Effect of lectins on the cobalamin-protein binding reactions: implications for the tissue uptake of cobalamin

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Plant lectins have been thought to impair nutrient absorption, both by specific and nonspecific interference in the absorptive process. The main objective of this investigation was to study the effect of lectins on the various binding reactions involving cobalamin (cbl)-protein complexes and their receptors, and to identify the rate-limiting step important in maintaining tissue levels of cobalamin. Among the lectins tested in vivo, only concanavalin A (ConA) was able to inhibit the transport of cobalamin to the tissues and caused a 70% to 75% inhibition of [^{57}Co] cobalamin transported to the liver and kidney. The inhibition of transport to the tissues was independent of route of administration of cobalamin, whether intragastric or systemic, and was not due to decreased gastrointestinal uptake. When tested in vitro, concanavalin A inhibited the binding of transcobalamin II-cbl to its receptor, but not the binding of cobalamin to intrinsic factor or intrinsic factor-cobalamin complex to the ileal receptor. These results suggest that late events during transcellular transport of cobalamin through the enterocytes is the rate-limiting step determining tissue levels of cobalamin and that ConA inhibits these latter events.

Keywords: Lectins; concanavalin A; cobalamin; uptake; intestine

Introduction

The gastrointestinal absorption and cellular uptake of cobalamin (cbl) is a complex process.[1,2] Cobalamin uptake into tissues is mediated by two binding proteins, one of which, intrinsic factor (IF), is a glycoprotein,[3] while the other, transcobalamin II (TC II), is not.[4] Cellular utilization of dietary cobalamin involves two separate receptor-mediated endocytotic events.[2] One, in the gastrointestinal lumen, involves IF and its receptor[5,6] which is located in the microvillus pit,[7] while the other, at the plasma/tissue interface, involves TC II and its receptor.[8] Moreover, the receptors that recognize both of these ligands are glycoproteins.[8,9] Thus, one might predict that lectins could certainly interfere with IF-cbl uptake, a system that involves two glycoproteins. The effect on TC II-cbl uptake would seem less probable, as TC II is not glycosylated.

Plant lectins are known to bind to membranes and to interact with many glycoproteins. These lectins are distributed widely and are common in many diets;[10,11] consequently, intestinal exposure to them is believed to be widespread.[11] Lectins are known to cause various changes within the gastrointestinal tract,[12-14] including malabsorption of nutrients.[15-17] Earlier studies have shown that rats fed with large doses of lectins for prolonged periods of time (21 days) malabsorbed cobalamin, as assessed by whole body counting.[18] A partial reversal was achieved both by either restoring rats to a lectin-free diet or by treatment with antibiotics. It was suggested that prolonged exposure to lectins caused increased mucosal adherence of bacteria and that the malabsorption was due to bacterial utilization of the vitamin. In support of this suggestion are studies in humans[19] with blind loop syndrome in whom competition for uptake of cobalamin by bacteria has been noted as a cause of malabsorption of cobalamin. In contrast to the above explanations for the cobala-

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min malabsorption noted with prolonged exposure to lectins, nothing is known about the effect of exposure to lectins for short-time intervals (3 to 5 hours), the period of time during which cobalamin is absorbed and appears in the tissue. Orally administered concanavalin A (ConA) is unaffected by digestive enzymes[20] and is largely recovered in the feces, but a small amount is absorbed intact by the intestine and rapidly excreted in the urine.[21] Thus, it is possible that ConA and dietary lectins could affect tissue uptake via the TC II receptor, as well as alter IF-cbl absorption from the lumen via the IF-cbl receptor.

The present work was undertaken to examine whether a single dose and a short exposure of lectins to the mucosa could be used to block one of the steps in the tissue uptake of cobalamin, i.e., either at the apical membrane level or in the process of transepithelial transport or at plasma/tissue interphase. Our in vivo and in vitro results show that gastrointestinal uptake is not affected by ConA, but the latter processes are delayed by ConA and could be reversed.

