The Effect of Deconjugated and Conjugated Bile Salts on the Intestinal Uptake of Radio-vitamin B_{12} *in Vitro* and *in Vivo*[1] (37238)

ANDREW CORONATO[2] AND GEORGE B. JERZY GLASS

Section of Gastroenterology and Gastroenterology Research Laboratory, Department of Medicine, New York Medical College, New York, New York 10029

Vitamin B_{12} and bile acids have their active transport mechanisms located in the terminal ileum both in man and in the guinea pig. It seemed reasonable to explore whether these transport mechanisms were separate or interrelated; and whether the presence of bile salts affected the absorption of vitamin B_{12}. This may have clinical significance in the "blind loop syndrome" where it has been shown that bacterial overgrowth in the small intestine deconjugates bile acids and inhibits vitamin B_{12} absorption.

Methods. In vitro, the guinea pig intestinal mucosal homogenate (GPIMH) assay described by Castro-Curel and Glass (1) 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 of ^{57}Co-B_{12} containing 2500 pg vitamin B_{12} (B_{12}), the specific activity of which provided 4–6 cpm/pg; (b) 1 ml suspension of GPI MH in normal saline containing 12 mg GPI MH; (c) 0.025 ml of GJ diluted to 1 ml with normal saline; and (d) 1 ml of the various bile acids in normal saline in a concentration of 1000 μg/ml. The bile acids (BA) were converted to their sodium salts by adding 0.1 *M* NaOH in dropwise fashion until all of the bile acid was seen to go into solution. We tested the two conjugated bile acids, glycocholic acid (GC) and glycodeoxycholic acid (GDC) and their deconjugated counterparts cholic acid (CA) and deoxycholic acid (DC). In test tubes which did not contain GJ or BA, 1 ml of normal saline was substituted instead. The reagents were mixed in different sequences as follows:

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

Each of the reagents was well mixed into the incubation solution prior to the addition of the next reagent. Sequences 1 and 2 aimed at studying the effect of the respective bile acids on the nonintrinsic factor-mediated uptake of radio-B_{12}. Sequence 3 acted as a control showing the increased uptake of B_{12} by the GPIMH with intrinsic factor (IF)-containing GJ. Sequences 4 through 7 aimed at studying the effect of the different bile salts on the various phases of the IF-mediated uptake of radio-B_{12} by the GPIMH.

The mixtures were incubated at 5° for 1 hr as described by England, Ashworth and Taylor (2) rather than at 37°. This was found to lower the non-IF-mediated uptake, but had no effect on 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.

Following incubation, the GPIMH was

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[2] U.S. Public Health Service Trainee in Gastroenterology.

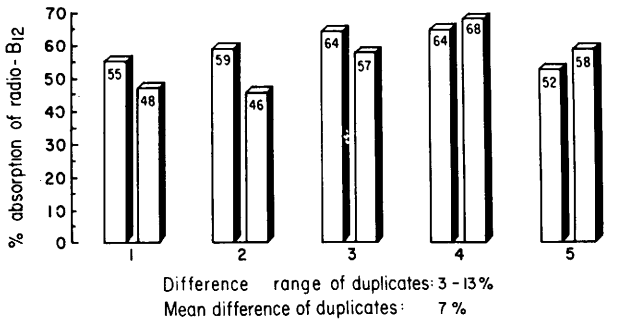

FIG. 1. The reproducibility of the double label hepatic uptake test performed twice in five consecutive normal adult volunteers.

centrifuged, washed, and counted in a heavily shielded well counter as previously described (1).

In vivo, the double label hepatic uptake test (DLHU) of Weisberg and Glass (3) was used. This test permits the quantitative measurement of radio vitamin B_{12} absorption. The test was performed by measuring the hepatic uptake 14 days after the oral administration of 1.0 μg ^{60}Co-B_{12} (0.51 μCi/μg) and comparing it with that of a simultaneously administered intravenous tracer dose (0.05 μg) of ^{57}Co-B_{12} (10 μCi/μg) after correcting for the different counting characteristics of the two cobalt isotopes. The reproducibility of the test in the same individual was determined by performing the test twice at 2 wk intervals. Five consecutive normal adult volunteers were studied. The mean of the difference in B_{12} absorption values of the duplicates was 7% and the range of the difference of the duplicates was 3–13% (Fig. 1). The test was then performed on two normal volunteers before and after oral administration of cholic acid orally, 250 mg three times daily for 3 days. Radio-B_{12} was given on Day 2 and the hepatic uptake was counted 14 days later.

