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INHERITED DEFECTS OF VITAMIN B_{12} METABOLISM

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INTRODUCTION

Vitamin B_{12} in this review is defined as a group of cobalamin compounds that act as intracellular coenzymes or that can be converted to these coenzyme forms by mammals. It thus excludes corrins (e.g. cobinamide), which may function in bacterial systems but not in mammalian cells.

Vitamin B_{12}–dependent reactions are absent from higher plants, which do not contain vitamin B_{12}. Many bacteria, fungi, and lower organisms synthesize the vitamin B_{12} they require. All of the naturally occurring vitamin B_{12} is eventually derived from such organisms.

Vitamin B_{12} coenzymes function in at least two intracellular reactions: (*a*) the conversion of methylmalonyl CoA to succinyl CoA, which in mammalian cells functions exclusively in the degradation of propionate and odd-chained fatty acids; and (*b*) the generation of methionine from homocysteine using 5-methyl-tetrahydrofolate as the major donor of the additional methyl group (95). The former reaction uses 5'-deoxyadenosyl cobalamin as coenzyme and is mitochondrial; the latter uses Cob(I)alamin and methyl cobalamin as coenzyme and is cytoplasmic. Evidence has been published that 5'-deoxyadenosyl cobalamin also acts as coenzyme for the interconversion of alpha and beta leucine in mammalian cells (89). Intracellular deficiency of vitamin B_{12} induces accumulation of the products of propionate metabolism (including methylmalonic acid and homocystine) and deficiency of methionine and substances derived from it. Patients deficient in vitamin B_{12} have altered morphology and function of a variety of cells, manifested as megaloblastic anemia, sore mouth and tongue, abnormal intestinal function, defective function of granulocytes and of the immune system, neurological disease including a characteristic form of neurological degeneration, dementia, and psychosis ("megaloblastic madness"), and failure to thrive.

Inherited defects in the metabolism and utilization of vitamin B_{12} have been instructive in identifying reactions affected by its deficiency.

BACKGROUND INFORMATION

Chemistry

Like porphyrins, corrins contain four pyrroline rings arranged in a planar configuration. Unlike porphyrins, two adjacent pyrrolines are linked directly; the other three interpyrroline linkages are through a carbon atom as in porphyrins (19, 61, 62). The corrins contain six conjugated double bonds. Cobalamins are corrin derivatives in which a complex side chain containing a nucleotide is attached to one of the pyrroline rings and to a metal atom bonded to the nitrogens of the pyrrolines. Three acetic acid side chains pointing in a single direction (away from the side chain containing the nucleotide) and four

propionic acid side chains pointing in the direction opposite to the acetates are attached to beta carbons of the pyrrolines; eight methyl groups are attached to the carbon atoms making up the periphery of the ring. In cobalamins defined as vitamin B_{12}, acetates and propionates are in the form of acetamides and propionamides, the metal is cobalt, and the side chain is 1-alpha-D-ribofuranosyl-5,6-dimethyl-benzimidazole-3'-phosphate linked through 1-amino-2-propanol to the propionamide on the D pyrroline ring.

Vitamin B_{12} is composed of two compounds with coenzyme activity (methyl-cobalamin and 5'-deoxyadenosyl-cobalamin) and the compounds cyanocobalamin, aquo- or hydroxocobalamin, cobalamin R [Cob(II)alamin], and cobalamin S [Cob(I)alamin]. Other cobalamins may also have vitamin B_{12} activity, including a glutathione-cobalamin complex and sulfitocobalamin, but their activity as vitamin B_{12} in mammals remains to be clarified. In cyano- , hydroxo-, and aquocobalamin, the cobalt is in the oxidized (3+) state, whereas in B_{12} R it is Co^{2+}, and in B_{12}S it is Co^{+}. Release of the methyl group from methylcobalamin generates Cob(I)alamin.

Cobalamins acquire a positive charge in acid solution associated with opening of the coordinate linkage between cobalt and the nucleotide and with protonation of the N-3 of the 5,6-dimethylbenzimidazole. Water is then coordinated to the cobalt to form the "base off" configuration of these compounds. Very strong acid is required for this reaction with aquocobalamin (pK -2.4), whereas alkyl cobalamins such as methyl cobalamin undergo this change readily (pK 2.7) (62).

Diet

The quantity of vitamin B_{12} available in the diet varies with its animal content. It can range from 0.1 μg/100 ml in human milk (20) to 30 μg/d in the diet of middle-class Americans (26). In some populations, intake among adults may be less than 1 μg/day (5).

Absorption

Transmembrane transport of vitamin B_{12} is efficient when the vitamin is bound to a specific transport protein, which then reacts with specific receptors on the cell surface. This mechanism permits transport of 50% or more of carrier-bound vitamin. In the absence of such specific carrier-receptor interaction, a small proportion of vitamin B_{12} (about 0.1–1%) will cross the cell membrane.

Absorption of physiologically significant quantities of vitamin B_{12} from the diet depends on the gastric intrinsic factor (IF) mechanism. Physiologically significant quantities are also absorbed from large oral doses of vitamin B_{12} (100 μg or more) without the gastric intrinsic factor. Ingested vitamin B_{12} probably binds to R binder in saliva and stomach (2, 68), and is digested free

of R binder in the small bowel by pancreatic enzymes (especially trypsin) where it binds to intrinsic factor. The IF-B_{12} complex binds to a specific receptor on the brush border of ileal cells (66) after which, by a process with maximum rate of entry into the portal vein 8–12 hours after ingestion (29, 33), the vitamin B_{12} is freed from the IF, binds to transcobalamin II (24, 101), and enters the portal venous plasma. There is some evidence that transport is mediated by endocytosis (65), that release of the IF is by lysozomal digestion, and that transcobalamin II is added to the cobalamin within or at the basal surface of the enterocyte (101). This transport does not require intracellular metabolism of vitamin B_{12} to a coenzyme form.

Storage

The majority of the vitamin B_{12} in the body is in the liver. Hepatic vitamin B_{12} appears to be cleared exponentially from the body, with a half-clearance time of about 400 days (1).

Although about 1000 ml of gastric juice are produced daily by normal adults, 2–4 ml of such normal juice fed with vitamin B_{12} permits absorption of enough vitamin B_{12} to prevent clinical deficiency in adults. Intrinsic factor secretion must decrease to less than 5% of normal to reduce absorption of vitamin B_{12} to a level that will produce deficiency (21). The gastric intrinsic factor is present at birth, and can be detected in amniotic fluid as early as the 16th week of gestation (119).

Plasma Binding of Vitamin B_{12}

Plasma and certain other body fluids contain glycoproteins with a high affinity for cobalamins (37). These vary in carbohydrate content but all cross-react immunologically and are chemically similar. They have been called by a variety of names including "R" binders, TC 1, TC 3, TC 0, cobalophyllin, and haptocorrin. Some of the different names relate to differences in carbohydrate content, with consequent differences in rates of clearance by hepatocytes from the plasma. They carry cobalamins of which some are derived from cells. The function of these "R" binders is unknown, but because the less sialated species are cleared by hepatocytes with excretion of their vitamin B_{12} into the bile and because they have a high affinity for a variety of corrins, it has been postulated that they clear the circulation of corrins without vitamin B_{12} configurations (102).

