during the infection and at varying times after recovery as indicated. When several tests were performed on the same subject, blank urine samples obtained before the next test contained no radioactivity. Serial tests were performed at intervals of at least one week.

Counting Technic. Samples were counted in a Geiger-Mueller Texas Allyn well type counter, using 75 ml. aliquots. The administered dose contained 50–60,000 counts per minute. For the normal group, samples counted at least three times background, with a counting error of less than 3%. Samples from patients with pernicious anemia contained

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little radioactivity and were counted for ten minutes. Four ml. plasma samples were counted in a thallium activated sodium iodide well type scintillation counter with a counting error of 3%.

Factors Effecting the Urinary Excretion Test

1. *Time of First Flushing Dose.* (table 1). When the first flushing dose was given three hours after the oral dose of $Co^{60}B_{12}$, 12–38% of the administered radioactivity was recovered in the subsequent 24 hour urine, in normal subjects. When tests were repeated in the same subject, repeated flushing doses given at two hour intervals did not significantly increase the urinary excretion when compared to a single flushing dose. In patient J.S., giving the flushing dose two hours before the oral dose of $Co^{60}B_{12}$ greatly reduced urinary excretion of radioactivity compared to two tests done one week before and one week after in which the flushing doses were given three hours after the oral radiovitamin. Delaying the first flushing does until six hours after the oral dose of $Co^{60}B_{12}$ did not significantly change the amount recovered in the subsequent 24 hour urine. However, when the first flushing dose was delayed until 24 hours after the oral dose, a decrease in urinary excretion of radioactivity was noted (patient P.M.). When the flushing dose was delayed until 48 hours, there was a further reduction, although 9% was still recovered (patient H.W.).

2. *Second Flushing Dose.* When a second flushing dose was given 24 hours after the oral dose, 3–10% of the administered dose was obtained in the subsequent 24 hour urine (table 1).

TABLE 1.—*Repeated Urinary Excretion Tests in Normal Subjects*

Patient	Age	Interval between oral and flushing dose in hours	% of dose in urine			
			24 hrs.	48 hrs.	72 hrs.	Total
P.M.	66	3	23	2	0	25
P.M.		3	30	1	0	31
P.M.		3	32	6	0	38
P.M.		3, 5, 7	23	6	—	29
P.M.		6, 8, 10	30	7	—	37
P.M.		24	0	15	1	16
P.M. with .5 mg. histamine		3	23	3	—	26
P.M.		3, 24	20	6	—	26
H.W.	50	3, 24	24	9	—	33
H.W. using .24 μg.		3, 24	38	8	—	46
H.W.		3, 24, 48	29	10	2	41
H.W.		48	0	1	9	10
I.J.	61	3, 24	20	6	—	26
I.J.		3, 24	12	3	—	15
J.S.	55	3, 5, 7	30	1	0	31
J.S.		−2, 0, 2	2	0	0	2
J.S.		3, 24	21	8	—	29
T.M.	53	3	17	2	—	19
T.M.		3	19	11	0	30
T.M.		3	25	1	—	26
T.M.		6, 8, 10	33	—	—	33
J.P.	61	3, 24	36	7	—	43
J.P.		6, 24	28	8	—	36

