JCI The Journal of Clinical Investigation

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J Clin Invest. 1977;59(1):51-58. https://doi.org/10.1172/JCI108621.

Research Article

Studies were designed to evaluate the binding of binding of vitamin B12 to cell membrane preparations from human placenta. The transcobalamin II-vitamin B12 complex (TCII-B12), which has a much greater affinity for the membranes than vitamin B12 alone, binds to a single saturable binding site with an approximate Ka = 7.2 mM-1. The binding requires a divalent cation and is temperature-dependent. Free TCII can compete with TCII-B12 for the binding site but has somewhat less affinity than does TCII-B12. Rat TCII-B12 has an affinity constant that is less than one-fifth that of human TCII-B12; human TCI-B12, bovine TCII-B12, hog intrinsic factor-B12 (IF-B12), and human IF-B12 do not bind to the membranes. Pretreating the membranes with trypsin causes a marked decrease in subsequent binding; this suggests the binding site includes a relatively exposed membrane protein. These data suggest that a specific cell surface receptor for the TCII-B12 complex exists in placenta. This TCII-B12 receptor can be solubilized with Triton X-100.

A Saturable High Affinity Binding Site for Transcobalamin II-Vitamin B_{12} Complexes in Human Placental Membrane Preparations

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ABSTRACT Studies were designed to evaluate the binding of vitamin B_{12} to cell membrane preparations from human placenta. The transcobalamin II-vitamin B_{12} complex (TCII-B_{12}), which has a much greater affinity for the membranes than vitamin B_{12} alone, binds to a single saturable binding site with an approximate K_a = 7.2 mM^{-1}. The binding requires a divalent cation and is temperature-dependent. Free TCII can compete with TCII-B_{12} for the binding site but has somewhat less affinity than does TCII-B_{12}. Rat TCII-B_{12} has an affinity constant that is less than one-fifth that of human TCII-B_{12}; human TCI-B_{12}, bovine TCII-B_{12}, hog intrinsic factor-B_{12} (IF-B_{12}), and human IF-B_{12} do not bind to the membranes. Pretreating the membranes with trypsin causes a marked decrease in subsequent binding; this suggests the binding site includes a relatively exposed membrane protein. These data suggest that a specific cell surface receptor for the TCII-B_{12} complex exists in placenta. This TCII-B_{12} receptor can be solubilized with Triton X-100.

INTRODUCTION

The mechanism by which vitamin B_{12} (B_{12}),[1] a large water-soluble molecule, is transported across biological membranes is only partially understood. In the normal in vivo situation it is a B_{12}-B_{12} binding protein complex that first binds to the membrane. For example, transport of B_{12} across the intestinal wall occurs after B_{12} forms a stable complex with intrinsic factor (IF-B_{12}; reference 1); this complex then binds to specific receptors on the membranes of intestinal microvilli (2). These receptors have been solubilized from the membranes of human and guinea pig intestinal mucosal cells (3, 4). B_{12} in human blood is carried by two major transport proteins (5), transcobalamin I (TCI) and transcobalamin II (TCII). TCI carries approximately 75% of the endogenous B_{12} in human plasma (6) and binds a small portion of B_{12} newly absorbed from the intestine (7). The functional role of TCI, however, remains unknown since a congenital deficiency of TCI in humans is asymptomatic and does not result in reduced levels of vitamin B_{12} in tissues (8). By contrast, congenital TCII deficiency in humans leads to severe manifestations of B_{12} deficiency; thus, TCII appears necessary for transport of B_{12} from the gut to peripheral tissues (9, 10). A number of studies have demonstrated that TCII promotes the uptake of B_{12} by human cells in vitro (11–14). Recently, specific saturable binding sites for rat TCII-B_{12} complexes on rat liver plasma membranes have been described (15).

A particularly interesting B_{12} transport mechanism is its placental transport. The vitamin, administered intravenously, is found within minutes in the placentas of pregnant rats or mice (16, 17) and, after a lag period of several hours, slowly enters the fetal circulation. In the experiments reported here we show evidence for the existence of a specific high affinity binding site for the transcobalamin II-vitamin B_{12} complex (TCII-B_{12}) in human placental membrane preparations. We have succeeded in solubilizing this receptor with the nonionic detergent Triton X-100.

METHODS

CM-Sephadex C50 was purchased from the Sigma Chemical Co. (St Louis, Mo.), hog IF from Calbiochem (San Diego, Calif.), and ^{57}Co-cyanocobalamin (200 μCi/μg) from Amersham-Searle Corp. (Arlington Heights, Ill.).

