Depression of the Intestinal Uptake of Radio-vitamin B_{12} by Cholestyramine[1] (37237)

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Since cholic acid depresses the intestinal uptake of radio-vitamin B_{12} *in vivo* and *in vitro* (1), it seemed reasonable to explore the effect of sequestering bile acids with cholestyramine (CH) on the absorption of radio-vitamin B_{12} in man.

Methods. In vivo, the double label hepatic uptake (DLHU) test (2) was used to quantitatively measure the absorption of radio-vitamin B_{12}. Following an overnight fast, background counts were taken over the liver for both ^{57}Co-B_{12} and ^{60}Co-B_{12}. Individuals were then given 1.0 μg of ^{60}Co-B_{12} (0.51 μCi/μg) orally and 0.05 μg of ^{57}Co-B_{12} (10 μCi/μg) intravenously. Fourteen days later, counting for both cobalt isotopes was repeated over the liver and the percentage absorption was calculated as previously described (2). The reproducibility of the DLHU test was previously demonstrated by performing the test twice at 2 wk intervals in five consecutive individuals (1).

The effect of CH on vitamin B_{12} absorption was studied in 10 normal volunteers. In the first six volunteers, control B_{12} absorption without CH was performed first. They were then given 4 g of CH orally four times a day for four consecutive days and the radio-B_{12} was administered on Day 3. In the last four patients, the sequence was reversed such that CH was given first and control absorption was performed 14 days later.

In vitro, the guinea pig intestinal mucosal homogenate (GPIMH) assay described by Castro-Curel and Glass (3) was used. Gastric juice (GJ) was collected from a normal volunteer by performing a maximal histamine stimulation test. The 30 and 60 min specimens were pooled, brought to pH 10 with 1.0 *M* NaOH, and then to pH 7.0 with 1.0 *M* HCl. The neutralized GJ was then strained through gauze, divided into 2 ml aliquots and frozen. Each assay was performed in duplicate in the following manner. To a 15 ml test tube containing 5 ml of Krebs–Henseleit bicarbonate glucose medium, the following were added: (a) 1 ml ^{57}Co-B_{12} containing 2500 pg vitamin B_{12}, the specific activity of which provided 4–6 cpm/pg; (b) 1 ml suspension of GPIMH in normal saline containing 12 mg GPIMH; (c) 0.025 ml of gastric juice (GJ) diluted to 1.0 ml with normal saline; and (d) 1 ml of a suspension of CH at a concentration of 15 mg/ml. In order to study the possible effect of CH on the various phases of the intrinsic factor (IF)-mediated uptake of B_{12} by the GPIMH, the reagents were mixed in different sequences:

1. B_{12} + GPIMH;
2. B_{12} + GJ + GPIMH;
3. B_{12} + GJ + CH + GPIMH;
4. B_{12} + CH + GJ + GPIMH;
5. CH + GJ + B_{12} + GPIMH;
6. GPIMH + CH + GJ + B_{12}.

The mixtures were incubated at 5° for 1 hr as described by England, Ashworth and Taylor (4) rather than at 37°. This was found to lower the non-IF-mediated uptake, but had no effect on the IF-mediated uptake thereby increasing the difference between the two 7- to 10-fold as opposed to incubating at 37° which showed a difference of 2- to 3-fold.

In order to further study the effect of CH

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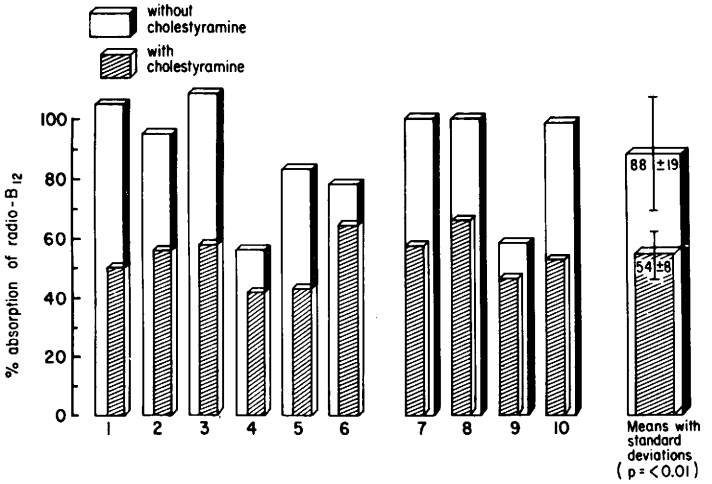

