The Neurology of Folate and Vitamin B_{12}

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INTRODUCTION

Folate and vitamin B_{12} are naturally occurring vitamins. The research delineating the mechanisms and cure of pernicious anemia were one of Medicine's great achievements of the 20th Century. Lindenbaum was perhaps the first to advocate for the addition of folate to foods in an effort to curtail anemia in alcoholics.[1] Folate supplementation, currently by prescription or over the counter use, will become universal in the United States with the addition of 140 μg folate/100 gm of grains and pastas.[2] The intent of folate fortification is the reduction of neural tube defects. Other beneficial effects might include reduced risk of vascular disease, anemia, neuropathy and subacute combined degeneration of the spinal cord (SCD). Concern has been enunciated that folate may aggravate epilepsy or "mask" neurologic disease caused by vitamin B_{12} deficiency. The concern that vitamin B_{12} deficiency is common in the elderly[3] is considered overstated by others.

Neurologists care for the patients with neural tube defects, SCD, neuropathy, stroke, and epilepsy. Thus, it is important for neurologists to contribute to the discussions and participate in monitoring the outcome of folate supplementation.

FOLATE AND VITAMIN B_{12} METABOLISM

FOLATE BIOCHEMISTRY (FIGURE 1)

Dietary folate, primarily as the polyglutamate derivative, is hydrolyzed in the intestine, fully reduced and methylated to form 5-methyl-tetrahydrofolate (5-methylene-THF). This form is the principal extracellular form involved in transport within the body, including cell membrane and blood-brain barrier transport systems.[4] Normally, urinary excretion averages about 10 μg/d or about 75% of intake. Folate is distributed throughout the cell. Upon entering the cell as 5-methylene-THF the methyl group is transferred to methionine and the THF converted to polyglutamates; these two products serve as a reservoir for methyl and THF moieties. Several tissues apparently utilize other reservoirs for methyl groups since they accumulate these compounds in great quantity. In brain, for instance, concentrations of choline moieties (1.6 mM/l; 3 methyl moieties) is comparable to those of creatin and creatin phosphate (6.4 mM).[5] However, unlike high energy phosphates, the methyl moiety of choline cannot return to its source (methionine) in the CNS. The route for this is through betaine:homocysteine methyltransferase (BHMT) [EC 2.1.1.5]. BHMT is absent from the human brain.[6,7] However, betaine can ameliorate CNS disease in some inborn errors, suggesting a mechanism for choline to participate in critical disease events.

Folate is present in relatively high concentrations within the brain[8] and the cerebrospinal fluid (CSF) level (normally 17-41 μg/l) is about three times that in blood (normally 7-20 μg/l).[9,10] The blood-brain barrier limits vitamin entry and specific active transport systems are utilized to move vitamins across the barrier.[11] Most organisms utilize tetrahydrofolic acid (THF) as the principal carrier of single carbon units in biosynthetic reactions. Single-carbon derivatives of THF are required in several metabolic pathways (purines, pyrimidines, histidine, and sulfated amino acids). Numerous methylation reactions utilize methionine or methylated folate derivatives. Deficiency of folate or vitamin B_{12} affects primarily synthesis of DNA precursors and

methylation processes. Megaloblasts reflect impaired DNA synthesis during hematopoiesis. Methylation is particularly important in the nervous system. Choline represents a reservoir of methyl moieties but is also important in apoptosis and for acetylcholine and sphingomyelin synthesis. Catecholamine catabolism requires methyl transfer reactions. Myelin formation involves several important methylation reactions. Myelin basic protein is methylated at Arg_{107} by a specific protein methylase [EC 2.1.1.126] and this appears necessary for formation of compact myelin. Lecithin is formed by methylation of ethanolamine and sphingomyelin incorporates a choline moiety. Methylation modulates DNA methylation and this is likely important in development.

Folate also enhances the storage of pantothenic acid, a metabolic precursor of coenzyme A, also an important compound to the nervous system.[12]

VITAMIN B_{12} BIOCHEMISTRY (FIGURE 2)

Vitamin B_{12} absorption requires gastric intrinsic factor and the ileal transport system. It is transported to tissues bound to transcobalamin II. Within the cell, vitamin B_{12} is converted into its two active coenzyme forms (Figure 2). Vitamin B_{12} (cobalamin) is a cofactor for only two enzymes, methionine synthase and L-methylmalonyl-CoA mutase.

Serum methylmalonic acid and/or total homocysteine concentrations are elevated in virtually every patient who has a clinical response to deficiency of vitamin B_{12}. The pool of vitamin B_{12} is between 1-12 mg, on average 2 mg. Deficiency syndromes develop when this pool shrinks to 10% of normal. Considering the severity of such deficiencies treatment of symptomatic patients requires high dose replacement. Vitamin B_{12} also has a blood-brain barrier and transport produces higher levels within the brain and CSF than the serum. However, this system presents a problem during therapy because the rate of transport limits flux into the CSF (and presumably the nervous system). This lead to suggestions for intrathecal therapy,[13] which did not gain wide acceptance.

Figure 1. Folate and vitamin B_{12} participate in the synthesis of DNA and protein. Folate is also one of the principal vehicle for one-carbon transfers. Numbers within circles in the figure refer to individual enzymes:
1. multiple transmethylation enzymes;
2. Adenosylhomocysteinase [EC 3.3.1.1];
3. Methionine adenosyltransferase [EC 2.5.1.6];
4. methionine synthase [EC 2.1.1.13];
6. glycine hydroxymethyl transferase [EC 2.1.2.1];
7. glutamate:formimino-THF transferase [EC 2.1.2.5];
8. methylene THF reductase [EC 1.5.1.20; EC 1.7.99.5];
9. formate-THF ligase [EC 6.3.4.3], formyl-THF deformylase [EC 3.5.1.10] or formyltransferases [EC 2.1.2.2 or 2.1.2.3];
10. dihydrofolate reductase [EC 1.5.1.3];
11. 5-formimino-THF cyclo-ligase [EC 6.3.3.2] or 5-formimino-THF cyclo-deaminase [EC 4.3.1.4];
12. 5,10-methylene THF dehydrogenase [EC 1.5.1.15; EC 1.5.1.5];
13. Methenyl-THF cyclohydrolase [EC 3.5.4.9];
14. cystathionine β-synthase [EC 4.2.1.22]
15. thymidylate synthase [EC 2.1.1.45];
16. dihydrofolate reductase [EC 1.5.1.3];
17. folate transport proteins.
Detailed information on these reactions is available[14]

Hypothesized sites of disruption during embryogenesis are indicated by the hashed lines (//////).[15] The valproate inhibitory site is indicated by the solid line (———).