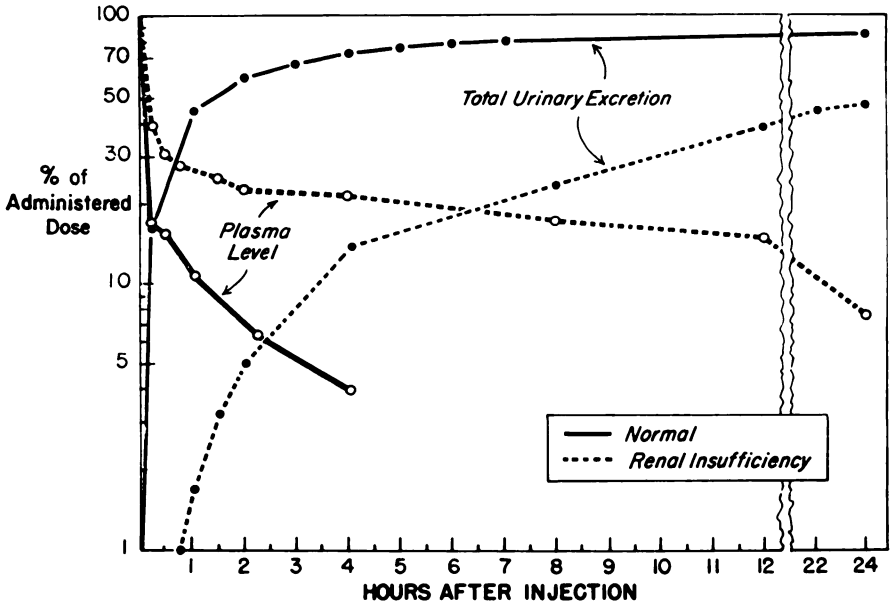

FIG. 1.—The plasma disappearance and urinary excretion of radioactivity following intravenous administration of 1,000 μg of cobalt 60 labeled vitamin B_{12} in a normal subject and a patient with renal insufficiency.

3. *Renal Excretion.* One normal subject and one patient with chronic renal insufficiency were studied by injecting 1,000 μg. of labeled vitamin B_{12} intravenously and measuring urinary excretion and disappearance from the plasma (fig. 1). In the normal the plasma level fell rapidly; 96% of the administered dose had left the plasma four hours after injection. In the patient with chronic renal insufficiency, the plasma level fell more slowly; four hours after injection, 78% of the injected dose had left the plasma. In the subsequent 24 hour urine, 47% of the injected dose was recovered, compared to 86% in the normal.

Modified Urinary Excretion Test

Because of the factors affecting the test, the following technic was employed for the duration of the study: an oral dose of 0.48 μg. of cobalt 60 labeled vitamin B_{12} (0.52 microcurie) was given in the fasting state. Two flushing doses were given three and 24 hours after the oral dose. Urine was collected for two 24 hour periods and the total radioactivity expressed as percentage of the administered dose. The lowest counting rate in samples from control subjects was eight times that of the highest rate in patients with pernicious anemia.

Application of the Modified Test

Normals: Sixteen control subjects were studied. Urinary excretion of radioactivity ranged from 21 to 48% of the administered dose (table 2).

Pernicious Anemia: Nine patients with pernicious anemia were studied. Urinary excretion of radioactivity ranged from 1–7% of the administered dose (table 2). In addition, three patients were studied (4 tests), who were thought to have combined system disease without anemia. Each of these patients has shown some improvement in neurologic status while receiving vitamin B_{12}, but their diagnoses were not certain. Urinary excretion of radioactivity ranged from 2–15% of the administered dose.

TABLE 2.—*Urinary Excretion Test in Control Subjects and Patients with Pernicious Anemia*
(Flushing Doses at 3 and 24 hours)

Patient	Age	% of dose in Urine		
		24 hrs.	48 hrs.	Total
		Controls		
A.L.	50	32	8	40
J.F.	68	33	6	39
H.S.	36	34	10	44
A.D.	56	24	8	32
M.S.	31	33	8	41
J.J.	24	39	9	48
P.O.	62	26	13	39
M.B.	24	33	9	42
H.W.	47	29	10	39
T.T.	58	25	9	34
A.H.	60	23	6	29
A.P.	61	36	7	43
C.G.	62	17	4	21
H.W.	66	29	7	36
H.S.	52	28	7	35
W.M.	61	26	5	31
Range				21–48
		Pernicious Anemia		
A.H.	63	2	1	3
A.B.	23	1	0	1
A.S.	60	3	0	3
C.W.	51	2	—	2
F.C.	66	3	1	4
H.E.	39	3	4	7
E.H.	32	4	0	4
J.S.	70	4	2	6
P.E.	60	1	0	1
Range				1–7
		Patients thought to have CSD, without anemia		
E.O.	60	2	0	2
E.B.	64	8	0	8
M.B.	58	12	3	15
		9	3	12
Range				2–15

Infection: Five patients with acute bacterial infections were studied. In three of these patients urinary excretion of radioactivity was decreased to the level found in pernicious anemia. During the convalescent period, the urinary excretion returned towards normal (table 3). In the two other patients with acute bacterial infections, the urinary excretion of the radioactivity was decreased, though not

to the pernicious anemia range. Two patients with infectious hepatitis and three patients with rheumatoid arthritis had normal urinary excretion.

