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Comparative Absorption of Vit. B_{12} Analogues by Normal Humans. III. 5,6-Dichlorobenzimidazole, 5,6-Desdimethylbenzimidazole and 5-Hydroxybenzimidazole Analogues. (23108)

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Preceding communications(1,2) have reported oral absorption of several non-cyanocobalamins to be inferior to cyanocobalamin. Furthermore, all cobalamins were equally effective, upon injection, in inducing excretion of orally administered cobalt-60 labeled forms. These findings indicate the importance of the cyanide group for cobalamin absorption, but offer no suggestion as to influence of structural modifications on oral absorption. It is known that pseudo-vit. B_{12} and its 2-methyl derivative(3), Factor III (3) and the desdimethyl analogue(3,4) of cyanocobalamin exhibit inferior growth activity in chicks. However, direct absorption tests of substituted benzimidazole derivatives in man remain to be performed. Accordingly, urinary excretion tests have been performed with 3 analogues of vit. B_{12} with altered substituents in the benzimidazole moiety. These compounds are (1) the 5,6-dichlorobenzimidazole analogue (DCBA)(5), (2) the 5,6-desdimethylbenzimidazole analogue (DDMBA)(5,6) and (3) 5-hydroxybenzimidazole analogue (Bernhauer's Factor III)(7).

Materials. Cobalt-60 labeled analogues (specific activity $\simeq$ 180 μc/mg) for oral administration were prepared by fermentation in medium containing cobalt-60 and appropriate precursors. Products were isolated by methods previously described for vit. B_{12}, followed by chromatography on cellulose(8).* The DCBA was prepared in the Merck Sharp & Dohme Research Laboratories.†

Method. Urinary excretion method of Schilling(9) was employed as index of absorption. This was(1,2) valid for cobalamins by comparison with 3 other test methods. An oral dose of 2 μg (except for 4 subjects as shown in Table I) of labeled derivative was fed in aqueous solution to normal young adult males, each receiving 1 mg injection (except where otherwise stated) of unlabeled derivative 2 hours after the oral dose. Urine was collected for 24 hours, and, in Study 6, for

* Non-isotopic modifications of DDMBA and Factor III were kindly supplied by Dr. E. L. Smith, Glaxo Laboratories, and by Prof. K. Bernhauer, Aschaffenburger Zellstoffwerke, respectively.

† Preparation of this compound by Drs. F. M. Robinson and I. M. Miller is gratefully acknowledged.

TABLE I. Comparative Urinary Excretion of Structural Analogues of Vit. B_{12} (24 Hr Responses).

Oral admin. Analogue	Dose, μg	Inj. analogue	mg	Study	No. subjects	Avg % of dose excreted ± S.D.
DCBA-Co^{60}	1.4	Cyanocobalamin	1	5*	2	3.9 ± .9
	2	*Idem*		3	4	3.7 ± 1.7
				6	3	3.4 ± .6
		DCBA	1	4	5	6.7 ± 1.4
				6	3	5.3 ± .5
		DDMBA	1	6	3	2.31 ± 1.72
DDMBA-Co^{60}	2	Cyanocobalamin	.2	4	3	.81 ± .11
		"	1	1	4	2.08 ± .91
				2	3	.74 ± .18
		DDMBA	1	2	3	.99 ± .60
				4	5	1.32 ± .63
				6	3	.95 ± .17
		DCBA	1	4	3	1.28 ± .04
		Factor III	1	4	3	1.06 ± .51
Factor III-Co^{60}	1	Cyanocobalamin	1	5*	2	≃ .7†
	2	Factor III	1	4	5	.30 ± .13

* Performed by Dr. L. R. Wasserman, Mt. Sinai Hospital. Personal communication.
† Actual avg is ≃ 1.2% of 1 μg dose. For 2 μg oral dose, response would have been ≃ .7% as recorded for group avg.

second and third days to obviate suspicion of slow elimination. In the latter instances, 1 mg injections of flushing analogues were administered daily to maximize excretion response. Radioactivity measurements have been described elsewhere(1,2). Urinary radioactivity was always calculated as percent of 2 μg dose. The relative effects of injecting the homologous unlabeled analogue and cyanocobalamin on urinary excretion of radioactivity was investigated with all 3 labeled materials. Additional analogues were injected after oral administration of labeled dichloro and desdimethyl derivatives.

