Isolation and Characterization of the Ileal Receptor for Intrinsic Factor-Cobalamin*

(Received for publication, September 2, 1980, and in revised form, December 19, 1980)

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The receptor for intrinsic factor-cobalamin (vitamin B12) has been purified 65,000-fold from 2.5 kg of canine ileal mucosa with a recovery of 26%. The initial purification steps involved solubilization of ileal mucosal homogenates with Triton X-100, followed by ethanolic precipitation and dialysis. The soluble receptor was then saturated with hog intrinsic factor-cobalamin and the receptor-hog intrinsic factor-cobalamin complex was adsorbed to anti-hog intrinsic factor-Sepharose. After extensive washing, the receptor was eluted with 5 mM EDTA at pH 5.0, leaving behind the hog intrinsic factor-cobalamin that remained bound to the anti-hog intrinsic factor-Sepharose.

Gel filtration on Bio-Gel A-5m with 0.1% Triton X-100 gave two peaks of receptor activity with apparent molecular weights of 7,500,000 and 5,000,000 indicating that the receptor aggregates under these conditions. Polyacrylamide disc gel electrophoresis with 0.1% Triton X-100 gave a single protein band that barely entered 4% gels, did not stain for carbohydrate, and coincided with the presence of intrinsic factor-cobalamin-binding ability. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate gave one major band with an apparent molecular weight of 180,000 which upon reduction with 2-mercaptoethanol gave two bands with apparent molecular weights of 59,000 and 42,000. Amino acid analysis indicated the presence of 55% hydrophobic amino acids, less than 1% amino sugars, and gave a value of 222,000 g of amino acid/mol of intrinsic factor-cobalamin-binding ability.

The purified receptor showed specific binding with human, hog, and canine intrinsic factor-cobalamin (Kd = 1 to 4 nM) but not with free cobalamin, free intrinsic factor, or R protein-cobalamin. Antibodies to the receptor have been raised in rabbits.

Cobalamin (vitamin B12) is synthesized only by microorganisms, but is required by man and other animals as an essential cofactor for several enzymatic reactions (1–3). Cobalamin is bound predominantly by R protein in gastric juice and becomes bound to intrinsic factor after the R protein moiety is partially degraded by pancreatic proteases in the jejunum (4–6). The intrinsic factor-Cbl complex then binds to receptors that are present on ileal brush borders, but not on jejunal brush borders (7–11). These receptors are specific for the intrinsic factor-Cbl complex and do not bind free Cbl, free intrinsic factor, or other protein-Cbl complexes (8). The binding of intrinsic factor-Cbl to these receptors markedly facilitates the absorption of Cbl although little is known about the subsequent steps in the absorptive process (12).

Ileal receptors for intrinsic factor-Cbl have been solubilized and partially purified but their characterization has been hindered because of the extremely small amount of receptor present in the ileum (4, 12–16). We have shown previously (8) that canine ileal mucosa has a somewhat higher number of intrinsic factor-Cbl receptors than most other species. We now report the successful purification of the intrinsic factor-Cbl receptor from this source using a newly developed immunofinity chromatography technique. This technique utilizes rabbit anti-hog intrinsic factor-Sepharose to bind the receptor-hog intrinsic factor-Cbl complex with the subsequent elution of the receptor alone, at pH 5.0, in the presence of EDTA. The receptor thus obtained is highly pure and this has enabled us to characterize a number of its physical properties.

EXPERIMENTAL PROCEDURES

Materials

CN-[15Co]Cbl and Na-125 were obtained from Amersham Corp., Arlington Heights, IL. Sepharose-4B, horse spleen ferritin, Escherichia coli B-galactosidase, beef liver catalase, human IgG, and ovalbumin were obtained from Sigma Chemical Co., St Louis, MO. Bio-Gel P-30, 100–200 mesh, Bio-Gel A-5m, 200–400 mesh, and Enzymobeads were obtained from Bio-Rad Laboratories, Richmond, CA. Hog intrinsic factor (17), human intrinsic factor (18), an abnormal human intrinsic factor (19), and hog R protein (17) were obtained as described previously. Canine intrinsic factor was purified from canine stomachs using a procedure similar to that employed for the purification of human intrinsic factor (18). Antibodies to various species of intrinsic factor were raised in rabbits and covalently coupled to Sepharose-4B as described previously (20). The equivalent of 2 ml of antiserum were coupled per ml of Sepharose.

