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# Correction of Cobalamin Malabsorption in Pancreatic Insufficiency with a Cobalamin Analogue that Binds with High Affinity to R Protein but not to Intrinsic Factor: *IN VIVO EVIDENCE THAT A FAILURE TO PARTIALLY DEGRADE R PROTEIN IS RESPONSIBLE FOR COBALAMIN MALABSORPTION IN PANCREATIC INSUFFICIENCY*

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In vitro studies indicate that [$^{57}$Co]cobalamin (Cbl) is preferentially bound to salivary R protein as opposed to intrinsic factor (IF) and that [$^{57}$Co]Cbl bound to R protein is not transferred to IF at either pH 2 or pH 8. Incubation of R protein-[$^{57}$Co]Cbl with pancreatic proteases causes a partial degradation of the R protein moiety and a rapid transfer of [$^{57}$Co]Cbl to IF. We have postulated that the etiology of Cbl malabsorption in pancreatic insufficiency is an inability to partially degrade R protein because of a lack of pancreatic proteases. We have tested this hypothesis by determining the ability of a nonradioactive Cbl analogue, bound with high affinity by R protein but not by IF, to correct the malabsorption of [$^{57}$Co]Cbl in patients with pancreatic insufficiency.

R protein bound the Cbl analogue known as cobinamide with affinities that were the same and only 14-fold lower than those for Cbl at pH 8 and pH 2, respectively. Cobinamide was bound by IF with affinities that were 600,000- and 10,000-fold lower than those for Cbl at pH 8 and 2, respectively. The addition of 125 pmol of nonradioactive cobinamide to 0.5 pmol of [$^{57}$Co]Cbl before being added to 1 pmol of R protein and 1 pmol of IF, markedly inhibited the ability of R protein to compete with IF for binding the […]



# Correction of Cobalamin Malabsorption in Pancreatic Insufficiency with a Cobalamin Analogue that Binds with High Affinity to R Protein but not to Intrinsic Factor

## IN VIVO EVIDENCE THAT A FAILURE TO PARTIALLY DEGRADE R PROTEIN IS RESPONSIBLE FOR COBALAMIN MALABSORPTION IN PANCREATIC INSUFFICIENCY

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ABSTRACT In vitro studies indicate that [$^{57}$Co]cobalamin (Cbl) is preferentially bound to salivary R protein as opposed to intrinsic factor (IF) and that [$^{57}$Co]Cbl bound to R protein is not transferred to IF at either pH 2 or pH 8. Incubation of R protein-[$^{57}$Co]Cbl with pancreatic proteases causes a partial degradation of the R protein moiety and a rapid transfer of [$^{57}$Co]Cbl to IF. We have postulated that the etiology of Cbl malabsorption in pancreatic insufficiency is an inability to partially degrade R protein because of a lack of pancreatic proteases. We have tested this hypothesis by determining the ability of a nonradioactive Cbl analogue, bound with high affinity by R protein but not by IF, to correct the malabsorption of [$^{57}$Co]Cbl in patients with pancreatic insufficiency.

R protein bound the Cbl analogue known as cobinamide with affinities that were the same and only 14-fold lower than those for Cbl at pH 8 and pH 2, respectively. Cobinamide was bound by IF with affinities that were 600,000- and 10,000-fold lower than those for Cbl at pH 8 and 2, respectively. The addition of 125 pmol of nonradioactive cobinamide to 0.5 pmol of [$^{57}$Co]Cbl before being added to 1 pmol of R protein and 1 pmol of IF, markedly inhibited the ability of R protein to compete with IF for binding the [$^{57}$Co]Cbl. Similar results were obtained with freshly aspirated gastric juice. This change was essentially indistinguishable from that observed previously when R protein or R protein-[$^{57}$Co]Cbl was incubated in vitro with trypsin. The oral administration of 100 nmol of nonradioactive cobinamide in Schilling tests was equivalent to trypsin in its ability to completely correct the malabsorption of 0.4 nmol of [$^{57}$Co]Cbl in three patients with pancreatic insufficiency.

The fact that both trypsin and nonradioactive cobinamide inhibit the ability of R protein to compete with IF for [$^{57}$Co]Cbl binding in vitro, and correct the malabsorption of [$^{57}$Co]Cbl in patients with pancreatic insufficiency in vivo, supports our hypothesis that the primary defect in Cbl absorption in this disease is an inability to partially degrade R protein because of a lack of pancreatic proteases.

