Absorption of Cyanocobalamin, Coenzyme B_{12}, Methylcobalamin, and Hydroxocobalamin at Different Dose Levels

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The whole body retention of radioactive cyanocobalamin, coenzyme B_{12}, methylcobalamin, and hydroxocobalamin was measured by whole body monitoring after oral doses of 1, 5 and 25 μg. At each dose level there were significant differences between the values for whole body retention of the different cobalamins.

Key-words: Absorption, small intestinal; cobalamin; coenzyme B_{12}

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The main naturally occurring form of vitamin B_{12} in mammalian tissues is coenzyme B_{12} (5:6 dimethylbenziminazolyl) 5′ — (deoxyadenosyl-cobamide) (14,17). It does not follow, however, that man's dietary intake of vitamin B_{12} is in coenzyme form. Indeed, it seems likely that this unstable, photosensitive form may be converted to other cobalamins in the course of the various preparative processes to which virtually all foods containing vitamin B_{12} are subjected. For this reason it seemed worth while studying the absorption of coenzyme B_{12} and other cobalamins in pure form at different dose levels as a baseline for further studies.

MATERIALS AND METHODS

Procedure and method of measurement of retained dose

The whole body retention of four radioactive cobolamins – cyanocobalamin, coenzyme B_{12}, methylcobalamin, and hydroxocobalamin – each given orally at dose levels of 1, 5, and 25 μg was measured by whole body monitoring. To minimize biological variation as well as logistic problems a double-tracer technique was used, each of the 63 patients studied receiving the same oral dose of two cobalamins, one incorporating ^{58}Co and the other ^{57}Co at intervals of 24 hours. Thus, at all dose levels, patients who were given ^{58}Co cyanocobalamin were also given ^{57}Co coenzyme B_{12} 24 hours later, and patients who were given ^{58}Co methylcobalamin were also given ^{57}Co hydroxocobalamin, 24 hours later. Doses were administered in water in a total volume of 100 ml in the fasting state, and food was withheld for 2 hours after the dose. Whole body radioactivity was measured in the Merlin whole body monitor (1), measurements being made shortly after each oral dose and again at 16 days after the first of the two doses, the fraction of the ^{58}Co counting rate detected in the energy band used for ^{57}Co being determined as necessary and an allowance being made for natural body radioactive decay.

Clinical material

Sixty-three subjects were studied. All were

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Table I. Mean values and, in parentheses, standard deviations of percentages of doses retained by each group of patients at each dose level

Oral dose	Number of subjects	Percentage retained from dose of: ^{58}Co cyanocobalamin	^{57}Co coenzyme B_{12}	^{58}Co methylcobalamin	^{57}Co hydroxocobalamin
1 μg	12	49.2(14.9)	33.7(11.1)	–	–
1 μg	10	–	–	44.4(10.0)	55.7(11.3)
5 μg	10	20.4(8.8)	12.9(10.7)	–	–
5 μg	10	–	–	18.8(7.1)	16.3(5.7)
25 μg	10	5.6(2.2)	7.9(3.6)	–	–
25 μg	11	–	–	6.1(2.3)	7.4(2.2)

ambulant hospital in or out patients, most being in the convalescent phase of their illnesses. All were aware of the nature and purpose of the tests. The patients had a wide variety of diseases but cases of hepatic disease, megaloblastic anaemia, malabsorption, and previous gastrointestinal surgery were excluded.

Preparation of cobalamins

^{58}Co cyanocobalamin and ^{57}Co hydroxocobalamin were obtained from the Radiochemical Centre, Amersham, and doses were prepared with commercially available unlabelled cyanocobalamin and hydroxocobalamin. ^{57}Co coenzyme B_{12}, unlabelled coenzyme B_{12}, ^{58}Co methylcobalamin, and unlabelled methylcobalamin were prepared by the methods of Johnson, Mervyn, Shaw & Smith (8). Manipulations of coenzyme B_{12} and methylcobalamin were carried out in a dim red light, and all solutions were stored in dark glass bottles shielded by heavy foil at 4° C. The activity of the doses ranged from 0.2 μCi for the 1 μg doses of ^{58}Co cobalamins to 1.0 μCi for the 25 μg doses of ^{57}Co cobalamins.

Statistical methods

Results obtained from patients given ^{58}Co cyanocobalamin and ^{57}Co coenzyme B_{12} at any one dose level and for patients given ^{58}Co methylcobalamin and ^{57}Co hydroxocobalamin at any one dose level were analysed by the Wilcoxon Test for matched pairs (2 tailed test). For other comparisons the Mann Whitney Test for independent groups (2 tailed test) was used.

RESULTS

The results are summarized in Table I, which shows the mean values and standard deviations of percentages of doses retained by each group of patients at each dose level.

