mother and thereby produce a somewhat different but possibly more permanent problem.

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Abnormalities of vitamin B_{12} and folic acid metabolism—their influence on the nervous system

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Recently Dr W. B. Castle of Harvard, one of the great figures of Medicine in this century, said, in the course of the first Sir Stanley Davidson Lecture of the University of Edinburgh, that little progress has been made in our knowledge of the causation of subacute combined degeneration of the cord, the neurological disorder that used to be such a striking complication of pernicious anaemia. Because of advances in therapy the condition is seldom seen now, but, despite all the progress that has been made in various fields of study of the megaloblastic anaemias, there

has not been a parallel increase in our knowledge of the precise cause of the neurological complications.

Causes of depletion of vitamin B_{12} or folic acid

First let me remind you that megaloblastic anaemia occurs when there is a considerable degree of deficiency of vitamin B_{12} or folic acid or both, but that such deficiency states cause widespread metabolic upsets throughout the body, the abnormalities by no means being limited to the bone marrow.

Vitamin B_{12} deficiency arises from the following main causes:

(a) Dietary deficiency, as in vegans.

(b) Lack of intrinsic factor (pernicious anaemia, partial or total gastrectomy, rarely after gastroenterostomy).

(c) Malabsorption in any of the 'sprue' group of diseases, or from organic disease of the lower ileum or its surgical removal.

(d) Bacteria in a blind loop or stagnant area of small intestine directly or indirectly depriving the patient of vitamin B_{12}. The fish tapeworm, *Diphyllobothrium latum*, a parasite in the intestine of people living on the shores of the Baltic, also does this.

Subacute combined degeneration of the cord has been reported most consistently in association with Addisonian pernicious anaemia, but it may occur in any condition that leads to vitamin B_{12} depletion (Woltman & Heck, 1937; Badenoch, 1954; Wokes, 1956; Harrison, Booth & Mollin, 1956; Richmond & Davidson, 1958; Bourne & Oleesky, 1960; Riley, 1966).

Folic acid depletion is found as a result of:

(a) Dietary deficiency. This is rarely seen in the United Kingdom other than in pregnancy, but may be found in geriatric patients, particularly in industrial areas.

(b) Malabsorption because of one of the 'sprue' group of diseases, or from extensive organic disease of the small intestine.

(c) Metabolic competition for folic acid from certain anticonvulsant drugs or from folic acid antagonists.

(d) Increased need for folic acid as in pregnancy, or from haemolysis, prolonged infection or widespread malignancy.

It is usually considered that folic acid deficiency by itself does not lead to subacute combined degeneration of the cord, but there have been reports suggesting that neurological disorders can be associated with depletion of folic acid alone (Hansen, Nordqvist & Sourander, 1964; Anand, 1964; Grant, Hoffbrand & Wells, 1965). Such reports are, however, few and usually not very convincing. The most interesting examples are seven patients reported by Grant *et al.* (1965) who came to a neurological clinic with peripheral neuropathy or spastic paraplegia and were found to have folate depletion (but with higher serum folate levels than are usually found in severe folate deficiency). It is possible that the neurological disorder led to folate deficiency from impaired appetites or feeding habits, and certainly there is no evidence that folate deficiency causes subacute combined degeneration of the cord.

Metabolic actions of folic acid and vitamin B_{12}

The precise metabolic activities of folic acid and vitamin B_{12} are not fully understood, and are a matter of great complexity. Much of what is believed to occur in man has been deduced from investigation carried out with bacteria or in animals, and, particularly as regards vitamin B_{12}, our knowledge is fragmentary.

Folic acid. There is reason to believe that in the cells of the body there is a 'pool' of 'one-carbon units' which are available for the synthesis of various substances. Forms of folate, called tetrahydrofolates because they contain four additional atoms of hydrogen, act as coenzymes. They receive single carbon units from the body pool and can donate them for the formation of various basic substances. The forms of tetrahydrofolate involved are the methyl, hydroxymethyl, methenyl, methylene, formyl and formimino derivatives. Cells containing folate have an enzyme system, named folic acid reductase, which permits reduction of folate to the tetrahydrofolate state.

The story is a very complicated one (Girdwood, 1959), but, in brief, derivatives of folic acid are involved in the formation of methionine from homocysteine and in the construction of purines and pyrimidines and hence of RNA and DNA. Thus folic acid is of fundamental importance in normal cell division and growth.

Vitamin B_{12}. So far as vitamin B_{12} is concerned, this substance has been found in micro-organisms and mammalian systems to be involved in the methylation of homocysteine to methionine, so that both it and folic acid are involved in methionine formation. There appear, however, to be a number of reaction mechanisms for vitamin B_{12}. In certain systems it is involved in isomerization reactions and, in others, in oxidation–reduction reactions. Unfortunately, too, the vitamin appears to carry out different functions in different organisms, and there are various active forms. Thus there is probably a methyl form which, in rats, is involved in the enzymatic methylation of soluble RNA to give methyl RNA (Venkataraman, Walerych & Johnson, 1967), and a dimethyl-benzimidazolylcobamide complex is possibly the storage form in man.