## INTRODUCTION

Intrinsic factor (IF)[1] binds cobalamin (Cbl; vitamin $B_{12}$) with high affinity from pH 1 to pH 10 (2). Because of this it has been assumed that Cbl is bound by IF in the acid environment of the stomach and that the IF-Cbl complex remains intact until sometime after it becomes bound to its ileal receptor. The conventional model of Cbl absorption ignores the fact that saliva, gastric juice, and bile contain significant amounts of a Cbl binding

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[1] *Abbreviations used in this paper*: Cbi, cobinamide; Cbl, cobalamin; IF, intrinsic factor.

protein known as R protein[2] (3) that might be able to compete with IF for Cbl binding.

In recent in vitro studies (4) we observed that human salivary R protein bound Cbl with affinities that were 3- and 50-fold higher than those of human IF at pH 8 and 2, respectively. Cbl bound to R protein was not transferred to an equal amount of IF at either pH 8 or pH 2. Cbl bound to IF was transferred to R protein with $t_{1/2}$'s of 90 and 2 min at pH 8 and 2, respectively, and within several hours respective ratios of R protein-Cbl/IF-Cbl of 2 and 50 were observed. Incubation of IF, IF-Cbl, R protein, and R protein-Cbl with high concentrations of pepsin at pH 2 failed to alter their molecular weights, affinities for Cbl, or abilities to compete for Cbl binding at either pH 2 or pH 8. Incubation of IF or IF-Cbl with pancreatic proteases at pH 8 also failed to cause changes in these parameters. Incubation of R protein and R protein-Cbl with pancreatic proteases did lead to alterations, however, that consisted of a 50% decrease in molecular weight, a 150-fold decrease in affinity for Cbl, and a rapid and essentially complete transfer of Cbl from R protein to IF.

Based on these studies we have postulated (4) that under normal conditions of gastric acidity, dietary Cbl enters the jejunum bound almost exclusively to R protein and does not become bound to IF until after the R protein moiety is partially degraded by pancreatic proteases in the small intestine. Our hypothesis is supported by the fact that more than 50% of patients with pancreatic exocrine insufficiency malabsorb Cbl based on Schilling tests (5–9) and by the fact that this abnormality can be corrected by the oral administration of pancreatic proteases (6, 8–11). The fact that partial correction is obtained with oral bicarbonate (6, 9) also supports our hypothesis because at pH 8 IF can partially compete with R protein for the initial binding and retention of Cbl.

If our hypothesis is correct, any means that inhibits the ability of R protein to compete with IF for Cbl binding should result in the correction of Cbl malabsorption in patients with pancreatic insufficiency. The present studies utilized a nonradioactive Cbl analogue that is bound with high affinity by R protein but not by IF and show that this analogue is capable of inhibiting the ability of R protein to compete with IF for [$^{57}$Co]Cbl binding in vitro at pH 2 and pH 8. We have tested our hypothesis by administering this analogue orally to patients with pancreatic insufficiency and determining its ability to correct the malabsorption of [$^{57}$Co]Cbl in Schilling tests.

## METHODS

*Materials.* [$^{57}$Co]Cbl (12.5 μCi/nmol) was obtained from Amersham-Searle Corp., Arlington Heights, Ill. Cobinamide (Cbi) was prepared and isolated as previously described (12). All in vitro and in vivo studies were performed with the same preparation of Cbi. Bovine pancreatic trypsin was obtained lyophilized in 50-mg sterile vials from Worthington Biochemical Corp., Freehold, N. J. Human IF and human salivary R protein were collected and equilibrated with 0.01 M Tris-HCl, pH 8, containing 0.15 M NaCl and 50 μg/ml of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as previously described (4). Rabbit anti-human IF (13) and anti-human R protein (14) sera, purified human IF (13), and human anti-IF blocking antibodies (13) were obtained as described previously. Cbl binding assays were performed as described previously (13).