Results. Table I lists the results of these excretion studies based upon 24 hours urinary excretion. Six studies, each performed at a different time, are reported. Group average results in terms of "% of Dose Excreted ± Stand. Dev." are reported for each study of each analogue, together with the number of subjects involved. Results of the extension of Study 6 to the second and third days are not tabulated.

Discussion. Results of Study 6 demonstrate that slow elimination of absorbed analogues is not a factor, since one- and 3-day values differ but little. Three-day excretions of DCBA-Co^{60} were 3.7%, 6.0% and 2.5% after injections of cyanocobalamin, DCBA and DDMBA, respectively; and the DDMBA-Co^{60} response was only 1% after flushing with DDMBA. The third day urine contributed <0.05%. For oral cyanocobalamin plus cyanocobalamin injection (*i.e.* standard test), the 1- and 3-day values would have totaled ≃ 12% and ≃ 17% of dose respectively(10,11).

It is evident from the 24-hr. responses (Table I) that none of the 3 substances tested attains the extent of absorption typifying cyanocobalamin. The most effective absorption indication obtained, *i.e.* average excretion of 6.2% ± 1.3% (stand. dev.) of oral dose of DCBA-Co^{60} after injection of 1 mg DCBA, was only half that characterizing cyanocobalamin; and still lower excretions of 3.6% (± 1.3%) and of 2.3% (± 1.7%) were noted after injections of cyanocobalamin‡ and DDMBA respectively.

Absorption indices for DDMBA were exceedingly low, over-all average responses amounting to 1.13% (± 0.56%) for DDMBA injection, 1.30% (± 0.86%) for

‡ Study 5 values are not included in these averages since the oral dose was other than 2 μg.

cyanocobalamin injection, and 1.28% (± 0.04%) and 1.06% (± 0.51%) for respective injections of DCBA and Factor III.

Factor III seemed the least readily absorbed of the 3 derivatives under investigation. The negligible value of 0.30% (± 0.13%) is indistinguishable from the average 0.5% figure reported for cyanocobalamin in pernicious anemia(12). The lower absorption observed of Factor III and DDMBA by normal humans accords with the behavior of these substances when fed to animals. Coates *et al.*(3) found growth stimulating effect of Factor III and DDMBA when fed to chicks to be only 4% and 25-27% respectively of that observed with cyanocobalamin; and a similar inferiority of DDMBA (9% as active as cyanocobalamin) in chicks was reported by Briggs and Fox(4).

The cobalamins and the structural analogues appear to differ in one additional respect. Whereas the extent of induced urinary excretion of the former was independent of cobalamin injected, excretion of DCBA indicates a dependence on the nature of the injected analogue. Unfortunately, this dependence could be extended neither to DDMBA nor to Factor III because of extremely low order of these responses.

Of the several cobalamins and structural analogues of vit. B_{12} studied, cyanocobalamin is most readily absorbed by humans, probably indicating involvement of the cyano-group in the mechanism of oral absorption process. Clearly, however, other factors likewise enter, since all 3 analogues are cyanide complexes. Of these, DCBA, with its 2 symmetrically distributed chlorine atoms, evinces the greatest absorption tendency. One is, accordingly, tempted to suggest that the 5,6 disubstitution is a favorable configuration, and that the difference between DCBA and cyanocobalamin lies in preference of the human organism for methyl rather than chloro groups.

Summary. 1. Urinary excretion tests of oral absorption were performed in normal humans with Co^{60}-labeled 5,6-dichlorobenzimidazole, 5,6-desdimethylbenzimidazole and 5-hydroxybenzimidazole analogues of vitamin B_{12}. 2. Excretion responses obtained with desdimethyl- and hydroxybenzimidazole derivatives are exceedingly low, indicating but slight oral absorption. 3. Behavior of labeled dichlorobenzimidazole analogue indicates good absorption, approximating one-half that of cyanocobalamin. In this case, maximal excretion is attained after flushing injections with unlabeled homologous compound.

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