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President C J Dickinson FRCP

with Section of Pathology

President G Dick MD

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Pathology of Vitamin Abnormality

Professor A V Hoffbrand
(Royal Free Hospital, Pond Street, London NW3 2QG)

Pathology of Folate Deficiency

Three main types of folate deficiency may affect man. The deficiency most frequently arises because of inadequate intake of the vitamin, and this is likely to occur when a diet deficient in fresh greens, fruit, nuts and liver is taken; particularly if demands for the vitamin are also increased, as in pregnancy, prematurity, chronic dialysis or in conditions of increased cell proliferation, e.g. hæmolytic anæmia, myelosclerosis, inflammatory diseases or malignancy. Malabsorption, e.g. in cœliac disease or tropical sprue, and anticonvulsant drug therapy, may also cause a general body deficiency of folate. A second type of folate deficiency, a metabolic defect, may be produced by treatment with a drug inhibiting folate metabolism, most commonly an inhibitor of the enzyme dihydrofolate reductase, e.g. methotrexate or pyrimethamine. These drugs deprive the body of the fully reduced (tetrahydro-) folates which form the active folate coenzymes needed for a variety of different biochemical reactions. The third type of deficiency is the metabolic defect of folate metabolism caused by lack of vitamin B_{12}, as in pernicious anæmia. Vitamin B_{12} deficiency, when severe, causes a secondary reduction in all the intracellular folate coenzymes. Although there is still discussion of the exact mechanism, it seems most probable that vitamin B_{12} deficiency causes 'trapping' of folate in its plasma form, methyltetrahydrofolate. This is thought to occur because vitamin B_{12} is needed as a cofactor in the homocysteine-methionine reaction by which methyltetrahydrofolate entering cells from plasma is 'demethylated' to release tetrahydrofolate, from which all the intracellular folate coenzymes are subsequently made (Lavoie *et al.* 1974).

A wide variety of tissues may be affected by all three types of folate deficiency (Table 1), so that a number of clinical lesions may occur. Folate is required in three reactions in DNA synthesis, one in pyrimidine synthesis (thymidylate synthesis) and two in purine synthesis. Thymidylate synthesis is a rate-limiting reaction in DNA synthesis and the most rapidly dividing tissues of the body suffer the main effects of the deficiency. The organs most affected are the bone marrow, the epithelial cell surfaces and the gonads. The defects produced in the bone marrow include a fall in red cell, white cell and platelet counts, due partly to death of their precursors in the marrow (ineffective hæmopoiesis) and partly to shortened survival of the cells that do reach the peripheral blood. The circulating

Table 1

General effects of folate deficiency

Well established
(1) Megaloblastic anæmia
(2) Impaired proliferation and macrocytosis of epithelial surfaces: glossitis, angular cheilosis, impaired intestinal absorption; and oral and gastrointestinal ulceration (established for therapy with dihydrofolate reductase inhibitors only)
(3) Sterility
(4) Skin pigmentation

Less well established
(1) Complications of pregnancy: anti- and post-partum hæmorrhage, congenital malformations, prematurity, &c
(2) Inhibition of growth in childhood
(3) Inhibition of liver regeneration
(4) Impaired bone turnover: established for vitamin B_{12} deficiency only
(5) Mental effects: dementia, schizophrenia-like state
(6) Neurological effects: protection from fits, peripheral neuropathy, spinal cord damage, mental retardation, brain damage

red cells are macrocytic and their precursors megaloblastic, the neutrophils show hypersegmented nuclei and there are giant metamyelocytes and hypersegmented megakaryocytes in the bone marrow. These changes are identical whether folate or vitamin B_{12} deficiency is the underlying cause.

The degree of anæmia, leukopenia and thrombocytopenia depends both on the severity of the deficiency and on its speed of onset. When the deficiency is of acute onset and severe, as with large doses of methotrexate, thrombocytopenia and leukopenia are more likely to occur than anæmia, because of the shorter life-span of neutrophils and platelets than red cells in the circulation. On the other hand, when the deficiency is of slow onset, anæmia is likely to occur before neutropenia or thrombocytopenia. The bone marrow precursors, transformed lymphocytes and dividing epithelial cells all show chromosome abnormalities: random breaks, centromere spreading and despiralization. A fall in peripheral lymphocytes as well as in granulocytes occurs in severe megaloblastic anæmia, presumably because lymphocyte formation is impaired, while a mild general reduction in immunoglobulins has been described and related to impaired proliferation of plasma cells. Selective nutrient deficiency affecting one cell line rather than another, e.g. lymphocytes and not bone marrow, has been described (Longo *et al.* 1975) and further studies of this phenomenon will be of considerable interest.

The changes in the epithelia show clinically as glossitis, angular cheilosis and in some cases as a malabsorption syndrome (Scott *et al.* 1968). Histologically, there are changes in the lining epithelium of the mouth, bronchi, bladder and cervix uteri. Again, changes localized to one tissue have been described (Lindenbaum *et al.* 1975). The jejunal mucosa also shows changes in B_{12} deficiency (Foroozan & Trier 1967) but probably not in pure nutritional folate deficiency, because of protection of the jejunum, which may have first use of what folate is ingested. Acute folate deficiency produced by methotrexate may cause ulceration of the mouth and of the upper intestinal mucosa, even before peripheral blood changes are marked, but whether folate or B_{12} deficiency cause ulceration of the buccal or gut mucosa is uncertain. Recent reports suggesting that the deficiencies may be associated with recurrent oral ulceration (*British Medical Journal* 1974) need to be confirmed. Tropical sprue in its early acute stage may be improved by folic acid therapy alone, but most patients also need antibiotic therapy for a complete cure. Whether folate deficiency simply predisposes to tropical sprue and aggravates the intestinal malabsorption syndrome due to some other primary cause, or whether it has a more fundamental role in the pathogenesis of the disease, remains obscure.