*Determination of association constants and distribution of bound Cbl.* Experiments performed at pH 8 employed 0.01 Tris-HCl, pH 8 and those at pH 2 employed 0.01 M phosphoric acid-NaOH, pH 2. All solutions contained 0.15 M NaCl and 50 μg/ml bovine serum albumin. Association constants ($K_a$) for CN-[$^{57}$Co]Cbl were determined by the charcoal adsorption technique as previously described (12). Values for the ratio $K_a$ Cbi/$K_a$ Cbl were determined by measuring the ability of nonradioactive Cbi to inhibit the binding of [$^{57}$Co]Cbl by the charcoal absorption technique as previously described (12). Values for $K_a$ Cbi were calculated from the values obtained for $K_a$ Cbl and $K_a$ Cbi/$K_a$ Cbl. The distribution of [$^{57}$Co]Cbl bound to R protein and IF was based on their precipitation with rabbit anti-human R protein and rabbit anti-human IF anti-sera in 40% $(NH_4)_2SO_4$ as previously described (12).

*Schilling tests.* Subjects were fasted for 12 h before and 2 h after the oral administration of 0.4 nmol of [$^{57}$Co]Cbl (0.5 μCi) in 15 ml of $H_2O$. Except where specifically indicated other components such as trypsin (20 mg freshly dissolved in 2 ml of 1 mM HCl), Cbi (100 nmol dissolved in 2 ml of $H_2O$), and purified human IF (0.8 nmol dissolved in 2 ml of $H_2O$) were mixed with the [$^{57}$Co]Cbl and incubated for 15 min at 4°C before being administered. An intramuscular injection of 1 mg of nonradioactive CN-Cbl was given immediately after the oral [$^{57}$Co]Cbl. Urine was collected for 24 h after the administration of the [$^{57}$Co]Cbl and was assayed for radioactivity with a Beckman Gamma 300 system (Beckman Instruments, Inc., Fullerton, Calif.). Schilling tests were performed at 3- to 10-day intervals. In the case of patients with pancreatic insufficiency, pancreatic extract was stopped for 36 h before the administration of the [$^{57}$Co]Cbl and was restarted 24 h later. Diarrhea was minimized by adherence to a low-fat diet during this period. Informed consent was obtained from normal subjects and patients in accord with the Declaration of Helsinki after approval by the Committee on Human Experimentation of Washington University School of Medicine.

### *Subjects*

*Normals.* Subjects I and II were 23- and 30-yr-old white males, with entirely negative medical histories.

*Patients.* Patient I was a 47-yr-old white male with a long history (over 20 yrs) of excessive alcoholic intake, and a 10-yr history of poor nutrition. He had undergone two laparotomies for drainage of pancreatic pseudocysts and abscesses. In 1972 he had a diverting choledochoduodenostomy for obstructive jaundice due to chronic edema of the head of the pancreas. In December of 1976 he presented with increasingly severe

[2] The term "R protein" was originally devised to denote a Cbl binding protein in human gastric juice that was devoid of intrinsic factor activity. It was designated as protein "R" because of its rapid mobility on electrophoresis. Subsequently, immunologically related proteins have been observed in a variety of human tissues and body fluids and have been referred to as the R proteins.

malnutrition, a 10-pound weight loss, and malodorous diarrhea. Physical examination revealed a wasted male with scattered ecchymoses, and ankle edema. No neuropathy was present. Values for serum chemistries included: bilirubin, 0.8 mg/dl; amylase, 72 dye U/ml (normal 45-200 dye U/ml); alkaline phosphatase, 181 M IU/ml; albumin, 2.1 g/dl; hemoglobin 14.1 g/dl; fasting sugar 93 mg/dl with a 2-h after-meal sugar of 209 mg/dl; folic acid, 16 ng/ml; and vitamin $B_{12}$, 1,520 pg/ml (normal 200–900 pg/ml). Upper GI series showed pancreatic calcification and compression of the second part of the duodenum by an extrinsic mass. Computerized axial tomography of the abdomen demonstrated two pseudocysts, including one in the head of the pancreas. The fat in the stool was graded 3+ on qualitative examination on a daily intake of 40 g of fat and was clearly increased. Jejunal culture for aerobes and anaerobes showed no growth. Pancreatin supplements were started (Viokase, VioBin Corp., Monticello, Ill.) at a dose of 5 tablets with meals, and 2 with snacks to a total of ~21 tablets per day. Within a few days stools returned to a formed or semiformed state, and within 2 wk weight gain had begun. Over 6 mo a total weight gain of 12 pounds was achieved. Mild diarrhea recurred during the periods off pancreatic supplements during the study.

