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Genetic defects of folate and cobalamin metabolism

Abstract Deficient activity of an enzyme can result from a defect in the conversion of the vitamin to a co-enzyme as well from an abnormal apo-enzyme or disturbed binding of co-enzyme to enzyme. Conversion of dietary vitamin to intracellular active co-enzyme can be complex and require many physiological and biochemical processes including stomach release of bound vitamin, intestinal uptake, carriers/transport, blood transport, cellular uptake, intracellular release and intracellular compartmentalisation. Disorders of malabsorption (food cobalamin malabsorption, intrinsic factor deficiency and abnormal enterocyte cobalamin processing) and transport proteins (transcobalamin II deficiency, R-binder deficiency) mostly lead to disturbed function of the two cobalamin requiring enzymes, methylmalonyl CoA mutase and methionine synthase.

Defects of early steps of intracellular cobalamin (cblF, cbl C/D) result in marked deficiencies of both cobalamin co-enzymes and homocystinuria combined with methylmalonic aciduria. Defective synthesis of adenosyl cobalamin in the cbl A/B defects leads to methylmalonyl CoA mutase. Isolated methionine synthase deficiency is also classified as a cobalamin disorder due to its associated deficient formation of methylcobalamin. Folate disorders include methylene-tetrahydrofolate reductase deficiency and glutamate formimino-transferase deficiency. In addition a hereditary disorder of intestinal folate transport has been described. Less well established are disorders of dihydrofolate reductase, methenyl-tetrahydrofolate cyclohydrolase, and defects of cellular folate uptake.

Key words Folate · Cobalamin · Homocysteine · Metabolism · Disorders

Abbreviations *TCII* transcobalamin II · *THF* tetrahydrofolate

Introduction

In this article the processes which are involved in the conversion of vitamin B_{12} (cobalamin) and folate to their active co-enyzmes, and the clinical and biochemical consequences of genetic disturbances of these processes are reviewed. Many, but not all of these disorders cause homocystinuria to a varying degree.

The conversion of a vitamin to its active co-enzyme and subsequent binding to an apo-enzyme producing active holo-enzyme are fundamental biochemical processes. Therefore deficient activity of an enzyme can result not only from a defect of the enzyme protein itself, which may involve interaction of a co-enzyme with an apo-enzyme, but also from a defect in the conversion of the vitamin to a co-enzyme.

Conversion of dietary vitamin to intracellular active co-enyzme can be complex and may depend on many physiological and biochemical processes. These processes include stomach release of bound vitamin, intestinal uptake and transport, blood transport, cellular uptake, intracellular release and intracellular compartmentalisation and metabolism.

Cobalamin co-enzymes are needed for just two reactions in man, those catalysed by mitochondrial methylmalonyl CoA mutase and cytosolic methionine synthase.

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Although vital reactions, their deficiency seems to be life-compatible and many of the potential disorders of cobalamin processing are known.

In contrast, folate co-enzymes play a multiple role in metabolism and a markedly deficient early step in processing is probably incompatible with life. Thus relatively few of the potentially many disorders are known.

Cobalamin defects

Absorption, transport and cellular uptake of cobalamin

Vitamin B_{12} (cobalamin) is a complex compound with a molecular weight of 1355 (cyano-form). It contains a corrin ring which binds a cobalt atom at its centre. Different groups can be bound to the cobalt atom and the nature of these groups determine its co-enzyme activity [11].

The processing of vitamin B_{12} depends on a number of protein classes; (1) intrinsic factors; (2) R-binders; (3) transcobalamins; (4) membrane receptors, and (5) intracellular binders which in fact mainly comprise the two cobalamin-requiring enzymes.

The nomenclature of these transport proteins is confusing since several names for the same component have been used [9]. Hansen [16] has reviewed the cobalamin transport proteins identifying the three main classes as: intrinsic factor (also known as S-binder); transcobalamin, (previously named as transcobalamin II (TC II), small molecular size binder, β-globulin binder); haptocorrin (also known as, for example, cobalophilin, R-binder, transcobalamin I, large molecular size binder, leucocyte binder, salivary binder). Intrinsic factor is a glycoprotein secreted by the gastric parietal cell. A human cDNA has been cloned which maps to chromosome 11, contains 1595 nucleotides and codes for a protein of 417 amino acid residues with six potential glycosylation sites [17]. Hapotocorrin (cobalophilin) exists in many physiological fluids and tissues and is heavily glycosylated, the carbohydrate content and nature varying considerably depending on its source. Its exact function remains unclear although in plasma it binds about 60% of the circulating cobalamin, albeit with a slow turnover, and a functional role in the protection of methylcobalamin from photolytic degradation has been recently proposed [12]. The glycoprotein TCII occurs in many tissues and fluids. In plasma it has a rapid turnover of 0.5 days and its gene has been cloned [32].

