Intrinsic factor receptor activity and cobalamin transport in bile duct-ligated rats

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Seetharam, Shakuntla, Kalathur S. Ramanujam, and Bellur Seetharam. Intrinsic factor receptor activity and cobalamin transport in bile duct-ligated rats. Am. J. Physiol. 262 (Gastrointest. Liver Physiol. 25): G210–G215, 1992.—The intrinsic factor (IF)-cobalamin (Cbl) receptor activity in the mucosal homogenates progressively decreased after bile duct ligation in the rat, and 80% of the receptor activity was decreased in 96 h after ligation. The activity was restored to normal values of 5.5–6 pmol of IF-[57Co]Cbl bound/g mucosa when the assays were performed with both conjugated and unconjugated bile acids. When [57Co]Cbl bound to intrinsic factor was orally administered, the tissue levels of [57Co]Cbl were decreased by 75–80% in bile duct-ligated rats. The apical membrane receptor activity was also decreased after bile duct ligation; however, the activity was stimulated twofold by the addition of ileal cytosol and threefold with the addition of both ileal cytosol and taurocholate (1 mM). Enhanced binding of IF-[57Co]Cbl to the apical ileal brush-border membrane occurred with the use of dialyzed ileal cytosol but not with cytosol isolated from duodenal or proximal jejunal mucosa. The enhanced binding obtained with ileal cytosol was abolished after its treatment with trypsin. These results suggest that luminal bile acids optimize the binding of IF-Cbl by the ileal membrane receptor via interactions with a cytosolic factor and thus influence the gastrointestinal absorption of cobalamin.

absorption; cytosolic factor; brush border membrane

THERE IS EVIDENCE based on studies using patients with obstructive jaundice and T-tube drainage (34) and bile fistula rats (4) that luminal bile is important in the intrinsic factor (IF)-mediated ileal absorption of cobalamin (Cbl). However, the exact step at which bile or bile acids regulate Cbl absorption is not known. The initial event in the gastrointestinal absorption of Cbl is the complexing of dietary Cbl by IF in the intestinal lumen (25). Previous in vitro studies (32, 33) have reported that bile and bile acids inhibit Cbl binding by IF. However, the inhibition noted has been shown to be due to the displacement of Cbl from IF by haptocorrin (R-type Cbl binder), which is present in both human bile and commercially available bile acids (28). Thus absence of bile in the intestinal lumen should enhance the amount of [57Co]Cbl bound to IF due to the absence of biliary R-type of Cbl binder. Recent studies (6) using bile fistula rats have shown this to be true. Because R-type Cbl binder could not be detected in rat gastric juice by gel filtration, this study concluded that in bile fistula rats enhanced binding of [57Co]Cbl by IF in the intestinal juice was unrelated to the absence of R-type Cbl binder. Despite the absence of R-type Cbl binder in rat bile, the reason for the high percentage of [57Co]Cbl bound to IF in the intestinal washings from the bile fistula rats is not known.

The actual process of Cbl absorption in adult rats is initiated by the complexing of IF-Cbl to the ileal receptor (21). Earlier studies have suggested that the binding of IF-[57Co]Cbl to crude ileal membranes (9) or ileal extracts (12) was increased by bile acids or bile although the effect of bile on the binding of IF-[57Co]Cbl to ileal extract was very marginal (12). Based on these studies, it was suggested that absence of bile in the intestinal lumen could affect the binding of IF-Cbl by the ileal receptor and thus could affect the absorption of Cbl.

The current study was undertaken to address the above hypothesis. Our studies using bile duct-ligated (BDL) rats show that ileal mucosal homogenate and apical membrane receptor activity declined by 80% and 60%, respectively. The intestinal transport of [57Co]Cbl to the tissues was also inhibited when IF-[57Co]Cbl was orally administered to BDL rats. The addition of bile salts to the mucosal homogenates but not to the apical membranes restored the receptor activity to normal levels. However, the addition of ileal cytosol and taurocholate to the apical membrane enhanced the binding of IF-[57Co]Cbl. We suggest that absence of luminal bile acids creates cryptic IF-Cbl binding sites on the membrane surface causing decreased binding and hence uptake of IF-[57Co]Cbl by the enterocytes resulting in an intraluminal accumulation of IF-[57Co]Cbl. The binding of bile acids to a cytosolic factor may unmask the IF-Cbl binding sites on the membrane and thus enhance the binding of IF-[57Co]Cbl to the membrane surface receptor.