Patient II was a 15-yr-old white female on whom a diagnosis of cystic fibrosis was made by sweat chloride test at age 3 mo. Her brother also had cystic fibrosis. She had developed malodorous loose stools from a very early age and required supplement with pancreatin (Viokase). Growth failure was noted early. Repeated pulmonary infection began at age 12 yr. Past antibiotics had included tetracycline at a dose of 1 g per day. During the 6 mo this drug was taken, no change in stool frequency or odor was noted. In April of 1977 she presented with a weight of 23.8 kg, and a height of 132 cm, both less than the third percentile of expected values. She was having 2–3 semisolid, greasy, malodorous stools per day. Chronic productive cough was present. On physical examination, the liver size was normal and no neuropathy was noted. Serum laboratory values included: carotene, 10 μg/dl (normal 70–250 μg/dl); alkaline phosphatase, 304 mIU/ml; hemoglobin 13.8 g/dl; and $Po_2$, 71 mm Hg. Her present medications included pancreatin (Viokase), six tablets per feeding (18–24 per day); Vitamin $K_3$ 10 mg/day; sulfasoxazole 2 g/day; cloxacillin 1 g/day; and *N*-acetyl cysteine by aerosol. Except for the pancreatin, all of these drugs were continued during all of the Schilling tests. Moderate diarrhea developed during the test periods off pancreatin.

Patient III was a 31-yr-old white female who had undergone a series of operations for abdominal pain which finally resulted in a total pancreatectomy. The operations included cholecystectomy in 1973, sphincterotomy and hemipancreatectomy in 1974, and total pancreatectomy with removal of duodenum, hemigastrectomy and vagectomy in 1976. After total pancreatectomy, loose malodorous stools developed, with particles of food frequently seen in the stool. A weight loss of 10 pounds occurred, and peripheral edema developed. Diabetes was well controlled with isophane insulin suspension (neutral protamine Hagedorn insulin) 23 U and regular insulin 6 U in the morning, and regular insulin 8 U before the evening meal. Physical examination revealed a chronically ill, malnourished patient with normal abdominal examination and no peripheral neuropathy. Pertinent laboratory values included: hemoglobin, 11.2 g/dl; vitamin $B_{12}$, 760 pg/ml; folic acid, 6.2 ng/ml; albumin, 2.7 g/dl, and fasting sugar 172 g/dl. Gastric analysis with Histalog (1.7 g/kg body weight) revealed a basal acid output of zero, and an maximal acid output of 1.07 meq. Jejunal culture for aerobes and anaerobes showed no growth. Treatment of malnutrition included a 40-g fat diet, pancreatin (Viokase, VioBin Corp.) five tablets with meals and three with snacks (24–27 per day), casein supplements (Casec, Mead Johnson & Co., Evansville, Ind.) 16 g three times daily, and multivitamins. On this program the patient gained 8 pounds and maintained a stable weight of 46 kg. Stools became formed and less frequent, although clinically significant steatorrhea recurred during the test periods when pancreatic supplements were withheld. Treatment with tetracycline, 1 g per day for 2 wk, resulted in no alteration in stool frequency or character.

## RESULTS

*Association constants of Cbl and Cbi for IF and R protein.* The structures of Cbl and Cbi are the same except that Cbi lacks the 5,6-dimethylbenzimidazole, ribose, and phosphate moieties. The association constants of Cbl and Cbi for IF and R protein determined at pH 8 and pH 2 are shown in Table I. For IF, Cbi had 6000,000-and 10,000-fold lower affinities than Cbl at pH 8 and pH 2, respectively. For R protein the affinities for Cbi were essentially the same and only 14-fold lower than those for Cbl at pH 8 and pH 2, respectively.

*Effect of nonradioactive Cbi on the ability of IF and R protein to compete for [$^{57}$Co]Cbl binding.* The data in Table II show the ability of 1 pmol of IF and 1 pmol of R protein to compete for binding 0.5 pmol of [$^{57}$Co]Cbl in the presence and absence of 125 pmol of nonradioactive Cbi at pH 8 and pH 2. At pH 8 in the absence of Cbi, IF was able to partially compete for the initial binding of [$^{57}$Co]Cbl, and some, but not all, of the [$^{57}$Co]Cbl was subsequently transferred to R protein. The addition of nonradioactive Cbi did not affect the binding of [$^{57}$Co]Cbl to IF but did completely inhibit the binding of [$^{57}$Co]Cbl to R protein. Nonradioactive Cbi completely inhibited the ability of R protein to compete with IF for the initial binding of [$^{57}$Co]Cbl and also completely inhibited the transfer of [$^{57}$Co]Cbl from IF to R protein.