Membrane receptors include an ileal enterocyte receptor for the intrinsic-factor-cobalamin complex [25] and receptors for the TCII-cobalamin complex which have been characterised from pig kidney [43] and human placenta [30].

These proteins and receptors all play important roles in the absorption, transport and cellular uptake of cobalamin which is summarised in Fig. 1. Protein-bound vitamin B_{12} from the diet is released in the stomach by the denaturation of protein due to increased acidity and by the action of peptidases.

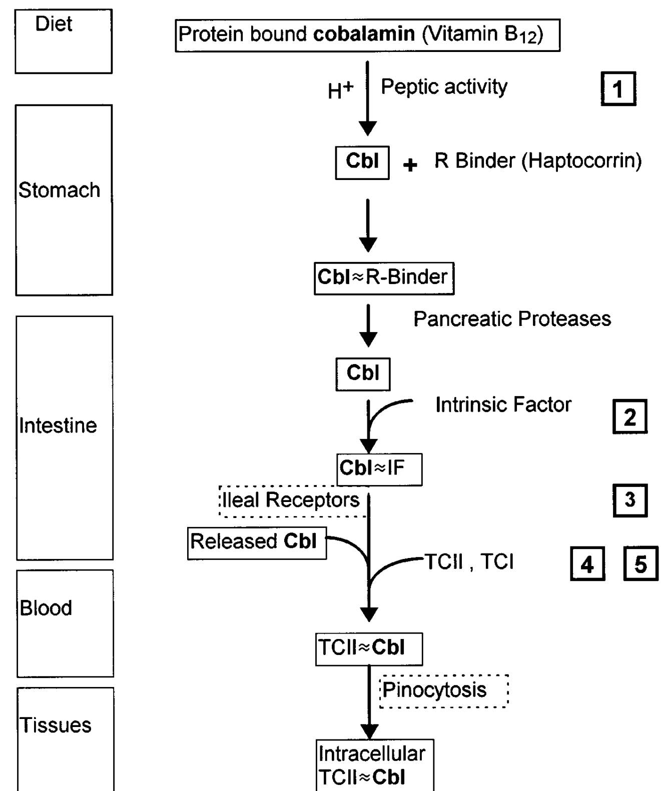

Fig. 1 Summary of the processes involved in absorption, transport and cellular uptake of cobalamin. The *numbers* refer to the sites of defects as follows: *1* food cobalamin malabsorption; *2* intrinsic factor deficiency; *3* enterocyte cobalamin malabsorption; *4* transcobalamin I (R-Binder) deficiency; *5* TC II deficiency. (*Cbl* cobalamin, *IF* intrinsic factor, *TC* transcobalamin)

Free cobalamin binds to haptocorrin (R-binder) in saliva and the stomach and is released by proteolytic hydrolysis in the small intestine where it binds to intrinsic factor.

Absorption of B_{12} is effected by binding of the intrinsic factor- B_{12} complex to ileal receptors followed by endocytosis then lysosomal digestion of the complex, releasing B_{12} which attaches to TCII and is released in bound form from the cell into the portal bloodstream [34]. It should be noted, however, that large oral doses of 100 μg or more result in absorption of some B_{12} without the involvement of gastric intrinsic factor [6]. All extracellular cobalamin is bound to TCII or to one of the closely related glycoprotein haptocorrin binders. Cobalamin bound to TCII enters cells by receptor mediated pinocytosis, is taken up by lysosomes and released from the complex by proteolytic digestion of TCII. When released cobalamin leaves the lysosomes and is converted to its active coenzyme forms.

Disorders of absorption, transport and cellular uptake of cobalamin

The localisation of the known disorders of cobalamin uptake and transport [31] is indicated in Fig. 1.

Table 1 Main features of genetic defects of processing of cobalamins in man (*MMA* methylmalonic acid, *tHcy* total homocysteine, *Neurol* neurological abnormalities)

Defect	Serum B_{12}	Clinical/Biochemical
Food cobalamin malabsorption	Low	Neurol. ± Anaemia / mild ↑ MMA/tHcy
Intrinsic factor deficiency	Low	Anaemia, delayed development / mild ↑ MMA/tHcy
Enterocyte cbl malabsorption (Imerslund-Gräsbeck)	Low	Anaemia, proteinuria delayed development / mild ↑ MMA/tHcy
Transcobalamin I (R-Binder) deficiency	Low	No abnormality / No ↑ MMA/tHcy
Transcobalamin II deficiency	Normal	Neurol. ± Anaemia failure to thrive / mild ↑ MMA/tHcy
Intracellular defects of cbl co-enzyme synthesis	Normal	Severe disease / ↑ MMA/tHcy

