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Historical Review

THE HISTORY OF FOLIC ACID

'Haematology has advanced more rapidly in the last 10 years than almost any branch of medicine. Current haematological literature is so prolific that it is increasingly difficult for anyone but a specialist to keep up to date'. These sentences, which many haematologists consider could have been written in any decade in the second half of the twentieth century, are the opening two sentences of the preface to the First Edition of Janet Vaughan's monograph 'The Anaemias' (Vaughan, 1934).

What were these advances in the period 1924–34? Largely they concerned the nutritional anaemias with the well-known discovery by Minot and Murphy of a cure for pernicious anaemia by feeding large amounts of liver, the description of 'intrinsic and extrinsic factors' by Castle, and the discovery by Lucy Wills (Fig 1) of a factor in yeast subsequently shown to be folate that corrected macrocytic anaemia of pregnancy.

The story of Lucy Wills has been reviewed by Daphne Roe (1978). She was born in England in 1898 and went to school at Cheltenham College for Young Ladies under the tutelage of Miss Beale and Miss Buss, pioneers of women's education. In 1911, she obtained a double first honours degree in Botany and Geology at Newnham College, Cambridge, and after the First World War, being interested in Freud's work, intended to specialize in Psychiatry. She became a medical student at the London School of Medicine for Women, later to become the Royal Free Hospital School of Medicine. She then worked in Chemical Pathology and in 1928, with a grant from the Tata Trust, went to Bombay to investigate macrocytic anaemia in pregnancy, prevalent in female textile workers. The fact that the anaemia was most frequent in poorer populations with diets deficient in protein, fruit and vegetables led Wills to study the effects of changes in diet on the macrocytic anaemia of albino rats produced by a deficient diet and Bartonella infection. The anaemia was prevented by yeast added to a diet otherwise lacking B vitamins (Wills & Mehta, 1930, 1931). Yeast or a yeast extract ('Marmite') was then found to correct the macrocytic anaemia in the pregnant Bombay patients (Wills, 1931). Lucy Wills continued active research in the field on her return to England in 1932. She kept hand-written day books (now in the possession of the Royal Free Hospital Archives) with detailed clinical and laboratory records of those patients with macrocytic anaemia who she treated with yeast extract (Fig 2) or, after 1945, with the newly synthesized folic acid, given to her by Tom Spies. She was a strict and stimulating teacher of medical students (all female) at the Royal Free School of Medicine. She is remembered as aristocratic, independent and radical in outlook, critical of established conservative medical and scientific committees. She rode to work on a bicycle rather than in a large car as did many of her colleagues. Lucy Wills retired soon after the War, developed a botanical garden and became a Labour Councillor in Chelsea. She died in 1964.

Janet Vaughan, who has been the subject of an earlier article in this Archive series (Firkin, 2000), found that children with coeliac disease and adults with idiopathic steatorrhoea suffering from 'megalocytic hyperchromic anaemia' also showed haematological responses to Marmite (Vaughan, 1932). Wills & Evans (1938) reported that patients with tropical macrocytic anaemia were cured by injections of crude liver extract ('Campolon') or by feeding autolysed yeast extract, after failing to respond to a purified liver extract ('Anahaemin') active in patients with Addisonian pernicious anaemia. Further evidence for the existence of a nutritional factor in liver other than that active in pernicious anaemia was provided when the observations on the efficacy of different liver preparations in the treatment of tropical macrocytic anaemia were confirmed in nutritional megaloblastic anaemia, megaloblastic anaemia of pregnancy and idiopathic steatorrhoea in non-tropical countries (Davidson *et al*, 1942; Fullerton, 1943; Watson & Castle, 1943).

Folic acid received its name in 1941 when it was isolated from spinach (folium = leaf (Lat.)] and was shown to be a growth factor for *Streptococcus lactis R* (*S. faecalis*) (Mitchell *et al,* 1941). A large number of other names were given (Table I). The compound was subsequently synthesized in pure crystalline form in 1943 by Bob Stokstad working at Lederle Laboratories (American Cyanid Company) in Pearl River, New York, and by Angier *et al* (1945). This proved that folic acid was composed of a pteridine ring, paraminobenzoic acid and glutamic acid, and was called 'pteroylglutamic acid' (PGA) (Fig 3). Soon after its synthesis, it became apparent that natural folates usually differed from pteroylglutamic acid in three respects: (1) additional glutamate residues ('polyglutamates'), (2) reduction to di- or tetra-hydroforms, and (3) additional single carbon units, e.g. methyl (–CH3), formyl-CHO, methylene = CH_2, methenyl = CH_4 attached to the N_5 or N_{10} nitrogen atoms (Fig 3). Folic acid (pteroylglutamic acid) is now used to denote the fully oxidized chemical compound, not present in natural foods. The term 'folate' is used to denote the large group of compounds possessing the same vitamin activity and includes natural folates and folic acid. Bob Stokstad returned to his Alma mater at Berkeley in 1963 where

