Genetic Defects of Cobalamin Metabolism

OWEN M. RENNERT, M.D.

Department of Pediatrics,
Department of Biochemistry and Molecular Biology,
University of Oklahoma Health Sciences Center,
Oklahoma Children's Memorial Hospital,
Oklahoma City, OK 73190

ABSTRACT

As a consequence of investigations on the pathogenesis of an inborn error of metabolism characterized by increased urinary and plasma levels of methylmalonic acid, the metabolism of vitamin B₁₂ and its functional roles have become elucidated. Four human mutations have been identified in which a defect in the metabolism of deoxyadenosinecobalamin or methylcobalamin occurs. These investigations have highlighted the functional significance of co-factors or coenzymes in the maintenance of health and have identified new approaches for the treatment of genetic diseases involving the use of pharmacologic doses of vitamins.

The study of the metabolism of vitamin B₁₂ or cobalamin has received its greatest impetus through investigations of the genetic disorder in man known as methylmalonic acidemia. This disorder is characterized by a profound failure to thrive, progressive microcephaly, and central nervous system deficit. Investigation of its pathogenesis has led to accrual of information on metabolic transformations of this co-factor and also into the genetic regulation of its metabolism. Clinical delineation of the biochemical consequences of vitamin B₁₂ deficiency has allowed us to define the molecular basis of the neurologic defect in pernicious anemia, the accumulation of odd-carbon-chain fatty acids and impaired fatty acid synthesis. It has provided the molecular basis for the effects of the crude animal protein factor isolated from Streptomyces griseus in 1949 by Folkers and associates and Smith and associates.

Chemical Considerations

Vitamin B₁₂ or cobalamin is unique amongst micromolecules in man since it contains cobalt bound with an organic ligand. It has a ring structure containing four pyrroles linked through their nitrogens in a fashion analogous to the linkage of iron in the center of the porphyrin rings of heme (figure 1). The cobalt is in the trivalent state and has two additional intramolecular ligands. Those are to the nitrogen of adenosine and an additional group which is perpendicular to the plane of the tetrapyrole structure. This molecular derivative defines the chemical form of cobalamin and consists of cyanide in cyanocobalamin, a methyl group in methylcobalamin, a hydroxyl group in naturally occurring vitamin B₁₂ and 5'-deoxy-5'-adenosyl substituent in adenosylcobalamin. It is unique that the covalent bond between the methyl group
of methylcobalamin and the deoxyadenosine of adenosylcobalamin are the organic carbon-cobalt linkages and functional components of this coenzyme. Vitamin B$_{12}$, in its role as a coenzyme, is involved in at least eight different reaction sequences. These include: reduction of the disulfide to sulfhydryl groups, activation of amino acids for protein synthesis, conversion of methylmalonyl CoA to succinyl CoA, conversion of $\beta$-methylaspartate to glutamic acid, reduction of formate to methyl groups, reduction of ribonucleotides to deoxyribonucleotides by ribonucleotide reductase, dismutation of vicinal diols to their corresponding aldehydes by a dehydrase reaction, and anaerobic degradation of lysine.$^{13}$ Though much information has been accumulated about the functional chemical characteristics of cobalamin, the precise molecular mechanism for its involvement in these reactions is still largely a matter of conjecture.

**Metabolic Conversions**

Though chemical interconversions in man determine the biochemical function of cobalamin, its availability, in large part, is dependent upon exogenous supply. The vitamin is present in significant concentration in milk, meat, fish, and eggs; however, it appears to be relatively absent in vegetable species. Its main natural occurring form is hydroxycobalamin. Cyanocobalamin is not found in nature but is a commercial byproduct obtained during its isolation. Hydroxycobalamin exists as a complex in the intestine with the gastric glycoprotein intrinsic factor which results in its interaction to ileal mucosa yielding a receptor specific transport sequence with subsequent release of free cobalamin into the portal circulation. Here it is bound to a serum protein (transcobalamin 2), carried to peripheral cells, and then released intracellularly through a process mediated by a specific receptor sequence. Intracellularly, it is converted to either methylcobalamin or adenosylcobalamin and subsequently utilized in metabolic processes.$^{1,21}$

