Human Plasma R-Type Vitamin $B_{12}$-binding Proteins

II. THE ROLE OF TRANSCOBALAMIN I, TRANSCOBALAMIN III, AND THE NORMAL GRANULOCYTE VITAMIN $B_{12}$-BINDING PROTEIN IN THE PLASMA TRANSPORT OF VITAMIN $B_{12}$*

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The normal human granulocyte vitamin $B_{12}$-binding protein, transcobalamin I, and transcobalamin III, have been labeled with $^{125}$I-labeled N-succinimidyl 3-(4-hydroxyphenyl)propionate and utilized for plasma clearance studies performed with rabbits. Both moieties of $^{125}$I-labeled granulocyte vitamin $B_{12}$-binding protein-$[^{57}$Co$]v$itamin $B_{12}$ were cleared rapidly from the plasma (>90% by 5 min) by the liver. After 80 min, the bulk of the $^{125}$I reappeared in the plasma in small molecular weight (<1000) form and was rapidly excreted in the urine. After 60 min the bulk of the $[^{57}$Co$]v$itamin $B_{12}$ reappeared in the plasma bound to rabbit transcobalamin II and was subsequently taken up by a variety of tissues. Approximately 15% of the $^{125}$I-labeled transcobalamin I-$[^{57}$Co$]v$itamin $B_{12}$ was excreted intact into the bile during the period from 10 to 80 min after injection. The hepatic uptake of the protein-vitamin $B_{12}$ complex was blocked by the prior injection of desialyzed fetuin but not by native fetuin. Similar results were obtained with $^{125}$I-labeled transcobalamin III-$[^{57}$Co$]v$itamin $B_{12}$. Approximately 90% of both moieties of $^{125}$I-labeled transcobalamin I-$[^{57}$Co$]v$itamin $B_{12}$ had prolonged plasma survivals similar to that of $^{125}$I-labeled bovine serum albumin. After treatment with neuraminidase, both moieties of the $^{125}$I-labeled transcobalamin I-$[^{57}$Co$]v$itamin $B_{12}$ complex were cleared rapidly from the plasma by the liver in a manner that was indistinguishable from that observed in the case of untreated granulocyte vitamin $B_{12}$-binding protein and transcobalamin III.

These observations indicate that desialyzed transcobalamin I and the native forms of the granulocyte vitamin $B_{12}$-binding protein and transcobalamin III are cleared from plasma by the mechanism elucidated by Ashwell and Morell (Ashwell, G., and Morell, A. G. (1974) Adv. Enzymol. 41, 99-128) that is capable of clearing a wide variety of asialoglycoproteins. These observations have implications concerning the function of the human R-type vitamin $B_{12}$-binding proteins, the nature of the enterohepatic circulation of vitamin $B_{12}$, the biologic significance of the mechanism described by Ashwell and Morell, and the etiology of the increased plasma concentration of human R-type protein that occurs frequently in chronic myelogenous leukemia and occasionally in hepatocellular carcinoma and other solid tumors.

Human plasma contains three vitamin $B_{12}$-binding proteins that are referred to as transcobalamin I, II, and III (1-5). Transcobalamin II is immunologically distinct from the other two proteins, has a molecular weight of 38,000 based on gel filtration (2, 3), and is not a glycoprotein (5). Transcobalamin II facilitates the cellular uptake of vitamin $B_{12}$ by a variety of cells (6-9). Transcobalamin II contains only 10 to 25% of the total plasma vitamin $B_{12}$ (1, 10), but $[^{57}$Co$]v$itamin $B_{12}$ bound to this protein is cleared rapidly (11, 12) from human plasma with a $t_{1/2}$ of 5 to 90 min. Recent studies (13) performed in rabbits with $^{125}$I-labeled rabbit and human transcobalamin II-$[^{57}$Co$]v$itamin $B_{12}$ indicate that both moieties of the transcobalamin II-vitamin $B_{12}$ complex are cleared from the plasma with a $t_{1/2}$ of 60 to 90 min, that the transcobalamin II moiety is degraded during this process, and that a significant amount of the vitamin $B_{12}$ recirculates. The importance of transcobalamin II in vitamin $B_{12}$ transport has been clearly established by the recent report by Hakami et al. (14) that congenital transcobalamin II deficiency results in a severe megaloblastic anemia that responds only to large, frequent injections of vitamin $B_{12}$.