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Fig 1. Lucy Wills – Honorary Consultant Pathologist to the Royal Free Hospital and graduate of Cambridge and the Royal Free Hospital School of Medicine. She worked in India for some years and was internationally recognized for her research on anaemias of pregnancy, one of which bears her name. She was independent and radical in outlook and a strict and stimulating teacher.

he and colleagues were the first to isolate, purify and characterize many of the mammalian enzymes involved in folate metabolism (Stokstad, 1979).

Soon after the synthesis of folic acid in 1945, it became apparent that it was effective in the treatment of megaloblastic anaemia of all types, but particularly those which had previously been found refractory to refined liver preparations, such as the megaloblastic anaemia of sprue (Hanes, 1942; Davidson *et al*, 1947), coeliac disease, pregnancy and malnutrition. It was also found to be temporarily effective in curing anaemia in Addisonian pernicious anaemia (Moore *et al*, 1945; Vilter *et al*, 1945), but it became apparent that the anaemia relapsed and neurological damage was not improved by folic acid (Amill & Wright, 1946) and was even made worse or precipitated by this therapy (Vilter *et al*, 1946; Hall, 1947; Heinle & Welch, 1947; Meyer, 1947).

ISOLATION OF VITAMIN B_{12}

The isolation of vitamin B_{12} (cobalamin) from liver (Rickes *et al*, 1948; Smith & Parker, 1948) soon led to the realization that this, not folic acid, was Castle's extrinsic factor and was the vitamin necessary for treatment of both the anaemia and neuropathy in Addisonian pernicious anaemia. It became clear that deficiency of either vitamin caused the same appearance of the bone marrow. The availability of folic acid and vitamin B_{12}, in pure form, both capable of producing a cure of megaloblastic anaemia, initiated the present phase of studies in the megaloblastic anaemias at a biochemical level.

FOLATE DEFICIENCY

Two laboratories and the research workers from them (some of whom appear in Fig 4), who subsequently set up their own research laboratories, dominated further research in folate in relation to haematological aspects. One was that of Victor Herbert, initially in Boston at the Thorndyke Laboratories where William Castle had demonstrated the presence of extrinsic and intrinsic factors, subsequently in New York at Mount Sinai and the Bronx Hospitals. The other was that of David Mollin at the Royal Postgraduate Medical School and Hammersmith Hospital in London. Herbert, a cousin of the Irish American composer of the same name, was born in the Bronx and served as a paratrooper in the US army. He was joined by a large number of talented researchers, many of whom went on to establish their own research in the field: Ralph Zalusky, Louis Sullivan (later to become Chief Advisor in Health to the US Government), Jack Metz, Sam Waxman, Ralph Green, Ralph Carmel, Neville Coleman, Dan Longo and others. This was the time when the microbiological assays for serum vitamin B_{12}, serum and red cell folate dominated the field. Victor Herbert performed the famous experiment in which he ate a folate-deficient diet for 4 months and monitored the sequence of events, haematologically and by assay for folate in blood. He showed that it took about 4 months for megaloblastic anaemia to develop. He incidentally developed severe potassium deficiency. He subsequently took a degree in law and became an expert in nutrition generally, particularly crossing swords with advocates of unproven 'health' additions to diet.

David Mollin, born in 1917 in a Welsh mining village, maintained his Welsh character, and was a charismatic, emotional and occasionally irascible radical. His unit, dedicated to research in the megaloblastic and sideroblastic anaemias, was in a small Nissen hut. Christopher Booth, Alan Waters, Jim Kohn, Israel Chanarin, Fred Klipstein, Victor Hoffbrand, Barbara Anderson and Beverley Jackson (née Newcombe) among others worked in this physically cramped and emotionally highly charged hut.

Other major centres for the study of folate grew up in London, Montreal, Israel and South Africa, and elsewhere. In Montreal, Louis Lowenstein, Bernard Cooper and Michael Whitehead published on maternal nutrition and inherited abnormalities of vitamin B_{12} and folate metabolism, leading to Montreal, under the direction of David Rosenblatt, now being the main centre for research in these rare but clinically and scientifically important defects.