In vitamin B_{12} deficiency in man there is an increased urinary excretion of methylmalonate, because methylmalonyl coenzyme A is isomerized by a vitamin B_{12}-mediated reaction into succinyl coenzme A. In lactobacilli, and perhaps in man, ribotides are converted to deoxyribotides by a vitamin B_{12}-mediated reaction. We do not, however, know a great deal about the functions of vitamin B_{12} in man, and so far as the metabolism of nervous tissue is concerned our ignorance in this respect is almost complete. It was suggested some years ago by Wokes (1956) that when there is a lack of vitamin B_{12}, the sulphydryl system is inhibited and this leads to abnormal metabolism of tryptophan and tyrosine with consequent accumulation of toxic products. Weaver & Neill (1954) reported that there is excessive urinary excretion of amino acids and taurine in pernicious anaemia, and Swendseid, Wandruff & Bethel (1947 *a*,*b*) and Abbot & James (1950) suggested that interference with the normal metabolism of tyrosine and tryptophan could lead to an

excessive excretion of phenolic bodies. Whether this has anything to do with neurological changes is by no means clear.

Findings in the cerebrospinal fluid

From time to time various workers have sought to investigate the neurological complications of vitamin B_{12} deficiency and other disorders by estimating the content of vitamin B_{12} or folic acid in the cerebrospinal fluid (CSF) (Herbert & Zalusky, 1961; Worm-Petersen, 1962; Gjertsen & Schrumpf, 1962; Simpson, 1964; Herbert, 1964; Wells, 1965). There have been certain discrepancies, but most authors agree that both in normal persons and in those with vitamin B_{12} deficiency the vitamin B_{12} level of the CSF is considerably less than that of the serum, whereas the folate level of the CSF is two or three times that of the serum, even in folate deficiency. A very recent paper (Wells & Casey, 1967) has shown a positive correlation between the *Lactobacillus casei* folate activities of serum and CSF in hospital control patients. This is not a subject that I have pursued very actively, but in Table 1 there are given the CSF vitamin B_{12} levels in a number of non-anaemic hospital patients whose serum vitamin B_{12} levels were within the normal range of 150–800 pg/ml.

Table 1. *Vitamin B_{12} levels in the cerebrospinal fluid of seventeen non-anaemic hospital patients*

Method of determination	Vitamin B_{12} concentration (pg/ml)								
Euglena gracilis	3	6	7	9	10	14	14	20	
Lactobacillus leichmannii	N	N	10	10	13	14	17	20	27

N, negligible.

The values in Table 1 are only approximate since, particularly with *L. leichmannii*, it is not possible to measure such small concentrations with any degree of precision.

I wondered whether the low levels might indicate the presence of some agent that inhibited the growth of the test organism, but efforts to dilute out a hypothetical inhibitor were quite unsuccessful.

Pathological changes in the central nervous system in vitamin B_{12} deficiency

Time does not permit me to deal with this, but the changes in the spinal cord should no longer be seen by pathologists because the condition should not develop. It is one of patchy degeneration in the white matter of the posterior and lateral columns. First there is swelling of the medullary sheaths, then fatty degeneration and disappearance of the axis cylinders. There is, conspicuously, an absence of neuroglial proliferation.

Clinical problems

As I see it, the clinical problems related to the nervous system that require consideration are:

Peripheral neuropathy. This may occur in association with any anaemia of moderate or severe degree and may cause loss of vibration sense which, however, is not extensive and responds to antianaemic therapy. Paraesthesiae may be a feature.

Posterior column degeneration. This may occur from deficiency of vitamin B_{12}. Paraesthesiae are a prominent feature, and there is pronounced loss of vibration sense, particularly in the lower limbs. Position sense is impaired, and there is likely to be peripheral neuropathy, depression of knee and ankle jerks and perhaps some loss of motor power.

Postero-lateral degeneration. This is now seldom seen in vitamin B_{12} deficiency, but, when it occurs, there is, in addition to the above features, spasticity and ataxia; extensor plantar responses are likely to be found. Postero-lateral degeneration may occur without anaemia, particularly if a diet with a high folate content is taken or, of course, if folic acid is given. It is not uncommonly found in vegans, and a possible instance of nutritional origin has been reported recently (Riley, 1966). There have been two reports of the condition progressing despite treatment with vitamin B_{12} (Ellis, Breur, Owen & Laszlo, 1966; Riley, 1967), but in both cases the patient had intestinal malabsorption in addition to possible pernicous anaemia, so the story is a complicated one.