Transcobalamin I and transcobalamin III belong to a group of immunologically indistinguishable proteins known as the R-type vitamin $B_{12}$-binding proteins (1-3). R-type proteins contain 33 to 40% carbohydrate and have apparent molecular weights in the range of 120,000 to 150,000 based on gel filtration. Transcobalamin I contains more sialic acid (18...
Particular attention was paid to the role of the liver in clearing plasma transport of vitamin B₁₂, transcobalamin I, transcobal-binding proteins. Apparent congenital deficiencies of all R-type vitamin B₁₂-binders who appeared hematologically normal despite having albumin (11, 12). Carmel and Herbert (15) have reported two plasma with a t₁/₂ of 9 to 12 days that is similar to that of albumin (11, 12). Artemis and Herbert (15) have reported two brothers who appeared hematologically normal despite having apparent congenital deficiencies of all R-type vitamin B₁₂-binding proteins.

In order to elucidate the role of the R-type proteins in the plasma transport of vitamin B₁₂, transcobalamin I, transcobalamin III, and the normal granulocyte vitamin B₁₂-binding protein have been labeled with ¹³¹I, saturated with [¹⁰⁰Co]vitamin B₁₂, and administered intravenously to rabbits. Particular attention was paid to the role of the liver in clearing these glycoprotein-vitamin B₁₂ complexes, since Ashwell and Morell (16) have described a process in which many asialoglycoproteins compete for hepatic uptake and subsequent catabolism and because an extensive literature exists (see Glass (17) for a review) that suggests, indirectly, that R-type vitamin B₁₂-binding proteins might play some role in the delivery of vitamin B₁₂ to the liver.

EXPERIMENTAL PROCEDURES

Materials

N-succinimidyl 3-(4-hydroxyphenyl)propionate was obtained from Pierce, Vibrio cholerae neuraminidase from Calbiochem, and fetuin and gelatin, type I, from Sigma. Other materials were obtained as described in the accompanying paper in this series (1) or from commercial sources.

Methods

Labeling of Proteins with ¹³¹I and ¹²⁵I—N-succinimidyl 3-(4-hydroxyphenyl)propionate was obtained from Pierce, Vibrio cholerae neuraminidase from Calbiochem, and fetuin and gelatin, type I, from Sigma. Other materials were obtained as described in the accompanying paper in this series (1) or from commercial sources.

Removal of Sialic Acid with Neuraminidase—All incubations were performed at 22° for 72 hours in a toluene atmosphere at pH 6.5 in 0.025 M Na₂HPO₄, 0.006 M citric acid, and 0.002 M CaCl₂. The concentration of Vibrio cholerae neuraminidase was 1.25 units/ml. Total sialic acid, i.e., bound and free, was assayed by the thiobarbiturate method of Warren (19) after hydrolysis in 1 N HCl for 1 min at 100°. Free sialic acid was assayed in the same way except that the hydrolysis step was omitted.

Fetuin was incubated at a concentration of 50 mg/ml. Greater than 95% of the sialic acid was in the free form at the end of the incubation. Fetuin utilized for control experiments was incubated in the absence of neuraminidase. Less than 5% of the total sialic acid was liberated under these conditions.

