Hepatic Uptake and Intestinal Absorption of Co^{58}-Labelled 5,6-dimethylbenzimidazolylcobamide Coenzyme.* (26606)

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The isolation of 5,6-dimethylbenzimidazolylcobamide coenzyme(1), (referred to here, for simplicity, as B_{12} coenzyme) has raised several questions regarding its turnover in the human body and its physiological significance in the metabolism of vit B_{12}. Although its role in the intermediate metabolism of amino acids has been established, very little has yet been published on the absorption, tissue distribution and urinary and fecal excretion of this substance(2-6). Twenty-four hours after intravenous injection of both labelled coenzyme and labelled cyanocobalamin to normal rats, more radioactivity was found in the kidneys than in the liver(5,6). Four to 5 days later, the reverse was true, *i.e.* more radioactivity was found in the liver than in the kidneys(5,6). This sequence is the opposite of that usually found, under similar conditions, after administration of cyanocobalamin(7,8). It was also noted that release of the parenterally administered coenzyme from the liver, through the bile, into the feces proceeds more slowly in rats than does that of cyanocobalamin(5). Hence the conclusion that the liver's affinity to coenzyme is greater than to cyanocobalamin(5, 6).

It was also demonstrated, by measurements of fecal radioactivity in the normal rat that there is much less absorption of orally administered coenzyme than cyanocobalamin at the same dosage(4-6). Comparative studies on humans have so far been few and fragmentary(2,3,5). The evidence available is scanty as to how much coenzyme, as compared with cyanocobalamin, is absorbed in the intestines and deposited in the liver of normals or patients with pernicious anemia. The present investigation was undertaken to answer some of these questions.

Material and method. For this study, we used 3 normal subjects and 3 patients with pernicious anemia in remission. The diagnosis of pernicious anemia was established by all available clinical and hematological criteria, including isotope assay of the intestinal absorption of labelled Vit. B_{12} and microbiological assay of the B_{12} serum levels. Co^{58}-labelled coenzyme of 0.616 $\mu c/\mu g$ specific activity and Co^{58}-labelled cyanocobalamin of 0.82 $\mu c/\mu g$ specific activity† were given orally to each subject at a dose of 2 μg after the radioactivity of the materials had been cut by physical decay by one-third to one-half of its initial potency. Each normal subject received (1) 2 μg of labelled cyanocobalamin and (2) 2 μg of labelled coenzyme at intervals of 15 to 20 days. At 2 to 2½ week intervals, each pernicious anemia patient received (1) 2 μg of labelled coenzyme; (2) 2 μg of labelled coenzyme together with 7.5 mg of hog intrinsic factor preparation # WES 727 (Lederle)‡; (3) 2 μg of labelled cyanocobalamin; (4) 2 μg of labelled cyanocobalamin together with 7.5 mg of the same intrinsic factor preparation. The sequence in which these 4 materials were given was changed from patient to patient.

The hepatic uptake of both labelled coenzyme and labelled cyanocobalamin was determined by the surface counting technic described earlier(9,10). Following oral administration of each of these materials, hepatic uptake of radioactivity was measured on 3 surface skin projections of the liver (anterior, antero-lateral and mid-lateral). In addition, 2 projections of the intestine (suprapubic and left lower quadrant) were counted as controls. The radioactivity over the left

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TABLE I. Hepatic Uptake of Radioactivity Following Administration of Co^{58}-coenzyme and Co^{58}-cyanocobalamin.

Name	Diagnosis	Cyanocobalamin, coenzyme B_{12}, administered	Hepatic uptake in cpm/μc Co^{58}	
			Cyanocobalamin	Coenzyme B_{12}
1. Clar.	Normal	Alone	309	172
2. Met.	"	"	371	457
3. Mla.	"	"	—	232
4. Sull.	Pernicious anemia	Alone	5	0
"	" "	With IF	397	254
5. Lea.		Alone	0	0
"	" "	With IF	330	119
6. Mea.	" "	Alone	0	0
		With IF	316	207

calf muscle was counted as body background. The counting areas were marked with indelible stain and adhesive tape to insure identical counting projections throughout the investigation. Each count was taken for at least 10 minutes, by means of a scintillation counter provided with a sodium iodide-thallium crystal ($3/4'' \times 1''$) and a gamma spectrometer, attached to a scaler, and were taken at the spectrometer's optimal energy setting for Co^{58}. The counting time was long enough to secure statistically significant counts(10). The hepatic and intestinal counts were averaged separately. The body background was deducted and the counts were corrected for physical decay of the material and for efficiency of the scaler with the use of a Co^{58} standard.

