The need to control cell division during periods of methionine deficiency may also explain why cells have evolved in such a way that 5-methyl-THF, the form in which folate is transported in plasma, must be demethylated before it can be incorporated into the cell.[19] Since in mammalian cells methionine is the only source of the homocysteine which is necessary for this process, its deficiency would limit folate uptake and reduce proliferation of rapidly dividing cells.

This requirement of the plasma transport form of folate (5-methyl-THF) to react with homocysteine before it can be incorporated into the cell means that, in B_{12} deficiency, the lack of MS, by decreasing this essential reaction, will decrease folate uptake (fig. 1). This will produce intracellular folate deficiency, which in turn enhances the megaloblastic changes. The proposal[20] that, for folate to be incorporated into cells, methionine must be degraded to formate and subsequently resynthesised to methionine seems improbable since such a process would result in no net gain in methionine.

It appears that in animals megaloblastic changes do not occur even after prolonged exposure to N_2O[13,14] but occur in human beings within hours.[10] Biochemical studies have shown that MS is inactivated in animals as well as human beings.[11,12] This species difference may be due to the higher levels of non-5-methyl-folates in the plasma of animals.[21] Since such folates need not be demethylated by the cell, they may permit the marrow cells to continue normal division even in the presence of B_{12} deficiency.

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Requests for reprints should be addressed to D. G. W., Trinity College Medical School, Sir Patrick Duns Hospital, Grand Canal Street, Dublin 2, Ireland.

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Hypothesis

THE METHYL FOLATE TRAP
A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid-induced exacerbation of subacute combined degeneration in pernicious anaemia

JOHN M. SCOTT DONALD G. WEIR

Departments of Biochemistry and Medicine, Trinity College, Dublin, Ireland

Summary It is suggested that in man the methyl folate trap is a normal physiological response to impending methyl group deficiency resulting from a very low supply of methionine. This decreases cellular S-adenosyl-methionine (SAM), which puts at risk important methylation reactions, including those required to maintain myelin. In order to protect these methylation reactions, the cell has evolved two mechanisms to maintain supplies of methionine and SAM as a first priority. (a) Decreased SAM causes the folate co-factors to be directed through the cycle involving 5-methyl-tetrahydrofolate (5-methyl-THF) and methionine synthetase and away from the cycles that produce purines and pyrimidines for DNA synthesis. This enhances the remethylation of homocysteine to methionine and SAM. In addition, by restricting DNA biosynthesis and with it cell division, competition for methionine for protein synthesis is reduced. Thus, whatever methionine is available is conserved for the vital methylation reactions in the nerves, brain, and elsewhere. (b) 5-methyl-THF, the form in which almost all folate is transported in human plasma, must react with intracellular homocysteine before it can be retained by the cell as a polyglutamate. Since homocysteine is derived entirely from methionine, methionine deficiency will cause intracellular folate deficiency, and the rate of mitosis of rapidly dividing cells will be reduced. Although these two processes have evolved as a response to methionine deficiency, they also occur in B_{12} deficiency, which the cell mistakenly interprets as lack of methionine. The resulting response is inappropriate and gives rise to a potentially lethal anaemia. In these circumstances the methylation reactions are also partly protected by the reduced rate of cell division. This explains why administration of folic acid, which induces cell division and use of methionine in protein synthesis, impairs methylation of myelin and precipitates or exacerbates subacute combined degeneration (SCD). During folate deficiency methionine biosynthesis is also diminished. As in methionine deficiency, the body responds to decreasing

PROFESSOR SCOTT AND OTHERS REFERENCES—*continued*

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availability of SAM by diverting folate away from DNA biosynthesis towards the remethylation of homocysteine to methionine and SAM. The selective use of available folate to conserve methionine, together with the ability of nerve tissue to concentrate folate from the plasma, explains the absence of SCD in folate deficiency.

