

Solubilized Receptor for Intrinsic Factor-Vitamin B₁₂ Complex from Guinea Pig Intestinal Mucosa

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ABSTRACT The absorption of vitamin B₁₂ in many animals requires its prior association with intrinsic factor (IF) and attachment to a specific receptor in the intestine. Employing Triton X-100, we have solubilized from guinea pig ileum a factor that binds intrinsic factor-vitamin B₁₂ complex (IF-B₁₂). This binding factor was soluble to the extent that it was not sedimented by centrifugation at 100,000 *g* for 1 h and was small enough to enter the included volume of a Sepharose 4-B column. Furthermore, the ileal extract contained no microfine particles of membrane upon electron microscopic search. When a portion of the extract was incubated with a mixture of gastric juice and ⁵⁷Co-labeled vitamin B₁₂, a portion of the radioactivity was excluded from a Sephadex G-200 column. When gastric juice from a patient with a congenital abnormality of IF that prevented its binding to intestine was substituted for normal human gastric juice, radioactivity was not excluded from the gel, indicating failure of this abnormal IF-B₁₂ to bind to the intestinal extract. These data suggested the presence of a specific binder of IF-B₁₂ in the ileal mucosal extract. The reactions of normal IF-B₁₂ with the solubilized binding factor and with the membrane-bound "receptor" had several characteristics in common, including calcium dependence, temperature independence, and pH optimum near neutral. Extracts from the distal intestine showed more activity than did those from the proximal. The solubilized binding factor seemed specific for IF-B₁₂ in that it was not blocked by prior incubation with excesses of either free vitamin B₁₂ or IF. Binding activity of the extract was decreased by incubation at pH 2.0, by heating to 56°C, and by incubation with chymotrypsin and dithiothreitol. Incubation with trypsin, neuraminidase, and sulphydryl blockers did not affect it.

The Triton X-100 extract of guinea pig ileal mucosa contains a specific binding factor that probably is the

receptor for IF-B₁₂. This appears to be a protein with function dependent on peptide and disulphide linkages.

INTRODUCTION

Dietary vitamin B₁₂ is absorbed from the small intestine of many animals, including man, by a complex process that begins when the vitamin is bound by intrinsic factor (IF)¹ (1), a glycoprotein that is synthesized and secreted by the gastric mucosa (2). In vitro studies have shown that preparations containing IF facilitate the uptake of vitamin B₁₂ by everted intestinal sacs (3), mucosal homogenates (4), and microvillous membranes (5) prepared from the distal small bowel. This uptake is dependent on pH and the presence of calcium ion (6). Similar preparations of proximal small bowel do not have this property. Thus, the mucosa of the distal small intestine was thought to contain a specific receptor to which vitamin B₁₂ would bind in the presence of normal IF (7). Uncertainty exists about the role of IF in subsequent events before the appearance of vitamin B₁₂ in portal vein blood. However, it seems clear that the first step in the intestinal phase of vitamin B₁₂ absorption involves the hypothesized receptor. Recently, Toskes, Hansell, Cerda, and Deren suggested that a pancreatic factor may be required for normal vitamin B₁₂ absorption, though it is not clear at which level this factor might have a role (8).

Our understanding of the mechanism of IF-mediated vitamin B₁₂ absorption might be facilitated by the isolation and study of the receptor for intrinsic factor-vitamin B₁₂ complex (IF-B₁₂). Several attempts to isolate this receptor have been reported (5, 9), but uncertainty exists about the validity of the claims of success (10).

Within the last decade, transport proteins from bacterial cell membranes have been successfully removed in soluble form (11, 12). In this report, we shall describe

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¹ *Abbreviations used in this paper:* IF, intrinsic factor; IF-B₁₂, intrinsic factor-vitamin B₁₂ complex.

the extraction of a factor from homogenates of guinea pig intestinal mucosa that bound normal IF-B₁₂. This factor was soluble to the extent that it was not sedimented by centrifugation at 100,000 *g* for 1 h and was small enough to enter the included volume of a Sepharose 4-B gel filtration column. Furthermore, an electron microscopic search of the mucosal extract did not reveal any identifiable membrane particles. We have studied some of the properties of this binding factor and suggest that it is the specific receptor for IF-B₁₂.

