Effect of Intrinsic Factor on Absorption of Vitamin B_{12} in Healthy Individuals

By LEON ELLENBOGEN, PH.D., BACON F. CHOW, PH.D.,* AND WILLIAM L. WILLIAMS, PH.D.

VARIOUS investigators have demonstrated that intrinsic factor causes the absorption of orally administered vitamin B_{12} in pernicious anemia patients.[1-8] The effect of added intrinsic factor on absorption of vitamin B_{12} by normal individuals who secrete intrinsic factor has been the subject of relatively fewer reports. Swendseid *et al.*[9] found that administering intrinsic factor concentrates (from hog stomach mucosa) or normal human gastric juice led to a decrease in absorption of vitamin B_{12} in three of four normal individuals. Chow *et al.*[10] used ninety-one young healthy males and demonstrated that a crude intrinsic factor preparation inhibited absorption, whereas a highly purified preparation and a different crude preparation increased absorption of vitamin B_{12}. Investigation of the effect of a variety of intrinsic factor preparations[11] revealed that of those tested many generally available preparations inhibit while a few augment absorption of vitamin B_{12} by normal individuals.

In patients with pernicious anemia, increasing the dosage of certain intrinsic factor preparations to two or four times the minimum amount effective in producing remission was reported to inhibit absorption of vitamin B_{12}.[4,5] An intrinsic factor preparation known to be inhibitory at certain levels with normal subjects[10,11] was found at the same levels to increase absorption of vitamin B_{12} in patients with pernicious anemia.[6,7]

In the above experiments[9-11] the intrinsic factor preparations were given in relatively large doses and suspended or dissolved in water with radioactive vitamin B_{12}. This differs from the usual method of administration of vitamin B_{12}, intrinsic factor and other supplements for oral therapy of anemia. The present experiments were designed to investigate the effects of intrinsic factor and vitamin B_{12} as combined in vitamin-mineral capsules with other substances.

METHODS AND MATERIALS

For the determination of absorption of vitamin B_{12} the urinary excretion method of Schilling[2] was used. The subjects were residents of a state mental institution. They were in good physical health and were not receiving any other medications at the time of the test. The hematocrit values and vitamin B_{12} serum levels of these patients were all within the normal range. The patients, whose ages ranged from 24–40 years, were housed in one large ward and received identical diets.

The patients were randomly allotted to groups consisting of from six to thirteen subjects. In each experiment one group was given capsules containing the intrinsic factor preparation (0.5 daily oral dose* per capsule) and the following other ingredients: radioactive vitamin B_{12}, 10 μg (0.17 μc); folic acid,

From the Nutrition and Physiology Section, Research Division, American Cyanamid Company, Pearl River, New York.

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

* We have defined a daily oral dose (DOD) of intrinsic factor as the minimum amount which causes satisfactory clinical improvement and an increase of about one million in red blood cell count in 21 days when given with 10 μg of vitamin B_{12} to a patient with pernicious anemia in relapse. Acceptance of the results obtained with a combination of one DOD and 15 μg of vitamin B_{12} or less by the U.S.P. Antianemia Advisory Board would permit reference to such a combination as a U.S.P. oral unit of vitamin B_{12} plus intrinsic factor.

1 mg; ascorbic acid, 50 mg; and $FeSO_4 \cdot 2H_2O$, 192 mg per capsule. A second group which served as the control received capsules with the same ingredients except intrinsic factor. Each subject received three capsules per day, one at 7, 8 and 9 a.m., followed by a flushing injection of 1 mg of unlabeled vitamin B_{12} at 10 a.m. after which urine was collected for 24 hours. A second flushing injection was then given and urine collected again for 24 hours. Radioactivity measurements were made on urine aliquots up to one liter using a specially designed beaker.[12]

Intrinsic factor concentrates were prepared by extraction of hog gastro-intestinal tissue with 2 per cent NaCl and precipitation with $(NH_4)_2SO_4$ as described elsewhere[11,13,14] and were similar to "crude AS fraction."[13] The various preparations were from different batches made by the same process.

RESULTS AND DISCUSSION

The results of seven consecutive experiments are summarized in Table I. The urinary excretion of radioactive vitamin B_{12} in the individual subjects of a typical experiment (Experiment No. 1) is presented in Table II. The data were submitted to Dr. F. W. Wilcoxon for statistical analysis. When the number of patients in each group did not exceed ten, exact probabilities were obtained from tables published by the Mathematical Centre, Amsterdam,[23] after the values had been ranked[24] through both groups. In other cases the normal approximation was used with standard deviation of rank total equal to: $\sqrt{\frac{\bar{T}n_1n_2}{6}}$ where $\bar{T}n_1$ is the expected rank total for the n_1 group, n_2 is the number of patients in the other group, while 6 is a constant.

