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How does cobalamin (vitamin B12) enter and traverse mammalian cells?

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Abstracts. This overview is intended to present recent information available on the work done in understanding the mode of entry of cobalamin into and out of mammalian cells. I have focused primarily on the uptake of cobalamin bound to 3 proteins: intrinsic factor, transcobalamin II and R-proteins. Even though the nature of these cobalamin binding proteins, and the receptors involved in the internalization of these proteins is known, a total picture on the intracellular trafficking of cobalamin and the binding proteins is far from complete.

Keywords. Cobalamin; uptake; cells; receptor; endocytosis; acidic vesicles.

Introduction

Cobalamin (cbl) a water soluble micronutrient, is taken up by mammalian cells across the lipid plasma membranes by receptor mediated endocytosis, a process which is common for the cellular utilization of proteins and peptides. Rapid strides have been made in our current understanding of how the various ligands such as peptide hormones, low density lipoproteins and others are taken up and sorted in the cells. However, such a detailed information on the uptake and cellular sorting of cbl is lacking mainly due to the complex nature of cbl and the proteins that mediate uptake. Despite this, work done in our laboratory and others have provided important clues regarding these processes.

Early events before absorption

In mammalian systems the only source of this vitamin is through diet, and the first cells which has to take up this vitamin are the ideal cells of intestinal mucosa. The sequence of events which lead up to the entry of cbl into blood is truly a complex, yet remarkably efficient process (Seetharam *et al*., 1982, 1985). Our current knowledge has come from *in vivo* and *in vitro* studies using various animal model systems. A very tiny amount (2–3 μg) of the vitamin is absorbed in humans at any given time. Before this amount can be absorbed by the ileal cells a series of complex binding reactions occur in the stomach and the intestinal lumen. The dietary cbl which is mostly bound to proteins in the food is released by the action of acid and pepsin. Bollowing this release cbl binds very strongly to a protein which is released from the salivary glands. Once cbl is bound to this protein, it can be released only when the protein is degraded by proteases. The cbl bound to the salivary binder cannot be utilized by the ileal cells. The reason for this paradox is due to the fact that in the

Abbreviations used: cbl, Cobalamin; M_r, molecular weight; TC-II, transcobalamin.

acid millieu of the stomach salivary binder has an affinity for cbl which is 50 times higher than for intrinsic factor, a glycoprotein secreted from the parietal cells of the stomach (Allen and Mehlman, 1973). Cbl bound to gastric intrinsic factor is very efficiently taken up by the ileal cells. During the normal course of events the dietary cbl which binds to salivary binder must be transferred to gastric intrinsic factor before it can be absorbed. Failure in such a transfer results in cbl malabsorption. Allen *et al.* (1978a) showed that cbl bound to salivary binder can be transferred to gastric intrinsic factor in the presence of pancreatic proteases such as trypsin, chymotrypsin and elastase. This simple *in vitro* demonstration helped provide a biochemical explanation for the cause of malabsorption of cbl in patients with pancreatic insufficiency (Toskes *et al*., 1971). Allen *et al.* (1978b) provided further *in vivo* evidence, for their *in vitro* observations when they corrected malabsorption of cbl in patients with pancreatic insufficiency either by oral administration of trypsin or an analog of cbl which bound to only salivary binder but not to gastric intrinsic factor. A schematic diagram of the sequence of events mentioned above is shown in figure 1.

Intestinal uptake of IF-cbl

Once cbl complexes with intrinsic factor, the complex attaches to a receptor which for some strange reason is located in the distal ileum in most animal species. This binding requires Ca^{2+} and a pH above 5 and below 7·5. The receptor which recognizes the ligand (IF-cbl) has been purified to homogenity (Seetharam *et al*., 1981), from canine ileal mucosa. The receptors purified from hog and human (Kouvonen *et al*., 1979) and canine ileal mucosa (Seetharam *et al*., 1981) have a molecular weight (M_r) around 230–240 $\times$ 10^3 and consist of two subunits. The canine receptor is located in the microvillus pits (Levine *et al*., 1984) and the IF-cbl binding domain is located on the lumenal side of the membrane (Seetharam *et al*., 1981b). The canine receptor is anchored to the plasma membrane *via* an anchor piece which is rather large (M_r 36,000) (Seetharam *et al*., 1982). Not all mammalian IF-cbl receptors are the same. The rat receptor does not cross-react with antiserum to canine receptor and can bind cbl only when complexed with rat IF but not IF prepared using human, hog and canine stomachs (Seetharam *et al*., 1983).