METHODS

High specific activity [⁵⁷Co]B₁₂ (100 μCi/μg), purchased from Amersham-Searle Radiochemical Laboratories (Buckinghamshire, England), was used for all the studies to be described. Gastric juice was obtained from human volunteers by intermittent hand suction through a nasogastric tube after the subcutaneous injection of 50 mg of betazole hydrochloride. It was kept on ice, filtered through gauze, and depepsinized (13). The total vitamin B₁₂-binding capacity and the IF content of gastric juice from each donor were measured by the albumin-charcoal method of Gottlieb, Lau, Wasserman, and Herbert (14). Specimens from four normal donors, in which IF content (46–54 ng U/ml) represented more than 90% of the total vitamin B₁₂-binding capacity (49–56 ng U/ml), were pooled and stored in aliquots at –20°C as a source of normal IF. The pooled gastric juice had a vitamin B₁₂-binding capacity of 55 ng/ml of which 52 ng were due to IF.

Gastric juice was obtained in similar fashion from a patient we recently described who secreted an immunologically identifiable IF that bound vitamin B₁₂ normally but did not facilitate its uptake by guinea pig or human distal intestinal mucosal homogenates (15). This patient's gastric juice did not promote the absorption of vitamin B₁₂ by a gastrectomized human volunteer. Its vitamin B₁₂-binding capacity was 56 ng/ml, of which 49 ng were due to IF, and it was used as a source of inert IF. Serum known to contain an antibody that bound IF-B₁₂ was obtained from a patient with pernicious anemia and used to establish the presence of IF-B₁₂ (16). IF-[⁵⁷Co]B₁₂ was prepared immediately before use as follows: [⁵⁷Co]B₁₂ was added in excess (30 ng) over the measured vitamin B₁₂-binding capacity to a portion (0.5 ml) of gastric juice. After 20 min at room temperature, residual free vitamin B₁₂ was removed by adsorption to albumin-coated charcoal (2.0 ml). After centrifugation at 20,000 *g* for 15 min at 0°C, the supernate was removed and counted, and an appropriate amount was used for the experiments of the day.

Protein content was estimated by the method of Lowry, Rosebrough, Farr, and Randall (17), employing crystalline bovine serum albumin (Nutritional Biochemicals Corp., Cleveland, Ohio) as a standard. Lipid content was estimated by extracting samples with ethanol-ether at –20°C by the method of Scanu, Lewis, and Bumpus (18), removing the precipitate by centrifugation at 20,000 *g*, and weighing the residue left after evaporation of the supernate to dryness. Radioactivity was measured in a gamma scintillation counter. Data were always presented in terms of the weight of vitamin B₁₂ bound, in nanograms or picograms.

Preparation of mucosal extracts. Male guinea pigs weighing approximately 250 g were fasted for 24 h, then sacrificed by a blow to the head. The small intestine, distal

to the ligament of Treitz, was removed, flushed immediately with 1 liter of cold (4°C) 0.15 M saline, and cut into quarters, which were then cut into manageable 15-cm lengths. The mucosa from each quarter was expressed in toothpaste fashion by pressure from the edge of a glass slide and placed immediately into separate beakers kept on melting ice and containing 100 ml of 0.03 M sodium phosphate-buffered 0.15 M saline of pH 7.4. Each batch of scrapings was disrupted in an ice-jacketed flask on a Virtis 45 homogenizer (Virtis Company, Gardiner, N. Y.) run at full power for 60 s. The resulting homogenates were washed three times with 20 ml of the same buffer, and the pellets obtained (approximately 3.0 ml/quarter/guinea pig) were resuspended separately in 20 ml of 0.003 M sodium phosphate buffer of pH 8.0, then further disrupted with a Branson Sonifier (Branson Instruments Co., Stamford, Conn.) run at full power for 60 s. The mucosal fragments from each quarter were placed in 1 liter of 0.003 M sodium phosphate buffer of pH 8.0 containing 0.05% (vol/vol) Triton X-100 and left overnight at 4°C. The fragments were then separated by centrifugation at 100,000 *g* for 1 h. Each supernate was collected, dialyzed for 48 h against two changes of 10 liters of deionized water, and concentrated by lyophilization. Extract so prepared was kept at 4°C until used (usually within 2 mo).

