ROLE OF B VITAMINS IN BIOLOGICAL METHYLATION

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ROLE OF B VITAMINS IN BIOLOGICAL METHYLATION

Methylation is the process of controlled transfer of a methyl group (one carbon atom and three hydrogen atoms) onto amino acids, proteins, enzymes, and DNA in every cell and tissue of the body to regulate healing, cell energy, genetic expression of DNA, neurological function, liver detoxification, immunity, etc. This process is one of the essential metabolic functions of the body and is catalyzed by a variety of enzymes. Many case histories and several scientific articles have demonstrated that this methylation process is responsive to environmental conditions and degrades with age, a process associated with a large variety of age-related disorders. Thus, with respect to the effect of methylation, it is a continuous struggle in life to adapt to the ever-changing environment. In fact, health and quality of life are highly dependent on the methylation process.

The process of methylation requires two cycles of events (see figure): the S-adenosyl methionine (SAM) cycle (cycle A) and the folate cycle (cycle B). The folate cycle is essential for inhibiting the SAM cycle and for keeping methionine available for producing SAM. The enzyme methionine synthase is an essential part of these cycles.

Methylation processes are central to the biochemical basis of the neuropsychiatry of folate and B12 metabolism. The *de novo* synthesis of methionine requires vitamin B12, which is involved directly in the transfer of the methyl group to homocysteine. In turn, methionine and adenosine triphosphate (ATP) are required in the synthesis of SAM, the sole donor in numerous methylation reactions involving proteins, phospholipids, and biogenic amines. The folate cycle requires folic acid, methyltetrahydrofolate (methyl-THF), vitamin B6, B12, and nicotinamide adenine dinucleotide phosphate (NADPH). Upon transfer of its methyl group, SAM is rapidly converted to S-adenosyl homocysteine (SAH) and subsequently hydrolyzed to homocysteine and adenosine. This hydrolysis is a reversible reaction that favors SAH synthesis. If homocysteine is allowed to accumulate, it will be rapidly metabolized to SAH, which is a strong inhibitor of all methylation

reactions, competing with SAM for the active site on the methyltransferase enzyme. The activities of the methyltransferase enzymes involved in these reactions are governed by the ratio of the tissue concentrations of SAM to SAH. Decreasing the SAM:SAH ratio reduces the activity of the SAM-dependent methyltransferase enzyme.

The biochemical basis of the interrelationship between folate and cobalamine is the maintenance of two functions, nucleic acid synthesis and the methylation reactions. The latter is particularly important in the brain and relies especially on maintaining the concentration of SAM, which, in turn, maintains the methylation reactions whose inhibition is considered to cause cobalamine deficiency-associated neuropathy. Defective methylation processes can lead to a number of serious health conditions. A simple abnormality in the methylation pathway, compounded with further assaults from environmental and infectious agents, can lead to a wide range of conditions including cardiovascular disease, neurotransmitter imbalances, cancer, diabetes, abnormal immune function, chronic fatigue syndrome (CFS), multiple sclerosis (MS), cognitive dysfunction in patients with dementia, neurological and psychiatric disorders, Alzheimers disease (AD), Down syndrome, autism, neural tube defects, chronic inflammation, etc.

In folate or vitamin B12 deficiency, the methionine synthase reaction is severely impaired. Vitamin B12, in particular, is the coenzyme required for the correct functioning of the methyl donation from 5-methyl-THF to THF, necessary for methionine synthase. Folate is a cofactor in one-carbon metabolism, during which it promotes the re-methylation of homocysteine - a cytotoxic and pro-oxidant sulfur-containing amino acid that can induce DNA strand breakage, increase the risk of heart disease, and cause various forms of vascular disease, oxidative stress, and apoptosis. Theoretically, in the key point of methylation previously underlined, it might be hypothesized that folic acid "obliges" the entire vitamin B12 to subserve as a coenzyme, thereby exacerbating the otherwise limited damage caused by the vitamin B12 defect per se. In the mammalian system, the primary remethylation pathway is catalyzed by methionine synthase with the help of vitamin B12 and folic acid. However, in a secondary pathway in kidney and liver cells, another enzyme known as betaine-homocysteine methyltransferase can also

re-methylate methionine by the transfer of a methyl group from betaine. Betaine is an oxidized form of choline.

