Vitamin B12, folic acid, and the nervous system

Edward Reynolds

Lancet Neurol 2006; 5: 949–60

Institute of Epileptology, King's College, Denmark Hill Campus, Cutcombe Road, London, SE5 6PJ, UK
(E Reynolds MD)
reynolds@buckles.u-net.com

There are many reasons for reviewing the neurology of vitamin-B12 and folic-acid deficiencies together, including the intimate relation between the metabolism of the two vitamins, their morphologically indistinguishable megaloblastic anaemias, and their overlapping neuropsychiatric syndromes and neuropathology, including their related inborn errors of metabolism. Folates and vitamin B12 have fundamental roles in CNS function at all ages, especially the methionine-synthase mediated conversion of homocysteine to methionine, which is essential for nucleotide synthesis and genomic and non-genomic methylation. Folic acid and vitamin B12 may have roles in the prevention of disorders of CNS development, mood disorders, and dementias, including Alzheimer's disease and vascular dementia in elderly people.

Historical background

In the late 19th century, Leichtenstern[1] and Lichtheim[2] wrote the earliest accounts of the neurological associations of megaloblastic anaemia; they described typical lesions in the posterior and lateral columns of the spinal cord of which Russell and colleagues[3] soon coined the term "subacute combined degeneration of the cord" (SCD). In the first third of the 20th century, before the availability of liver therapy, the neuropsychiatry and neuropathology of megaloblastic anaemia was thoroughly documented by many authors.[4–6] They recognised that the nervous-system complications could be very varied and included peripheral-nerve and psychiatric disorders as well as the classic SCD and that there was commonly substantial dissociation between the haematological and neuropsychiatric symptoms, either of which could precede the other. In the absence of treatment, nearly all patients with megaloblastic anaemia eventually developed some nervous-system involvement before death. Before the discovery of vitamin B12 or folic acid, the separation of megaloblastic anaemias had not begun and most were regarded as "pernicious anaemia", the diagnosis of which relied on the demonstration of achlorhydria. Kinnier Wilson[6] noted that acid was found in the stomachs of up to 25% of patients with neurological symptoms.

Folic acid was synthesised in 1945, 3 years before the isolation of vitamin B12, and was immediately used in the treatment of pernicious anaemia as the possibly deficient dietary factor.[7] These trials were encouraged by some initially promising improvement in the megaloblastic anaemia. However, over the next 5 years, there were several disturbing reports of aggravation or precipitation of neurological complications of pernicious anaemia by folic acid.[7,8] The vitamin was also commonly associated with later deterioration in the anaemia after the initial improvement;[8] and in some reports there was some temporary improvement in neurological symptoms before the more florid deterioration.[9,10]

These developments in 1945–50 had a profound effect on subsequent concepts. The introduction of vitamin-B12 treatment with its beneficial effects on both blood and nervous system coincided with the height of concern about folic acid. In the third quarter of the 20th century, the neuropsychiatric symptoms of megaloblastic anaemia were erroneously assumed to be caused solely by deficiency of vitamin B12 and not of folic acid.[10]

In the final third of the 20th century, these deeply held misconceptions were slowly eroded with the use of vitamin B12 and folate assays and other techniques to assess patients with neuropsychiatric disorders with and without megaloblastic anaemia[7,11] and were reinforced by the introduction of homocysteine assays in the 1990s.[12] Such is the interest now in the role of folates, vitamin B12, and homocysteine in brain metabolism and function at all ages, especially in relation to nervous-system development, repair, mood, ageing, cognitive function, and dementia,[12–21] that some have questioned whether folic acid ever had any harmful effects on the nervous system:[22] another exaggerated swing of the pendulum.[23]

Vitamin B12 and folate metabolism

Reviews of the structure, binding, absorption, transport, metabolism, function, and interaction of vitamin B12 and folates, and the polymorphisms of folate-related enzymes can be found elsewhere.[12,13,17,21,24–27] A key interaction is that between vitamin B12 and folate in the synthesis of methionine from homocysteine by methionine synthase, in which both 5-methyl-tetrahydrofolate and methyl-vitamin-B12 are cofactors (figure 1), a reaction that can be inhibited by nitrous oxide. The folate cycle, which synthesises methyl groups, is essential for many genomic and non-genomic methylation reactions, via S-adenosylmethionine and, indirectly, for the synthesis of purines and thymidine and, therefore, of nucleotides, DNA, and RNA.

Neurology of vitamin-B12 deficiency

Kinnier Wilson wrote the best review of the older detailed description of the overlapping syndromes of peripheral neuropathy, SCD, autonomic dysfunction, optic atrophy, mood and behaviour changes, psychosis, memory impairment, and cognitive decline.[6] Recent studies have concentrated on early diagnosis and treatment based on the modern techniques for the separation of the megaloblastic anaemias.

Patients may present to haematologists and physicians with megaloblastic anaemia or to neurologists and psychiatrists with predominantly nervous-system

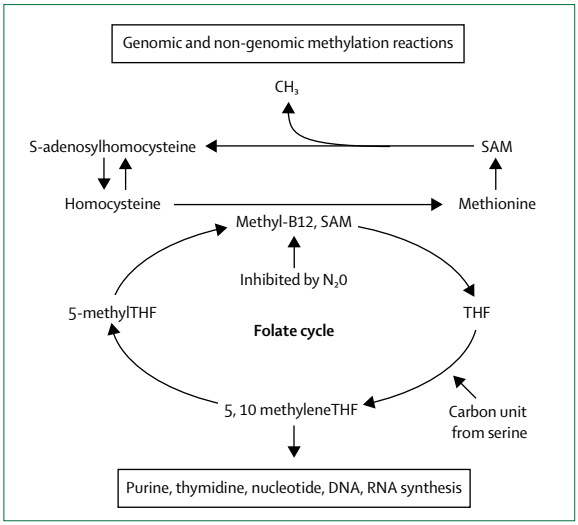

Figure 1: **Associations between the folate cycle, vitamn B12, methylation, and nucleotide synthesis**
SAM=S-adenosylmethionine. THF=tetrahydrofolate.

symptoms.[6,7,28–32] In a prospective study, my colleagues and I described 50 patients with vitamin-B12-deficient megaloblastic anaemia admitted to medical wards (table 1).[29] The commonest finding was peripheral neuropathy; SCD was uncommon. About a quarter of patients had either cognitive impairment or an affective disorder but a third had no detectable nervous-system involvement. In two-thirds the cause of vitamin-B12 deficiency was pernicious anaemia.

Healton and co-workers[31] retrospectively identified 369 patients with low serum vitamin-B12 concentrations at two New York hospitals over 17 years. 50% had neurological symptoms or signs or both. Some of these patients had disorders that were thought to be unrelated to the deficiency (eg, Alzheimer's disease, stroke), which suggests an overall incidence of 40% with related nervous system involvement. Within this group, nearly a fifth had no evidence of either anaemia or macrocytosis;[30] 25% had peripheral neuropathy and 11% SCD, but an additional 40% had mild sensory or autonomic symptoms or signs of possible peripheral-nerve or cord origin. SCD has been described with disorders of vitamin-B12 binders, sometimes with normal or high serum concentrations of vitamin B12.[33,34]

	Vitamin-B12 deficiency (N=50)	Folic acid deficiency (N=34)
Neuropsychiatric findings (%)		
Normal	32	35
Cognitive change	26	27
Affective disorder	20	56
Subacute combined degeneration	16	0
Peripheral neuropathy	40	18
Optic atrophy	2	0
Cause of anaemia (number of cases)		
Pernicious anaemia	32	..
Coeliac disease	..	16
Dietary	8	8
Gastrointestinal	7	..
Malabsorption	..	8
Unexplained	3	2

Table 1: **Neuropsychiatric findings in patients presenting to physicians with megaloblastic anaemia[29]**

Patients with cord or peripheral-nerve syndromes invariably present with symmetrical distal sensory symptoms and signs usually beginning in the feet, spreading to the hands, and accompanied by varying degrees of ataxia..[6,7,29,31,32] The commonest neurological signs are diminished vibration sense and proprioception in the legs and can include impaired distal cutaneous sensation. Limb reflexes may be exaggerated, diminished, or absent depending on the relative involvement of the cord. Lateral column signs of a spastic paraparesis may occur, accompanied by autonomic bladder, bowel, or sexual symptoms. The differentiation of the sensory symptoms and signs of peripheral-nerve involvement from those related to pathology in the posterior columns of the cord can be difficult. Peripheral neuropathy may be the only neurological syndrome, but if cord signs are present there is invariably some electrical evidence of a predominantly axonal neuropathy.[29,35]

