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VITAMIN B_{12}–FOLATE INTERRELATIONSHIPS

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BACKGROUND

The interrelationship between vitamin B_{12} and folate metabolism in man is best illustrated by the hematologically indistinguishable, macrocytic megaloblastic anemia resulting from a deficiency of either vitamin. Large pharmacological doses of either vitamin will elicit a hematological response in patients suffering from a deficiency of either or even both vitamins (61, 119). Large doses of folate cause a temporary or partial hematological remission in pernicious anemia patients but fail to correct the neurological lesions that arise from prolonged vitamin B_{12} deprivation. The relationship can also be demonstrated biochemically in man and in experimental animals by the common deficiency symptoms of elevated urinary excretion of formiminoglutamate, aminoimidazolecarboxamide, and formate, all of which indicate a primary defect in folate metabolism. Methionine is also involved in this interrelationship, as its administration normalizes many of the biochemical indicators of folate deficiency in vitamin B_{12} deficiency in man and experimental animals. However, methionine exacerbates the megaloblastic changes in the bone marrow of vitamin B_{12}–deficient patients.

The interrelationships among folate, vitamin B_{12}, and methionine metabolism have been the subject of several reviews (22, 40, 90, 93, 100). This review updates recent information on the metabolic relationships involved. A brief background on those areas of folate metabolism that bear directly on the subject is presented.

FOLATE METABOLISM

Folate coenzymes serve as acceptors or donors of one-carbon units in a variety of reactions involved in amino acid and nucleotide metabolism. Some of these reactions, known as one-carbon metabolism, are shown in Figure 1. The coenzyme forms of the vitamin are the tetrahydro derivatives (Figure 2). These can accept one-carbon units at the oxidation level of formate (from formiminoglutamate, a histidine catabolite, or formate) and at the level of formaldehyde (from serine). Formate, in the form of 10-formyl-H_4PteGlu[1], is utilized in the de novo biosynthesis of the purine ring, while formaldehyde, in the form of 5,10-methylene-H_4PteGlu, is utilized in the synthesis of thymidylate from deoxyuridylate. 5,10-Methylene-H_4PteGlu, which is freely interconvertible with 5,10-methenyl- and 10-formyl-H_4PteGlu, can also be reduced to 5-methyl-H_4PteGlu. The methyl group of this compound is used in the biosynthesis of methionine from homocysteine. Figure 1 depicts the interconversion and

[1]Abbreviations used: PteGlu, pteroylglutamic acid, folic acid; $H_4PteGlu_n$, tetrahydropteroyl-poly-γ-glutamate, where n indicates the number of glutamate residues.

metabolism of pteroylmonoglutamates. However, practically all the folates in mammalian tissues, with the exception of plasma, are present as conjugated folylpolyglutamate derivatives (Figure 2). Most, if not all, of the reactions outlined would use these polyglutamate forms as substrates under physiological conditions.

Amino Acid Interconversions

Serine hydroxymethyltransferase, a pyridoxal phosphate-containing enzyme, catalyzes the reversible transfer of formaldehyde from serine to H_4PteGlu (Figure 1, reaction 3) to generate 5,10-methylene-H_4PteGlu and glycine. In mammalian tissues the β-carbon of serine is the major source of one-carbon units for folate metabolism.

5,10-Methylene-H_4PteGlu can be metabolized in a number of directions. A major pathway in mammalian tissues involves its reduction to 5-methyl-H_4PteGlu (Figure 1, reaction 10) followed by the transfer of the methyl group to homocysteine to form methionine and regenerate H_4PteGlu (Figure 1, reaction 11). The reduction of 5,10-methylene-H_4PteGlu is catalyzed by the flavoprotein methylenetetrahydrofolate reductase and NADPH is required to reduce enzyme-bound FAD. The reaction is essentially irreversible under physiological conditions, making it the first committed step in methionine biosynthesis. Methionine exerts feedback control over the reaction via adenosylmethionine inhibition of the reductase (55, 111).

Methionine synthetase catalyzes the transfer of the methyl group from 5-methyl-H_4PteGlu to homocysteine to form methionine. The mammalian enzyme contains tightly bound cobalamin, which is methylated by the folate substrate. The methyl group is then transferred from methylcobalamin to homocysteine to generate methionine (106). This is the only reaction known in mammalian tissues for the metabolism of 5-methyl-H_4PteGlu with the subsequent regeneration of H_4PteGlu. Adenosylmethionine and a reducing system are required in vitro for an initial priming of the enzyme-bound cobalamin. Whether adenosylmethionine is required in vivo to methylate the cobalamin has not been established.