Elevated levels of homocysteine in the blood predispose to arteriosclerosis and stroke. Indeed, it has been recently estimated that as many as 47% of patients with arterial occlusions manifest modest elevations in plasma homocysteine. Among the many causes are genetic alterations in enzymes such as cystathionine beta-synthase, a defect found in 1-2% of the general population, and deficiencies in vitamins B6, B12, and folate, whose intake is suboptimal in perhaps 40% of the population. The strength of the association between homocysteine and cerebrovascular disease appears to be greater than that between homocysteine and coronary heart disease or peripheral vascular disease. During stroke or head trauma, disruption of the blood-brain barrier results in exposure of the brain to near plasma levels of amino acids, including homocysteine and glycine. Homocysteine is detoxified by either of the two separate mechanisms described below:

1. **Homocysteine can be re-methylated by active folic acid, methyl-THF, to make methionine. The process of re-methylation simply adds a methyl group to the sulfur atom of the homocysteine molecule, thereby modifying its overall structure and function. It is important for two reasons. The first reason is that it helps to lower total homocysteine levels. The second, perhaps more significant reason, is that the molecule formed by re-methylation is utilized to make SAM, which methylates DNA, RNA, proteins, phospholipids, and many other essential biochemical molecules.**

2. **Homocysteine can also bind with the amino acid serine to form cystathionine. Cystathionine breaks down to cysteine and homoserine via the vitamin B6-dependent transsulfuration pathway. This reaction results in the loss of methionine and gain in cysteine. This cysteine is the precursor of glutathione.**

Both folic acid and trimethylglycine (TMG) have been used for nearly two decades to lower homocysteine. These two pathways are independent. Some people are better at using TMG to lower homocysteine and others are better at utilizing folic acid. That is why it is better to use both. We

found a combined approach in conjunction with vitamin B6 and B12 can normalize homocysteine in most cases.

BIOLOGICAL METHYLATION

In this section we will discuss only DNA, protein, and phospholipid methylation.

Gene or DNA methylation **in humans occurs at cytosine-phospho-guanine (CpG) sites where the triplet for cytosine is directly adjacent to the triplet for guanine in the DNA sequence coding for these amino acids; this methylation results in the conversion of cytosine to 5-methyl cytosine. DNA methylation can lead to changes in gene expression and function without altering the primary sequence of DNA. Methylation can be affected by dietary levels of methyl-donor components, such as folic acid. This may be an important mechanism for environmentally-induced changes in gene expression. While methylation of cytosine is usually associated with the silencing of harmful genes, it may also activate genes in some instances. In other words, methylation systems are not only essential for proper cell function but also have a major influence on how genes are used by cells. Moreover, methylation also plays a role in the histone acetylation process by which it hypoacetylates histone H3 and H4 within the transgene. This suggests that methylated genes repress local transcription with the help of the enzyme histone deacetylase. In addition to this activity, methylation also plays a central role in maintaining and establishing an inactive state of a gene by presenting the chromatin structure unavailable to the transcription process. Structurally CpG sites are uncommon in mammalian genomes but often found at higher density near mammalian gene promoters where they are collectively referred to as CpG islands. Any change in the methylation state of these CpG sites of DNA can have a major impact on gene activity/expression. Although DNA methylation is necessary for normal mammalian embryogenesis, both hypo-and hypermethylation of DNA are frequently observed in carcinogenesis and other pathological disorders. DNA hypermethylation silences the transcription of many tumor suppressor genes, resulting in immortalization of tumor cells, whereas DNA hypomethylation may cause oncogenic mutation by promoting chromosomal instability. In general,**

tumor cells show two concommitant opposing changes in gene methylation. Globally, the genome is hypomethylated but selected CpG islands become densely hypermethylated. A number of human diseases such as Down syndrome, neural tube defect, repeated miscarriages, atherosclerosis, carcinogenesis, etc. has been associated with aberrant gene methylation patterns in the vascular tissue and peripheral blood cells. At present there is enough evidence that global DNA hypomethylation coexists with hyperhomocysteinemia in advanced atherosclerosis. In our experience, the altered gene methylation processes in atherosclerosis are mainly secondary to a decrease in factors essential for the synthesis of SAM, such as folic acid and vitamin B12, or to homocysteine-induced blocking of SAM biosynthesis.