Methods

Receptor Assays—Membrane-bound receptor present in canine...
ileal homogenates was assayed as described previously (8). Soluble receptor was assayed by a modification of the method of Cotter and Rothenberg (13). Test tubes contained the following in a final volume of 1.0 ml: Tris-HCl, pH 7.5, 10 mM; NaCl, 140 mM; CaCl₂ or EDTA, 10 mM; hog intrinsic factor-[³⁵⁰Co]Cbl, 0 to 1.2 pmol; Triton X-100, 0.1% (v/v); and various amounts of soluble receptor. After incubating for 1 h at 22°C, aliquots were pipetted into a 4°C centrifuge tube, centrifuged at 10,000 g for 30 min, and 50 µl of supernatant was added to assay tubes. 

Fractionation of Receptor—The mucosa from the distal halves of canine intestines was scraped off with glass slides and stored at −70°C for up to 2 weeks. Mucosa, 2.5 kg, was thawed, 10 liters of 5 mM potassium phosphate, pH 7.4, containing 140 mM NaCl and 5 mM KCl were added, followed by homogenization in a Waring blender for 1 min at top speed at 4°C. Triton X-100 was added to give a final concentration of 1% (v/v), and after stirring for 16 h at 4°C, the homogenate was centrifuged at 200,000 × g for 30 min. Ethanol stored at −20°C was added slowly to the supernatant fraction to give a final concentration of 30% (v/v). After stirring for 1 h at 4°C the samples were centrifuged at 200,000 × g and the pellets were suspended in 3 liters of the buffer solution described above and dialyzed against 10 liters of this solution for 18 h with three changes of dialysate. The dialyzed material was then treated with Triton X-100 to give a final concentration of 1% (v/v) and allowed to stir overnight at 4°C followed by centrifugation for 1 h at 150,000 × g and collection of the supernatant. The ethanol precipitation step was then repeated as just described except that the initial pellets were resuspended in only 500 ml of buffer solution. 

Assays of receptor activity following the second ethanol precipitation step using a value of 10–15 nmol of total hog intrinsic factor-[³⁵⁰Co]Cbl binding activity. Based on the actual value obtained for a given preparation at this stage, a 2-fold excess of hog intrinsic factor-[³⁵⁰Co]Cbl, i.e. 20–30 nmol, was added to the samples which had been adjusted to contain 2.5 mM CaCl₂ and 1.25 mM MgSO₄. After incubating for 1 h at 22°C, the sample was cooled to 4°C and applied to a 2.5 cm diameter and 1 cm tall column of rabbit anti-hog intrinsic factor-Sepharose which had a capacity to bind approximately 40 nmol of hog intrinsic factor-Cbl. The column was washed immediately before the sample was applied with the following solutions at a flow rate of 30 ml/hr: wash A, 250 ml of 5 mM potassium phosphate-HCl, pH 7.4, 140 mM NaCl, 5 mM KCl, 5 mM EDTA, and 1% Triton X-100; wash B, 250 ml of potassium phosphate, pH 7.4, 140 mM NaCl, and 1% Triton X-100; and wash C, 250 ml of potassium phosphate, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂, 1.25 mM MgSO₄, and 1% Triton X-100. The sample was applied at a flow rate of approximately 20 ml/hr at 4°C. The pH was immediately adjusted to 7.4 with 1 M NaOH and 1.0 M CaCl₂ was added to give a concentration of 5 mM. Receptor was stored at 4–5°C for up to 1 month without loss of activity. 

RESULTS

Assay for Soluble Receptor—Fig. 1 illustrates the (NH₄)SO₄ precipitation assay utilized for measuring soluble receptor and demonstrates that the receptor-intrinsic factor-[³⁵⁰Co]Cbl complex precipitates at lower concentrations of (NH₄)SO₄ than does unbound intrinsic factor-[³⁵⁰Co]Cbl. Routine assays utilized 1.5 M (NH₄)SO₄, which precipitated 92% of the receptor-intrinsic factor-[³⁵⁰Co]Cbl complex while precipitating only 3% of the unbound intrinsic factor-[³⁵⁰Co]Cbl. When CaCl₂ was replaced by EDTA in assays containing receptor and intrinsic factor-[³⁵⁰Co]Cbl, the amount of intrinsic factor-[³⁵⁰Co]Cbl precipitated was equal to that obtained with intrinsic factor-Cbl alone. The ability of EDTA to inhibit the binding of intrinsic factor-Cbl to membrane-bound (8) and solubilized preparations (4) of crude receptor has been shown previously. 