Methionine synthetase is one of three mammalian enzymes known to require vitamin B_{12} as a cofactor, the others being methylmalonyl-CoA mutase, which contains bound 5-deoxyadenosylcobalamin, and leucine 2,3-aminomutase (78). Although a B_{12}-independent enzyme was reported in mammalian tissues, more recent studies aimed at detecting the B_{12}-independent activity have not been successful (17). The methionine synthetase reaction is subject to inhibition by methionine although methionine is only a weak inhibitor of the mammalian enzyme.

Folate is also involved in the metabolism of formiminoglutamate, a histidine catabolite (Figure 1, reaction 12). Formiminotransferase catalyzes the transfer

of the formimino group to H_4PteGlu to generate 5-formimino-H_4PteGlu and glutamate. The formimino group is at the oxidation level of formate. Formimino-H_4PteGlu is metabolized by deamination to 5,10-methenyl-H_4PteGlu (Figure 1, reaction 13) in a reaction catalyzed by a cyclodeaminase. In mammalian tissues, formiminotransferase and cyclodeaminase activities are associated with a single polypeptide (59). Under conditions of folate deficiency, formiminoglutamate catabolism is impaired and it is excreted in elevated amounts by experimental animals and humans.

Thymidylate Synthesis

Although one-carbon metabolism is not involved in the de novo synthesis of pyrimidines, folate is required for the synthesis of thymidylate (Figure 1, reaction 9). The reaction is catalyzed by thymidylate synthetase, and involves the transfer of formaldehyde to the 5-position of deoxyuridylate. The pyrazine ring of H_4PteGlu supplies the reducing component for the reduction of the transferred formaldehyde to methanol, which results in the oxidation of H_4PteGlu to H_2PteGlu. The formation of deoxynucleotides, mediated by thymidylate synthetase and ribonucleotide reductase, is considered to be the rate-limiting step in DNA synthesis. Mammalian cells can also synthesize thymidylate via the thymidine kinase–mediated salvage pathway.

H_2PteGlu formed in the thymidylate synthetase reaction is functionally

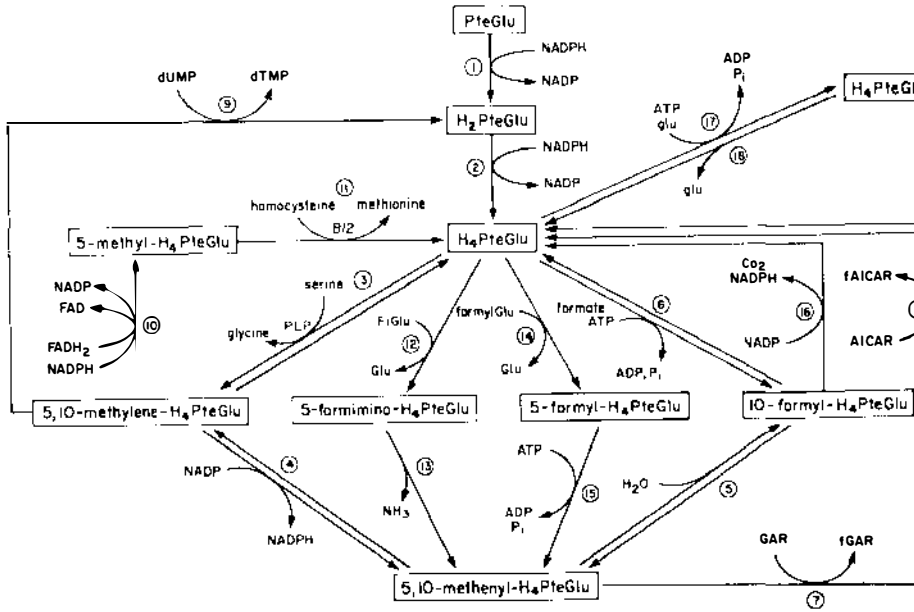

Figure 1 Metabolic reactions of one-carbon metabolism in the mammalian cell cytoplasm.