Purification of binding proteins. For most studies, TCII was partially purified from human serum by adsorption to and elution from CM-Sephadex. For this procedure (performed at 4°C), serum (100–200 ml) was diluted with 3 vol 0.05 M

Received for publication 16 January 1976 and in revised form 27 September 1976.

[1] *Abbreviations used in this paper*: B_{12}, vitamin B_{12}; TCI, transcobalamin I; TCII, transcobalamin II; IF, intrinsic factor.

sodium phosphate, pH 5.8, and dry CM-Sephadex (1 mg/ml of diluted serum) was added. The mixture was stirred for 60 min. The CM-Sephadex, collected by centrifugation at 3,000 g for 30 min, was then washed once with 200 ml of the same buffer. TCII was eluted in 1/20 the original volume with 0.05 M sodium phosphate, pH 7.4, containing 1 M NaCl. The CM-Sephadex contains about 50% of the B_{12}-binding capacity of the serum and has been purified about eight-fold relative to serum. After binding radioactive B_{12} to aliquots of these preparations, it could be shown that the B_{12}-binding activity, when chromatographed on Sephadex G100, eluted as a single peak (see Fig. 5 *a*) in the same volume found for highly purified human TCII (see below). TCI elutes near the void volume and is clearly separated from TCII on this column; TCI contamination of these TCII preparations amounts to less than 0.2% based on total B_{12}-binding capacity. The B_{12}-binding capacity is stable when the TCII is stored as aliquots frozen at −20°C. TCII from serum of other species was also purified in this way.

For several experiments a small amount of highly purified human TCII was obtained from the human plasma concentrate Cohn fraction III, kindly supplied by Dr. Joseph Baughman, Ortho Diagnostics, Raritan, N. J., using the affinity chromatography method of Allen and Majerus (18). The TCII eluted from the Sepharose-B_{12} affinity column was carried through the DEAE-cellulose chromatography step, and the preparation, which was about 40% pure as judged by polyacrylamide disk gel electrophoresis, bound 8–10 μg B_{12}/mg protein. The preparation was divided into aliquots, bound to B_{12}, and stored at −80°C (18).

For experiments in which TCII free of B_{12} was used, an aliquot of TCII-B_{12} was thawed, and the B_{12} removed by dialysis against 7.5 M guanidine-hydrochloride and then against buffer as described by Allen and Majerus (18). The B_{12} binding capacity of this TCII was about 5 μg/mg protein.

Human TCI, free of TCII, was prepared from fresh human serum using DEAE-cellulose chromatography (19); human IF was partially purified from human gastric aspirate by the method of Flood and West (20).

Preparation of membranes. A human placental membrane fraction, containing both microsomal and surface membranes, was prepared from placental homogenates using the method described by Posner (21). Human chorionic villous surface membranes were prepared by the method of Smith et al. (22). In the latter preparation, no homogenization is performed; rather, a portion (about 5 g) of freshly delivered human placenta is spread out manually in a Petri dish and washed rapidly, first with ice-cold isotonic $CaCl_2$ solution and then with ice-cold Krebs-Ringer physiological saline. After placing the tissue in ice-cold 0.9% NaCl and stirring for 30 min, the saline wash is poured off and centrifuged at 800 g for 10 min (to remove fragments of tissue and remaining erythrocytes). The supernate is centrifuged at 10,000 g for 5 min to remove larger particles, and a higher speed centrifugation (100,000 g for 60 min) pellets the villous surface membrane. Electron microscopy (22) of the resuspended low-speed pellet shows that it consists of intact trophoblast cells which have lost their villous extensions, while electron microscopy of the high-speed pellet shows that it consists of membrane-bound bodies resembling microvilli. Although it contained microsomal membranes, the preparation from placental homogenate was used for most of our experiments because it was a more ready source of large quantities of membrane.

Binding assays. The B_{12}-binding capacities of TCII and IF preparations were assayed by the charcoal absorption method of Gottlieb et al. (23).