Renal Insufficiency: One patient with terminal uremia, studied four weeks and again five days before death, excreted negligible urinary radioactivity over a 48 hour period. Two other patients with chronic renal insufficiency (NPN-100), excreted low normal amounts of radioactivity in the total collection. However,

TABLE 3.—*Urinary Excretion Test in Patients with Various Diseases*
(Flushing doses at 3 and 24 hours)

Patient	Age	Disease	% of dose in urine		
			24 hrs.	48 hrs.	Total
		Infection			
A.M.	28	Pneumonia	1	8	9
A.M.		5 wks. afebrile	47	—	47
A.W.	28	Pneumonia	1	0	1
A.W.		4 wks. afebrile	21	7	28
H.J.	23	Strep. Pharyngitis	7	0	7
H.J.		10 days afebrile	17	2	19
R.M.	34	Pneumonia	16	2	18
W.G.	36	Lung Abscess	17	3	20
L.J.	31	Inf. Hepatitis	21	3	24
L.J.		6 wks. after jaundice	21	6	27
A.S.	26	Inf. Hepatitis	24	8	32
A.S.		4 wks. after jaundice	16	11	27
		Renal Disease			
C.R.	70	Uremia—mild	36	15	51
O.T.	28	Uremia—mod.	14	15	29
H.J.	24	Uremia—mod.	12	15	27
J.D.	40	Uremia—Terminal	0	3	3
J.D.			0	0	0
		Miscellaneous			
W.S.	33	Severe Rheum. Arthritis with psoriasis	20	5	25
J.C.	45	Rheum. Spond.—mod.	27	9	36
W.O.	48	Rheum. Spond—mod.	15	7	22
R.H.	28	Rheum. Spond.—mild	14	16	30
M.O.	62	Severe Hodgkin's Disease	35	8	43
A.P.	56	Hodgkin's Disease	20	5	25
J.O.	44	Multiple Myeloma	27	9	36
D.H.	50	Iron Def. Anemia	41	9	50
H.C.	58	Hypertension on Hexamethonium	18	8	26
F.K.	57	Aplastic Anemia	18	13	31
F.S.	30	Myxedema	27	9	36
H.G.	29	Myxedema, Diabetes	14	11	25
J.D.	58	Myxedema	29	12	41
R.H.	47	Sprue	13	4	17
H.H.	58	Sprue, Cirrhosis	11	7	18
H.P.	47	Exfol. Dermatitis on Cortisone	14	5	19

more radioactivity was found in the second 24 hour period than in the first. One patient with chronic renal disease (NPN-50), refractory anemia, and cholecystitis, had normal excretion (table 3).

Miscellaneous: Other patients with various disorders were tested and the results are listed in table 3. Included in those who had normal tests were patients with severe iron deficiency anemia, multiple myeloma, Hodgkin's Disease and myxedema.

Repeated testing (using similarly timed flushing doses) resulted in decreased urinary recovery in 2 out of 5 normal controls (table 1).

Discussion

I. Factors Affecting the Urinary Excretion Test

A. *Time of First Flushing Dose:* Maximum absorption of the orally administered dose is desirable in order to obtain maximum urinary excretion. The high serum levels of vitamin B_{12} following flushing doses tend to decrease absorption of the radiovitamin; illustrated by patient J.S. (table 1), and noted by others.[4, 5] Therefore, it would seem logical to delay the flushing dose until absorption of the oral dose was complete. However, the time necessary for complete absorption of vitamin B_{12} is not known. There is evidence that following oral administration of radioactive vitamin B_{12} in doses of 1 microgram and less, radioactivity begins to appear in the plasma after about three hours, and reaches a peak in 8–12 hours.[6, 7] If this represents absorption from the intestine, the time sequence is unlike most orally administered materials, and the mechanisms accounting for this delay are obscure. From the small amount of data presented in this study, it seems that giving the first flushing dose six hours after the oral dose did not significantly effect the urinary recovery, when compared to three hours. On the other hand, delaying the flushing dose until 24 or 48 hours, greatly decreased urinary recovery. From the practical standpoint the first flushing dose can be given any time between three and six hours after the oral dose.