The conversion of: hydroxycobalamin $\rightarrow$ methylcobalamin is catalyzed by the enzyme $\text{N}^5$-methyltetrahydrofolate: homocystine-S-methyltransferase.$^{20}$ This enzymatic sequence results not only in the formation of methylcobalamin but is the mammalian mechanism for the conversion of homocysteine to methionine. It is a one carbon transfer reaction functional in nucleic acid synthesis and is catalyzed by the soluble cytosolic enzyme.$^{15}$

The transformation of: hydroxycobalamin $\rightarrow$ adenosinecobalamin involves a complex sequence of transformations. Through a sequence of two steps involving the loss of two electrons catalyzed by cobalt reductase enzymes, the valence state of cobalt is converted from cobalt (III) to cobalt (I). Then in the presence of an adenosyl transferase and ATP, hydroxycobalamin containing cobalt (I), conversion to adenosine-
cobalamin and release of inorganic pyrophosphate occurs.

Our functional knowledge of the consequences of cobalamin deficiency was, in part, an outgrowth of studies of megaloblastic anemia and pernicious anemia. These disorders, however, represented deficiencies that were a consequence of deficient bioavailability of vitamin B12. In 1967 through 1968, significant insights and advances occurred in our knowledge about the metabolism of cyanocobalamin and its functional role in metabolic homeostasis as a consequence of the description and recognition of the genetic disorder methylmalonic acidemia.

Clinical Correlations

Two primary reaction sequences serve as the molecular foundation for investigations which led to an understanding of cobalamin metabolism and of the regulation of its interconversions. These are:

\[
\text{Homocysteine} + N^5\text{-methyltetrahydrofolate} \xrightarrow{\text{methylcobalamin}} \text{methionine} + \text{tetrahydrofolate.}
\]

This reaction occurs in the cytosolic intracellular compartment; and

\[
\text{L-methylmalonyl CoA} \xrightarrow{\text{adenosylcobalamin}} \text{Succinyl CoA.}
\]

This reaction occurs within the mitochondrial compartment and is catalyzed by a soluble mutase enzyme containing cobalamin as its coenzyme.

The classic clinical features of methylmalonic aciduria are: failure to thrive, profound central nervous system dysfunction, recurrent metabolic acidosis, microcephaly, and early death when unrecognized. It is a prototype for clinicians, since it is a neonatal metabolic emergency requiring prompt diagnosis and institution of therapy. The diagnosis is dependent upon the demonstration of increased concentrations of methylmalonic acid in blood and urine. The simplest method for the detection of this compound in biological fluids is based upon the original method of Gorgio and Plant which demonstrates the increased excretion of methylmalonic acid in pernicious anemia. More recent techniques involve the utilization of gas chromatographic techniques.

Pathophysiology

The pathophysiology of methylmalonic acidemia is a consequence of the failure of conversion of methylmalonic acid to succinyl CoA, thus, compromised energy metabolism and formation; however, the accumulation of analogues of citric acid derived from methylmalonyl CoA (methylcitrate) also further compromises metabolism.

Initial investigations of the pathogenesis of this disorder examined ileal transport of cobalamin. These studies revealed that patients had normal serum concentrations of cobalamin and an intact capacity to absorb and transport orally administered cobalamin. These observations led to postulations of three potential molecular mechanisms as to its etiology: (1) defective mutase apoenzyme; (2) defective cobalamin metabolism; (3) defective inter- or intracellular cobalamin transport. Further evidence for multiple etiologies came from the recognition of clinical heterogeneity: patients with increased levels of methylmalonic acid, a variant associated with increased levels of homocysteine. It was possible that a defect occurred in cobalamin binding to a subunit of its apoenzyme so that there could be increased excretion of methylmalonic acid and homocysteine. Such a clinical variant could also be due to a common step in the formation of adenosylcobalamin and methylcobalamin. Yet another postulation was a regulator defect controlling the production of both the methyltransferase enzyme and the mutase enzyme.

Investigation of these two well-defined clinical variants of methylmalonic
acidemia led to the delineation of further phenotypes and enhanced knowledge on the biotransformation of cobalam.

