

# Clinical Reports

## Effect of Food on the Absorption of Vitamin B<sub>12</sub>

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THE vitamin B<sub>12</sub> urinary excretion test<sup>1</sup> has been used frequently for the measurement of absorption of vitamin B<sub>12</sub>. The procedure of this test involves the oral administration of a test dose of the labeled vitamin, followed by the intramuscular injection of a large dose of unlabeled vitamin in order to "flush out" the absorbed radioactive vitamin. Measurement of radioactivity in urine specimens collected for twenty-four hours, or longer, is used as an index of absorption. Such data yield arbitrary but reproducible values if the test conditions are standardized and if the physiologic state of the volunteer subjects is essentially uniform. Although this test has been used by a number of investigators, the effects of slight variation of the test conditions, such as prefeeding shortly prior to the administration of radioactive vitamin B<sub>12</sub>, have not been carefully studied. The purpose of this report is to study the influence of prefeeding on the urinary excretion of radioactivity from labeled vitamin B<sub>12</sub> in man and on the deposition of radioactivity in selected

tissues in rats. Inferences about absorption will be made from the tracer studies.

### MATERIALS AND METHODS

The test procedure used is essentially that described in a previous paper.<sup>2</sup> Three experiments with human subjects were carried out. In one, twenty male volunteer subjects (inmates of a state penal institution) twenty-four to forty years of age, clinically healthy and with substantially uniform dietary histories over the recent past, were assigned at random to one of two groups. One group (ten subjects) was offered breakfast consisting of liberal amounts of dry cereal with milk, pancakes and bacon; the other (ten subjects) was fasted from 4 P.M. of the previous day.

Within thirty minutes after breakfast subjects in each of the two groups, were given capsules containing 50 µg. of labeled vitamin B<sub>12</sub>. Two hours later, each subject was given an intramuscular injection of 1,000 µg. of unlabeled vitamin B<sub>12</sub> and the urinary output of each was collected for the next twenty-four hours. Half of each sample was evaporated on the steambath to less than 50 ml. and transferred quantitatively to a brown bottle calibrated at 50 ml. Radioactivity was measured in a gamma scintillation counter.

In the second study, twenty-three subjects were divided into two groups. Subjects in group A (twelve subjects) were fasted; those in group B (eleven subjects) were given breakfast as described. Two hours later all subjects were given 10 ml. of aqueous solution containing 50 µg. labeled vitamin B<sub>12</sub>. Quantitative ingestion of the dose was assured by repeated rinsing of the containers with 10 ml. of water. Each subject drank four such washings. In the third study, two groups of five subjects were used.

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TABLE I  
Studies in Man  
(Urinary Excretion of Radioactivity\*)

Study	A (with breakfast)	B (without breakfast)	A:B
1	1.60 ± 0.072(10)†	0.82 ± 0.054(10)	1.94
2	2.72 ± 0.24(11)	1.39 ± 0.07(12)	1.96
3	2.00 ± 0.20(5)	1.22 ± 0.20(5)	1.64

\* Values are expressed in per cent of the administered dose of 50 µg./Co<sup>60</sup>B<sub>12</sub>.

† Numbers in parentheses denote number of subjects (human) used.

A third experiment on man was conducted differing from the first two in that the breakfast of the prefed group (five subjects) consisted of a large portion of corn flakes, milk and sugar, *ad libitum* (Table I). Immediately after breakfast, they were given 50 µg. radioactive vitamin B<sub>12</sub> orally. They excreted 2.00 ± 0.2 per cent of the ingested radio-vitamin in the twenty-four-hour urine. For comparison, the fasted group was fed the same amount of vitamin B<sub>12</sub> and the five subjects excreted 1.22 ± 0.20 per cent only. The 66 per cent increase in excretion is again statistically significant ( $P < 0.025$ ).

To study the effect of prefeeding on the absorption of vitamin B<sub>12</sub> in rats, male adults were fasted overnight. On the following morning, half of them were offered 5 gm. portions of a casein diet. Feed cups were withdrawn half an hour later. Any unconsumed food was then weighed. The feed intake varied from 1 to 5 gm. per rat. Precisely thirty minutes later, 50 mµg. of radioactive vitamin B<sub>12</sub> was given by stomach tube to both fasted and fed animals. Feces were collected for four days, after which the animals were sacrificed and their livers and kidneys removed. The feces and organs were solubilized with concentrated H<sub>2</sub>SO<sub>4</sub> or KOH solution for radioactivity measurement.