MATERIAL AND METHODS

The following chemicals were purchased commercially as indicated: [57Co]cyanocobalamin (15 µCi/µg, Amersham, Arlington Heights, IL) and bile acids (Sigma Chemical, St. Louis, MO). Pure rat gastric IF was prepared as described earlier (27). Binding of rat IF [57Co]Cbl to the ileal homogenates or apical membranes in the presence of bile acids (1 mM) or cytosolic fractions (500 µg protein) was determined as described earlier (26).

Briefly, ileal homogenates (1–2 mg protein) or apical brush-border membrane (500–600 µg protein) was incubated for 1 h at 22°C in a total volume of 1 ml containing (in µmol) 10 tris(hydroxymethyl)aminomethane (Tris)-HCl at pH 7.4, 10 CaCl2, or 10 EDTA, as well as 2 pmol rat IF-[57Co]Cbl and additions as indicated above. The Ca2+-specific binding of IF-[57Co]Cbl was calculated by subtracting the amount bound in the presence of EDTA from the amount bound in the presence of CaCl2. In some assays, the apical membranes were washed with EDTA at pH 5 to remove endogenous IF-Cbl, because the binding of IF-Cbl occurs optimally at pH 7.4 and requires Ca2+.

Male adult rats (150–175 g) were used for bile duct ligation. The common bile duct was ligated under anesthesia essentially according to Kaplan and Righetti (14). Briefly, adult male rats were lightly anesthetized, subjected to a midline incision, and the common bile duct was isolated and tied off as close to the liver as possible to minimize damage to the pancreatic ducts. After surgery, the animals were given free access to food and...
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water. The animals were alert and feeding normally 12-24 h after the operation. To ascertain the absence of any damage to the pancreatic secretions, the total proteolytic activity in the luminal washings (0.9% NaCl) was determined by assessing the hydrolysis of casein at pH 7.4 as described earlier (17). Some rats were sham operated with laparotomy and the internal manipulation that were required with the BDL rats. At various times after bile duct ligation in some rats, the entire length of the intestine was removed and the lumen was washed with ice-cold saline. Mucosa was scraped with a glass slide from duodenum, proximal jejunum, distal jejunum, and ileum. The mucosa was homogenized using (in mM) 50 mannitol, 2 HCl buffer at pH 7.4 containing 140 NaCl, 0.1 phenylmethylsulfonyl fluoride (PMSE), and 2 benzamidine, as well as 0.3 aprotonin (buffer A). Apical brush-border and internal membranes from control and BDL rats (96 h) were isolated by the Ca2+-aggregation method of Schmitz et al. (24) as modified by Kessler et al. (15) and suspended in buffer A. This method has been validated in the rat intestine (7) to quickly separate the apical and internal membranes by differential centrifugation of the CaCl2-treated homogenates. The Ca2+-precipitate obtained at low-speed centrifugation contained the intracellular membranes, predominantly endoplasmic reticulum and/or Golgi. The apical membranes were enriched for alkaline phosphatase, a brush-border marker by 20- to 25-fold with a recovery between 25 and 30%. The cytosolic fraction from different anatomic regions of the intestine from BDL (96 h) or control rats was prepared by centrifuging a 10% (wt/vol) homogenate prepared in sucrose (0.25 M) and EDTA (1 mm) for 1 h at 105,000 g. The clear supernatant below the top fluffy lipid layer was carefully removed.

The IF-[57Co]Cbl-binding activity in the mucosal homogenates or isolated apical membranes was determined as follows. An aliquot of mucosal homogenate (1-1.2 mg protein) was first preincubated with bile acids (1 mM) for 30 min at 22°C before the addition of rat IF-[57Co]Cbl (2 pmol). The effect of cytosol (500-600 μg protein) on the binding of IF-[57Co]Cbl (2 pmol) to the apical brush-border membrane (500-600 μg protein) was carried out first by a preincubation of the apical membranes with the cytosol in the presence or absence of taurocholate (1 mM) for 30 min at 22°C. The binding reaction was initiated with the addition of rat IF-[57Co]Cbl (2 pmol) and allowed to incubate for an additional 60 min at 22°C. For some experiments, the dialyzed cytosol used was obtained after dialysis of cytosol (20 ml) against 10 ml of 10 mM Tris-HCl buffer pH 7.4 for 24 h with a 6-liter change after 12 h. For some assays, the cytosol treated with tosyl-phenylalanine chloromethyl ketone (TPCK)-trypsin (1 mg cytosol protein with 0.1 mg TPCK-trypsin) for 4 h at 37°C was used. The trypsin digestion of the cytosol was terminated by addition of a mixture of protease inhibitors. The final concentration of these inhibitors was 0.1 mM for PMSE and 1 mM for benzamidine.