The first variant described by Morrow and associates was characterized by one to 12 percent of the mutase activity in homogenates of liver, leukocytes, and fibroblasts obtained from patients with methylmalonic acidemia. In vitro studies delineated a second variant based upon the finding of deficient mutase activity; however, incubation of the cell homogenate with 500 to 1,000 fold concentrations of adenosinecobalam in corrected the deficient enzymatic activity. The second variant was shown to be heterogeneous since two clinical phenotypes were definable within this subgroup. The first of these was associated with increased accumulation and excretion of methylmalonic acid, and the second with increased levels of methylmalonic acid and homocystine in blood and urine. Further in vitro investigations identified not only defective mutase activity in these patients but also 10 percent or less of the normal methyltransferase activity. Addition of methylcobalam in to cell-free homogenates corrected methyltransferase activity to approximately 45 percent of control values.

These observations served as the impetus for the pioneering work of Rosenberg and associates on the transformation of cobalam in man. Investigations on skin cultured fibroblasts of patients who had methylmalonic acidemia but who responded to adenosinecobalam in therapy resulted in the following observations. Adenosinecobalam in levels in fibroblasts and leukocytes are significantly diminished in these patients. Incubation of cells with co-cobalam in followed by isolation of radiolabelled derivatives and their identification by thin layer chromatography resulted in the demonstration of decreased adenosinecobalam in synthesis. Following the development of a cell free assay method to measure the conversion of cobalam in (CoII) to cobalam in (Col) as well as the formation of adenosinecobalam in, Mahoney and associates demonstrated that cell-free systems from patients with this variant were incapable of producing adenosinecobalam in.

Further exploitation of these biochemical breakthroughs led to the identification of four phenotypes of methylmalonic acidemia. The first was a defect in the production of the mutase enzyme itself; the second was a defect in adenosinecobalam in synthesis; the third a defect in adenosine and methylcobalam in synthesis; the fourth a defect in adenosinecobalam in synthesis in intact cells but which could not be demonstrated in cell-free systems.

Simultaneously to the application of chemical methods to the study of these phenotypes, Rosenberg and associates employed yet another approach. Employing inactivated Sendai virus, heterokaryons were formed from tissue cultured fibroblasts of patients. Initial studies resulted in the definition of four complementation groups. These data indicated that four independent genetic loci were responsible for the molecular defect: (1) a mutase defect, Cbl A; (2) a strain characterized by defective adenosinecobalam in synthesis in intact cells not demonstrable in a cell-free system (Cbl A); (3) a strain with absent adenosinecobalam in synthesis (Cbl B); (4) defective adenosinecobalam in and methylcobalam in synthesis (Cbl C). Modification of techniques by Willard and Morrow yielded a fifth complementation group (Cbl D) which consists of a subset of two patients with methylmalonic acidemia and homocystinuria who do not have the capacity to correct the defect in the Cbl C group cells when a heterokaryon is formed.

Applying the technique developed by Youngdall-Turner, a system to study cobalam intracellular transport and utilization was available. It is based upon the observation that the cobalamin-transcobalam in 2 complex interacts at the cell receptor, in the presence of calcium,
so that absorptive endocytosis occurs with subsequent lysosomal intracellular release of cobalamin and its metabolic utilization. In the Cb1 C and D variants, it can be shown that all of these processes were normal. By these techniques,\textsuperscript{18} it was demonstrated that the cobalamin binding activity of cells was, in fact, synonymous with their methyltransferase and adenosine triphosphate (ATP) transferase activity. Further investigations have shown that the probable defect in the Cb1 C and Cb1 D variants is a defect in the cobalamin III reductase system rather than in any binding proteins per se. The precise mechanism for this defect is unclear; however, it is clear that different loci appear to be responsible for the genetic mutation.

Recent work\textsuperscript{7} has lead to the recognition that the methyltransferase gene resides on chromosome 1. It is through the application of such biochemical and cell biology techniques that the mapping of all the loci involved in the regulation of cobalamin metabolism and its function as a coenzyme in a variety of enzymatic systems will be accomplished.

References