Mental symptoms in patients presenting to haematologists and physicians are commonly but not always accompanied by peripheral-nerve or cord signs.[29] In cases presenting to neurologists or psychiatrists with psychiatric syndromes this association seems to be less common.[30,36] Any combination of neurological and psychiatric syndrome is possible in addition to clinically isolated syndromes.[6,29,30,31,36]

Recent studies confirm the frequent dissociation of nervous system and haematological signs noted in the early research.[6,8,28–31] Healton and co-workers[31] found a significant inverse correlation between the degree of anaemia and the severity of neurological involvement and this was independent of the duration of symptoms. Patients without anaemia or macrocytosis tend to have the most severe nervous-system involvement. The reasons for this finding are unclear—they are not related to serum concentrations of vitamin B12,[31] but might be related to higher serum folate concentrations.[37,38]

Diagnosis

Neurological disorders due to vitamin-B12 deficiency typically occur in both sexes between age 40 and 90 years with a peak at age 60–70 years.[6,31,32] A few patients can present before age 40 years. These disorders are usually symmetrical and progressive, but the evolution can be variable and uneven in rate.

The diagnosis is usually clear in the presence of typical neuropsychiatric syndromes associated with megalo-

blastic anaemia or macrocytosis and a low serum concentration of vitamin B12. Difficulties are encountered in the absence of anaemia or macrocytosis, if the serum vitamin-B12 concentration is borderline, or in the presence of purely psychiatric syndromes.[30,31,36,38] If the serum vitamin-B12 concentration is equivocal, a raised plasma homocysteine or methylmalonic acid concentration confirms the presence of a significant deficiency.[12,26,27]

In patients presenting with peripheral-nerve or cord syndromes the differential diagnosis may rarely include diabetic or alcoholic neuropathies, paraneoplastic syndromes, cervical spondylosis, neurosyphilis, HIV-related neurological disorders, and nitrous-oxide abuse.[6,31,32]

Nitrous-oxide abuse

A myeloneuropathy indistinguishable from SCD occurs after chronic recreational or occupational exposure to nitrous oxide, usually in dental or hospital personnel. Layzer[39] described 15 patients in 1978, and Savage and Lindenbaum[32] reviewed a further 15 by 1995. As in SCD, the variable clinical picture can include neuropathy and mental changes.

Single or repeated exposure to nitrous oxide during surgical procedures has also been suspected to precipitate neurological complications in several patients with underlying vitamin-B12 deficiency.[40]

Nitrous oxide rapidly and irreversibly inactivates methionine synthase, which is vitamin-B12 dependent,[41] and megaloblastic changes in the bone marrow can be detected after 8–24 h of continuous exposure during surgery.[42,43] Intermittent abuse requires several months to cause neuropsychiatric disturbances. Only a few patients have any haematological abnormality and most have normal serum vitamin-B12 concentrations.[32,39]

Multiple sclerosis and vitamin-B12 deficiency

Rarely a first episode of multiple sclerosis with prominent symmetrical posterior column sensory symptoms can be confused with vitamin-B12 deficiency. A few patients with undoubted multiple sclerosis also have vitamin-B12-deficient megaloblastic anaemia or macrocytosis.[44] The latter may be detected at any stage in the clinical progression of multiple sclerosis, including at onset. Patients with multiple sclerosis without anaemia but very mild degrees of macrocytosis or borderline low serum vitamin-B12 concentrations are more common,[45–47] some of whom have high plasma homocysteine.[48,49]

The age of these patients is typical of multiple sclerosis (ie, young adults), which is unusual for vitamin-B12 deficiency. The cause of the deficiency is usually unclear. Few patients have pernicious anaemia. As is typical in multiple sclerosis but surprising in vitamin-B12 deficiency these patients do not have peripheral neuropathy.[44,46] The nature of the relation between multiple sclerosis and vitamin-B12 deficiency is also unclear.[50] Is the association the result of overlapping autoimmune disorders? Does the deficiency reflect an increased demand for vitamin B12 for myelin repair? Or is there a more direct causal relation?

Neurology of folic-acid deficiency

The first reports of megaloblastic anaemia due to folate deficiency associated with spinal-cord, peripheral-nerve, and mental disorders appeared in the mid-1960s.[51,52] These were soon followed by accounts of folate-responsive neuropsychiatric disorders in patients deficient in folate with or without anaemia or macrocytosis.[53–56]

The reported neuropsychiatric effects of folate deficiency are remarkably similar to those described for vitamin-B12 deficiency. Shorvon and colleagues[29] compared the neuropsychiatric complications of megaloblastic anaemia presenting with either folate or vitamin-B12 deficiency to haematologists or physicians. The incidence of nervous system involvement was similar occurring in about two-thirds of each series (table 1). About a quarter of each group had cognitive decline. However, peripheral neuropathy was twice as common in vitamin-B12 deficiency than in folate deficiency. By contrast, depression was more than twice as common in folate deficiency than in vitamin B12 deficiency. SCD was uncommon in vitamin B12 deficiency, but it was not seen at all in 34 patients with folate deficiency. Pincus[57] reviewed 25 patients with SCD caused by folate deficiency and concluded five were convincing and 20 probable. Electrical studies of peripheral-nerve function in folate deficiency confirm that the predominantly sensory axonal neuropathy is similar to that described for vitamin-B12 deficiency.[58,59]

Neuropsychiatric disorders without anaemia or macrocytosis

About a third of patients with vitamin-B12 or folate deficiency severe enough to produce megaloblastic anaemia do not have neuropsychiatric disorders when first seen.[29] Conversely, about a fifth of patients presenting with nervous-system disorders caused by vitamin-B12 deficiency and about a quarter of patients with folate-responsive neuropsychiatric syndromes do not have anaemia or macrocytosis.[28,30,60]

Up to a third of psychiatric and especially psychogeriatric admissions have low serum or red-cell folate concentrations, mostly without anaemia or macrocytosis.[15,20,60,61] The corresponding incidence of low serum vitamin-B12 concentrations is usually up to 5%[12,28,36] but between 10% and 20% in elderly patients.[62] Folate deficiency has been consistently associated with evidence of depression[15,20,28,29,53,56,60,63,64] and cognitive decline,[15,28,53–56,60,61,65,66] whereas low vitamin-B12 concentrations have been mainly associated with cognitive impairment.[29,31,36,67,68]

The cause of folate deficiency has been variously ascribed to poor diet, chronic illness, drugs (eg,

barbiturates, alcohol), malabsorption, increased demand, or unknown.[15,60,67] One reason for the apparently high incidence of folate deficiency in elderly people is that folate concentrations in serum and CSF fall and plasma homocysteine rises with age,[69–71] perhaps contributing to the ageing process.[15,72]

Neuropsychological studies have found general and specific impairments of intellectual function—including attention, episodic and visuospatial memory, and abstract reasoning—that were attributed to folate deficiency.[15,73] In the Kungsholmen (Stockholm) community ageing and dementia project, the pattern of cognitive dysfunction resulting from folate deficiency is said to resemble that in normal ageing—ie, impairment in tasks that involve little structure, are unfamiliar and attention demanding, and involve complex processing of information.[72,74]

There has been considerable debate about the significance of folate deficiency without anaemia or macrocytosis in the presence of psychiatric illness. For those who have continued to doubt the existence of neuropsychiatric symptoms of folate deficiency it has been all too easy to assume that the deficiency is secondary to the mental illness for dietary reasons, especially as apathy, withdrawal, and anorexia are common symptoms in depression and dementia. However, nutritional studies have not confirmed this oversimplistic interpretation,[60,67] and others have pointed out that even when folate deficiency is secondary to mental illness it is an aggravating factor that leads to a vicious circle of decline.[28,63,66,67] Furthermore, impaired motivation and social withdrawal due to deficiency are some of the most folate-responsive symptoms.[23,53,55,56] In the past 15 years, evidence of a more direct causal link between folate metabolism and some depressions and dementias has been reinforced by studies of homocysteine metabolism.