Under physiological conditions, all of the extracellular vitamin B_{12} in the body is bound to either transcobalamin II (TC 2) or to one of the R binders. The former is found in plasma, spinal fluid, semen, and extracellular fluid; the latter is in most secretions, plasma, in the cytoplasm of a variety of cells including granuylocytes and their precursors, erythrocytes, and platelets, and in extracts of tissues (37). TC 2 is synthesized by a number of different cells, e.g. enterocytes (24), fibroblasts (9), endothelial cells, and amniocytes (99),

and by myocardium, kidney, spleen, and several other tissues (37, 54), but not by lymphocytes (52).

The R binders contain different quantities of carbohydrate and have different isoelectric points due to variable sialic acid content. Transcobalamin II appears to be free of carbohydrate but the gene products of its different alleles have different isoelectric points (30, 38). Both types of binder are single-chain molecules coded by single pairs of alleles, and both bind vitamin B_{12} tightly ($K_a \approx$ 1–10 pM). The R binders bind vitamin B_{12} more tightly than does TC 2 but are less selective for the cobalamin and corrin structure (37, 68).

Circulating levels of TC 2 are markedly increased in patients with Gaucher's disease (44) and in those with antibodies against TC 2 (88). Levels of R binder are increased in patients with chronic myelogenous leukemia and in some with other hematological neoplasms, hepatoma, and metastatic carcinoma. The chemistry, distribution, and physiology of these binders has been reviewed (37).

Vitamin B_{12} bound to transcobalamin II associates with receptors on the cell surface (84) and the complex is internalized into endosomes. The transcobalamin II is rapidly released from the cobalamin and leaves the cell as iodinated fragments when radioactive iodine is used to label the transcobalamin. Release and digestion of the complex and penetration of the vitamin B_{12} into the cytoplasm of the cell is prevented by chloroquine (128), which accumulates in endosomes and prevents generation of low pH. At low pH, vitamin B_{12} is released from transcobalamin II (43). There is no information about how vitamin B_{12} penetrates the endosomal or lysozomal membrane. Data have been published suggesting the presence on the surface of fibroblasts of a saturatable receptor for unbound cobalamin; this may represent a transport system for free vitamin B_{12} (9). In *Escherichia coli,* a complex system coded by several genes functions to transfer cobalamin. Utilization of vitamin B_{12} by *E. coli* consists of transport across the outer membrane into the periplasmic space followed by transport across the inner membrane into the cytoplasm. The first of these processes involves the binding of vitamin B_{12} to the btu B protein of the CBL receptor, followed by proton motive force– and ton B–dependent release of vitamin B_{12} into the periplasmic space. The second process involves the inner membrane–associated btu C and btu D gene products and possibly a third product (btu E). The function and relationship of these gene products to a 22,000-dalton protein with high affinity for vitamin B_{12} that is released from the periplasmic space by osmotic shock are unknown (10, 31, 41).

The human placenta effectively transfers vitamin B_{12} to the fetus using transcobalamin II and its receptors on the placenta (40, 107). As indicated below, all of the TC 2 in cord blood is fetal and intact maternal TC 2 is not found in the fetus. The vitamin B_{12} content of blood in the intervillous spaces

of the human placenta exceeds that in maternal or fetal plasma (47). The vitamin B_{12} content of umbilical venous and arterial blood is greater than that of the mother (126), although much of fetal plasma cobalamin is bound to a binder of the R type and so its availability is unknown (112). The human placenta also is permeable to passive diffusion of vitamin B_{12} when unbound vitamin is present in very high concentrations in the mother (85, 126).

Entry of Cobalamin into the Cytoplasm of the Cell

Some processing of vitamin B_{12} is required before it can accumulate in cells. More than 95% of intracellular cobalamin is bound to the two intracellular enzymes: methionine synthase and methylmalonyl CoA mutase. Methionine synthase from human placenta and from *E. coli* appear to bind Cob(III)alamin poorly (113, 117). Accumulation of vitamin B_{12} in cell cytoplasm might therefore require reduction of Cob(III)alamin to Cob(II)alamin.

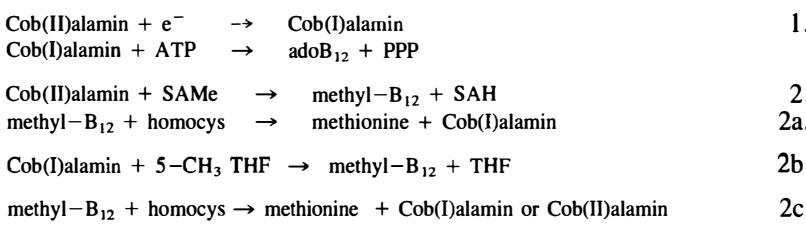
Methionine synthase is a cytoplasmic enzyme effecting transfer of a methyl group to homocysteine from 5-methyl-tetrahydrofolate. Polyglutamyl folate is only slighty preferentially utilized over monoglutamyl. Since the folate available to mammalian cells in vivo is 5-methyl-tetrahydrofolyl monoglutamate, folate entering the cell usually must traverse this pathway, although it can be bypassed by exposing the cells to folic acid or 5-formyl-tetrahydrofolate. The methyl group transferred from the folate converts Cob(I)alamin to methyl cobalamin, with subsequent methyl group transfer to homocysteine to generate methionine and enzyme-Cob(I)alamin (80, 113). After a number of such cycles, the enzyme-Cob(I)alamin oxidizes spontaneously to enzyme-Cob(II)alamin [or Cob(III)alamin] and apparently requires a reducing system and S-adenosyl cobalamin to regenerate methyl cobalamin and continue to be active. In *E. coli,* this reducing system apparently consists of two peptides that are separate from the synthase enzyme itself (42). The mammalian enzyme contains iron [(117); unlike the bacterial enzyme, which contains copper (80)] and may contain its own reducing system related in some way to the iron bound to the enzyme.

Methylmalonyl coenzyme A mutase (MMA) is bound in mitochondria and converts methylmalonyl CoA to succinyl CoA. It functions to convert products of propionate metabolism to easily metabolized products. This pathway is not a quantitatively important source of succinate in mammalian cells. Defective enzyme activity causes methylmalonic acid to accumulate, with consequent acidosis, ketosis, hyperglycinemia, hyperglycemia, and certain other metabolic abnormalities. The enzyme binds its cofactor 5'-deoxyadenosyl cobalamin tightly. The cobalamin is required for activity. Mitochondria also contain Cob(I)alamin: ATP-adenosyl transferase, which generates 5'-deoxyadenosyl cobalamin.