Between six and 24 hours after oral administration, an increasing proportion of the absorbed radiovitamin becomes nonexchangeable with the parenterally administered vitamin, and cannot be flushed. The exchangeable radioactivity cannot be accounted for by free vitamin B_{12} in the plasma, since free vitamin B_{12} is rapidly excreted by the kidney.[8, 9] It is more likely that after 48 hours, the parenterally administered vitamin B_{12} exchanges with intracellular radiovitamin and a small amount bound to plasma proteins.

B. *Second Flushing Dose:* The second flushing dose at 24 hours increased total urinary excretion of radioactivity. Similar results have been reported by Ellenbogen et al.[10]

C. *Size of Oral Dose:* There is adequate evidence that with increasing oral doses of vitamin B_{12} above 0.5 micrograms, a decreasing proportion of the dose is absorbed.[1, 5] For testing purposes, it would seem reasonable to use the smallest dose consistent with accurate counting. At the present time, doses of less than 0.5 micrograms result in urinary radioactivity which cannot be counted accurately.

Results Obtained with Modified Test

In the present study, urinary recovery of administered radioactivity in the normals is higher than has been previously reported.[2, 10, 11] This results in counting rates in normals which are far greater than those obtained in patients with pernicious anemia. This separation is obviously desirable in the interest of diagnostic accuracy. The range which we have obtained in patients with known pernicious anemia confirms that these patients absorb small amounts of vitamin B_{12} and that this varies from patient to patient.[1] In the group suspected of having combined system disease without anemia, values ranged up to 15% of the administered dose, a value approaching that found in other systemic disorders.

Of great interest was the temporary decrease in urinary radioactivity during acute infections, simulating values obtained in patients with pernicious anemia. Whether or not this is due to temporary decrease in the absorption of the vitamin cannot be answered. A slight decrease in absorption of cobalt 60 labeled vitamin B_{12} during viral infections has been reported.[1, 12] On the other hand, a decrease in both the serum levels and urinary excretion of vitamin B_{12} during acute infection has also been reported.[13] Further work is needed to clarify this problem. In any case, the urinary excretion test may be invalid in the presence of acute infection.

In the presence of renal insufficiency, two variations from the normal were found. Delayed excretion of radioactivity was found in mild to moderate uremia, with the total amount being normal. This delay was further shown by the intravenous study, and emphasizes the need for collecting urine beyond 24 hours. In severe uremia, the urinary excretion was markedly decreased to the range found in pernicious anemia. As with infection, the urinary excretion test may be invalid in the presence of renal insufficiency.

Of the other disease states studied, only those patients with sprue and a patient with exfoliative dermatitis receiving cortisone had values which were clearly below the normal range. As more studies are carried out, it is likely that other diseases will be encountered which give urinary excretion tests below the normal range. Also, since pernicious anemia probably does not develop abruptly, one would expect to find low values in persons who are clinically free of disease but who may go on to develop the clinical manifestations of pernicious anemia. There is probably another group of subjects with low values who represent the normal variation found in any biologic phenomenon. Because of these biologic factors, plus the ever present possibility of incomplete urine collections, a test giving a low value is at best only presumptive evidence of impaired absorption of vitamin B_{12} at the time of testing. On the other hand, a high urinary excretion is reliable evidence against impaired vitamin B_{12} absorption.

Summary

1. Factors relating to the urinary excretion test for oral vitamin B_{12} absorption were studied. A modified procedure is presented giving a high percentage of urinary recovery of radioactivity in the normal.

2. Values in the pernicious anemia range were found in the presence of acute infection and severe renal insufficiency. Other limitations in the interpretation of the test are discussed.

SUMMARIO IN INTERLINGUA

1. Esseva studiate factores que interessa le test de excretion urinari pro le determination del absorption de oral vitamina B_{12}. Es presentate un technica modificate que resulta in alte procentages de recovramento urinari in subjectos normal.

2. In le presentia de acute infection e de sever insufficientia renal, valores de magnitudes characteristic de anemia perniciose esseva obtenite. Altere limitationes in le interpretation del test es discutite.

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