To determine whether a factor that altered the radius of IF-B₁₂ was present in the extract of ileal mucosa, a mixture of extract and IF-[⁵⁷Co]B₁₂ was passed through a Sephadex G-200 column as follows: a 25-mg portion of lyophilized ileal extract was dissolved in 2 ml of 0.10 M Tris-HCl-buffered 0.15 M saline, pH 7.4, containing 0.001 M CaCl₂. IF-[⁵⁷Co]B₁₂ was prepared as described above, and 2.0 ng of this was added to the extract. The mixture was made up to a total volume of 5 ml with the CaCl₂-containing buffer. After incubation on ice for 60 min, the sample was applied to the top of a jacketed Sephadex G-200 column (60 × 2.5 cm) that had previously been standardized with blue dextran 2000, bovine serum albumin, and glucose. The sample was filtered with 0.03 M Tris-HCl-buffered 0.15 M NaCl solution of pH 7.4 at a flow rate of 12 ml/h. Filtration at 5.4 ml/h did not change the results obtained. To prevent disassociation of the IF-B₁₂, all filtration was done at 3°C (19). 3.5-ml fractions were collected in a refrigerated automatic fraction collector. The quantity of radioactive vitamin B₁₂ shifted from the included volume of the column, where IF-B₁₂ normally emerged, to the excluded volume was taken as a measure of the amount of "receptor" activity present in the portion of extract, under the conditions of the experiment. Filtration assay results were reproducible, always within 5%, in repeated runs employing the same experimental conditions.

RESULTS

Fig. 1 shows a Sephadex G-200 elution pattern obtained when ileal mucosal extract (25 mg) was incubated with 2.0 ng of IF-[⁵⁷Co]B₁₂ prepared with normal human gastric juice. A distinct peak of radioactivity was noted in the excluded volume of the column marked by blue dextran 2000. This contrasted with the elution pattern obtained when the same experiment was done with 2.0 ng of [⁵⁷Co]B₁₂ bound to gastric juice obtained from the patient with biologically inert IF. There was no radioactivity present in the excluded volume of the latter

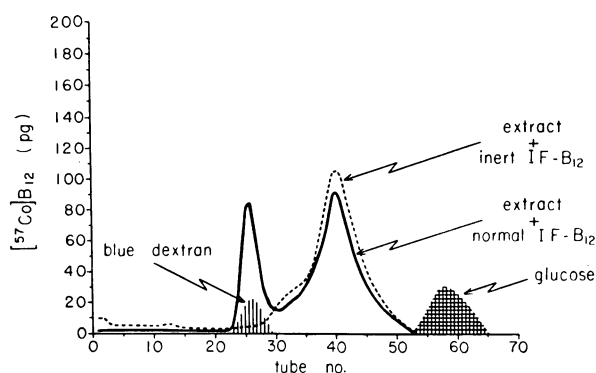


FIGURE 1 Elution pattern from Sephadex G-200 column of an extract from guinea pig ileal mucosa incubated with IF- ^{57}Co B $_{12}$ (neutralized, depepsinized gastric juice incubated with ^{57}Co B $_{12}$). With normal IF, some radioactivity emerged in the void volume (marked by blue dextran 2000), while with biologically inert IF, all the radioactivity emerged in the included volume. The small peak that emerged at fraction number 33 probably represents the salivary vitamin B $_{12}$ binder.

column. The only difference from normal which we had in the past been able to demonstrate in the inert IF lay in its inability to facilitate B $_{12}$ uptake by ileal mucosa (15). Thus, the data suggested that a specific binder of normal IF-B $_{12}$ was present in the Triton X-100 extract of ileal mucosa.