1. A specific malabsorption of cobalamin due to its defective release from protein has been postulated as a cause of low plasma cobalamin although the aetiology remains unclear [4].
2. Intrinsic factor deficiency has been known for a long time in the elderly but can also exist in childhood [44].
3. Disordered cobalamin processing in enterocytes (Imerslund/Gräsbeck syndrome) is a further cause of functional cobalamin deficiency in childhood. Some cases have a deficiency of the intrinsic factor-cobalamin receptor which has been demonstrated in ileal biopsy specimens [14]. Many features of this disorder are illustrated by the dog model described by Fyfe et al. [13].
4. Deficiency of TCII, a cause of functional cobalamin deficiency in the early months of life, is paradoxically characterised by normal plasma cobalamin levels due to the presence of haptocorrin [19]. Neurological abnormalities are rarely present on initial presentation but may result from inadequate treatment [15]. Recently two mutant alleles associated with this deficiency have been identified in one family [21].
5. Deficiency of haptocorrin (TC1, R-Binder deficiency) is thought not to be associated with disease or functional cobalamin deficiency in spite of low serum levels of the vitamin [3].

The B_{12} levels in serum and the main clinical and biochemical features in disorders of cobalamin processing are summarised in Table 1. While markedly elevated levels of plasma total or free homocyst(e)ine and urinary methylmalonic acid have been reported in the intracellular defects, direct evidence of increases of these levels in the absorption and transport defects, using current sensitive methods, is scarce. However, such abnormalities seem likely on the basis of findings in nutritional cobalamin deficiency as well as in elderly subjects with pernicious anaemia [37].

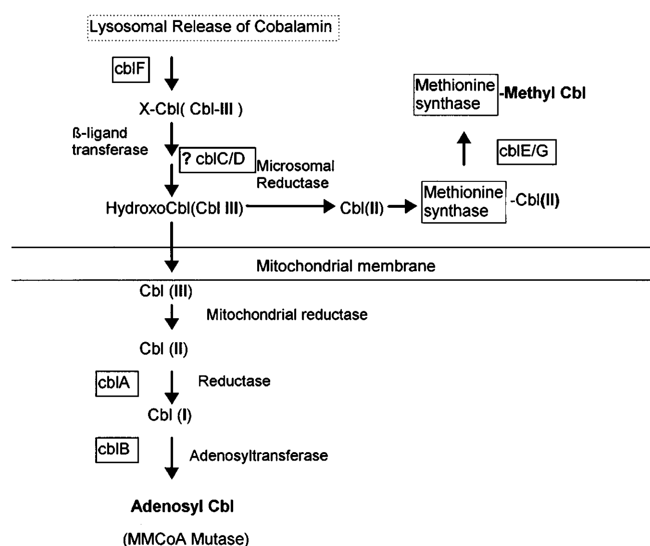

Fig. 2 Summary of the intracellular processing of cobalamins. The pathway includes all reactions described in the text, some of which are not definitely proven to occur in man and possible unknown steps remain to be discovered. The probable sites of genetic defects are indicated as follows: *1* cblF; *2* cblC/D (exact site not proven); *3* cblE/G; *4* cblA; *5* cblB. (*Cbl* cobalamin, *MMCoA* methylmalonyl CoA mutase)

The intracellular processing of cobalamins

The intracellular processing of cobalamins is summarised in Fig. 2. Following lysosomal digestion of the TCII-carrier protein complex, cobalamin is released into the cytoplasm. In this form of cobalamin the cobalt atom is trivalent (cob[III]) and must be reduced before it can bind to the respective enzymes [34]. Although it remains unclear in which form (cob[II]) or cob[III]), cobalamin enters mitochondria [34] there is much evidence for the existence of reductive reactions which are necessary for the formation of both co-enzymes of cobalamin. Pezacka [28] recently identified and characterised two enyzmes involved in intracellular cobalamin metabolism. Cyanocobalamin β-ligand transferase is a cytosolic flavine adenine dinucleotide and NADPH-requiring enzyme which catalyses the formation of glutathionyl cobalamin, a naturally occurring intracellular cobalamin. A microsomal, NADH-linked cob(III)alamin reductase produces cob(II)alamin as does a similar but mitochondrial associated enzyme. Thus the proposed early steps of a β-ligand removal reaction followed by reduction of the cobalt atom from the 3 to 2 valence state could be the site of the defect in the combined deficiency. In this model the microsomal cob(III)alamin reductase would participate in methylcobalamin formation while the inner mitochondrial membrane reductase would participate in the adenosylcobalamin synthesis pathway. The role of cytochrome b_5 and cytochrome c (P-450) reductases [36] which may be able to reduce cob(III)alamin as a secondary substrate is unclear.