Protein methylation **is an important and reversible post-translation modification (PTM) of proteins which governs cellular dynamics and plasticity. The process serves an important role in modulating signal transduction pathways. As an example, not only does protein phosphatase 2A, a key regulator of many signal transduction cascades, undergo this modification, but the methylation process itself may be regulated by various cellular stimuli or states. Beside methylation, types of PTMs include phosphorylation and the backbone or side-chain methylation (n-methylation) in several types of amino acids such as lysine, arginine, histidine, alanine, asparagines, etc. Also, methylation occurs at cysteine residues known as S-methylation. We will only focus on modifications of lysine and arginine residues in this chapter. Lysine can be methylated once, twice or three times by histone lysine methyltransferase (HKMT). The methylation of lysine has been mostly studied in H3 and H4 histone proteins, which play essential roles in many biological processes such as X-chromosome inactivation and transcriptional silencing or activation. Moreover, HKMTs also modify a variety of non-histone proteins with diverse functions. For example, methylation of P53-dependent genes *in vivo* increases their stability and regulates the expression of p53-dependent genes.**

Protein methylation can also occur on the guanidino nitrogen atom of arginine. It can be methylated once or twice, with either both methyl groups on one terminal nitrogen or one on each nitrogen by

peptidylarginine methyltransferase (PRMTF). Although arginine methylation can also modify the core histones and form "histone codes" together with lysine methylation, the substrates of PRMTFs are much more diverse than HKMTs. Thus, protein arginine methylation may be involved in more functional processes, such as RNA processing, transcriptional regulation, signal transduction, and DNA repair. A typical example of protein methylation is methylation of myelin. Myelin is a multilamellar compacted membrane structure that surrounds and electrically insulates the axon, facilitating the conduction of nerve impulses. Myelin coating of nerves is important for proper neurotransmission. Myelin basic protein (MBP) constitutes 30% of the total myelin protein in the central nervous system (CNS), but it is a lesser constituent of the peripheral nervous system (PNS) myelin. Methylation of amino acids in MBP helps to stabilize it against degradation. The enzyme responsible for methylating MBP is protein arginine methyltransferase (PRMT) which is distinct from the histone-methylating enzyme. So far 5 PRMTs are known. It has been postulated that the introduction of a methyl group to the side chain of arginine induces the hyrophobicity of the protein, thus influencing the interaction of MBP and phospholipid molecules in the formation of the myelin sheath. Myelin acts as a sheath, and for faster transmission of electrical impulses this insulation is absolutely essential. When the methylation cycle is interrupted, as it is during vitamin B12 deficiency, the clinical consequence is the demyelination of nerve cells resulting in a neuropathy which may lead to ataxia, paralysis, and, if untreated, ultimately death. Not only can nerves not myelinate without proper methylation, but they also cannot re-myelinate after any environmental damage or viral infection. Regeneration of nerves is also disrupted due to the lack of methylation of the myelin sheath. Methylarginine and methylated proteins have been connected to a number of diseases. Monomethylarginine and asymmetric dimethylarginine are potent inhibitors of nitric oxide synthase (NOS), and increased levels of these species have been found in patients with various cardiovascular and noncardiovascular disorders. Several targets for arginine methylation have been implicated in autoimmune disease. Anti-myelin basic protein antibodies have been found in MS patients.

Phospholipids methylation **in the cell membrane, which involves the sequential n-methylation of phosphatidylethanolamine (PE), provides a mechanism for increasing membrane fluidity, as well as contributing approximately 10% to the overall synthesis of phosphatidylcholine (PC). In this process, SAM serves as a methyl donor, and the key enzyme is PE-methyltransferase (PEMTF). This is absolutely essential for various biological functions of the cell, especially for the timely signaling of the immune system and protecting the cell from nerve damage. The other function of phospholipids methylation is to sustain both synthesis of PC in the liver and production of choline moiety derived from PC catabolism during starvation. Lack of membrane fluidity has been found to be the cause of various diseases such as AD, MS, and amyotrophic lateral sclerosis (ALS). Histamine as well as dopamine are known to be involved in the methylation process of phospholipids. Histamine stimulates the enzymatic synthesis of PC from PE in the presence of SAM. Dopamine is also involved in a receptor-mediated system. Excessive methylation has been proposed to be involved in the pathogenesis of Parkinsons disease via a mechanism that involves phospholipids methylation. On the other hand, reduced methylation of red blood cell (RBC) membrane components in sickle cell anemia has been reported, which is 50% less than that of normal RBCs. This reduced methylation may be due to altered membrane conformation. Globally, however, phospholipids methylation is extremely rare in comparison to protein-carboxyl methylation, which represents the bulk of the membrane methylation.**