#### RESULTS

Table I summarizes the findings of our first experiment with human volunteer subjects. The 94 per cent increase in excretion of radioactivity attributable to ingestion of food prior to test is statistically significant ( $P < 0.001$ ).

In the second experiment (Table I), the ratio of excretion of radioactivity in the prefed group (eleven subjects) to the fasted group (twelve subjects) was found to be 1.96. The 96 per cent increase attributable to feeding is statistically significant ( $P < 0.001$ ).

TABLE II  
Studies in Rats  
(Radioactivity\* in Livers and Kidneys)

Study	Liver			Kidneys		
	Fasted	Fed	Ratio	Fasted	Fed	Ratio
A (5/5)†	0.72	1.49	2.07	1.50	2.90	1.93
B (5/5)	1.05	2.24	2.13	1.17	3.11	2.66
C (6/6)	1.27	2.44	1.92	2.53	4.79	1.89
D (6/5)	1.08	2.33	2.16	4.01	7.73	1.93
Mean			2.07			2.10

\* Radioactivity expressed as geometric mean millimicrograms of labeled vitamin B<sub>12</sub> in the organ.

† Numbers in parentheses correspond to numbers of subjects (rats) in fasted and fed groups, respectively.

Four separate rat studies were completed in which the radioactivity in liver and kidneys was determined. Table II is a summary of the data and the meaningful ratios. With respect to both liver and kidney values, the ratios did not differ significantly from experiment to experiment. The mean ratios, 2.07 for liver levels and 2.10 for kidney levels, correspond to increases in the fed animals greater than 100 per cent over the fasted animals. These are statistically very highly significant ( $P < 0.001$ ).

In experiment A, the fecal excretion of radioactivity was determined. In the fed group this excretion was 30 per cent less than in the fasted group. This difference is compatible with the hypothesis that feeding enhances absorption, but the data are too few to be statistically significant.

#### COMMENTS

The vitamin B<sub>12</sub> molecule is poorly absorbed from the gastrointestinal tract of even clinically healthy subjects. Numerous attempts have been made to increase the absorption of the orally administered vitamin and the literature contains conflicting reports on the measure of success attained.

Unfortunately, the absorption of vitamin B<sub>12</sub> is extremely difficult to measure explicitly, and substantially all the conclusions referred to, including those deduced from this communication, rest on inferences drawn from more readily measurable data. It is logical to assume that the absorption of the vitamin B<sub>12</sub> molecule parallels the absorption by tissues or

urinary excretion of the radioactive tag, usually cobalt<sup>58</sup> or cobalt<sup>60</sup> in labeled vitamin B<sub>12</sub>, and there are at least a few experiments which support the validity of inferences so drawn.

The majority of the studies rely for their conclusiveness on the ability of the person carrying out the experiment to make certain statements about the formal statistical significances of the reported findings. Almost uniformly, however, these statements beg the question whether the observed differences are physiologically meaningful and indeed we have little or nothing in the way of yardsticks by which we can measure the physiologic significance of any of the differences reported. No doubt the best criteria are those seen in studies of patients with pernicious anemia in whom reticulocytosis, increases in hemoglobin level and clinical improvement are less obscure evidences of beneficent changes. However, the population available for such studies is small and the applicability of findings in such populations to the population at large is questionable, because of the known defect in intrinsic factor output in the patient with pernicious anemia.

Nevertheless, and even with all these limitations in mind, the data presented in this communication demonstrate quite clearly that there is a statistically significant increase in the apparent absorption of vitamin B<sub>12</sub> from the gastrointestinal tract following the oral administration of the vitamin to clinically healthy human subjects and to rats, when the vitamin is offered after feeding rather than on an empty stomach. Curiously, the conclusions

reported herein with respect to human subjects are contradictory to those reported earlier by Swenseid et al.,<sup>3</sup> and this difference still requires reconciliation. Direct comparison of the studies cannot be made because of differences in methods and dose range. However, despite their conclusions, Swenseid et al., did show an enhancement of absorption by food at a 0.5  $\mu$ g. dose and speculate that secretion of intrinsic factor may be stimulated by food.

#### SUMMARY

Data involving the measurement of absorbed radioactivity following the oral administration of labeled vitamin B<sub>12</sub> are presented which show greater apparent absorption of the vitamin in puffed human subjects or rats, using as a criteria urinary excretion in man and organ (liver and kidneys) uptake or excretion in rats.

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