Adenosylcobalamin is formed from hydroxocobalamin intramitochondrially by successive reductions of the cobalt atom catalysed by flavoprotein NADH requiring reductases followed by adenosyl group transfer in an ATP-requiring reaction catalysed by an adenosyl transferase (EC 2.5.1.7) [8]. Methylcobalamin is formed from hydroxocob(II)alamin in the cytoplasm in a complex reaction which is an integral part of the catalytic action of methionine synthase, involving several substrates and cofactors.

Disorders of intracellular cobalamin metabolism

The localisation of defects of intracellular cobalamin metabolism [31] is shown in Fig. 2. The various defects have been classified, on the basis of complementation analysis and other biochemical findings and fibroblast assays, as cobalamin disorders [8]. So far seven different complementation classes, cblA to cblG, have been described. Defects in the early steps of this pathway result in deficient activity of both cobalamin requiring enzymes, methylmalonyl CoA mutase and methionine synthase and are characterised by homocystinuria combined with methylmalonic aciduria. Defective lysosomal release of cobalamin, the cblF defect, has so far been described only in five cases. The more common cblC/D defect is also due to disturbance of a step common to both co-enzymes but the exact mechanism remains unproved. The recently reported cyanocobalamin β-ligand transferase or microsomal, NADH linked cob(III)alamin reductase are candidates for the abnormality in this defect. However the finding that both of these activities in fibroblasts were low in cblC mutant fibroblasts and the use of the non-physiological cyanocobalamin in the assay of the β-ligand transferase indicate that deficiency of these enzymes may not represent the primary defect [28]. The isolated deficiency of methionine synthase is also classified as a cobalamin disorder due to its association with low levels of methylcobalamin both in vivo and in cell culture. Megaloblastic changes and growth retardation, psychomotor retardation, neurological abnormalities together with hyperhomocysteinaemia and hypomethioninaemia are associated with deficient methionine synthase activity in the cblC/D, cblF, cblE, cblG defects. The clinical and biochemical features of the cblE and cblG defects have been reviewed [33]. For details of combined homocystinuria and methylmalonic aciduria (cblC/D) see Ogier de Baulny [26].

Deficient formation of adenosylcobalamin can be caused by one of two distinct enzyme deficiencies, either cobalamin (II) reductase or cobalamin (I): adenosyltransferase, both leading to a deficiency of methylmalonyl CoA mutase. In common with other forms of methylmalonic aciduria the cblA and cblB defects are characterised clinically by vomiting, severe metabolic decompensation, poor growth and with inadequate treatment, psychomotor retardation. Excretion of methylmalonic acid is markedly increased and response to high dose, intramuscular hydroxocobalamin treatment has been reported in most patients, particularly those with the cblA defect [8].

Folate defects

Absorption, transport and cellular uptake of folate

Folic acid is a complex molecule comprising pteroic acid and between one and nine glutamic acid residues linked by amide bonds [33]. A number of different 1-carbon units related to its co-enzyme function are linked to the nitrogen atoms at position 5 and/or 10. Dietary folates exist mainly in the polyglutamate forms which probably do not cross cell membranes [11] and must be hydrolysed by folylpolyglutamate conjugase to the monoglutamyl form in the intestine before absorption can occur. The conjugases are widely distributed and the human enzyme has been isolated from jejunal mucosa and clears both terminal and internal glutamate linkages [41] and is located in lysosomes.

Intestinal uptake of folylpolyglutamate is effected by specific transport systems, located in basolateral and brush border membranes principally in the duodenum and jejunum. Transport can be passive or active, the latter being highly pH dependent with an optimum of pH 5.8. Active transport exhibits uptake against a concentration gradient, inhibiton by folate analogues, sodium and glucose dependence and is temperature dependent [33].

Recently the functional expression and mRNA distribution of a cDNA involved in folate transport has been described [35]. Following intestinal uptake folates are carried to the liver by the hepato-portal circulation, methylated and then enter the bloodstream. Cellular uptake is mediated by specific transport systems. Two have been characterized from various sources including liver [11] and L1210 mouse leukaemia cells. One has a high capacity and low affinity and is driven by anion gradients, the other exhibits high affinity for folate but has low capacity.

Specific folate receptors, which have been described in three iso-forms and are expressed variably in different tissues, participate in cellular uptake. Two forms are widely distributed while one seems to be specifically associated with placenta and is likely to be important in maternal-fetal transfer of folate [29, 38]. Once transported into cells, monoglutamyl folate is converted to its polyglutamyl forms by folylpoly-γ-glutamate synthetase. The polyglutamyl forms of folate are better retained in cells and can be more effective as co-enyzmes than the monoglutamate forms [27].

Folate metabolism

Folic acid in its various forms is the cofactor in 1-carbon unit metabolism. One-carbon unit metabolism plays a role in many processes including the synthesis of purines and pyrimidines, methionine, glycine and serine metabolism, and in the breakdown of histidine [33].