The transport of [57Co]Cbl to the tissues in control and BDL (96 h) rats was carried out as described earlier (21). Briefly IF-[57Co]Cbl (3.7 pmol) was directly instilled in the stomach of rats through a feeding tube. After 1 or 4 h following the instillation of the label, the animals were killed. The intestine was carefully removed from the pylorus to the ileocecal end, cut into 10 equal segments, and counted for [57Co]Cbl. The liver and kidneys were removed, rinsed with ice-cold saline, blotted dry, weighed, cut into small pieces, and counted for [57Co]Cbl using Beckman gamma 4,000 counter. Alkaline phosphatase in the mucosal homogenates and the isolated apical brush-border membrane was assayed using p-nitrophenylphosphate (PNPP) as a substrate (11) (expressed as pmol of PNPP hydrolyzed·min-1·mg protein-1). The protein concentrations in the various fractions were determined according to Lowry et al. (18) using bovine serum albumin as standard.

RESULTS

Effect of bile duct ligation on luminal protease and mucosal receptor activity. The luminal proteolytic activity was measured in the washings from the sham-operated and the BDL rats to confirm that the ligation had not affected pancreatic secretions into the lumen. Total proteolytic activity expressed as trypsin varied from animal to animal but was 75-95 μg/ml in sham-operated rats and 65-110 μg/ml in rats 4 days after BDL. In the BDL rats, the mucosal receptor activity sharply declined to 50% of the control value in ~24 h, and further decline (75-80%) of the activity occurred slowly for up to 96 h (Fig. 1). In contrast, the alkaline phosphatase activity rose by 100% in 12 h and declined to 50% of the control value 96 h after the ligation (Fig. 1). Because the maximum loss of the receptor activity occurred between 72 and 96 h after bile duct ligation, all subsequent studies were performed using rats whose bile duct was ligated 96 h before the experiments.

Effect of bile duct ligation on tissue levels of IF-[57Co]Cbl. In BDL rats, 4 h after the instillation of IF-[57Co]Cbl in the stomach, the transport of [57Co]Cbl in the liver and kidney was lowered by 80% and 90% of the values from sham-operated rats (Table 1). The transport of [57Co]Cbl to the tissues depends on the uptake of IF-[57Co]Cbl by the enterocytes. For this to occur, the IF-[57Co]Cbl

<table>
<thead>
<tr>
<th>Time (h) After Bile Duct Ligation</th>
<th>Rati0-IF-[57Co]Cbl Bound (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>48</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>72</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>96</td>
<td>0.2 ± 0.05</td>
</tr>
</tbody>
</table>

Alkaline Phosphatase (unitised pmol) 

Table 1. Effect of bile duct ligation on transport of [57Co]Cbl

<table>
<thead>
<tr>
<th>Transported to</th>
<th>fmol/g Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>45±8</td>
</tr>
<tr>
<td>Kidney</td>
<td>195±15</td>
</tr>
</tbody>
</table>

Values reported represent means ± SD from 6 control and 5 bile duct-ligated (BDL) rats. Rat intrinsic factor (IF)-[57Co] cobalamin (Cbl) (3.7 pmol) was instilled into stomachs of BDL and sham-operated rats. Animals were killed 4 h later, and the indicated tissues were removed. Transport of [57Co]Cbl to the liver and kidney was measured. Other details on the tissue removal and radioactivity measurements are provided in METHODS.
administered orally must travel to the sites of maximum absorption along the horizontal axis of the small intestine. When \(^{57}\text{Co}\)Cbl radioactivity was measured in unwashed segments of the intestine after 1 h (Fig. 2, top) or 4 h (Fig. 2, bottom) of oral administration of IF-\(^{57}\text{Co}\)Cbl, the relative distribution of the radioactivity in the BDL rats and sham-operated rats along the horizontal axis of the gut was the same. However, the amount of \(^{57}\text{Co}\)Cbl/segment was higher at both 1 h and 4 h in BDL rats, and in 4 h the \(^{57}\text{Co}\)Cbl/g intestine was 270 and 110 fmol/g in BDL and sham-operated rats, respectively. By 4 h the maximum \(^{57}\text{Co}\)Cbl was observed in the seventh segment and in BDL rats a nearly 2–2.5 times higher amount of \(^{57}\text{Co}\)Cbl was present in segments 7–10. These results suggested that BDL had not affected either the gastric emptying or the transit of IF-\(^{57}\text{Co}\)Cbl through the intestinal lumen. The disproportionately higher amounts of \(^{57}\text{Co}\)Cbl in the BDL rats relative to sham-operated rats suggested that binding of IF-\(^{57}\text{Co}\)Cbl to the mucosal receptor might be affected, thus causing decreased uptake and transport of \(^{57}\text{Co}\)Cbl to the tissues. Therefore, the effect of bile duct ligation on the IF-\(^{57}\text{Co}\)Cbl binding to the isolated apical brush border was tested.

