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

II. EFFECT OF CRUDE AND PURIFIED INTRINSIC FACTOR PREPARATION

By BACON F. CHOW, PH.D., WILLIAM L. WILLIAMS, PH.D., KUNIO OKUDA, M.D., AND RALPH GRASBECK, M.D.

CASTLE postulated the presence in normal gastric juice of a substance which he called intrinsic factor. The biological activity of this substance is shown by the response of subjects with pernicious anemia to its co-administration with oral vitamin B_{12}. While this hypothesis is now well established, the effect of this substance on the absorption of the vitamin by normal individuals has not been studied in detail. Rosenblum[1] and Chow[2] and their associates demonstrated that certain intrinsic factor preparations of hog origin may even interfere with the absorption of orally administered radioactive (Co^{60}) vitamin B_{12} by rats, in that the addition of this agent would actually increase the fecal excretion of radioactivity. These results were interpreted to indicate a possible inhibition of absorption of vitamin B_{12} given by mouth due to the administration of crude concentrates to rats. Swendseid[3] reported a similar adverse effect of intrinsic factor concentrates in totally gastrectomized subjects. No systematic study has yet shown whether or not intrinsic factor aids vitamin B_{12} absorption by clinically healthy humans.

We have recently reported on the variability of the urinary excretion test[4,5] among normal persons and it was, therefore, of interest to apply this procedure to such a study. In this communication we wish to present the results of feeding various intrinsic factor preparations to healthy, young individuals, as measured by the urinary excretion of the radioactivity following the administration of labeled vitamin B_{12}. Our results show that the inhibitory effect of intrinsic factor material shows itself when certain crude, but not purified, preparations are used.

From the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

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EXPERIMENTAL

Test Procedure

The procedure employed is essentially that described in our previous communication.[5] Two hours after the oral administration of the desired amount (2 or 50 μg) of cobalt^{60}-labeled vitamin B_{12}[6] with or without the intrinsic factor preparation being studied, 1 milligram of crystalline vitamin B_{12} in physiologic saline solution was injected intramuscularly. Urine specimens were collected for a period of 24 or, in some instances, 48 hours, under carefully supervised conditions. An aliquot of each collection, equivalent to one-quarter or one-half of the total volume collected, was evaporated to 50 ml in a beaker containing 50 μg of unlabeled vitamin B_{12} as carrier. The concentration was measured by scintillation counting, using a thallium-activated sodium iodide crystal. The radioactivity measured in this fashion was corrected to the original volume of urine and computed as per cent of oral dose excreted. The activity of one microgram was equivalent to about 25,000 to 30,000 counts per minute. Although it is not certain to what extent the urinary excretion test represents a measure of true absorption of vitamin B_{12}, this

method is convenient and useful both for qualitative determination of intrinsic factor preparations and effective comparisons of materials.

Choice of Subjects

All subjects used in the present study were residents of a state penal institution for at least three years and had comparable dietary histories. They were clinically healthy and free from acute infectious diseases or metabolic disturbances at the time of the tests. During the first twelve hours of the urine collection periods they were required to stay in one room and were denied toilet facilities without the consent of the supervisor. They were then allowed to sleep in their dormitories. On the following morning they were again requested to stay in one room in order to complete the 24- or 48-hour collections. The comparability of the nutritional state of the test subjects, which might be inferred from the controlled dietary of the institution, and the satisfactory condition for the urine collection provide a very desirable and, indeed, necessary prerequisite for a study of normal metabolism. To insure complete urinary collections, some specimens were analyzed for creatine nitrogen and for microbial activity of vitamin B_{12}. Usually at least 90 per cent of the injected vitamin B_{12} or 1.8 g creatine nitrogen were found in the urine.

Intrinsic Factor Preparation

Two batches of intrinsic factor preparations obtained by the extraction of hog stomach and intestines with ammonium sulfate or alcohol were used. Their intrinsic factor potencies, as established in patients with pernicious anemia in relapse, were found to be such that about 25 and 50 milligrams, together with 15 micrograms of vitamin B_{12}, comprised one U.S.P. unit, for preparations *A* and *B*, respectively. A highly purified preparation (C), less than 1 mg of which together with 15 μg of crystalline vitamin B_{12} by mouth gave a satisfactory response in suitable test subjects, was also used.