Purification of the Receptor—A summary of a typical purification of receptor from 2.5 kg of canine ileal mucosa is shown in Table I. The final purification was approximately 65,000-fold with a yield of 26% and gave approximately 2 mg of receptor which appeared homogeneous, as described below. The initial purification steps were performed primarily to obtain a solution of receptor that would pass over the anti-
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Table I
Purification of the canine ileal receptor for intrinsic factor-Cbl

| Step                                    | Volume (ml) | Total protein (mg) | Total intrinsic factor-Cbl-binding ability (nmol) |
|-----------------------------------------|-------------|--------------------|-----------------------------------------------|
| Triton-treated ileal homogenate         | 12,300      | 600,000            | 23.2                                          |
| 1st ethanol precipitation               | 3800        | 30,000             | 17.7                                          |
| 2nd ethanol precipitation              | 900         | 9,700              | 14.7                                          |
| Immunoaffinity chromatography on anti-intrinsic factor-Sepharose | 65         | 2.4                | 6.05%                                        |

*This value represents a recovery of 26% and a purification of 65,000-fold. The final specific activity was 2.52 nmol of hog intrinsic factor-Cbl bound/mg of protein.

FIG. 1. Assay for solubilized ileal receptor for intrinsic factor-Cbl. Assays were performed as described under “Methods” in which 1 pmol of hog intrinsic factor (IF)-[15Co]Cbl (specific activity, 0.3 to 1.0 µCi/µg of intrinsic factor) was incubated alone, or together, with 0.23 pmol of purified canine ileal receptor for intrinsic factor-Cbl. Various concentrations of (NH₄)₂SO₄ were then added to determine the amount of hog intrinsic factor-[15Co]Cbl precipitated under these conditions. The inset illustrates the values obtained for the differences between samples containing receptor and those not containing receptor at each (NH₄)₂SO₄ concentration.

Polyacrylamide Disc Gel Electrophoresis—Evidence for receptor aggregation was also obtained with polyacrylamide disc gel electrophoresis in the presence of 0.1% Triton X-100 since, as shown in Fig. 3, the receptor barely entered a 4% gel. A single protein band was observed approximately 2 mm from the top of the gel with diffuse staining occurring toward the cathode over an adjoining 6 mm. Both the major band and the diffuse area contained receptor activity as determined by measuring intrinsic factor-[15Co]Cbl-binding ability in individually eluted gel slices (see Fig. 3). The band and diffuse area did not stain for carbohydrate (data not shown) suggesting that the receptor has a very low or absent carbohydrate content. Attempts to obtain a sharper protein band by varying the time and voltage conditions of electrophoresis, by varying the concentration of Triton X-100, or by preincubating the receptor with Triton X-100 for 24 h prior to electrophoresis, proved ineffective.

SDS-Polyacrylamide Gel Electrophoresis—When Triton X-100 was removed from preparations of purified receptor and the receptor was subjected to polyacrylamide gel electrophoresis with 1% SDS, one protein band was observed as shown in Fig. 4 which had an apparent molecular weight of 180,000. Upon reduction with 1% 2-mercaptoethanol, the receptor gave two protein bands with apparent molecular weights of 59,000 and 42,000. These same bands should also be demonstrated when the receptor fraction was treated with a protease inhibitor before reduction and treatment with SDS (data not shown). The value of 180,000 is close to the value of 222,000 g of amino acid/mol of intrinsic factor-Cbl-binding and 5,000,000 and indicated that the receptor had aggregated under the conditions utilized for the gel filtration. Similar elution profiles were observed for receptor-hog intrinsic factor-[15Co]Cbl (data not presented) and for [125I]-labeled receptor (Fig. 2C).