Folate, homocysteine, depression, dementia, and ageing

Hyperhomocysteinaemia has long been identified as a risk factor for vascular disease and the lowering of homocysteine concentrations by treatment with folic acid, or possibly vitamin B12 and vitamin B6, might reduce the risk of both cardiovascular and cerebrovascular disease.[75,76] Long-term prophylactic studies with folic acid are therefore underway.[77]

After the earlier reports of an association between folate deficiency, depression, and dementia several mainly community or cross-sectional studies have suggested that hyperhomocysteinaemia is a risk factor for depression and especially dementia, including Alzheimer's disease and vascular dementia.[16,19–21,71,75,78–89] Among homocysteine studies reviewed by Morris,[16] only two did not show a link with dementia. The large prospective Framingham community study confirmed that a high plasma homocysteine concentration doubled the risk of developing either Alzheimer's disease or other dementias.[71] Likewise a Swedish community study suggested low concentrations of serum folate or vitamin B12 doubled the risk of Alzheimer's disease.[90] A prospective Italian population-based study confirmed that high plasma homocysteine and low serum folate concentrations were independent predictors of dementia and Alzheimer's disease, whereas the association with vitamin B12 was not significant.[19] In a retrospective study of the survivors of the Scottish Mental Surveys of 1932, which included childhood IQ, plasma homocysteine concentration accounted for 7–8% of the variance in cognitive performance.[91]

There is substantial overlap between Alzheimer's disease and vascular dementia, and the separation of these two diseases from each other and from other dementias is not easy in life even with the most sophisticated techniques.[92] Therefore neuropathological studies are of particular importance. In a case–control study of 164 patients with Alzheimer's disease, 76 of which were confirmed neuropathologically, Alzheimer's disease was significantly associated with high plasma homocysteine and low serum folate and vitamin B12.[93] Higher plasma homocysteine was associated with a more rapid atrophy of the medial temporal lobes over 3 years. In 12 patients, high homocysteine was also significantly associated with confirmed vascular dementia.[93] In people without dementia, plasma homocysteine was inversely related to MRI measures of hippocampal and cortical volume.[94] However, poor cognitive ability associated with high homocysteine concentrations was independent of structural brain changes on MRI.[95] In a prospective study of 30 nuns from the same environmental and nutritional background who died at age 78–101 years, half had neuropathological confirmation of Alzheimer's disease. Of 18 nutritional factors examined, only serum folate was correlated with atrophy of the neocortex, especially in the 15 nuns with Alzheimer's disease but also in those with minimal atherosclerosis and no infarcts.[96]

Raised plasma homocysteine concentrations have been observed in up to 30% of patients with severe depression.[20,86,97–99] Bottiglieri and colleagues[97] have described a biological subgroup of depressed patients with high plasma homocysteine concentrations, folate deficiency, and impaired monoamine neurotransmitter metabolism. A meta-analysis has confirmed the association of the thermolabile variant of 5-methyltetrahydrofolate reductase with depression.[64] There is also interest in the association of low serum and high plasma homocysteine concentrations with the "negative" symptoms of schizophrenia.[100,101]

Folic acid and epilepsy

Folate deficiency induced by phenytoin or barbiturates is commonly associated with mental changes, especially depression, apathy, psychomotor retardation, and cognitive decline.[28,53] Folate deficiency in patients with epilepsy is now much less common with the availability of newer antiepileptic drugs.

Treatment of 26 patients with epilepsy who had folate deficiency with 5 mg folic acid daily for 1–3 years resulted in improved drive, initiative, alertness, concentration, mood, and sociability in most and an increase in seizure frequency in some.[53] Controlled trials of folate therapy for up to 3 months produced conflicting results, but there is abundant experimental evidence that folate derivatives have excitatory properties, especially when the efficient blood–brain barrier mechanism for the vitamin is circumvented.[28,102,103] In laboratory animals intravenous sodium folate will only induce seizures in very large doses; but if the blood–brain barrier is damaged locally by trauma or a heat lesion, the dose required for an epileptogenic effect is much lower.[103,104] If the blood–brain barrier is circumvented by intraventricular or intracortical administration all folate derivatives are highly convulsant.[103,104] Furthermore, the vitamin enhances the kindling model of epilepsy and can even be used to kindle seizures directly.[105] How folates lead to excitation is unknown, but they may do so by blocking or reversing GABA-mediated inhibition.[106] Epileptic phenomena produced by folates resemble those induced by disinhibitory compounds such as bicuculline, penicillin, or picrotoxin.[23]

The risk of aggravating epilepsy seizures in patients is small because the blood–brain barrier limits entry of folic acid[23,107] but probably increases with larger doses over longer times.[23,28] The vitamin can also lower blood phenytoin concentrations.[108]

Treatment issues

Vitamin-B12 deficiency

Two-thirds of patients with vitamin-B12 deficiency and neurological complications are still functionally independent at the time of diagnosis, and only about 10% are severely disabled.[31,32] The severity of the neurological disorder correlates with the duration of symptoms but also inversely with the haemoglobin concentration.[31]

The treatment of neuropsychiatric disorders is empirical, based mainly on haematological experience rather than neurological study.[7,32] Although remission of megaloblastosis can be induced within weeks by small doses of parenteral vitamin B12, the haematological principle is to saturate body stores of the vitamin so that if the injections are abandoned, as occurs in 10–20% of patients, it will take longer for relapse to occur.[7,32] Therefore, weekly injections of 1000 µg of hydroxycobalamin or cyanocobalamin have been recommended for the first 3 months followed by a maintenance injection every 3 months. The same principle has been applied in the absence of anaemia or macrocytosis. Whether this is the most appropriate regimen for neuropsychiatric manifestations of vitamin-B12 deficiency is unknown. Claims that the nervous system requires larger doses for longer periods than the blood have not been adequately studied or confirmed.[32,38]

Published experience suggests that sensory symptoms begin to improve more quickly than motor symptoms, within the first 6 weeks.[7,32] No improvement within 3 months may reinforce doubts about the significance of a low serum vitamin-B12 concentration in relation to the neurological disorder. The duration and degree of neurological recovery is correlated with the duration of symptoms and the severity of disability before treatment and therefore early diagnosis and treatment is imperative.[31,32] In a large series, just less than 50% of patients made a complete recovery.[31] About 90% of patients can expect at least 50% improvement in disability and up to 10% may be left with moderate to severe disability.[31] Increasing age may raise the risk of residual disability.

Folic acid in vitamin-B12 deficiency

The 1945–50 experience indicates that treatment of patients with vitamin-B12-deficiency with folic acid is inappropriate because it may precipitate or aggravate neurological complications or allow them to progress by masking the anaemia (figure 2).[7,8,10]

Larger doses of folic acid for longer periods are more likely to lead to neurological progression. Savage and Lindenbaum[32] reviewed 38 cases of vitamin-B12 deficiency treated with less than 1 mg folic acid, which had little haematological effect. Only six patients had neurological deterioration, but remarkably they had been treated for much longer than the remaining cases, illustrating the importance of duration of treatment in relation to the nervous system.[32]

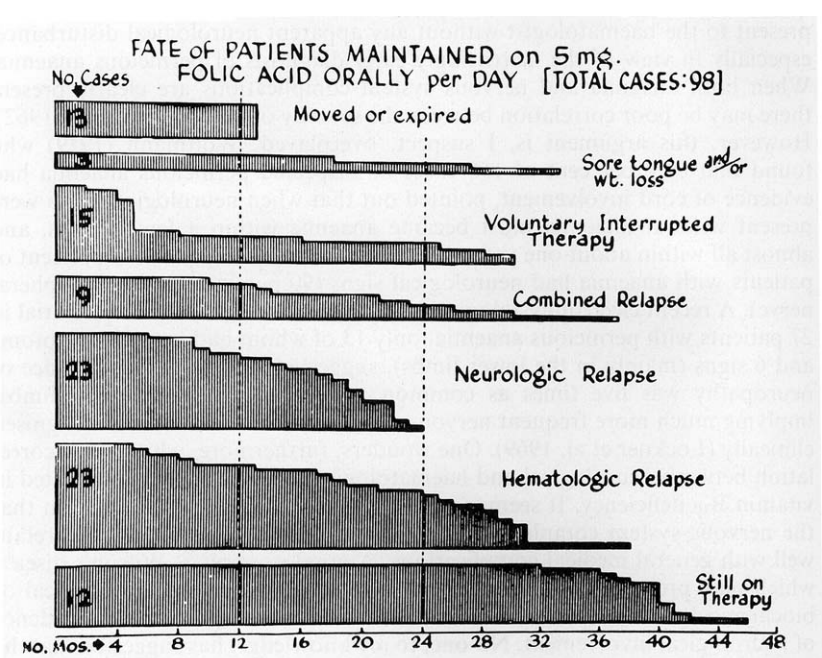

Figure 2: **Neurological and haematological relapse patterns in patients with pernicious anaemia treated with folic acid**
Described by Schwartz and colleagues in 1950.[8]