INTRODUCTION

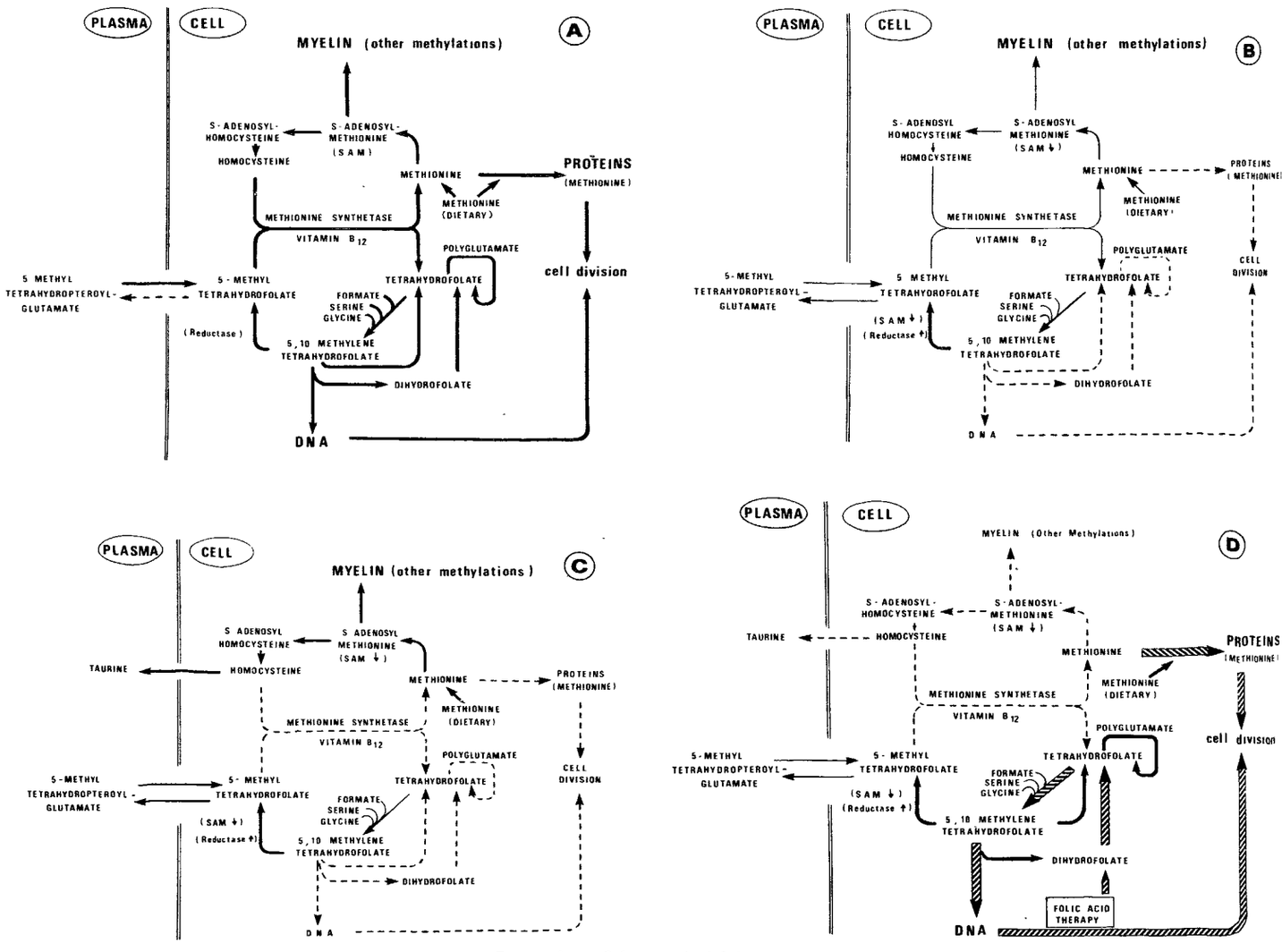
THE methyl folate trap hypothesis was first put forward twenty years ago.[1,2] Although controversial,[3] it explains many of the experimental findings associated with vitamin B_{12} deficiency and consequently is accepted as being broadly correct.[4] A most important question not yet answered is why such a potentially damaging process, which may result in pernicious anaemia and a life-threatening lack of erythropoiesis, should exist in human cells.

Our recent studies have shown that subacute combined degeneration (SCD) can be prevented or diminished in B_{12}-compromised monkeys by increasing the dietary intake of methionine.[5] This finding prompted us to reconsider this area. We suggest that in man the methyl folate trap is an essential physiological response to methionine deficiency and results in an inappropriate response to B_{12} deficiency.

PHYSIOLOGICAL RESPONSE TO METHIONINE DEFICIENCY

During the biosynthesis of purines and pyrimidines, it is unnecessary for the recycling folate co-factors to pass through the 5-methyltetrahydrofolate (5-methyl-THF) form to continue their biosynthetic activity (figure, A). Clearly this form provides a channel through which methyl groups can pass from formate and from the aminoacids, serine, glycine and histidine, via methionine synthetase (MS) and S-adenosylmethionine (SAM) to be available to the different methylating enzymes found in mammalian cells.[6]

As dietary methionine intake diminished below a critical level, the concentration of SAM would begin to fall and reactions vital for the methylation of myelin[5] and similar methylation reactions in the brain and elsewhere would be placed at risk.[6] The falling level of SAM together with a reduction in the level of S-adenosylhomocysteine (SAHC) would decrease catabolism of homocysteine[7] and enhance its remethylation to methionine by increasing the activity of 5,10-methylene-tetrahydrofolate reductase (methylene-THF-reductase) and thus the conversion of cellular folates into 5-methyl-THF (figure, B).[8,9] The intracellular folate co-factors would be channelled towards the remethylation cycle and away from DNA biosynthesis, thus permitting available methionine to be efficiently recycled, and decreasing cell division and competition for methionine for protein synthesis. Any folate emerging from the trap would, after receiving a carbon unit from glycine, serine, or formate, be directed back into the trap and away from DNA biosynthesis



Operation of the methyl folate trap:

A, Normal; B, Methionine or folate deficiency; C, B_{12} deficiency; D, B_{12} deficiency after folic acid therapy.

for as long as the level of SAM remained low and methylation reactions were at risk.