RESULTS AND DISCUSSION

The results of five separate trials (a–e) in which 2.0 μg of radioactive vitamin B_{12} were given to 41 young male individuals, and which were carried out over a period of seven months, are collected in Table I. Each trial reported in this communication is designated by a letter. Similar letters indicate that the trials were carried out concurrently. At least three batches of radioactive vitamin B_{12} were used. A striking finding is that with 2-μg doses of radioactive vitamin B_{12} the mean urinary excretion of radioactivity varied only from 204 to 240 millimicrograms within 24 hours. Fifty μg of radioactive vitamin B_{12}* were given orally to another 26 subjects in four trials (b, d, e, f). The urinary output of radioactivity increased to a range of means of 442–491 millimicrograms in three separate trials; in the fourth (study b), an unusually large variation was encountered because of the low specific activity of the labeled vitamin B_{12} fed, and there was insufficient activity in the urine for accurate measurement.

TABLE I

Urinary Excretion of Radioactive Vitamin B_{12} after Oral Administration

Amount of B_{12} given (μg)	Study	Labeled vitamin B_{12} in urine (24 hr) mμg ± S.E. of the mean
2	a (12)	204 ± 18
	b (7)	244 ± 38
	c (8)	240 ± 44
	d (8)	216 ± 24
	e (6)	210 ± 20
50	b (6)	502 ± 52
	d (7)	454 ± 35
	e (7)	442 ± 30
	f (6)	491 ± 32

( ) Denotes the number of subjects used.

In Table II are tabulated the results of three trials involving the use of 59 different individuals. In each trial (d, e, f), a separate control group was used. Fifty-microgram doses of radioactive vitamin B_{12} were fed orally with or without the simultaneous administration of one or another of the intrinsic factor preparations. In trial (d) the administration of 50 μg of vitamin B_{12} resulted in the urinary excretion of approximately 454 ± 35 mμg of radioactive

* Generously supplied by Dr. Rosenblum of Merck and Company, Rahway, New Jersey.

vitamin B_{12}. In the same trial the administration of 4 U.S.P. equivalents of intrinsic factor preparation *A*, together with 50 mμg of labeled vitamin B_{12} resulted in a decrease to 332 ± 46 mμg. The difference between the mean urinary excretion is statistically significant ($P < 0.05$). Similar decreases were obtained in two other trials (e and f) using the same preparation; they were likewise statistically significant.

TABLE II

Effect of Intrinsic Factor Preparations (I.F.P.) on Urinary Excretion of Orally Administered Radioactive Vitamin B_{12}*

Trials	Treatment Vit. B_{12} (μg) + I.F.P. (mg)		No. of Subjects	Radioactivity in 24-hr urine mμg ± S.E.
d	50	0	7	454 ± 35
	50	100 (A)	7	332 ± 46
e	50	0	7	442 ± 30
	50	100 (A)	6	354 ± 20
	50	200 (B)	5	365 ± 36
	50	12 (C)	6	525 ± 40
f	50	0	6	491 ± 32
	50	100 (A)	8	364 ± 26
	50	12 (C)	7	609 ± 33

* All subjects were between 22 and 33 years old.
(A), (B), (C) denote preparations of intrinsic factor concentrates used (see text).

Table II also demonstrates clearly that another crude preparation, *B*, was equally inhibitory to oral absorption, causing a decrease in urinary excretion of labeled vitamin B_{12} of from 442 to 365 mμg. To test the possibility that the decrease in radioactivity in the urine may have been due to a delay in excretion, the collections of urine in some trials were extended from 24 to 48 hours. The second 24-hour period collection failed to increase the recovered urinary radioactivity by more than 5 per cent either in the control or the experimental group (i.e., with and without intrinsic factor). Therefore, delayed excretion was not a factor. These data, taken as a whole, therefore indicate that under our test conditions, both crude intrinsic factor concentrates in some way decreased the urinary excretion of subjects receiving radioactive vitamin B_{12}.* It cannot be predicted whether such effects could be observed if the ratio of vitamin B_{12} to intrinsic factor preparations were altered. In trial (e), 50 μg of vitamin B_{12} were also given to two additional groups of 8 subjects each, together with 1 or 16 U.S.P. units equivalent of preparation *A*. Our results showed that one U.S.P. unit equivalent caused no inhibitory effect (450 ± 25 mμg), but 16 U.S.P. units equivalent decreased the urinary excretion to 225 ± 20 mμg from a control value of 442 ± 30 mμg.