¹²⁵I-Labeled transcobalam I—[¹⁰⁰Co]vitamin B₁₂ was incubated at a concentration of bound [¹⁰⁰Co]vitamin B₁₂ of 4 μg/μl (approximately 0.2 mg of protein/ml). The sample was subsequently subjected to gel filtration on Sephadex G-150 as described above. The elution profiles of ¹²⁵I and ¹⁰⁰Co were indistinguishable from those observed with ¹²⁵I-labeled transcobalam I—[¹⁰⁰Co]vitamin B₁₂ incubated in the absence of neuraminidase, except that the apparent molecular weight of the treated sample (based on both ¹²⁵I and ¹⁰⁰Co) was 138,000, as compared with a value of 150,000 observed with both unincubated and control incubated preparations. Sufficient transcobalam I was not available for direct measurements of free and bound sialic acid. It has been demonstrated previously (20) that the incubation conditions employed here do result in the release of greater than 90% of the sialic acid bound to the human milk and saliva R-type vitamin B₁₂-binding proteins; less than 10% release was observed when these proteins were incubated in the absence of neuraminidase.

Other Methods—¹²⁵I, ¹⁰⁰Co, and ¹⁳¹I were assayed on a dual channel Packard Auto-Gramma counter as described elsewhere (13). All other methods were performed as described in the accompanying paper in this series (1).

RESULTS

Labeling of Proteins with ¹²⁵I and ¹³¹I—When the ¹²⁵I-labeled human granulocyte vitamin B₁₂-binding protein was saturated with [¹⁰⁰Co]vitamin B₁₂ and applied to a column of Sephadex G-150 the elution profile presented in Fig. 1A was obtained. Similar profiles were observed with corresponding preparations.
of transcobalamin I and transcobalamin III (data not presented). Greater than 98% of the $^{125}$I and $^{55}$Co eluted in an asymmetrical peak that centered on Fraction 28. Greater than 98% of the $^{125}$I and $^{55}$Co present in Fractions 25 to 29 were adsorbed by rabbit anti-human milk R-type vitamin B$_{12}$-binding protein-Sepharose; less than 5% were adsorbed by rabbit anti-human transcobalamin II-Sepharose, or by vitamin B$_{12}$-Sepharose. Fractions 25 to 29 were pooled and utilized for in vivo experiments. Similar fractions with identical adsorption characteristics were pooled for transcobalamin I and transcobalamin III. The respective molar ratios of $^{125}$I to $^{55}$Covitamin B$_{12}$ were 0.18, 0.21, and 0.20 for these preparations of double-labeled granulocyte vitamin B$_{12}$-binding protein, transcobalamin I, and transcobalamin III. Rechromatography of portions of these preparations on Sephadex G-150 resulted in single symmetrical peaks of radioactivity that contained both the $^{125}$I and the $^{55}$Co-vitamin B$_{12}$ as shown in Fig. 1B for the granulocyte vitamin B$_{12}$-binding protein. The apparent molecular weight of all three protein-vitamin B$_{12}$ complexes, based on $^{125}$I and $^{55}$Co-vitamin B$_{12}$, was 150,000 which is the same value that was observed previously (1) for all three of the noniodinated protein-$^{55}$Co-vitamin B$_{12}$ complexes.

Examination of Fig. 1A reveals that an excess of $^{55}$Co-vitamin B$_{12}$ over $^{125}$I was present in Fractions 30 to 35. Greater than 90% of the $^{125}$I, but only approximately 50% of the $^{55}$Co-vitamin B$_{12}$, was adsorbed by rabbit anti-human R-type protein-Sepharose. Neither isotope in this region was adsorbed by vitamin B$_{12}$-Sepharose, or rabbit anti-human transcobalamin II-Sepharose. When Fractions 30 to 35 were rechromatographed on Sephadex G-150, approximately 50% of the $^{55}$Co-vitamin B$_{12}$, eluted as free vitamin B$_{12}$. The nature of this phenomenon has not been established.

$^{125}$I-Labeled preparations of apo-human granulocyte vitamin B$_{12}$-binding protein, apo-transcobalamin I and apo-transcobalamin III eluted from Sephadex G-150 as single symmetrical peaks of radioactivity with apparent molecular weights of 150,000. Greater than 90% of the $^{125}$I in each preparation was selectively adsorbed by rabbit anti-R-type protein-Sepharose. From 60 to 70% of the $^{125}$I was adsorbed by vitamin B$_{12}$-Sepharose; less than 10% was adsorbed when a 2-fold excess of free vitamin B$_{12}$ was added to the proteins 30 min before they were applied to vitamin B$_{12}$-Sepharose. The inability to achieve 100% binding of apo-R-type protein to vitamin B$_{12}$-Sepharose is consistent with previous observations (1, 21) that indicate that a portion (30 to 50%) of all R-type proteins are denatured, i.e., they lose the ability to bind vitamin B$_{12}$ when they are renatured from guanidine in the absence of vitamin B$_{12}$.