At pH 2, IF and R protein were both capable of binding [$^{57}$Co]Cbl although the superiority of R protein was such that [$^{57}$Co]Cbl was bound exclusively to R protein regardless of the order with which [$^{57}$Co]Cbl, IF, and R protein were mixed together. The addition of nonradioactive Cbi did not affect the binding of [$^{57}$Co]Cbl to IF. It did cause a marked inhibition of [$^{57}$Co]Cbl binding to R protein although the inhibition observed at pH 2 was

TABLE I

*Affinity of IF and R Protein for Cbl and Cbi at pH 8 and pH 2*

| | $K_a$Cbl | | $K_a$Cbi | | $K_a$Cbl/$K_a$Cbi | |
|---|---|---|---|---|---|---|
| | IF | R | IF | R | IF | R |
| | *per pM* | | | | | |
| pH 8 | 0.6 | 1.6 | 0.000001 | 1.3 | 600,000 | 1 |
| pH 2 | 0.02 | 1.0 | 0.000002 | 0.07 | 10,000 | 14 |

TABLE II

*Effect of 125 pmol of Nonradioactive Cbi on the Ability of 1.0 pmol of IF and 1.0 pmol of R Protein to Compete for the Binding of 0.5 pmol of [$^{57}$Co]Cbl*

| pH | First addition | Time of incubation | Second addition | Time of incubation | Precipitation of [$^{57}$Co]Cbl | |
|---|---|---|---|---|---|---|
| | | | | | Anti-IF | Anti-R |
| | | *min* | | *min* | % | % |
| 8 | [$^{57}$Co]Cbl + IF | 30 | None | 60 | 97 | 1 |
| 8 | [$^{57}$Co]Cbl + R | 30 | None | 60 | 2 | 98 |
| 8 | [$^{57}$Co]Cbl + (IF + R)* | 30 | None | 60 | 26 | 71 |
| 8 | [$^{57}$Co]Cbl + IF | 30 | R | 60 | 69 | 27 |
| 8 | [$^{57}$Co]Cbl + R | 30 | IF | 60 | 1 | 98 |
| 8 | ([$^{57}$Co]Cbl + Cbi)* + IF | 30 | None | 60 | 97 | 5 |
| 8 | ([$^{57}$Co]Cbl + Cbi)* + R | 30 | None | 60 | 0 | 0 |
| 8 | ([$^{57}$Co]Cbl + Cbi)* + (IF + R)* | 30 | None | 60 | 96 | 0 |
| 8 | ([$^{57}$Co]Cbl + Cbi)* + IF | 30 | R | 60 | 97 | 3 |
| 8 | ([$^{57}$Co]Cbl + Cbi)* + R | 30 | IF | 60 | 96 | 0 |
| 2 | [$^{57}$Co]Cbl + IF | 30 | None | 60 | 88 | 9 |
| 2 | [$^{57}$Co]Cbl + R | 30 | None | 60 | 0 | 98 |
| 2 | [$^{57}$Co]Cbl + (IF + R)* | 30 | None | 60 | 0 | 98 |
| 2 | [$^{57}$Co]Cbl + IF | 30 | R | 60 | 0 | 98 |
| 2 | [$^{57}$Co]Cbl + R | 30 | IF | 60 | 0 | 98 |
| 2 | ([$^{57}$Co]Cbl + Cbi)* + IF | 30 | None | 60 | 95 | 4 |
| 2 | ([$^{57}$Co]Cbl + Cbi)* + R | 30 | None | 60 | 1 | 30 |
| 2 | ([$^{57}$Co]Cbl + Cbi)* + (IF + R)* | 30 | None | 60 | 80 | 16 |
| 2 | ([$^{57}$Co]Cbl + Cbi)* + IF | 30 | R | 60 | 90 | 9 |
| 2 | ([$^{57}$Co]Cbl + Cbi)* + R | 30 | IF | 60 | 72 | 23 |

Incubations were performed at 37°C in a volume of 0.5 ml.