Cobalamin entering the cell must enter the mitochondria for conversion to 5'-deoxyadenosyl cobalamin and to permit functioning of the MMA mutase

enzyme. Studies with mitochondria from rat liver (34, 35) have revealed that Cob(III)alamin (as hydroxocobalamin) will passively enter swollen rat liver mitochondria to produce 5'-deoxyadenosyl cobalamin without addition of exogenous reducing agents, but it does require adequate intra-mitochondrial ATP and glutamate. Measurable quantities of 5'-deoxyadenosyl cobalamin were generated by rat mitochondria incubated in less than 0.1 nM of Cob(III)alamin (34). The entry of Cob(III)alamin into mitochondria swelled by incubation appears to be passive, does not require proton pumping, and does not involve a demonstrable transport system. The above have been extensively reviewed by Sennett and Rosenberg (36, 109). Whether Cob(II)alamin penetrates mitochondria more effectively than does Cob(III)alamin, and whether Cob(II)alamin or Cob(I)alamin can leave mitochondia and enter the cytoplasm of cells, are unknown.

The above would suggest that defective activity of MMA mutase enzyme could be due to (*a*) defective mutase enzyme, either absent or kinetically abnormal (83, 125); (*b*) Cob(I)alamin:ATP-adenosyl transferase enzymes, or (*c*) abnormal penetration of cobalamin into mitochondria or its intramitochondrial reduction to Cob(I)alamin. Cobalamin-mediated reactions in mammalian cells include the following:



1.
2.
2a.
2b.
2c.

where $adoB_{12}$= 5-deoxyadenosyl cobalamin; PPP=triphosphate; methyl$-B_{12}$= methyl cobalamin; homocys= homocysteine; and $5-CH_3$ THF= N−5 methyl-tetrahydrofolate (mono- or polyglutamate).

RECOGNITION AND INVESTIGATION OF DEFICIENCY OF VITAMIN B_{12}

Clinical manifestations of deficiency differ in the infant and in the adult. In infants, deficiency may induce delayed growth, diarrhea, vomitting, failure to thrive, and pancytopenia. In older patients, symptoms may be limited to those of anemia, a sense of being unwell, paresthesias, or weakness. In the infant, methylmalonic aciduria with or without homocystinuria is usually present and may be associated with other acidurias including ketone bodies and glycinuria

(79). In defects affecting methionine synthase, megaloblastic anemia is often present.

LABORATORY FINDINGS Deficiency of the vitamin is usually associated with a low concentration of vitamin B_{12} in serum; this is not observed in most patients with metabolic defects. Determination of vitamin B_{12} concentration in serum either microbiologically or using radiodilution assay will detect the former. Low levels of vitamin B_{12} in serum do not, however, prove deficiency; decreased concentrations are also found in a small proportion of subjects not deficient in vitamin B_{12}.

Anemia with macrocytic erythrocytes (mean corpuscular volume, MCV, 106 or greater) may be present, but in children with certain inherited defects, macrocytosis of erythrocytes may be absent. Neutropenia and thrombocytopenia are regularly present in children with vitamin B_{12}–dependent homocystinuria, and some neutropenia and thrombocytopenia may be observed in children with defects in methylmalonyl CoA mutase activity (79, 95).

Methylmalonic acid is found in urine in large quantities (grams per gram of creatinine) in children with defects of intracellular metabolism of cobalamin, and in smaller quantities in those with deficiency of the vitamin. Measurement of methylmalonic acid in serum may provide a reliable test for deficiency of vitamin B_{12}, and should be explored (cited in 87). In patients with deficiency or abnormal metabolism of methyl cobalamin, a positive deoxyuridine suppression test on bone marrow cells proves deficiency of vitamin B_{12} (123).

Recognizing deficiency in the newborn is often difficult, but delay in diagnosis and institution of therapy can be devastating to the infant. Treatment with large doses of vitamin B_{12} (1000 μg/day by injection) should be instituted as soon as megaloblastic anemia is identified and specimens of bone marrow, blood, and urine are collected for analysis. Determination of transcobalamin II level is difficult if plasma or serum contains very large quantities of vitamin B_{12}, and so should be determined on plasma collected before treatment with vitamin B_{12}. Subsequent identification of the defect will utilize tests of absorption and cultured fibroblasts from a skin biopsy. These are not adversely affected by prior treatment of the patient. Inherited defects of cobalamin metabolism are summarized in Table 1, and a scheme of their location within the metabolism of the cell is illustrated in Figure 1.

DEFECTS IN THE ENTRY OF VITAMIN B_{12} INTO THE BODY

Children Born of Mothers Deficient in Vitamin B_{12}

Although deficiency of vitamin B_{12} may be associated with sterility, there is no evidence that deficiency of this vitamin in the mother affects the in-

Table 1 Inherited defects of cobalamin metabolism[a]

	Defective IF	Gastrointestinal absorption	TC 2 defect	Cobalamin A	B	C	D	E	F
Clinical sign									
Megaloblastic anemia	A	A	A	N	N	A	A	A	A
Developmental delay	A	A	A	A	A	A	A	A	A
Earliest onset (mo.)	12	12	1	1	1	1	150	2	2
Homocystinuria	—	—	N	N	N	A	A	A	N
Methylmalonic aciduria	—	—	+ −	A	A	A	A	N	A
Defects detectable in cultured fibroblasts	0	0	+	+	+	+	+	+	+
Whole cells									
TC 2 synthesis			A	N	N	N	N	N	N
Propionate uptake			N	A	A	A	A	N	A
CH_3−THF uptake			N	N	N	A	A	A	A
B_{12} uptake			N	N	N	A	A	N	incr
CH_3−B_{12} content			N	N	N	A	A	A	A
Ado-B_{12} content			N	A	A	A	A	N	A
Lysozomal B_{12} efflux			N	N	N	N	N	N	A
Extracts									
Activity of holoenzyme of methionine synthase				N	N	A	A	N[b]	A
MMA mutase				A	A	A	A	N	A

[a]N = normal; A = abnormal. [b]Normal in excess reducing conditions.

trauterine development of the child. The possible contribution of deficiency of vitamin B_{12} to neural tube defects has been queried (103), especially because anencephaly in rats has been described among offspring of vitamin B_{12}–deficient dams. No requirement for vitamin B_{12} has been demonstrated for the human fetus until birth.

Several reports (23, 58, 64) have described deficiency of vitamin B_{12} appearing in infants born of mothers deficient in vitamin B_{12} and subsequently breast fed by them. Symptoms were first noted after 3–6 months, with diagnosis made about 3 months later. Symptoms appeared earlier (6–10 weeks) in infants born of mothers with pernicious anemia than in children of vegetarians. A variety of neurologic abnormalities have been described in these children, with mental development often remaining delayed after therapy.