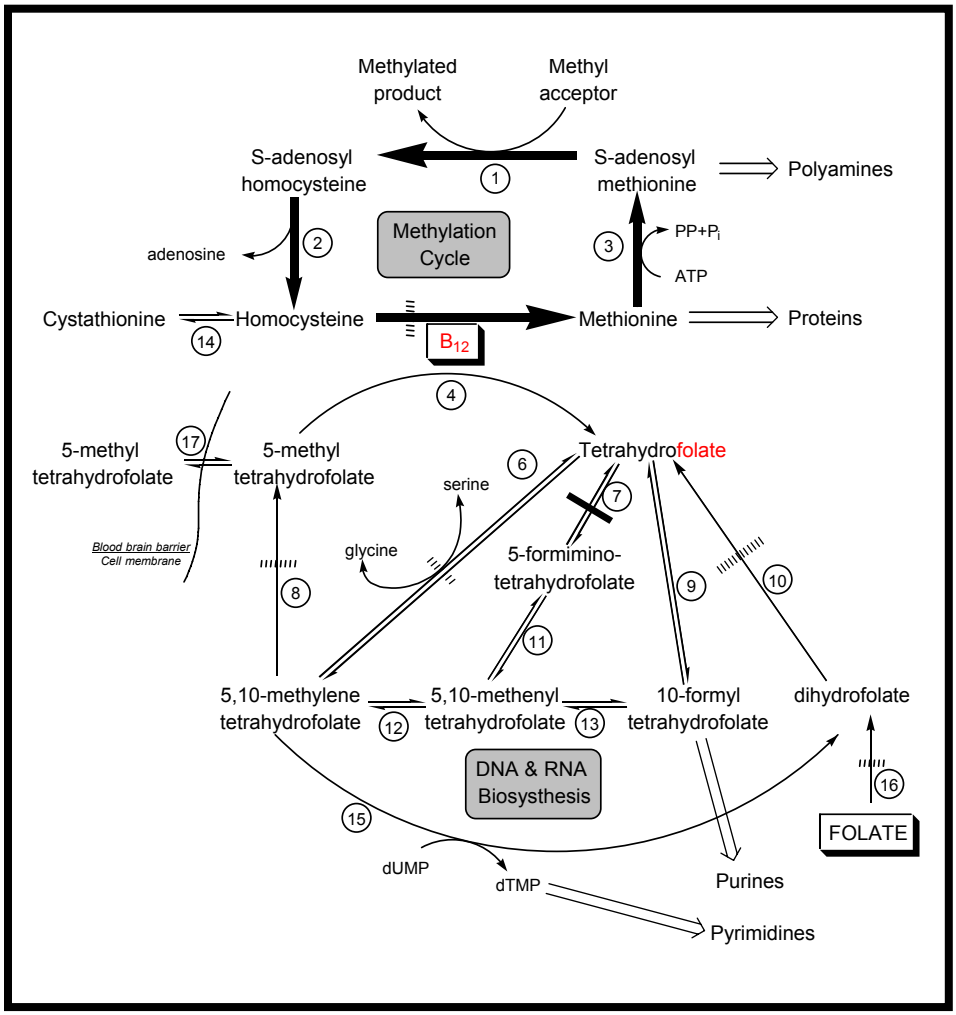
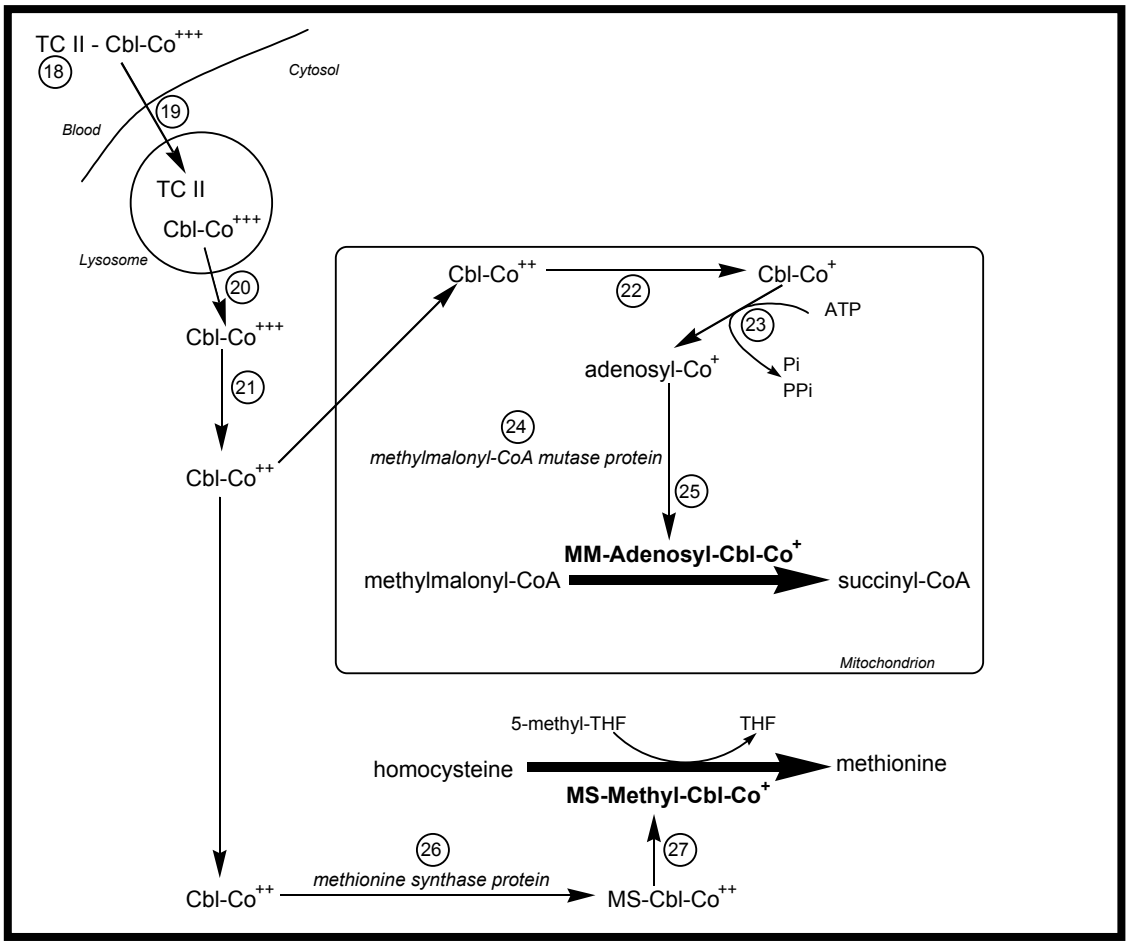


Figure 2. Vitamin B_{12} is incorporated into two enzymes as a covalently bound cofactor. The steps leading to this are:
18. Transcobalamin transport proteins (three types: TC-I which is an R-protein, TC-II, and intrinsic factor);
19. Cellular transport systems;
20. lysosomal efflux;
21. cob(III)alamin reductase [EC 1.6.99.8];
22. cob(II)alamin reductase [EC 1.6.99.9];
23. cob(I)alamin adenylotransferase [EC 2.5.1.17];
24. apomethylmalonyl-CoA mutase [EC 5.4.99.2];
25. holomethylmalonyl-CoA mutase [EC 5.4.99.2];
26. apomethionine synthase [EC 2.1.1.13]; and
27. holomethionine synthase [EC 2.1.1.13].