The binding of B_{12}, TCII-B_{12}, or IF-B_{12} to placental membranes was determined by retention of the complex on filters. In 0.5 ml, ^{57}Co-B_{12} alone or after binding to carrier protein was added to a quantity of placental membrane in 0.1 M Tris, pH 7.4, containing 0.006 M $CaCl_2$. After incubation, 5 ml of ice-cold 0.154 M NaCl was added to the reaction mixture which was then immediately filtered under reduced pressure through a Millipore EGWP (cellulose acetate, 0.2 μm pore size) filter (Millipore Corp., Bedford, Mass.). The filter was washed twice with 5 ml cold saline and assayed for radioactivity using a Nuclear-Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.) with 85% efficiency for ^{57}Co. The filters had been pretreated by soaking them in a 25-mg/ml solution of bovine serum albumin. Immediately before application of the diluted reaction mixture, the filter was washed with 5 ml of cold saline under reduced pressure. This treatment was essential to lower the nonspecific binding of B_{12} and TCII-B_{12} to the filter.

All reported data are the average of duplicate determinations from which have been subtracted the radioactivity retained on the filter from a reaction mixture lacking membranes. These control values were always less than 5% of the radioactivity retained from a complete reaction mixture and equal to those obtained from incubations with membranes in the presence of EDTA.

The binding capacities of both kinds of membrane preparations were stable for at least 1 mo as determined from aliquots stored at −20°C. The binding capacities for TCII-B_{12} of membrane preparations from different placentas were found to vary by as much as 50%.

Solubilization of the placental TCII-B_{12} receptor. The conditions used are essentially those described by Katz and Cooper (3) for the solubilization of a human ileal receptor for IF-B_{12}. Membranes (25 mg protein) were suspended in 50 ml of 0.003 M sodium phosphate, pH 8.0, containing 0.05% Triton X-100. The suspension was stirred slowly for 24 h at 4°C and then dialyzed against cold distilled water (2 liters) for 48 h with two changes of water. The nondialyzable material was centrifuged at 100,000 g for 1 h, and the supernate was lyophilized to dryness and stored as the dried powder at −20°C until use. It was then dissolved in 3.5 ml 0.1 M Tris, pH 7.4, and the resulting solution was again centrifuged at 100,000 g for 1 h. An electron microscope examination of the supernate after negative staining with phosphotungstic acid revealed no clearly identifiable membranous structures.

Assay for solubilized receptor. Either radioactive TCII-B_{12} alone or aliquots of the above supernate plus radioactive TCII-B_{12} were incubated for 60 min at 25°C and then applied to a Sephadex G-100 column (1.5 × 95 cm) which was equilibrated with 0.03 M Tris, pH 7.4, containing 0.154 M NaCl, 0.006 M $CaCl_2$, and 0.05% Triton X-100 at 4°C. The column was developed with the same buffer at a flow rate of 6 ml/h. Fractions (2.5 ml) were collected and counted for radioactivity. The radioactivity appearing with the void volume when TCII-B_{12} was incubated with the supernate was considered bound to the receptor based on a number of criteria discussed in Results.

Protein determination. Protein was measured by the method of Lowry et al. (24), using bovine serum albumin as the standard. Solubilization of membrane protein was achieved by heating at 100°C for 30 min in 1 N NaOH.

RESULTS

Binding of TCII-B_{12} to membranes appears to be saturable (Fig. 1). Consistent with saturation is the observation that a 10-fold excess of unlabeled TCII-B_{12}, added to the reaction mixture before the addition of membranes, causes at least an 87% decrease in radioactivity retained on the filter at the highest level of TCII-B_{12} tested (360 pg B_{12}). This indicates that nonspecific binding accounts for less than 5% of the total radioactivity retained by the filter at any level of TCII-B_{12} tested. To test whether the TCII-B_{12} binding curve in Fig. 1 is an artifact of the filter assay, we compared this assay to one in which the membranes were collected by centrifugation for 60 min at 100,000 g. At each point the radioactivity in the pellet obtained from centrifugation equaled the radioactivity retained on the filter.

The affinity of B_{12} for the membranes is less than that of TCII-B_{12}; in fact the binding may take place at a different site on the membranes (Fig. 1). When radioactive B_{12} is mixed with a 10-fold excess of unlabeled TCII-B_{12}, binding of the radioactive B_{12} is equal to that in the absence of TCII-B_{12}. Also in accord with a second binding site is the observation that a 50-fold excess of unlabeled B_{12} does not interfere with the binding of labeled TCII-B_{12}. During the prior incubations in these experiments, there is no exchange of free B_{12} with B_{12} already bound to TCII.