Defective Absorption of Vitamin B_{12}

DEFECTIVE INTRINSIC FACTOR The usual cause of intrinsic factor deficiency is immunological destruction of the gastric mucosa, which causes pernicious anemia. Achlorhydria is regularly observed in these patients be-

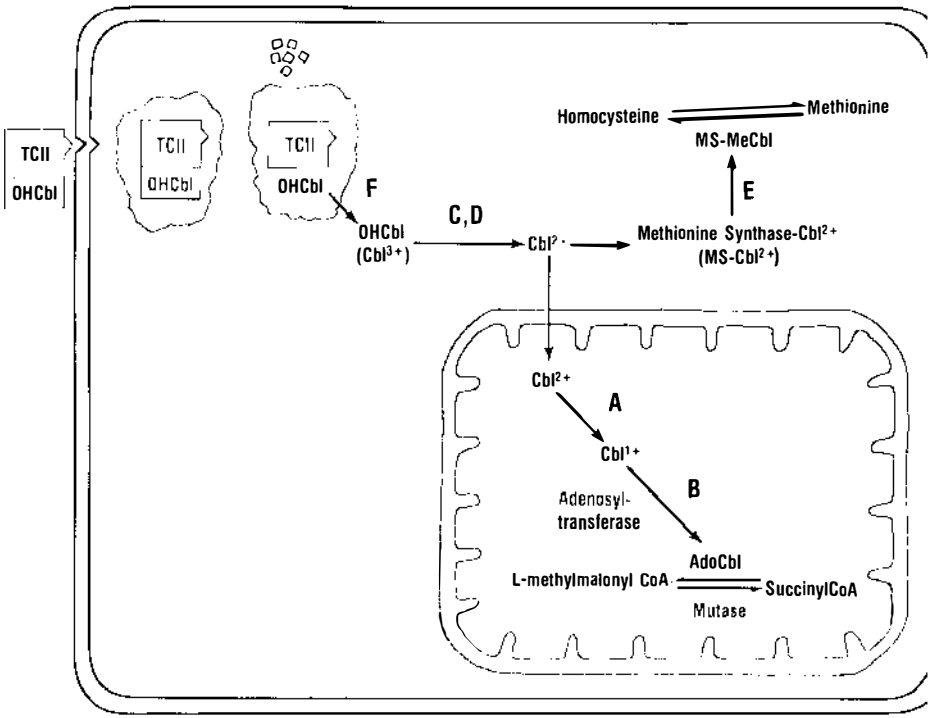


Figure 1 Intracellular metabolism of vitamin B_{12} (Cbl). Scheme of entry and utilization of vitamin mammalian cells. The letters refer to cobalamin mutant classes and place the defect in these wi metabolic scheme.

cause the gastric IF is synthesized and stored in the gastric parietal cell—the cell that produces acid (60).

In a number of children, absence of effective intrinsic factor has been reported in the presence of normal acid secretion and mucosal cytology (reviewed in 127). In these children, absorption of vitamin B_{12} is abnormal but becomes normal when the vitamin is mixed with normal human gastric juice as a source of normal intrinsic factor. Since the first demonstration that one such case was caused by an abnormal intrinsic factor with reduced affinity for the ileal IF-B_{12} receptor (67), some, but not all, have been shown to synthesize immunologically reactive but physiologically inert IF (15, 71, 127). The lability to destruction by acid and pepsin of intrinsic factor not binding vitamin B_{12} (49) has been emphasized by a report of a labile IF molecule with low affinity for vitamin B_{12} (127). Deficiency of vitamin B_{12} in these children appears after the first year of life and usually before age 5. In some with only partially defective IF molecules, illness has appeared as late as age 12 (67).

DEFECTIVE TRANSPORT OF VITAMIN B_{12} BY ENTEROCYTES Because the IF-B_{12} receptor on the brush border of ileal enterocytes is required for the absorption of vitamin B_{12}, it is logical to assume that some cases of defective absorption are due to abnormalities of this structure (22, 76). At least 60 cases of selective malabsorption of vitamin B_{12} from the gut with normal intrinsic factor have been described. The illness appears to be more common in inbred populations, and is particularly common in Finland (50) and among North African Jews (22). All of those investigated have had normal intrinsic factor in their gastric juice, normal transcobalamin II and absence of antibodies against IF in plasma, and normal intestinal morphology and gastrointestinal function except for malabsorption of vitamin B_{12} that was not corrected by adding intrinsic factor. In some, proteinuria was present and in a few was described as of the "tubular" type. Most patients have developed clinical manifestations of deficiency of vitamin B_{12} between the ages of 1 and 15 years, but the deficiency has occasionally appeared later. In one achlorhydric patient who may have had this illness, clinical deficiency first appeared at age 41 years (25).

Three brothers with this syndrome had normal quantities of IF-B_{12} receptor in ileal biopsies (homogenates bound IF-B_{12} normally), which suggests that their defective transport of vitamin B_{12} was due to a defect other than absence of the IF-B_{12} receptor (76). The transport of vitamin B_{12} following binding to the IF-B_{12} receptor is complex, as demonstrated by the fact that some analogues of cobalamin bound to IF will bind to the IF-B_{12} receptor in rabbit intestine but will not cross the intestine (68). Apparent absence of the intestinal receptor for the IF-B_{12} complex in some of these patients has been described (12). Although the exact physiology of this "Immerslund-Grasbeck" syndrome is unknown, patients respond completely to periodic injections of vitamin B_{12}, but proteinuria persists.

Histological examination of renal biopsies from these patients has not been helpful. Some mesangial thickening, slight thickening of basement membrane, and minimal proliferative changes have been described in glomeruli; electron microscopy and immunochemistry reveal some deposits staining for IgG in mesangial areas and along the basement membrane of glomeruli, with "moon craters" and membranous convoluted structures in glomerular basement membrane, extracellular round particles between epithelial cells and basement membrane (28).

Defective absorption of vitamin B_{12} due to lack of tryptic digestion of R binder may be caused by pancreatic insufficiency and is observed in diseases of the small bowel. As described below, functional transcobalamin II is required for completion of the absorptive process, so that patients without detectable transcobalamin II in plasma do not absorb vitamin B_{12} from the gut.

TRANSCOBALAMINS

Transcobalamin II

The physiological importance of transcobalamin II (TC 2) has been proved by descriptions of and studies on patients with congenital deficiency of transcobalamin II. These are described below.

CLINICAL SYNDROME We are aware of 17 published, documented cases of congenital deficiency of TC 2 (Table 2). Dr. Charles Hall (52) has accumulated information about an additional 10 unpublished cases in six families, which makes a total of 27 proved cases. Two patients were dizygotic twins, younger siblings of patient AFr (86), and were diagnosed soon after birth and before symptoms developed. They were treated at age 3–4 weeks when leucocyte and platelet counts decreased, and so their symptoms cannot be evaluated. Several probable cases have been reported but these are excluded from this analysis.

The defect is recessive and thus requires transmission of defective genes from both parents. Some (but not all) patients were products of consanguinous marriages, but more than one case has occurred in at least four families.

An electrophoretic method has been described that defines five normal isotypes of transcobalamin II and three others that may be defective and cause deficiency (30, 38). In normal plasma, four bands are observed: one pair representing one each of maternal and paternal types. By this technique it has been demonstrated that the TC 2 in the plasma of infants is not derived from maternal TC 2 but is the infant's own TC 2. Children with deficiency of TC 2 are normal at birth despite evidence that maternal TC 2 is not detected in cord blood and thus apparently does not enter the fetus in detectable quantities (8). One patient with severe deficiency of transcobalamin II (108) bore two normal children.