**IF-Cbl receptor activity in brush-border membranes.**

The apical brush-border membrane of control and BDL rats bound between 140 and 150 and 55 and 60 fmol of IF-\(^{57}\text{Co}\)Cbl/mg protein, respectively. The recovery of receptor activity expressed as a percent of total homogenate receptor activity in the internal membrane (Table 2, parentheses) was the same (±60%) for both control and BDL rats. However, the recovery of the receptor activity in the apical membranes from BDL rats was only 8%, whereas in the membranes from sham-operated rats it was 15–18%. When the apical membranes were

**Table 2. Effect of bile duct ligation on distribution of receptor activity between apical and internal membranes**

<table>
<thead>
<tr>
<th>Membranes</th>
<th>IF-(^{57}\text{Co})Cbl Bound, fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Apical brush border</td>
<td>14±10 (18%)</td>
</tr>
<tr>
<td>Internal membranes</td>
<td>40±10 (60%)</td>
</tr>
<tr>
<td>EDTA pH 5 washed apical membranes</td>
<td>155±7</td>
</tr>
</tbody>
</table>

Values reported represent means ± SD of 4 control and 6 bile duct-ligated (BDL) animals. Values in parentheses indicate receptor activity as a fraction of total activity present in homogenate. Apical and internal membrane isolation and IF-\(^{57}\text{Co}\)Cbl binding to these membranes were carried out as described under METHODS.

**Table 3. Effect of bile acids on binding of IF-\(^{57}\text{Co}\)Cbl**

<table>
<thead>
<tr>
<th>Bile Acid, 1 mM</th>
<th>Mucoosal Homogenate, pmol/g</th>
<th>Brush Border Membrane, pmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>42±b</td>
<td>45±b</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>5.9±0.5</td>
<td>47±6</td>
</tr>
<tr>
<td>Cholate</td>
<td>5.6±0.3</td>
<td>49±6</td>
</tr>
<tr>
<td>Glycolate</td>
<td>5.0±0.4</td>
<td>52±7</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>5.9±0.4</td>
<td>54±5</td>
</tr>
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</table>

Values reported represent means ± SD of duplicate assays using fractions from 5 bile duct-ligated (BDL) rats. Brush-border membrane (500–600 µg protein) or crude homogenate (100–150 µg protein) from BDL rats were used for the binding of intrinsic factor (IF)-\(^{57}\text{Co}\)Cbl homogenates (Cbl) (2 pmol). Bile acids (1 mM) were used as sodium salt. washed with EDTA at pH 5, the binding of exogenously added IF-\(^{57}\text{Co}\)Cbl did not change significantly. These results suggested that the low receptor activity in the apical membranes from BDL rats was not due to increased amounts of endogenous IF-Cbl bound to it, but somehow IF-\(^{57}\text{Co}\)Cbl binding was affected in the absence of endogenous bile. Thus the receptor activity in the mucosal homogenates and the apical brush border was assayed in the presence of various bile acids.

**Effect of bile acids on binding of IF-\(^{57}\text{Co}\)Cbl to mucosal homogenates and isolated apical brush border from BDL rats.**

The addition of bile acids to the assay system increased the binding of IF-\(^{57}\text{Co}\)Cbl to the mucosal homogenates from 0.8 to 3–6 pmol/g mucosa (Table 3). All the bile acids tested were equally effective. The activity restored with taurochenodeoxycholate was very close to the activity of 6 pmol/g noted in the absence and between 6 and 7 pmol/g noted in the presence of bile salts noted using homogenates from the sham-operated animals (data not shown). None of the bile acids tested had any effect on the binding of IF-\(^{57}\text{Co}\)Cbl by the brush borders. These results indicated that the bile salt-mediated binding of IF-\(^{57}\text{Co}\)Cbl binding to the homogenates from BDL rats may be mediated by a cytosolic factor that is present during the assay for the receptor using the mucosal homogenates but not using the apical membranes. This hypothesis was tested directly by a series of in vitro reconstitution experiments using cytosol fraction isolated from homogenates prepared using mucosa from various regions of the gut.