Solubilization studies. Initial experiments were designed to determine the best conditions for solubilization of the membrane-bound receptor. An operational definition of solubility was adopted: soluble receptor was considered to be that specific binding activity that remained in the supernate when a suspension of membrane particles was centrifuged at 100,000 g for 1 h. Several extraction procedures were evaluated. These are listed in Table I along with the results obtained on gel filtration assay of each extract mixed with 10 ng of normal IF- ^{57}Co B $_{12}$. The greatest yield was obtained with extracting fluid of low ionic strength and high pH, containing Triton X-100. The requirement for Triton X-100, however, was not absolute. Excellent recovery of solubilized receptor was also obtained when distal intestinal mucosal homogenates not disrupted by sonification were suspended in large volumes of 0.003 M sodium phosphate buffer of pH 8.0 without Triton X-100. Fig. 2 shows the results obtained when equal portions of washed ileal mucosal homogenates were suspended in increasing volumes of 0.003 M sodium phosphate buffer of pH 8.0. The quantity of binding activity that was recovered in the supernate after centrifugation at 100,000 g increased as the volume of buffer used in the extraction procedure increased.

When an aliquot of supernate was concentrated to half its volume with Carbowax (Union Carbide Corp.,

TABLE I
Effect of Physical Methods on Release of IF-B $_{12}$ Receptor from Guinea Pig Ileal Mucosal Homogenates*

Composition of extracting fluid	Yield of receptor activity	
	Volume ml	pg
Sodium phosphate buffer, 0.003 M, pH 8.0 with 0.05% Triton X-100	1,000	505
Sodium phosphate buffer, 0.003 M, pH 8.0 without Triton X-100	80	51
with 0.05% Triton X-100	80	150
with 0.05% Triton X-100, homogenate sonified†	80	155
with 5.00% Triton X-100, homogenate sonified	80	440
NaCl solution, with 0.05% Triton X-100; homogenate sonified		
0.15 M, buffered to pH 8.0 with 0.003 M Tris-HCl	80	362
1.5 M, buffered to pH 8.0 with 0.003 M Tris-HCl	80	260
Tris-HCl buffer, pH 8.0 with 0.05% Triton X-100; homogenate sonified	80	205

* Ileal mucosal scrapings were first homogenized and washed three times with 20 ml of 0.03 M sodium phosphate-buffered 0.15 M saline of pH 7.4. Equal aliquots were then subjected to the procedure listed. The resultant extracts were then incubated with 10 ng of IF- ^{57}Co B $_{12}$ and assayed for receptor activity by the gel filtration method.

† In addition, as indicated, some aliquots of mucosal homogenate were further disrupted by sonification before being added to the extracting fluid.

New York), then recentrifuged at 100,000 g , a reduction of receptor activity from that expected was observed. This reduction could be eliminated by adding Triton X-100 (0.05%) before the concentration and recentrifugation procedures. These data suggested that the receptor, when present in the solvent in greater than a

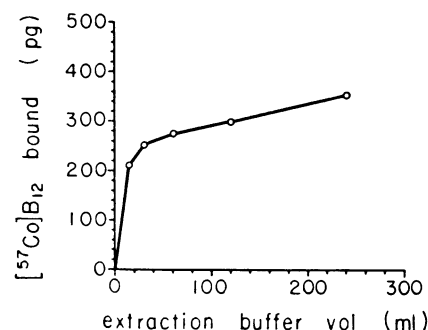


FIGURE 2 Effect of increased volumes of 0.003 M sodium phosphate buffer of pH 8.0 (without Triton X-100) on the yield of solubilized IF-B $_{12}$ -binding activity obtained from equal aliquots of guinea pig ileal mucosal homogenates. As the volume of buffer in which the mucosal homogenate was suspended increased, the receptor activity remaining in the supernate (100,000 g) increased.

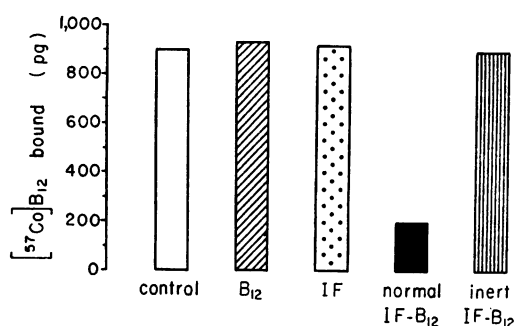


FIGURE 3 Effect of preincubation of ileal extract with excess (fivefold) normal IF, [⁵⁷Co]B₁₂ (nonradioactive), normal IF-[⁵⁷Co]B₁₂, and inert IF-[⁵⁷Co]B₁₂ on the uptake of normal IF-[⁵⁷Co]B₁₂ by receptor. Only the nonradioactive normal IF-B₁₂ was able to block receptor activity.

critical concentration, aggregated to form larger particles that could be sedimented by centrifugation at 100,000 *g*. At least a part of the effect of Triton X-100, then, was to reduce this tendency of the solubilized receptor to form aggregates that could sediment.