* Items in parentheses were mixed together before being added to assay tubes.

not as great as at pH 8. Nonradioactive Cbi markedly inhibited the ability of R protein to compete with IF for [$^{57}$Co]Cbl binding and the transfer of [$^{57}$Co]Cbl from IF to R protein, although the degree of inhibition was slightly less than at pH 8.

Similar results were observed with freshly aspirated basal gastric juice as shown in Table III and rules out the possibility that R protein or IF have been altered during their partial purification or storage.

*Effect of trypsin and Cbi on [$^{57}$Co]Cbl absorption in normal subjects.* The results of Schilling tests performed on two normal subjects are shown in Fig. 1. In subject I, 18.9% of the [$^{57}$Co]Cb! was excreted in the urine when [$^{57}$Co]Cbl was administered alone. Respective values of 24.1 and 19.3% were observed when 20 mg of trypsin and 100 nmol of nonradioactive Cbi were added. A base-line value of 15.7% was obtained with the second normal subject. Values of 11.2 and 14.7% were obtained with trypsin and Cbi, respectively. These results indicate that neither trypsin nor Cbi affects the absorption of [$^{57}$Co]Cbl in normal subjects.

*Effect of trypsin and Cbi on the absorption of [$^{57}$Co]Cbl in patients with pancreatic insufficiency.* The results of Schilling tests performed on two patients with pancreatic insufficiency are shown in Fig. 2. In patient I a base-line value of 1.7% was obtained and this increased to 27.5% upon the administration of trypsin. A second base-line value of 2.5% was obtained followed by a value of 22% when Cbi was added. In patient II the initial base-line value was 1.8% and this increased to 11.4% when trypsin was added. The second base-line value was 2.3% and this increased to 15.8% upon the addition of Cbi. These results indicate that 20 mg of trypsin and 100 nmol of nonradioactive Cbi are equivalent in terms of their ability to correct Cbl malabsorption in patients with pancreatic insufficiency.

The results of Schilling tests performed in a patient with a total pancreatectomy and marked hypochlorhydria secondary to a hemigastrectomy are shown in Fig. 3. A base-line value of 0.8% was obtained but in this patient the addition of trypsin resulted in a value of only 1.1% suggesting that she was also deficient in IF. This possibility was confirmed by the value of 14.7% obtained when IF and trypsin were added to the [$^{57}$Co]Cbl before its administration. When IF alone was added to [$^{57}$Co]Cbl before its administration, an intermediate

TABLE III

*Effect of 1,000 pmol of Nonradioactive Cbi on the Binding of 4 pmol of [$^{57}$Co]Cbl by R Protein and IF in Freshly Aspirated Basal Gastric Juice from Two Subjects Undergoing Endoscopy for Evaluation of Peptic Ulcer Disease*

| Subject | Gastric juice: Volume aspirated | pH | Cbl binding ability | | Incubation: Gastric juice | [$^{57}$Co]Cbl | Cbi | Precipitation of [$^{57}$Co]Cbl: Anti-IF sera | Anti-R sera | Control sera |
|---|---|---|---|---|---|---|---|---|---|---|
| | *ml* | | *pmol/ml* | *%IF** | *ml* | *pmol‡* | *pmol‡* | *%* | *%* | *%* |
| A | 34 | 1.8 | 43.4 | 54 | 1.5 | 4 | 0 | 0 | 97 | 2 |
| | | | | | 1.5 | 4 | 1,000 | 73 | 29 | 1 |
| B | 32 | 3.1 | 25.9 | 95 | 1.5 | 4 | 0 | 52 | 46 | 1 |
| | | | | | 1.5 | 4 | 1,000 | 97 | 1 | 0 |

Gastric juice, 1.5 ml, was incubated immediately after aspiration with Cbi and [$^{57}$Co]Cbl in 0.05 ml of 0.15 M NaCl for 30 min at 37°C. The samples were then cooled to 4°C, neutralized by the addition of 0.1 ml of 1.0 M Tris-HCl, pH 9.2, and the amount of [$^{57}$Co]Cbl bound to R protein and IF was measured by precipitation with specific antisera.

* Based on the decreased binding observed in the presence of anti-IF blocking antibody.