Folic-acid deficiency

Some of the treatment issues are similar to those for vitamin-B12 deficiency, others are unique to folic acid. More patients with neuropsychiatric complications of folate deficiency present without anaemia or macrocytosis than occurs with vitamin-B12 deficiency, probably because body folate stores are more rapidly depleted by dietary and other mechanisms than those of vitamin B12.[7,13] Low red-cell folate, especially if accompanied by high plasma concentrations of homocysteine, is a better guide to the degree and significance of deficiency than serum folate.[7,13,60] Considering that a third of patients with folate or vitamin B12 deficiency severe enough to produce anaemia have no immediate nervous-system involvement,[29] the significance of folate deficiency (or vitamin-B12 deficiency) in the presence of neuropsychiatric disorders is not always clear, especially in the elderly with depression or dementia.[15,28,60] However, if this deficiency, whether primary or secondary, is not already affecting the nervous system it is highly likely to do so if left untreated.[15,23,60]

As with vitamin B12, the response to folate treatment is slow over many months, at least in part because of the efficient blood–brain barrier mechanism for this vitamin, which limits entry, perhaps because of its excitatory properties.[23,60,103,104,107] Treatment should be for at least 6 months, but some improvement should be detected within 3 months. Again, the response and the degree of residual disability will be related to the duration and severity of nervous-system complications before treatment.

In patients with depression, folate deficiency is associated with a poorer response to standard antidepressant therapy.[63,109] Several controlled trials for up to 1 year have confirmed an effect of folates on mood, cognitive function, and social recovery either directly or in addition to psychotropic treatment in psychiatric patients (table 2).[110–114] Which folate formulation is best for the nervous system is unknown—ie, folic acid, folinic acid, or perhaps methyl folate, the transport form across the blood–brain barrier.[23,60] Small doses over the long term may be preferable to larger doses in the short or long term, not least because of risks to the nervous system, especially in vitamin-B12 deficiency and epilepsy.[23] There is some evidence that folates can improve mood and mental function in the absence of a deficiency state and that the psychological response may be related to serum or red-cell folate concentrations.[60,110] An additional rare side-effect of excessive doses is sleeplessness, overactivity, arousal, and even hypomania, as can occur with any anti-depressant and has been noted with the closely related metabolite, S-adenosylmethionine.[115,116]

Nervous system development

Neural-tube defects

Inadequate maternal folate intake and status is one of several well-established factors that can increase the risk of neural-tube defects, especially spina bifida and anencephaly.[14,114,117,118] Impaired vitamin-B12 status and high plasma homocysteine are also suspected to be additional or related risk factors.[119,120] Periconceptional preventive treatment with 400 µg folic acid significantly reduces the risk of such defects but at least a third of neural-tube defects are not preventable with the vitamin.[121,122] The mechanisms of the preventive action of folic acid are uncertain but possibly relate to methionine and nucleotide biosynthesis and to genetic vulnerability resulting in part from several common polymorphisms of folate-dependent enzymes involved in the folate cycle.[14,17]

Some countries, including the USA, Canada, and Chile have introduced mandatory fortification of flour, resulting in significant reductions in neural-tube defects but also a higher intake of folic acid than predicted.[123–126] Others, including the UK, have not done so due to concerns about the masking of vitamin-B12 deficiency in the elderly.[127] The debate about the most effective public-health policy continues.[118]

Disorders of vitamin-B12 and folate metabolism in infancy and childhood

Reviews of the many different inherited or acquired disorders of the absorption, transport, or metabolism of either vitamin are available.[24,128,129] The neurological features vary with the age of presentation (panel).[130] Haematological involvement is also variable or absent, as in adults with deficiencies.

	Patients	N	Trial design	outcome
Botez et al[110]	Depression, folate deficiency	24	Folic acid 15 mg daily *vs* placebo; 4 months	Improved mood, Wechsler IQ memory scale, and Kohs' block design
Coppen et al[111]	Manic depression, taking lithium	102	Folic acid 200 µg daily *vs* placebo; 1 year	Lower affective morbidity index associated with higher end-of-trial serum folate concentration
Godfrey et al[112]	Depression, schizophrenia, red-cell folate <200 µg/L, on standard psychotropic medication	41	Methyl folate 15 mg daily *vs* placebo; 6 months	Enhanced clinical social recovery in depression and schizophrenia increasing over time
Passseri et al[113]	Elderly depression with moderate dementia	96	Methyl folate 50 mg daily *vs* trazadone 100 mg daily; 8 weeks	Similar outcome in mood (Hamilton Scale) in both groups.
Coppen, Bailey[114]	Depression, taking fluoxitine	127	Folic acid 500 µg daily *vs* placebo; 10 weeks	Enhanced mood outcome, especially in women

Table 2: **Controlled clinical trials of folates in neuropsychiatric disorders**

A new syndrome of idiopathic cerebral folate deficiency has recently been described by Ramaekers and co-workers,[131] perhaps due to high-affinity blocking autoantibodies to membrane-bound folate receptors on the choroid plexus.[132]

Metabolic mechanisms

Dissociation

An issue that has puzzled many for a century is the lack of association between the haematological and neuropsychiatric manifestations of vitamin-B12 or folate deficiencies.[6–8,10,29–31] This dissociation has led to repeated suggestions that the nervous-system disorders must have a different mechanism to the megaloblastic anaemia.[7,10,30,31] To some extent, the dissociation is illusory, reinforced by the relatively early modern diagnosis of these disorders. In the first third of the 20th century, before any treatment was available, patients would eventually progress at different rates to nearly 100% association between anaemia and neuropsychiatric disorder.[4–6] Furthermore, the dissociation is not unique to vitamin-B12 and folate deficiency but seems common to all general metabolic disorders that also affect the nervous system.[28] For example, Wilson's disease may present either to a neurologist or hepatologist with predominant cerebral or hepatic involvement; likewise hypothyroidism may present to an endocrinologist, neurologist, or psychiatrist. There are several possible reasons for these divergences, including the highly specialised structure, environment, and function of the nervous system in comparison to other organs, but they need not and usually do not imply any fundamental difference in the metabolic basis of the neural manifestations.[28,38]

Folic acid and vitamin-B12 associations

The inverse correlation between the degree of anaemia and of neurological disability in vitamin-B12 deficiency[31] might be linked to the effect of folic acid on the blood and nervous system in the presence of vitamin-B12 deficiency. In the presence of vitamin-B12 deficiency, folic acid is harmful to the nervous system but can improve the anaemia.[7,10] Some enthusiasts of folic-acid fortification of food have doubted the adverse effect of folic acid on the nervous system on the grounds that it was not proven by a controlled trial;[22,133] however, no controlled trial has been done to prove benefit of vitamin B12. These enthusiasts accept, however, that folic acid may improve the anaemia thus "masking" and delaying the diagnosis of vitamin-B12 deficiency, allowing the neurological disorder to progress. None of these views accurately reflect what was reported in the late 1940s and early 1950s. Careful review of that research shows that after giving folic acid to treat "pernicious anaemia" there was sometimes brief temporary symptomatic neurological improvement before the more florid and sometimes explosive deterioration,[9,28] and after the obvious but suboptimal haematological improvement there was commonly a later insidious haematological relapse.[8,28] A similar number of patients have neurological and haematological relapse, although often dissociated (figure 2).[8] In other words, both the nervous system and the blood may show improvement and relapse but to different degrees and at different rates, which may in turn reflect profound differences in structure, function, and cellular turnover in the two tissues.

Patients with neurological complications of vitamin-B12 deficiency have significantly higher serum folate concentrations than those without such CNS disorders.[37,38] The inverse correlation between anaemia and neurological disability in vitamin-B12 deficiency may reflect a more harmful effect of folic acid on the nervous system or greater masking effect on the blood. In fruit bats deficient of vitamin B12, pretreatment with folates speeds up the onset of nitrous-oxide induced SCD.[134]

Folate cycle, homocysteine, methylation, DNA, and epigenetic mechanisms

The key to the metabolic understanding of both the neurology and haematology of vitamin-B12 and folate deficiency is the synthesis of methionine from homocysteine by methionine synthase in which both 5-methyl-tetrahydrofolate and vitamin B12 act as cofactors (figure 1). Failures in the folate cycle and the supply of methyl groups or in the availability of vitamin B12 will have similar and overlapping consequences on both blood and nervous system.