Suspension of 4.0 g (wet weight) of sonified ileal mucosal homogenate in 1 liter of 0.003 M sodium phosphate buffer of pH 8.0 containing 0.05% Triton X-100 resulted in the recovery of some 250 mg of extract. The protein content of the extract as determined by the method of Lowry et al. (17) was 9.0 mg/25-mg portion. When 25 mg of extract was subjected to ethanol-ether treatment (18), the protein precipitate weighed 9.5 mg. When the supernate was evaporated to dryness, the residue weighed 14 mg. The extract thus appeared to be about one-third protein and two-thirds lipid. Electron microscopic examination of the extract dissolved in water and negatively stained with phosphotungstic acid (20) revealed no microfine particles of membrane.^a Receptor activity was stable upon storage of the extract in lyophilized form at 4°C for at least 4 mo.

To determine whether the radioactive vitamin B₁₂ bound by the receptor and emerging in the excluded volume of a Sephadex G-200 assay column was still attached to IF, the three earliest fractions containing part of the excluded volume were pooled, and Na₂EDTA was added to a final concentration of 0.005 M. Chelation of divalent cations by Na₂EDTA is known to free the vitamin B₁₂ taken up by mucosal homogenates when normal IF is present (6). After incubation on melting ice for 30 min, an aliquot of the Na₂EDTA-treated sample was refiltered on a Sephadex G-200 column, and two peaks of radioactivity were noted, one in the excluded volume, representing some 50% of the starting counts, and the second in the included volume, where

IF-B₁₂ normally emerged. To prove that this second peak was indeed IF-B₁₂, a second aliquot of the starting material was incubated with serum, known to contain an antibody that binds to IF-B₁₂, from a patient with pernicious anemia. After standing at 0°C for 30 min, this mixture was filtered on Sephadex G-200. Only one peak of radioactivity was noted in this experiment, and it emerged in the excluded volume. This suggested that the second peak had been recognized and bound by antibody to IF-B₁₂, rendering it sufficiently large to be excluded from the Sephadex G-200 gel.

Mucosal homogenates take up IF-B₁₂ only when divalent cations are present. Chelators of divalent cations, such as Na₂EDTA, have been shown to block this uptake entirely (6). The effect of Na₂EDTA on the binding of radioactive IF-B₁₂ by ileal mucosal extract was studied by assaying 25 mg of extract dissolved in buffer containing 0.005 M Na₂EDTA. Receptor activity was reduced to 10% of that of a control assayed without Na₂EDTA. This experiment was repeated employing the same Sephadex G-200 column and the same concentration of Na₂EDTA, but this time with CaCl₂ added to a final concentration of 0.006 M. Receptor activity under these conditions was identical to that of a control. Thus, binding of IF-B₁₂ by mucosal extract was Na₂EDTA inhibitable and calcium dependent. Other divalent cations were not studied.

Homogenates of distal ileal mucosa have been shown to have a much greater affinity for vitamin B₁₂ when it is bound to IF than they have for either free vitamin B₁₂ or IF alone (3). We compared the capacity of a fivefold excess of free nonradioactive vitamin B₁₂, free IF (as gastric juice), and nonradioactive IF-B₁₂ to block the uptake by mucosal extract of subsequently added IF-[⁵⁷Co]B₁₂. The results are shown in Fig. 3. Only the nonradioactive IF-B₁₂ was able to block receptor activity. When similar experiments were done with a fivefold excess of biologically inert IF-B₁₂, no blocking of receptor activity was noted. These data demonstrate the great specificity of the solubilized binder for IF-B₁₂.