THE NEUROLOGY OF VITAMIN B_{12} AND FOLATE DEFICIENCY

NEUROPATHY

Peripheral neuropathy is common in neurologic practice. While often familial (genetic) or caused by diabetes, nutritional problems (alcoholism, gastrointestinal or other disease), or chemotherapy, it is frequently idiopathic. Serum vitamin B_{12} and folate are *routinely* obtained in individuals with clinical manifestations of neuropathy. Vitamin E levels are also appropriate, but not routinely utilized at present. Apparently some generalists regard vitamin B_{12} deficiency as a cause of neuropathy, but not folate deficiency.[16]

OPTIC NEUROPATHY

Optic neuropathy is a known complication of vitamin B_{12} deficiency,[17] but can also occur with isolated folate deficiency. Cyanide accumulation may play a critical role. Alcohol and smoking are common accompaniments, leading to the term tobacco-alcohol ambylopia.[18]

The centrocecal scotoma characteristic of tobacco-alcohol ambylopia is generally attributed to demyelination. Methanol poisoning also produces optic neuropathy. Folic acid reduces the formic acidemia in methanol intoxication and might well be a treatment adjunct.[19,20]

SUBACUTE COMBINED DEGENERATION

Subacute combined degeneration of the spinal cord (SCD) is a myeloneuropathy affecting primarily the posterior columns of the spinal cord and the large myelinated fibers in the peripheral nervous system (PNS). Untreated, the disorder progresses and also involves the lateral columns of the cord and the spinocerebellar and pyramidal tracts. This pathology correlates with the clinical progression typically observed (Table 1). Thus, afferent sensory pathways are affected early in SCD and proprioceptive modalities are most involved. The disorder is readily detectable by simple bedside techniques well before patients become symptomatic (i.e., subjectively aware of the disorder). The tool for this is a 256 cps ("middle C") tuning fork. However, current practice is not adequate. Most physicians do not routinely test vibratory sensation. When they do, they generally generate a maximum vibration, touch the tuning fork to the toe and immediately ask for a report from the patient: "Do you feel this?" Quantitative sensory testing is far more reliable and involves continuing to hold the tuning fork to the toe and asking for an additional report: "Tell me when it stops." It is quite easy to standardize ones personal tuning fork and thereby recognize the abnormal patient. Implementing this as a new practice standard requires physician education. In a recent series of patients using *non*-quantitative testing, vibratory sensory loss was present in 88%.[21]

Table 1. Stages in the evolution of subacute combined degeneration.

Stage	Clinical Findings	Historical frequency	
		Bethell[22]	Wieck[23]
Pre-symptomatic	Asymptomatic; vibratory sensory loss on quantitative testing	33%	7
Initial symptomatic	Paresthesias; vibratory loss, decreased reflexes	16	15
Early ataxia	Added impairment of sense of motion and mild ataxia	36	51
Moderate-severe ataxia	Increased ataxia and severe or total vibratory sensory loss	10	12
Non-ambulatory	Ataxia limiting walking; spasticity and paraplegia	5	15

The relative contribution of peripheral nerve and posterior column dysfunction is difficult to determined because they both can produce the abnormal symptoms and examination. Only sophisticated and expensive laboratory testing can ascertain the contribution of each lesion. Thus far these studies are limited but suggest both cord and PNS involvement.[24,25] Fortunately, it is not clinically necessary to make this distinction. Patients require vitamin therapy regardless of the relative contributions.

The more disabling phase of deterioration begins with the onset of ataxia. This too has several components. Severe neuropathy limits proprioceptive input. But the lateral column degeneration affects spinocerebellar connections. Finally, as the pyramidal track is involved, patients develop spasticity and paraplegia (spastic paraplegia).

The older literature documents important features concerning therapy for SCD. Most improvement in neurologic function occurs in the first few months after initiating therapy, but may continue for one year.[22] Complete or dramatic recovery was the rule with symptom duration of less than three months. Residua were present with longer or more severe symptoms. For instance, only a moderate improvement occurred with severe neuropathy present for a year or longer. SCD was typically a disease in middle and old age with peak incidence in the late sixties.[26] Delay in diagnosis was frequent and therefore most patients had significant disease (Table 1). A modern series of patients is far different, with less than 15% progressing beyond the second stage! Furthermore, treatment responsiveness was greater with only 6% having moderate to severe disability.[21]

SCD was the leading cause of death in pernicious anemia in the pretreatment era. Its incidence in pernicious anemia was variably reported: from 10%[26] to 90%.[27] The incidence of SCD is high with some causes; for instance, 79% in patients with fish tapeworm.[26] N_2O anesthesia can induce an irreversible oxidation of cobalamin Co^{+} to Co^{+++}, metabolic changes of vitamin B_{12} deficiency, and a rapid onset of SCD[28,29]. N_2O was more commonly used in prior years. One study linked an HLA haplotype to an increased risk of SCD in pernicious anemia.[30]

The pathology of SCD is curious. Demyelination begins as discrete foci, more common in the cervical cord, which coalesce and spread leading to larger lesions with older and newer processes. The vessels are involved, demonstrating regressive wall changes. A lacunar state can develop affecting the glial mesh and axons. The literature on treated disease is limited, but suggests that lesions remit and transform into a diminutive circatricial stage.[26] MRI recognizes these lesions[31] and also indicates that they resolve during therapy.[32] The brain can have similar pathologic lesions.[33]

Pernicious anemia

True pernicious anemia (Addison-Biermer syndrome) is an autoimmune disorder affecting gastric mucosa, producing atrophy, loss of parietal cells, achlorhydria and inadequate glycoprotein intrinsic factor. These factors limit vitamin B_{12} ("extrinsic factor") absorption. Folate and vitamin B_{12} may both be deficient. Patients develop megaloblastic anemia and SCD. Serum vitamin B_{12} is reduced. Folate may induce an immediate, albeit generally not permanent, remission in hematologic abnormalities. Folate doses as low as 0.1 mg/d can restore erythropoiesis,[34] but doses as high as 0.4 mg/d may not induce reticulocytosis in pernicious anemia.[27] Treating the nervous system requires both vitamin B_{12} and folate. Herein lies a clinical dilemma: the hematologic improvement may lure the physician into complacency while neurologic disease progresses. Because this has occurred in the past, concern is appropriate. However, the solution lies not in withholding folate out of fear of "masking" neurologic disease. Rather, physicians need better information on the early detection of SCD and the need to evaluate both vitamins.

Folate and/or Vitamin B_{12} Deficiency and Subacute Combined Degeneration

Classical SCD patients have low serum vitamin B_{12} associated with clinical disease. They also have elevated serum homocysteine and methylmalonate. Treatment of SCD with vitamin B_{12} without attention to folate can be dangerous.

Vitamin B_{12} alone will not ameliorate SCD when there is concomitant folate deficiency. Thus, patients will continue to progress despite vitamin B_{12} therapy.[35] Such patients emphasized that SCD can occur without any prior abnormality in vitamin B_{12}.[16] Indeed, such patients have been identified.[36,37,38,35] They are best characterized by finding low folate and elevated homocysteine and normal vitamin B_{12} and methylmalonate.[38]

It is a common misperception that SCD is due exclusively to vitamin B_{12} deficiency. Current wisdom cautions about the masking of SCD by folate therapy.[39] However, this ignores the equally dangerous masking of folate deficiency by vitamin B_{12} therapy. It is important to address both vitamins in patients with pernicious anemia, SCD or neuropathy. The interrelationship of folate and vitamin B_{12} in producing SCD should be evident.