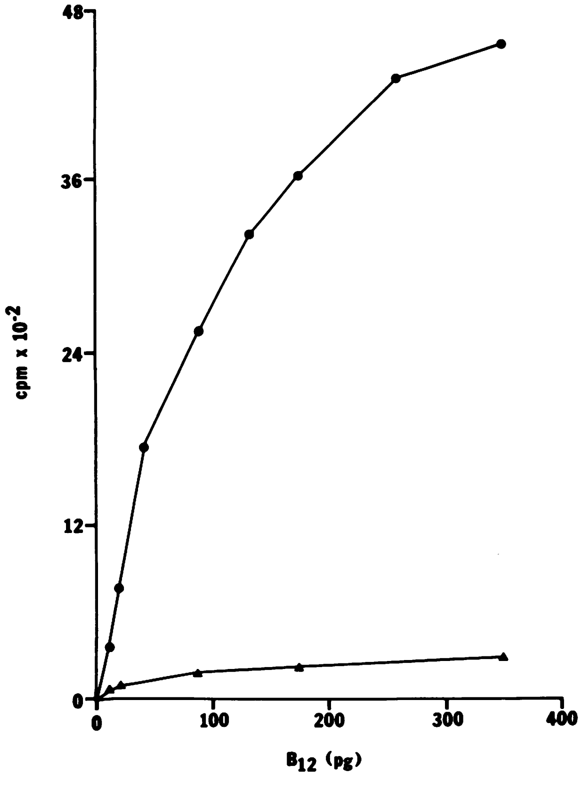

FIGURE 1 The concentration dependence of the binding of TCII-B_{12} and B_{12} to human placental membranes. Placental membranes (240 μg) were incubated for 60 min at 25°C with either TCII-B_{12} or B_{12} alone in 0.5 ml of 0.1 M Tris, pH 7.4, containing 0.006 M $CaCl_2$. Concentrations are expressed as picograms B_{12} either bound to TCII or free, and binding is expressed as the radioactivity of B_{12} retained on the filter. The partially purified TCII preparation in this experiment had a protein concentration of 3.9 mg/ml, 1 ml bound 1,730 pg B_{12} (2.61×10^5 cpm). (●) TCII-B_{12}; (▲) B_{12} alone.

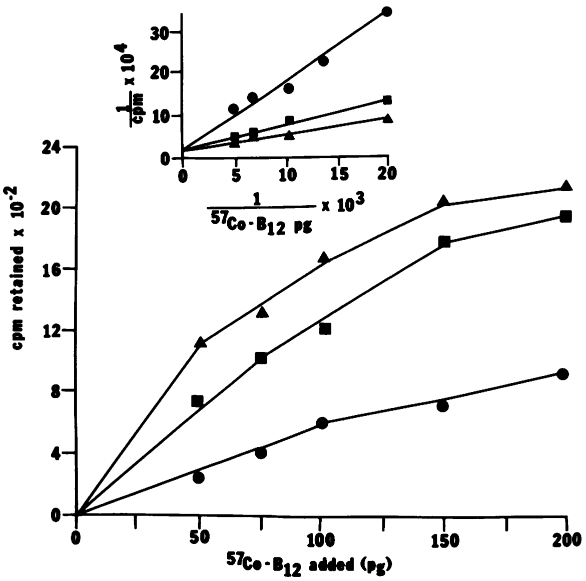

FIGURE 2 Inhibition of the binding of radioactive TCII-B_{12} by either excess free TCII or nonradioactive TCII-B_{12}. Conditions are as in Fig. 1 with the following exceptions: 100-μg placental membranes were used; the TCII used was the 40% pure preparation described in Methods; bovine serum albumin (7 mg/ml) was present. TCII-^{57}Co-B_{12} was either incubated alone at the levels indicated or was premixed at each of these levels with nonradioactive TCII-B_{12} (500 pg B_{12}) or with free TCII (B_{12} binding capacity = 500 pg). (▲) TCII-^{57}Co-B_{12} alone; (■) + free TCII; (●) + nonradioactive TCII-B_{12}. *Inset*, double reciprocal plot of the data.