Gel Filtration of the Receptor—As shown in Fig. 2, two peaks of intrinsic factor-[15Co]Cbl-binding ability were observed when partially purified (Fig. 2A) or purified (Fig. 2B) receptor were chromatographed on Bio-Gel A-5m in the presence of 0.15% Triton X-100. Samples consisted of: A, partially purified receptor after the second ethanol precipitation step containing 30 pmol of hog intrinsic factor (IF)-[15Co]Cbl-binding ability; B, purified receptor after the immunoaffinity chromatography step containing 30 pmol of hog intrinsic factor-[15Co]Cbl-binding ability; and C, purified receptor after the immunoaffinity chromatography step that had been labeled with [125I] and contained 10⁴ dpm. The void volume of the column was determined with Blue Dextran 2000. The two peaks observed in A and B have apparent molecular weights of 7,500,000 and 5,000,000 as determined with proteins of known molecular weights as described under “Methods.”
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Isoelectric Focusing—When purified receptor was subjected to isoelectric focusing in the presence of 4 M urea and 2% Triton X-100, two protein-staining bands were observed with $P_I$ values of 4.9 and 5.4. These two bands could not be subdivided further when focusing was done for longer periods of time.

Amino Acid and Amino Sugar Composition—The results of the amino acid and amino sugar analyses are presented in Table II. The purified receptor contained 222,000 g of amino acid and only 1,300 g of amino sugar/mol of intrinsic factor-Cbl-binding ability. The low value obtained for amino sugars suggests that the receptor contains at most a small amount of carbohydrate. The value of 222,000 g of amino acid/mol of intrinsic factor-Cbl-binding ability is close to the molecular weight of 180,000 obtained by SDS-polyacrylamide gel electrophoresis (see above) and indicates that the receptor contains a single intrinsic factor-Cbl binding site. The purified receptor contains approximately 57% of hydrophobic amino acids.

Affinity and Specificity of Purified Receptor—As shown in Fig. 5, purified canine receptor bound canine intrinsic factor-[15Co]Cbl, hog intrinsic factor-[15Co]Cbl, and human intrinsic factor-[15Co]Cbl with association constants of 1.2 nM, 3.2 nM, and 4.0 nM, respectively. These values are similar to those observed previously (8) with crude membrane-bound receptor. When studied under similar conditions, binding was not observed with [15Co]Cbl or with [15Co]Cbl bound to canine, hog, or human R protein. Abnormal human intrinsic factor-[15Co]Cbl (19) was bound but with an affinity that was approximately 50-fold less than that observed with normal human intrinsic factor-[15Co]Cbl, a difference that is similar to that observed with membrane-bound human and canine

![Graph](image1)

**FIG. 3.** Polyacrylamide disc gel electrophoresis of purified canine ileal receptor for intrinsic factor (IF)-Cbl. **Top,** 12.5% labeled receptor (106 dpm) was applied and individual gel slices were assayed for 125I. **Middle,** 35 mg of receptor protein was applied and individual gel slices were eluted and assayed for hog intrinsic factor-[15Co]Cbl-binding ability. Although recoveries cannot be measured with precision, about 5-10% of the applied activity was eluted from the gel. **Bottom,** 35 mg of receptor protein was applied and the gel was stained for protein with Coomassie brilliant blue. In each case, the direction of electrophoresis was from top to right.

![Graph](image2)

**FIG. 4.** SDS polyacrylamide gel electrophoresis of purified canine ileal receptor for intrinsic factor-Cbl performed in the absence, left, and in the presence, right, of 2-mercaptoethanol. Samples contain 25 mg of receptor protein and gels were stained for protein with Coomassie brilliant blue. The apparent molecular weights of the protein bands were determined with proteins of known molecular weight, as described under "Methods." The direction of electrophoresis was from top to bottom.

ability obtained by amino acid analysis (see below) and supports the concept that the much higher molecular weights observed by gel filtration (see above) are due to receptor aggregation. The receptor appears to consist of two different subunits that are joined by disulfide bonds although the number of each subunit present is not known.

![Table](image3)

**TABLE II**

Amino acid and amino sugar analysis of the canine ileal receptor for intrinsic factor-Cbl and of hog intrinsic factor