SCD has occurred in the presence of normal serum vitamin B_{12} and folate. The problem in these patients is clarified by the elevated serum homocysteine and methylmalonate. They may have abnormal vitamin B_{12} transport systems.[40]

DEMENTIA

Vitamin B_{12} and folate deficiency are associated with dementia and, in severe situations, coma.[41] The older literature refers to this a pernicious encephalopathy.[42] Cerebral effects of vitamin B_{12} deficiency are evident in EEG studies during treatment of SCD.[26] Both dementia and vitamin deficiencies are more common in the elderly, and many studies have noted an association. Dementia is heterogeneous and few studies differentiate them. Alzheimer disease patients have serum vitamin B_{12} and folate similar to healthy controls; homocysteine and methylmalonate levels, while increased in comparison to healthy controls, are still similar to hospitalized controls.[43]

Vascular dementia will likely correlate with folate deficiency inasmuch as folate is relevant to cerebrovascular disease.

CONGENITAL ANOMALIES

Folate and embryogenesis

Folate affects embryo development. Neural tube defects (anencephaly and meningomyelocele) correlate with low maternal folate and high homocysteine. Substantial data indicate that folate supplementation reduces the risk of these serious anomalies. Further insight results from studies of teratogens that interfere with folate metabolism and increase the risk of neural tube defect. Of course, these data must be interpreted with an appreciate for the inherent or background risks of malformations.

Fetal loss

Fecundability in humans is relatively low and about 25% per menstrual cycle. Between 25 and 50% of early embryos are lost,[44,45] most subclinically. Malformations usually result in spontaneous abortion. Neural anomalies are present at a very high rate (75% or more) in early aborted embryos. The prevalence of neural tube defects decreases during gestation. Most fetuses with meningomyelocele do not survive until term (Table 2). Holoprosencephaly is 70 to 100 times more common in fetuses.[48] Statistics in liveborn newborns will therefore greatly under estimate the frequency of anomalies.

Table 2. The incidence of Meningomyelocele in fetuses and newborns

Cohort	Incidence	References
Early gestation (3-6 mm CRL)	2.5-2.9%	**46,47**
Spontaneous abortions†	1.8-4.9%	**48,49,50,51**
Induced abortions	0.4%	**48**
Live born	0.06%	**46**
% survival to term	2%-20%	**46,49,51**

† about 40% will have chromosomal abnormalities.

Prevention

Few prevention strategies are currently available, but they illustrate the possibilities for the future. Most rigorous scientific data concerns neural tube defects, but similar approaches may be applicable for other anomalies. Primary prevention involves actions directed at preventing a malformation from occurring. Folic acid administered to women with prior children with neural tube defects reduced the risk of recurrence by 72%.[52] Current recommendations are for 4 mg/d *peri*conceptionally (when attempting to become pregnant) in these higher risk women and 0.4 mg/d interconceptionally in these and all other fertile women.[53,54] Folate fortification of grains, rather than pharmaceutical sources,

addresses the entire population without requiring a change in behaviors[55,56] and is a cost effective approach.[57] Drugs which interfere with folate metabolism, such as valproic acid,[58] should be administered with supplemental folate. Folate metabolites are substrates for methionine synthase (enzyme 4, Figure 1). Mothers of children with neural tube defects have high homocysteine values.[59] Also, 677C→T frequency is increased in mothers, fathers and patients with neural tube defects.[60] Homocysteine is embryotoxic, but vitamin B_{12} ameliorates this toxicity.[61] Methionine is required for neurulation and supplementation reduces the incidence of neural tube defects in animal model studies,[62] including those evaluating drug teratogenicity.[63] Because vitamin B_{12} is also required for homocysteine metabolism, the most effective prophylaxis may require vitamin B_{12} or methionine supplementation,[59] in addition to folate. Other nutrients, including inositol are important in neurulation.[64,65] Free radicals also impair neurulation and ascorbate and vitamin E are protective in animal models.[66]

Antiepileptic drug teratogenicity

Meningomyelocele occurs in between 1 and 5% of infants born after *in utero* exposure to valproate[67,68] and is also slightly more common in women taking carbamazepam.[69] Minimal risk is attributable to phenytoin, barbiturates or primidone.[69] AEDs account for less that 1% of all meningomyeloceles in a general population.[70]

Antiepileptic drugs (AEDs) and their various metabolites (see Table 1) have differing teratogenic potential.[74] If two metabolites have strikingly different teratogenic potential, then changes produced by both are probably irrelevant. Valproate disrupts folate metabolism but the non-teratogenic 2-en analog does not.[41] This supports the hypothesis that folate is relevant in valproate teratogenicity. Individual variation in drug metabolism is recognizable by determining AED metabolites and this may have value in predicting teratogenicity in humans.[76] Presumably genetic variation between patients is responsible.

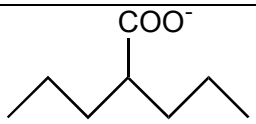
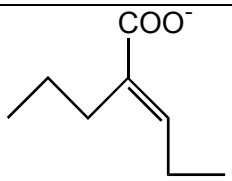
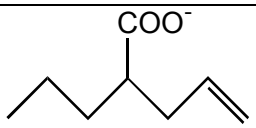
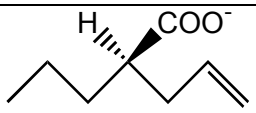
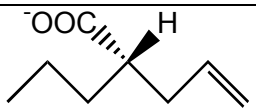
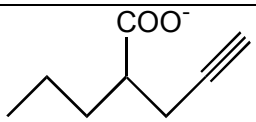
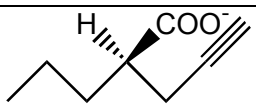
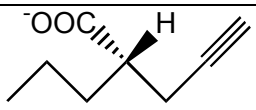
In animals, 2-en-valproate is less teratogenic and the 4-en metabolite more teratogenic than the parent compound.[77,78,79] Furthermore, the 4-en teratogenicity appears specific for the S-4-yn-enantiomer which is over 7 times more teratogenic than its antipole, the R-4-yn enantiomer.[80] The basis for the elantoselective differences relates directly to teratogenic effects because transplacental transport and pharmacokinetics are not different. Both enantiomers have equivalent antiepileptic effects and less toxicity.[81] Thus, less teratogenic metabolites (2-en-valproate or R-4-yn-valproate) represent potential future improvements in therapy.