In Israel, Moshe Rachmilewitz, Professor of Medicine at the Haddassah Medical School in Jerusalem, with his colleagues Nathan Grosowicz and Gabriel Izak recognized the value of red cell folate as a measure of folate status and performed various studies of folate deficiency, including

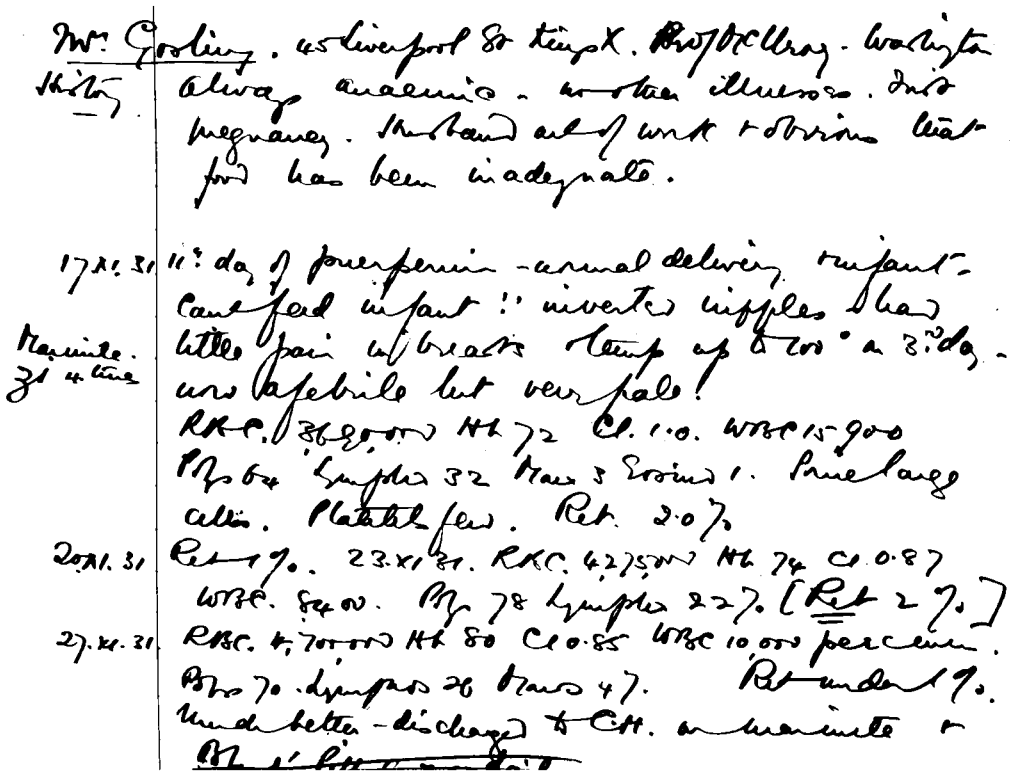

Fig 2. A page in Lucy Wills' handwritten notebook of 1931 in which she documents treatment with Marmite in a patient with anaemia in pregnancy.

studies in Burmese pregnant women with subsequent major work on the *trans*cobalamins.

Jack Metz became Director of the South African Institute for Medical Research. With associates he performed substantial studies of folate deficiency in pregnant and lactating women and infants. They showed that prophylactic folic acid reduced the incidence of prematurity in undernourished populations.

Israel Chanarin, originally from South Africa but subsequently based in London, made major contributions to knowledge in all aspects of folate metabolism, particularly showing that increased demands for folate underlie folate deficiency in pregnancy and many bone marrow and other disorders. He and Irwin Rosenberg in the USA showed that dietary folates are reduced, methylated and deconjugated to 5-methyl tetrahydrofolate (THF) during absorption. He

Table I. The discovery of folic acid.

1930, 1931	Wills & Mehta	Dietary anaemia in rats prevented by yeast extract
1931	Wills	Yeast or Marmite prevents macrocytic anaemia of pregnancy
1932	Vaughan	Marmite corrects anaemia of coeliac disease
1938	Wills & Evans	Purified liver extracts do not correct nutritional, pregnancy or macrocytic anaemia
1938	Day *et al*	Vitamin M corrects nutritional anaemia in monkeys
1938	Stokstad & Manning	Factor 'S', vitamin B_C present in yeast, prevents
1940	Hogan & Parrott	nutritional anaemia in chickens
1940	Snell & Peterson	Norit eluate factor – factor absorbed from yeast or liver is growth factor for *Lactobacillus casei*
1941	Mitchell *et al*	'Folic acid' receives name and shown to be a growth factor for *Streptococcus lactis R* (*S. faecalis*)
1943	Fullerton; Watson & Castle	Idiopathic steatorrhoea megalobastic anaemia responds to crude liver extracts or yeast extract
1943	Wright & Welch	Enzyme hydrolysing folate polyglutamates to microbiologically active forms (monoglutamates) – folate conjugase
1944	Binkley *et al*	Yeast extracts potent as a source of vitamin B_C only 2–5% as active for *L. casei*. Need enzymatic digestion to equalize activity
1945	Angier *et al*	Synthesis of folic acid and called pteroylglutamic acid
1945	Day *et al*	Purified *L.casei* factor is vitamin M
1946	Pfiffner *et al*	Naturally occurring folate in liver is a heptaglutamate