Following the attachment of IF-cbl complex to the ileal receptor how the vitamin gets in and gets out of the ileal cell is not foully understood. However, it is fairly clear that IF-cbl complex is internalized (Kapadia *et al*., 1983). Following this internalization analysis of intracellular cobalamin has revealed that cbl is bound to free IF, If which is still attached to the receptor, and finally to a transport protein (Transcobalamin II; TC-II) which shuttles cbl to various tissues. The exact vesicle where the release of cbl occurs, and the mechanisms by which this release is brought about has been a subject of intensive research. Most ligands which enter a cell by receptor mediated endocytosis are initially present in the endocytic vesicles. These vesicles are acidic and are called receptosomes (Willingham and Pastan, 1980). Most ligands are dissociated from their receptor due to low pH of these prelysosomal vesicles. The receptor in some cases recycles to the membrane or it can get degraded. The ligands are delivered *via* golgi to the lysosomes for eventual degradation. In some cases both the receptor and the ligand return to the cell surface as the case following internalization of transferrin (Ciechanover *et al*., 1983).

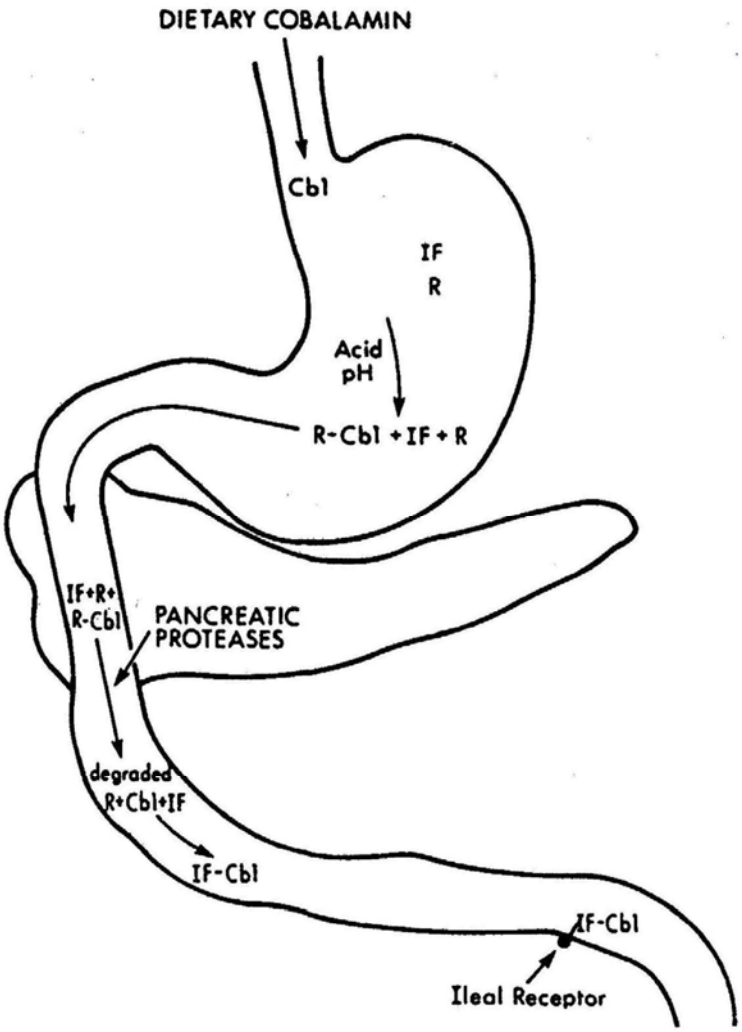

Figure 1. Proposed scheme for the absorption of dietary cobalamin. Reproduced with permission from Annual Reviews of Nutrition: Seetharam and Alpers (1982a).