Materials and methods

Materials

[^{57}Co]cobalamin (specific activity, 15 to 220 μCi/μg) and Na[^{125}I]-specific activity (17 Ci/mg) were purchased from Amersham (Arlington Heights, IL, USA). Lectins were purchased from Sigma Chemical Company (St. Louis, MO, USA). The ConA used in these studies was determined to be pure by nondenaturing gel electrophoresis using ^{125}I-ConA.

Concanavalin A and IF were iodinated using Na[^{125}I] by the Enzymo-Bead technique,[22] producing a specific activity of 7,500 dpm/μg and 500 dpm/fmol (^{57}Co)cbl binding, respectively.

Rat IF was purified from rat stomachs as previously described.[23] Rabbit TC II was partially purified according to Allen and Majerus.[4] Rat kidney basolateral membranes were prepared by the method of Molitoris and Simon.[24] The membranes were enriched 10-fold for Na^+K^+ ATPase. Rat intestinal brush border membranes were prepared according to Kessler et al.[25] The enrichment of TC II-cbl receptor in the kidney basolateral membranes and of IF-cbl receptor in the intestinal brush border membranes was 7- and 10-fold, with a recovery of 8% and 15%, respectively.

In vitro receptor binding assays. Binding of [^{57}Co]cbl by IF was measured by the method of Gottlieb et al.[26] Binding in the presence of lectins (50 μg) in a 3-ml volume was assessed by the addition of [^{57}Co]cbl following a 10-minute incubation of IF with various lectins. Calcium-dependent binding of IF-[^{57}Co]cbl to ileal brush border membranes and of TC-II[^{57}Co]cbl to kidney basolateral membranes was determined according to Seetharam et al.[23] and Seligman and Allen,[8] respectively. When lectins (10 to 100 μg/ml) were used, they were added to the membranes and preincubated for 30 minutes at 22°C before the addition of the ligand. When α-methyl mannoside, mannan, myo-inositol, and antiserum to the receptor were added, the membranes were preincubated with these reagents for 1 hour at 22°C, followed by the addition of ConA (50 μg), and further incubated for 15 minutes at 22°C. [^{57}Co]cbl-labeled ligands were added last, and the membranes were incubated for 1 more hour at 22°C.

In vivo distribution of cobalamin

Fecal and urinary excretion. Male adult rats were force-fed 3.7 pmol of rat IF-[^{57}Co]cbl in phosphate-buffered saline mixed with 5 mg of either lectin or bovine serum albumin. Each animal was housed in a separate metabolic cage. Urine and feces were collected over a 72-hour period and counted. Feeding was carried out using a Pharmaseal R 31 feeding tube (15" long) connected to a 3-ml syringe. After the rats were fasted for 24 hours to ensure that their stomachs were empty, the volume administered was between 1 to 1.5 ml. In some instances, rats were given an intramuscular injection of cobalamin (7.4 nmol) 15 to 30 minutes following oral administration of the traces of IF-[^{57}Co]cbl.

Intestinal uptake of cobalamin

Intestinal uptake of [^{57}Co]cbl was followed after oral administration of [^{125}I] IF-[^{57}Co]cbl in the presence and absence of lectins (5 mg). Three hours following feeding, the animals were killed, the liver and kidney were perfused free of blood with 0.9% saline, and the intestine was washed. The intestinal mucosa was treated with 5 mM EDTA, pH 5.0, to remove surface-bound radioactivity. The mucosal radioactivity following this treatment represented the internalized IF-cbl.

Uptake into the internal organs after intraluminal intrinsic factor-cobalamin. Tissue uptake of [^{57}Co]cbl was also followed at various times, up to 24 hours. For these experiments, IF-[^{57}Co]cbl (3.7 pmol) alone or mixed with [^{125}I]-ConA was administered under light ether anesthesia into the lumen of the mid-gut. The gut loop was gently returned into the peritoneum and the incision was stapled. After regaining consciousness, the animals were allowed water and food until they were killed, after which the tissues were removed, cleaned with 0.9% saline, and blotted dry, and the radioactivity was estimated in a dual-channel Beckman Gamma 4000 counter.