Phenytoin and carbamazepam teratogenicity appear related to arene oxide metabolites generated by the P450 pathways.[82,83] Agents inhibiting the P450 system reduce malformations in these animal models.[83] Interestingly, however, ethoin, which is not so metabolized, remains teratogenic in animals[84] and others dispute phenytoin's action via epoxides.[85] These agents can affect folate.[38]

Primary prevention includes folate supplementation and utilizing the minimum necessary dose of valproate. Folate supplementation (5 mg/d) is recommended.[86,87,88] Folate reduces meningomyelocele in a variety of circumstances,[89,90,52] but its role in mothers treated with valproate is not yet firmly established. Considering the block induced by valproate (enzyme 7, Figure 1) and the role of methionine, supplementation

by 5-formimino-tetrahydrofolate appears more rational.[75] Animal studies demonstrate discrepancies between *in vivo* and *in vitro* systems,[91] also pointing to the possible importance of folate metabolites in preventing terata. Secondary prevention of AED induced meningomyelocele includes α-fetoprotein and ultrasound screening and termination of affected pregnancies if acceptable to the parents.[87, 77] Caution is advised because serum α-fetoprotein testing alone may be less sensitive in women taking valproate than those screened from other cohorts.[92] Thus, serum screening alone is insufficient. Ultrasound testing is required and should also include the limbs because the radial reduction syndrome occurs with valproate therapy.[93]

Table 3. Teratogenicity of valproate (VPA), its metabolites, and related compounds.

Compound	Structure	Exencephaly (%)	Dose (mmol/kg)	Reference
VPA		44	3.00	71
E-2-en-VPA		0	3.70	72
(±)-4-en-VPA		33	3.00	73,58
R(+)-4-en-VPA		17	3.00	58
S(-)-4-en-VPA		70	3.00	58
(±)-4-yn-VPA		92	2.47	58
R(+)-4-yn-VPA		1	3.00	58
S(-)-4-yn-VPA		65	1.05	58

CEREBROVASCULAR DISEASE H

Four lines of evidence implicate folate as a risk factor for stroke: genetic, mechanistic, epidemiologic and intervention studies. The latter are largely in progress and therefore we must reserve judgment about the importance of folate in stroke prevention. The literature is very enthusiastic about folate as a preventive strategy. There are, however, significant issues to encourage skeptics and stimulate the serious scientist to further investigation. Clearly this is an exciting area for study with potentially great importance. The literature on coronary and peripheral vascular disease is even more extensive and beyond this review.[94]

Genetic disorders and polymorphisms

Classic homocystinuria, caused by a deficiency of cystathionine β-synthase [EC 4.2.1.22] (OMIM[95] 236200; enzyme 14 in Figure 1), first called attention to the association of homocysteine and vascular disease. Patients have a higher incidence of some types of vascular disease. Parents and grandparents have a tendency towards increased risk of stroke and myocardial infarction, but data sets are too small to establish this definitively.[96] Heterozygosity or polymorphism for this gene was not observed in patients from the general population with premature vascular disease.[94]

Homocysteine is not the only risk factor for stroke in homocystinuria. Patients with this disorder originate from a selected genetic pool which demonstrates founder effects. Mutations in factor V Leiden, although segregating independently, are also increased in this genetic pool. In homocystinuric patients, stroke was found exclusively in patients with mutations in both cystathionine β-synthase and factor V Leiden.[97] Thus, a "double hit" appeared necessary to produce an infarct. While suggested that this association does not appear in the general population,[98] it was also observed in the Physician's Health Study.[99] Nonetheless, this is not a trivial issue because anticoagulation is appropriate with factor V Leiden deficiency.[97]

Patients with deficiency of methylene-THF reductase (OMIM 236250; enzyme 8 in Figure 1) develop strokes at an early age.[100,101] Polymorphism was identified in studies of this gene and they were subsequently investigated in vascular patients. Several reports note not increase in the thermolabile variant (677C→T). But another found it present in 12%-15% of European and Japanese individuals and is associated with almost a doubling of serum homocysteine. 677C→T is present in 28% of individuals with premature vascular disease, suggesting that it plays an important role in these disorders.[94] Folate lowers serum homocysteine in individuals with this polymorphic variant.[102] This raises the hope that up to 50,000 *coronary* vascular deaths a year could be avoided by folate fortification.[94,103]

Neuroepidemiological studies

Homocysteine was extensively investigated in the general population as a risk factor for coronary, peripheral and cerebrovascular disease. In the former, a relative risk for hyperhomocystinemia was a least 2 in most studies. This ascribes a significance to elevated homocysteine similar to smoking and hypertension.

Carotid stenosis was associated with elevated homocysteine (relative risk=2.0).[104] But, this study defined carotid stenosis as >25%, whereas risk of stroke is generally associated with much greater stenosis.

Elevated homocysteine correlated with ischemic stroke in a series of case-controlled studies.[105,106,107,108,109] Low serum folate also correlates with high homocysteine. Finally, low serum folate was correlated with ischemic stroke, but the relative risk was low and confidence intervals overlapped with normal.[108] The small number of stroke events in these studies mandated a more comprehensive investigation.

The European Concerted Action Project (ECAP)[110] firmly established that elevated homocysteine is a risk factor for stroke in the general population. Furthermore, this study found erythrocyte folate and vitamin B_{12} inversely related to serum homocysteine. The small number of adults taking folate, pyridoxine and vitamin B_{12} were at lowest risk of stroke. They concluded that *homocysteine conferred a risk of stroke that was similar to smoking or hyperlipidemia* and that a controlled study of folate in stroke prevention was needed. A critical evaluation of these ECAP data provides an assessment of the importance of homocysteine. The relative risk of elevated fasting homocysteine for stroke was 2.0.

Several challenges to homocysteine as a risk factor merit consideration: 1) The independence of homocysteine as a risk factor is not clear. a) Factor V Leiden as a concomitant risk was addressed earlier. Other "double hit" mechanisms involving coagulation, vessels or other systems may be operative. b) Homocysteine blood levels are highly correlated with renal function. Early renal vascular disease may increase homocysteine. Recent studies attempt to control for this utilizing serum creatinine. However, careful kinetic studies in renal failure suggest that the primary basis for high homocysteine is not directly related to renal function, but to tissue methylation.[111] The effects of hypertension and smoking on kidney or tissue methylation may impact homocysteine; if so, they are not independent risk factors. 2) A large, population-based Finnish study failed to identify folate as a risk factor.[112] The population-based study design has advantages over the case control methodology of all other studies. However, different populations might also carry different risks for elevated homocysteine.

Considering the population at large is important in epidemiologic studies. The case-control studies identify risks. The relative risk of homocysteine in stroke approximates 2 in most studies. The attributable risk is *not* easily inferred from these studies. Some *tenuous assumptions* are necessary to perform such calculations, including variation in age stratification and proportion of thrombotic events between the study population and the reference data available on the entire population. Nonetheless, some population estimate may help. The attributable risk (AR)=A/(1+A) where A=prevalence x (relative risk-1).[113] Thus, with a RR of 2.0 and prevalence of 20%, the AR is 16.7%. Similar calculations have attributed 10% of myocardial infarctions to high homocysteine.[94]

Mechanistic investigations

While observations from genetic and epidemiologic investigations suggest a role of folate and homocysteine in stroke, precise underlying mechanisms are not clearly defined. Several lines of investigations address this important issue. Non-human

primates feed high homocysteine, high fat, high cholesterol diets have more pronounced atherosclerosis. Human studies also correlate intimal thickening, putatively reflecting atherosclerosis, to plasma homocysteine.[114] Early reports concerning stroke in homocystinuria also noted arteriosclerosis and is was commonly assumed that this caused stroke. The concomitant factor V Leiden mutations raised significant new issues.

