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

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ABSTRACT Studies were designed to evaluate the binding of vitamin B\textsubscript{12} to cell membrane preparations from human placenta. The transcobalamin II-vitamin B\textsubscript{12} complex (TCII-B\textsubscript{12}), which has a much greater affinity for the membranes than vitamin B\textsubscript{12} alone, binds to a single saturable binding site with an approximate $K_d = 7.2$ mM$^{-1}$. The binding requires a divalent cation and is temperature-dependent. Free TCII can compete with TCII-B\textsubscript{12} for the binding site but has somewhat less affinity than does TCII-B\textsubscript{12}. Rat TCII-B\textsubscript{12} has an affinity constant that is less than one-fifth that of human TCII-B\textsubscript{12}; human TCII-B\textsubscript{12}, bovine TCII-B\textsubscript{12}, hog intrinsic factor-B\textsubscript{12} (IF-B\textsubscript{12}), and human IF-B\textsubscript{12} do not bind to the membranes. Pre-treating 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\textsubscript{12} complex exists in placenta. This TCII-B\textsubscript{12} receptor can be solubilized with Triton X-100.

INTRODUCTION

The mechanism by which vitamin B\textsubscript{12} (B\textsubscript{12})\textsuperscript{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\textsubscript{12}-B\textsubscript{12} binding protein complex that first binds to the membrane. For example, transport of B\textsubscript{12} across the intestinal wall occurs after B\textsubscript{12} forms a stable complex with intrinsic factor (IF-B\textsubscript{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\textsubscript{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\textsubscript{12} in human plasma (6) and binds a small portion of B\textsubscript{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\textsubscript{12} in tissues (8). By contrast, congenital TCII deficiency in humans leads to severe manifestations of B\textsubscript{12} deficiency; thus, TCII appears necessary for transport of B\textsubscript{12} from the gut to peripheral tissues (9, 10). A number of studies have demonstrated that TCII promotes the uptake of B\textsubscript{12} by human cells in vitro (11–14). Recently, specific saturable binding sites for rat TCII-B\textsubscript{12} complexes on rat liver plasma membranes have been described (15).

A particularly interesting B\textsubscript{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\textsubscript{12} complex (TCII-B\textsubscript{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 $\mu$Ci/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
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 B12-binding capacity of the serum and has been purified about eight-fold relative to serum. After binding radioactive B12 to aliquots of these preparations, it could be shown that the B12-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). TCII eluted near the void volume and is clearly separated from TCII on this column; TCII contamination of these TCII preparations amounts to less than 0.2% based on total B12-binding capacity. The B12-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-B12 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 B12/mg protein. The preparation was divided into aliquots, bound to B12, and stored at −80°C (18).

For experiments in which TCII free of B12 was used, an aliquot of TCII-B12 was thawed, and the B12 removed by dialysis against 7.5 M guanidine-hydrochloride and then against buffer as described by Allen and Majerus (18). The B12 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 CaCl2 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 B12-binding capacities of TCII and IF preparations were assayed by the charcoal absorption method of Gottlieb et al. (23).

The binding of B12, TCII-B12, or IF-B12 to placental membranes was determined by retention of the complex on filters. In 0.5 ml, 57Co-B12 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 CaCl2. 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 57Co. 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 B12 and TCII-B12 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-B12 of membrane preparations from different placentas were found to vary by as much as 50%.

Solubilization of the placental TCII-B12 receptor. The conditions used are essentially those described by Katz and Cooper (3) for the solubilization of a human ileal receptor for IF-B12. 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-B12 alone or aliquots of the above supernate plus radioactive TCII-B12 were incubated for 60 min at 25°C and then applied to a Sephadex G-100 column (1.5 x 95 cm) which was equilibrated with 0.03 M Tris, pH 7.4, containing 0.154 M NaCl, 0.006 M CaCl2, 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-B12 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-B12 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.

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 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). (C) TCII-B$_{12}$; (A) 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 pre-mixed at each of these levels with nonradioactive TCII-B$_{12}$ (500 pg B$_{12}$) or with free TCII (B$_{12}$ binding capacity = 500 pg). (A) TCII-$^{57}$Co-B$_{12}$ alone; (●) + free TCII; (○) + nonradioactive TCII-B$_{12}$. Inset, double reciprocal plot of the data.
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 ($K_a$ = 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

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<td><strong>Comparison of the Binding of TCII-B$<em>{12}$ and B$</em>{12}$ by the Resuspended Microsomal Preparation and by the Placental Villous Surface Membrane Preparation</strong></td>
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| 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.
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-B₁₂ 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-B₁₂ than for B₁₂ alone. In addition, they bind about 10% more TCII-B₁₂ per milligram protein and only about two-thirds as much free B₁₂ per milligram protein as does the microsomal preparation. When the binding of the surface villous membranes to different concentrations of TCII-B₁₂ was assayed and the results subjected to Scatchard analysis, a single class of binding sites with an approximate Kₘ = 6.7 nM⁻¹ was observed. Thus, these surface villous membranes appear to have the same receptor for TCII-B₁₂ 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-B₁₂ 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 B₁₂-B₁₂ binding protein complexes. With rat TCII-B₁₂ the affinity constant for human placental membranes is less than one-fifth that of human TCII-B₁₂. Bovine TCII-B₁₂, human TCII-B₁₂, hog IF-B₁₂, and human IF-B₁₂ 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-B₁₂ from human intestine (10), we have solubilized the TCII-B₁₂ receptor from a placental membrane preparation (Fig. 5). When the solubilized membrane preparation is incubated with radioactive TCII-B₁₂ 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-B₁₂ 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-B₁₂, no radioactivity appears in

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<th>Enzymes</th>
<th>Concentration</th>
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<tr>
<td>None</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>100</td>
<td>105</td>
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<td>Neuraminidase</td>
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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⁻²⁵⁰Co B₁₂ (125 pg B₁₂ bound; 29,180 cpm), 0.05 M NaCl saline, and 0.006 M CaCl₂ 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.
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

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**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 $\mu$Ci/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%.
was not significantly enhanced by including Ca\textsuperscript{2+} in the incubation (15). In our incubations, omission of Ca\textsuperscript{2+} caused only a 10–15% decrease in binding. Since EDTA abolished binding and since addition of Ca\textsuperscript{2+} 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\textsubscript{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\textsubscript{12} interaction, the observation that EDTA abolishes the binding of the solubilized receptor with TCII-B\textsubscript{12} suggests a more direct role.

Marginal B\textsubscript{12} intake during gestation in the rat causes lowered birth weights in the newborns compared to newborns from mothers supplemented with B\textsubscript{12}; this difference persisted throughout the 1st yr even if all newborns received adequate dietary B\textsubscript{12} (29). At 1 yr the offspring of mothers not supplemented with B\textsubscript{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\textsubscript{12} transport may result in fetal wastage and anomalies. The presence of a congenital defect in intestinal transport of IF-B\textsubscript{12} (31, 32) suggests that similar defects might be present in placental transport of TCII-B\textsubscript{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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