***Panel:* Clinical neurological features in remethylation defects related to age of presentation[130]**

Neonatal and early infancy (<3 months)
Poor feeding
Lethargy
Hypotonia or hypertonia
Seizures
Coma

Late infancy and early childhood (>3 months to <10 years)
Slow development
Lethargy
Mental deterioration
Encephalopathy
Seizures
Spastic paresis (subacute combined degeneration)
Extrapyramidal signs
Neuropathy

Late childhood and early adulthood (>10 years)
Previous mild retardation
Mental deterioration
Behaviour disturbance
Encephalopathy
Myelopathy (subacute combined degeneration)
Neuropathy

The megaloblastic anaemia in either deficiency state is due to impairment of DNA synthesis, integrity, and transcription, associated with failures in the synthesis of purines and especially thymidine.[7,12,13,26,27] In vitamin-B12 deficiency, especially pernicious anaemia, there is a block in the utilisation of methyl folate, commonly leading to a rise in plasma folate, the so-called methyl-folate trap; in folate deficiency there is a failure of delivery of methyl folate. Either mechanism leads to morphologically indistinguishable megaloblastic anaemia.[37,38]

I have suggested that similar mechanisms may apply to the neurological disorders in these two deficiency states.[28,38] However, the nervous system is also much more complex and hierarchical than the haemopoietic system and includes metabolic pathways that have little or no role in the blood (eg, in relation to myelin or mood). Nor should we assume that all neuropsychiatric complications have the same metabolic basis. There is much interest and speculation in this topic but most of it does now relate to the folate cycle and its involvement in nucleotide and DNA synthesis, to the homocysteine-methionine cycle and its involvement in numerous methylation reactions through the methyl donor, S-adenosylmethionine, including the methylation of DNA, which plays a vital part in gene expression and other epigenetic mechanisms.[14,15,17,135,136] The role of homocysteine in vascular and neurotoxic mechanisms is also a subject of current research in stroke, vascular dementia, and Alzheimer's disease.[12,75,76,137,138]

An important model is that of nitrous oxide, which in man can produce megaloblastic changes in bone marrow within hours of anaesthesia and the full range of neuropsychiatric complications within weeks or months of abuse,[32,39,43] and which has been studied in several species, including monkeys, pigs, rats, and fruit bats.[134,139–141] By oxidising the cobalt atom of vitamin B12, nitrous oxide mimics vitamin-B12 deficiency, rapidly inactivating methionine synthase (figure 1). Methionine protects against nitrous oxide induced SCD implying that methylation processes are important in this disorder,[140,141] as is supported by results of studies of demyelination in inborn disorders of remethylation.[24,142,143] I suggest that the euphoriant laughing-gas effect of nitrous oxide in man is due to the rapid raising of methyl-folate concentrations in the nervous system, consequent upon the almost instantaneous inactivation of vitamin-B12.[115,139]

Table 3 summarises postulated mechanisms not only for the neuropsychiatric complications of vitamin-B12 and folic-acid deficiency but also for the possible protective effect of folate in some disorders not primarily due to deficiency. Prophylactic folic acid can reduce the incidence of neural-tube defects in the early embryonic period, even in the absence of folate deficiency.[14,17,121,122] Recently Iskandar and colleagues[18] reported that folic acid significantly improved the regrowth of sensory axons in a spinal cord regeneration model and improved neurological recovery from spinal cord contusion injury in rats. They suggest that folic acid can influence repair mechanisms in the adult nervous system as well as the developing nervous system. Folates seem to be of fundamental importance in brain growth, differentiation, development, repair, mood, cognition, and ageing.[13–15,17,18,115,135,136,144] These functions and their breakdown in folate and vitamin-B12 deficiency are probably primarily mediated through nucleotide synthesis, DNA integrity and transcription, and epigenetic mechanisms, including gene expression, relating to DNA methylation.[17,135,136] As in megaloblastosis, these mechanisms are probably involved in most if not all the neurological complications of deficiencies of vitamin B12 and folic acid—a kind of "megaloblastosis" of the nervous system. However, in addition there is widespread failure of non-genomic methylation involving potentially over 100 S-adenosylmethionine-mediated methylation reactions in numerous neural pathways.[13,17,115,116,145] Possible examples are myelin basic protein and membrane phospholipids, which may perhaps contribute to demyelination,[140–143] and monoamines, the turnover of which is increased by both folic acid and S-adenosylmethionine, and which are implicated in depression.[60,115,116] Furthermore, there is some experimental evidence that homocysteine, in addition to being implicated in vascular disease, can induce DNA damage in the CNS.[138,144] It is therefore of interest in relation to ageing, cognitive decline, and various forms of dementia, including vascular and Alzheimer's disease, that serum and CSF folate concentrations fall and serum homocysteine concentrations rise with age.[69–71] Some authors also propose that homocysteine-related impairment of glutathione metabolism and oxidative stress,[146,147] or impaired DNA methylation and associated epigenetic mechanisms, may increase amyloid-β-peptide production and toxicity, for example in Alzheimer's

	Clinical implications of vitamin-B12 or folate deficiency* or inborn errors	Possible metabolic mechanisms
Embryo, fetus infant, child	Disorders of CNS growth and development	Impaired DNA synthesis and transcription, impaired genomic methylation and epigenetic mechanisms, homocysteine-mediated DNA damage
Adult	SCD or neuropathy	As above and impaired non-genomic methylation (eg, myelin proteins, phospholipids)
	Depression or psychiatric disorders	Impaired non-genomic methylation (eg, turnover of monoamines)
Old age	Brain ageing, cognitive decline, dementia	All the above mechanisms including DNA synthesis, genomic and non-genomic methylation, and homocysteine/metabolite neurotoxicity; failure of repair mechanisms
Other	Cerebrovascular disease and stroke	Homocysteine-related vascular mechanisms
	Alzheimer's disease	All the above mechanisms and oxidative stress, impaired glutathione metabolism, and increased amyloid-β peptide

*In some disorders (eg, AD or stroke) the deficiency may be secondary to the underlying neuropsychiatric disorder.

Table 3: **Proposed metabolic mechanisms of vitamin-B12 or folate disorders**

Search strategy and selection criteria

References for this review were identified through searches of PubMed most recently done on June 1, 2006, and of the authors' files from 1966. The search terms were "vitamin B12", "folic acid", "folate", "nervous system", "neuropathy", "dementia", "epilepsy", "multiple sclerosis", "nitrous oxide". Relevant textbooks were also used. The final reference list was generated on the basis of originality and relevance to each of the subtopics reviewed.

disease.[138,144,148] Activation of NMDA receptors by homocysteine or its oxidation metabolites may have some neuropsychiatric implications.[149] Whether the involvement of the methylmalonyl-succinyl CoA pathway contributes to neurological damage in vitamin-B12 deficiency is unresolved.[24,41]

Vulnerability to the above mechanisms will be increased in relation to both the severity and duration of either deficiency,[15,31,32,60] in the presence of predisposing genetic factors, including some polymorphisms of folate and vitamin B12 dependent enzymes, especially if the latter are additionally compromised by dietary factors (nutrigenomics),[17,21,64,135,150] and in the presence of associated metabolic disorders, such as malabsorption, or pharmacological stress—eg, folate antagonists.[28,29,53,60]

Conclusions

Vitamin-B12 and folic-acid deficiency and related inborn errors of metabolism may result in similar megaloblastic anaemias and overlapping neuropsychiatric complications. In the early stages there is often dissociation between the neuropsychiatric and haematological manifestations, as occurs in other general metabolic disorders that affect the CNS. The occurrence of CNS complications is influenced by the duration as well as the severity of either deficiency, by predisposing genetic factors, including polymorphisms of folate or vitamin-B12 dependent enzymes, and by any associated metabolic disorders. The administration of folic acid in the presence of vitamin-B12 deficiency may be harmful to the nervous system, after brief temporary improvement, and ultimately harmful to the blood, after more striking but suboptimal temporary improvement. In the CNS, as in the blood, failure or blocking of the supply of methyl folate will result in impaired purine, thymidine, nucleotide, and DNA synthesis, as well as disruption of DNA transcription, methylation, gene expression, and other epigenetic mechanisms affecting tissue growth, differentiation, and repair. There is now substantial interest in the role of folic acid, vitamin B12, and related pathways in nervous-system function and disease at all ages and the potential use of the vitamins, especially folic acid, in the prophylaxis of disorders of CNS development, mood, and cognitive decline, including some dementias.