‡ These items were mixed together before being added to the gastric juice.

value of 8.1% was obtained. This intermediate value is consistent with our in vitro studies because in the absence of gastric acidity some, but not all, of the [$^{57}$Co]Cbl would be transferred to endogenous R protein during the several hours required for the administered IF-[$^{57}$Co]Cbl to reach the terminal ileum. When [$^{57}$Co]Cbl was given alone 3 min before the IF, a value of only 1.6% was obtained indicating that the [$^{57}$Co]Cbl was rapidly rendered unavailable for binding to the IF. When Cbi was mixed with the [$^{57}$Co]Cbl and administered 3 min before the IF, a value of 13.6% was obtained indicating that the [$^{57}$Co]Cbl was now available for binding to the IF.

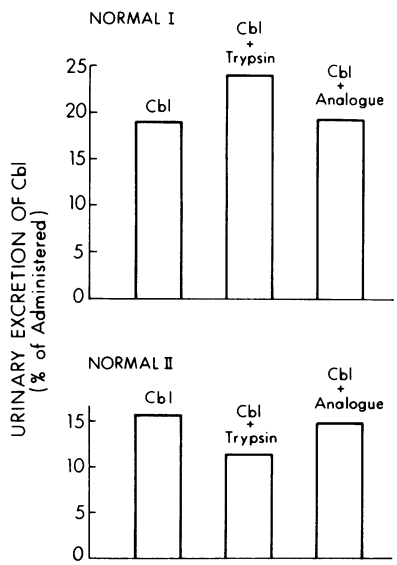


FIGURE 1 The effect of trypsin (20 mg) and a nonradioactive Cbl analogue (100 nmol of Cbi) on the absorption of 0.4 nmol of [$^{57}$Co]Cbl in normal subjects as measured by Schilling tests. Trypsin and analogue were mixed with the [$^{57}$Co]Cbl and incubated for 15 min at 4°C before their oral administration. Schilling tests were performed in order from left to right.

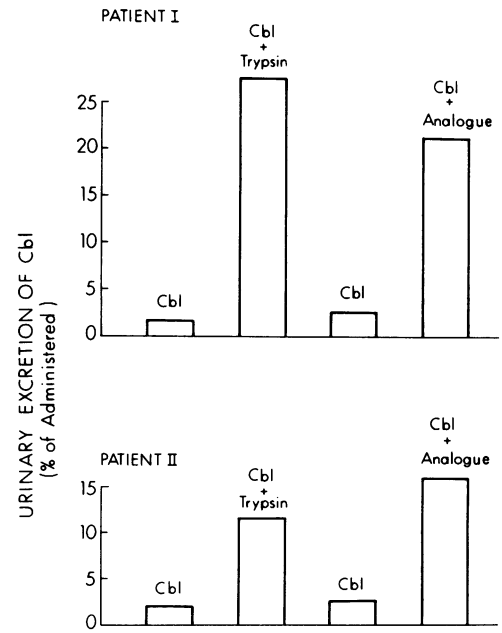


FIGURE 2 The effect of trypsin (20 mg) and a nonradioactive Cbl analogue (100 nmol of Cbi) on the absorption of 0.4 nmol of [$^{57}$Co]Cbl in patients with pancreatic insufficiencey as measured by Schilling tests. Trypsin and the analogue were mixed with the [$^{57}$Co]Cbl and incubated for 15 min at 4°C before oral administration. Schilling tests were performed in order from left to right.