One portion of each tissue was sonicated and centrifuged at 20,000 × *g* for 30 minutes and the supernatant fraction was precipitated with trichloroacetic acid (final concentration, 5%). This precipitate was assayed for radioactivity as an estimate of the amount of intact ConA absorbed into the tissues.

Uptake into internal organs after intracardiac cobalamin. The uptake of [^{57}Co]cbl into the liver and kidney was followed after giving ConA and phytohemagglutinin orally. Three hours later, [^{57}Co]cbl was administered by intracardiac injection. The animals were

killed 30 minutes and 24 hours following injection, at which times the liver and kidney were perfused (via the aorta) with normal saline until the venous effluent was clear, and radioactive [^{57}Co]cbl was measured as described above. Total urinary excretion of [^{57}Co]cbl was measured over the 24-hour period.

Statistics

All results are given as mean ± SD. Significance between different samples were assessed by the unpaired Student's *t* test.[27]

Results

Effect of lectins on in vitro cobalamin-protein–binding reactions

The gastrointestinal uptake of dietary cobalamin is mediated by two binding reactions; cobalamin by IF and IF-cbl by its intestinal receptor. We tested the effect of lectins on each of these binding reactions in vitro, because it was not possible to eliminate the presence of endogenous cobalamin and IF in vivo, both of which would interfere with accurate assessment of their binding reactions. The binding of equimolar [^{57}Co]cbl by IF (*Figure 1*, top panel) and of rat IF-[^{57}Co]cbl by rat ileal brush border membranes (*Figure 1*, middle panel) was not inhibited by ConA or other lectins. However, ConA increased the binding of IF-[^{57}Co]cbl to ileal membranes threefold (*Figure 1*, middle panel). This increase was Ca^{2+} dependent and rose sharply up to ConA concentration of 100 μg in the incubation mixture. Further increases in ConA concentration resulted in a sharp decline in the Ca^{2+}-dependent binding due to a large increase in Ca^{2+}-independent nonspecific binding of IF-[^{57}Co]cbl (*Figure 2*). The binding of [^{125}I]-Con A to the membranes was calcium independent (data not shown). The effects on brush border interactions were reversed by α-methyl mannoside and mannan, but not by *myo*-inositol (*Figure 1*, middle panel).

Following absorption, cobalamin bound to TC II is taken up by the TC II receptor located on the cell surface of various tissues. In the rat kidney, the binding of TC II-cbl occurred preferentially to basolateral