Fig. 2. Distribution of intrinsic factor (IF)-\(^{57}\text{Co}\)cobalamin (Cbl) in gut in normal and bile duct-ligated rats. Rat IF-\(^{57}\text{Co}\)Cbl was instilled into the stomachs of bile duct-ligated and sham-operated rats. Animals were killed 1 (top) and 4 h (bottom) later, the intestine was removed from the pyloric to the ileocecal end, cut into 10 equal segments, and the radioactivity was counted. Values represent means ± SD from 6 normal (●) and bile duct ligated for 96 h (■) rats.
Effect of cytosolic factor on taurocholate-mediated binding of IF-$^{57}$CoCbl. The binding of IF-$^{57}$CoCbl to the ileal brush-border membrane isolated from BDL rats was not affected by the addition of taurocholate (Fig. 3). When the ileal cytosolic protein in the ratio (1:1) of membrane protein was added, taurocholate increased the binding by nearly threefold when the source of the cytosol was either ileal jejunum or ileum. Taurocholate-mediated binding of IF-$^{57}$CoCbl (180 fmol/mg protein) was very close to control values (150 fmol/mg protein) with the cytosolic fraction obtained from ileum but not from either duodenum or proximal jejunum. The cytosolic fraction from distal jejunum was moderately effective in the presence of taurocholate. When the ileal cytosol was dialyzed, its ability to enhance the binding of IF-$^{57}$CoCbl was unaffected. However, trypsin treatment abolished the activation of IF-$^{57}$CoCbl binding whether taurocholate was present or not (Fig. 3). Rat ileal cytosol used for reconstitution with the apical membranes had intrinsic $^{57}$CoCbl-binding activity of only 40–50 fmol/ml, an amount too small to contribute to the enhanced binding noted with taurocholate and this fraction. The replacement of taurocholate with other detergents such as Triton X-100 (0.2–1%), NP-40 (1%), and sodium dodecyl sulfate (0.5%) was not effective (data not shown). Ileal cytosol from BDL rats was equally effective as ileal cytosol from sham-operated animals in restoring the receptor activity. (Fig. 3).

**DISCUSSION**

The gastrointestinal absorption and transport of dietary and biliary Cbl are extremely complex events involving several binding and transfer reactions in the lumen of the gut, on the surface membrane, and within the absorptive enterocytes (25). The luminal factors that influence the absorption of Cbl include the pancreatic proteases (1, 2) and bile (34). Although the mechanism by which the pancreatic proteases mediate Cbl absorption is known (1, 19), the exact step at which bile or components of bile regulate Cbl absorption is not clear. Studies by Bergesen et al. (3, 5) have shown that Cbl malabsorption in rats with bile fistula is not due to bacterial overgrowth or to impairment of epithelial function or mucosal hypoplasia. One study (19) has indicated that bile acids may act as a “releasing factor” to release Cbl from IF. However, it is important to note that IF-Cbl is internalized via its receptor (13, 20–22, 30) and the release of Cbl occurs within the enterocyte. Moreover, the release of Cbl from IF in vitro by bile or bile acids is due to the presence of haptocorrin (R-type Cbl binder), which is present in both bile and commercially available bile acids (28). Thus it is highly unlikely that bile acids have a role in the intraluminal or intracellular release of Cbl from IF.

Earlier studies (9, 12, 28) have suggested that bile acids may have a role in modulating IF-Cbl binding by the receptor, an important step before the entry of IF-Cbl into the cell and the exit of Cbl from the enterocytes. The mechanism by which the binding is affected is not known. The current studies have addressed this issue using rats whose bile ducts were ligated. In this model, the pancreatic function was not affected, because the secretion of proteolytic activity into the lumen of the gut was not decreased. In the current study, we chose to use bile-obliterated rats rather than bile fistula rats. One disadvantage with this model is that, although bile acid levels are decreased on the luminal side of the enterocytes and in the mucosa, their levels in other tissues are increased. Despite this shortcoming, the bile-obliterated rats have been extensively used to study the effect of bile on intestinal mucosal enzyme activity levels (8, 16, 31).