Results. In vitro, GC and GDC had no effect on the non-IF- and IF-mediated uptake of B_{12} by the GPIMH as is shown in Figs. 2 and 3. DC had no effect on non-IF-mediated uptake of B_{12} but caused a slight decrease in the IF-mediated uptake when added in the sequences 4, 5, and 7 (see Methods) shown in Fig. 4. This decrease was not statistically significant. CA, however, produced a 50–55% decrease in both non-IF- and IF-mediated uptake of B_{12} regardless of the sequence in which it was added (Fig. 5).

In vivo, the effect of CA on the absorption of B_{12} in two normal individuals, as measured by the DLHU test, was studied. The first individual was given CA as previously described during the first measurement and none during the second. The sequence was reversed in the second patient. In the first patient the absorption of ^{60}Co-B_{12} was decreased from 48 to 15% while on CA, and in the second patient it was decreased from 66 to 40% (Fig. 6).

Discussion. These studies indicate that cholic acid, the unconjugated bile acid, inhibits the uptake of radio-B_{12} by the GPI MH and decreases the absorption of radio-B_{12} in 2 normal volunteers. The fact that it inhibited the uptake of radio-B_{12} by the GPI MH regardless of the sequence in which it was added suggests it produces its effect by a direct action on the ileal mucosa rather than by interfering with the formation of the intrinsic factor-vitamin B_{12} complex.

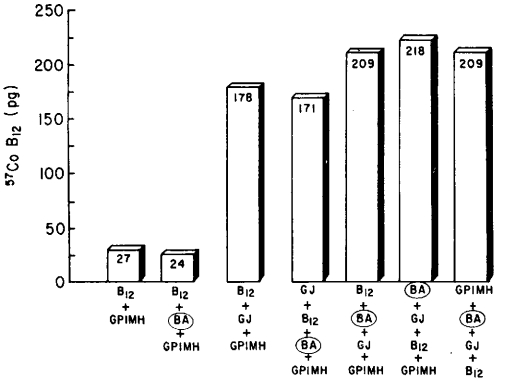

FIG. 2. The effect of glycocholic acid on the uptake of ^{57}Co-B_{12} by the GPIMH.

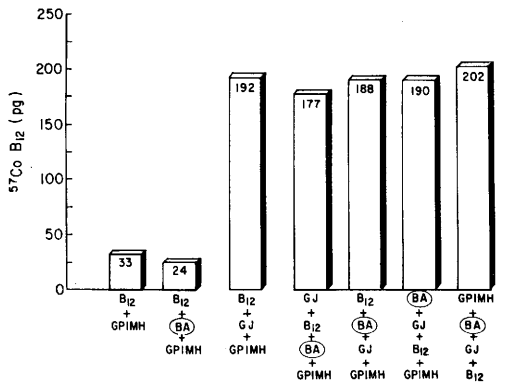

FIG. 3. The effect of glycodeoxycholic acid on the uptake of ^{57}Co-B_{12} by the GPIMH.

Low-Beer, Schneider and Dobbins (4) have described significant morphological changes in the intestine of the guinea pig and hamster following *in vitro* incubation and *in vivo* perfusion with sodium cholate. Unconjugated bile salts have also been shown to inhibit several intestinal mechanisms concerned with the transport of water-soluble nutrients by the rat jejunum *in vitro* which was not the case with conjugated bile salts (5). Thus, it would seem reasonable to assume that the decrease in absorption of B_{12} produced by CA is the result of a direct toxic effect on the ileal mucosa.