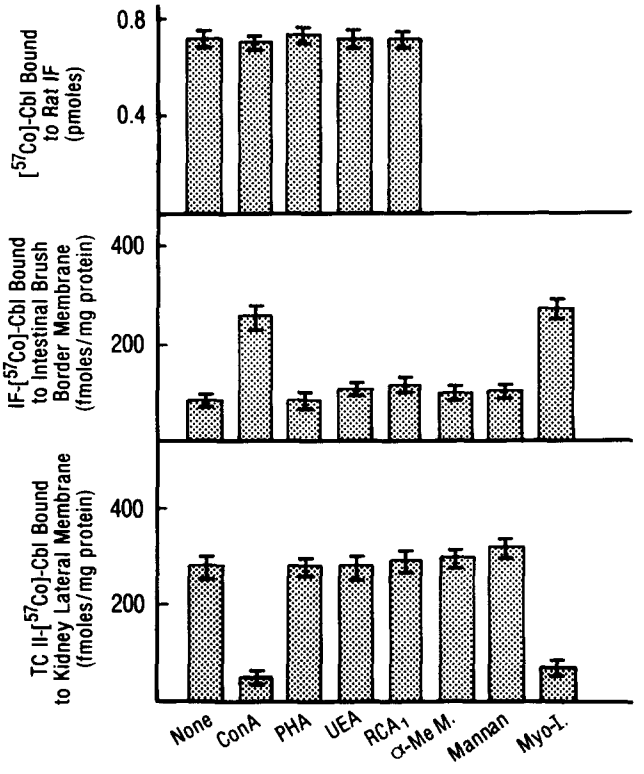

Figure 1 In vitro effect of lectins on cobalamin-binding protein interactions. (*Top panel*): Binding of cobalamin by IF. (*Middle panel*): Binding of IF-cbl by intestinal brush border membranes. (*Bottom panel*): Binding of TC II-cbl by kidney basolateral membranes. Diluted rat IF fractions were assayed in the presence of 50 μg lectins for the binding of [^{57}Co]cbl (0.74 pmol). The amount of IF added was determined to be sufficient to saturate cobalamin mole for mole. The bound and unbound cobalamin was separated, as discussed in the Materials and Methods section. Intestinal apical brush border and kidney basolateral membranes (300 μg) were incubated with or without 50 μg of lectins. Binding was assessed using 1 ng of IF-[^{57}Co]cbl or TC II-[^{57}Co]cbl, respectively. Membranes were preincubated with α-methyl mannoside (10 mM), mannan (1 mg), and *myo*-inositol (10 mM) for 30 minutes at room temperature before addition of the ligand. The results are the mean value ± SD of six assays using two separate membrane preparations.

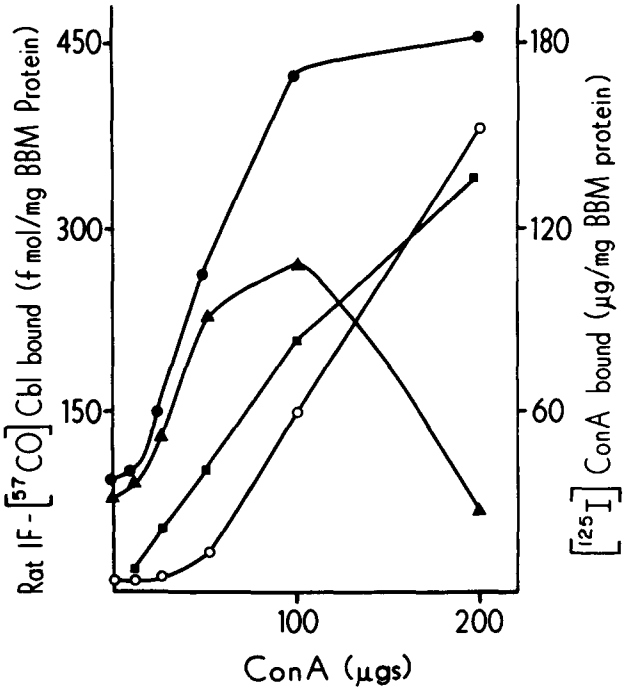

Figure 2 Effect of ConA concentration on the Ca^{2+} dependent and independent binding of rat IF-[^{57}Co]cbl to the ileal brush border membrane. Brush border membrane (250 to 300 μg protein) was preincubated with various amounts of [^{125}I]-ConA (specific activity, 7,500 dpm/μg) in a reaction mixture of 800 μl containing 10 mM Tris-HCl, pH 7.4, with 20 mM $CaCl_2$ or EDTA. After preincubation for 30 minutes, rat IF-[^{57}Co]cbl (0.74 pmol) was added and the reaction mixture was brought to 1 ml and incubated for 2 hours at 22°C. Other details are provided in the Materials and Methods section. The values represent an average of four assays using two separate membrane preparations. IF-[^{57}Co]cbl bound; total (●); Ca^{2+} independent (○); Ca^{2+} dependent (▲); ^{125}I-ConA bound (■).

compared with apical brush border membranes (data not shown). The binding of TC II-cbl to rat kidney basolateral membranes was inhibited only by ConA, but not by other lectins (*Figure 1*, bottom panel). This effect was reversed by α-methyl mannoside and mannan, but not by *myo*-inositol.