VITAMIN B12 AND ITS CLINICAL FUNCTION

The diagnosis of vitamin B12 deficiency has traditionally been based on low serum vitamin B12 levels, usually less than 200 pg per ml (150 pmol per L), along with clinical evidence of disease. However, studies indicate that older patients tend to present with neuropsychiatric symptoms in the absence of hematologic findings. Furthermore, measurements of metabolites such as methylmalonic acid and homocysteine have been shown to be more sensitive in the diagnosis of vitamin B12 deficiency than the measurement of serum B12 levels alone. In a large study of 406 patients with known vitamin B12 deficiency, 98.4% had elevated serum methylmalonic acid levels, as only one

patient out of 406 had normal levels of both metabolites, resulting in a sensitivity of 98% when methylmalonic acid and homocysteine levels are used for diagnosis. Twenty-eight per cent of the patients in this study had normal hematocrit levels and 17% had normal mean corpuscular volumes. This finding suggests that methylmalonic acid and homocysteine levels can be early markers for tissue vitamin B12 deficiency, even before hematologic manifestations occur. If increased homocysteine or methylmalonic acid levels and a normalization of these metabolites in response to replacement therapy are used as diagnostic criteria for vitamin B12 deficiency, approximately 50% of these patients have serum vitamin B12 levels above 200 pg per ml. This observation suggests that use of a low serum vitamin B12 level as the sole means of diagnosis may miss up to one half of patients with actual vitamin B12 deficiency. Vitamin B12 or folic acid deficiency can cause the homocysteine level to rise, so folic acid levels should also be checked in patients with isolated hyperhomocysteinemia. In addition, folic acid deficiency can cause falsely low levels of vitamin B12 in serum. Looking at the reactions that use vitamin B12, an elevated methylmalonic acid level is clearly more specific for vitamin B12 deficiency than an elevated homocysteine level. However, methylmalonic acid levels can be elevated in patients with renal disease; thus, elevated levels must be interpreted with caution.

NEUROPSYCHIATRIC SYMPTOMS: ROLE OF VITAMIN B12

Data obtained from the literature state that vitamin B12 is somehow bound to cognition and to the implementation of active strategies to coordinate and problem solve. Larner et al. published a review of the literature, and it emerges that the actual number of vitamin B12 defect-dementia patients is extremely small. However, elderly individuals with cobalamine deficiency may present with neuropsychiatric or metabolic deficiencies without frank macrocytic anemia. Psychiatric symptoms attributable to vitamin B12 deficiency have been described for decades. These symptoms seem to fall into several clinically separate categories: slow cerebration, confusion, memory changes, delirium with or without hallucinations and/or delusions, depression, acute psychotic states, and, more rarely, reversible manic and schizophrenia-like states.

A higher prevalence of low serum vitamin B12 levels has been found in subjects with AD, other dementias, and in people with different cognitive impairments, as compared with controls. In contrast, other cross-sectional studies have failed to find this association. The most recent study on the topic examined the relationship between vitamin B12 serum levels and cognitive and neuropsychiatric symptoms in dementia; in AD, the prevalence of low vitamin B12 serum levels is consistent with that found in community-dwelling elderly persons in general but is associated with greater overall cognitive impairment.

Furthermore, some intervention studies have shown the effectiveness of vitamin B12 supplementation in improving cognition in demented or cognitively impaired subjects. Chronic dementia responds poorly but should nevertheless be treated if there is a metabolic deficiency (as indicated by elevated homocysteine and/or methylmalonic acid levels). These data have been confirmed by other studies. However, a treatment effect was demonstrated among the patients presenting with cognitive impairment, improving when compared to matched patients on the verbal fluency test. On the contrary, other studies have failed to confirm this optimistic result when testing executive functions (tests of cognitive function by the Mini Mental State Examination).

Our conclusion could be that vitamin B12 treatment may improve frontal lobe and language function in patients with cognitive impairment, but rarely reverses dementia.

HOMEOSTATIS OF FOLIC ACID & METHYLATION PROCESS

Let us discuss the contribution of folic acid in the methylation pathway. As we have mentioned in the folate cycle, not only is folic acid involved in methylation, but many derivatives of folic acids also play crucial roles in the methylation pathway under different physiological conditions. These derivatives are THF, 10-formyl THF, 5-formyl THF, 5-methyl THF, etc.

