The Urinary Excretion Test for Absorption of Vitamin B_{12}

I. REPRODUCIBILITY OF RESULTS AND AGEWISE VARIATION

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SEVERAL radioactive tracer methods have been proposed for the determination of the extent of vitamin B_{12} absorption by humans. These methods employ oral test doses of 0.5–2 μg of vitamin B_{12} labeled with radioactive cobalt.[1–3] Absorption may be estimated from radiometric measurements of feces,[4] scintillation counting of liver projections,[5] or determination of urinary radioactivity estimated after injection of a massive dose of non-radioactive vitamin B_{12}.[6]

The urinary excretion test[6] is useful for the study of the effect of intrinsic factor on the absorption of vitamin B_{12} by patients with pernicious anemia; the application of this test to those without an extreme derangement in absorption mechanism is hampered by the lack of information on the reproducibility of the results obtained in clinically healthy subjects. To make a systematic study, a group of young volunteers from a single penal institution was used. These subjects were all males between 19 and 30 years of age. They had a similar dietary history and all had lived in the same environment for at least three years. These uniform conditions, together with the careful supervision of urine collections, are desirable and, indeed, essential, in quantitative studies, and have rarely been presented in previous reports.

In addition, older subjects were drawn from three homes for the aged, each providing medical supervision. All subjects were ambulatory and were apparently in good health and free from acute infectious diseases and metabolic disturbances. Twenty-four hour nursing service was provided specifically to assure a complete urine collection. Some of these persons were subjected to repeated tests in order to study variations in individual responses. A total of 40 normal adult men, ranging in age from 19 to 85 years, were studied. These experiments provide information concerning the variation in absorption of vitamin B_{12} between individuals of various age groups living under somewhat comparable conditions.

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EXPERIMENTAL

Test Procedure

The procedure employed is essentially that outlined by Schilling.[6] Two hours after the oral administration of 2 μg of labeled vitamin B_{12} to the test subjects, one milligram (1000 μg) of crystalline vitamin B_{12} in physiologic saline solution was injected intramuscularly, and urine specimens were collected for a period of 24 hours. In all but three cases, 24-hour collections were made in two successive periods of (1st period) 0–8 or 0–12 hours and (2nd period) 8–24 or 12–24 hours. In nine cases, a second 24-hour collection was made. An aliquot of each collection, equivalent to one-quarter of the total urine collected, was evaporated to 50 ml in a beaker containing 50 μg of vitamin B_{12} as carrier, and the radioactivity of the concentrate was measured by scintillation counting[7] using a thallium-activated sodium iodide crystal. The radioactivity measured in this manner was corrected to the total volume of urine, and computed as per cent of oral dose excreted. The activity

of the 2-μg dose measured in this manner was about 25,000 counts per minute. In 31 cases an aliquot of the original urine was assayed microbiologically for total vitamin B_{12} content using *L. leichmannii*.[3]

Intrinsic Factor Used

In view of the known efficacy of intrinsic factor concentrates in promoting the absorption of orally administered vitamin B_{12} by patients with pernicious anemia, eight individuals were tested with two oral intrinsic factor concentrates to explore the effect of such preparations in normal persons. One preparation (I) was known to be clinically active at 40 mg per day and the second preparation (II) was proved active at 2 mg or less per day. An oral dose of 40 mg of preparation I or 2 mg of preparation II was administered with the tracer vitamin.

RESULTS

Age Group Averages

Responses obtained by the Schilling urinary excretion test are reported in Table I. The results are presented by age groupings. The radioactivity and microbiologic activities excreted in the second 24-hour interval were usually well below 1 per cent of the oral dose and are not included in these figures. Urinary excretion of the injected dose of 1 mg of crystalline vitamin B_{12} was essentially complete in 24 hours, subsequent elimination being inappreciable. In the last two columns of Table I are listed group average percentages of the total 24-hour output which appeared in the urine during the first collection period for both radioactivity and microbiological measurement.