These in vitro results suggested that ConA, but not other lectins, might affect in vivo cobalamin uptake by tissues. To test the effect of ConA on transcellular transport across the enterocyte and the uptake of circulating cobalamin by the tissues, we administered to animals both lectins orally, and [^{57}Co]cbl orally and systemically.

Effect of lectins on the tissue uptake of [^{57}Co]cbl

Intestinal uptake. When lectins were mixed with IF-[^{57}Co]cbl and administered orally, there was no significant change in the amount of [^{57}Co]cbl internalized by the enterocytes (*Table 1*). Nearly 80% to 94% of IF-cbl was internalized within 3 hours in the presence of the six different lectins. The results suggest that ConA and other lectins, when administered in vivo, did not affect gastrointestinal uptake. Further confirmation of this result was obtained from the assessment of total body cobalamin absorption.

The amount of [^{57}Co]cbl absorbed (amount administered minus fecal excretion) in a 72-hour period was not significantly affected by the oral administration of ConA or phytohemagglutinin (PHA) (*Table 2*). When tissue uptake was prevented by saturating the body with cold cobalamin, the amount of [^{57}Co]cbl excreted in the urine was 4.2% of the absorbed dose. When the tissues were not first saturated with cobalamin, only 0.6% of the absorbed dose escaped tissue uptake and was excreted in the urine. With the addition of oral ConA, the urinary excretion of [^{57}Co]cbl rose from 0.6% to 1.6%. Excretion did not reach levels seen in the control animals, suggesting that ConA was not as effective an inhibitor of tissue uptake as unlabeled cobalamin. The effect of oral ConA on urinary excretion of cobalamin could be interpreted as the result of decreased tissue uptake of [^{57}Co]cbl. On the other hand, when PHA was used, urinary excretion of cobalamin was 0.8%, suggesting that tissue uptake of [^{57}Co]cbl was normal in the presence of this lectin. The [^{57}Co]cbl excreted in the urine of rats fed with the lectins is assumed to be due to lectin effect alone, as these rats were not treated with cold cobalamin earlier.

Table 1 Effect of lectins on the intestinal uptake of IF-[^{57}Co]cbl

Lectin added	[^{57}Co]cbl internalized (fmol/g mucosa)
None	80 ± 10
ConA	85 ± 5
WGA	90 ± 5
RCA1	94 ± 5
UEA	85 ± 5
PNA	79 ± 5
HPA	90 ± 4

Abbreviations: WGA, wheat germ agglutinin; RCA, *Ricinus communis*; UEA, Ulex europaeus; PNA, peanut agglutinin; HPA, helix-pomatia agglutinin

Lectins (5 mg) were mixed with rat IF-[^{57}Co]cbl (3.7 pmol) and administered orally through a feeding tube. The animals were killed 3 hours following oral administration. The intestine was washed with cold saline, and the intestine was cut into 5-cm lengths and opened longitudinally. The mucosal surface was exposed to 5 mM EDTA at pH 5.5 for 30 minutes at 22°C. The mucosa was then scraped and weighed, and the radioactivity was assessed. The results indicated are the mean values ± SD using five animals for each experiment

Table 2 Effect of concanavalin A on the gastrointestinal absorption and urinary excretion of [^{57}Co]cbl

Lectin added to orally administered IF-[^{57}Co]cbl	Total body absorption (% of administered)[b]	Urinary excretion (% of absorbed)[c]
None[a]	50 ± 5	4.2 ± 0.5
None	47 ± 5	0.6 ± 0.1
+ ConA	55 ± 3	1.6 ± 0.3 (0.001)
+ PHA	45 ± 5	0.8 ± 0.2 (0.01)