Of the 15 published cases who developed clinical illness, all apparently were normal at birth, except for immunological deficiency in one (59). Symptoms (usually vomitting, pallor, weakness) appeared during the first month of postnatal life in eight patients, during the second month in three, late in the first year in two, and after this in one. Immunologically reactive TC 2 was absent from the plasma or serum of 9 of the 11 patients tested in this group. Illness appeared at the ages of 26 days and 12 years in the two with immunologically reactive TC 2 in plasma or serum.

Most patients deficient in transcobalamin II usually have had pancytopenia and megaloblastic anemia (Table 3). In one child, only erythroid hypoplasia was noted (86). Patients have sometimes developed combined system disease (subacute combined degeneration of the spinal cord), the neurological abnormality characteristic of vitamin B_{12} deficiency. Neurological disease has

Table 2 Published cases of transcobalamin II deficiency

Patient	Age[a] (days) Symptomatic	Age[a] (days) At diagnosis	MCV	Marrow	MMA	Neurologic abnormalities	TC 2	Prescription (mg/w) B_{12}	Prescription (mg/w) Folate	Ref.
AnS	17	24		meg[b]	neg[c]		0	2		51
AmS	3	35	92	meg			0	3.5		51
AB	30	160		meg			0	3	15	59
	90	270		meg				2–3		116
MN	9	45		meg	neg[c]	+	0.7	0.7	105	11
	42	34 yr		meg			+	1		108
NA	60	60	89	meg	pos			[d]		75
KP	30	15 yr		meg			0	1	5	45, 118
HM	30	60		meg			0	0.5		39
	42	56	91	±[e]			0	1		90
AFr	30	75	90	[f]				1		86
AFv	26	7 yr		meg	neg	+	+	2	42	115
JW	60	120	89	meg	neg			3.5		18
MS	100	165	90	[g]		+	0	1	1	81
KC F	10	150		meg	pos		0	2	2.5[h]	129
CB	12 yr	34 yr		meg	neg[c]	+		1		56, 57
XFr[i]										8
UFr[i]										38

[a]When symptoms noted and when B_{12} deficiency recognized.
[b]meg = Megaloblastic
[c]Tested after relapse from withdrawal of therapy.
[d]"Massive parenteral doses of hydroxocobalamin."
[e]Bone marrow erythroid hypoplasia megaloblastic + 9% blasts.
[f]1% normoblastic erythroid cells, "myeloid series macrocytic with cytoplasmic vacuoles and evidence of myeloid maturation arrest."
[g]16.5% blast cells, 26.5% promyelocytes, 1% erythroid, giant metamyelocytes.
[h]Transient response to 12.5 μg/day of B_{12}, maintained on oral B_{12}.
[i]Dizygotic twins, siblings of AFr, treated before they became symptomatic.

Table 3 Characteristics of published patients with TC 2 deficiency

Megaloblastic bone marrow	14/15
Erythroblastopenia	3/15
Increased myeloblasts present	2/15
MMA before any B_{12} therapy	2/3
Immunologically reacting TC 2 present	3/14[a]
Neurological signs	3/10
Mouth ulcers	6/12
Infection	4/12
Response to folate	5/5[b]

[a]Additional cases studied by Hall (51a) and by Frater-Schröder (38).
[b]One of these relapsed during two weeks of folinic acid

occurred 5, 9, and 16 months after the beginning of illness (11, 81, 115), and was associated with treatment with folic acid in one of these (115). Some children with absent TC 2 have been shown to have severe immunological deficiency with defective humoral and cellular immunity, and one has had defective granulocyte function (59). The immunological, hematological, and systemic symptoms have responded to treatment with vitamin B_{12}.

Homocystinuria has not been described in any of the case reports,[1] but in most its excretion was not determined when patients were first seen. In one patient "urine screening for amino acids was negative" (90) before treatment, and in two others, homocystinuria was sought and not found (18, 129). Homocystinuria was not found in three patients studied following discontinuation of therapy (11, 106, 118), but in one of these, low plasma methionine level was reported (11). The excretion of methylmalonic acid has been measured in seven patients: four before initial therapy with vitamin B_{12} and three in hematological relapse following discontinuation of therapy. Abnormal MMA excretion was observed in two of the four tested before initial therapy, and in one of the three tested during relapse. The quantity of MMA excreted has been much smaller than in children with inherited intracellular metabolic defects. It has been suggested that the general symptoms and hematological abnormalities in this disease do not correlate with MMA or homocystine excretion, and a role for cobalamin delivery to hepatic cells by R binders has been postulated (68, 73). Measurements of hepatic vitamin B_{12} and more measurements of methionine and homocystine in plasma and urine are required to determine if a phenomenon requiring explanation exists.

Patents deficient in TC 2 have been effectively treated by maintaining serum vitamin B_{12} at very high levels. In some effectively treated patients, levels of serum vitamin B_{12} have been described as 1000–3000 but in others a

[1]A later additional report about one patient (115) states that homocystinuria was present (60a).

level of 10,000 pg/ml or more was observed. These levels were achieved with therapy ranging from oral hydroxo- or cyanocobalamin twice weekly (500–1000 μg) to injections of 1000 μg of hydroxocobalamin weekly or more frequently. Treatment dosages must be titrated against blood counts, symptoms, and immune function. In at least two patients, the dosage of vitamin B_{12} required to correct hematological parameters was less than required to correct neutrophil function or hypogammaglobulinemia (59, 90).

Folic acid was effective in correcting the hematological abnormality in most patients. Of five patients so treated, four received folic acid at doses of 2 mg daily by mouth, 15 mg per week by injection, 15 mg per day by mouth, or a single dose of 15 mg by mouth. All of these dosages increased granulocyte and platelet counts. One of these patients relapsed in two weeks while treatment with an unspecified dosage of folinic acid was continued. Two patients remained hematologically well for 16 and 70 months receiving folic acid orally at 15 mg per day (11) and folinic acid orally at 6 mg per day (115) respectively. Because of the probability of hematological relapse and the danger of neurological damage, folate should not be administered to these patients without effective doses of vitamin B_{12} (105).

Bone marrow was megaloblastic in most patients examined, but in three patients, severe erythroid hypoplasia was present. In one of these, megaloblastic features were not noted in the 1% of residual erythroid cells and many blasts and promyelocytes were present. Had the giant metamyelocytes not been recognized, this patient might have been diagnosed as having acute leukemia. An older sibling of one patient was reported to have died of "promyelocytic leukemia" (118).

In all but one of the patients, transcobalamin II was absent from plasma when tested by its capacity to bind vitamin B_{12}. In 12 patients without B_{12} binding by TC 2 in plasma, immunologically reacting transcobalamin was sought. It was present in three patients. The absorption of vitamin B_{12} from the gut apparently requires the synthesis of transcobalamin II. Of the six patients in whom vitamin B_{12} absorption was evaluated, it was normal in only two (56, 108), both of whom had immunologically measurable TC 2 in plasma, whereas absorption was abnormal in all four patients tested in whom no immunologically reactive TC 2 was present (11, 51, 59, 129). This suggests that although transcobalamin II synthesis by enterocytes probably is required for intrinsic factor–mediated transport of vitamin B_{12} across the enterocyte, this may not require a functionally normal transcobalamin molecule.