Several coagulation proteins are altered by high homocysteine and folate deficiency.[115] Homocysteine also interferes with endothelial antithrombin III binding and activation of thrombomodulin, the latter being important in activating protein C.[116,117] Platelet aggregation is also enhanced by homocysteine, in part because of induction of thromboxane biosynthesis.[118]

Vessel size is carefully regulated. Vasodilatation is mediated, in part, by nitrous oxide (NO). NO also has antithrombotic effects. This reactive molecule also interacts with sulfur amino acids, including homocysteine, forming nitrosothiols and thionitrates.[119] In monkeys, physiologic dilatation of vessels is impaired by high homocysteine.[120] Homocysteine impaired responses to endothelial derived relaxing factor, perhaps by reducing synthesis of S-NO formation.[121]

The possible mechanistic links between high homocysteine and stroke have implications for intervention. Atherosclerosis develops over long time intervals and may be less amenable to secondary prevention. Coagulation and vessel chemistry and size change over short time spans and may respond to secondary prevention.

Clinical trials

In the United States and Canada a large trial multivitamins with folate, pyridoxine and vitamin B_{12} (Vitamin Intervention in Stroke Prevention or VISP) will evaluate their efficacy in preventing a recurrent stroke.[122] Neurologists are thus positioned to provide the first controlled data on folate as a preventive agent in vascular disease. This study plans to enroll 3,600 patients with non-disabling ischemic stroke. A recurrence of 12% in the first two years is anticipated in the control group and the study is designed to detect a 30% treatment effect over a two year interval of observation.[123]

EPILEPSY

Folate and Antiepileptic Drugs (AEDs)

AEDs can affect folate metabolism and these affects may contribute to some of their side effects. Most concerning are potential teratogenic effects on embryos (see above).

Folate effects on Epilepsy

Several authors reported that folate therapy in patients with epilepsy increased the frequency of seizures.[124,125,126,127] Other observers noted no such association.[128,129,130,131,132,133,134] Reynolds, one of the influential proponents of an adverse role, later revised his opinion.[135] AEDs and folate affect each others metabolism.[136,137,138,139] Folate has direct excitatory effects on the nervous system.[140,141] The LD_{50} of folate in rats is very high (450-500 mg/kg), but death occurs with status epilepticus.[142] Some opined that AEDs exerted their anti-seizure effect by lowering folate.[142] However, folate deficiency also increased susceptibility in animals models of

seizures.[143] Currently many epilepsy patients receive folate without any obvious adverse effects.

Folate deficiency, due to a genetic transport deficiency, produced severe seizures. Folate was not an effective neurologic treatment, but the seizures responded to methionine therapy.[144] This important observation suggests a another mechanism whereby folate deficiency can reduce the seizure threshold. However, this does not appear operative in most patients with epilepsy.

If folate has an adverse effect on epilepsy, it is probably by virtue of its effects on AED metabolism.[145] Providing continuous folate supplements virtually eliminates the risk of folate deficiency. Supplements will also maximize the folate effects on AED metabolism and, by eliminating this variable, enhance the predictability of AEDs after adjustments in their dose. In other words, folate will facilitate the management of AEDs.

METABOLIC-CLINICAL CORRELATIONS

MIRACLE CURE AND MEDICINE'S WRONG TURN

Anemia and SCD are independent manifestations of vitamin deficiency. SCD progresses independent of hematologic disease.[27] Thus, SCD may occasionally become apparent long before hematologic symptoms.[26] Furthermore, with better folate nutritional status, anemia as a presenting sign of folate and/or vitamin B_{12} deficiency may be greatly reduced. This seems to have concerned hematologists who are now the principal proponents about the risks of this "masking." It seems rather peculiar that hematologists would advise against fortification with folate while agreeing that it will virtually eliminate a serious cause of anemia. But others do not share this opinion.[101,146] It is not logical to utilize anemia as the initial sign of SCD or neuropathy.

There is an irrational passion evident in critics of fortification. There are two essential elements to their logic. The first element relates to the changing clinical picture induced by folate supplementation. But the second goes well beyond this, bringing an inflammatory tenor of concerns about toxicities of folate which seems to have arisen from circumstances of history.

Medicine took a wrong turn concerning folate a half century ago. The recognition by Minot and Murphy in 1926 that liver and gastric extracts could cure pernicious anemia and SCD was a tremendous advance, rewarded with the Nobel Prize in 1934. Castle proposed the model of conditioned nutritional deficiency. An essential "extrinsic factor" from the diet interacted with a constituent of normal gastric juice ("intrinsic factor") to produce the hemopoietic factor. Investigation focussed on identifying the active ingredients. In 1939 yeast extracts containing folate were found helpful in treating the anemia. Synthetic folate became available by 1945 and it was reported as effective as liver extract. By 1947 reports began to appear about progressive neurological disease in some patients treated with folate; it was not the extrinsic factor. A scathing editorial in the *New England Journal of Medicine*, written anonymously, blasted folate as a dangerous and ineffective agent and encouraged return to the use of liver extract.[147] In 1948 vitamin B_{12} was isolated and became available. Clinical assays of vitamin B_{12} did not become available until 1952. In the seven years between the introduction of folate and the ability to assay vitamin B_{12} there was ample opportunity to err by inducing remissions with folate while being unable to assess vitamin B_{12} status. By 1952,

vitamin B_{12} was recognized as the extrinsic factor and folate, considered unnecessary, was banished.[148]

Neurologists didn't help matters. Widely respected neurologists continued into the 1950s to point out the difficulty of making the diagnosis of SCD in the absence of anemia, pointing out the wide differential diagnosis of spinal cord disease and the limited diagnostic testing of the era. This was a bit of overstatement since they also pointed out that the serum vitamin B_{12} level provided a "refined" diagnostic method that was particularly helpful when there was no anemia.[27] Also emphasized was the notion that folate might accelerate the onset of SCD.

But folate effects did raise some questions. Before metabolic pathways were delineated, it was assumed there was an interaction between vitamin B_{12} and folate, with the former being of primary importance. This logic was later incorporated into a new hypothesis, the methyl-folate trap.

There are some lessons from this history that merit emphasis. Medicine uncovered a tremendous cure. In the excitement of implementation, a few details were overlooked. Therapy was given without assays to quantitate the deficiencies or to monitor the effects of therapy. The theoretical framework of the day, enunciated by Castle, emphasized absorption, accommodated extrinsic factor (vitamin B_{12}) and did not require folate. Folate was further pushed aside to a secondary role by the methyl-folate trap theory. Physicians seemingly impressed with their cure overlooked the pernicious nature of the disease and ascribed malignant SCD to folate toxicity rather than treatment failures.[146]

It could have gone the other way if vitamin B_{12} had an equivalent historical jump on folate! We now know that vitamin B_{12} therapy can also "mask" folate deficiency. It is critically important in current discussion to recognize that we are no longer hampered by the important limitations that set the tenor of articles from this era. We now measure both vitamin B_{12} and folate and, increasingly, homocysteine and methylmalonate. We will see more neurologic disease without anemia; this is a common experience for neurologists, but other physicians may need further education to recognize this scenario.

