Effect of Hog Intrinsic Factor on the Absorption of the Coenzyme Form of Vitamin B_{12} (5,6-dimethylbenzimidazolylcobamide)

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THE isolation of 5,6-dimethylbenzimidazolylcobamide (Co-B_{12}),[1,2] the coenzyme of vitamin B_{12}, prompted us to investigate whether or not this compound, when given orally to patients with pernicious anemia or to clinically healthy subjects, is readily absorbed and whether or not the absorption can be improved by the coadministration of intrinsic factor. To these ends, the radioactive coenzyme of vitamin B_{12} or vitamin B_{12} alone (cyanocobalamin) was administered orally to subjects, with or without intrinsic factor with known activity. The absorption was estimated by the urinary excretion test of Schilling.[3] The results of these studies are now reported.

EXPERIMENTAL

Urinary Excretion Test

Two micrograms of radioactive vitamin B_{12}** or Co-B_{12} labeled with cobalt^{58} was administered orally to six clinically health subjects who were residents of a state penal institution, and to five patients with proved pernicious anemia with typical features of megaloblastic anemia and histamine-refractory achlorhydria after overnight fasting. Two hours later, 1,000 μg. of unlabeled vitamin B_{12} was given intramuscularly to the test subjects regardless of the radioactive material given. Twenty-four-hour urine collections were made. Half of the total urine was concentrated on a steam bath and transferred quantitatively into a brown bottle previously calibrated to 50 ml. for the radioactivity measurement with a scintillation counter. To test the effect of intrinsic factor on the absorption of vitamin B_{12}, or its coenzyme, 120 mg. of an intrinsic factor preparation was mixed with 2 μg. of the test radiovitamin in 10 ml. water, and administered orally to the test subjects, in the manner just described.

Materials Used

The radioactive coenzyme of vitamin B_{12}†† was transported to us in a light-proof container. To minimize the degradation of this compound due to light, the preparations were kept in the dark and under refrigeration until the compound was administered. The specific activity of both radioactive compounds was diluted isotopically to contain 0.15 μc. per ug. The activity of the intrinsic factor preparation (WES 816) according to the manufacturer‡‡ was such that 60 mg. was equivalent to 1 N.F. unit.

RESULTS

The total amounts of radioactive material in the twenty-four-hour urine specimens of five patients with pernicious anemia receiving 2 μg. radioactive (coenzyme) Co-B_{12} (Table 1) ranged from 5 to 8 mμg. Three months later, the same volunteer subjects received 2 μg.

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†† The radioactive cyanocobalamin was kindly supplied by Merck & Co.

‡‡ Supplied by Lederle Laboratories.

TABLE I

Urinary Excretion* of Radioactivity by Patients With Pernicious Anemia Following Oral Administration of Co^{58}-labeled Coenzyme Co-B_{12} With or Without Intrinsic Factor

Subject	Treatment		
	Co-B_{12} Alone (A)	Co-B_{12} plus 2 Units Intrinsic Factor (WES 816) (B)	B/A
1	7	58.6	8.4
2	8	21.6	2.7
3	7	14.9	2.1
4	5	47.2	9.4
5	4	38.9	9.7
Mean	6.2	36.2	6.6

* All results are expressed as mμg. Co-B_{12} equivalent in twenty-four-hour urine.

TABLE II

Urinary Excretion* of Radioactivity by Patients With Pernicious Anemia Following Oral Administration of Co^{58}-labeled Vitamin B_{12} With or Without Intrinsic Factor

Subject	Treatment		
	Vitamin B_{12} Alone (A)	Vitamin B_{12} plus 2 Units Intrinsic Factor (WES 816) (B)	B/A
1	18	192	10.7
2	20	316	5.8
3	10	210	21.0
4	14	146	10.4
Mean	15.5	216	14.5

* All results are expressed as mμg. vitamin B_{12} equivalent in twenty-four-hour urine.

of this isotope together with 2 units of intrinsic factor. The urinary excretion of the radioactive substance increased in every instance. We believe this average increase of more than sixfold is significant despite the small number of subjects studied.