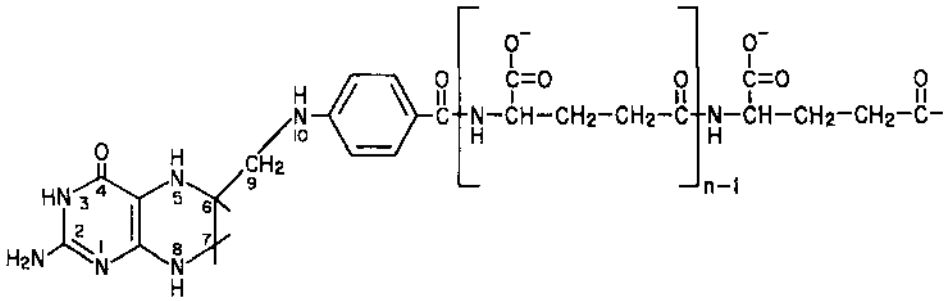

Figure 2 Tetrahydropteroylpoly-γ-glutamate ($H_4PteGlu_n$).

inactive as a coenzyme and has to be reduced to $H_4PteGlu$ before it can participate in one-carbon transfer reactions (Figure 1, reaction 2). This reduction is catalyzed by dihydrofolate reductase, an enzyme that also catalyzes the reduction of PteGlu. Normally, PteGlu is not found in unsupplemented foods and the major role of dihydrofolate reductase appears to be to reduce $H_2PteGlu$ formed in the thymidylate synthetase reaction.

Purine Biosynthesis

The C-8 and C-2 positions of the purine ring are derived from the one-carbon pool (Figure 1, reactions 7, 8) in reactions catalyzed by glycinamide ribonucleotide (GAR) transformylase and 5-amino-4-imidazolecarboxamide ribonucleotide (AICAR) transformylase, respectively. 10-Formyl-$H_4PteGlu$ is the one-carbon donor for both reactions (96).

FOLYLPOLYGLUTAMATES AND FOLATE HOMEOSTASIS

The role of folylpolyglutamates was the subject of several recent reviews (21, 50, 66). Folylpolyglutamates are the major intracellular forms of the vitamin and are the natural substrates for the enzymes of one-carbon metabolism. They are as effective as, and in some cases more effective than, pteroylmonoglutamates as substrates for the enzymes of one-carbon metabolism.

Some of the enzymes of one-carbon metabolism (Figure 1) are present as multifunctional proteins in mammalian tissues. Substrate channelling with polyglutamate substrates has been observed for the bifunctional protein formiminotransferase-cyclodeaminase (60) without release of the intermediate product. This phenomenon, which is not observed with the monoglutamate substrate, increases the local concentration of the intermediate 5-formimino-$H_4PteGlu$ product and also prevents the accumulation of this nonfunctional intermediate. Folylpolyglutamates may also play an important role in the regulation of one-carbon metabolism. Recent studies show that they effectively

inhibit a number of enzymes of one-carbon metabolism, while the corresponding monoglutamate derivatives are ineffective or weak inhibitors of the same enzymes (50, 66).

Folylpolyglutamates do not cross or are only poorly transported across cell membranes. Consequently, metabolism of pteroylmonoglutamates to polyglutamate forms allows the cell to concentrate folates at much higher levels than in the external medium. Pteroylmonoglutamates are the transport form of the vitamin. Dietary folates are hydrolyzed in the gut prior to absorption in a reaction catalyzed by γ-glutamylhydrolase (conjugase; Figure 1, reaction 18). The monoglutamate derivatives are transported in plasma to the liver or peripheral tissues where they are reconjugated in a reaction catalyzed by folylpolyglutamate synthetase (Figure 1, reaction 17).

The importance of polyglutamate formation has been demonstrated in cultured Chinese hamster ovary cell mutants that lack folylpolyglutamate synthetase activity. Although folate transport by these cells is unimpaired, intracellular folate levels are reduced by over 90%. The mutant cells contain pteroylmonoglutamates while pteroylhexa- and heptaglutamates predominate in the wild type (30, 63, 105). The mutant cells require exogeneous methionine, glycine, purines, and thymidine for growth, while the wild type will grow in the absence of these compounds provided sufficient folate, vitamin B_{12}, and homocysteine are supplied in the medium.

Mammalian folylpolyglutamate synthetase was recently purified to homogeneity from hog liver (20) and partially purified from rat (67) and mouse liver (70). The enzyme catalyzes the sequential addition of glutamate moieties to a variety of folate substrates, although H_4PteGlu is the preferred substrate. The major endogenous polyglutamate derivative differs from tissue to tissue and between different animals. Pentaglutamates predominate in rat liver (89) while octaglutamates predominate in human fibroblasts (31). The actual distribution in a particular tissue is defined primarily by the specificity of the enzyme for its folylpolyglutamate substrates (21).