The ability of TCII alone to inhibit the binding of TCII-B_{12} to the membranes was tested (Fig. 2). As shown, TCII inhibits the binding of TCII-B_{12} but is somewhat less effective as an inhibitor of the binding of the radioactive TCII-B_{12} than is unlabeled TCII-B_{12}. The expected competitive nature of the inhibition is shown in the double reciprocal plot (inset, Fig. 2). These data indicate that B_{12} increases the affinity of TCII for the membrane binding site either by altering the conformation of the TCII or by directly interacting with the binding site or both. A decrease in the Stokes radius of both IF and TCII upon binding B_{12} has been reported (25). It should be noted that, while B_{12} increases the affinity of TCII for the membranes, TCII alone still has appreciable affinity for the binding site; in fact, an approximate affinity constant of about 2 nM^{-1} can be calculated from the data in Fig. 2. This K_a is within a factor of 4 of the K_a for TCII-B_{12} (See below).

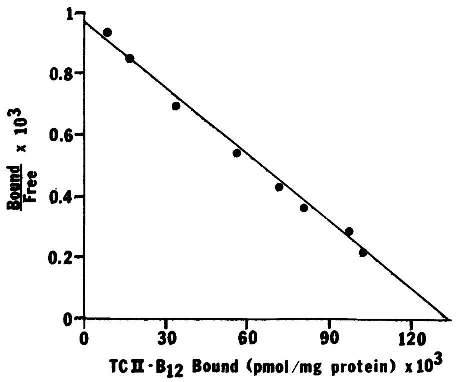

FIGURE 3 Scatchard analysis of TCII-B_{12} binding curve of Fig. 1. The membrane-bound TCII-B_{12} in picomoles per milligram membrane protein divided by free TCII-B_{12} in picomolar concentration was plotted on the ordinate against membrane-bound TCII-B_{12} on the abscissa.

When the placental membranes are preincubated for 10 min at 37°C with 0.006 M EDTA, binding is abolished in the subsequent incubation with TCII-B_{12}, suggesting a divalent cation requirement. The need for divalent cations is demonstrated by the retention of 80–90% of binding if 0.012 M $CaCl_2$ is included in the incubations. A similar requirement has been demonstrated for the binding of IF-B_{12} to ileal microvilli (26). If, after the usual incubation of TCII-B_{12} with membranes, EDTA (0.006 M) is added for an additional incubation at 37°C for 30 min, no binding is found; thus, it appears that the membrane TCII-B_{12} complex is destroyed in the presence of EDTA.

A Scatchard analysis of the data in Fig. 1 indicates a single class of binding sites with high affinity for TCII-B_{12} (K_a = 7.2 nM^{-1}; Fig. 3). Since the TCII preparation used certainly contains some TCII complexed in vivo with nonradioactive B_{12}, the total amount of TCII-B_{12} assayed at each point actually equals the sum of the TCII-^{57}Co-B_{12} plus the nonradioactive complex. Although it can be calculated from the yield of B_{12}-binding capacity in the TCII preparation and from reported values for B_{12} levels and B_{12}-binding capacity in human serum (19) that the contribution of endogenous TCII-B_{12} is almost certainly less than 20% of the total B_{12}-binding capacity of the TCII preparation, since the endogenous TCII-B_{12} has not been considered in the calculations, the affinity constant should be regarded as an approximation. That it is a close approximation is suggested by the fact that a less detailed binding curve, obtained with the TCII eluted from Sepharose-B_{12} (and thus, free of endogenous TCII-B_{12}), gave a K_a of about 7–10 nM^{-1}. An approximate K_a = 6 nM^{-1} for the binding of IF-B_{12} to the receptor in human intestinal mucosa (3) and one of 5.5 nM^{-1} for the binding of rat TCII-B_{12} to rat liver plasma membranes have been reported (15).

The closeness of the K_a of the placental microsomal membrane preparation and the surface membrane preparation of rat liver is compatible with the interpretation that the binding of TCII-B_{12} is to placental surface membrane. Such receptors for the large, water-soluble TCII-B_{12} complex may well exist in the surface membranes of many or all cell types. Nonetheless, the microsomal membrane preparation used here contains other membranes (especially endoplasmic reticulum) than surface membranes; thus, several experiments were done in an attempt to document that binding to placental surface membrane is occurring. One approach involved measuring the binding of radioactive B_{12} and TCII-B_{12} to intact trophoblast cells after 5-min incubations in isotonic saline at 25°C. Here, too, at least 10 times more TCII-B_{12} (calculated in picograms B_{12}) bound to the cells than did B_{12} alone. While the experiments demonstrated the presence of a saturable binding site for TCII-B_{12}, there was much more nonspecific (unsaturable) binding with both B_{12} and TCII-B_{12}, amounting to as much as 50–75% of the total radioactivity bound. This (and the fact that intact cells internalize the bound label) made only a rough measurement of affinity possible ($K_a \approx$ 2–3 nM^{-1}).