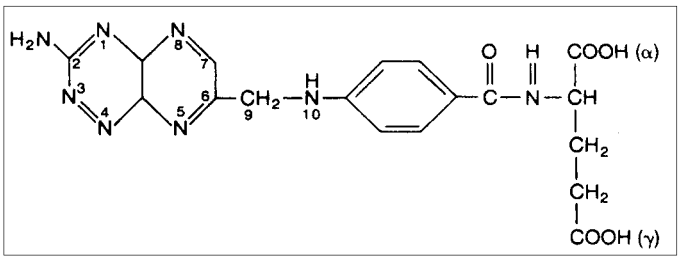

Fig 3. The structure of folic (pteroylglutamic) acid.

wrote three editions of the major monograph on the megaloblastic anaemias (Chanarin, 1990).

The 1950s and 1960s saw the elucidation of the biochemical reactions involving folates in single carbon unit transfer in amino acid conversions including homocysteine conversion to methionine and in purine and pyrimidine synthesis (reviewed by Blakley, 1969) (Fig 5). One of these reactions emerged as particularly important in DNA synthesis: thymidylate synthesis in which deoxyuridine monophosphate (dUMP) is methylated by 5,10 methylene THF to thymidine monophosphate (dTMP). In Denmark, Sven Killmann (Killmann, 1964) introduced the deoxyuridine suppression test that assesses the integrity of this reaction. This was used in the Herbert, Chanarin, Hoffbrand, Metz, Vander Weyden and Wickramasinghe laboratories among others as a differential test for folate and vitamin B_{12} deficiency, and to investigate vitamin B_{12}/folate inter-relations *in vitro*. In Mollin's laboratory, the test was also used to show that exposure to nitrous oxide inactivated vitamin B_{12}. Scott and Weir subsequently showed that nitrous oxide inhibited methionine synthase (reviewed by Weir & Scott, 1995).

Noronha & Silverman (1962) and Herbert & Zalusky (1962) hypothesized that there was failure of conversion of methyl THF to THF in vitamin B_{12} deficiency with a consequent increase in methyl THF in plasma but a shortage of other folate forms in cells, the 'methyl folate trap' hypothesis. Subsequent research showed, albeit indirectly, that there was a failure of folate polyglutamate synthesis in vitamin B_{12} deficiency and that this was as a result of a lack of the correct (THF) substrate for glutamate addition (Lavoie *et al*, 1974). They hypothesized that methyl THF (entering cells from plasma) was not a suitable substrate for folate polyglutamate synthesis. Shane's laboratory subsequently showed directly that the substrate for the mammalian enzyme folyl-polyglutamate synthase was either THF or formyl THF but not methyl THF (Shane, 1989). Polyglutamate forms of folate are the active intracellular folate coenzymes (including those for thymidylate synthesis) and are also needed to retain folates in the cell. Assays for the immediate DNA precursors, the deoxynucleoside triphosphates, in human cells were established for the first time. Surprisingly, the concentration of deoxythymidine triphosphate (dTTP) was not selectively lower in megaloblastic compared with normal cells even though the anti-folate methotrexate lowered the level rapidly (Hoffbrand *et al*, 1974). Nevertheless, Hoffbrand and co-workers hypothesized that the DNA defect in folate (or vitamin B_{12} deficiency) arose from thymidylate starvation, suggesting that replicating segments of DNA (replicons) opened up during mitosis but remained incomplete through lack of dTTP. Wickramasinghe & Hoffbrand (1980) subsequently suggested that the apparent paradox was as a result of compartmentalization, lack of dTTP at the DNA replicating fork being masked by dTTP in the rest of the cell, acquired largely from salvage of preformed thymidine derived from dying cells. Goulian, Luzzatto, Wickramasinghe and others have suggested, on the other hand, that

Fig 4. Many of the research workers in folate gathered at the NIH, Bethesda, USA. Front row: Drs. Helmut Mueller, John M. Scott, Charles E. Butterworth, Jr., Samuel Waxman, Victor Hoffbrand, Sheldon E. Rothenberg, Joseph R. Bertino. Middle row: Drs. E. L. Robert Stoksad, Ronald H. Girdwood, Charles H. Halstead, Conrad Wagner, Harry P. Broquist, ––, V. Michael Whitehead, I, Charnarin, Carlos L, Krumdieck. Back row: Drs. Victor Herbert, Edward R. Eichner, John Lindenbaum, Erik M. Magnus, Donald W. Horne, Bernard A. Cooper, K. Hoppner. Photograph by Dr. Neville Colman.

