Intrinsic Factor Mediated Cobalamin Absorption

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ABSTRACT

The physiological absorption of vitamin B₁₂ is a complex process which requires the interaction of several macromolecules. Mediated by the glycoprotein, intrinsic factor (IF), this process requires formation of a primary complex between vitamin B₁₂ and IF (IF-B₁₂), the recognition and binding of this complex to specific ileal receptors and the transport of vitamin B₁₂ across the ileal cell. As a measure of this overall process, the vitamin B₁₂ absorption test has helped to identify abnormal vitamin B₁₂ absorption in patients with exocrine pancreatic insufficiency and familial vitamin B₁₂ malabsorption (ImmerslunG-grasbeck syndrome). Progress into understanding the role of proteolytic enzymes in promoting vitamin B₁₂ absorption as well as the molecular events of vitamin B₁₂ transport across the ileal cell has been brought about by recent investigation based upon this determination.

Introduction

Since the original studies of Castle⁷ in 1928, the role of intrinsic factor (IF) in the absorption of vitamin B₁₂ has been the subject of numerous investigations. The glycoprotein IF, which is an obligate requirement³ for the absorption of physiologic amounts of vitamin B₁₂, has been isolated, purified and characterized by several laboratories.²¹⁸ IF avidly binds vitamin B₁₂ with the formation²⁰ of a stable complex (Kₐ = 1.0 × 10⁹ 1·mole⁻¹), which has specific requirements for structural determinants in the basic corrinoid ring.⁵

Several steps must occur during the absorption of vitamin B₁₂. As outlined by Grasbeck,¹⁶ the sequence of IF mediated vitamin B₁₂ absorption begins with IF secretion from the gastric parietal cell. IF then binds vitamin B₁₂ released from hydrolysis of ingested proteins, and forms a stable complex (IF-B₁₂). This complex passes to the terminal ileum where it binds to specific, high-affinity receptors.¹¹ By an energy dependent process,¹⁷ vitamin B₁₂ then enters the blood stream. A lag period of between three and four hours is observed from the time of IF-B₁₂ binding to the ileal surface until the appearance of vitamin B₁₂ in the portal circulation.⁴

Although 90 percent of the absorbed vitamin B₁₂ is bound to the plasma transport protein, transcobalamin II (TC II), it remains unclear as to whether or not TC II is required for the normal absorption¹³ of vitamin B₁₂. Patients deficient in TC II have been shown to have a defect in vitamin B₁₂ absorption.⁶ In contrast to these observations, perfusion of ileal loops with
plasma does not enhance the absorption of vitamin B₁₂ from such preparations.²² This conflicting data might be explained by the presence of vitamin B₁₂ deficiency, a state known to interfere with vitamin B₁₂ absorption, in patients lacking TC II. Once absorbed into the plasma, vitamin B₁₂ is distributed between TC II and another transport protein, transcobalamine I (TC I). TC I does not appear to be required for vitamin B₁₂ absorption.⁴ The distribution of vitamin B₁₂ between TC II and TC I is directly dependent upon their concentrations in serum and their binding constants.²³

A useful tool to determine the overall integrity of this process is the vitamin B₁₂ absorption test (Schilling Test).²⁴ Classically, the Schilling Test has been used for the identification of pernicious anemia. Deficiency of IF in this disorder leads to failure of vitamin B₁₂ absorption which can be corrected by the addition of exogenous IF. In conjunction with studies of other events in vitamin B₁₂ absorption, however, the Schilling Test is presently yielding much information regarding the molecular events of this complex absorption process. At least two areas deserve discussion: the role of proteolytic enzymes in the absorption of vitamin B₁₂; and the nature of the molecular events required for the transport of vitamin B₁₂ across the ileal cell.

Proteolytic Enzymes and Vitamin B₁₂ Absorption

The work of Toskes and Deren has been fundamental in understanding the role of proteolytic enzymes in the absorption of vitamin B₁₂. In patients with exocrine pancreatic insufficiency, vitamin B₁₂ absorption is abnormally low and cannot be corrected by the addition of exogenous IF. Earlier hypotheses to explain this observation, such as a decrease in intraluminal pH, were carefully excluded by these investigators.⁸ In addition, there was no evidence in such patients for antibodies against IF, ileal disease or bacterial overgrowth. IF from these patients, in the form of gastric juice, was fully effective in promoting vitamin B₁₂ absorption in subjects with pernicious anemia and normal pancreatic function. Thus, in the presence of normal pancreatic secretion, IF from such patients was capable of complete function.

Toskes, et al further reported that incubation of crude IF preparations with either insolubilized trypsin or chymotrypsin could correct the absorptive defect in these patients.¹⁵ In these studies, IF was incubated with proteolytic enzymes, complexed to vitamin B₁₂ and administered to patients with pancreatic insufficiency. Absorption of vitamin B₁₂ was entirely normal. Care was taken to remove the proteolytic enzymes by centrifugation, and the supernatants were shown to have no proteolytic activity prior to administration. Biochemical studies of these trypsin treated crude IF preparations failed to identify the nature of this physiological alteration. The binding of vitamin B₁₂, apparent molecular weight as determined with both Sephadex and gel chromatography, as well as ileal receptor binding parameters were identical to those of untreated IF preparations.