Deficiency of R Binder

Six individuals in five families have been described with deficient or absent R binders in plasma, saliva, and leukocytes (Table 4) (13, 14, 17, 63, 110). Although neurological disease of unknown cause was present in two of these

patients, no evidence has been found of adverse effects from their R binder deficiency. Serum vitamin B_{12} levels are in the deficient range in such persons but they are not deficient in vitamin B_{12}, and TC 2–B_{12} levels are normal. It is of interest that in three patients, sickle hemoglobin or beta thalassemia trait were present and that the ethnic origins of the patients described were Puerto Rican, Corsican, and Asian Indian; two had hemoglobin sickle cell trait. It is possible that this abnormality may be more common in non-Caucasian groups.

Defective Retention or Mobilization of B_{12}

One patient has been described in whom mild deficiency of vitamin B_{12} was associated with a rapid decrease of vitamin B_{12} associated with transcobalamin II in plasma after injection of cyanocobalamin (7). Detailed studies revealed slightly decreased R binder levels and no abnormality in the affinity of the transcobalamin II for vitamin B_{12} or for the TC 2–B_{12} receptor. The affinity of TC 2–B_{12} receptors on the patient's lymphocytes for normal TC 2–B_{12} was also normal. The patient was 39 years old, and holo–TC 2 levels in her four children were normal. It is unclear whether loss of vitamin B_{12} from her body was accelerated or whether mobilization from the liver was defective.

DISORDERS OF UTILIZATION

Inherited defects in the cellular utilization of vitamin B_{12} have been defined alphabetically as mutations A through F.

In mutant classes A and B, only the synthesis of 5'-deoxyadenosyl cobalamin is abnormal. Methyl-cobalamin-dependent reactions including bone marrow morphology are normal.

In mutant classes C and D, accumulation of cobalamin by cells is defective, and cellular metabolism utilizing both 5'-deoxyadenosyl cobalamin and

Table 4 Reported cases of haptocorrin (R binder) deficiency

Patient	Sex	Age	Serum B_{12} (pg/ml)	Associated conditions	Ref.
PB	m	47	0–74	subtotal gastrectomy	17
WB	m	46	34–93	multiple sclerosis	17
NC	m	77	<50	AS hemoglobin[a]	13
—	m	64	196	AS hemoglobin	14
—	f	34	<50	beta thalassemia	63
ER	m	45	144	neurologic disease	111

[a]Incomplete deficiency of haptocorrin; AS hemoglobin = sickle hemoglobin trait.

methyl cobalamin is abnormal. Cobalamin E patients have a defect in intracellular methionine synthesis with normal activity of methionine synthase in extracts. Several patients with similar clinical syndromes have reduced activity of methionine synthase in cell extracts and represent a distinct complementation group.[2]

Cobalamin F is a defect in transport of cobalamin from endosomes or lysozomes into the cytoplasm of the cell.

Cobalamin Mutant Classes A and B

Children with defects in MMA mutase enzyme have been described (95) and are not discussed here. Those with defective or absent activity of Cob(I)alamin:ATP-adenosyl transferase have been described as Cobalamin mutant class B (77), in which synthesis of 5'-deoxyadenosyl cobalamin is defective in intact fibroblasts or extracts of fibroblasts, and methylmalonic acid accumulates in plasma and is excreted in the urine. The clinical syndrome is similar to that of children with defective MMA mutase enzyme.

A group of patients has been described with methylmalonic aciduria and defective synthesis of 5'-deoxyadenosyl cobalamin in intact fibroblasts but normal synthesis of 5'-deoxyadenosyl cobalamin in extracts of fibroblasts (77). The defect is unknown and differs from that in Cobalamin B disease. It is possible that the defect is in reduction of Cob(II)alamin or Cob(III)alamin to Cob(I)alamin in mitochondria, or it may be due to some other defect. The latter cases have been named "Cobalamin mutant class A" or Cobalamin A disease.

Many of these defects are incomplete, and in these treatment with large doses of vitamin B_{12} may permit some intracellular synthesis of 5'-deoxyadenosyl cobalamin and thereby reduce the accumulation of methylmalonic acid, with consequent clinical improvement. It should be noted that in isolated rat mitochondria, transport of cyanocobalamin is inferior to that of hydroxocobalamin (34, 35), and so treatment with the former is theoretically preferred. Cyanocobalamin is, however, effective treatment for patients.

CLINICAL STUDIES In normal subjects, methylmalonic acid in urine usually is less than 15–20 μg per gram of creatinine, whereas affected children usually excrete more than 100 mg and up to 2 grams of MMA per day. In adults with pernicious anemia, median daily MMA excretion is about 200 mg (range about 20–1500).

Of twelve patients, with Cobalamin A disease (79), illness began by day 7 in five, after 1 year of age in one, and between age 1 month and 1 year in six.

[2]These have been named "mutant classes Cobalamin G" (100a).

Of eight patients with mutant class Cobalamin B disease, symptoms began before the age of 1 month in four, and after the age of 1 year in one. Symptoms were similar in both Cobalamin A and B patients and in patients with inherited deficiency of the mutase enzyme. Symptoms were related to accumulation of methylmalonic acid, with consequent acidosis and ketosis. In descending order of frequency these were lethargy, failure to thrive, vomitting, dehydration, respiratory distress, hypotonia, developmental retardation, hepatomegaly, and coma. Laboratory
nemia in 80%, increased blood ammonia in 65%, leukopenia and/or thrombocytopenia in 65%, and anemia in 30%.

Of these Cobalamin A patients 70% responded to therapy and remained well, 23% survived but were impaired, and only one died. Of 10 Cobalamin B patients, three died and three were alive and well. Response to therapy with vitamin B_{12} differed between mutant classes A and B: 10 of 11 Cobalamin A patients responded to therapy whereas only 3 of 8 Cobalamin B patients responded with marked change in methylmalonic acid excretion ($P < 0.05$).

Methylmalonic acid is poorly metabolized when released from CoA, so that mothers bearing infants with these defects may excrete methylmalonic acid. Prenatal diagnosis of the defect and treatment with vitamin B_{12} is therefore possible if suspected (3). Because, however, the infants appear to be born normal, it is unclear if prenatal treatment is better than initiation of treatment at birth.

Several children excreting small quantities of methylmalonic acid (1–3.4 μg of MMA/mg of creatinine) without clinical or other biochemical abnormalities have been described (70). There has been some challenge to the specificity of the assay used (87), although complementation studies provided additional evidence of the defect in two the eight children reported. Methylmalonic acid excretion did not respond to treatment with cyanocobalamin in the six children treated. Two adults in a family were found to excrete methylmalonic acid (16 and 115 mg/day) as a result of a partial and asymptomatic defect in intracellular synthesis of 5'-deoxyadenosyl cobalamin (46).