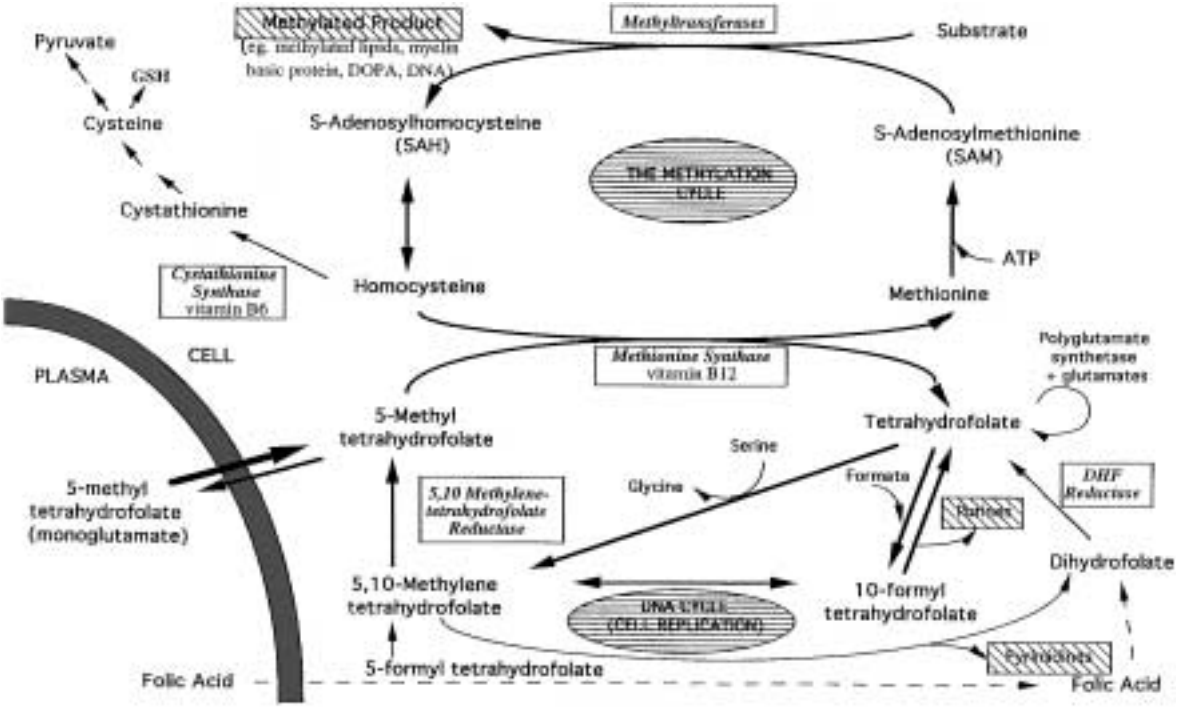

Fig 5. The metabolism of folate. DHF, dihydrofolate (courtesy of Professor John Scott).

megaloblastic DNA is defective through the misincorporation of uracil instead of thymine into DNA because of the pile-up of dUMP and dUTP in cells in which thymidylate synthesis is blocked (Wickramasinghe, 1995).

ANTI-FOLATES

The observation soon after its discovery that folic acid therapy enhanced the growth of tumours led to research into folate antagonists at the laboratories of Nobel Laureates G. H. Hitchings and G. B. Elion. Aminopterin was the first antagonist given to humans by Sydney Farber and colleagues (Farber *et al,* 1948), followed by its close analogue methotrexate. His demonstration that methotrexate was effective in childhood acute lymphoblastic leukaemia led to the modern post-war development of a wide variety of anti-cancer agents that inhibit normal metabolic reactions. Methotrexate inhibits the enzyme dihydrofolate reductase (DHFR), which is required to return folate, oxidized by the thymidylate reaction, from the metabolically inactive DHF to the active THF state. Subsequent work at the Wellcome Laboratories developed trimethoprim, which selectively inhibits bacterial DHF. It is particularly effective as an antibacterial agent when combined with a sulphonamide that inhibits bacterial synthesis of the para-amino benzoic acid ring needed for synthesis of folate. Pyrimethamine, another DHFR inhibitor, which is effective particularly against the malarial enzyme, was also introduced.