**Hepatic Uptake of $^{125}$I-Labeled Vitamin B$_{12}$-binding Proteins Containing Bound $^{55}$Co-vitamin B$_{12}$**—The data presented in Table I reveals that both moieties of the $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein-$^{55}$Co-vitamin B$_{12}$ complex and the $^{125}$I-labeled transcobalamin III-$^{55}$Co-vitamin B$_{12}$ complex were cleared rapidly (>80% by 5 min) from rabbit plasma by the liver. The hepatic uptakes of $^{125}$I and $^{55}$Co were markedly inhibited by the prior injection of desialyzed fetuin, but not by native fetuin. $^{55}$Co-vitamin B$_{12}$ bound to the unpurified vitamin B$_{12}$-binding protein released from granulocytes by incubation with LiCl at 24°C (1) was taken up by the liver in a manner (data not presented) that was indistinguishable from that of $^{55}$Co-vitamin B$_{12}$ bound to the $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein.

**Hepatic Release of $^{125}$I and $^{55}$Co Moieties of $^{125}$I-Labeled Vitamin B$_{12}$-binding Protein-$^{55}$Co-vitamin B$_{12}$ Complexes**—The data presented in Fig. 2A reveal that both moieties of the $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein-$^{55}$Co-vitamin B$_{12}$ complex reappeared in the plasma after their initial uptake by the liver. The $^{55}$Co moiety began to reappear 30 min after injection and was rapidly excreted in the urine. The peak plasma level and the period of maximal urinary excretion of $^{125}$I both occurred between 45 and 75 min. The $^{55}$Co moiety did not begin to reappear in the plasma until 60 min after injection and did not reach its peak plasma level until 120 min after injection. The plasma level of $^{55}$Co declined slowly after 120 min; only negligible amounts were excreted in the urine. Similar results were observed with the $^{125}$I-labeled transcobalamin III-$^{55}$Co-vitamin B$_{12}$ complex (Fig. 2B) and with the desialylated $^{125}$I-labeled transcobalamin I-$^{55}$Co-vitamin B$_{12}$ complex (Fig. 2C).

The data presented in Fig. 2A reveal that the prior injection of 92 mg of desialyzed fetuin markedly prolonged the plasma survival of the $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein-$^{55}$Co-vitamin B$_{12}$ complex and markedly inhibited the urinary excretion of the $^{125}$I moiety. Similar, but less marked inhibition, was observed (Fig. 2B') when 73 mg of desialyzed fetuin were injected prior to the injection of the $^{125}$I-labeled transcobalamin III-$^{55}$Co-vitamin B$_{12}$ complex. The data presented in Fig. 2C reveal that only approximately 10 to 15% of the $^{125}$I-labeled transcobalamin I-$^{55}$Co-vitamin B$_{12}$ complex was missing from the plasma 5 min after injection and that approximately 17% of the $^{125}$I was excreted in the urine during the subsequent 180 min. The remaining material (85 to 90%) disappeared slowly at a rate equal to that of $^{125}$I-labeled bovine serum albumin. This similarity in the clearance rates of bovine serum albumin and transcobalamin I continued for at least 5 days (data not presented).