It should be pointed out (Table I) that on the average the bulk of the *orally* administered radioactive vitamin which is flushed out is eliminated in the second collection of the 24-hour period, whereas microbiologic assays indicate a preponderant excretion of *injected* vitamin B_{12} during the first collection period (0–12 hours). Thus, group averages ranging between 9 and 36 per cent of the total radioactivity from the orally administered labeled vitamin B_{12} excreted in 24 hours appear in the first period urines, while elimination of the unlabeled vitamin B_{12} ranged from 41 to 89 per cent in the same period after the intramuscularly administered dose. In only 4 of the 40 cases reported in Table I did the radioactivity of the first period urine exceed that in the second collection, and then by very little; even in these cases total vitamin excretion was more rapid than the excretion of radioactivity. Similarly, four cases were noted in which the quantity of unlabeled vitamin B_{12} in the initial urine was less than that in the second collection; but these subjects still exhibited a relatively much greater initial elimination of nonradioactive vitamin than of the radioactive form. The elimination of the injected vitamin B_{12} is practically complete (average 91 per cent) in 24 hours, compared to the limited excretion (average 11.4 per cent) of the oral dose

TABLE I

Excretion of Vitamin B_{12} by Normal Subjects of Different Ages

Age (yr)		No. of subjects	% of Radioactivity excreted	Average % of 24-hr total in first period urine (0–8 or 0–12 hr)	
Range	Mean		Mean and Range	Radioactive	Microbiol.
19–25	22.3	12	10.2 ± 2.8* (4.5–13.8)	36	89
26–40	30	5[a]	13.0 ± 2.8 (8.4–17.7)	31	77
41–55	49.5	6	13.4 ± 4.7 (5.3–19.7)	29	58
56–70	60.4	5[b]	8.2 ± 3.7 (2.3–13.3)	9	41
71–85	77.1	12	12.3 ± 6.2 (1.8–25.6)	25	63

* Values following ± symbol are standard errors of the means.

[a] In three of these cases, a single 24-hour collection was made. Microbiologic assays were performed only for the other two subjects. Ratios apply only to these two subjects.

[b] The apparent lower excretion rate shown by the 56–70 year groups is due to the shorter duration of the first collection period, i.e., 8 hours compared to 10 to 12 hours.

of the radioactive vitamin. The rapid and quantitative elimination of large doses of parenteral vitamin B_{12} has been reported elsewhere.[9,10]

Reproducibility

To study the reproducibility of results on repeated tests with the same individuals, a group of eight subjects, age 20 to 26, were given 2 μg of radioactive vitamin B_{12} by mouth in two successive studies conducted two months apart. The results of repeated tests are shown in Table II. The maximum difference between the first and second tests was no more than 2 per cent of the administered dose and the variation with time was random. By and large, the duplicate tests were reproducible to within 10 to 15 per cent of the initial value.

TABLE II

Repeated Tests of Urinary Excretion of Radioactive Vitamin B_{12} in Young Subjects

Subject's age	First trial	Second trial	Difference (First trial − second)
yr	%	%	%
20	8.9	9.4	−0.5
21	12.0	10.2	+1.8
21	7.7	8.6	−0.9
24	10.2	10.6	−0.4
24	7.5	8.2	−0.7
26	11.8	9.8	+2.0
26	8.6	8.2	+0.4
26	11.1	12.3	−1.2

A group of eight subjects, aged 34 to 85 years, were also given 2 μg of radioactive vitamin B_{12} orally in successive tests. Furthermore, the effect of intrinsic factor concentrate was examined in ten experiments. Results of successive tests, again expressed as per cent of dose excreted, are shown in Table III. These values were not included in the averages shown in Table I.

The results in Table III demonstrate that the largest difference in the per cent of excretion of radioactivity in the urine of the test individuals in the two successive trials without intrinsic factor was 6 per cent of the administered dose (subject: age, 85), although in a majority of cases, the mean absolute difference was only 2.3 per cent.

Of the seven subjects who were tested some two weeks later with an intrinsic factor preparation at the time of the tracer study, three responded with an increased output, whereas four had a decreased excretion. A second test with the more potent preparation (II) was also equivocal. Such variations are not unexpected, since our "older" individuals cannot be considered as homogeneous subjects.

TABLE III

Repeated Tests with and without Intrinsic Factor Concentrate

Per cent of orally administered radioactivity in urine

Subject's age	First trial		Second trial	
	No IF (3/25/54)	Plus IF (4/9/54)	No IF (5/18/54)	Plus IF (7/13/54)
yr				
34	13.1	16.9 (I)*	—	—
53	14.3	12.3 (I)	14.7	—
56	8.8	3.1 (I)	9.1	—
61	2.3	4.8 (I)	3.8	9.1 (I)
63	6.6	4.4 (I)	8.7	5.9 (I)
77	14.6	—	9.4	—
83	1.8	9.2 (II)*	2.4	6.2 (II)
85	13.2	9.2 (II)	19.2	—

* I or II in parentheses denote the intrinsic factor (IF) preparations used (see text) and all figures are expressed as per cent of the radioactive vitamin administered orally.