METABOLISM OF HOMOCYSTEINE

Homocysteine (tHcy) was initially isolated by Du Vigneaud

Fig 6. James D. Finkelstein.

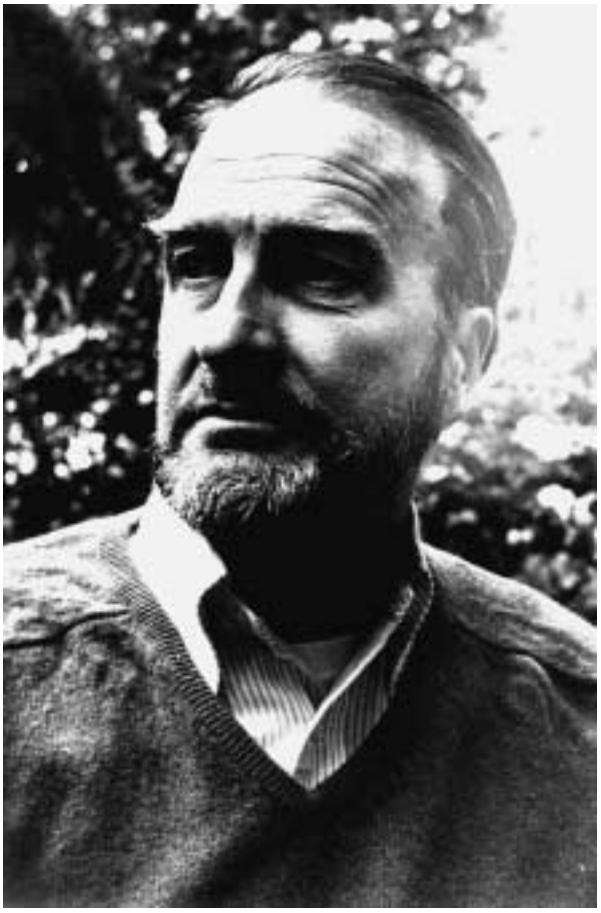

Fig 7. Harvey S. Mudd.

(1950). However, its clinical significance was only discovered in 1972 when Nina Carson and her colleagues in Northern Ireland (Carson & Neill, 1962) and Gerristen and Weisman in Wisconsin (Gerritsen *et al*, 1962) described the clinical syndrome associated with homocysteinuria, namely mental retardation, skeletal malformation and previous thromboembolic disease.

Events then moved to the National Institute of Health, Bethesda, where Leonard Laster was making a particular study of patients who had 'inborn errors of metabolism'. One such patient was an eight-year-old girl with homocysteinuria associated with high methionine levels. This girl was referred to a team consisting of James Finkelstein (Fig 6), Harvey Mudd (Fig 7) and Fil Irreverre to investigate (Mudd *et al*, 1964). They demonstrated that the cause of the homocysteinuria was deficiency of cystathionine synthetase, which controls the *trans*-sulphuration pathway for homocysteine metabolism. What they also showed was that, as well as the *trans*-sulphuration pathway, tHcy was being remethylated to methionine, which produced the high levels of methionine in their patient. The remethylation pathway was via methyl THF- and vitamin B_{12} (cobalamin)-dependent methionine synthase. Although this pathway had been described by Du Vigneaud and Mary Bennett (Bennett, 1950), its significance in the metabolism of tHcy had not been appreciated. Finkelstein, working on rats, and Mudd, working subsequently in humans, went on to demonstrate that remethylation was the dominant pathway for tHcy metabolism, the *trans*-sulphuration pathway being used at times of methionine excess, such as immediately after a meal (as reviewed in Mudd, 1988). The structure and mode of action of methionine synthase has subsequently been elucidated by Rowena Matthews and Ruma Bannerjee and their colleagues (Bannerjee & Matthews, 1990).

The function of methyl THF is the remethylation of homocysteine. It is synthesized by methylenetetrahydrofolate reductase (MTHFR) which, under physiological conditions, is a one-way reaction. The fundamental work on the structure and function of this enzyme has been described (Matthews & Haywood, 1979). The function of this enzyme had been shown by Stokstad to be allosterically regulated by the product of the remethylation pathway, S-adenosyl methionine (SAM), the so-called 'universal methylator' of internal metabolism (Kutzback & Stokstad, 1967). Conrad (Connie) Wagner went on to show that methyl THF also inhibits glycine methyl transferase, thus acting as a method of controlling excess SAM-dependent transmethylation at times of methionine excess (Wagner *et al*, 1985). The clinical manifestations of the MTHFR mutations have been described elsewhere (Rosenblatt, 1989).