The other methyl donor, choline, and its degradation product, betaine, pass methyl groups to homocysteine: since all the cell's homocysteine is derived from methionine, the value of this pathway is limited. Also, for the plasma form of folate, 5-methyl-THF, to be retained by cells, it must be demethylated by reaction with homocysteine.[10,11] Since homocysteine is limited in methionine deficiency, the growth rate of rapidly dividing cells dependent on uptake of folate from the plasma is diminished. This further reduces competition for methionine for incorporation into new proteins.

Clinical evidence to support the above hypothesis is available from many studies of kwashiorkor.[12] Megaloblastic anaemia is either always[13] or frequently[13-18] present. It is generally assumed that the megaloblastic anaemia is due to folate deficiency, since the serum B_{12} level is frequently normal,[13,16,18] and the anaemia responds to folic acid therapy[18] (see later). However, whereas folate deficiency undoubtedly occurs in some of these malnourished groups, in many cases the folate level is normal.[13,16,18] In one study of eleven cases, all serum folate and B_{12} levels were normal but all eleven had megaloblastic marrows.[16] In another study,[19] the results of the formimino glutamic acid (FIGLU) excretion test were abnormally high in 80% of cases, but folic acid therapy reduced them to normal in only one-third of these patients.

We suggest that in these patients megaloblastic anaemia is produced by reducing DNA biosynthesis in order to conserve methionine for essential methylation reactions.

RESPONSE TO VITAMIN B_{12} DEFICIENCY

Whereas the response of the cell to methionine deficiency appears appropriate, its response to B_{12} deficiency, which causes a life-threatening anaemia, is inappropriate. It seems there has been no pressure on human cells to evolve a response to B_{12} deficiency and they interpret it as impending methionine deficiency. Thus, as the level of B_{12} falls, the activity of MS and the level of SAM diminish. This causes diversion of folate co-factors away from DNA biosynthesis into remethylation of homocysteine. Although this mechanism conserves methionine when it is deficient, in B_{12} deficiency conversion of the intracellular folates into 5-methyl-THF does little to maintain methylation reactions because the activity of MS is reduced. It does, however, divert folates away from DNA biosynthesis and cell division (figure, C). The result is the appearance of megaloblastic changes in rapidly proliferating cells such as the bone marrow.

In addition, before 5-methyl-THF, the plasma transport form of folate, can be retained by cells, it must be demethylated by the B_{12}-dependent MS.[10-11] In B_{12} deficiency, rapidly dividing cells, such as marrow cells, are deprived of folate, since they are dependent on continuous folate uptake from the plasma. This further restricts DNA biosynthesis, enhancing the megaloblastic changes. As in methionine deficiency, decreased cell turnover reduces competition for methionine for incorporation into proteins and ensures a preferential supply of methionine for SAM biosynthesis and methylation reactions. However, since the activity of MS, essential for the recycling of homocysteine back into methionine, is decreased in B_{12} deficiency, methionine is wasted and catabolised to taurine (figure, C).

The clinical evidence for the above hypothesis in B_{12}-deficient states is as follows:

Megaloblastic erythropoiesis resulting from B_{12} deficiency is clearly due to diminished DNA biosynthesis,[20] the marrow picture being identical to that seen when DNA synthesis is inhibited by DNA antimetabolites.[21] Flooding the DNA cycle with folates other than 5-methyl-THF produces a normal marrow.[20]

Negative nitrogen balance has been reported frequently;[22-25] following B_{12} therapy this rapidly becomes positive.

Aminoaciduria, which also responds to therapy, has been reported.[26-29] Most patients with pernicious anaemia have taurinuria[27,28] which, significantly, is not found in megaloblastic anaemia due to folate deficiency. The muscle myopathy associated with pernicious anaemia, and previously thought to be due solely to myelopathy, may result partly from decreased availability of methionine. The cell's inability to convert 5-methyl-THF into a polyglutamate leads to decreased cellular folate uptake in B_{12} deficiency, as shown by reduced red cell folate levels.[10,20] Eventually, methylating reactions become limiting, causing SCD[5] and other disorders.[31,32]

Treatment with B_{12} quickly reverses the above process as follows. Improved MS activity allows methyl groups from the trap, and from serine, glycine, and formate to elevate the SAM level. This liberates folates from the trap and allows them to proceed towards DNA biosynthesis. Since the homocysteine produced in the methylation reaction can now be remethylated by MS, the excessive catabolism to taurine and methionine wastage ceases. The return of normal MS activity permits demethylation of 5-methyl-THF by reaction with homocysteine and conversion to polyglutamate, thus returning the intracellular folate and red cell folate levels to normal. Increased DNA synthesis and availability of methionine return cell division and protein biosynthesis to normal, improve the anaemia, and reverse the negative nitrogen balance, aminoaciduria, and muscle myopathy.