Cobalamin Mutant Classes C and D

The amount of vitamin B_{12} in fibroblasts cultured from most patients with Cobalamin C and D abnormalities is below normal, and these cells accumulate little vitamin B_{12} when cultured in vitro. Of the cobalamin in such fibroblasts cultured in cyanocobalamin, the majority remains as cyanocobalamin, which suggests that this cobalamin form could not be processed. Hydroxocobalamin [Cob(III)alamin] is utilized slightly better by these cells (34, 53), but intracellular accumulation usually is less than in normal cells.

The specific defect causing this abnormality is unknown. As indicated

above, Cob(III)alamin passively enters swollen mammalian mitochondria. Cells of patients with Cobalamin C disease are deficient in both intracellular methyl cobalamin and 5'-deoxyadenosyl cobalamin. If the processing of vitamin B_{12} [Cob(III)alamin] required for intracellular accumulation is limited to reduction to Cob(II)alamin, then either (*a*) entry of vitamin B_{12} into mitochondria within cells requires such reduction, unlike swollen mitochondria in vitro, or (*b*) these cells must contain an additional defect.

Because of the intracellular deficiency of methyl cobalamin and 5'-deoxyadenosyl cobalamin, in such patients homocystine and methylmalonic acid may both be found in plasma and are excreted in the urine. Their symptoms, caused by defects in these pathways, include acidosis etc as in Cobalamin A and B disease, megaloblastic anemia or sometimes severe hypoplastic anemia, and neurological abnormalities ranging through combined system disease, psychosis and delirium, and cerebrovascular disease (which may be secondary to homocystinemia).

CLINICAL SYNDROME On the basis of complementation studies performed using fibroblasts from affected individuals, patients with combined methylmalonic aciduria and homocystinuria have been divided into two classes designated Cobalamin C and Cobalamin D (124). Accumulated data on patients reported thus far are listed in Table 5, compiled by Mitchell and colleagues (82). It was originally thought that Cobalamin C patients presented soon after birth with lethargy, failure to thrive, feeding difficulties and megaloblastic anemia, and that the Cobalamin D patients were more mildly affected, presenting with behavioral problems and mild mental retardation. However, more recent reports describe patients with Cobalamin C presenting later in childhood or even at adolescence. Thus, Cobalamin C has more genetic heterogeneity than was originally believed.

All symptomatic patients with Cobalamin C or Cobalamin D presented with abnormal neurologic or psychiatric signs. Of the 11 infantile-onset patients for which data are available, eight had lethargy or delayed mental development, four had microcephaly, and two had convulsions. In contrast, all three patients with later-onset disease presented with psychosis or delirium. Spasticity and electroencephalographic slowing were documented in two of them, and at least one had cerebral atrophy. In three patients, a retinal abnormality associated with perimacular pigmentation was described (16, 82, 94) and responded to treatment with vitamin B_{12}.

The initial signs of Cobalamin C disease have been nonspecific, especially if not accompanied by pigmentary retinopathy or megaloblastic anemia, and diagnosis has depended on urinary amino acid and organic acid determinations. In at least two instances, neonatal methylmalonic acid screening has been the method by which the diagnosis was made. Retinopathy has been

Table 5 Cobalamin mutant classes C and D: cases reported[a]

Reference	onset	death	birth wt.	Hematology Hb g/dl	Marrow (megaloblastic)	Retinal Pigmentation	CLINICAL SIGNS Neuropsychiatric	PRETREATMENT BIOCHEMICAL VALUES MMA	Hcys	Met	Vit.B_{12}	Folate	Ethnic Origin	Other Features
INFANTILE ONSET														
72	2d.	2 mo.	2840	11.4	-	NR	Lethargy, convulsion, craniosynostosis	(u) 0.2 mmol/24h	(pl) 70 µmol/1 (u) 19 µmol/24h	(pl) 5 µmol/1 (u) 0	NR	NR	NR	GI Bleeding, hematuria, *azotemia*
6	2 wk.	4 mo.	2670	6.5	+	NR	Neuropathology normal, microcephaly	(u) 8.9-61.9 mmol g. creat.	(u) 2.38 12.65 µmol g. creat.	NR	285	5.1	NR	multiple thromboses "hemolytic uremic syndrome"
94	1 mo.	-	2160	"anemia	NR	+	microcephaly developmental delay	(pl) and (u) present	(pl) 10 µmol/1 (u) present	NR	"normal"		NR	failure to thrive
82	1 mo.	-	2720	8.5	-	+	Lethargy, poor feeding	(pl) 51 µmol/1 (u) 18.1 mmol/g creat.	(u) present	(pl) 13µmol/1	1475	45	French Canadian	
74	1 mo.	-	2160	NR	+	NR	Microcephaly	(u) increased	(pl+u) increased	(pl+u) decreased	1160	9.2	NR	
16	6 wk.	-	2780	8.3	+	+	Convulsion	(u) 3.4-42.6 µmol/24h	(pl) increased	(pl) 7 µmol/1	610	greater than 50	Mexican American	GI Bleeding
93	6 wk.	-	3100	9.8	NR	NR	Somnolence, Retardation, Microcephaly, Nystagmus	(u) 1.4 mmol/24h	(u) 0.132 mmol/24h	(s) 10 µmol/1	707	35.7	NR	

Table 5 *(Continued)*

32	2 mo.	7 yr.	NR	10.3	+	NR	Apathy, Lethargy, Neuropathology: subacute combined degeneration	(u)4.0 - 6.2 mmol/24h	(u)23–47 µmol/ 24h	(pl)18 µmol/1	1000	15.6	NR	cachexia infections
91	2 mo.	-	NR	an-emia	NR	NR	NR	(u)48 mmol/g creat.	3010 µmol/g creat.	NR	"elevated"		NR	
78	8 mo.	-	2970	an-emia	yes	abnormal evoked potentials	Lethargy, hypotonia	(u) 3.9 mmol/24h	(u) 36 µmol/24h	NR	2523	60	His-panic	Hatchet-shaped head; arachno-dactyly
27	8 mo.	-	NR	NR	NR	no	Hypotonia, ataxia developmental delay (CT): dilated lateral ventricles	(u) increased	(u) and (pl) increased	NR	increased		Aust-ralian	Epileptiform eye movements Sibling died at 5 years; coma & fever
4	18 mo.	-	NR	"nor-mal"	NR	NR	Ataxia Pyramidal signs Developmental delay	(u) 7.95 mmol/24h	(pl)11 µmol/1	(pl)5 µmol/1	1505	19.6	Aust-ralian	Asymptomatic affected 12 mo. old brother
CHILDHOOD ONSET														
82	4 yr.	-	5390	12.7	+	no	delerium, spas-ticity, behavior problems CT: atrophy EEG: slowing and epileptic spikes	(u) 1.88 mmol/g creat.	12 µmol/1	(pl) 16.8 µ mol/1	1350	17.2	Port-uguese	2 siblings died in infancy in poorly defined circumstances

[a]Abbreviations: u = urine, pl = plasma, s = serum.