Another significant advance was the work of Kang and Wong and their colleagues who initially defined folate protein binding and then went on to describe the thermolabile form of MTHFR which occurs in around 10% of the Caucasian population (Kang *et al*, 1988). This has been shown to have important associations with a variety of clinical conditions (*vide infra*). The genetic defect for this mutation was subsequently described by Rima Rozen and colleagues in Canada and the Netherlands (Frosst *et al*, 1995).

VASCULAR DISEASE

Finkelstein and Mudd went on to investigate a further patient with another form of homocysteinuria in the Massachusetts Grand Hospital. This patient had homocysteinuria, methylmalonic aciduria and, paradoxically, a low methionine level. The patient had impaired cobalamin function, which caused malfunction of methionine synthase and, accordingly, defective remethylation of homocysteine (Mudd *et al*, 1969). This child subsequently died and the autopsy performed by Kilmer McCully (McCully, 1969) demonstrated marked vascular pathology, which led him to postulate an association between vascular disease and hyperhomocysteinaemia. This was followed by the observations of Bridget & David Wilken (Wilken & Wilken, 1976) who found abnormal methionine metabolism in 20% of their patients with unexplained atherosclerotic cardiovascular disease.

Further evidence had to await the development of methodology for the measurement of total homocysteine (tHcy) in the plasma (Stabler *et al*, 1987; Refsum *et al*, 1989). Atherosclerotic disease of the cerebral, coronary and peripheral vasculatures was subsequently shown to be

associated with a mild to moderate elevation of serum tHcy by Lars Brattstrom and colleagues in Sweden (Brattstrom *et al,* 1984), Malinow et al (1990) and Jacob Selhub and colleagues (Selhub *et al,* 1995) in the USA, Clarke *et al* (1991), Graham *et al* (1997) in Ireland, Ubbink *et al* (1991) in South Africa, and many others (see reviews by Welch & Loscalzo, 1998; Hankley & Eikelboom, 1999; Malinow *et al*, 1999; Christen *et al,* 2000; Perry, 2000). Low serum folate levels have also been associated with arterial occlusive disease (see reviews by Green & Jacobsen, 1995; Morrison *et al*, 1996; Rosenberg, 1996; Verhoef *et al*, 1996). Meta-analysis shows that the polymorphism in the enzyme 5,10 methylene tetrahydrofolate reductase is not associated with cardiovascular disease. Lars Brattstrom showed that taking folic acid was associated with significant falls in the serum tHcy (Brattstrom *et al*, 1988). Boushey *et al* (1995) compared the probable benefits of lowering serum homocysteine by giving folic acid with that of lowering serum cholesterol with lipid lowering agents. However, it has yet to be shown that folic acid has any primary preventative function in these diseases, although preliminary data are encouraging (Verneulen *et al*, 2000). The results of ongoing trials of folic acid in the prevention of cardiovascular disease are eagerly awaited.

BRAIN DISEASE

Recent evidence from Robert Clarke's laboratory in Oxford has suggested that hyperhomocysteinaemia may be associated with disease of the microvasculature supplying the hippocampal areas of the brain, resulting in vascular dementia, Alzheimer's disease and Parkinson's disease (Clarke *et al*, 1998). In a further paper from the Nun study, the degree of brain damage in these patients was found to be inversely related to blood folate levels, suggesting, but not proving, a cause and effect relationship (see review Weir & Molloy, 2000).

Whether folate deficiency can cause the type of neurological disease found in patients with cobalamin deficiency has long been a contentious issue. However, Robert Surtees *et al* (Surtees *et al,* 1981) have produced evidence that malfunction of any of the three enzymes involved with the synthesis of S-adenosyl methionine, the universal methylator of internal metabolism, namely methylene reductase, methionine synthase and methionine adenosine transferase, induces a brain disease that is similar to that seen in cobalamin deficiency. Evidence was also produced suggesting that treatment with the products of these enzymes, namely methylfolate, methionine and S-adenosyl methionine, respectively, could ameliorate the neurological lesion. This supported earlier work by John Scott and Donald Weir in Dublin that nitrous oxide (N_2O) inhalation in monkeys and, subsequently, in pigs induced a neuropathy similar to cobalamin deficiency neuropathy in man. It was shown that N_2O inhibited methionine synthase and that the neurological lesion could be ameliorated by taking oral methionine (see review Weir & Scott, 1995).

NEURAL TUBE DEFECTS (NTDS)

The evidence that folate deficiency plays an important part in the pathogenesis of NTDs is now beyond doubt. The original suggestive evidence came from case reports of patients receiving anti-folate chemotherapy during pregnancy being associated with an NTD-affected pregnancy. This was followed by studies in a variety of experimental animals, which confirmed the tetratogenicity of anti-folates. Simple folate deficiency, however, did not produce NTDs in mice.