MASKING: AN UPDATED PERSPECTIVE

Folate and vitamin B_{12} are metabolically intertwined. They certainly affect each other and the processes they modulate together. However, the concept of "masking" is misleading, based on skewed interpretations and selective use of data. It may be helpful to dissect out various conclusions or points, the observations that seemingly initiated them, and the more seasoned perspective that we now can bring to the discussion.

Point #1: Vitamin B_{12} deficiency is the *sole* cause of SCD

Observation: SCD responded transiently to folate but vitamin B_{12} produced a lasting effect.

Historical view: During the historical window between 1945 and 1952 this conclusion was tenable. Initially, three premises were reasonable: folate appeared to be the extrinsic factor, patients' anemia responded and no neurologic disease developed. No assays were available to verify or challenge conclusions. Then each premise was challenged by contrary data. The entire construct was discarded. Vitamin B_{12} alone was viewed as curative for all SCD.

Commentary: There are now well delineated patients with normal vitamin B_{12}, folate deficiency and response to folate therapy alone. Indeed some of these patients failed to respond to vitamin B_{12} therapy. Some patients were even treated with vitamin B_{12} alone, even though known to have low folate;[35,36] such was the influence of this perspective.

Point #2: Folate therapy hastens neurologic disease

Observation: SCD began fulminately in some patients treated with folate.

Historical view: Fulminate SCD during folate therapy was presumed to be caused by the folate. This notion pervades the literature of the 1950s and continues to the present.

Commentary: SCD was often a bad disorder before folate therapy was available;[146] thus the name "pernicious" anemia. Physicians were better hematologists than neurologists. Delays in diagnosis of neurologic disease were frequent, often in the face of months of symptoms. By then, a crisis was brewing. It is debatable whether folate actually aggravated the situation; but it was a good scapegoat. One notable patient had his exacerbation very shortly after dental extractions (possibly using N_2O), failed to respond initially to liver extract and also had pancreatitis flare within a short time.[149] Certainly the claim of exacerbation is unproven.[150] Vitamin B_{12} therapy is also associated with a deterioration in some SCD patients.[21] Perhaps the well recognized fluctuations in SCD are erroneously attributed to therapeutic agents coincidentally associated with deteriorations early in therapy.

The independence of hematologic and neurologic disease is evident. We should diagnose the problem regardless of the mode of presentation. The tuning fork probably requires more use in standard evaluations.

Point #3: Folate "masks" vitamin B_{12} deficiency

Observation: During folate therapy SCD may progress.

Historical view: This point states that folate treats the anemia and obscures the ability to recognize significant neurologic disease.

Commentary: This is the most important historical point. It has been the most evincing argument provoking reluctance to implement folate supplementation. The perspective is illogical, not entirely factual and the allegory muddled. At least 20% to 40% of vitamin B_{12} deficient patients already present with neurologic disease without hematologic symptoms.[150, 21] The "mask" is the elimination of the blood smear as the route to diagnosing neurologic disease: "The clinician may be faced with the challenge of diagnosis of neuropathy in the absence of anemia."[39] This is a minor handicap if one employs a tuning fork and follows up abnormal results with some serum assays. Folate does not put a mask of obscurity on anemia. It may successfully treat it; that's desirable. Also, hematologic remission was just as common as neurologic remission in a large study during the mid-1940s.[151] Smaller studies appear similar.[152] Folate does not obscure neurologic disease. In some patients, where there is primarily folate deficiency, it will also treat neurologic disease. In others, including those with vitamin B_{12} deficiency, it may be ineffective — a treatment failure.

If there is a remaining truth to this perspective, it speaks more to our shortfalls as physicians than those of folate. The older literature is replete with patients whose neurologic disease progressed far too long before diagnosis.[26] The only strategy for this dilemma is education. Patients should be taught to report paresthesias and physicians should use their tuning fork in routine evaluations or screening. Fortunately, SCD is now uncommon due to greater vigilance.[153] Less than 10% now have moderate to severe neurologic disability after treatment.[21] Also, as the associations of homocysteine with vascular disease and malformations are better known, we will see an increasing number of patients screened for deficiencies or genetic polymorphisms.

BIOCHEMICAL PATHOPHYSIOLOGY OF NEUROPATHY

Folate neuropathy falls in the category of demyelinating neuropathies. Peripheral nerve biochemistry is not available. But myelin contains lecithin and ethanolamine; S-adenosyl-methionine provides the methyl groups in synthesizing lecithin from ethanolamine. Elevated ethanolamine and reduced lecithin has been observed in folate deficiency in rats,[154] but direct studies of human nerve are not available. Interference with nerve methylation may cause neuropathy.[155,101] Vitamin B_{12} deficiency is also generally regarded as causing a demyelinating neuropathy. Severe deficiency of folate or vitamin B_{12} can produce an axonal degeneration.[41]

Other direct evidence of the importance of folate to peripheral nerves comes from the effects of methotrexate chemotherapy. This agent, a folate antagonist, produces a neuropathy in virtually all recipients.

BIOCHEMICAL PATHOPHYSIOLOGY IN THE CENTRAL NERVOUS SYSTEM

Methylation abnormalities are also considered paramount in demyelination in the CNS when folate or vitamin B_{12} metabolism are disrupted. CSF S-adenosylmethionine (SAM) is reduced in patients with demyelination and deficiency or inborn errors of metabolism folate/vitamin B_{12}.[156,153,157] This includes patients with SCD.[37,153]

Other mechanism may also be operative. Folate deficiency may impact biopterin metabolism and therefore catechol- or indole-amine pathways.[157] Homocysteine is metabolized to several amino acid neurotransmitters. Excitatory amino acids are important in neural injury. Homocysteic acid and cysteine sulfinic acid are considered endogenous excitatory ligands for NMDA glutamate receptors. Taurine and hypotaurine may act as inhibitory neurotransmitters.[158] Folate itself has excitatory properties similar to the classic toxin, kainic acid.[159] Folate and 5-formimino-THF are more toxic than methylene-THF in these animal models.

SAM is available as a pharmacologic agent in Europe and has diverse effects on patients. It promotes remyelination in inborn errors of metabolism, exacerbates psychosis in schizophrenia, and ameliorates depression. Betaine also improves myelination and metabolic abnormalities. Betaine has a potential advantage over SAM because it lowers S-adenosylhomocysteine (SAH) and homocysteine. SAH has important regulatory feedback effects as an inhibitor of many methylation reactions.

Transport of folate and vitamin B_{12} into the CNS require specific transport systems. If these systems are impaired, systemic disease may respond while CNS disease progresses. Because pharmaceutical folate is not natural folate, there was some concern

expressed that these forms of folate might block transport of natural folates into the CNS, thereby producing a deficiency and disease.[160] Folinic acid (5-formimino-THF) or methyl folate[37] may be more effective than folic acid in some patients. This potential block of transport by artificial folate has also been offered as a mechanism whereby folate might exacerbate or worsen neurologic disease.