**Properties of $^{125}$I-Labeled Material and $^{55}$Co-vitamin B$_{12}$ Released from Liver**—An 80-min plasma sample was obtained from a rabbit that had been administered $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein-$^{55}$Co-vitamin B$_{12}$ and the sample was subjected to gel filtration on Sephadex G-150. The elution profile obtained is presented in Fig. 1C. The elution profiles of $^{125}$I and $^{55}$Co-vitamin B$_{12}$ both differed markedly from their preinjection profiles (Fig. 1B) and from each other. Less than 2% of either isotope eluted in the 150,000 apparent molecular weight position of intact $^{125}$I-labeled granulocyte vitamin B$_{12}$ binding protein-$^{55}$Co-vitamin B$_{12}$. The $^{55}$Co eluted in two major peaks with apparent molecular weights of 70,000 (30%) and less than 1,000 (70%). None of the $^{125}$I present in these positions was adsorbed by rabbit anti-R-type protein-Sepharose or vitamin B$_{12}$-Sepharose. The nature of the 70,000 apparent molecular weight $^{125}$I-labeled material is unknown although the $^{125}$I appears to be covalently bound to some component, possibly rabbit albumin, since it is not dialyzable when it is dialyzed against 1% sodium dodecyl sulfate or 7.5 M guanidine HCl in the presence of 1% 2-mercaptoethanol. The
fact that similar material has been observed in experiments in which $^{125}$I-labeled rabbit and human transcobalamin II were studied in rabbits (13) suggests that it is formed after the catabolism of a number of similarly $^{125}$I-labeled proteins. The nature of the small molecular weight (<1000) $^{125}$I is also unknown although free $^{125}$I and $^{125}$I-labeled N-succinimidyl 3-(4-hydroxyphenyl) propionate, either free or attached to one or a few amino acids, are likely possibilities. All of the $^{125}$I excreted in the urine in the experiments shown in Fig. 2 had a similar apparent molecular weight of less than 1000 (data not presented).

All of the $[^{57}Co]$vitamin B$_{12}$ present in the 80-min plasma sample (Fig. 1C) eluted from Sephadex G-150 with an apparent molecular weight of 40,000. This value is the same as the apparent molecular weight of rabbit transcobalamin II which accounts for >80% of the total, and >95% of the unsaturated, vitamin B$_{12}$-binding protein present in rabbit plasma (13). The $[^{57}Co]$vitamin B$_{12}$ also resembled rabbit transcobalamin II-$[^{57}Co]$vitamin B$_{12}$ in that it was precipitated by chicken anti-human transcobalamin II sera but was not precipitated by rabbit anti-human transcobalamin II sera or rabbit anti-human R-type protein sera.

Gel filtration results similar to those described above were also observed with a plasma sample obtained 180 min after the injection of $^{125}$I-labeled granulocyte vitamin B$_{12}$-binding protein-$[^{57}Co]$vitamin B$_{12}$ except that there was less $^{125}$I in the small molecular weight region. Indistinguishable results were observed with a plasma sample obtained 180 min after the injection of $^{125}$I-labeled transcobalamin III-$[^{57}Co]$vitamin B$_{12}$.

In both experiments it was possible to calculate that less than 2% of the injected $^{125}$I and $[^{57}Co]$vitamin B$_{12}$ were still present in the plasma in their preinjection form. In the case of a plasma sample obtained 180 min after the injection of $^{125}$I-labeled transcobalamin I-$[^{57}Co]$vitamin B$_{12}$, the elution profile revealed that 91% of the total $^{125}$I in the sample had an apparent molecular weight of 150,000, while 3% and 6%, respectively, were in the 70,000 and <1000 apparent molecular weight positions. In the case of $[^{57}Co]$vitamin B$_{12}$, 83% and 17% of the total $[^{57}Co]$vitamin B$_{12}$ eluted with apparent molecular weights of 150,000 and 40,000, respectively. The ratio of $^{125}$I to $[^{57}Co]$vitamin B$_{12}$ in the 150,000 apparent molecular weight region was not significantly different from the preinjection ratio and >95% of both isotopes in this region were specifically adsorbed by rabbit anti-human R-type protein-Sepharose. The $^{125}$I-labeled bovine serum albumin used in these experiments eluted from Sephadex G-150 in a symmetrical peak, with an apparent molecular weight of approximately 70,000, both before and 180 min after it was administered intravenously to rabbits.