FIG. 1. The effect of cholestyramine on radio-B_{12} absorption in normal adult volunteers as measured by the double label hepatic uptake test.

on the IF activity of GJ, 1 ml dilutions of GJ containing 0.025 ml of GJ were extracted with increasing amounts of CH (1, 10, 30, and 66 mg). The extractions were performed by shaking the GJ and CH thoroughly, centrifuging and using only the supernatant GJ in the subsequent assays.

Results. In vivo, CH was found to lower the absorption of radio-B_{12} in all 10 volunteers as shown in Fig. 1. The mean absorption of the controls was 88 $\pm$ 18.9%, while that of the individuals treated with CH was 54 $\pm$ 7.6%. This difference was significant by the t test applied to paired comparisons, with the $p < 0.01$. CH administration also diminished the absorption of radio-B_{12} in a patient with pernicious anemia (Fig. 2). When B_{12} was given together with IF, CH decreased the percentage absorption from 50 to 34%. When B_{12} was given without IF, CH decreased the percentage absorption from 21 to 15%, values well within the limits of reproducibility.

In vitro studies performed by means of the GPIMH assay were done to explore the mechanism of CH depression of B_{12} absorption. In the GPIMH system, CH produced a marked reduction in radio-B_{12} uptake when allowed to react with GJ prior to the addition of radio-B_{12} [Fig. 3, sequences 5, 6 (see Method)]. This did not occur when CH was added after B_{12} and GJ had been previously mixed (Fig. 3, sequence 3), or if CH was allowed to react with B_{12} prior to the addition of GJ (Fig. 3, sequence 4).

Since this suggested that CH decreases the radio-B_{12} uptake by binding a portion of the IF in GJ and thereby preventing the formation of the IF-B_{12} complex, we attempted to decrease the IF activity of GJ by previously extracting it with CH.

GJ was extracted with CH as previously described. The extracted GJ showed an asymptotic decrease in IF activity as it was extracted with increasing amounts of CH (Fig. 4).

Discussion. The results of this study indi-

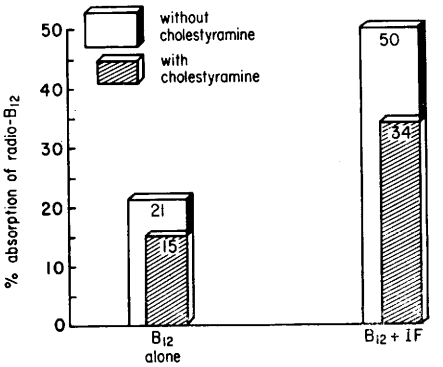

FIG. 2. The effect of cholestyramine on the intestinal absorption of radio-B_{12} in a pernicious anemia patient as measured by the double label hepatic uptake test.

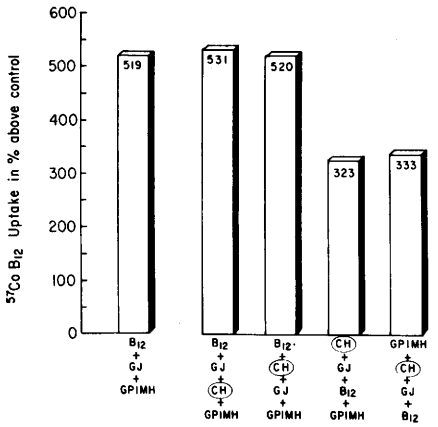

FIG. 3. The effect of cholestyramine on the IF-mediated uptake of ^{57}Co-B_{12} by GPIMH.

cate that CH decreases the intestinal absorption of radio-B_{12} in normal volunteers and in a patient with pernicious anemia. The mechanism of this inhibition was explored *in vitro* using the GPIMH assay. The results of the *in vitro* studies suggest that CH decreases the uptake of radio-B_{12} probably by binding a portion of the same binding sites on the IF molecule which normally bind vitamin B_{12} and thereby preventing the formation of the IF-B_{12} complex. There was no evidence to suggest that this inhibition occurred as a result of CH binding free vitamin B_{12}, the IF-B_{12} complex, or receptor sites on the ileal mucosa. It had previously been shown that cholic acid and possibly deoxycholic acid decreased the absorption of radio-B_{12}, while glycocholic acid and glycodeoxycholic acid had no effect on radio-B_{12} absorption (1). It seemed unlikely then, that the CH decrease in radio-B_{12} absorption was mediated via the sequestration of bile acids.