Because metabolism of folates to polyglutamate derivatives is required for the cellular retention of folates, factors that affect the expression of folylpolyglutamate synthetase activity can regulate the level of folate in the cell. Folylpolyglutamates with long chain length, the type that predominate in cells, retain high affinity for the enzyme but have little or no substrate activity; this suggests they can act as end-product inhibitors of the enzyme (20, 30, 67). If this is the case, then accumulation of folylpolyglutamates would be expected to inhibit further synthesis and lead to a steady-state folate concentration in the cell. The actual steady-state folate level would be dependent on the concentration of exogenous vitamin, as increasing the monoglutamate concentration would disturb this equilibrium and lead to further polyglutamate synthesis until a new steady state was reached. Studies with cultured mammalian cells suggest this is the case.

The net accumulation of labelled folate by cultured cells, which is a reflection of folylpolyglutamate synthesis, remains constant on a per cell basis irrespective of culture time, which indicates that folate is accumulated to a steady-state level and then accumulation ceases; the intracellular folate concentration is proportional to the medium pteroylmonoglutamate concentration (30, 42, 98, 99, 114). Polyglutamylation of folates is more rapid in dividing cultures than in stationary-phase cells, presumably because of the decreased intracellular folate concentration after cell division (71). Similarly, mammalian cells that are starved for folate have a greatly increased capacity for accumulating exogenous vitamin (30, 34, 71), a reflection that the synthetase enzyme is not inhibited. Slightly longer folylpolyglutamate derivatives are found in folate-depleted cultured cells and in the livers of folate-depleted rats (15). The rate of folylpolyglutamate synthesis can also be affected by changing the folate one-carbon distribution in the cell. This is elaborated on in a later section. Recent studies indicate that hormones such as insulin and dexamethasone stimulate polyglutamate synthesis, while cAMP has the opposite effect (33, 48). The mechanism responsible for these effects is not known. Under normal physiological conditions, the levels of glutamate in the cell are insufficient to saturate folylpolyglutamate synthetase, and it is possible that these hormones act indirectly by modifying cellular glutamate levels.

METHYL TRAP HYPOTHESIS

The only known metabolic pathway common to folate, vitamin B_{12}, and methionine is the methionine synthetase reaction (39). Based on this, and on initial observations of (*a*) an increased proportion of folate in the 5-methyl-H_4PteGlu form in B_{12}-deficient rats, and of (*b*) elevated serum folate levels (also presumed to be 5-methyl-H_4PteGlu) in B_{12}-deficient patients, Noronha & Silverman (73) and Herbert & Zalusky (41) advanced the "methyl trap" hypothesis. This postulates that under conditions of vitamin B_{12} deficiency the activity of the vitamin B_{12}–dependent methyltransferase is significantly diminished and folate is trapped as the 5-methyl derivative, which cannot be reoxidized via the methylenetetrahydrofolate reductase reaction because this reaction is essentially irreversible under physiological conditions (47). A functional folate deficiency ensues and this results in a decrease in the tissue levels of other folate coenzymes and a consequent impairment of folate-dependent reactions. The slowdown in thymidylate and purine biosynthesis, and consequently of DNA synthesis, results in megaloblastosis. The original methyl trap hypothesis has been expanded to account for the ameliorating effect of methionine on some of the biochemical symptoms of vitamin B_{12} deficiency. The sparing effect of methionine can be explained by adenosylmethionine inhibition of methylenetetrahydrofolate reductase (55), which would decrease the synthesis of 5-methyl-H_4PteGlu and prevent its subsequent trapping. The hypothe-

sis has also been expanded to account for the lowered folate levels in the tissues of vitamin B_{12}–deficient animals. This is attributed to 5-methyl-H_4PteGlu being a poor substrate for folylpolyglutamate synthetase. A decreased rate of synthesis of folylpolyglutamates would result in decreased folate retention by tissues (92).

Other theories have also been presented for the interrelationships among folate, vitamin B_{12}, and methionine metabolism. These include direct effects of vitamin B_{12} and/or methionine on the membrane transport of folates and on the activity of folylpolyglutamate synthetase.