The main features of folate metabolism are summarised in Fig. 3. The reactions involved in the synthesis of the different 1-carbon units are central to these path-

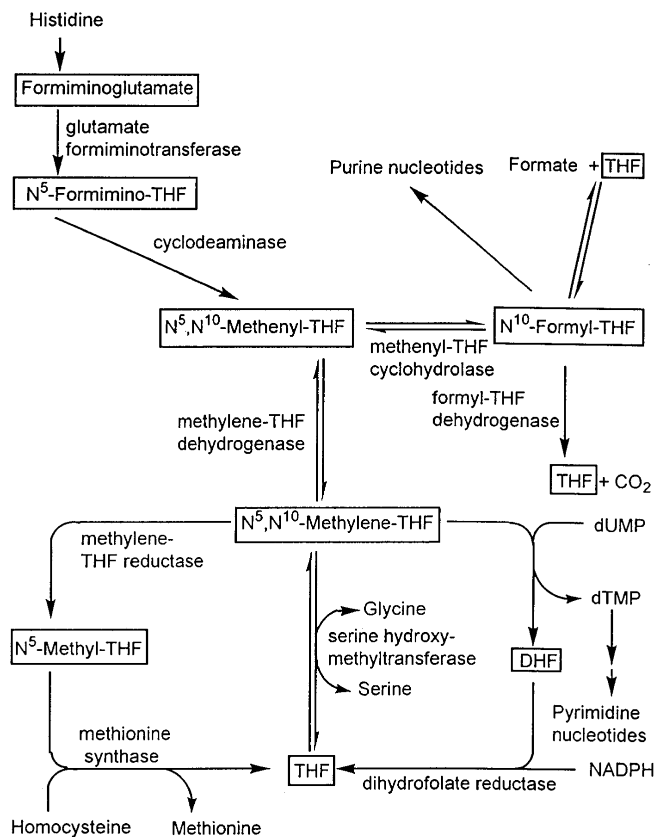

Fig. 3 Summary of the major reactions of folate. See text for description. (*dUMP* deoxy-uridine phosphate, *dTMP deoxy-thymidine phosphate*, *DHF* dihydrofolate)

ways. N^{10}-formyl-tetrahydrofolate (THF) is formed from THF and formate, by 10-formyl-THF synthetase (EC 6.3.4.3) then converted to N^5 N^{10}-methenyl-THF by 5,10-methenyl-THF cyclohydrolase (EC 3.5.4.9). N^5, N^{10}-methenyl-THF is converted to N^5, N^{10}-methylene-THF by 5, 10-methylene-THF dehydrogenase (EC 1.5.1.5). These three enzymes constitute the multi-enzyme complex, C_1-THF synthetase. THF can be regenerated from N^5, N^{10}-methylene-THF by serine hydroxymethyltransferase (EC 2.1.2.1) which converts glycine to serine thereby completing the conversion of formate to serine [39]. N^5N^{10}-methylene-THF can also be reduced to 5-methyl-THF by 5, 10-methylene-THF reductase (EC 1.1.1.68). Once formed, 5-methyl-THF must be converted to THF by transferring its methyl group to homocysteine in the methionine synthase catalysed reaction which links folate metabolism intricately to homocysteine. This 1-carbon unit cycle is linked to (1) pyrimidine nucleotide synthesis through the participation of 5,10-methylene-THF in the formation of thymidylate and (2) purine synthesis by the role of 10-formyl-THF in the reactions catalysed by 10-formyl-THF: 5 aminoimidazole-4-carboxamide ribonucleotide formyltransferase (EC 2.1.2.3) and 10-formyl-THF: glycinamide ribonucleotide formyltransferase (EC 2.1.2.2). Histidine catabolism involves two folate enzymes, glutamate formimino transferase (EC 2.1.2.5) and N^5 formimino-THF cyclodeaminase (EC 4.3.1.4) which exist as an octameric enzyme complex.

Glycine-serine interconversion involving serine hydroxymethyl transferase (above) and the glycine cleavage system are central to folate metabolism. The importance of the number of glutamate residues of folate co-enzymes is illustrated by the variation of affinity of formyl-THF synthetase for substrates with 1–6 glutamate residues. The Michaelis constant for the monoglutamyl form of formyl-THF is 150 times higher than that for the substrate with five glutamate residues [39]. The possible role of folylpolyglutamate synthetase in the regulation of folate metabolism and the relationship of glutamate content of the different folate co-enzymes to enzyme activity has been reviewed in detail [20, 22, 23, 27].