It is noteworthy that the two subjects (ages 61 and 83) who exhibited low initial responses excreted more radioactive vitamin B_{12} in the urine after the administration of intrinsic factor, whereas the remaining subjects with essentially normal initial excretion received no benefit from the preparation. The lack of effect on the latter group is an indication of the probable normalcy of absorption, which apparently cannot be enhanced by exogenous intrinsic factor preparation.

DISCUSSION

Variations among individuals on repeated tests may occur under certain test conditions. For example, if the injection of the "flushing" dose of 1 mg of vitamin B_{12} is carried out at $^1/_2$ or 3 hours instead of 2 hours after the oral dose, the urinary excretion may be decreased by 50 per cent. Furthermore, the amount of radioactivity in the urine cannot be increased by more frequent "flushing" with a larger total

dose of injected vitamin B_{12}. Thus, injection of a total of 4 mg of crystalline vitamin B_{12} every two hours resulted in excretion of 10.8 ± 2.2 per cent (10 subjects) of radioactivity in 24 hours. The same amount was excreted after a single injection of 1 mg after two hours.

Whether this test is an important index of vitamin B_{12} absorption remains to be answered by further experiments. However, available data demonstrate that the radioactivity "flushed out" in the urine represents only a fraction of the oral vitamin B_{12} actually absorbed by a subject. It has been shown that absorbed vitamin B_{12} is retained in large part by rats[11] or man[12] for a long period of time, at least 6 months. Glass, Boyd, and Stephanson[12] found by means of the hepatic uptake method that about 40 per cent of a 2 μg oral dose is absorbed. This is in substantial agreement with the value of 50 per cent reported by Swenseid, Gasster, and Halsted[13] using the fecal excretion method. On this basis our overall average value of 11.4 per cent flushed out in the urine corresponds to about one-fourth of the total absorbed vitamin. Despite the fact that absorbed vitamin B_{12} circulates through the blood stream and is retained in certain organs, the amount excreted still represents a large enough fraction to constitute a significant measure of the total amount absorbed.

The data in Table I do not indicate an abnormal variation of the group average response with age; nor do individual responses among the persons examined fluctuate markedly. Such responses may be considered an index of the absorptive capacity of the test subjects. If we assume that these responses are a measure of absorption, we are justified in using urinary excretion in comparing absorption of vitamin B_{12} as a function of age. Such an assumption does not recognize the possible differences in renal function and in tissue retention for vitamin B_{12} between subjects of different ages. Examination of the data in Table I fails to reveal any significant difference in response between the several age groups.*

* Since this paper was written a report by Schilling (*Am. J. Clin. Nutrition* 3: 45, 1955) was published in which the same conclusion was made.

It is of interest to point out that there were some isolated cases of extremely low absorption. One subject, aged 61 years, gave a response of only 2.3 per cent, while another subject, aged 81, excreted only 1.8 per cent of the oral radioactive dose. Besides these unusually low responses, one case each of 5 per cent excretion was noted in the 19–25 and 41–55 age groups. It is conceivable that these individuals suffered from some incipient dysfunction which had not yet fully developed, and that the low urinary excretions observed need not reflect poor absorption due to aging *per se*.

In a previous communication[14] it was reported that, following the oral administration of a test dose of 1000 μg of vitamin B_{12}, a positive response (i.e., a rise in vitamin B_{12} level in plasma) is less frequent in subjects 65 years or older than in persons between 20 and 35 years old. While these findings might be construed as indicating a defective absorption mechanism in the aged, it must be recognized that the dose of 1000 μg was far in excess of physiologic requirements. The current study, which reduces the test dose to a more physiologic level, appears on the surface to contradict the observations based upon the plasma assays. However, the discrepancy probably results from the 500-fold difference in oral dose. It is plausible that the older individuals may possess enough intrinsic factor to aid the absorption of 2, but not 1000, micrograms.

SUMMARY

A urinary excretion test using radioactive vitamin B_{12} was applied to a group of young male adults with similar dietary histories and living conditions and under conditions which minimize errors in urine collections, as well as to a group of ambulatory elderly individuals. The variation of the results obtained from this group of normal subjects and the reproducibility of the tests performed on the same individuals are presented. The results failed to reveal a significant variation of absorption with age. Of interest is the small incidence of low absorption in the aged group. It is not certain to what extent these measurements represent true absorption, since urinary ex-

cretion of orally administered vitamin B_{12} may be influenced by tissue retention and kidney function. The simultaneous administration of intrinsic factor concentrates to subjects without pernicious anemia does not appear to enhance the absorption of vitamin B_{12}. Variations observed were not greater than the normal variation exhibited by a given subject.

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