At the 1 μg dose level the highest mean value for percentage of dose retained was after hydroxocobalamin (55.7 per cent), the others in descending order being after cyanocobalamin (49.2 per cent), methylcobalamin (44.4 per cent) and coenzyme B_{12} (33.7 per cent). The values for hydroxocobalamin were significantly higher than those for methylcobalamin ($P < 0.01$) and coenzyme B_{12} ($P < 0.02$), those for cyanocobalamin were significantly higher than for coenzyme B_{12} ($P < 0.01$), and those for methylcobalamin significantly higher than for coenzyme B_{12} ($P < 0.05$).

At the 5 μg dose level the order was different, the mean values in descending order being, after cyanocobalamin 20.4 per cent, after methylcobalamin 18.8 per cent, after hydroxocobalamin 16.3 per cent and after coenzyme B_{12} 12.9 per cent. The values for cyanocobalamin and methylcobalamin were both significantly higher than those for coenzyme B_{12} ($P < 0.02$).

At the 25 μg dose level the order changed again, the greatest mean value retained being after coenzyme B_{12} (7.9 per cent) followed by hydroxocobalamin (7.4 per cent), methylcobalamin (6.1 per cent) and cyanocobalamin

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(5.6 per cent). The values for coenzyme B_{12} were significantly greater than those for cyanocobalamin ($P < 0.05$).

DISCUSSION

On the assumption that the whole body radioactivity 16 days after oral administration of a radioactive cobalamin is a measure of the amount of radioactive cobalamin absorbed, it seems reasonable to conclude from the results that the fraction of the dose which is absorbed is a function of both the mass and the structure of the cobalamin, in particular to the nature of the ligand occupying sixth place on the cobalt atom. The importance of the mass of the dose in relation to the fraction absorbed by normal subjects was shown by Glass, Boyd & Stephanson (3), Swendseid, Gasster & Halsted (16), and Callender & Evans (2). The conclusion that structure was a factor was reached by Rosenblum et al. (12, 13), who found that cyanocobalamin was better absorbed than chlorocobalamin, sulphitocobalamin, nitrocobalamin, and thiocyanatocobalamin, at dose levels of 0.5 to 2.0 μg, by Herbert & Sullivan (6), who suspected that cyanocobalamin was better absorbed than coenzyme B_{12} at 2.0 μg doses, and by Heinrich & Gabbe (4), who concluded that the proportions of cyanocobalamin and hydroxocobalamin absorbed were similar and greater than coenzyme B_{12} at dose levels of 0.1 to 1.0 nMol. The interrelationship of the two factors, mass and structure, vis-à-vis fraction absorbed has not, however, been described previously.

The reasons for the differences at any one dose level, far less at different dose levels, are not obvious. The possibility that traces of cyanide in the gastric juice may be relevant (13) cannot be discarded but seems unlikely to be important in view of the pattern of results. Differences in electrolyte nature and attendant solubility properties do not seem important, at least at the 2 μg dose level (13), and tightness of binding of the cyano group in the cobalt co-ordination sphere was thought by Rosenblum et al (13) to account for the better absorption of cyanocobalamin compared with chlorocobalamin, sulphitocobalamin, nitritocobalamin, and thiocyanatocobalamin, at the 2 μg dose level when absorption would depend on the intrinsic factor mechanism. This explanation would be in keeping with our result at the 1 and 5 μg dose levels but not with those at 25 μg dose levels. There is some evidence, however, that absorption of cyanocobalamin, at least, occurs independently of the intrinsic factor mechanism at a 30 μg dose level (5), and it may be that factors, such as tightness of binding of the ligand, which are important when absorption is intrinsic factor dependent are less important, or are compensated for, when absorption is independent of the intrinsic factor mechanism. Support for this possibility comes from the degrees of statistical significance which, in general, are high at the 1 μg dose level and become less impressive with greater doses until they only just reach the 5 per cent level in one pair of 25 μg dose results.

Whether our findings have any physiological significance is open to question. Although results of absorption tests with meat-bound cobalamin(s) were comparable with those with pure cyanocobalamin (7), and although suggestions that liver-bound cobalamins are more readily absorbed than pure cyanocobalamin (9, 11) have not been confirmed (10, 15), we are hesitant about extrapolating from a relatively simple, unphysiological study of the absorption of pure cobalamins without any gastric secretory stimulation to the complex field of absorption from food. This hesitancy is heightened by the marked individual variation in amounts retained, apparent in the results in the standard deviations; and although this factor was considerably reduced by the use of a double-tracer technique in the current study, it was not possible to eliminate the problems inherent in comparison of results from different groups of subjects. It does seem reasonable, however, to conclude from the results that the absorption of vitamin B_{12} appears to be an increasingly complex subject.

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