| Item          | Canine ileal receptor for intrinsic factor-Cbl | Hog intrinsic factor |
|---------------|-----------------------------------------------|---------------------|
|               | mol/mol intrinsic factor-Cbl binding ability | mol/mol Cbl-binding ability |
| Amine acid    |                                               |                     |
| Lysine        | 91                                            | 4.4                 |
| Histidine     | 78                                            | 3.8                 | 9.2          |
| Arginine      | 81                                            | 3.9                 | 2.7          |
| Aspartate     | 210                                           | 10.2                | 50           |
| Threonine     | 126                                           | 6.1                 | 37           |
| Serine        | 140                                           | 6.8                 | 36           |
| Glutamate     | 187                                           | 9.0                 | 48           |
| Proline       | 110                                           | 5.3                 | 31           |
| Glycine       | 193                                           | 9.3                 | 25           |
| Alanine       | 178                                           | 8.6                 | 32           |
| Valine        | 143                                           | 6.9                 | 26           |
| Isoleucine    | 114                                           | 5.5                 | 25           |
| Leucine       | 182                                           | 8.8                 | 43           |
| Tyrosine      | 60                                            | 3.0                 | 9            |
| Phenylalanine | 80                                            | 3.9                 | 14           |
| Methionine    | 11                                            | 0.5                 | 10           |
| Half-cystine  | 78                                            | 3.5                 | 9            |
| Tryptophan    | --                                            | --                  | 6            |
| Total amino acids | 2064a                                   | 99.8  | 439 | 99.8 |
| Amino sugar   |                                               |                     |
| Galactosamine | 0                                             | 9                   |
| Glucosamine   | 8                                             | 25                  |

a From Ref. 18.

b These values may be falsely low because of oxidation during hydrolysis.

c Not determined.

d This corresponds to a molecular weight of 222,000 g of amino acid/mol of intrinsic factor-Cbl-binding ability.
ileal receptors (19). The binding of hog intrinsic factor-
$[^{57}Co]Cbl$ was not inhibited by a 100-fold excess of nonradioactive Cbl nor by a 100-fold excess of hog intrinsic factor
devoid of Cbl (data not presented). These studies indicate
that binding to the purified canine receptor is specific for the
intrinsic factor-Cbl complex as has been shown previously (8)
for the crude membrane bound canine and human ileal receptors.
Free Cbl or intrinsic factor or abnormal intrinsic factor-
Cbl has very little or no affinity for purified receptor.

Studies with Anti-receptor Antibody—The data in Fig. 6 demonstrate first that $^{125}$I-labeled receptor was precipitated,
with only 0.0625 $\mu l$ of rabbit anti-canine receptor antiserum.
Approximately 75% of the $^{125}$I-labeled receptor was precipitated
with 0.75 $\mu l$ of anti-receptor antiserum. In a separate
experiment, when unlabeled receptor was used, hog intrinsic factor-$[^{57}Co]Cbl$-binding ability was also inhibited with only
0.0625 $\mu l$ of rabbit anti-canine receptor serum and 100% of the
hog intrinsic factor-$[^{57}Co]Cbl$-binding ability was inhibited
with 0.75 $\mu l$ of anti-receptor antiserum. Approximately 50%
predipitation and inhibition were observed with 0.125 $\mu l$
of anti-receptor antiserum.

The anti-receptor antiserum contained a small amount of
anti-intrinsic factor activity with $5 \mu l$, $15 \mu l$, and $75 \mu l$
of antiserum giving 50% precipitation in 1.5 M (NH$_4$)$_2$SO$_4$
with canine intrinsic factor-$[^{57}Co]Cbl$, hog intrinsic factor-$[^{57}Co]$-
Cbl, and human intrinsic factor-$[^{57}Co]Cbl$, respectively. This
anti-intrinsic factor activity may be due to the fact that the
final preparation of purified receptor may have contained traces of intrinsic factor. This possibility is supported by the
fact that the anti-receptor antiserum has more activity for
canine and hog intrinsic factors than it does for human intrin-
sic factor, since canine intrinsic factor may have been present
in the initial ileal homogenates while hog intrinsic factor was
utilized directly in the purification of the receptor. It is also
possible that the receptor for intrinsic factor-Cbl and intrinsic
factor share at least one common antigenic determinant.

In order to explore this latter possibility, we covalently
coupled rabbit anti-canine intrinsic factor antiserum, rabbit
anti-hog intrinsic factor antiserum, rabbit anti-human intrin-
sic factor antiserum, and rabbit control serum to Sepharose.
Individual columns containing 1 ml of each of these materials
did not adsorb any purified receptor when 1 ml containing 5
pmol of purified receptor was passed over them in the presence
of either 5 mM CaC$_2$ or 5 mM EDTA, based on assays of the
effluents for hog intrinsic factor-$[^{57}Co]Cbl$-binding ability.
Control serum-Sepharose also failed to adsorb any of 5 pmol
of canine, hog, or human intrinsic factor-$[^{57}Co]Cbl$ while all
three species of intrinsic factor-$[^{57}Co]Cbl$ were adsorbed
(>90%) by all three species of anti-intrinsic factor-Sepharose.
These results suggest that the canine receptor for intrinsic
factor-Cbl does not share antigenic determinants with canine,
hog, or human intrinsic factor although they do not rule out
this possibility.