Once IF-cbl is internalized the intracellular sorting which results in the transfer of cbl from IF to TC-II is a complex process and is poorly understood. Our areas of ignorance include the following: (a) the intracellular vesicle (endosomes or lysosomes) where the actual release of cbl occurs; (b) the mechanism (proteolytic cleavage or acidic pH) by which this release occurs; (c) the fate of IF and its receptor (degraded or recycled) and finally the origin of TC-II (mucosal or blood).

The answers to some of these questions have begun to emerge. Recent work by Seetharam *et al.* (1985) and Robertson *et al.* (1985a,b) have suggested that cbl is probably released due to acidification of IF-cbl complex and not due to proteolytic degradation of IF. In the presence of chloroquine there was an inhibition in the amount of cbl transferred to TC-II from IF suggesting that acid alone can bring about this transfer. Even though cbl bound to TC-II has been demonstrated in the

mucosal extracts, following gastrointestinal uptake of cbl, a direct demonstration of a *de novo* synthesis of TC-II or its presence in a cell free homogenate in the absence of IF-cbl uptake has never been demonstrated. In direct evidence (Chanarin *et al*., 1978; Rothenberg *et al*., 1978) has suggested that TC-II may be produced by ileal cells in response to the uptake of IF-cbl by these cells. A schematic diagram showing the uptake of IF-cbl by the enterocytes and the areas of ignorance is shown in figure 2.

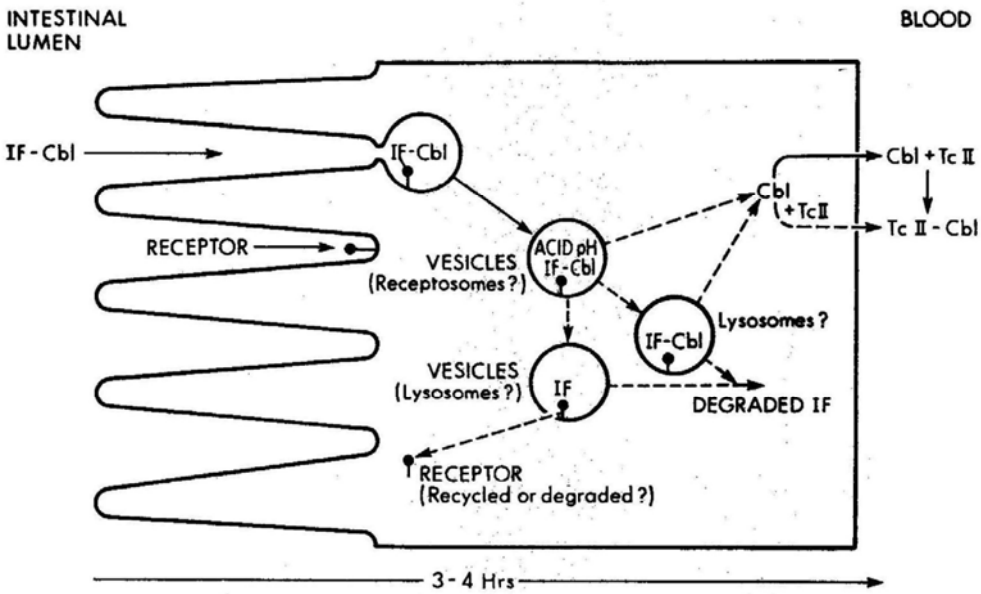

Figure 2. Possible pathways for movement of IF and cbl within the enterocyte. Established pathways are marked by solid lines. Pathways that are incompletely established or proposed are marked by broken lines.