In several animals and in man, workers have demonstrated that mucosal uptake of IF-B₁₂ occurs mainly in the distal small bowel and is only minimal in the jejunum (21, 22). We prepared homogenates of intestinal mucosa from successive descending quarters of the small bowel of a fasted guinea pig. The membrane fragments were sedimented at 20,000 *g*, and the supernates so obtained were each extracted with Triton X-100 as described above. An aliquot of the homogenate prepared from each quarter was assayed for binding activity by the method of Sullivan, Herbert, and Castle (4), and the remainder from each quarter was subjected to the Triton X-100 extraction procedure. All of the resultant extracts were then assayed by the Sephadex G-200

^a We are grateful to Mrs. M. Gomersall, Department of Microbiology and Immunology, McGill University, for preparation of the specimens for electron microscopy.

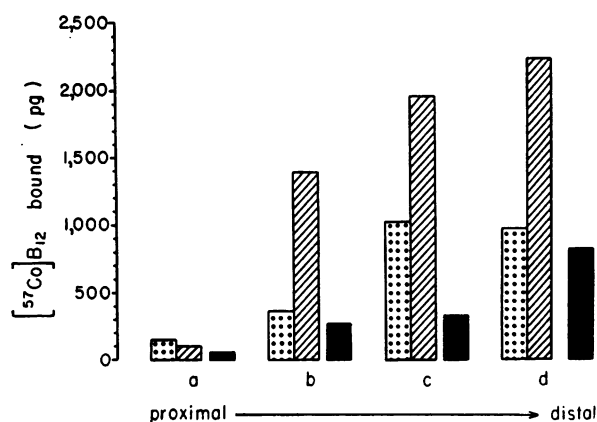


FIGURE 4 IF- B_{12} receptor activity of successive descending quarters of the small bowel of a fasted guinea pig, measured by the mucosal homogenate uptake method of Sullivan et al. (4) and by Sephadex G-200 gel filtration of extracts prepared from the same homogenates. Receptor activity in cell sap and particles not sedimented by centrifugation at 20,000 g after the homogenization procedure was also measured by gel filtration assay. Dotted bars indicate activity of membrane particles by mucosal homogenate assay; striped bars indicate activity of membrane particles by gel filtration assay; and solid bars indicate activity of supernates by gel filtration assay.

method for IF- B_{12} receptor activity. The results were corrected to reflect the total receptor activity present on the mucosa obtained from each quarter of the small intestine. The data are shown in Fig. 4. It was noted that, as expected, most of the receptor activity was associated with the membrane portion of the mucosa. The increase in receptor activity was similar by both homogenate uptake and gel filtration assay techniques as the more distal segments of the small bowel were studied.

To demonstrate the effect of the concentration of IF- $[^{57}\text{Co}]B_{12}$ on receptor activity, increasing quantities of it were added to 25 mg of distal intestinal extract dissolved in 2 ml of CaCl_2 -containing buffer. The total volume of the reaction mixture was immediately made up to 5 ml with Tris-HCl-buffered saline, pH 7.4, containing 0.001 M CaCl_2 . The reaction was permitted to proceed for 60 min at 0°C before gel filtration on Sephadex G-200. The results obtained are shown in Fig. 5a. The IF- B_{12} -binding factor could be saturated by increased concentrations of IF- B_{12} . The association constant obtained by plotting the data by the Scatchard method (see Fig. 5b) was $0.76 \times 10^9 \text{ M}^{-1}$. The number of binding sites available in 25 mg of extract was calculated as 2.7×10^{13} .

The effect of pH on the uptake of IF- B_{12} by ileal mucosal extract was studied by incubating portions of the extract with IF- B_{12} in Tris-HCl-buffered saline at various pH's from 2 to 11, then filtering on a Sephadex G-200 column that had been previously equilibrated