Methionine therapy in pernicious anaemia has been shown to be ineffective.[33] Although a high level of methionine might be expected to prevent folate from entering the trap by elevating the SAM level, it could do nothing to liberate methyl folate already trapped by inactive MS (figure, C). Similarly, in the absence of MS activity, elevated methionine cannot increase the cell's capacity to take up 5-methyl-THF from the plasma. The seemingly contradictory effects of methionine[34] both in vivo and in vitero may be caused by elevation of the SAM level by methionine, which prevents new folate entering the trap, thus increasing the availability of folate co-factors and enhancing DNA biosynthesis.

RESPONSE TO FOLIC ACID THERAPY IN PERNICIOUS ANAEMIA (OR OTHER B_{12}-DEFICIENT STATES)

Folic acid in pharmacological quantities initially circumvents the trap, eliciting in many cases full remission of symptoms.[35] In most cases this is not sustained and frequently such patients relapse with SCD within months.[35-39] The onset of the symptoms of neurological relapse is occasionally very rapid,[37-39] leading to the belief that the folic acid therapy exacerbated the neurological lesion. It is difficult to see at first how the administration of folic acid could do other than increase both 5-methyl-THF levels and MS activity, thus improving the supply of methionine and methyl groups for myelin biosynthesis.[5] However, since MS is inactive without B_{12}, folic acid has little effect. On the other hand, by directly producing folate co-factors which participate in the biosynthesis of DNA, such therapy allows

increased cell division and diverts methionine into synthesis of proteins such as haemoglobin (figure D). Methylation reactions are thus deprived of the diminishing amounts of methionine, leading to impaired myelin biosynthesis and giving clinical signs of exacerbation of the SCD. Other methylation reactions in the brain and elsewhere are probably also affected.[6] Folic acid at the concentration found in multivitamin preparations can apparently produce this effect.[40] It seems, therefore, that when folic acid is to be given prophylactically to large sections of the population for long periods,[41] methionine should also be given. Furthermore, fortification of the diet of malnourished populations with folic acid[42] would also be inappropriate unless methionine were also given. The use of methionine to minimise the toxic affects of methotrexate should be investigated.

RESPONSE TO FOLATE DEFICIENCY

We suggest that the methyl folate trap also operates in folate deficiency. As the level of folate co-factors in the cell declines, the level of SAM falls and endangers the methylation reactions (figure, B). This in turn diverts folates into the trap, restricting DNA biosynthesis further. The passage of folate through the trap will depend upon the availability of homocysteine, the product of the methylation reactions. Furthermore, even when folate is released from the trap during the methylation of homocysteine, it will quickly obtain another methyl group from serine, glycine, or formate and, because of the low level of SAM, it will be directed back to the remethylation cycle and away from DNA biosynthesis. Thus, whatever folate is available will be concentrated in the methylation cycle at the expense of DNA biosynthesis. This process, by optimising remethylation and depressing competition for methionine, together with the ability of nerve tissue to concentrate folate, explains why SCD is not a feature of folate deficiency.[20]

METHYL FOLATE TRAP IN OTHER SPECIES

Despite countless studies, even those using nitrous oxide, a very effective blocker of B_{12}-dependent MS, megaloblastic changes have not been found in experimental animals,[5] whereas folate deficiency has resulted in such changes.[43] As pointed out earlier, the inability of B_{12}-deficient human bone marrow to demethylate the transport form of folate (5-methyl-THF) results in decreased retention of folate by rapidly dividing cells. The consequent folate deficiency of these cells may cause their megaloblastic changes. There is evidence that higher concentrations of non-methyl folates are found in animal plasma.[44] These do not require demethylation before incorporation into the cell and are independent of MS activity. Sufficient folate to maintain normal erythropoiesis could, therefore, be supplied to animal marrows even during nitrous oxide exposure or severe B_{12} deficiency.

In solving all good detective stories one must always look for the motive. We suggest in the case of the methyl folate trap hypothesis that the motive has been found: perhaps it now warrants the name of "methyl folate trap process".

Requests for reprints should be addressed to D. G. W., Trinity College Medical School, Sir Patrick Duns Hospital, Grand Canal Street, Dublin 2, Ireland.

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