Cholestyramine is a basic anion exchange resin with a strong affinity for binding bile acids in the intestinal tract and thereby decreasing their absorption (5). A number of other agents including warfarin, phenylbutazone, chlorothiazide, and phenobarbital can be bound by cholestyramine *in vitro* and their absorption can be delayed *in vivo* when they are administered simultaneously with a single dose of cholestyramine (6). Bergman, Heedman and Van der Linden (7) found that cholestyramine impaired the absorption of orally administered thyroxine in Syrian hamsters and inhibited the transport of thyroxine across the wall of isolated rat intestinal sacs. It also significantly decreased the absorption of thyroxine in patients receiving both drugs, and caused the clinical relapse of the patient's hypothyroid state (8). Thomas, McCullough and Greenberger (9), demonstrated that CH could bind both inorganic and organic iron *in vitro* and could significantly impair the absorption of both in rats.

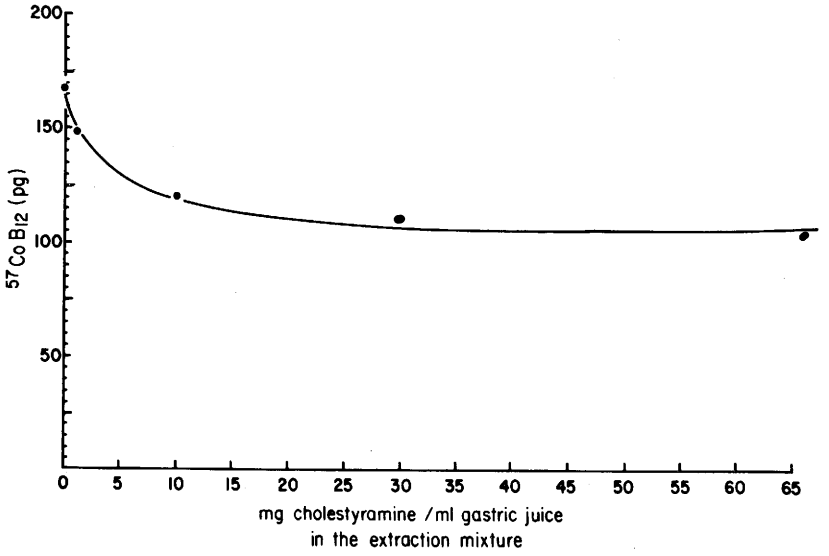

FIG. 4. Uptake of ^{57}Co-B_{12} by GPIMH after cholestyramine extraction of gastric juice.

The question of whether long-term treatment with CH can deplete vitamin B_{12} stores and lead to megaloblastic anemia cannot be answered at this time. One can speculate, however, that it seems unlikely that decreasing an individual's normal absorption of vitamin B_{12} from 90 to 55% would deplete his vitamin B_{12} stores. However, if an individual with previously borderline vitamin B_{12} absorptive capacity (*e.g.*, patients with subtotal gastrectomy, atrophic gastritis, or inflammatory disease of the small bowel) were placed on long-term CH therapy, it is reasonable to assume that this individual's stores would eventually be depleted and macrocytic anemia could develop.

Summary. Cholestyramine decreased the intestinal absorption of radio-B_{12} as measured by the double label hepatic uptake test in 10 consecutive normal adult volunteers, and in a patient with pernicious anemia. The mechanism of this inhibition was explored *in vitro* using the guinea pig intestinal mucosal homogenate assay. The *in vitro* studies suggested that cholestyramine decreased intrinsic factor-mediated B_{12} absorption probably by binding to a portion of the same binding sites on the intrinsic factor molecule which normally bind B_{12}. This would impair the formation of the intrinsic factor-B_{12} complex.

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