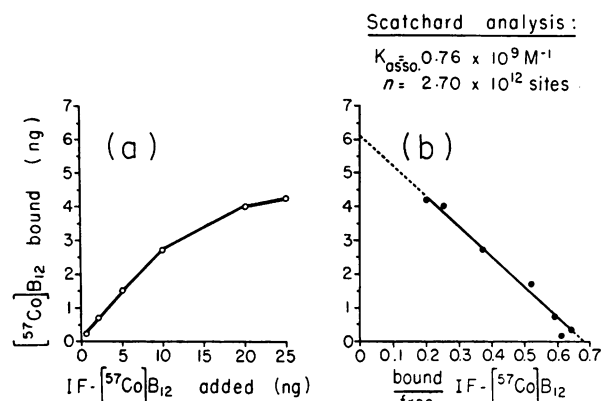


FIGURE 5 Receptor activity in a fixed quantity of ileal extract (25 mg) studied with increasing amounts of IF- $[^{57}\text{Co}]B_{12}$ (a) in a total volume of 5 ml. The data plotted by the Scatchard method are shown on (b).

with buffer to the same pH. The results are shown in Fig. 6 and indicate that the optimum pH for binding is between 6 and 9.5.

IF- B_{12} -receptor complex was filtered on a Sepharose 4-B gel column (60 \times 2.5 cm diameter) that had been standardized with blue dextran 2000, immunoglobulin M, albumin, and glucose. The resulting elution pattern showed a peak of binding activity emerging after blue dextran 2000, indicating that the IF- B_{12} -receptor complex was indeed small enough to enter the included volume of the column.

The effect of temperature on the uptake of IF- B_{12} by receptor in ileal mucosal extract was studied as follows: Extract was incubated with IF- B_{12} at 0°, 37°, and 56°C for 60 min before gel filtration on Sephadex G-200. Uptake at 0°C was 90% of that at 37°C, and at 56°C, there was no apparent binding. Incubation of extract at 56°C for 30 min, followed by cooling to 0°C, addition of IF- $[^{57}\text{Co}]B_{12}$, and assay by gel filtration demonstrated that receptor activity had been destroyed by heating.

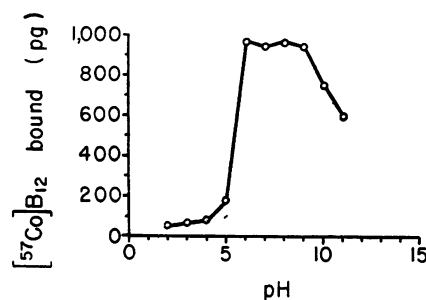


FIGURE 6 Effect of pH on the uptake of IF- $[^{57}\text{Co}]B_{12}$ by solubilized receptor. Ileal extract was dissolved in CaCl_2 -containing 0.03 M Tris-buffered 0.15 M NaCl, of various pH's, and incubated with IF- B_{12} at the same pH before gel filtration on Sephadex G-200. Elution was with buffer at the same pH as that used for the incubation.

TABLE II
Studies of the Properties of the IF-B₁₂ Binder

Lability of receptor activity	Treatment*	Activity†
		% of control
To heat	0°C, 30 min	100
	37°C, 30 min	80
	56°C, 30 min	3
To pH	pH 2, 30 min	1
	pH 4, 30 min	42
	pH 6, 30 min	100
	pH 8, 30 min	100
To enzymes	trypsin (Worthington Biochemical Corp., Freehold, N. J.), 37°C, 60 min	90
	chymotrypsin (Worthington Biochemical), 37°C, 60 min	35
	phospholipase A (Russell's viper, Sigma Chemical Co., St. Louis, Mo.): 37°C, 60 min	100
	neuraminidase (<i>Vibrio cholerae</i> , Sigma Chemical Co.), 37°C, 60 min	100
	To -SH reagents	17
	N-ethyl maleimide (0.002 M, 30 min)	
	dithiothreitol (0.002 M, 30 min)	

* In each of the experiments, equal aliquots of ileal extract were dissolved in buffer, subjected to the treatment listed, then placed on melting ice, the pH adjusted to 7.4, and receptor activity assayed by the gel filtration method as described in the text.

† The results are expressed as the percent of a control experiment in which an aliquot of the ileal extract was assayed for receptor activity as described in the text.

When IF-B₁₂ (10 ng) was mixed with extract (25 mg) on melting ice for 30, 60, 90, and 180 min before gel filtration, receptor activity was maximal by 60 min, and it remained constant.