The folates also play critical roles in amino acid metabolism and cell replication, prevent cardiovascular disease, and are essential for homocysteine metabolism. The chemical lability of all naturally-occurring

folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation. Half or even three quarters of initial folate activity may be lost during these processes. Although natural folates rapidly lose activity in foods over periods of days or weeks, the synthetic form of this vitamin, folic acid, is almost completely stable for months or even years. During absorption and transport across the gut, folic acid undergoes a four-electon reduction to THF. One-carbon units at the oxidation level of formic acid and formaldehyde can enzymatically combine with THF to form a half-dozen or so one-carbon congeners, some of which are essential for the *de novo* synthesis of purine thymidylate. Thus, DNA synthesis and cell replication are dependent on folate as a mediator of one-carbon metabolism. The complexity of intracellular forms is multiplied by the conversion of folates to folypoly-γ-glutamates, containing as many as six additional glutamate residues. In serum, however, the predominant form of folate is 5-methyl-THF. Folypoly-γ-glutamates, mostly in the form of N-methyl-THF-poly-γ-glutamate, are seen in RBCs. The polyglutamate tail is required for intracellular retention of folates. It also constitutes an important recognition site for folate-dependent enzymes; many such enzymes exhibit much lower Km values for folate polyglutamate substrates than for the corresponding monoglutamates. Most mammalian cells receive exogenous folate as the monoglutamate of 5-methyl-THF, the form of folate that is transported in the blood stream. There is an active transport process for methyl folate across the blood-brain barrier such that folic acid levels in cerebrospinal fluid (CSF) in humans are approximately three times greater than in the serum. The bioavailability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete, thereby reducing the bioavailability of natural folate by as much as 25-50%. In contrast, synthetic folate appears to be highly bioavailable, approximately 85% or greater. The biomarker plasma homocysteine has been identified as a very sensitive indicator of folate status. RBC folate values are thought to represent tissue concentration, whereas serum folate values reflect recent dietary intake. At Vitamin Diagnostics we have experienced increased erythrocyte folate and low plasma folate in women who are homozygous for thermolabile methyl THF-reductase (MTHFR) (*n* = 128). Neutral tube defect risk has been observed to be associated with the concentration of RBC folate in a continuous dose

response relationship (n = 17). Our laboratory has developed a data base which shows that both genetic and acquired factors are responsible for intracellular folate deficiency (n = 168).

THE CLINICAL INDEPENDENT ROLE OF FOLIC ACID

One of the most recent reviews on folic acid clearly states its importance in neuropsychiatric disorders. Dietary folate is required for the normal development of the nervous system, playing important roles in regulating neurogenesis and programmed cell death. Recent epidemiological and experimental studies have linked folate deficiency and resultant increased homocysteine levels with several neurodegenerative conditions, including stroke, AD, and Parkinsons disease. Folate deficiency sensitizes mice to dopaminergic neurodegeneration and motor dysfunction caused by neurotoxin methyl phenyl tetrahydropyridine (MPTP). Additional experiments indicate that this effect of folate deficiency may be mediated by homocysteine. These findings suggest that folate deficiency and hyperhomocysteinemia might be risk factors for Parkinsons disease.

Depression is more common in patients with folate deficiency, and subacute combined degeneration with peripheral neuropathy is more frequent in those with vitamin B12 deficiency. Several clinical research studies have suggested that nearly one-third of their severely depressed inpatients have folate deficiency, as indicated by an RBC folate concentration below 150ug/l. Experience from the early part of the 20th century suggests that of the one-third of patients with anemia who have no psychiatric disorder, most would go on to develop such complications if left untreated.

To examine the effects of folic acid supplementation with or without vitamin B12 on elderly healthy and demented people in preventing cognitive impairment or retarding its progress, a review has been published. All double-blind placebo-controlled randomized trials, in which supplements of folic acid with or without vitamin B12 were compared with placebo for elderly healthy people or people with any type of dementia or cognitive impairment, were reviewed. Analysis of the included trials found no benefit from folic acid

with or without vitamin B12 in comparison with placebo on any measures of cognition or mood for healthy or cognitively impaired or demented people. Folic acid plus vitamin B12 was effective in reducing serum homocysteine concentrations. Folic acid was well tolerated, and no adverse effects were reported. The available studies are limited in size and scope but provide no evidence that folic acid, with or without vitamin B12, has a beneficial effect on cognitive function or mood of health or cognitively impaired older people.