The effect of severe folate deficiency on the gonads is to cause sterility in both males and females. Methotrexate is indeed a powerful abortifacient. Less well established effects of folate deficiency in pregnancy are an increased frequency of congenital malformations, antepartum and postpartum hæmorrhage, abruptio placentæ, and prematurity. It is unlikely, however, that folate deficiency of the clinical severity usually encountered in pregnancy causes any of these effects except prematurity (Baumslag *et al.* 1970, Pritchard *et al.* 1970). The widespread use of prophylactic folic acid therapy in pregnancy has, in any case, substantially reduced the opportunity to study these associations further.

A more firmly established but poorly understood clinical effect of folate deficiency is to cause reversible, widespread pigmentation affecting the skin generally, most marked in the skin creases and round the nail-beds. The biochemical basis for this pigmentation is not clear. Pteridine cofactors are required for tyrosine and tryptophane hydroxylation, but no definite role for folate in these reactions or any other reaction concerned with melanin formation has been established.

The overall function of a previously normal liver is not impaired in clinical folate or vitamin B_{12} deficiency, although some disturbances, e.g. impaired breakdown of formininoglutamic acid (Figlu), can be detected by sensitive biochemical techniques. In two special circumstances, however, a clinically significant effect on liver function has been described. Regeneration of cirrhotic liver has been shown to be impaired in folate-deficient alcoholics in the USA (Leevy 1966) and numerous studies have documented fibrosis of the liver in patients with psoriasis given prolonged courses of methotrexate (Muller 1970, Warin *et al.* 1975).

Vitamin B_{12} deficiency has been found to cause a fall in serum alkaline phosphatase to subnormal levels, with correction to normal when vitamin B_{12} is administered (van Dommelen *et al.* 1964). Since the effect was particularly marked in a patient with Paget's disease of bone, it was postulated that alkaline phosphatase from bone was particularly lowered, implying that vitamin B_{12} deficiency reduced osteoblast activity. As yet, a similar phenomenon has not been shown for 'pure' folate deficiency, nor has a fall in intestinal alkaline phosphatase in vitamin B_{12} or folate deficiency been adequately excluded.

A single report has suggested that children with severe folate deficiency due to a hæmolytic anæmia fail to grow correctly until folate is administered (Watson-Williams 1965), and it might be supposed that a similar situation could result from folate deficiency in cœliac disease. However, in no single

case has a growth response to folic acid been documented in the absence of other therapy, e.g. antibiotics for associated infections in sickle-cell disease or a gluten-free diet in cœliac disease.

Finally, it is worth while to review briefly the question of what effect, if any, folate deficiency has on the nervous system. Vitamin B_{12} deficiency is known to cause an organic neuropathy, and although the biochemical basis for this remains unknown, it is not generally considered that the mechanism for the neuropathy is the same as that for anæmia. Indeed, patients with vitamin B_{12} neuropathy may have little anæmia and often show high serum folate levels, while folic acid therapy aggravates, rather than improves, the neuropathy.

The various neurological syndromes that have been described in patients with folate deficiency or a defect of folate metabolism include: mental retardation associated with inborn errors of folate metabolism (Arakawa 1970, Tauro *et al.* 1976), a peripheral neuropathy or posterior lateral column lesion associated with nutritional folate deficiency, often in epileptics or the aged (Grant *et al.* 1965, Manzoor & Runcie 1976), brain and spinal cord damage associated with intrathecal methotrexate therapy (Kay *et al.* 1972), an organic dementia associated with nutritional folate deficiency in the elderly (Strachan & Henderson 1967), a schizophrenia-like illness associated with homocystinuria in a child with an inborn error of folate metabolism (Freeman *et al.* 1975), and an impaired psychological state with relative protection from fits associated with folate deficiency in epileptics (Reynolds 1973). The extensive literature on all these aspects has recently been comprehensively reviewed by Reynolds (1976) and the reader is referred to that article for an excellent survey of this subject.

The best substantiated of these effects is the neurological damage caused by methotrexate. Whether nutritional folate deficiency, even when severe, causes more than minor psychological changes remains an open question. Double-blind trials have not shown that folate therapy increases fit frequency in epilepsy, while a cause-and-effect relation has not yet been satisfactorily demonstrated between nutritional folate deficiency and organic neurological damage. Moreover, the suggestion that methyltetrahydrofolate may be involved in the brain in the methylation of catecholamines and indole-amines (Leysen & Laduron 1974) has not been confirmed as a physiological pathway (Meller *et al.* 1975). S-adenosylmethionine remains the most likely methyl donor in the brain and, as yet, no special important role for folate in the central nervous system has been proven.

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Dr J Andrews
(West Middlesex Hospital, Isleworth)

Nonscorbutic Effects of Vitamin C Deficiency: Clinical Aspects

In scurvy produced experimentally, the more complete the dietary deficiency of vitamin C the more quickly the first signs of scurvy present themselves (Crandon *et al.* 1940, Hodges *et al.* 1969). Scurvy observed clinically generally presents as part of a mixed deficiency.

Russell *et al.* (1968) have suggested that low vitamin status may play a part in maintaining