### Table I

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nonradioactive material injected 5 min prior to injection of radioactive material</th>
<th>Radioactive material injected at time zero</th>
<th>Distribution of radioactive material 5 min after its injection</th>
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* The amount of fetuin or desialyzed fetuin injected was 92 mg.

* The amount of I-labeled protein injected was approximately 75 ng. Vitamin B$_{12}$-binding proteins were saturated with approximately 1.5 ng of vitamin B$_{12}$.

* Per cent of total amount administered.

* Assumed to be 100% and used to calculate the plasma volume.
the liver at a faster rate than \([^{57}\text{Co}]\text{vitamin B}_{12}\), and that the \(^{131}\text{I}\) reaches its peak concentration in the kidney at the same time as its maximal excretion in the urine in the form of small molecular weight (<1000) fragments (see above). \(^{131}\text{I}\) does not accumulate in the heart or lung at any time period. \([^{57}\text{Co}]\text{-vitamin B}_{12}\) accumulates in the kidney, heart, and lungs, but only after its release from the liver and its subsequent binding to rabbit transcobalamin II in the plasma (see above). This late tissue distribution of \([^{57}\text{Co}]\text{vitamin B}_{12}\) is the same, with the exception of the intestine (see below), as that observed when rabbit transcobalamin II-\([^{57}\text{Co}]\text{vitamin B}_{12}\) is injected intravenously into rabbits (13).

The time course of appearance and distribution of \(^{131}\text{I}\) and \([^{57}\text{Co}]\text{vitamin B}_{12}\) in the small intestine is distinct from that of the other organs (see Fig. 3). Both moieties appear in equal amounts in the proximal small intestine between 5 and 30 min after injection and reach their maximal values by 60 min. At later time periods (120 to 180 min) \([^{57}\text{Co}]\text{vitamin B}_{12}\) is present in excess of \(^{131}\text{I}\) and the bulk of both moieties are present in the distal small intestine, which is where vitamin B_{12} appears to be absorbed from the gastrointestinal tract in rabbits (22). The \(^{131}\text{I}\) and \([^{57}\text{Co}]\text{vitamin B}_{12}\) enter the intestine via the bile, since when the common bile duct was cannulated in rabbits the level of both moieties in the intestine at 60 and 180 min fell by over 90%. The time course of appearance of \(^{131}\text{I}\) and \([^{57}\text{Co}]\text{vitamin B}_{12}\) in the bile is shown in Fig. 4. When a pooled sample of bile collected from 0 to 180 min after injection was subjected to gel filtration on Sephadex G-150, greater than 90% of the \(^{131}\text{I}\) and \([^{57}\text{Co}]\text{vitamin B}_{12}\) were present in a denatured state since it does not bind to vitamin B_{12} holoprotein. The significance of this difference has not been determined although it could reflect a difference in susceptibility to lysosomal proteases within the hepatocyte. Apo-transcobalamin I was cleared from plasma twice as fast as holo-transcobalamin I, although both forms were cleared much more slowly than their granulocyte vitamin B_{12} binding protein and transcobalamin III counterparts. It should be noted that all of the differences observed between the apo- and holo-forms of these three proteins could be related to the fact that 30 to 40% of each apo-protein preparation appears to be present in a denatured state since it does not bind to vitamin B_{12}-Sepharose (see above).

**DISCUSSION**

Ashwell and Morell and their associates (23) have described and elucidated a mechanism by which a large number of asialoglycoproteins, including the asialo forms of orosomucoid, fetuin, ceruloplasmin, haptoglobin, \(\alpha-2\)-macroglobulin, thyroglobulin, chorionic gonadotropin, follicle-stimulating hormone, and luteinizing hormone are cleared from plasma and catabolized by the liver. (For a review see Ashwell and Morell (16)).
Terminal galactose residues appear to be required for glycoprotein binding to common receptors that are present on hepatocyte plasma membranes since binding is abolished by modification or removal of galactose residues from the glycoprotein or by the attachment of sialic acid distal to galactose on the glycoprotein. Within minutes after binding to plasma membrane receptors, asialoglycoproteins appear to enter hepatocytes intact, presumably by pinocytosis, and are degraded by lysosomal enzymes over the ensuing 30 to 90 min. The biological function of this phenomenon has been difficult to elucidate since all of the proteins listed above appear to be present normally in plasma in their fully sialated forms. It has been postulated that sialic acid is slowly released from these proteins in vivo by neuraminidase but this has not been demonstrated.