A number of drugs have been shown to interfere with the absorption of vitamin B_{12} by direct injury to the ileal mucosa. Jacobsen, Chodos and Faloon (6) demonstrated that the administration of neomycin sulfate for 6 to 9 days reduced the urinary excretion of

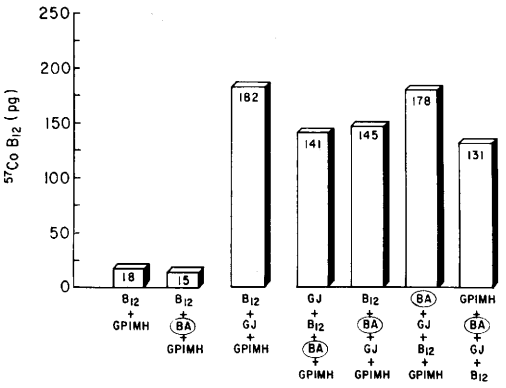

FIG. 4. The effect of deoxycholic acid on the uptake of ^{57}Co-B_{12} by the GPIMH.

^{60}Co-B_{12} by 20 to 60% in four of six subjects. Dobbins, Herreo and Mansback (7) subsequently demonstrated abnormal small intestinal morphology in neomycin-induced malabsorption. Lindenbaum and Lieber (8) demonstrated impaired absorption of vitamin B_{12} in subjects given 158 to 253 g of ethanol daily for 2 wk along with adequate protein and vitamin intake. This impaired absorption was accompanied by striking ultrastructural changes in an ileal biopsy obtained from one subject.

Our findings further clarify the possible mechanism of B_{12} malabsorption in "the blind loop syndrome." Schjönsby *et al.* (9) have demonstrated that certain enteric organisms such as Bacteroides and Enterobacteria are capable of partly binding the IF-

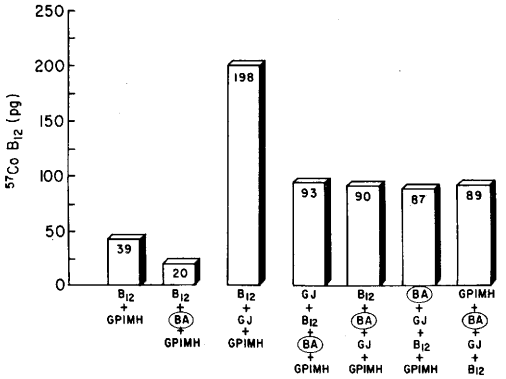

FIG. 5. The effect of cholic acid on the uptake of ^{57}Co-B_{12} by the GPIMH.

B_{12} complex *in vitro*. They showed that in patients with bacterial contamination of the small bowel following a small oral dose of IF-bound ^{57}Co-B_{12}, a considerable amount of radioactivity (43–72%) is recoverable from ileal aspirates. Less than 11% could be recovered from control subjects. This was taken to indicate that B_{12} malabsorption occurred in cases of the blind loop syndrome because of the bacterial binding of the IF-B_{12} complex. In light of the present information, it could equally be concluded that the increased radioactivity in the ileal aspirates and malabsorption of B_{12} may be the result of bacterial deconjugation of bile salts and their subsequent inhibition of B_{12} absorption by their direct toxic effect on the ileal mucosa.

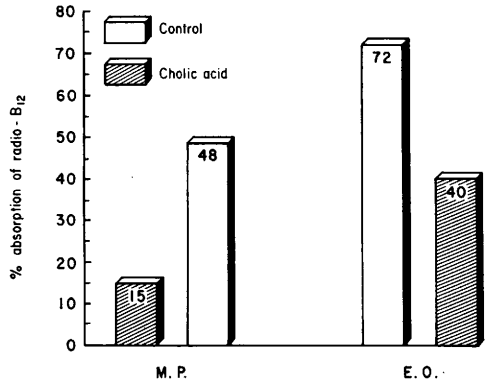

FIG. 6. The effect of cholic acid on the absorption of radio-B_{12} in normal volunteers as measured by the double label hepatic uptake test.

Summary. Deconjugated cholic acid decreased the intestinal uptake of radio-B_{12} by the guinea pig intestinal mucosal homogenate by 50 to 55%. It also decreased the absorption of radio-B_{12} in 2 normal adult volunteers as measured by the double label hepatic uptake test. Cholic acid was felt to produce this decrease by a direct toxic effect on the ileal mucosa. Deoxycholic acid was found to produce a slight but not statistically significant decrease in the uptake of radio-B_{12} by the guinea pig intestinal mucosal homogenate. Glycocholic acid, and glycodeoxycholic acid had no statistically significant effect on radio-B_{12} uptake by the guinea pig intestinal mucosal homogenate.

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