In another series of experiments a study of the binding of TCII-B_{12} to placental subcellular fractions was attempted. The fact that human placenta contains both cytotrophoblast and syncytiotrophoblast cells with different sized organelles and distinct villous surfaces (27) makes subcellular fractionization most difficult. Despite this, it has been possible to obtain highly enriched nuclear and mitochondrial fractions (as judged by electron microscope and enzyme marker criteria). When equivalent amounts of membrane protein were incubated with TCII-B_{12}, the nuclear fraction had less than 5% and the mitochondrial fraction less than 12% of the TCII-B_{12}-binding capacity of the microsomal membrane preparation. The microsomal pellet which contains the binding site for TCII-B_{12} is composed of small pieces of

TABLE I

Comparison of the Binding of TCII-B_{12} and B_{12} by the Resuspended Microsomal Preparation and by the Placental Villous Surface Membrane Preparation

Membrane source	Binding, *cpm* retained on filter: TCII-B_{12}	B_{12} alone
Resuspended microsomal pellet	3,330	179
Placental villous surface membranes	3,661	121

Each incubation contained 165 μg of membrane protein and 100 pg B_{12}, either free or bound to TCII. Assay conditions were as described in Fig. 1.

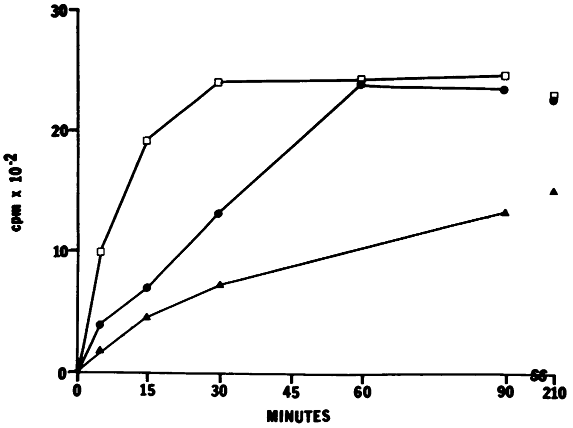

FIGURE 4 Temperature dependence of binding of TCII-B_{12} to placental membranes. Conditions were as in Fig. 1 except that incubations were carried out at 0° (▲), 25° (●), and 37°C (■). The TCII-B_{12} added to each incubation was 230 μg protein which bound 100 pg B_{12} (1.57×10^4 cpm).

endoplasmic reticulum as well as small pieces of cell surface membrane from both trophoblast cell types. Many attempts to obtain an enriched cell surface membrane preparation from this fraction, using discontinuous sucrose density gradient centrifugation, were unsuccessful. An alternative method for preparing human placental villous surface membrane, involving a simple salt extraction of the tissue (22), was used, and its affinity for TCII-B_{12} was compared to that of the resuspended microsomal pellet (Table I). As shown, the villous surface membranes also have a much greater affinity for TCII-B_{12} than for B_{12} alone. In addition, they bind about 10% more TCII-B_{12} per milligram protein and only about two-thirds as much free B_{12} per milligram protein as does the microsomal preparation. When the binding of the surface villous membranes to different concentrations of TCII-B_{12} was assayed and the results subjected to Scatchard analysis, a single class of binding sites with an approximate $K_a = 6.7\ nM^{-1}$ was observed. Thus, these surface villous membranes appear to have the same receptor for TCII-B_{12} as do the membranes obtained from the resuspended microsomal pellet. Whether an indistinguishable (perhaps identical) binding site is present on the endoplasmic reticulum in the microsomal preparation cannot be determined from the data.

The temperature dependence of binding of TCII-B_{12} to the membranes is shown in Fig. 4. Maximum binding is reached at 30 min when incubation is at 37°C and by 60 min when at 25°C. At 0°C maximum binding is not achieved during incubations as long as 210 min.

The specificity of the interaction of human TCII to the placental membrane preparation was examined by testing the binding to the membranes of other B_{12}–B_{12}-binding protein complexes. With rat TCII-B_{12} the affinity constant for human placental membranes is less than one-fifth that of human TCII-B_{12}. Bovine TCII-B_{12}, human TCI-B_{12}, hog IF-B_{12}, and human IF-B_{12} do not bind to the human placental membranes.