A question of importance is whether the decreased urinary excretion is due to intrinsic factor *per se* or to some inhibitory substances present in the concentrate. To this end, preparation *C* derived from preparation *A* was tested. In the ultracentrifuge and by electrophoresis, it contains a major unsymmetrical peak (75%) and a minor one (25%). The administration of 12 mg of this purified substance caused a statistically significant increase in urinary excretion above that seen when labeled vitamin B_{12} was given alone, or with a more crude extract (*A*) (Table II). These data, therefore, suggest that the inhibitory effects on urinary excretion noted with crude preparations are, perhaps, associated with substances other than intrinsic factor itself; these appear to have been removed during the purification procedure used to prepare substance *C*.

Conversely, it should be pointed out that not all crude intrinsic factor preparations contain inhibitory substances. For example, it was found that the co-administration of 4 units of a preparation† (50 mg/U.S.P. unit) with 50 μg of radioactive vitamin B_{12} resulted in an increase of 24-hour urinary excretion from a mean value of 510 ± 40 mμg for the control group (8 subjects) to 660 ± 30 mμg (8 subjects). The difference is statistically significant. These data indicate then that this preparation is not inhibitory.

The amount of radioactivity excreted in the urine of our test subjects is dependent not only on the absorption but also on the tissue retention and renal function. Thus, the decrease in urinary excretion following the administration of certain intrinsic factor preparations can

* Made available to us through the courtesy of Dr. William Williams of Lederle Laboratories.

† "Neotinic" made available to us by Stuart Co., Burbank, California.

be considered only as suggestive evidence that such concentrates may inhibit absorption of orally administered vitamin B_{12}. Although we do not believe that differences in tissue retention or in renal function existed among our subjects in randomly selected groups, supporting evidence of the inhibitory effect must await the studies using other independent criteria, such as the determination of fecal excretion of orally administered radioactive vitamin B_{12} or the oral tolerance test for changes in blood vitamin B_{12} levels following prolonged oral feeding of unlabeled vitamin B_{12}.

TABLE III

Vitamin B_{12} Binding Power of Intrinsic Factor Preparations Used

Preparation	mg/unit*	Millimicrograms vitamin B_{12} bound/mg I.F.P.	
		Microbiologic method	Dialysis
Crude A	50	37.5	53.0
Crude B	200	49.0	68.0
C (Purified A)	1 or less	54.0	65.0

* Antipernicious anemia unit as defined—U.S.P.

It is also of interest to point out that the highly purified preparation *C* is still capable of binding vitamin B_{12}, as determined by our microbial test[8] or by dialysis.[9,10] The data in Table III demonstrate that the binding power of the purified material was equal to or even greater than that of the crude preparations (consisting of more than 6 electrophoretic components) when expressed on the basis of vitamin B_{12} bound per milligram of dry powder. These facts thus provide additional suggestive evidences for our hypothesis[11] that intrinsic factor binds vitamin B_{12}, although not all binding substances in the extracts of hog stomach or intestine possess intrinsic factor activity.

SUMMARY

A systematic study involving the use of 91 young, healthy volunteers demonstrated that certain crude intrinsic factor preparations decreased the urinary excretion of orally administered radioactive vitamin B_{12}. On the other hand, a highly purified preparation derived from the crude concentrate or a special preparation with no inhibitory substance increased the urinary output of radioactivity. Thus, the observed decrease in urinary excretion is not due to intrinsic factor *per se*, but to some interfering substances present in our crude concentrates. The purified preparation is still capable of binding vitamin B_{12} according to the microbial absorption procedure or by the dialysis method.

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