Hydroxocobalamin. VI. Comparison of Intestinal Absorption in Man Of Large Doses of Hydroxocobalamin and Cyanocobalamin.* (31041)

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Intestinal absorption in man of large doses of vit. B_{12} is not intrinsic factor dependent. Several investigators(1-3) have therefore utilized cyanocobalamin in daily oral doses of 300 to 1000 μg in the maintenance therapy of pernicious anemia and at these dosages have maintained serum vit. B_{12} levels in the normal range for prolonged periods.

Hydroxocobalamin is retained longer in the human body than cyanocobalamin(4-6), presumably because of its strong binding to serum and tissue proteins(7-9). At low oral doses, intestinal absorption of hydroxocobalamin and cyanocobalamin has been shown to be about equal(3,4,9-11), but no quantitative information is yet available on the intestinal absorption of hydroxocobalamin at large dosages. If the intestinal absorption of large oral doses of hydroxocobalamin were equal to that of cyanocobalamin, the greater body retention of hydroxocobalamin would make it advantageous in the oral treatment of vit. B_{12} deficiency. The present study was therefore undertaken to quantitate the intestinal absorption of hydroxocobalamin and compare it with that of cyanocobalamin at large oral dosages of 100 to 1000 μg.

Since at these large dosages vit. B_{12} absorption proceeds similarly in normal individuals and pernicious anemia patients(12,13), normal subjects were used in this study.

Methods. 1. *Fecal excretion studies.* The differential excretion of the 2 cobalamins in feces following their simultaneous oral administration was studied by means of a double label isotope technique. Co^{57}-labeled cyanocobalamin and Co^{60}-labeled hydroxocobalamin† were administered simultaneously to 3 pairs of normal individuals in identical oral doses of 100, 500 and 1000 μg and the first 3 to 5 whole stool specimens collected. The ratio of the 2 isotopes in each stool specimen was then compared with the ratio of the 2 isotopes in the administered dose.

Radioactivity in individual whole stool samples was counted in a large-sample-volume well-type scintillation detector (Armac) connected to a gamma spectrometer and scaler. In this instrument the counting chamber is enveloped in a liquid scintillator which is surrounded by 6 radially arranged photomultiplier tubes. Variations in the geometry of individual stool samples were of no significance since the position of the standard and each stool sample in the counting chamber was identical for counting at both Co^{57} and Co^{60} settings and only the ratio of the 2 isotopes in each was considered in the calculation of results.

2. *Quantitative hepatic uptake studies.* Intestinal absorption of hydroxocobalamin and cyanocobalamin was quantitated and compared in the same individual by a modification of the previously reported double label hepatic uptake test(14). In this method intestinal vit. B_{12} absorption is quantitated by comparing the hepatic uptake of orally administered $Co^{60}B_{12}$ with that of a simultaneously administered intravenous tracer dose of Co^{57}-B_{12}.

Proper corrections were made for the unequal penetration in body tissue and unequal counting efficiency in a sodium iodide crystal of Co^{57} and Co^{60} gamma radiation as described in the original method(14). With the use of a conversion factor derived from the regression equation $Y = 1230 + .53\ X$ (where Y = hepatic counts from the dose of $Co^{60}B_{12}$ given orally and X = hepatic counts from the dose of $Co^{57}B_{12}$ given intravenous-

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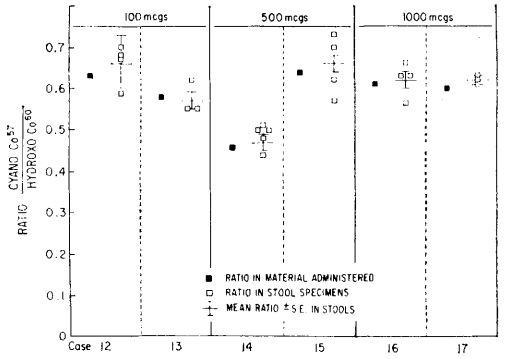

FIG. 1. Ratio of cyanocobalamin Co^{57}/hydroxocobalamin Co^{60} in successive whole stool specimens compared with cyanocobalamin Co^{57}/hydroxocobalamin Co^{60} ratio in administered dose.

ly), intestinal vit. B_{12} absorption in per cent was calculated as:

$$\frac{(Co^{60}\text{ hepatic counts})}{1230 + .53\ (Co^{57}\text{ hepatic counts})} \times 100.$$

In the present study the method was adjusted so that intestinal absorption of sequentially ingested Co^{57}-labeled cyanocobalamin and hydroxocobalamin was quantitated by comparing in each subject the hepatic uptake after the oral dose of each of the 2 cobalamins with the hepatic uptake of an intravenous tracer dose of 0.15 μg ($\approx$ 0.2 μc) Co^{60}-labeled cyanocobalamin administered simultaneously with the first oral dose. In each case, the second oral dose, which was cyanocobalamin in half the tests and hydroxocobalamin in the other half, was administered after peak hepatic radioactivity from the first oral dose was reached. The latter was used as a base line for calculating the increment in radioactivity following the second oral dose. Hepatic radioactivity was measured using a heavily shielded 2 $\times$ 2-inch sodium iodide crystal probe detector coupled to a gamma spectrometer and scaler. In 24 studies on 11 normal subjects the tests were performed with oral doses of 100, 500 and 1000 μg hydroxocobalamin and cyanocobalamin.