Allen and coworkers subsequently reported an effect of trypsin on the binding of vitamin B₁₂ to both IF and R-binders (the non-intrinsic factor vitamin B₁₂ binding proteins found in gastric juice).¹ A decrease was reported in the binding of vitamin B₁₂ to R-binders following trypsin treatment. Allen and coworkers then postulated that this might explain the enhanced effect of crude IF preparations following trypsin treatment.

Toskes, et al have now shown that it is not the tryptic activity of these enzyme preparations which has the effect of increasing vitamin B₁₂ absorption.¹⁴ Crude IF preparations were incubated with trypsin plus trypsin inhibitor. Under these conditions, treated IF still promoted vitamin B₁₂ absorption in patients with
pancreatic insufficiency. In addition, more highly purified trypsin preparations could not reproduce this effect. The nature of this agent and its effect upon IF still remain to be defined. It would be of great interest to study the effect of these enzyme preparations upon highly purified IF. Such experiments would address the question as to whether or not this effect is mediated upon the IF molecule itself, or upon some other agent in crude IF preparations.

Transport of Vitamin B$_{12}$ Across the Ileal Cell

The events which occur between the binding of IF-B$_{12}$ to the ileal receptor and the entry of vitamin B$_{12}$ into the portal circulation remain unclear. The overall process requires at least three to four hours$^4$ and some portion or portions are energy dependent.$^{17}$ At least two distinct aspects of this problem have been studied: the fate of IF after the binding of IF-B$_{12}$ complex to the ileal receptor; and the molecular events required for the transport of vitamin B$_{12}$ across the ileal cell.

Although early studies by Harris, Hines and Rosenberg$^{19}$ as well as those by Hoffbrand and Peters$^{21}$ have suggested that IF remains bound to the brush border following vitamin B$_{12}$ transport, more recent studies have suggested that either IF, or a portion thereof, may be absorbed into the ileal cell. Donaldson and Mackenzie$^9$ postulated that IF was split at the luminal surface to produce a small peptide fragment. They suggested that this peptide accompanied vitamin B$_{12}$ across the ileal cell. The work of Ficarra, Rothenberg and Weisberg$^{12}$ would support this theory. Using ileal loops, they harvested mucosal cells after incubation with IF-B$_{12}$ complex. The mucosal cells were disrupted and separated into different subcellular compartments. In the cell debris, which included brush borders, both IF and vitamin B$_{12}$ were found. In addition to this, however, both vitamin B$_{12}$ and IF were found in the mitochondrial fraction. Another protein, to which vitamin B$_{12}$ bound, was also identified in the mitochondria. This protein could be precipitated by the addition of 15 percent Na$_2$SO$_4$.

Although other workers have suggested that ileal loops lose their structural integrity after prolonged incubation,$^4$ this study nevertheless suggests that vitamin B$_{12}$ may be bound by one or more proteins during transport across the ileal cell. It was originally thought that this accumulation of vitamin B$_{12}$ in the mitochondria was required for molecular interconversion from one form of cobalamin to another.$^4$ Subsequently, it was shown by Peters,$^4$ that other vitamin B$_{12}$ analogs proceed through this step as well. The reason for concentration of vitamin B$_{12}$ within the ileal mitochondria remains unclear.

The group of patients with Immer- slung-Grasbeck syndrome provides an excellent model for study of the events required for transport of vitamin B$_{12}$ across the ileal cell. In this disorder, vitamin B$_{12}$ absorption is low and cannot be corrected by the addition of exogenous IF. As shown by MacKenzie, et al,$^{10}$ IF isolated from these patients is fully active in promoting vitamin B$_{12}$ absorption in other systems. Ileal biopsy material from these patients has also been used to assay receptor binding activity. The binding of IF-B$_{12}$ to receptor sites in these preparations is entirely normal. Thus, the molecular derangement in this disorder is thought to relate to uptake and perhaps processing of vitamin B$_{12}$ within the ileal cell.

The vitamin B$_{12}$ absorption test, with and without exogenous IF, has helped to identify patients with vitamin B$_{12}$ malabsorption owing to both pancreatic insufficiency and the Immerslung-Grasbeck syndrome. In addition, it is now apparent that this test, when used in conjunction with studies of other aspects of vitamin B$_{12}$ absorption, can help to define the mecha-
nisms of vitamin B₁₂ absorption and transport. The effects of exogenous IF, antibiotics, pH correction and determination of ileal receptor binding activity when employed along with the vitamin B₁₂ absorption test may help to localize an absorptive defect within or outside of the ileal cell. By this process, factors necessary to correct vitamin B₁₂ absorption may be identified in a given patient and may further define the mechanisms by which pancreatic enzymes, and possibly intracellular peptides, effect vitamin B₁₂ absorption.

References