Folate deficiency has been described in epileptic patients: treatment of folate deficient epileptic patients with folic acid daily for one to three years resulted in improved drive, initiative, alertness, concentration, mood, and sociability in most. The highest incidence of folate deficiency as measured by serum and RBC folate concentrations is in elderly populations. A close association with dementia and the apparent depression, apathy, withdrawal, and lack of motivation has been noted. One reason for a high incidence of folate deficiency in elderly people is that folate concentrations in serum and CSF fall and plasma homocysteine rises with age, perhaps contributing to the ageing process. Recent epidemiological studies have shown an association between low serum folate levels and risk of vascular disease, including stroke and various types of vascular cognitive impairment. This was supported by data from the Canadian Study of Health and Ageing. After adjusting for covariates, the risk estimate for an adverse cerebrovascular event associated with the lowest folate quartile compared with the highest quartile was odds ratio (OR) 2.42 (95% confidence interval [CI]; 1.04-5.61). Results from stratified analyses also showed that relatively low serum folate was associated with a significantly higher risk of an adverse cerebrovascular event among female subjects (OR 4.02, 95%CI; 1.37-11.81). There is a concern that low folate status may represent a proxy for low socio-economic status or some related status. In general medical patients admitted acutely to hospital, 71% of those with severe folate deficiency had organic brain syndrome, compared with 31% of a control group. In a prospective community-based study of 370 healthy elderly Swedish subjects, folate or B12 deficiency doubled the risk of subsequently developing AD. In a survey of nutritional status and cognitive functioning in 260 healthy elderly subjects aged 60 to 94 years in the community, there was a significant relation between impaired abstract thinking ability and memory and lower folate levels. Recently, the much larger and longer Framingham community-based study confirmed that a

raised plasma homocysteine (bound to low folate level) concentration doubled the risk of developing AD and non-AD dementia. On the basis of neuroimaging, another study concluded that chronic folate deficiency could induce cerebral atrophy. In a relatively small sample, serum folate had a strong negative association with the severity of atrophy of the *neocortex*. In the Kingsholmen ageing and dementia project in Stockholm, impaired episodic memory was related to low serum concentrations of folate. In other case-control studies in patients with AD, cognitive decline was significantly associated with raised plasma homocysteine and lowered serum folate (and vitamin B12) concentrations. In open studies reviewing experiences with folic acid, authors emphasized the effects of the vitamin on mood and cognitive function. An ad-hoc double blind, controlled versus placebo pilot study to evaluate the efficacy of folic acid in aged patients with abnormal cognitive decline and low serum folate demonstrated a significant improvement in both memory and attention deficiency in patients treated, when compared with a placebo group. Above all, the intensity of memory improvement positively correlated with the initial severity of folate deficiency. Our conclusions can be that co-relational studies show an association between low folate and psychiatric disorders, even if this association does not mean causality. Only prospective studies can show causality. Co-relational studies show an association between low folate and psychiatric disorder, but the available prospective studies are limited in size and scope and provide no evidence that folic acid, with or without vitamin B12, has a beneficial effect on cognitive function or mood of healthy or cognitively impaired older people.

Folinic acid, also known as 5-formyl THF, is an active and reduced form of a group of vitamins known as folates. In contrast to folic acid, a synthetic form of folate, folinic acid, is one of the forms of folate found naturally in foods. In the body folinic acid may be converted into any of the other active forms of folate, and it is an immediate precursor to 5, 10 methylene THF. Supplying the body with folinic acid bypasses many of the required metabolic steps, and it is rapidly converted to 5-methyl-THF. It is transported into the brain readily. Folinic acid has been observed to promote higher levels of glutathione and dopamine in autistic children. Some children with developmental delay, psychomotor regression, seizures, mental retardation, and autistic features had improved motor skills after treatment with folinic acid.

Since folinic acid does not require the actions of dihydrofolate reductase for its conversion, it is unaffected by inhibition of this enzyme by drugs such as methotrexate, trimethoprim, and pyrimethanine. In our laboratory we have observed the benefit of folinic acid when used as an adjunct to selective serotonin reuptake inhibitor (SSRI) treatment in SSRI-treatment refractory depressed individuals with normal or high folic acid levels. It is more stable than folic acid, has a longer half-life in the body, bypasses first-pass metabolism by the liver, and readily crosses the blood-brain barrier.

VITAMIN B12 AND FOLIC ACID: A CLINICAL RELATIONSHIP

Potential benefits of food folic acid fortification for an elderly population might be relevant, but there is the risk of precipitating clinical manifestations related to vitamin B12. Cyanocobalamin deficiencies should be excluded before folate supplementation is commenced; if in doubt, it may be safer to supplement folate and vitamin B12 together. These ideas have been rejected by the study conducted by Dickinson et al. Actually, however, general guidelines recommend that only specific and separate dosage of folate and B12 should be done and, whenever possible, a correct implementation of the depletion status should be effected in order to avoid, if not certain, at least possible cognitive impairment. Many different studies have tried to describe a possible consequence of the combined defect of vitamin B12 and folate. Riggs et al. investigated the relationship between plasma concentrations of folate, vitamin B12, vitamin B6, and homocysteine and scores on a battery of cognitive tests in 70 men, aged 54-81, participating in the Normative Aging Study. Lower folate and vitamin B12 concentrations were associated with poorer spatial copying skills. In addition, plasma homocysteine concentration, which is inversely correlated with plasma folate and vitamin B12 concentration, was a stronger positive predictor of spatial copying performance than either folate or vitamin B12 concentrations. Among markers of cobalamin/folate status, plasma homocysteine shows the best association with neuropsychiatric dysfunction.