**DISCUSSION**

Using immunoaffinity chromatography as the major puri-
fication technique, we have isolated the canine ileal receptor
for intrinsic factor-Cbl in apparent homogeneous form.
The final preparation bound 2.5 nmol of hog intrinsic factor-
$[^{57}Co]Cbl$/mg of protein and this represents a 10-fold and 25-
fold higher specific activity than previous purifications of the
hog (15) and guinea pig (14) ileal receptors, respectively. The
availability of milligram amounts of purified receptor has
enabled us to perform both SDS-gel electrophoresis experi-
ments and quantitative amino acid analyses. These experi-
ments demonstrate that the receptor has a molecular weight
of approximately 200,000, that it contains a single binding site
for intrinsic factor-Cbl, that it contains little if any carbohy-
drate, and that the molecular weight values in excess of
1,000,000 obtained by gel filtration are due to receptor aggre-
gation. The purified receptor is essentially identical with
preparations of unpurified membrane-bound receptor in terms
of its affinity and specificity for intrinsic factor-Cbl (8) and in
terms of its inhibition by EDTA (8). These properties indicate
that the receptor has not been altered appreciably during any
of the purification procedures.

Our studies indicate that reduction with 2-mercaptoethanol
dissociates the canine ileal receptor for intrinsic factor-Cbl
into two subunits with molecular weights of approximately
59,000 and 42,000 based on SDS electrophoresis. A previous
study (15) has suggested that the hog ileal receptor for intrin-

![FIG. 5. Double reciprocal plots for binding of intrinsic factor (IF)-$[^{57}Co]Cbl$ to 1 pmol of purified canine ileal receptor. The species of intrinsic factor were: ●, canine intrinsic factor; ▲, hog intrinsic factor; and ●, human intrinsic factor.](image-url)
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Previous studies (21, 27) have shown that intrinsic factor, R protein, transcobalamin II, and the transcobalamin II receptor resemble one another in terms of having single polypeptide chains with molecular weights of approximately 40,000 for their amino acid portions, single binding sites, and similarities in amino acid composition. These similarities have suggested that proteins involved in Cbl transport may have evolved from a common ancestral gene, although differences in amino acid composition, isolated congenital deficiencies of these proteins, and an apparent lack of immunologic cross-reactivity demonstrate that each is now coded for by a separate gene (27). The canine ileal receptor for intrinsic factor-Cbl is clearly different from intrinsic factor since the receptor has a molecular weight of approximately 200,000, contains two polypeptide chains that appear to be joined via disulfide bonds, and differs markedly from intrinsic factor in its content of several amino acids and amino sugars. One of the subunits does, however, have a molecular weight of 42,000 and it would be interesting to study this subunit in more detail to determine its amino acid composition and whether this subunit possesses the binding site for intrinsic factor-Cbl.

We have prepared anti-canine receptor antibodies in rabbits and have shown that they both precipitate the canine ileal receptor and inhibit its binding of intrinsic factor-Cbl. A small amount of activity is observed against intrinsic factor from various species, but it appears likely (see “Results”) that small amounts of anti-intrinsic factor antibody were raised against trace amounts of intrinsic factor that may have been present in the purified receptor preparations, since three different antisera raised against canine, hog, and human intrinsic factor did not react with the canine ileal receptor. A previous study (15) has suggested that anti-intrinsic factor antibodies may cross-react with at least one component present in purified hog ileal receptor preparations, but details concerning these experiments were not provided and the possibility that the component recognized was actually intrinsic factor itself was not excluded. In any event, it appears likely that partially purified or adsorbed preparations of anti-receptor antibodies will be useful in future studies designed to elucidate the subcellular localization of the receptor for intrinsic factor-Cbl and the role that the receptor plays in the cellular uptake of Cbl by the ileum and its subsequent release into plasma.

Acknowledgments—We thank Dr. Ralph Bradshaw for assistance in performing the amino acid and amino sugar analyses, and Ms. Maria Ignacio and Ms. Sharon Calahan for assistance in preparing the manuscript.

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