I should perhaps at this point refer to a paper by Cox (1962) who reported on the findings in 478 patients with untreated pernicious anaemia—a reasonable number to merit consideration. Twelve had only mental disturbances and no other neurological features. Of the remaining 466, 39% had no neurological features, 23% had paraesthesiae, 12% had posterior column signs, and 26% had evidence of postero-lateral degeneration. In contrast, Davidson (1957) reported paraesthesiae in 38% of 250 patients, ataxia in 6% and spasticity in 1%, while Cabot (1908) found paraesthesiae in 100% of 1200 patients, ataxia in 7% and spasticity in 4%.

Psychiatric disorders in vitamin B_{12} depletion. When folic acid was first introduced as a haemopoietic agent and its inability to control the neuro-psychiatric features of pernicious anaemia was not realized, I gave it to six patients who showed a gratifying haematological improvement, but one attempted to murder his wife!

The mental abnormalities of vitamin B_{12} deficiency can occur without anaemia (McAlpine, 1929; Strachan & Henderson, 1965), and possible features include confusion, forgetfulness, intellectual deterioration, delusions, paranoia, mania, incontinence and possibly epileptiform attacks. There has been controversy about the best way to screen psychiatric patients for vitamin B_{12} deficiency (Henderson, Strachan, Beck, Dawson & Daniel, 1966; Hansen, Rafaelson & Rødbro, 1966; Varadi, 1966), but a recent prospective controlled investigation suggests that routine screening is not necessary (Shulman, 1967). The number studied was, however, small, and it is certainly important to remember that mental change in a relative of a patient with pernicious anaemia may be due to vitamin B_{12} deficiency. Diffuse abnormalities may be found in the electroencephalograms of vegans, but, unlike those of pernicious anaemia, the abnormalities are not corrected by treatment with cyanocobalamin (West & Ellis, 1966).

Psychiatric disorders in folate depletion. This has not been accepted as a feature in pregnancy or malabsorption, but was reported by a normal subject himself suffering from experimental folate deficiency, as consisting of sleeplessness, forgetfulness and irritability (Herbert, 1962). It has also been described in elderly people suffering

from folate depletion (Strachan & Henderson, 1967), but there are problems of complicating arteriosclerosis and of the difficulties involved in confirming folate deficiency (Girdwood, Thomson & Williamson, 1967). It seems that in some areas of Great Britain folate deficiency in the aged is a considerable problem (Varadi & Elwis, 1967), whereas in others it is not, possibly because of differing dietetic habits. Nevertheless, it must always be borne in mind when an elderly person becomes disorientated or demented.

Possible neurological changes in folate depletion. Reference has already been made (p. 102) to the rarity of this.

Optic atrophy and retrobulbar neuritis. In deficiency of vitamin B_{12} or folic acid there may occur haemorrhagic retinopathy, impairment of visual acuity or disturbance of colour vision (Adams, Chalmers, Foulds & Withey, 1967; Gorrell, 1967). In addition, however, Heaton, McCormick & Freeman (1958), Heaton (1960) and Freeman & Heaton (1961) showed that the mean serum vitamin B_{12} level was reduced in tobacco amblyopia and suggested that the optic neuritis which is sometimes found in pernicious anaemia is tobacco amblyopia. Smith (1961) suggested that the neurological lesion in 'tobacco amblyopia' is due to chronic cyanide intoxication from tobacco smoke but it can occur in non-smokers (Adams *et al.* 1967), possibly from another source of cyanide. The condition is not related to the other neurological complications of vitamin B_{12} depletion. It is logical and more effective to treat 'tobacco amblyopia' or retrobulbar neuritis of vitamin B_{12} deficiency with hydroxocobalamin rather than cyanocobalamin (Chisholm, Bronte-Stewart & Foulds, 1967). It is possible that optic atrophy in prisoners of war in Japanese POW camps was due to vitamin B_{12} deficiency together with smoking, but although I saw many of these prisoners (Girdwood, 1950) I did not ask them about smoking habits.

Anticonvulsant drugs and megaloblastic anaemia. Finally, I must remind you that there may be megaloblastic anaemia responding to folic acid in patients under treatment with anticonvulsants, particularly primidone or phenytoin. Moreover, phenytoin is said to be neurotoxic (Utterback, 1958). The serum folate level is frequently low in patients treated with anticonvulsants, but there may be no significant difference between the levels in those with normoblastic and those with megaloblastic marrows (Reynolds, Milner, Matthews & Chanarin, 1966). The CSF folate level may also be low in drug-treated epileptic patients (Wells & Casey, 1967), but the significance of all this is uncertain.

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Copper: one man's meat is another man's poison

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Copper is widely distributed in nature and indeed the rich copper ores of the eastern Mediterranean made possible the brilliant civilizations of Egypt, of Crete and of Mycenae. Even away from areas of economically valuable copper deposits the metal is still present in most if not all soils and hence in the plants that grow on them. Copper is also present in the sea and it was probably its ready availability that led, early in evolution, to its use both for oxygen transport and as the final electron transfer oxidase for the reduction of molecular oxygen to water. Copper thus early became, and has since remained, an essential constituent of all living things (Adelstein & Vallee, 1961; Schubert, 1964); hence it is present in the normal diet in