A recent study examined the relationship between low levels of serum vitamin B12 and folic acid and cognitive functioning in very old age. In general, the effects of folic acid exceeded those of B12.

Vitamin Diagnostics offers testing of the entire methylation pathway. Various factors of the pathway can be measured in whole blood, RBC, plasma, or urine. Details are provided in Table 1.

SIMPLE TEST FOR HYPO- AND HYPERMETHYLATION

HYPOMETHYLATION

Since clearance of histamine takes place by methylation, elevated histamine level in whole blood indicates hypomethylation. Moreover, depleted levels of serotonin, dopamine, and norepinephrine in platelets are also indications of hypomethylation. Hypomethylated individuals respond to methionine, SAM, magnesium (Mg), calcium (Ca), zinc (Zn), vitamin B6, inositol etc. positively but react very poorly to folic acid, vitamin B12, choline, dimethylethylamine (DMEA), etc. Often plasma folate is high in those individuals. Positive response to the supplementation is usually slow; it takes almost 3 to 6 months to observe any progress. Normalization of histamine level takes even longer. Sometimes antihistamine (Dilantin, Benedryl, etc.) can be helpful as they deplete the folates in serum.

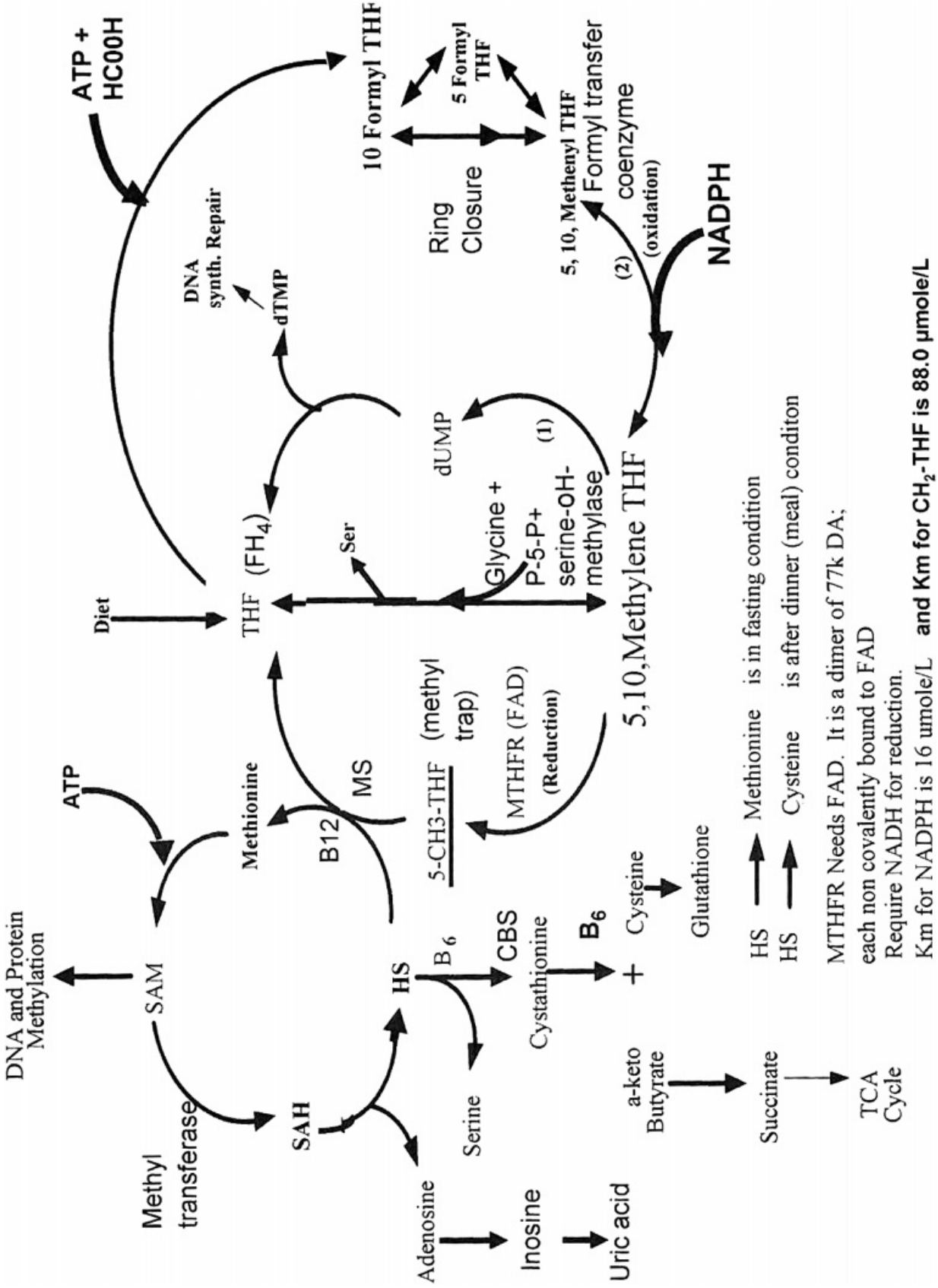
HYPERMETHYLATION

Usually hypermethylated individuals cannot handle SSRI drugs very well. They are anxiety-prone and exhibit hyperactivity, paranoid schizophrenia, obsessive compulsion disorder (OCD), bipolar disorder, etc. Many of these individuals have moderately low histamine in whole blood, high RBC copper, low or high platelet dopamine, norepinephrine and low folic acid and vitamin B12. Supplementation of folic acid as well as vitamins B12, B5, and B3 can eliminate most of the problems of hypermethylated individuals. These individuals are less tolerant to methionine and SAM and have high chemical sensitivities such as to perfumes.

ROLE OF B VITAMINS IN BIOLOGICAL METHYLATION

URINE	**N-methyl nicotinamide (NMN)**	**Thio-sulphate**