The effects of prior treatment of the membranes with enzymes were examined (Table II). Trypsin (10 μg/ml) caused a marked decrease in subsequent binding, while neuraminidase (50 μg/ml) caused a modest decrease. Phospholipase C has no effect. The observations are quantitatively similar to those reported for insulin receptors in the placenta and suggest that the binding site includes a relatively exposed membrane protein (4).

Using a procedure similar to that described for the solubilization of the receptor for IF-B_{12} from human intestine (10), we have solubilized the TCII-B_{12} receptor from a placental membrane preparation (Fig. 5). When the solubilized membrane preparation is incubated with radioactive TCII-B_{12} and the reaction mixture passed over a Sephadex G-100 column, a significant amount of radioactivity appears in the void volume of the column (Fig. 5B). This indicates binding since TCII-B_{12} itself is included completely by Sephadex G-100 (Fig. 5A). If EDTA is included in the incubation of the solubilized membrane preparation with radioactive TCII-B_{12}, no radioactivity appears in

TABLE II

Effect of Enzyme Pretreatment of the Placental Membranes on Their Subsequent Interaction with TCII-B_{12}

Enzymes	Concentration	Specific binding, % of control
	μg/ml	%
None	—	100
Trypsin	10	14
Phospholipase C	100	105
Neuraminidase	50	85

Placental membranes (3.9 mg) were incubated in a final volume of 5 ml of 0.1 M Tris buffer, pH 7.4, with the various enzymes at the concentrations indicated. After 60 min at 37°C, the reaction mixtures were cooled on ice and then centrifuged at 100,000 *g* for 45 min. Soybean trypsin inhibitor (30 μg/ml) was added before centrifugation to the trypsin-containing incubation. The membranes were resuspended in 5 ml of the buffer, and their binding capacities assayed in an incubation mixture containing 0.2 ml of the membrane suspension, 0.1 ml TCII-^{57}Co B_{12} (125 pg B_{12} bound; 29,180 cpm), 0.05 M NaCl saline, and 0.006 M $CaCl_2$ in a volume of 0.45 ml. Binding was determined by the filtration assay after incubation for 30 min at 37°C. The membrane samples without enzyme (controls) bound 2,700 cpm. Controls to which soybean trypsin inhibitor (30 μg/ml) was added before centrifugation also bound 2,700 cpm.