Table 5 *(Continued)*

Reference	onset	death	birth wt.	Hematology Hb g/dl	Marrow (megaloblastic)	CLINICAL SIGNS Retinal Pigmentation	Neuropsychiatric	PRETREATMENT BIOCHEMICAL VALUES MMA	Hcys	Met	Vit.B_{12}	Folate	Ethnic Origin	Other Features
ADOLESCENT ONSET														
110	14 yr.	-	NR	12.6	no	NR	psychosis, gait disturbance, cortical atrophy (CT) EEG slowing	(u) increased	46 µmol/1	(pl) trace	824	16.8	NR	Asymptomatic 12 year old and 8 week old siblings
CBL D														
48	12 yrs	-	NR	14.1	-	NR	psychosis, psychomotor retardation, nystagmus	(u) 1.54-17.95 mmol/d	(pl) undetectable (u) 6.1-87.0 µmol/day	(pl) 20 µmol/l	766	17.2	NR	cbl d group asymptomatic 2.5 year old brother
Normal Values								(pl)undetectable (u)0.035±0.010mmol/g. creat. (30) or 0-0.10 mmol/24h (5)	(pl)&(u) undetectable	(pl) 10-41 µmol/l (u) 0-2 µmol g. creat.	200-900 pg/ml	4-20 ng/ml		

[a]Abbreviations: u = urine, pl = plasma, s = serum.

reported in three children with Cobalamin C disease. Fundoscopy can strongly indicate the diagnosis in advanced cases, but initial changes have been subtle. Hematological abnormalities have sometimes been absent: only 8 of 11 infantile-onset patients were reported to have macrocytosis, anemia, or megaloblastic changes, and only one of the older-onset patients had mild megaloblastic changes. Elevated serum cobalamin and folate levels have been observed in several but not all patients.

TREATMENT Most Cobalamin C patients have improved with hydroxocobalamin treatment (1 mg daily or twice weekly, usually by injection). The homocystinuria and methylmalonic aciduria have usually decreased but have not been completely eliminated, and the megaloblastic changes in bone marrow usually have reversed. In one patient (74) homocystinuria was not affected by treatment with hydroxocobalamin but decreased during treatment with 0.5 mg of folic acid per day, increased during treatment with methionine, and then responded partially to betaine to disappear when folic acid (4 mg/day) was administered.

The form of cobalamin recommended for therapy is unclear. In fibroblasts (35), HeLa cells, and certain other cells cultured in vitro (53), hydroxocobalamin was more effectively utilized than was cyanocobalamin. In two studies, injections of 500 μg of OH-B_{12} or CN-B_{12} did not decrease methylmalonic aciduria (32, 82), whereas 1000 μg did. 5'-Deoxyadenosyl cobalamin appeared to be no more effective than hydroxocobalamin (cited in 32). In one child treated with 500 μg of methyl cobalamin twice weekly, methylmalonic aciduria may have decreased more than with hydroxocobalamin but the effect was transient (74).

Cobalamin Mutant Classes E and G

COBALAMIN E Mutant class E is a putative defect in the methionine-synthase-associated reducing system, characterized by homocystinuria without methylmalonic aciduria and defective biosynthesis of methionine despite normal activity of methionine synthase in cell extracts under standard assay conditions (96, 104). The defect appears related to maintaining vitamin B_{12} bound to methionine synthase in the reduced state.

The clinical findings in the original patient with Cobalamin E disease included vomitting and poor feeding, beginning at 9 weeks of age, associated with pancytopenia, megaloblastic anemia, homocystinuria, homocystinemia, and hypomethioninemia. In the affected sibling of this patient, the defect was identified in utero (97) by studies of cultured amniocytes. We are aware of at least two additional unpublished cases of Cobalamin E disease.

Diagnosis was based on homocystinuria that was responsive to vitamin B_{12}

in the absence of methylmalonic aciduria. Diagnosis was confirmed by studies of cobalamin forms and methionine synthase measurements in extracts of cultured fibroblasts under different reducing conditions. In these fibroblasts, cobalamin uptake from the medium and accumulation in cells was normal. The distribution of intracellular cobalamins in these cells was abnormal, with decreased concentrations of intracellular methyl cobalamin, normal 5'-deoxyadenosyl cobalamin, and increased proportion of intracellular hydroxocobalamin compared with normal fibroblasts. In obligate heterozygotes, we have observed hydroxocobalamin excess in some as the only abnormality.

Treatment requires large doses of vitamin B_{12} (1 mg/day to 1 mg/week), titrated to maintain normal levels of plasma methionine and the absence of homocystinuria. The siblings so treated have developed normally since institution of vitamin B_{12} therapy at birth.

COBALAMIN G Subsequently, several unrelated additional patients have come to our attention with similar defects (55, 114, 121). It appears that they fall into two classes on the basis of complementation analysis (121, 122). The two classes appear to correlate with normal or reduced activity of methionine synthase in cell extracts under standard reducing conditions. Methyl cobalamin in cell extracts of patients in the new subgroup was decreased whereas adenosyl cobalamin levels were normal, as with Cobalamin E cells. These children developed megaloblastic anemia with homocystinuria early in life and responded to injections of hydroxocobalamin. It appears that these children have an abnormality genetically different from Cobalamin E children; their abnormality has been called "mutant class Cobalamin G" (cblG).

Cobalamin F

One infant with a phenotype similar to Cobalamin C has been found to accumulate unbound vitamin B_{12} in lysozomes or endosomes, without significant entry of the cobalamin into the cytoplasm of the cell (98, 100). This has been labeled mutant class Cobalamin F (cblF).

Accumulation of cobalamin by this patient's fibroblasts from cyanocobalamin in the culture medium was increased above normal, and 90% or more of this was found to be free cyanocobalamin. The cyanocobalamin was associated with the mixed lysozomal-endosomal fraction of the cells during subcellular fractionation. When these fibroblasts were incubated in cyanocobalamin with chloroquine, the cyanocobalamin remained associated with transcobalamin 2, as observed in normal fibroblasts treated with chloroquine.

The defect in metabolism in this child is thus localized to transfer from endosome/lysozome into cytoplasm. Whether this represents a defect in a receptor or in some other aspect of transport is unknown. This disorder

appears analogous to the defects in lysozomal transport of cystine in cystinosis, and of neuraminic acid in Salla disease (92).

CLINICAL SYNDROME An infant girl developed stomatitis, glossitis, convulsions, and hypotonia at age 12 days; at age 3 weeks she developed methylmalonic aciduria that was responsive to therapy with 1 mg of cyanocobalamin (100). No data about homocystine or methionine excretion before therapy were available. The child has developed normally, receiving hydroxocobalamin 1 mg orally twice daily. Anemia, macrocytosis, and megaloblastic anemia were absent. CT scan of the head revealed enlargement of all CSF-containing spaces.

Cultured fibroblasts and Epstein-Barr-virus-transformed lymphocytes from this patient were found to accumulate unbound vitamin B_{12} in lysozomes or endosomes, without significant entry of the cobalamin into the cytoplasm of the cell. The mother subsequently bore a normal, unaffected child.

Several patients have been described with what appear to be inborn errors of cobalamin or folate metabolism (69, 120). Subsequent evaluations of these patients with newer tests have not been done. The cause of these illnesses cannot be determined at this time.

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