Results. The results are tabulated in Fig. 1 and 2 and in Table I. In normals, absorption of coenzyme B_{12} in the intestine and its deposition in the liver was, in one case, 23% higher, and, in another, 40% less, than that of cyanocobalamin (Fig. 1). In the pernicious anemia patients, when cyanocobalamin and coenzyme were given without an additional source of intrinsic factor (Table I), neither material was absorbed in the intestine and deposited in the liver in appreciable amounts.

When cyanocobalamin and coenzyme were given alone to 3 normals, or with IF preparation to the 3 pernicious anemia patients, both materials were absorbed in the intestine and deposited in the liver (Fig. 1). In all pernicious anemia cases, hepatic uptake of cyanocobalamin, when given with IF, was in a range similar to that observed in the normal subjects who had received a similar dose of cyanocobalamin without the additional source of IF (Fig. 1). In these pernicious anemia patients, the range of hepatic uptake

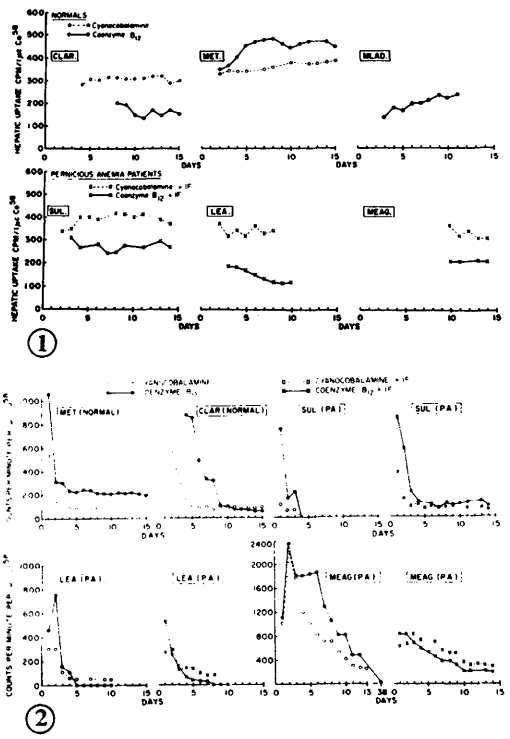

FIG. 1. Hepatic uptake of radioactivity in normals and pernicious anemia patients following oral administration of Co^{58} labelled cyanocobalamine and coenzyme B_{12} alone and together with intrinsic factor (IF).

FIG. 2. Radioactivity counts over intestine in 2 normal and 3 pernicious anemia patients (P.A.) following oral administration of Co^{58} labelled cyanocobalamine and coenzyme B_{12} alone and together with intrinsic factor (IF).

was from 300 to 400 cpm per 1 μc of $Co^{58}B_{12}$, which is the normal range of hepatic uptake of $Co^{58}B_{12}$ observed previously (11).

Hepatic uptake of the labelled coenzyme, when given with IF to the same pernicious anemia patients, was only 36-64% of that observed following a similar dose of cyanocobalamin with the same amount of the same IF preparation. Its range was 109-254 cpm per 1 μc Co^{58} as compared to 316-397 cpm per 1 μc Co^{58} hepatic uptake of cyanocobalamin.

As shown by the data listed in Fig. 2, the surface counts over the intestinal projections of normals and patients with pernicious anemia after administration of coenzyme alone were much higher and more prolonged than after administration of corresponding doses of cyanocobalamin. The differences were especially marked during the first few days after administration of the material, but in some instances they extended over the entire 2-week period of observation. After administration of coenzyme together with IF preparation to PA patients, the differences between cyanocobalamin and coenzyme in intestinal counts were less consistent, however, and were seen in only one of the 3 cases tested. In most instances, prolonged intestinal counts masked hepatic uptake for a much longer time than when labelled cyanocobalamin was administered.