Radioactive vitamin B_{12} was also administered, with or without intrinsic factor, to four different subjects with pernicious anemia in remission. The results demonstrate a marked increase in radioactivity in the urine (Table II). They further demonstrate that the urinary radioactivity excretion of patients with pernicious anemia receiving cyanocobalamin alone was slightly but significantly ($p < 0.05$) higher than those receiving coenzyme-B_{12}. This difference is of doubtful physiologic importance. It is of interest that the increase in average urinary excretion of radioactive cyanocobalamin due to coadministration of intrinsic factor is nearly fifteenfold, i.e., from 15.5 to 216 mμg.

We have also administered radioactive cyanocobalamin or the coenzyme to clinically healthy subjects with no absorptive defects and measured their urinary excretion of these compounds. The results of the study (Table III) demonstrate that the amount of radioactivity found in the urine of subjects receiving cyanocobalamin is about three times greater than that in those receiving an equal amount of the coenzyme form.

COMMENTS

The isolation of coenzymes of vitamin B_{12} by Barker and associates,[1] constitutes another important contribution toward our understanding of the physiologic role of vitamin B_{12}. It appears to be reasonable to postulate that since cyanocobalamin is probably stored as coenzyme-B_{12} in the tissues, e.g., liver, the native form may be absorbed more readily. Indeed, it has been reported[5] that the absorp-

TABLE III

Urinary Excretion Tests With Young Healthy Persons Using Co^{58}-Labeled Vitamin B_{12} and Coenzyme-Vitamin B_{12} (Co-B_{12})

Group (dose)	No. of Subjects	Urinary Excretion in 24 Hours (mμg.)
Vitamin B_{12} (2 μg.)	6	212 ± 20.8
Co-B_{12} (2 μg.)	6	68 ± 9.6

NOTE: Unlabeled cyanocobalamin, 1 mg., was injected intramuscularly for "flushing" in both groups.

tion of coenzyme-B_{12} is less dependent upon intrinsic factor than is vitamin B_{12}. This claim however, lacks sufficient experimental support.

The purpose of this study was to determine whether absorption of coenzyme-B_{12} can be improved by the coadministration of intrinsic factor and to compare the urinary excretion of these two compounds following the oral administration to healthy subjects. Our data obtained, admittedly from a study with a limited number of available subjects with pernicious anemia, lead us to conclude that the administration of intrinsic factor improved the urinary excretion of coenzyme-B_{12}, although this increase in terms of percentage of test material administered orally was only a third of that seen with cyanocobalamin. Furthermore, our data show that the amount of radioactivity of the coenzyme form excreted by healthy persons without known defect in intrinsic factor production following oral administration, was also much lower than that of vitamin B_{12} as the cyanocobalamin. From such data one may not conclude that there is necessarily a difference in absorbability of these two compounds. For example, on the basis of the fecal excretion test, Okuda and Chow[4] found that rats, when given these two compounds orally, absorbed them to almost the same extent. However, they excreted more cyanocobalamin than its coenzyme in the urine. It will be unwise to assume that these results are necessarily applicable to human beings.

SUMMARY AND CONCLUSIONS

Our studies demonstrate that only a negligible amount of radioactive coenzyme-vitamin B_{12} (Co-B_{12}) given orally to five subjects with pernicious anemia was excreted in the urine, according to the results of the urinary excretion test. The coadministration of an intrinsic factor preparation increased the urinary excretion of radioactivity of this compound, but not to the same extent as that observed when cyanocobalamin was the test substance. On a weight-for-weight basis more cyanocobalamin (vitamin B_{12}) is excreted in normal subjects, or patients with pernicious anemia receiving intrinsic factor, than the coenzyme form, 5,6-dimethylbenzimidazolylcobamide, when administered orally and studied by radioactive-labeled cobalt.

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