The studies presented here demonstrate that transcobalamin I is included among those glycoproteins whose desialylated forms are cleared rapidly from plasma by the mechanism of Ashwell and Morell. The studies concerning the granulocyte vitamin B₁₂-binding protein and transcobalamin III provide additional evidence for the concept that the latter protein is secreted from cells in a form such that the native protein is present in the cystic ducts, although the mechanism by which this occurs also has not been demonstrated. The observation that R-type proteins vary markedly in their plasma survival in normal human subjects (12) is compatible with the view that the R-type vitamin B₁₂-binding protein in human plasma is a protein that is present in plasma in its fully sialated forms.

Approximately 80 to 90% of the [³¹⁷Co]vitamin B₁₂ that enters the hepatocyte bound to R-type protein has a prolonged plasma survival in normal human subjects (12). Direct evidence that some human R-type proteins are cleared rapidly from human plasma by the liver is not available although several studies (27, 28) can be interpreted in a way that supports this possibility. The fact that human bile contains 3 to 9 µg of vitamin B₁₂ per day (29) and the fact that the unsaturated vitamin B₁₂-binding protein in human bile is an R-type protein (30) indicate that significant amounts of R-type protein-vitamin B₁₂ may be cleared from human plasma by the liver in vivo. Much if not all of the R-type protein could be derived from granulocytes since the granulocytes that require the vitamin for growth. A similar antibacterial function might serve to bind vitamin B₁₂ and prevent its utilization by bacteria that require the vitamin for growth. A similar function has been well documented for the iron-binding protein lactoferrin (34, 35) which is present in granulocytes (36) and a number of secretions. The significance of our observation that the
granulocyte vitamin B₁₂-binding protein is cleared rapidly from plasma by the liver has not been established although it indicates the presence of a mechanism by which vitamin B₁₂ present in areas of cell necrosis and infection within the body can be delivered exclusively to the liver rather than to cells throughout the body as would occur if such vitamin B₁₂ were bound by transcobalamin II (13). This mechanism could be important if the liver controls the amount or coenzyme form of vitamin B₁₂ bound to transcobalamin II in plasma or if the liver contains a mechanism for distinguishing native vitamin B₁₂ from vitamin B₁₂ analogs which are synthesized by bacteria (37) and might be harmful to certain cells within the body. The latter mechanism is suggested by the fact that R-type proteins contain a mechanism for preferentially secreting them into the bile. The fact that intrinsic factor binds a much narrower range of vitamin B₁₂ analogs (38, 39) indicates that many such analogs can be delivered exclusively to the liver rather than to cells throughout the body as would occur if such vitamin B₁₂ were bound by transcobalamin II (13). This mechanism could be important if the liver controls the amount or coenzyme form of vitamin B₁₂ bound by transcobalamin II in plasma or if the liver contains a mechanism for distinguishing native vitamin B₁₂ from vitamin B₁₂ analogs which are synthesized by bacteria (37) and might be harmful to certain cells within the body. The latter mechanism is suggested by the fact that R-type proteins contain a mechanism for preferentially secreting them into the bile. The fact that intrinsic factor binds a much narrower range of vitamin B₁₂ analogs (38, 39) indicates that many such analogs would not be reabsorbed from the intestine. It is of interest, in regard to this type of protective mechanism, that one of the two brothers with congenital R-type protein deficiency has a poorly defined neurologic illness that is clinically similar to multiple sclerosis (15).

The existence of a granulocyte-mediated mechanism for the transport of vitamin B₁₂ exclusively to the liver suggests a possible biologic function for the process described by Ashwell and Morell and suggests that similar mechanisms may exist for iron and for other vitamins and metals. Transport of this kind could serve to regulate various metabolic systems within the liver as well as serve as a scavenger function.

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