Rat IF-[^{57}Co]cbl (3.7 pmol) was orally administered with or without lectins as indicated

[a] Cold cobalamin (7.4 nmol) was administered intramuscularly 15 to 30 minutes following the oral dose

[b] Radioactivity administered minus fecal excretion in 72 hours

[c] Radioactivity in urine collected over 72 hours

The values represent the mean ± SD for four rats in each experiment. The numbers in parentheses represent probability values compared with rats fed with IF-[^{57}Co]cbl

Hepatic and Renal Uptake. When [^{125}I] IF-[^{57}Co]cbl was instilled in the intestinal lumen, hepatic accumulation of [^{57}Co]cbl reached a plateau in 12 hours, while uptake by the kidney continued to rise even at 24 hours (*Figure 3*). In both the liver and kidney, almost 75% of the Triton X-100 extracted ^{57}Co radioactivity was acid precipitable at all times, while over 90% of ^{125}I radioactivity in the tissue extracts was not precipitated with acid (data not shown). These results demonstrated a complete transfer of [^{57}Co]cbl to tissue proteins and that ^{125}[I]-IF was degraded, although the site of degradation (lumen or within the enterocyte) could not be determined. When cold ConA (containing tracer amounts of [^{125}I]-ConA) was mixed with the IF-[^{57}Co]cbl complex and orally administered to rats, there was a 65% to 75% decrease in the hepatic and renal levels of [^{57}Co]cbl (*Table 3*), and this inhibition was reversed by 24 hours (*Figure 3*). Concanavalin A was equally effective in causing lower (60% to 75%) levels of [^{57}Co]cbl in other tissues, such as the lungs, heart, muscle, brain, and spleen (data not shown). Analysis of [^{125}I]radioactivity in blood and kidney extracts revealed that it was precipitable by acid and probably represented intact ConA. Concentrations of ConA in blood and kidney were 5 μg/ml and 4 μg/g, respectively; no ConA was detected in the liver. When other lectins were orally administered with IF-[^{57}Co]cbl, there was no inhibition in the uptake of

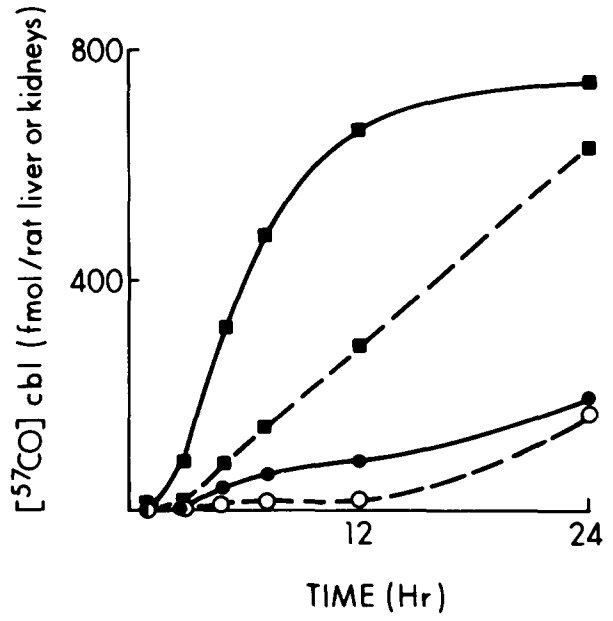

Figure 3 Effect of ConA on the uptake of [^{57}Co]cbl by the liver and kidney. [^{125}I]IF-[^{57}Co]cbl (3.74 pmol; 1 ml) containing 5 mg of either bovine serum albumin or ConA was instilled into the intestinal lumen near the midjejunum of control (—) and experimental (----) rats, respectively. At indicated time intervals, the rats were killed and their entire liver and both kidneys were removed and perfused with saline. The tissues were blotted dry, cut into small pieces, and radioactive [^{57}Co]cbl was assessed in the liver of control (●) and experimental (○) rats, and in the kidneys (■) of these rats.