Conflicts of interest

I have no conflicts of interest. I have been actively involved in research of this topic since 1965 and have received research grants from the UK Medical Research Council and Bioresearch (Milan).

References

1 Leichtenstern O. Progressive perniciöse anämie bei tabeskranken. *Dtsch Med Wochenschr* 1884; **10:** 849–50.
2 Lichtheim L. Zur kenntniss der perniciösen anämie. *Munch Med Wochenschr* 1887; **34:** 301–06.
3 Russell JSR, Batten FE, Collier J. Subacute combined degeneration of the spinal cord. *Brain* 1900; **23:** 39–110.
4 Woltmann HW. The nervous symptoms in pernicious anaemia: an analysis of 150 cases. *Am J Med Sci* 1919; **157:** 400–09.
5 Ahrens RS. Neurologic aspects of primary anaemia. *Arch Neurol Psychiatry* 1932; **28:** 92–109.
6 Kinnier Wilson SA. Neurology. London: Arnold, 1941: 1339–58.
7 Chanarin I. The megaloblastic anaemias. Oxford: Blackwell, 1969.
8 Schwartz SO, Kaplan SR, Armstrong BE. The long-term evaluation of folic acid in the treatment of pernicious anaemia. *J Lab Clin Med* 1950; **35:** 894–98.
9 Hall BE, Watkins CH. Experience with pteroylglutamic (synthetic folic) acid in the treatment of pernicious anaemia. *J Lab Clin Med* 1947; **32:** 622–34.
10 Reynolds EH. Folic acid, vitamin B12 and the nervous system: historical aspects. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry, and internal medicine. New York: Raven Press, 1979: 1–5.
11 Botez MI, Reynolds EH, eds. Folic acid in Neurology, Psychiatry and Internal Medicine. New York, Raven Press 1979.
12 Carmel R, Jacobsen DW, eds. Homocysteine in health and disease. Cambridge: Cambridge University Press, 2001.
13 Bailey LB, ed. Folate in health and disease. New York: Marcel Dekker, 1995.
14 Massaro EJ, Rogers JM, eds. Folate and human development. Totowa, New Jersey: Humana Press, 2002.
15 Reynolds EH. Folic acid, ageing, depression, and dementia. *BMJ* 2002; **324:** 1512–15.
16 Morris MS. Homocysteine and Alzheimer's disease. *Lancet Neurol* 2003; **2:** 425–28.
17 Lucock M. Is folic acid the ultimate functional food component for disease prevention? *BMJ* 2004; **328:** 211–14.
18 Iskandar BJ, Nelson A, Resnick D, et al. Folic acid supplementation enhances repair in the adult central nervous system. *Ann Neurol* 2004; **56:** 221–27.
19 Ravaglia G, Forti P, Maioli F, et al. Homocystine and folate as risk factors for dementia and Alzheimer disease. *Am J Clin Nutr* 2005; **82:** 636–43.
20 Bottiglieri T. Homocysteine and folate metabolism in depression. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2005; **29:** 1103–12.
21 Durga J, van Boxtel MPJ, Schouten EG, Bots ML, Kok FJ, Verhoef P. Folate and the methylenetetrahydrofolate reductase 677C-T mutation correlate with cognitive performance. *Neurobiol Aging* 2006; **27:** 334–43.
22 Dickinson CJ. Does folic acid harm people with vitamin B12 deficiency? *Q J Med* 1995; **88:** 357–64.
23 Reynolds EH. Benefits and risks of folic acid to the nervous system. *J Neurol Neurosurg Psychiatry* 2002; **72:** 767–71.
24 Surtees R. Cobalamin and folate responsive disorders. In: Baxter P, ed. Vitamin responsive conditions in paediatric neurology: international review of child neurology series. London: MacKeith Press, 2001: 96–108.
25 Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab* 2000; **71:** 121–38.
26 Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program* 2003; **1:** 62–81.
27 Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. *Annu Rev Nutr* 2004; **24:** 105–31.
28 Reynolds EH. Neurological aspects of folate and vitamin B12 metabolism. In: Hoffbrand AV, ed. Clinics in haematology, volume 5. London: Saunders, 1976: 661–96.

29 Shorvon SD, Carney MWP, Chanarin I, Reynolds EH. The neuropsychiatry of megaloblastic anaemia. *BMJ* 1980; **281:** 1036–38.

30 Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anaemia or macrocytosis. *N Engl J Med* 1988; **318:** 1720–28.

31 Healton EB, Savage DG, Brust JCM, Garrett TJ, Lindenbaum J. Neurologic aspects of cobalamin deficiency. *Medicine* 1991; **70:** 229–45.

32 Savage DG, Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects *Bailliere's Clin Haematol* 1996; **8:** 657–78.

33 Sigal SH, Hall CA, Antel JP. Plasma R binder deficiency and neurologic disease. *N Engl J Med* 1987; **317:** 1330–32.

34 Reynolds EH, Bottiglieri T, Laundy M, et al. Subacute combined degeneration with high serum vitamin B12 level and abnormal vitamin B12 binding protein: new cause of an old syndrome. *Arch Neurol* 1993; **50:** 739–42.

35 Fine EJ, Soria E, Paroski MW, Petryk D, Thomasula L. The neurophysiological profile of vitamin B12 deficiency. *Muscle Nerve* 1990; **13:** 158–64.

36 Zucker DK, Livingston RL, Nakra R, Clayton PJ. B12 deficiency and psychiatric disorders: case report and literature review. *Biol Psychiatry* 1981; **16:** 197–205.

37 Waters AH, Mollin DL. Studies on the folic acid activity of human serum. *J Clin Pathol* 1961; **14:** 335–44.

38 Reynolds EH. Interrelationships between the neurology of folate and vitamin B12, deficiency. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York: Raven Press, 1979: 501–15.

39 Layzer RB. Myeloneurophathy after prolonged exposure to nitrous oxide. *Lancet* 1978; **2:** 1227–30.

40 Admir H, Krzysztof G, Sanborn KV, Thys DM. Severe neurologic deficit after nitrous oxide anaesthesia. *Anaesthesiology* 1995; **83:** 863–66.

41 Riedel B, Fiskerstrand T, Refsum H, Ueland PM. Co-ordinate variations in methylmalonyl-CoA mutase and methionine synthase, and the cobalamin cofactors in human glioma cells during nitrous oxide exposure and the subsequent recovery phase. *Biochem J* 1999; **341:** 133–38.

42 Amess JAL, Burkman JF, Rees GM, Nancekievill DG, Mollin DL. Megaloblastic haemopoiesis in patients receiving nitrous oxide. *Lancet* 1978; **2:** 339–42.

43 Nunn JF. Clinical aspects of the interaction between nitrous oxide and vitamin B12. *Br J Anaesth* 1987; **59:** 3–13.

44 Reynolds EH, Linnell JC, Faludy JE. Multiple sclerosis associated with vitamin B12 deficiency. *Arch Neurol* 1991; **48:** 808–11.

45 Crellin RF, Bottiglieri T, Reynolds EH. Multiple sclerosis and macrocytosis. *Acta Neurol Scand* 1990; **81:** 388–91.

46 Reynolds EH, Bottiglieri T, Laundy HNC, Crellin RF, Kirker SG. Vitamin B12 metabolism in multiple sclerosis. *Arch Neurol* 1992; **49:** 649–52.

47 Sandyk R, Awerbuch GI. Vitamin B12 and its relationship to age of onset of multiple sclerosis. *Int J Neurosci* 1993; **71:** 93–99.

48 Besler HT, Comogleu S. Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. *Nutr Neurosci* 2003; **6:** 189–96.

49 Vrethem M, Mattsson E, Hebelka H, et al. Increased plasma homocysteine levels without signs of vitamin B12 deficiency in patients with multiple sclerosis assessed by blood and cerebrospinal fluid homocysteine and methylmalonic acid. *Mult Scler* 2003; **9:** 239–45.

50 Reynolds EH. Multiple sclerosis and vitamin B12 metabolism. *J Neuroimmunol* 1992; **40:** 225–30.

51 Grant HC, Hoffbrand AV, Wells DG. Folate deficiency and neurological disease. *Lancet* 1965; **2:** 763–67.

52 Reynolds EH, Chanarin I, Matthews DM. Neuropsychiatric aspects of anti-convulsant megaloblastic anaemia. *Lancet* 1968; **1:** 394–97.