LESSONS FROM ANIMAL MODELS OF SCD

Animals develop SCD while fed vitamin B_{12} deficient diets, following total gastrectomy, and after administration of metabolic inhibitors. Demyelination is the initial event in the cord in primates, with axonal damage occurring later.[161] These models have shown reduced methylation of myelin lipids and of myelin basic protein.[162,163] Deficiency of S-adenosylmethionine appears particularly important in pathogenesis.[160] Inhibitors of methionine-homocysteine metabolism induce SCD.[164,165]

Toxic compounds might contribute to neurodegeneration. Formate dysmetabolism occurs in some models.[166] Rats will not develop SCD with low vitamin B_{12} induced by a cobalamin deficient diets. But gastrectomy will induce vitamin B_{12} deficiency and SCD. Ornithine decarboxylase induction occur in rats after total gastrectomy, suggesting a role for polyamines in the neurodegeneration.[167,168] Factors other than mere vitamin B_{12} deficiency are operative in these animals.

Treatment of these animals with SCD demonstrates a positive response to increase dietary methionine.[169]

INBORN ERRORS OF FOLATE AND VITAMIN B_{12} METABOLISM

Folate metabolism is complex and thus there are numerous inborn genetic errors of folate metabolism. Megaloblastic anemia commonly occurs. Nervous system disease takes several forms, including malformations, mental retardation, and SCD.

Transport defects

Severe folate deficiency occurs with transport defects that block intestinal absorption. Serum and probably systemic tissue levels are corrected with parenteral folate, but entry into the CSF and brain is also affected in transport defects and parenteral therapy may not correct the nervous system deficiency.[170,100] Thus, the megaloblastic anemia is correctable, but neurological disease may persist.

Vitamin B_{12} inborn errors of transport affect both methylmalonic and one-carbon metabolism. There are several "R-proteins" involved in vitamin B_{12} transport and deficiency (OMIM 193090) can produce neurologic disease. Transcobalamin II (TCII) deficiency (OMIM 275350 and OMIM 277410; protein 18 in figure 2) has produced neurologic disease. In some reports this followed folate treatment which corrected the anemia.[171,172]

Methylene-THF reductase

Deficiency of methylene-THF reductase (OMIM 236250; enzyme 8 in Figure 1) permits normal DNA synthesis and megaloblastic anemia does not develop. Neurologic symptoms are quiet variable and include SCD or hypo- or dys-myelination. This disorder

reduces levels of methylene-THF and abnormal methylation occurs.[157,160] Folate was reported as ineffective in these severe deficiencies and may cause a deterioration.[173] Some patients do respond positively to folate.[174] Betaine treatment appears most helpful and provides methyl moieties to regenerate methionine via betaine methyltransferase [EC 2.1.1.5].[175] Biopterin metabolism is intertwined with that of folate and these patients can have Parkinsonian symptoms with reduced concentrations of CSF biopterin and catechol and indole amine metabolites.[173] Patients can develop strokes at an early age.[100,101] The gene for methylene-THF reductase has been cloned, sequenced and at least nine mutations identified for the severe deficiency state.[176]

Homocstinuria

Classic homocystinuria is an inborn error of metabolism due to deficiency of cystathionine synthase (OMIM 236200; enzyme 14 in Figure 1). It does not directly impact folate or vitamin B_{12} metabolism but, like vitamin deficiencies elevates serum homocysteine. This permits an assessment of homocysteine's effects independent of vitamin deficiency. Importantly, homocstinuria patients have premature vascular disease and stroke as discussed above.

Methionine levels are not reduced in this disorder and methylation systems remain intact. Demyelination does not occur.

Homocstinuria with methylmalonic aciduria

Defects early in the metabolic processing of vitamin B_{12} affect both methionine synthase (Enzyme 4 in Figure 1 and Enzyme 27 in Figure 2) and methylmalonyl-CoA mutase (Enzyme 25 in Figure 2). Upon entry into the cell, lysosomes cleave TCII-cobalamin, releasing col(III)amin into the cytosol. A defect in this process is designated clbF (OMIM 277380; process 20 in Figure 2). A hypothesized defect in cytosolic reduction of col(III)amine is present in cblC (OMIM 277400; enzyme 21 in figure 2) and cblD (OMIM 277410; enzyme 21 in figure 2).

Homocstinuria without methylmalonic aciduria

Homocstinuria in the absence of methylmalonic aciduria results from impaired formation of methylcobalamin. Methionine synthase (OMIM 156570; enzyme 4 in Figure 1) is abnormal and a metabolic block affects the transfer of methyl groups to methionine as well as DNA synthesis. Hypomylinization occurs. Neurologic and hematologic features are prominent.[177,100,101]

Two forms of homocystinuria without methylmalonic aciduria exist. CblE disease has low methionine synthase activity in the absence of a reducing agent and cblG (OMIM 250940; enzyme 4 in Figure 1) has normal activity with or without the agent. These patients have megaloblastic anemia and neurologic disease which responds to vitamin B_{12}. It is hypothesized that in cblE patients there is a defect in one of the two redox proteins that activate methionine synthase. Further heterogeneity in these two groups was suspected.[178] Indeed, subsequently cblG subgroups also were distinguished by assay reducing agent conditions.[179] Mutations in cblG clarify these data. Mutations in the 3´-half of the gene encoding for the cobalamin and adenosylmethionine binding

domains reduce enzyme activity in the absence of reducing agents. Mutations in the other half seem to affect transcription or stability of mRNA.[179]

Methylmalonic aciduria

Classic methylmalonic aciduria results from a mutation in the methylmalonyl-CoA mutase apoprotein (OMIM 251000; enzyme 24 in Figure 2). Relevant to this discussion are two vitamin B_{12} responsive forms in which the apoprotein is normal. Two defects are recognized. In cblA there is an inability to reduce col(II)amine within the mitochondrion (OMIM 251100; enzyme 22 in Figure 2). In cblB (OMIM 251110; enzyme 23 in Figure 2) the reduced cobalamin is not adenylated due to a deficiency of the adenylotransferase. These disorders are important because these patients do not develop myelinization abnormalities.[160]

Other Genetic Disorders

Glutamate formiminotransferase deficiency (OMIM 229100; enzyme 7 in Figure 1) is a block in histidine metabolism and has a variable hematologic and neurologic phenotype.[100]

Dihydropteridine reductase (DHPR) deficiency interferes with folate metabolism. Reaction 10 in Figure 1 can also be catalyzed by DHPR. Dihydropteridine, which accumulates in DHPR deficiency, is a competitive substrate for Enzyme 8 in Figure 1.[100] The principal defects in DHPR deficiency involve the metabolism of phenylalanine and catecholamines. In this situation folate can cause some deterioration of neurologic status while folinic acid (5-formimino-THF) results in improvement.[180]

A single, but interesting, patient with a the Leber hereditary optic neuropathy mt-DNA mutation (OMIM 535000) developed blindness while vitamin B_{12} deficient and responded to cobalamin therapy.[181]

Patients with Machado-Joseph disease (SCAIII; OMIM 109150), who have a dominantly inherited multisystem disease, were serendipitously noted to improve on sulfamethoxazole-trimethoprim therapy. Trimethoprim inhibits both dihydrofolate reductase and dihydropteridine reductase. A larger study confirmed these findings and noted significant changes in biopterin and neurotransmitter metabolites.[182]

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