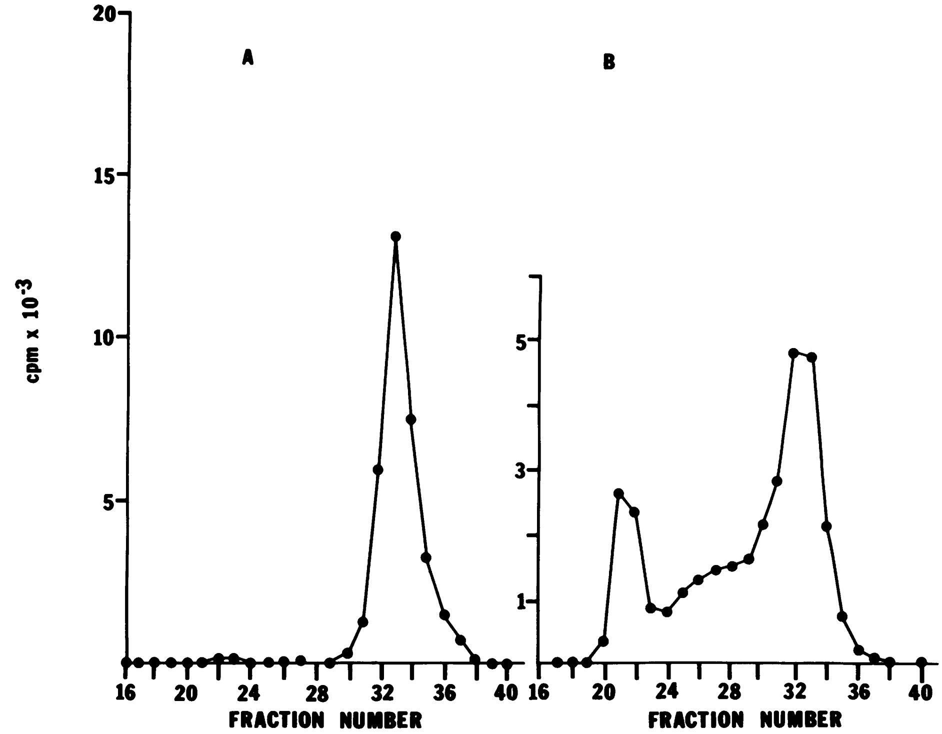

FIGURE 5 Column chromatography of TCII-B_{12} or of a mixture of TCII-B_{12} with a Triton X-100-treated placental membrane preparation. Conditions were as described in Methods. (A) Radioactive TCII-B_{12} alone; 550 pg B_{12} with a specific activity of 220 μCi/μg was prebound to 1.2 mg TCII protein which was applied to the column in a vol of 1 ml. (B) Radioactive TCII-B_{12}, the same concentration as in A plus supernate (0.5 ml containing 0.98 mg protein) from the solubilization procedure described in Methods. The recovery of radioactivity applied in both A and B was 80%.

the void volume. About 85% of the binding activity of an aliquot of placental membranes is recovered in an equivalent aliquot of the solubilized preparation. If radioactive B_{12} alone is incubated with the solubilized membrane preparation, the radioactivity elutes from the Sephadex G-100 column as a single free B_{12} peak. This indicates not only that the binding of B_{12} to the soluble receptor requires TCII as well as a divalent cation but also suggests that the radioactivity seen in the void volume in Fig. 5B does not result from the shifting of B_{12} bound to TCII to a binding site on a larger intracellular B_{12}-binding protein which might have been carried with the membranes during their preparation.

DISCUSSION

Because of its molecular weight and water solubility, vitamin B_{12} should not easily diffuse through cell membranes. A specific receptor in the intestine for IF-B_{12} has been reported that mediates transport at this site (2), and a receptor for TCII-B_{12} complexes exists in rat liver plasma membranes (15). The high affinity-specific binding site for TCII-B_{12} present in membrane preparations from human placenta resembles the liver membrane receptor in that the K_a is similar and it is the only high affinity receptor for the TCII-B_{12} complex present in either membrane preparation.

One difference between the two preparations is that the rat liver plasma membrane preparation binds as much free B_{12} as TCII-B_{12}, while free B_{12} has little affinity for either of the placental membrane preparations reported here. The binding of free B_{12} by the rat liver membranes was specific and saturable but apparently irreversible. We have not studied the nature of the binding of free B_{12} to the placental membranes but it appears to be saturable (Fig. 1). The reason for the greater binding of free B_{12} by the rat liver membrane preparation is not apparent. As stated by Fiedler-Nagy et al. (15), since virtually all (99%) of the B_{12}, in the blood of rats is bound to TCII (28), rat liver cells contact little free B_{12} and, while there may well be a second mechanism for the uptake of free B_{12} it is quantitatively unimportant. That such a mechanism also exists in humans is indicated by the fact that patients with congenital deficiency of TCII can be successfully treated with large doses of free B_{12} (10).

The binding of TCII-B_{12} to the rat liver membranes was not significantly enhanced by including Ca^{++} in the incubation (15). In our incubations, omission of Ca^{++} caused only a 10–15% decrease in binding. Since EDTA abolished binding and since addition of Ca^{++} in excess of EDTA restores it, our membrane preparations presumably contain sufficient divalent cation to give nearly maximal binding. The rat liver membranes may also contain sufficient divalent cation to allow for maximum TCII-B_{12} binding; thus, incubations with EDTA, which were not reported for the rat liver membranes (15), would have to be performed to demonstrate a divalent cation requirement. The role the divalent cation plays is unknown. While it may alter membrane conformation and indirectly facilitate receptor-TCII-B_{12} interaction, the observation that EDTA abolishes the binding of the solubilized receptor with TCII-B_{12} suggests a more direct role.

Marginal B_{12} intake during gestation in the rat causes lowered birth weights in the newborns compared to newborns from mothers supplemented with B_{12}; this difference persisted throughout the 1st yr even if all newborns received adequate dietary B_{12} (29). At 1 yr the offspring of mothers not supplemented with B_{12} had less resistance to infection by *Salmonella typhimurium* and possessed less liver aminopyrine demethylase and glucose-6-phosphatase activities. Ullberg (30) has speculated that defects of placental B_{12} transport may result in fetal wastage and anomalies. The presence of a congenital defect in intestinal transport of IF-B_{12} (31, 32) suggests that similar defects might be present in placental transport of TCII-B_{12}.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grant HD 08884 from the National Institute of Child Health and Development. Dr. Friedman is the recipient of a Research Career Development Award from the National Institute of Child Health and Development (U. S. Public Health Service grant HD 00023).

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