53 Reynolds EH. Mental effects of anticonvulsants, and folic acid metabolism. *Brain* 1968; **91:** 197–214.

54 Reynolds EH, Rothfeld P, Pincus J. Neurological disease associated with folate deficiency. *BMJ* 1973; **2:** 398–400.

55 Manzoor M, Runcie J. Folate-responsive neuropathy: a report of ten cases. *BMJ* 1976; **1:** 1176–78.

56 Botez MI, Fontaine F, Botez T, Bachevalier J. Folate-responsive neurological and mental disorders: report of 16 cases. *Eur Neurol* 1977; **16:** 230–46.

57 Pincus J. Folic acid deficiency: a cause of subacute combined degeneration. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York: Raven Press, 1979: 427–33.

58 Botez MI, Peyronnard JM, Charron L. Polyneuropathies responsive to folic acid therapy. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York, Raven Press, 1979: 401–12.

59 Shorvon SD, Reynolds EH. Folate deficiency and peripheral neuropathy. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York: Raven Press, 1979: 413–21.

60 Bottiglieri T, Crellin R, Reynolds EH. Folate and neuropsychiatry, In: Bailey LB, ed. Folate in health and disease. New York: Marcel Dekker, 1995: 435–62.

61 Engelborghs S, Vloeberghs E, Maertens K, et al. Correlations between cognitive, behavioural and psychological findings and levels of vitamin B12 and folate in patients with dementia. *Int J Geriatr Psychiatry* 2004; **19:** 365–70

62 Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2004; **33:** 34–41.

63 Reynolds EH, Preece JM, Bailey J, Coppen A. Folate deficiency in depressive illness. *Brit J Psychiatry* 1970; **117:** 287–92.

64 Lewis SJ, Lawlor DA, Davey Smith G, et al. The thermolabile variant of MTHFR is associated with depression in the British Women's Heart and Health Study and a meta-analysis. *Mol Psychiatry* 2006; **11:** 352–60.

65 Ramos MI, Allen LH, Mungas DM, et al. Low folate status is associated with impaired cognitive function and dementia in the Sacramento area Latino study on aging. *Am J Clin Nutr* 2005; **82:** 1346–52.

66 Sneath P, Chanarin I, Hodkinson HM, McPhearson CK, Reynolds EH. Folate status in a geriatric population and its relation to dementia. *Age Ageing* 1973; **2:** 177–82.

67 Carney MWP, Sheffield BF. Associations of subnormal folate and vitaminB12 values and effects of replacement therapy. *J Nerv Ment Dis* 1970; **150:** 404–11.

68 Starr JM, Pattie A, Whiteman MC, Deary IJ, Whalley LJ. Vitamin B12, serum folate, and cognitive change between 11 and 79 years. *J Neurol Neurosurg Psychiatry* 2005; **76:** 291–92.

69 Bottiglieri T, Reynolds EH, Laundy M. Folate in CSF and age. *J Neurol Neurosurg Psychiatry* 2000; **69:** 562.

70 Serot JM, Barbe F, Arning E, et al. Homocysteine and methylmalonic acid concentrations in cerebrospinal fluid: relation with age and Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2005; **76:** 1585–87.

71 Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002; **346:** 476–83.

72 Wahlin TBR, Wahlin A, Winblad B, Backman L. The influence of serum vitamin B12 and folate status on cognitive functioning in very old age. *Biol Psychol* 2001; **56:** 247–65.

73 Goodwin JS, Goodwin JM, Garry PJ. Association between nutritional status and cognitive functioning in a healthy elderly population. *JAMA* 1983; **249:** 2917–21.

74 Hassing L, Wahlin A, Winblad B, Backman L. Further evidence on the effects of vitamin B12 and folate levels on episodic memory functioning; a population-based study of healthy very old adults. *Biol Psychiatry* 1999; **45:** 1472–80.

75 McIlroy SP, Dynan KB, Lawson JT, Patterson CC, Passmore AP. Moderately elevated plasma homocysteine, methylenetetrahydrofolate reductase genotype, and risk for stroke, vascular dementia, and Alzheimer disease in Northern Ireland. *Stroke* 2002; **3:** 2351–56.

76 Clarke R, Collins R, Lewington S. Homocysteine and risk of heart disease and stroke: a meta-analysis. *JAMA* 2002; **288:** 2015–22.

77 Spence JD. Homocysteine: call off the funeral. *Stroke* 2006; **37:** 281–82.

78 Nilsson K, Gustafson L, Faldt R, et al. Hyperhomocysteinaemia: a common finding in a psychogeriatric population. *Eur J Clin Invest* 1996; **26:** 853–59.

79 Riggs KM, Spiro A, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 1996; **63:** 306–14.

80 McCaddon A, Davies G, Hudson P, Tandy S, Cattell H. Total serum homocysteine in senile dementia of Alzheimer type. *Int J Geriatr Psychiatry* 1998; **13:** 235–39.

81 Leblhuber F, Walli J, Artner-Dworzak E, et al. Hyperhomocysteinemia in dementia. *J Neural Transm* 2000; **107:** 1469–74.

82 Morris MS, Jacques PF, Rosenberg IH, Selhub J. Hyperhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 2001; **73:** 927–33.

83 McCaddon A, Hudson P, Davies G, Hughes A, Williams JHH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. *Dement Geriatr Cogn Disord* 2001; **12:** 309–13.

84 Ravaglia G, Forti P, Maioli F, et al. Homocystine and cognitive function in healthy elderly community dwellers in Italy. *Am J Clin Nutr* 2003; **77:** 668–73.

85 Teunissen CE, Blom AHJ, van Boxtel PJ, et al. Homocysteine: a mark of the cognitive performance? A longitudinal follow-up study. *J Nutr Health Aging* 2003; **7:** 153–59.

86 Almeida OP, Lautenschlager N, Flicker L, et al. Association between homocysteine, depression, and cognitive function in community-dwelling older women from Australia. *J Am Geriatr Soc* 2004; **52:** 327–28.

87 Quadri P, Fragiacomo C, Pezzati R, et al. Homocysteine, folate and vitamin B12 in mild cognitive impairment, Alzheimer's disease, and vascular dementia. *Am J Clin Nutr* 2004; **80:** 114–22.

88 Mizrahi EH, Bowirrat A, Jacoben DW, et al. Plasma homocysteine, vitamin B12 and folate in Alzheimer's patients and healthy Arabs in Israel. *J Neurol Sci* 2004; **227:** 109–13.

89 Quadri P, Fragiacomo C, Pezzati R, Zanda E, Tettamanti M, Lucca U. Homocysteine and B-vitamins in mild cognitive impairment and dementia. *Clin Chem Lab Med* 2005; **43:** 1096–100.

90 Wang H-X, Wahlin A, Basun H, Fastbom J, Winblad B, Frattelioni L. Vitamin B12 and folate in relation to the development of Alzheimer's disease. *Neurology* 2001; **56:** 1188–94.

91 Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr* 2002; **75:** 908–13.

92 Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. *Lancet* 2001; **357:** 169–75.

93 Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998; **55:** 1449–55.

94 den Heijer T, Vermeer SE, Clarke R, et al. Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain* 2003; **126:** 170–75.

95 Prins ND, Den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly: the Rotterdam scan study. *Neurology* 2002; **59:** 1375–80.

96 Snowdon DA, Tully CL, Smith CD, Riley KP, Markesbery WR. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the nun Study. *Am J Clin Nutr* 2000; **71:** 993–98.

97 Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MWP, Reynolds EH. Homocysteine, folate, methylation and monoamine metabolism in depression. *J Neurol Neurosurg Psychiatry* 2000; **69:** 228–32.

98 Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B12, and homocysteine in major depressive disorder. *Am J Psychiatry* 1997; **154:** 426–28.

99 Reif A, Pfuhlmann B, Lesch KP. Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychosis. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2005; **29:** 1162–68.

100 Goff DC, Bottiglieri T, Arning E, et al. Folate, homocysteine, and negative symptoms in schizophrenia. *Am J Psychiatry* 2004; **161:** 1705–08.

101 Muntjewerff JW, Kahn RS, Blom HJ, den Heijer M. Homocysteine, methylenetetrahydrofolate reductase and risk of schizophrenia: a meta-analysis. *Mol Psychiatry* 2006; **11:** 143–49.