Disorders of absorption, transport and cellular uptake of folate

Hereditary folate malabsorption

This condition has been described in only 13 cases and is characterised by megaloblastic changes, diarrhoea, failure to thrive and neurological signs in the first few months of life. Folate is deficient in serum and CSF and responds poorly to oral folates. Increased formiminoglutamic acid excretion following histidine loading may occur [7].

Large dose oral folate treatment (up to 100 mg/d) may improve serum levels and the anaemia but complete correction of CSF folate deficiency may require parenteral, intravenous or even intrathecal administration of the vitamin. Information on the exact molecular mechanism is scarce but the recent discovery of the cDNA for a folate receptor may help to resolve this question [35].

Other disorders of folate transport

Branda et al. [2] described a possible defect of uptake of 5-methyl-THF into bone marrow cells in a kindred of four generations with 34 similarly symptomatic subjects. Reduced 5-methyl-THF uptake into erythrocytes was described in one patient with dyserythropoiesis [18].

A defect of a folate binding protein of the choroid plexus has been proposed as the cause of low folate levels in one adult with progressive neurological symptoms including sensorineural hearing loss, dysarthria, dysgraphia and gait ataxia [42].

Glutamate formiminotransferase/ formimino-THF-cyclodeaminase deficiency

This has been described in about 20 subjects so far, some of whom were clinically affected with neurological abnormalities including three patients with associated malignancy [40]. Some have been asymptomatic and it remains to be established whether this biochemical entity has di-

rect clinical consequences. Increased excretion of formiminoglutamic acid has been found but only after histidine loading in some patients. This has improved on high dose folate treatment in some but not all patients. The enzymatic basis remains to be fully explained which is hindered by the need for liver biopsy specimens. In 5 patients liver enzyme activities ranged from 14%–54% of control values [33].

Methylene-THF reductase deficiency

This is a well established inherited disorder of metabolism constituting the second most common homozygous enzyme deficiency which causes severe hyperhomocysteinaemia. For biochemical and diagnostic aspects of the disorder see Fowler and Jakobs [10]. The molecular basis is covered in Matthews et al. [24] and the association of the common thermolabile variant of methylene THF reductase (C677T mutation) with moderate hyperhomocysteinaemia and vascular disease in adulthood and an increased incidence of neural tube defects is covered by Blom [1].

The defect is characterised by deficient formation of methyl-THF leading to reduced remethylation of homocysteine to methionine [33]. The condition can present severely in early childhood as early as 23 days or much more mildly as late as 16 years. The clinical features of methylene-THF reductase deficiency are illustrated by two cases known to the author, one presenting in early life the other much later. The first, a male infant was born by normal delivery to first cousin parents. At 12 days of age poor feeding with vomiting was observed. By 6 weeks of age gross hypotonia and hypothermia were evident but there was no anaemia. Poor development continued with poor head control, nystagmus, inability to focus, and absent MORO and ASTVR reflexes. Several therapeutic approaches were tried including betaine, pyridoxine and folate but these were unsuccessful and the child died at 9 months of age due to bronchopneumonia. The presentation in the second patient was much different. This male infant was born at full-term by normal delivery to unrelated parents. There were no problems during the neonatal period. The developmental history was unremarkable, with walking by 15 months of age but poor speech, clumsiness and dysarthria, became apparent. There were no visual problems and no joint stiffness. When seen at 13 years of age the patient showed mental delay with an IQ of about 60, there was a definite Marfanoid appearance but with normal eyes and evidence of generalised osteoporosis on X-ray. This patient showed a marked response of sulphur amino acid levels to treatment with large doses of folate (30 mg/d) leading to a clear clinical improvement indicated by increased mobility due to improved tonicity, increased power of concentration and better overall performance at school. The response of methionine synthesis from labelled formate (see Fowler and Jakobs [10]) to supplementation of the culture medium with 100 mg/L folate in cultured fibroblast of this patient provides an in vitro explanation of the in vivo folic acid responsiveness.

In fact most cases of this deficiency do not respond to high dose vitamin treatment although a few cases which responded to folic acid have been reported [33] and one riboflavin-responsive case is known (Fowler, unpublished data). In the non-responsive cases, treatment with betaine or methionine together with unspecific reduction of homocysteine with pyridoxine, folic acid and cobalamin can improve the abnormal amino acid levels to some degree although the long-term prognosis is poor.

An animal model for 10 formyl-THF dehydrogenase deficiency [5]

A mouse model was discovered for this enzyme deficiency following irradiation of male mice with fission-spectrum neutrons which caused deficient expression of a number of liver proteins due to gene deletions. One deleted protein proved to be 10-formyl-THF dehydrogenase and breeding of such mice led to establishment of animals homozygous for this enzyme deficiency. In such mice with undetectable liver activity of the enzyme, the total folate pool and THF level was decreased in liver. The affected mice appeared completely healthy questioning an important physiological role for this enzyme in the light of other THF generating reactions. One effect of the deletion however was an unusual breeding pattern whereby greatly extended breeding times were observed in mating pairs of mice homozygous for this enzyme deficiency.