The data presented here demonstrate that the absence of luminal bile resulted in decreased binding of IF-$^{57}$CoCbl to the surface membrane receptor (Table 1) and the noted Cbl transport to the tissues (Table 2) may be related to this inhibition. The bile acids in the presence of a ileal cytosolic factor may unmask the ligand (IF-Cbl) binding sites of membrane-bound receptor. Several lines of evidence support such a conclusion. First, BDL caused a progressive loss of the receptor activity in the ileum (13, 20–22, 30) and the binding of Cbl to the apical membrane and hence in increased amounts to the surface receptor. The recovery of the receptor in the internal membranes from both control and BDL rats was the same suggesting that absence of bile acids did not result in decreased amount of receptor delivered to the apical membrane and hence in the accumulation of intracellular receptor (Table 2). Second, bile acids activated IF-$^{57}$CoCbl binding to the mucosal homogenates but not to the apical membrane. This observation is in sharp contrast to our earlier ob-

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servations in dogs that in vitro bile acids activated the binding of hog IF-[57Co]Cbl to the canine ileal membrane. The reason for the noted in vitro differential effect of bile acids on the binding of IF-[57Co]Cbl to the ileal membrane receptor from the rat and dog is not apparent. However, it is possible that the difference is real and is related to the fact that canine and rat IFCR do differ in many properties (27, 29). Third, absence of luminal bile either due to bile obstruction or bile diversion (6) resulted in higher amount of [57Co]Cbl bound to intestinal washings. Because BDL did not affect either the gastric-emptying or intestinal transit time of IF-[57Co]Cbl (Fig. 3), the most logical explanation for the higher level of IF-[57Co]Cbl recovered in the intestinal washings is that there is decreased binding of IF-[57Co]Cbl to the receptor. Finally, the amount of IF-[57Co]Cbl bound to the ileal membrane was ~180 fmol/mg protein in the presence of both taurocholate and ileal cytosol (Fig. 3). This value is close to the value of 150 fmol of IF-[57Co]Cbl bound/mg protein using the apical membranes from sham-operated rats.

Although the nature of cytosolic factor that optimizes IF-[57Co]Cbl binding to the apical membranes isolated from BDL rats is not known, it is nondialyzable, trypsin sensitive, and its expression is regulated along the horizontal axis of the gut (Fig. 3). Another cytosolic protein whose expression has recently been shown to be limited in the ileum is the lipid/bile acid binder (23). It is interesting to speculate whether functional topography of the IF-Cbl receptor is influenced by the binding of bile acids to its carrier proteins. The cytosolic fraction used in this study bound very small amounts [57Co]Cbl (40–50 fmol/ml), an amount not sufficient to contribute to the noted effect of cytosol in enhancing the binding of exogenously added IF-[57Co]Cbl. Thus the observed increase in the binding of IF-[57Co]Cbl by the membrane receptor in the presence of ileal cytosol is not an artifact of the assay. Moreover, in the assay system all the [57Co]-Cbl was bound to IF and the amount (2 pmol) of IF-[57Co]Cbl was enough to saturate the receptor (data not shown). Although the ileal cytosol by itself enhanced the binding of IF-[57Co]Cbl by nearly twofold, the addition of taurocholate had an additional effect. Thus it seems that both the bile salts and the cytosolic factor are needed for the optimal binding of IF-[57Co]Cbl by the membrane receptor. How this is accomplished in vivo is not known. This study is another example of the effect of bile acids in modulating the activity of intestinal membrane proteins. Bile acids have been shown to affect the activity of several intestinal apical membrane enzymes (8, 16, 31). In some instances, such as γ-glutamyltranspeptidase, the bile acids modulate the activity by directly acting on the enzyme molecule (10). We have shown previously (28) that bile acids enhanced the binding of IF-[57Co]Cbl to the canine ileal membrane but not to the pure receptor. Thus the role of bile acids on the receptor activity is a membrane event and could have implications in the normal absorption of Cbl.

In conclusion, the results of the present study show that in the absence of luminal bile orally administered IF-[57Co]Cbl accumulates in the intestinal lumen because of decreased binding to the surface receptor resulting in decreased transport of [57Co]Cbl to the tissues. Although these studies suggest a role for the luminal bile in optimizing IF-Cbl and its receptor interactions, further studies are needed to define the role of cytosolic protein and bile acids in such an interaction.

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