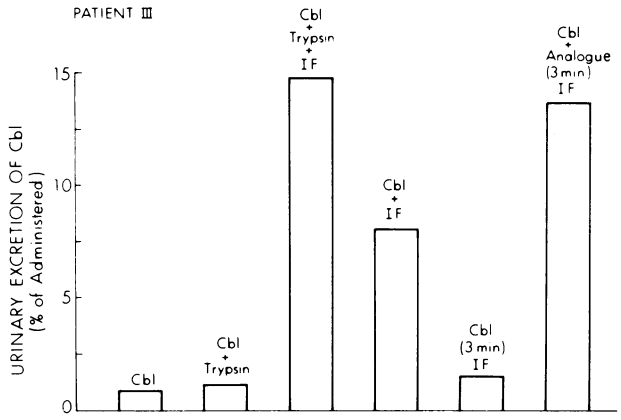


FIGURE 3 The effect of trypsin (20 mg), purified human IF (0.8 nmol), and a non-radioactive Cbl analogue (100 nmol of Cbi) on the absorption of 0.4 nmol of [$^{57}$Co]Cbl in a patient with a total pancreatectomy and marked hypochlorhydria secondary to a hemigastrectomy, as measured by Schilling tests. Except for the last two Schilling tests, trypsin and IF were mixed together with the [$^{57}$Co]Cbl and incubated for 15 min at 4°C before their oral administration. In the last two Schilling tests, [$^{57}$Co]Cbl and [$^{57}$Co]Cbl plus analogue were administered orally 3 min before the oral administration of IF. The Schilling tests were performed in order from left to right.

## DISCUSSION

In the present study we have shown that 100 nmol of nonradioactive Cbi does not alter the absorption of 0.4 nmol of [$^{57}$Co]Cbl in normal subjects and that it corrects the malabsorption observed in patients with pancreatic insufficiency. Based on our in vitro studies and on the association constants determined for IF and R protein, 100 nmol of Cbi would not be expected to inhibit the binding of [$^{57}$Co]Cbl to IF but should markedly inhibit the binding of [$^{57}$Co]Cbl to the 1 nmol and 0.2 nmol of R protein that are secreted in the saliva and bile, respectively, each hour. The fact that Cbi and trypsin are equivalent in their ability to correct the malabsorption of [$^{57}$Co]Cbl in pancreatic insufficiency provides in vivo support for our hypothesis (4) that the primary defect in Cbl absorption in this disease is a lack of pancreatic proteases and a resultant inability to partially degrade R protein and allow Cbl to become bound by IF.

The fact that Cbi was able to correct Cbl malabsorption in a patient with a total pancreatectomy makes it extremely unlikely that pancreatic proteases play any additional role in Cbl absorption such as causing a subtle but essential alteration in the IF-Cbl molecule before or after it attaches to its ileal receptor. The observation that the absorption of [$^{57}$Co]Cbl bound to purified IF was only moderately decreased in a patient with achlorhydria and pancreatic insufficiency supports our hypothesis that normal gastric acidity is required for the occurrence of severe Cbl malabsorption in pancreatic insufficiency. Because it appears likely that achlorhydria is frequently combined with pancreatic insufficiency (4) and because gastric acid secretion is not stimulated in routine Schilling tests it appears likely that variations in basal gastric acidity are responsible for many of the differences in Cbl absorption observed among patients with pancreatic insufficiency and for the differences observed for individual patients when studied on multiple occasions (11). Differences in the R protein content of basal gastric juice may also be involved.

Cbl in bile enters the jejunum bound to R protein (3), and our studies indicate that this is also true for dietary Cbl. This suggests that the initial binding of Cbl to R protein may be of functional importance although its significance at the present time is unknown. R proteins in secretions and in granulocytes appear to serve an antibacterial function (3, 16) by binding Cbl and preventing its uptake by bacteria that require Cbl for growth. If Cbl bound to R protein is less available to bacteria than Cbl bound to IF, this could explain the initial binding of Cbl to R protein in gastric juice and bile. The granulocyte R protein binds a variety of inactive and potentially toxic, naturally occurring Cbl analogues, and transports them exclusively to hepatocytes which retain these analogues and prevent their dissemination to other tissues (12). The initial binding to R protein in gastric juice and bile could function to prevent the absorption of naturally occuring Cbl analogue if (*a*) R protein-Cbl analogue complexes are less susceptible to degradation by pancreatic proteases than R protein-Cbl or if (*b*) Cbl analogues bound to partially degraded R protein are transferred to IF at a slower rate than Cbl bound to partially degraded R protein.

Although Cbl malabsorption in pancreatic insufficiency can be corrected by the oral administration of Cbi, it is important to point out that Cbi is not indicated as therapy for these patients. One reason is that Cbl malabsorption is already corrected by the pancreatic extract that the vast majority of these patients take to control the diarrhea associated with their disease, and this appears to account for the fact that true Cbl deficiency occurs rarely in this disease (15). A second reason is the uncertainty concerning the safety of long-term oral administration of Cbi. Because of this, a monthly injection of Cbl would be the treatment of choice for those patients who do not require, or cannot take, oral pancreatic extract.

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