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References

1. Blom HJ (1998) Mutated 5,10-methylenetetrahydrofolate reductase and moderate hyperhomocysteinaemia. Eur J Pediatr (this issue)
2. Branda RF, Moldow CF, Macarthur JR, Wintrobe MM, Anthony BK, Jacob HS (1978) Folate-induced remission in aplastic anemia with familial defect of cellular folate uptake. N Engl J Med 298 : 469–475
3. Carmel R (1988) Plasma R binder deficiency. N Engl J Med 318 : 1401
4. Carmel R, Sinow RM, Siegel ME, Samloff M (1988) Food cobalamin malabsorption occurs frequently in patients with unexplained low serum cobalamin levels. Arch Intern Med 148 : 1715–1719
5. Champion KM, Cook RJ, Tollaksen SL, Giometti CS (1994) Identification of a heritable deficiency of the folate-dependent enzyme 10-formyltetrahydrofolate dehydrogenase in mice. Proc Natl Acad Sci 91 : 11338–11342
6. Cooper BA, Rosenblatt DS (1987) Inherited defects of vitamin B_{12} metabolism. Annu Rev Nutr 7 : 291–320
7. Corbeel L, Van den Berghe G, Jaeken J, Van Tornout J, Eeckels R (1985) Congenital folate malabsorption. Eur J Pediatr 143 : 284–290
8. Fenton WA, Rosenberg LE (1995): Inherited disorders of cobalamin transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. 7th edn. McGraw-Hill, New York, pp 3129–3150