	Methylmalonic acid (MNA)	**Thio-cyanate**
	Formiminoglutamic acid (FIGLU)	**Kryptopyrrole**
	Total sulphate	**Sulphite**
	Free sulphate	
WHOLE BLOOD	**Histamine**	**Riboflavin**
	Folinic acid	**Niacin**
RBC	**Glutamate oxalo-transminase (GOT)**	
	Glutathione peroxidase (GP)	
	Glutathione reductase (GR)	
	Super-oxide dismutase (SOD)	
	Catalase	
PLASMA	**THF**	**FAD**
	5-methyl THF	**FNM**
	5-formyl THF	**Total sulphate**
	10-formyl THF	**Free sulphate**
	Homocysteine	**Adenosine**
	Cysteine	**Inosine**
	Taurine	**Uridine**
	Cystathionine	**NADPH**
	Glycine	**TMG**
	Methionine	**DMG**
	Serine	
PLASMA & RBC	**Glutathione**	**SAM**
	Oxidized glutathione	**SAH**
	Folic acid	**Vitamin B12**
	Pyridoxal-5-phosphate	**Vitamin B6**

FOLATE METABOLISM: ALTERNATIVE PATHWAYS



ABBREVIATIONS

AD	Alzheimers disease
ALS	amyotrophic lateral sclerosis
ATP	adenosine triphosphate
CFS	chronic fatigue syndrome
CI	confidence interval
CNS	central nervous system
CpG	cytosine-phospho-guanine
CSF	cerebral spinal fluid
DMEA	dimethylethylamine
DNA	desoxyribose nucleic acid
FAD	flavin adenine dinucleotide
FIGLU	formiminoglutamic acid
FNM	flavin mononucleotide
GOT	glutamate oxalo-transminase
GP	glutathione peroxidase
GR	glutathione reductase
HKMT	histone lysine methyltransferase
MBP	myelin basic protein
MNA	methylmalonic acid
MPTP	methyl phenyl tetrahydropyridine
MS	multiple sclerosis
MTHFR	methyl tetrahydrofolate reductase
NADPH	nicotinamide adenine dinucleotide phosphate
NMN	N-methyl nicotinamide
NOS	nitric oxide synthase
OCD	obsessive compulsive disorder
OR	odds ratio
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEMTF	phosphatidylethanolamine methyltransferase
PNS	peripheral nervous system
PRMT	protein arginine methyltransferase
PRMTF	peptidylarginine methyltransferase
PTM	post-translation modification

RNA	ribonucleic acid
RBC	red blood cell
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SOD	super-oxide dismutase
SSRI	selective serotonin reuptake inhibitor
THF	tetrahydrofolate
TMG	trimethylglycine