Cellular uptake of TC-II-cbl

The cbl which exists the ileal mucosa must be delivered to the tissues. This is accomplished by TC-II, a protein of M_r 38,000. Cobalamin bound to TC-II is taken up by receptor mediated endocytosis *via* a receptor which is located in the plasma membrane of all tissues. The receptor for TC-II has been purified from human placenta (Seligman and Allen, 1978). The receptor has a M_r 50,000 and has very similar amino acid composition as its ligand TC-II, suggesting the possibility that TC-II and its receptor might have originated from a common gene. The TC-II receptor binds both apo TC-II and holo TC-II, but the association constant (k_a) for holo TC-II is twice that of apo TC-II. The plasma clearance (Schnider *et al*., 1976) of [^{125}I]-TC-II was 30% faster than the clearance for [^{125}I]-TC-II-cbl; suggesting that *in vivo* apo TC-II may have higher affinity for the receptor than holo TC-II. This discrepancy might simply reflect the results one might obtain when one calculates ligand-receptor binding *in vitro* with pure proteins and the actual values one may

Obtain when one studies in vico uptake from the circulation by receptors which are membrane bound.

Within the cells internalized TC-II-cbl complex is degraded by the lysosomes Evidence for direct lysosomal degradation has been obtained using cultured fibroblastrs (Turner *et al*., 1978), and rat kidney (Newmark *et al*., 1970).

Hepatic uptake of cbl bound to nonintrinsic factor (R-proteins)

In addition to IF and TC-II a family of cobalamin binding proteins have been identified and in some cases been purified. These proteins have been identified in leukocytes, plasma and other biological fluids. These proteins are all immunologically identical, but because of their rather rapid mobility during electrophoresis, they are called R-proteins. The R-proteins purified from human milk, saliva and normal granulocytes have a M_r around $60–66 \times 10^3$.

The physiological significance for the presence of R-proteins in mammalian secretion is not clear. It has been suggested (Allen, 1975) that potentially harmful analogs of cbl (which might be present either in the diet or produced by bacteria), are cleared by binding with very high affinity to these proteins. The cbl or cbl analogs bound to R-proteins is cleared in the liver *via* asialoglycoprotein receptor which is present in the hepatic plasma membrane. This receptor recognizes glycoprotein ligands once their sialic acid is removed, exposing the terminal galactose (Ashwell and Morell, 1974). The plasma clearance of cbl bound to R-proteins is very fast ($T_{½}$= 5min) (Burger *et al*., 1975). The cbl or their analogs are released in the lysosomes and secreted through the bile. When the cbl analogs are cleared through bile, they are not reabsorbed since intrinsic factor binds these analogs with lower affinity than cbl.

Cobalamin deficiency due to defective intracellular sorting of cbl

Cbl deficiency in man and other animals can occur due to a number of causes (Kapadia *et al*., 1985) during its complicated pathway of absorption, transport and cellular utilization (Rosenberg 1983). In addition to these causes cbl deficiency could also occur due to defective sorting within the cells. Imerslund (1960) and Grasbeck *et al.* (1960) described a familial disorder in which megaloblastic anemia developed in children due to a failure to absorb cbl. These children contained normal amounts of intrinsic factor. Since this earlier finding, more cases in children have been reported (MacKenzie *et al.*, 1972). In these patients there was normal amounts of ileal receptor and normal absorption of fat and xylose. It is possible that the defect could be due to an intracellular transport of the newly synthesized receptor to apical plasma membranes. On the other hand, the defect might be due to the inability of intracellular acidic vesicles to release cbl bound to proteins.

A defect in the handling of TC-II-cbl complex by cultured fibroblasts from a patient has been suggested to be due to a failure in a possible transport system associated with the lysosomes (Rosenblatt *et al*., 1985). The exact reasons for a defect in the intracellular sorting or cbl in these patients will be clearer once all the pathways in the intracellular trafficking of cbl in a normal cell is established.

Summary

Cellular entry and exit of cbl is a complex process. The story of cbl and its relation to anemia has been known for 50 years, but the slow unravelling of the complexities related to intracellular trafficking has been mainly due to a variety of reactions involved, complexity of cbl structure and the possible influence of structure on binding to intracellular proteins and hence on cellular entry and exit, and to the extremely low levels of tissue proteins which delayed their isolation. Now that all the binding proteins, and their receptors are isolated, and our knowledge in cell biology of intracellular trafficking has greatly increased the future of cbl research appears to be bright. It is hoped that a total picture on the cellular movement and turnover of cbl and its analogs will help in a clearer understanding of the pathogenesis in some forms of cbl deficiency.

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