Table 3 Effect of lectins on hepatic and renal levels of [^{57}Co]cbl

Lectin added to oral IF[^{57}Co]cbl	[^{57}Co]cbl (fmol/g)	
	Liver	Kidney
None	9 ±2	45 ± 5
ConA	3 ± 1[a]	12 ± 3[b]
PHA	12 ± 2	50 ± 2
RCA1	13 ± 1	49 ± 3
UEA	13 ± 3	50 ± 3

Lectins (5 mg) were mixed with rat IF-[^{57}Co]cbl (3.7 pmol), and administered orally through a feeding tube. The rats were anesthetized after 3 hours, the liver and kidney were perfused with 0.9% NaCl, and the radioactivity in the tissues was assessed as described in the Materials and Methods section. The results are expressed as the mean value ± SD for four rats

[a] $P \leq 0.05$

[b] $P < 0.01$ versus the group with no lectin administered

cobalamin by the liver and the kidney, examined 3 hours later (*Table 3*).

The observation that small amounts of ConA had been absorbed suggested that ConA might be inhibiting the tissue uptake of circulating [^{57}Co]cbl. To study tissue uptake of cobalamin more directly, ConA or PHA was administered orally and, 3 hours later, [^{57}Co]cbl was administered by intracardiac injection. Inhibition in the uptake by the liver and kidney was seen 30 minutes following the entry of [^{57}Co]cbl directly into the circulation (*Table 4*). After 24 hours, however, the levels in the kidney and liver and the urinary excretion of [^{57}Co]cbl were nearly the same for control and ConA-treated animals. Similar experiments with PHA did not reveal any change in the uptake of [^{57}Co]cbl by the liver and kidney at 30 minutes following cobalamin administration (*Table 4*).

Discussion

The eventual cellular utilization of dietary cobalamin is dependent on the luminal binding of cobalamin by IF, and IF-cbl by the receptor. This is followed by transepithelial transport of cobalamin bound to TC II. Cobalamin bound to TC II is delivered to the tissues that need cobalamin and TC II-cbl is taken up by a specific receptor located on the surface membranes of many cells. The present results show that ConA specifically lowers the tissue level of cobalamin at stages beyond the entry of cobalamin into the enterocytes. This conclusion is based on the following evidence: First, neither ConA nor any other lectin tested affected the binding of cobalamin by IF (*Figure 1*, top panel). Second, none of the lectins tested inhibited the binding of IF-cbl to the ileal receptor (*Figure 1*, middle panel), nor did they affect the enterocyte uptake of IF-cbl (*Table 1*). Total body cobalamin absorption was the same in both control and lectin-fed rats (*Table 2*). Thus, lectins do not seem to inhibit cobalamin absorption during the first 3 hours after ingestion of cobalamin. Third, ConA mediated decreased uptake of [^{57}Co]cbl by both the liver and kidney (*Figure 3*), and simultaneously increased urinary excretion (*Table 3*). Other lectins tested did not share this property (*Table 4*). Fourth, ConA administered orally blocked the uptake by liver and kidney of circulating [^{57}Co]cbl (*Table 4*). The reversal of this inhibition by 24 hours was probably due to clearance in the urine of the absorbed and circulating lectin. Absorption of [^{125}I]-ConA was found, confirming earlier reports,[21] and probably accounts for the fact that systemic ConA inhibits cobalamin tissue uptake, thereby increasing urinary excretion (*Table 2*). Absorption of [^{125}I]-IF by guinea pig intestine has been proposed based on autoradiographic localization of ^{125}[I] radioactivity over the infoldings of the lateral membrane.[28] In our studies, we were unable to precipitate ^{125}[I] radioactivity in the tissue extracts by either acid or antibody to IF. These results suggested that [^{125}I]IF is degraded even though the precise site of degradation cannot be determined.