TABLE I

Effect of Intrinsic Factor on Urinary Excretion of Vitamin $B_{12}Co^{56}$ by Normal Persons

Exp. No.	Patient group	Number of patients in group	Intrinsic factor concentrate in capsules	Average urinary excretion of vit. $B_{12}Co^{56}$ in 48 hours	Probability (P)
			Preparation No.	mμg.	
1	I	6	—	977	0.265
	II	7	182	1162	
2	III	6	—	990	0.314
	IV	7	182	1062	
3	II	6	—	1038	0.294
	I	6	87–3	1129	
4	IV	8	—	985	0.422
	III	6	87–3	1013	
5	V	13	—	814	0.258
	VI	12	87–2	849	
6	VI	13	—	711	0.215
	V	13	98–1	808	
7	VII	13	—	916	0.312
	VIII	13	635	1017	
				Combined Probability	0.23

TABLE II

Urinary Excretion of Vitamin $B_{12}Co^{56}$ in Normal Individual Subjects*

Without intrinsic factor		With intrinsic factor	
Subject	Excretion mμg in 48 hours	Subject	Excretion mμg in 48 hours
S.M.	902	J.A.	1022
J.T.	1070	F.B.	1249
J.T.	1371	J.B.	1170
D.V.	688	M.B.	892
E.W.	770	M.D.	982
J.W.	1058	R.D.	1456
		D.H.	1366

* Experiment No. I.

The deviation of a rank total from the expected value Tn_1 was divided by the standard deviation and the probability obtained from Normal Tables. The expected total is the average rank times the number of items in the group. The average rank is $\frac{n_1 + n_2 + 1.}{2}$

The probabilities were combined according to Fisher[25] and are included in Table I.

Although the combined probabilities, $P = 0.23$, show only slight significance, intrinsic factor preparations administered in capsules together with vitamin B_{12} caused a small increase in the urinary excretion of vitamin B_{12} in seven consecutive experiments. Probably the reason that the effect of exogenous intrinsic factor was small was the fact that the absorption without intrinsic factor was high. Absorption without intrinsic factor in these experiments is about twice as high as reported by Chow *et al.*[10] using 50 μg of vitamin B_{12}.

Probably of greater importance was the lack of inhibition of absorption in contrast to reports of experiments with other intrinsic factor preparations.[10,11] Vitamin B_{12} serum levels have been reported to be decreased in persons who are iron-deficient,[15] elderly[16,17] or pregnant.[18–20] It is, therefore, desirable that measures be taken to assure the absorption of vitamin B_{12} by patients in these categories.

It has been reported that the absorption of vitamin B_{12} from the gastrointestinal tract of individuals with normal gastric function is limited. Glass *et al.*[21] and Swendseid *et al.*[9] have both found that the upper limit of absorption is 1.5 μg even after oral doses of 50 μg of the vitamin. This emphasizes the importance of non-inhibition or of augmentation of vitamin B_{12} absorption. Judging from the observed ratio that the amount of vitamin B_{12} absorbed is three times the 24-hour urinary excretion value or two times the 48 hour value,[22] the average amount of vitamin B_{12} absorbed by patients receiving intrinsic factor in this study was 2.0 μg. This compared closely with the upper limit of 1.5 μg reported by Glass *et al.*[21] and Swendseid *et al.*[9]

TABLE III

Effect of Intrinsic Factor on Vitamin B_{12} Serum Levels

No intrinsic factor μμg/ml	With intrinsic factor μμg/ml
Initial vitamin B_{12} serum level	Initial vitamin B_{12} serum level
240 ± 18.6	237 ± 25.8
at 6 weeks	at 6 weeks
248 ± 25.2	388 ± 37.4
at 12 weeks	at 12 weeks
239 ± 20.4	420 ± 31.5

± Standard deviation of the mean.

Capsules with essentially the same formula, but with nonradioactive vitamin B_{12} and with and without intrinsic factor were administered daily over a 12-week period to two groups of 13 elderly patients. Vitamin B_{12} serum levels were determined at 6 and 12 weeks. The results are shown in Table III. The increase in serum levels and hence the increase in absorption of vitamin B_{12} which was stimulated by intrinsic factor, was highly significant. $P = <.001$ at 6 and 12 weeks.

SUMMARY

Capsules containing several hematinic agents including intrinsic factor and vitamin B_{12} were administered to clinically healthy males. The intrinsic factor concentrate studied did not inhibit absorption of vitamin B_{12} as has been reported for some other preparations, but caused a slight increase in absorption of the vitamin, in seven consecutive experiments as measured by the urinary excretion tests. In a separate experiment the capsules containing intrinsic factor administered over a period

of 12 weeks caused a marked and statistically significant increase in vitamin B_{12} serum levels.

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