9. Fernandes-Costa F, Metz J (1982) Vitamin B12 binders (transcobalamins) in serum. Crit Rev Clin Lab Sci 18 : 1–30
10. Fowler B, Jakobs (1998) Post-and prenatal diagnostic methods for the homocystinurias. Eur J Pediatr (This issue)
11. Friedrich W (1988) Vitamins. Walter de Gruyter, Berlin-New York
12. Frisbie SM, Chance MR (1993) Human cobalophilin – the structure of bound methylcobalamin and a functional role in protecting methylcobalamin from photolysis. Biochemistry 32 : 13886–13892
13. Fyfe JC, Giger U, Hall CA, Jezyk PF, Klumpp SA, Levine JS, Patterson DF (1991) Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. Pediatr Res 29 : 24–31
14. Gueant JL, Saunier M, Gastin I, Safi A, Lamireau T, Duclos B, Bigard MA, Grasbeck R (1995) Decreased activity of intestinal and urinary intrinsic factor in Gräsbeck-Imerslund disease. Gastroenterol 108 : 1622–1628
15. Hall CA (1992) The neurologic aspects of transcobalamin-II deficiency. Br J Haematol 80 : 117–120
16. Hansen M (1990) Cobalamin-binding proteins in human blood. In: Guéant JL, Nicolas JP (eds) Cobalamin and related binding proteins in clinical nutrition. Elsevier, Amsterdam, pp 69–79
17. Hewitt JE, Gordon MM, Taggart RT, Mohandas TK, Alpers DH (1991) Human gastric intrinsic factor – characterization of cDNA and genomic clones and localization to human chromosome-11. Genomics 10 : 432–440
18. Howe RB, Branda RF, Douglas SD, Brunning RD (1979) Hereditary dyserythropoiesis with abnormal membrane folate transport. Blood 54 : 1080–1090
19. Kaikov Y, Wadsworth LD, Hall CA, Rogers PCJ (1991) Transcobalamin-II deficiency – Case report and review of the literature. Eur J Pediatr 150 : 841–843
20. Kim JS, Lowe KE, Shane B (1993) Regulation of folate and one-carbon metabolism in mammalian cells 4. Role of folylpoly-gamma-glutamate synthetase in methotrexate metabolism and cytotoxicity. J Biol Chem 268 : 21680–21685
21. Li N, Rosenblatt DS, Kamen BA, Seetharam S, Seetharam B (1994) Identification of two mutant alleles of transcobalamin II in an affected family. Hum Mol Genet 3 : 1835–1840
22. Lin BF, Huang RFS, Shane B (1993) Regulation of folate and one-carbon metabolism in mammalian cells 3. Role of mitochondrial folylpoly-gamma-glutamate synthetase J Biol Chem 268 : 21674–21679
23. Lowe KE, Osborne CB, Lin BF, Kim JS, Hsu JC, Shane B (1993) Regulation of folate and one-carbon metabolism in mammalian cells 2. Effect of folypoly-gamma-glutamate synthetase substrate specificity and level on folate metabolism and foylpoly-gamma-glutamate specificity of metabolic cycles of one-carbon metabolism. J Biol Chem 268 : 21665–21673
24. Matthews RG, Sheppard C, Goulding C (1998) Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. Eur J Pediatr (this issue)
25. Nicolas JP, Gueant JL (1995) Gastric intrinsic factor and its receptor. Baillieres Clin Haematol 8 : 515–531
26. Ogier de Baulny H, Gérard M, Saudubray JM, Zittoun J (1998) Remethylation defects: guidelines for clinical diagnosis and treatment. Eur J Pediatr (this issue)
27. Osborne CB, Lowe KE, Shane B (1993) Regulation of folate and one-carbon metabolism in mammalian cells 1. Folate metabolism in Chinese hamster ovary cells expressing Escherichia-coli or human folylpoly-gamma-glutamate synthetase activity. J Biol Chem 268 : 21657–21664
28. Pezacka EH (1993) Identification and characterization of two enzymes involved in the intracellular metabolism of cobalamin – cyanocobalamin beta-ligand transferase and microsomal cob(III)alamin reductase. Biochim Biophys Acta 1157 : 167–177
29. Prasad PD, Ramamoorthy S, Moe AJ, Smith CH, Leibach FH, Ganapathy V (1994) Selective expression of the high-affinity isoform of the folate receptor (FR-alpha) in the human placental syncytiotrophoblast and choriocarcinoma cells. Biochim Biophys Acta Molecular Cell Res 1223 : 71–75
30. Quadros EV, Sai P, Rothenberg SP (1994) Characterization of the human placental membrane receptor for transcobalamin II-cobalamin. Arch Biochem Biophys 308 : 192–199
31. Qureshi AA, Rosenblatt DS, Cooper BA (1994) Inherited disorders of cobalamin metabolism. Crit Rev Oncol/Hematol 17 : 133–151
32. Regec A, Quadros EV, Platica O, Rothenberg SP (1995) The cloning and characterization of the human transcobalamin II gene. Blood 85 : 2711–2719
33. Rosenblatt DS (1995) Inherited disorders of folate transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds): The metabolic and molecular bases of inherited disease. (7th edition) McGraw-Hill Book Co, New York, pp 3111–3128
34. Rosenblatt DS, Cooper BA (1990) Inherited disorders of vitamin B_{12} utilization. Bioessays 12 : 331-334
35. Said HM, Nguyen TT, Dyer DL, Cowan KH, Rubin SA (1996) Intestinal folate transport: identification of a cDNA involved in folate transport and the functional expression and distribution of its mRNA. Biochim Biophys Acta Biomembranes 1281 : 164–172
36. Saido H, Watanabe F, Tamura Y, Funae Y, Imaoka S, Nakano Y (1993) Mitochondrial NADPH-linked aquacobalamin reductase is distinct from the NADPH-linked enzyme from microsomal membranes in rat liver. J Nutr 123 : 1868–1874
37. Savage DG, Lindenbaum J, Stabler SP, Allen RH (1994) Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med 96 : 239–246
38. Shen F, Ross JF, Wang X, Ratnam M (1994) Identification of a novel folate receptor, a truncated receptor, and receptor type beta in hematopoietic cells – cDNA cloning, expression, immunoreactivity and tissue specificity. Biochemistry 33 : 1209–1215
39. Strong WB, Schirch V (1989) In vitro conversion of formate to serine: effect of tetrahydropteroyl-polyglutamates and serine hydroxymethyltransferase on the rate of 10-formyltetrahydrofolate synthetase. Biochemistry 28 : 9430–9339
40. Van Gennip AH, Abeling NGGM, Nijenhuis AA, Voute PA, Bakker HD (1994) Formiminoglutamic/hydantoinpropionic aciduria in three patients with different tumours. J Inherit Metab Dis 17 : 642–643
41. Wang TTY, Chandler CJ, Halsted CH (1986) Intracellular pteroylpolyglutamate hydrolase from human jejunal mucosa: isolation and characterization. J Biol Chem 261 : 13551–13555
42. Wevers RA, Hansen SI, Hubar JLMV, Holm J, Hoiermadsen M, Jongen PJH (1994) Folate deficiency in cerebrospinal fluid associated with a defect in folate binding protein in the central cervous system. J Neurol Neurosurg Psychiatr 57 : 223–226
43. Yamada S, Riittinen L, Majuri R, Fukuda M, Grasbeck R (1991) Studies on the transcobalamin receptor in hog kidney. Kidney Int 39 : 289–294
44. Yang YM, Ducos R, Rosenberg AJ, Catrou PG, Levine JS, Podell ER, Allen RH (1985) Cobalamin malabsorption in three siblings due to an abnormal intrinsic factor that is markedly susceptible to acid and proteolysis. J Clin Invest 76 : 2057–2065