In our studies, we chose to administer cobalamin in the form of Cyano-cbl (CN-cbl), although the nature of the modification of CN-cbl during its transit through the enterocyte and other tissues is not known. Moreover, Kolhouse and Allen[29] have shown that tissue dissemination of cobalamin analogs occurs by more than one mechanism, and excretion in urine is dependent both on the structure of cobalamin and on the nature of the proteins to which CN[^{57}Co]cbl is bound (R type versus TC II). Thus, the magnitude of the effects of ConA on tissue uptake in the first few hours and on urinary excretion at 24 hours and beyond are difficult to com-

Table 4 Effect of intragastric administration of lectins on the uptake of circulating [^{57}Co]cbl

Lectin fed	Time after cobalamin injection (hr)	Tissue uptake (fmol/gm) Liver	Kidney	*P* value	Urinary excretion (% administered dose)
None		25 ± 2	80 ± 5		—
ConA	0.5	4 ± 1	12 ± 3	<0.01	—
PHA		35 ± 4	92 ± 5	NS	—
None		45 ± 5	447 ± 20		4 ± 1
ConA	24	37 ± 3	400 ± 15	NS	3.7 ± 0.3
PHA		47 ± 5	395 ± 17	NS	4.2 ± 0.7

ConA or PHA (5 mg) was administered orally through a feeding tube. Three hours later, [^{57}Co]cbl (3.7 pmol) was injected into the left ventricle. The rats were killed 30 minutes and 24 hours later, liver and kidney were perfused, and the radioactivity was measured as described in the Materials and Methods section. The results are the mean value ± SD of three rats. The lectin-fed rats were compared with nonfed animals for statistical evaluation

pare, because tissue uptake and urinary excretion may be influenced by differences in the structure and binding of plasma cobalamin. The role of ConA in decreasing tissue levels of cobalamin in the first few hours, however, was confirmed by in vitro inhibition of TC-II[^{57}Co]cbl binding to basolateral membranes (*Figure 1*, bottom panel). This effect is probably due to blocking of some of the mannose residues present on the TC II receptor,[8] as this effect was reversed by α-methyl mannoside and mannan. Concanavalin A has also been demonstrated to bind to the inositol moiety of membrane phospholipids in both native and artificial membranes.[30] However, the inability of inositol to block the effects of ConA on the binding of TC II-cbl and IF-cbl to kidney and ileal membranes suggests that inositol is not involved in this particular ligand-binding event.

At lower concentrations of ConA, the calcium-dependent IF-cbl receptor binds more IF-cbl (*Figure 1*, middle panel). The enhanced binding of IF-cbl to isolated ileal membranes was blocked by preincubation with α-methyl mannoside and mannan, suggesting that ConA was bound to membrane surface sugars and that this site and the IF-cbl receptor might be located near one another on the ileal membrane surface. While the binding of ConA to brush border membranes is Ca^{2+} independent, much of the enhanced binding of IF-[^{57}Co]cbl at lower ConA concentrations is dependent on Ca^{2+} (*Figure 2*). Once saturation of the receptor by IF-cbl has occurred, additional IF-cbl binds to the membrane independent of Ca^{+}. This nonspecific Ca^{2+}-independent binding may be due to IF-cbl-ConA complex bound artificially to the membrane via ConA. Whatever the mechanism of enhanced binding, it does not result in enhanced uptake by enterocytes (*Table 1*), and thus seems not to be mediated by a direct effect on the IF-cbl receptor.

In conclusion, our results show that a single dose of ConA administered orally decreased the [^{57}Co]cbl levels in the kidney and liver, irrespective of the route of administration of [^{57}Co]cbl (intragastric or systemic). The decrease was observed for several hours and was reversed by 24 hours, and was due at least in part to delayed transit through the enterocytes and in part to blocking of TC II-cbl binding to the TC II receptor. It is intriguing to wonder whether the cobalamin deficiency seen in adult ovo-lacto vegetarians might be hastened by a lectin-mediated inability to use small amounts of dietary cobalamin.

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