Discussion. These results indicate that orally administered coenzyme B_{12}, in the absence of intrinsic factor, is not absorbed in the intestine and therefore cannot be used alone and orally in treatment of pernicious anemia. Moreover, it appears that intestinal absorption of orally administered coenzyme B_{12} in pernicious anemia is less influenced by intrinsic factor preparations derived from animal sources than is that of cyanocobalamin. This occurs in spite of its entering into a bond with these IF preparations(12). Consequently, when these materials are given orally with intrinsic factor preparations from hog stomach to pernicious anemia patients, less coenzyme than cyanocobalamin is deposited in the liver.

Since, in rats, the affinity of coenzyme to the liver is greater than that of cyanocobalamin(4,6), the smaller hepatic deposition of coenzyme in pernicious anemia patients, when administered orally with intrinsic factor preparations, is probably not due to its having less affinity to the liver than cyanocobalamin. There is also no reason to assume that coenzyme exerts a cathartic effect upon the intestine resulting in its more rapid removal from the gut. The smaller hepatic deposition of coenzyme, as compared to cyanocobalamin, is therefore possibly due to its relatively less efficient absorption in the intestine. The same is known to be true for other B_{12} derivatives and analogues as well, such as chloro-, sulpho- or nitrocobalamin (13), or 5,6-dichlorobenzimidazole, 5,6-desdimethylbenzimidazole and 5-hydroxybenzimidazole(14).

In normal subjects, the radioactivity over the intestine was more prolonged after oral administration of labelled coenzyme alone than after cyanocobalamin. The same was also observed in pernicious anemia patients in spite of the fact that no intestinal absorption of this material occurred in absence of IF. This finding may indicate that the attachment of coenzyme to the intestinal mucosa prior to its absorption is easier and stronger than that of cyanocobalamin. This attachment may not depend on the ultimate fate of coenzyme, *i.e.*, its passage through the intestinal mucosa (in normals) or its return to the intestinal lumen (in PA patients).

Summary and conclusions. Measurements of the hepatic uptake of orally administered Co^{58}-labelled 5,6-dibenzimidazolylcobamide coenzyme suggest that it cannot be absorbed in the intestine in the absence of intrinsic factor and, consequently, cannot be used alone in oral treatment of pernicious anemia, Under similar conditions, less coenzyme than cyanocobalamin is deposited in the liver of patients with pernicious anemia when it is orally administered with active IF preparations from animal stomach. This may be due either to the lessened effect of animal IF preparations on intestinal absorption of coenzyme or to the diminished efficiency of in-

testinal absorption of coenzyme as compared with that of cyanocobalamin.

Surface counting of intestinal areas after oral administration of labelled coenzyme alone and labelled cyanocobalamin to normal subjects and patients with pernicious anemia shows the radioactivity of the intestine to be much more prolonged after administration of coenzyme than after cyanocobalamin. This may be interpreted as indicating that, of the 2, coenzyme forms the easier and stronger attachment to the intestinal mucosa prior to its absorption, regardless of whether in the presence of intrinsic factor it ultimately passes through the intestinal mucosa or, in the absence of intrinsic factor, returns to the intestinal lumen.

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Lipoprotein Pre-Staining and Ultracentrifugal Analysis in a Density Gradient.* (26607)

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Pre-staining methods, used for analysis of serum lipoproteins by paper electrophoresis (1-4) and ultracentrifugal flotation(5,6), are not quantitative. Lipids dispersed on celite have been solubilized by agitation with serum lipoproteins(7). A modified procedure, pre-staining with a sudan black B (SBB) - celite slurry, is used here for the ultracentrifugal analysis of serum lipoproteins in a density gradient(8).

Methods. SBB - celite slurry. Two g SBB† was dissolved in 200 ml methyl cellosolve and added to 10 g celite 535. Distilled water, 500 ml, followed by 100 ml 2 *M* NaCl were then added with constant stirring. The suspension was filtered on a sintered glass filter, the cake washed with 300 ml 0.15 *M* NaCl, transferred to a glass stoppered flask, and suspended in 100 ml 0.15 *M* NaCl. *Staining.* Three ml of thoroughly mixed slurry was added to 1 ml of serum in a test-tube and agitated gently on an automatic shaker for 15 hr at room temperature. The tube was then centrifuged for 30 min at 1000 $\times$ *g* and stained diluted serum withdrawn.

Ultracentrifugation. Three ml of the stained serum was placed in a 13.5 ml centrifuge tube containing 1 ml 2 *M* NaCl and mixed. Five ml 0.15 *M* NaCl was layered over this solution and the tube centrifuged

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