Some of the properties of the binding activity in extracts of ileal mucosal homogenates were studied. In each of the experiments reported in Table II, the extract was dissolved in the buffer and subjected to the condition listed. The pH was then adjusted to 7.4 and the sample placed on melting ice before 10 ng of IF-[⁵⁷Co]B₁₂ was added and binding activity was assayed by the gel filtration method. Receptor activity was heat labile, as described, above, and acid labile. Incubation with chymotrypsin decreased receptor activity relative to that of a control incubated without enzyme; incubation with trypsin and neuraminidase changed this activity only minimally; incubation with N-ethyl maleimide, a sulphydryl-blocking agent, slightly increased it in relation to that of a control; and incubation with 0.002 M dithiothreitol markedly reduced it.

DISCUSSION

The mechanism of selective membrane permeability has been the subject of much recent work employing bac-

terial cells. Developments have included the isolation and partial purification of some of the components of the specific sugar transport system of *Escherichia coli* (12, 23). In similar fashion, the isolation in pure form of some of the components of the system with which animals absorb vitamin B₁₂ might facilitate our understanding of this intriguing transport mechanism. Recently, IF has been purified in good yield from hog and human gastric juice (24). We have converted membrane-bound receptor for IF-B₁₂ from guinea pig ileum into a form which fulfills current criteria for solubility.

The previously well-documented characteristics of the membrane-bound receptor for IF-B₁₂, such as localization in the distal small bowel, calcium dependence, Na₂EDTA inhibitability, and pH dependence, were all established for the solubilized binder obtained from guinea pig ileal mucosa. Vitamin B₁₂ bound to inert IF was not taken up by this binder. Only vitamin B₁₂ bound to normal IF was recognized by the solubilized binder, as evidenced by the blocking experiments illustrated in Fig. 3. The identity and specificity of the binding factor in intestinal extracts was thus firmly established. Since IF-B₁₂ could be identified in the excluded volume of a typical gel filtration experiment, it seems probable that it is the entire complex and not vitamin B₁₂ alone that is bound by the receptor.

Donaldson, Mackenzie, and Trier reported the presence of a factor that bound IF-B₁₂ in the supernate obtained when hamster microvillous membranes were sedimented at 30,000 *g* (5). Centrifugation at 54,500 *g* of a similar preparation resulted in the loss of this activity. Rothenberg similarly reported the presence of a binder of IF-B₁₂ in the supernate obtained when guinea pig mucosal membranes were centrifuged at 30,000 *g* (9). Several authors have suggested that Rothenberg may not have been sedimenting tiny particles of membrane and that the binder he studied may actually have been particulate (10, 25). In 1970, Pastan, Blanchett-Mackie, and Pricer reported that even centrifugation at 100,000 *g* does not sediment all particulate matter (26). A recently published definition of a soluble molecule includes the following characteristics (27): (a) cannot be sedimented by centrifugation at 100,000 *g* for 1 h, (b) enters the included volume of a Sepharose 4-B column, and (c) contains no microfine particles of membrane on electron microscopy. By each of these criteria, the binder we have studied is soluble.

In its solubilized form, the receptor for IF-B₁₂ was amenable to studies of its composition. The heat and acid lability of the receptor suggested that it is a protein. Furthermore, receptor activity was reduced by incubation with chymotrypsin. Treatment with neuraminidase did not destroy it, suggesting that sialic acid residues are not critical to function; and treatment with

sulphydryl blockers had little effect on it, which indicates that sulphydryl groups are not involved in normal function. Finkler and Hall have demonstrated that the membrane-bound receptor for IF-B₁₂ is destroyed by dithiothreitol (28). This was also the case when the solubilized receptor was incubated with the same concentrations of dithiothreitol that they used. The effect of Triton X-100 in increasing the yield of soluble receptor of IF-B₁₂ suggests, but of course it does not prove, that it is a lipoprotein.

Cooper, studying uptake of IF-B₁₂ by everted sacs of guinea pig intestine, reported an association constant for the reaction of $0.68 \times 10^6 \text{ M}^{-1}$ (3). The association constant which we obtained with solubilized receptor was $0.76 \times 10^6 \text{ M}^{-1}$. This similarity to Cooper's data implies that the presence of residual Triton X-100 in the extract, which seems likely by extrapolation from previous reports with other systems (29), did not measurably alter at least this one parameter of receptor function.

ACKNOWLEDGMENTS

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