Results. 1. *Fecal excretion studies.* Fig. 1 shows, for each of 6 subjects, the ratio of cyanocobalamin Co^{57}/hydroxocobalamin Co^{60} in successive whole stool specimens compared with the ratio of the 2 isotopes in the administered doses of 100, 500 and 1000 μg. The mean ratio (with standard error) of both radioactivities in all stool specimens for each subject is also shown. In each case the mean ratio of cyanocobalamin to hydroxocobalamin excreted did not differ significantly from their ratio in the administered dose at each dosage level.

2. *Quantitative hepatic uptake studies.* Fig. 2 shows the individual and mean per cent intestinal absorption (with standard error) of cyanocobalamin and hydroxocobalamin in 4 subjects at the 100 μg level. Fig. 3 shows the same in 4 subjects at the 500 μg level and 3 subjects at 1000 μg. Mean hydroxocobalamin absorption at doses of 100, 500 and 1000 μg was 7.2 $\pm$ 0.9%, 3.8 $\pm$ 1.1% and 2.2 $\pm$ 0.7%, respectively, *vs* 5.1 $\pm$ 0.7%, 2.5 $\pm$ 1.2% and 2.5 $\pm$ 1.2%, respectively, for cyanocobalamin. These differences between hydroxocobalamin and cyanocobalamin absorption were not statistically significant.

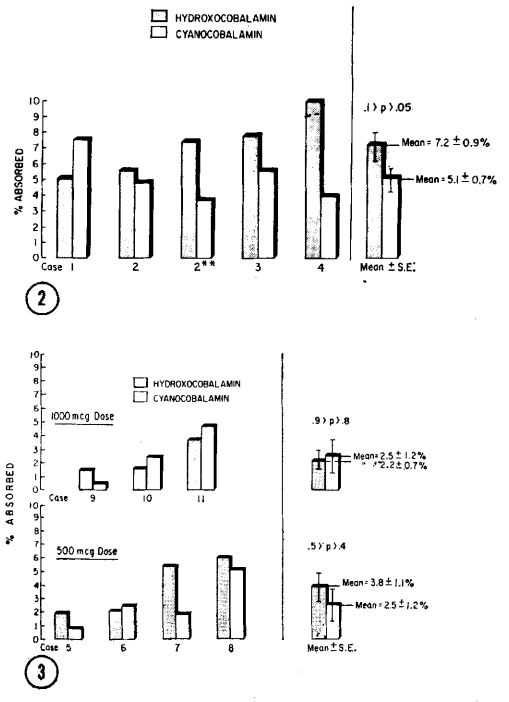

FIG. 2. Comparison of per cent intestinal absorption of 100 μg hydroxocobalamin Co^{57} and cyanocobalamin Co^{57} as assayed by the double label hepatic uptake test in 4 normal subjects. ** Repeat test.

FIG. 3. Comparison of per cent intestinal absorption of 500 μg and 1000 μg hydroxocobalamin Co^{57} and cyanocobalamin Co^{57} as assayed by the double label hepatic uptake test in 7 normal subjects.

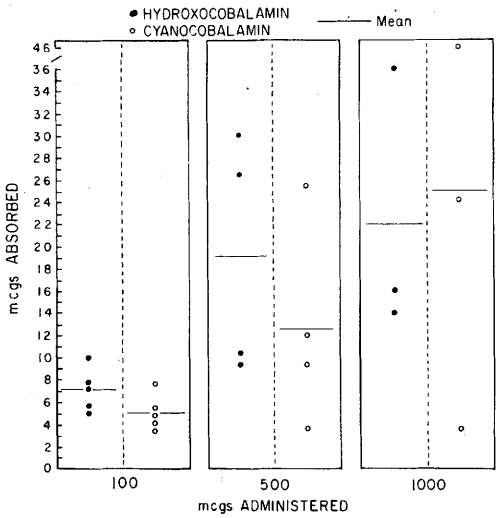

FIG. 4. Comparison of amounts of hydroxocobalamin Co^{57} and cyanocobalamin Co^{57} absorbed from intestine after oral doses of 100, 500 and 1000 μg.