The original suggestion that folate deficiency, apart from causing megaloblastic anaemia, might also play a part in producing NTDs was made by Brian Hibbard (1964), who demonstrated an association between folate deficiency and NTDs. This was supported by others including Smithells et al (1976) who showed that patients with megaloblastic anaemia of pregnancy had a high incidence of NTDs. Both Hibbard and Smithells worked as obstetricians and gynaecologists among the poor communities of Liverpool. They were the first to suggest that folate deficiency might lead to complications of pregnancy other than megaloblastic anaemia. These included abruptio placentae, antenatal haemorrhage and prematurity, as well as NTDs (anencephaly, encephalocoele and spina bifida) in the fetus. This was supported by a small folate intervention trial run by Laurence *et al* (1981). The final proof of the value of folic acid in prevention of NTDs was the Medical Research Council (MRC) trial (MRC Vitamin Study Research Group, 1991). This trial established that folic acid therapy, 4 mg daily, given periconceptually afforded about 75% protection to pregnant women who had already given birth to an NTD-affected infant from having a second child with the same defect. Prevention by folic acid of a first occurrence of NTD when given periconceptually was subsequently proven by Czeizel & Dudas (1992) in Hungary. Scott and Weir in Dublin went on to relate the incidence of first-time NTDs to blood folate and vitamin B_{12} levels in the mother (Kirke *et al*, 1993; Scott *et al*, 1995). They showed a decreasing incidence of NTDs with higher maternal serum and red cell folate levels well into the accepted normal ranges. They also showed that the addition of 400 μg of folic acid into the diet is needed to raise red cell folate levels to those associated with a 75% reduction in NTD incidence in at-risk mothers (Daly *et al*, 1998). They (Whitehead *et al*, 1995) and Van der Putt et al (1995) in the Netherlands have shown that the common mutation of the enzyme MTFHR C677T occurs more frequently in the affected NTD fetus and, therefore, in their parents than in controls, and is also associated with lower serum and red cell folate levels than controls (Shields *et al*, 1999). Wald, Scott and Hoffbrand have recently served on the Working Group on Folic Acid for the Committee on Medical Aspects (COMA) of Food and Nutrition policy of the Department of Health (DOH). This has recommended fortification of the British diet with folic acid added to flour (240 μg/100 g flour) as a means of reducing the incidence of NTDs. Fortification of food with folic acid at a lower dose is already carried out in the USA. The results of this fortification on the folate status of the USA population and

the evidence on NTD incidence are now being analysed (Jacques *et al*, 1999; Mills, 2000). The relationship between folate status and NTDs remains unclear, but there is some evidence that accumulation of homocysteine and S-adenosyl homocysteine in the fetus may be relevant. The role of folate in pregnancy has been fully reviewed elsewhere (Scott & Weir, 1998).

OTHER CLINICAL CONDITIONS ASSOCIATED WITH FOLATE DEFICIENCY AND HYPERHOMOCYSTEINAEMIA

Hyperhomocysteinaemia has recently been associated with a number of clinical conditions that have an increased risk of vascular disease. These include chronic renal failure, disseminated lupus erythematosis, rheumatoid arthritis and inflammatory bowel disorders. With the exception of renal failure, the hyperhomocysteinaemia in these conditions has been shown to be related to folate deficiency and to respond to folic acid therapy (see Weir & Scott, 1998a).

Colonic cancer may also be related to folate deficiency causing DNA hypomethylation and malincorporation of uracil during DNA synthesis. Meir Stampfer, Walter Willet and their colleagues from Harvard Medical School showed convincingly that nurses who took multivitamin tablets containing folic acid had a progressive decline in the incidence of colon cancer over a period in excess of 15 years (Giovannucci *et al*, 1998). This is supported by findings of folate deficiency in dysplastic, colonic mucosal cells, and in abnormalities associated with the MTHFR polymorphism, suggesting that impaired DNA hypomethylation may be a significant pathogenetic factor (see Weir & Scott, 1998b).

CONCLUSIONS

In the 70 years since Lucy Wills' original discovery that yeast extract contained a factor to which macrocytic anaemia of pregnancy responds, many aspects of folate metabolism and the role of folate deficiency in causing megaloblastic anaemia have been elucidated. The exact role of folate deficiency or abnormal metabolism in the pathogenesis of neurological and vascular diseases, neural tube defects and certain types of cancer now present important problems for research to take into the 21st century.

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