102 Reynolds EH, Gallagher BB, Mattson RH, Bowers M, Johnson AL. Relationship between serum and cerebrospinal fluid folate. *Nature* 1972; **240:** 155–57.

103 Hommes OR, Hollinger JL, Jansen MJT, Schoofs M, Vanderweil T, Kok JCN. Convulsant properties of folate compounds; some considerations and speculations. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York, Raven Press, 1979: 285–316.

104 Hommes OR, Obbens EAMT, Wijfels CCB. Epileptogenic activity of sodium-folate and the blood brain barrier in the rat. *J Neurol Sci* 1973; **19:** 63–71.

105 Miller AA, Goff D, Webster RA. Predisposition of laboratory animals to epileptogenic activity of folic acid. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York, Raven Press, 1979: 331–34.

106 Davis J, Watkins JC. Facilitatory and direct excitatory effects of folate and folinate on single neurons of cat cerebral cortex. *Biochem Pharmacol* 1973; **22:** 1667–68.

107 Spaans F. No effect of folic acid supplement on CSF folate and serum vitamin B12 in patients on anticonvulsants. *Epilepsia* 1970; **11:** 403–11.

108 Rivey MP, Schottelius DD, Berg MJ. Phenytoin-folic acid: a review. *Drug Intell Clin Pharm* 1984; **18:** 292–301.

109 Papakostas GI, Petersen T, Lebowitz BD, et al. The relationship between serum folate, vitamin B12, and homocysteine levels in major depressive disorder and the timing of improvement with fluoxetine. *Int J Neuropsychopharmacol* 2005; **8:** 1–6.

110 Botez MI, Botez T, Leveille J, Beilmann P, Cadotte M. Neuropsychological correlates of folic acid deficiency; facts and hypotheses. In: Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York: Raven Press, 1979: 435–61.

111 Coppen A, Chaudhry S, Swade C. Folic acid enhances lithium prophylaxis. *J Affect Disord* 1986; **10:** 9–13.

112 Godfrey PSA, Toone BK, Carney MWP, et al. Enhancement of recovery from psychiatric illness by methyl folate. *Lancet* 1990; **336:** 392–95.

113 Passeri M, Cuciniotta D, Abate G, Senin U, Ventura A. Oral 5-methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. *Ageing Clin Exp Res* 1993; **5:** 63–71.

114 Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *J Affect Disord* 2000; **60:** 121–30.

115 Reynolds EH, Carney MWP, Toone BK. Methylation and mood. *Lancet* 1984; **2:** 196–98.

116 Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademethionine (S-adenosylmethionine) in neurological disorders. *Drugs* 1994; **48:** 1137–52.

117 Mitchell LE, Adzick NS, Melchionne J, Pasquariello PS, Sutton LN, Whitehead AS. Spina bifida. *Lancet* 2004; **364:** 1885–95.

118 Eichholzer M, Tönz O, Zimmermann R. Folic acid: a public-health challenge. *Lancet* 2006; **367:** 1352–61.

119 Ray JG, Blom HJ. Vitamin B12 insufficiency and the risk of foetal neural tube defects. *Q J Med* 2003; **96:** 289–95.

120 Rosenquist T, Ratashak SA, Selhub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. *Proc Natl Acad Sci USA* 1996; **93:** 1527–32.

121 MRC Vitamin Study Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991; **338:** 131–37.

122 Czeizel AE, Dudas I. Prevention of the first occurrence of neural tube defects by peri-conceptional vitamin supplementation. *N Engl J Med* 1992; **327:** 1832–35.

123 US Department Health and Human Services, Food and Drug Administration. Food standards: amendments of the standards of identity for enriched grain products to require addition of folic acid. *Federal Register* 1996; **61:** 8781–807.

124 Quinlivan EP, Gregory JF. Effect of food fortification on folic acid intake in the United States. *Am J Clin Nutr* 2003; **77:** 221–25.

125 Choumenkovitch SF, Selhub J, Wilson PWF, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. *J Nutr* 2002; **132:** 2792–98.

126 Shane B. Folate fortification: enough already? *Am J Clin Nutr* 2003; **77:** 8–9.

127 Report of the Committee on Medical Aspects of Food and Nutrition Policy, Folic Acid and the Prevention of Disease. London: Stationery Office, 2000.

128 Rosenblatt DS, Fenton WA. Inherited disorders of folate and cobalamin transport and metabolism. In: Scriver CS, Beaudet AL, Sly WS, Calle D, eds. The Metabolic Basis of Inherited Disease, 8th edn. New York: McGraw-Hill, 2001: 3897–933.

129 Whitehead VM. Acquired and inherited disorders of cobalamin and folate in children. *Br J Haematol* 2006; **134:** 125–36.

130 Ogier de Baulny H, Gerard M, Saudubray JM, Zittoun J. Remethylation defects: guidelines for clinical diagnosis and treatment. *Eur J Pediatr* 1998; **157:** S77–83.

131 Ramaekers VT, Blau N. Cerebral folate deficiency. *Dev Med Child Neurol* 2004; **46:** 843–51.

132 Ramaekers VT, Rothenberg P, Sequeira JM, et al. Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N Engl J Med* 2005; **352:** 1985–91.

133 Oakley GP. Let's increase folic acid fortification and include vitamin B12. *Am J Clin Nutr* 1997; **65:** 1889–90.

134 Van der Westhuyzen J, Fernandes-Costa F, Metz J. Cobalamin inactivation by nitrous oxide produces severe neurological impairment in fruit bats: protection by methionine and aggravation by folates. *Life Sci* 1982; **31:** 2001–10.

135 Friso S, Choi S-W. Gene nutrient interactions and DNA methylation. *J Nutr* 2002; **132:** 2382S–7S.

136 Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci* 2003; **26:** 137–46.

137 Nagy ZS, Smith MZ, Esiri MM, Barnetson L, Smith AD. Hyperhomocysteinaemia in Alzheimer's disease and expression of cell cycle markers in the brain. *J Neurol Neurosurg Psychiatry* 2000; **69:** 565–66.

138 Irizarry MC, Gurol ME, Raju S, et al. Association of homocysteine with plasma amyloid β protein in aging and neurodegenerative disease. *Neurology* 2005; **65:** 1402–08.

139 Lumb M, Perry J, Deacon R, Chanarin I. Changes in plasma folate levels in rats inhaling nitrous oxide. *Scand J Haematol* 1981; **26:** 61–64.

140 Scott JM, Dinn JJ, Wilson NVB, Weir DG. Pathogenesis of subacute combined degeneration: a result of methyl group deficiency. *Lancet* 1981; **2:** 334–37.

141 Scott JM, Molloy AM, Kennedy DG, Kennedy S, Weir DG. Effects of the disruption of transmethylation in the central nervous system; an animal model. *Act Neurol Scand* 1994; **154:** 27S–31S.

142 Surtees R, Leonard J, Austin S. Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. *Lancet* 1991; **338:** 1550–54.

143 Surtees R. Demyelination and inborn errors of the single carbon transfer pathway. *Eur J Pediatr* 1998; **157:** 118S–21S.

144 Kruman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *J Neurosci* 2002; **22:** 1752–62.

145 Bottiglieri T, Godfrey P, Flynn T, Carney MWP, Toone BK, Reynolds EH. Cerebrospinal fluid S-adenosylmethionine in depression and dementia; effects of treatment with parenteral and oral S-adenosylmethionine. *J Neurol Neurosurg Psychiatry* 1990; **53:** 1096–98.

146 McCaddon A, Regland B, Hudson P, Davies G. Functional vitamin B(12) deficiency and Alzheimer disease. *Neurology* 2002; **58:** 1395–99.

147 McCaddon A, Hudson P, Hill D, et al. Alzheimer's disease and total plasma aminothiols. *Biol Psychiatry* 2003; **53:** 254–60.

148 Fuso A, Seminara L, Cavallaro RA, D'Anselmi F, Scarpa S. S-adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. *Mol Cell Neurosci* 2005; **28:** 195–204.

149 Gortz P, Hoinkes A, Fleischer W, et al. Implications for hyperhomocysteinemia: not homocysteine but its oxidized forms strongly inhibit neuronal network activity. *J Neurol Sci* 2004; **218:** 109–14.

150 Rampersaud GC, Kauwell GPA, Hutson AD, Cerda JJ, Bailey LB. Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. *Am J Clin Nutr* 2000; **72:** 998–1003.