The absolute quantities of the vitamins absorbed at each of these dosage levels are shown in Fig. 4. An average (with standard error) 7.2 $\pm$ 0.9 μg hydroxocobalamin and 5.1 $\pm$ 0.7 μg cyanocobalamin was absorbed from a single oral dose of 100 μg, 19.0 $\pm$ 5.5 μg hydroxocobalamin and 12.5 $\pm$ 6.0 μg cyanocobalamin from a single oral dose of 500 μg, and 22.0 $\pm$ 7.0 μg hydroxocobalamin and 25.0 $\pm$ 12.0 μg cyanocobalamin from a single oral dose of 1000 μg. These amounts are well in excess of the highest estimated daily requirements for vit. B_{12}(15-17).

Discussion. The results of this study demonstrate that both hydroxocobalamin and cyanocobalamin are absorbed from the intestine in similar amounts irrespective of the dose given. These findings therefore extend those of others in rat and man at low oral doses of hydroxocobalamin and cyanocobalamin(3,4, 10,11) to the high dosage range as well.

The absorption figures obtained in this study for large oral doses of cyanocobalamin are higher than those reported by others for this dosage range with the urinary excretion test(3,12). Their results, however, were indirect and their conclusion based on the assumption that ⅓ of the vit. B_{12} absorbed is excreted in the first 24-hour urine. This assumption has not yet been validated at such high oral dosages. The results of the present study were obtained using a more direct method of quantitation.

Thus, since hydroxocobalamin is retained longer in the body than cyanocobalamin(4-6) and the build-up and maintenance of vit. B_{12} blood levels after parenteral administration are more easily obtained with hydroxocobalamin than with cyanocobalamin(4), therapeutic trials with large daily oral doses of hydroxocobalamin appear worthwhile in the treatment of pernicious anemia and other vit. B_{12} deficiency states. These trials are currently under way in our service.

Summary. Intestinal absorption of large oral doses of hydroxocobalamin and cyanocobalamin (100, 500 and 1000 μg) was compared and quantitated in 17 normal subjects using a double label isotope technique.

In 3 pairs of normal individuals, Co^{57}-labeled cyanocobalamin and Co^{60}-labeled hydroxocobalamin were administered simultaneously in oral doses of 100, 500 and 1000 μg and the ratio of the 2 isotopes in whole stool specimens was compared with that in the administered dose. No significant differences were found between the ratios administered and excreted at each dosage level. In 11 subjects intestinal absorption was quantitated by a modification of the double label hepatic uptake test in which the hepatic uptake of orally administered cyanocobalamin Co^{57} and hydroxocobalamin Co^{57} was given simultaneously with the first oral dose. At oral doses of 100, 500 and 1000 μg, mean hydroxocobalamin and cyanocobalamin absorption were not significantly different.

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Inhibitory Effect of ACTH and Related Peptides on Extinction of Conditioned Avoidance Behavior in Rats. (31042)

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ACTH administered during avoidance learning delays extinction of a shuttle-box avoidance response in intact as well as in adrenalectomized rats(1,2). The removal of the posterior and intermediate lobe of the pituitary (posterior lobectomy) in rats facilitates extinction of a similar avoidance response, and the rapid rate of extinction can be inhibited by the treatment with ACTH, α-MSH and pitressin(3). These results indicate that ACTH and related peptides play an important role in maintenance of avoidance conditioned behavior.

Since synthetic ACTH as well as fragments of this peptide hormone have become available it was deemed of interest to study the effect of these peptides on the extinction of an avoidance response in intact rats in order to obtain information about the active part of the ACTH molecule responsible for this behavioral effect.

Materials and methods. Male white rats from an inbred strain weighing 140-180 g were used. Avoidance conditioning was studied in two different situations.

a. *Shuttle-box experiment.* Conditioning was performed in a 2-compartment box, divided into 2 equal compartments by a 5 cm barrier. The conditioned stimulus (CS) was the sound of a buzzer presented for 5 seconds prior to the unconditioned stimulus (US) of shock, delivered through the feet of the rat (40 V; 1.8 mA). If the animal crossed the barrier within 5 seconds, the CS was terminated and the rat avoided shock. Ten conditioning trials were given each day with a fixed intertrial interval averaging 60 seconds presented in a predetermined random sequence (4). Conditioning trials lasted till the rat had achieved criterion, *i.e.*, 80% or more avoidances during 3 consecutive days. Those rats which did not reach the criterion within 14 days were dropped from further participation in the experiment. The day after the criterion was reached extinction trials were run with the schedule and procedure as in conditioning, except that the US was never presented, and the CS, terminated after 5 seconds, if a barrier crossing had not occurred. Extinction was studied for 14 days.