Serum Folate Levels

Folates in plasma or serum are pteroylmonoglutamates, predominantly 5-methyl-H_4PteGlu. One of the initial observations leading to the methyl trap postulate was the detection of elevated levels of *L. casei*–active folates, presumed to be 5-methyl-H_4PteGlu, in sera from vitamin B_{12}–deficient patients (41). This observation has been confirmed in a number of studies except for patients suffering from conditions that result in decreased folate intake (113). Elevated serum 5-methyl-H_4PteGlu levels, which have also been observed in vitamin B_{12}–deficient animals (93), were corrected by vitamin B_{12} administration. The clearance rate of serum 5-methyl-H_4PteGlu, following an intravenous dose of PteGlu or labeled 5-methyl-H_4PteGlu, was significantly reduced in vitamin B_{12}–deficient patients compared to the clearance rate in patients after vitamin B_{12} therapy, provided that only a small loading dose was administered. In addition, the clearance rates of nonmethyl folates were increased in the untreated subjects. These data support the concept of a 5-methyl-H_4PteGlu trap resulting in decreased levels of other folate coenzymes (41).

Histidine, Serine, and Formate Metabolism

Histidine, serine, and formate catabolism are impaired under conditions of folate deficiency, and formiminoglutamate and formate are excreted in urine (12, 80). The C-2 of histidine and C-3 of serine normally enter the folate one-carbon pool to be utilized in biosynthetic reactions, and excess one-carbon units are oxidized to CO_2 via the 10-formyltetrahydrofolate dehydrogenase reaction (Figure 1, reaction 16). The oxidation of histidine, formate, and serine to CO_2 is decreased in folate-deficient animals and humans. Similar defects in the metabolism of these compounds have been observed in vitamin B_{12}–deficient subjects and experimental animals, which indicates that vitamin B_{12} deficiency induces a secondary folate deficiency (101).

Urinary formiminoglutamate excretion after a histidine loading dose is elevated in vitamin B_{12}–deficient patients, and some investigators report that urinary formiminoglutamate levels are highest in those patients with the severest anemia (51). The urinary levels of this abnormal metabolite can be

reduced by vitamin B_{12} or folic acid administration, and also by methionine. A similar pattern has been observed in experimental animals. Rats fed a diet supplemented with both vitamin B_{12} and methionine excreted very low levels of formiminoglutamate (19). Depleting the diet of vitamin B_{12} or methionine increased formiminoglutamate excretion approximately 10-fold, while depletion of both compounds caused a further 40-fold elevation in formiminoglutamate excretion to levels similar to those observed in folate-depleted animals. Histidine and formate oxidation to CO_2 are also decreased in the vitamin B_{12}/methionine-deficient rat. Methionine injections prior to the labeled histidine or formate dose corrected the abnormally low oxidation of these compounds by the deficient animals. Glycine, sarcosine, and serine oxidation are also impaired in the vitamin B_{12}-deficient rat, phenomena that are at least partially reversible by methionine administration (13). Methionine also decreases formiminoglutamate excretion in folate-depleted animals (94).

Low histidine oxidation and a build up of formiminoglutamate are also observed in the perfused rat liver and in rat hepatocyte suspensions, irrespective of whether the donor animals were vitamin B_{12} deficient or not (14, 53). In each case, the abnormal metabolism of histidine was corrected by the addition of methionine to the medium. Hepatocytes and the perfused liver of fully supplemented animals demonstrated a metabolic pattern associated with vitamin B_{12}–deficient animals because they were methionine deficient. This was due to methionine leakage during their preparation and to their rapid utilization of endogeneous methionine.

Folate Uptake and Metabolism

One of the bases for the original methyl trap hypothesis was the observation that 5-methyl-H_4PteGlu levels were increased in the livers of vitamin B_{12}–deficient rats and were restored to normal by the administration of methionine (73). This vitamin B_{12}–deficiency-induced increase in the proportion of folate in the 5-methyl-H_4PteGlu form has been confirmed in a large number of studies using a variety of experimental animals, and the effect is more pronounced if the animals are also methionine deficient (95, 108). 5-Methyl-H_4PteGlu$_n$ is the predominant folate derivative in the livers of vitamin B_{12}– and methionine-deficient rats and sheep; it is the major detected folate derivative in the urine of deficient rats after a dose of [^{3}H]folic acid, accounting for as much as 90% of the total vitamin (112). Supplementation of the animals with vitamin B_{12} and/or methionine increased the proportion of hepatic and urinary H_4PteGlu$_n$. The effect of methionine was most pronounced. Large doses of methionine, or smaller doses of ethionine, shifted the folate coenzyme pattern to the extent that H_4PteGlu$_n$ became the predominant folate in liver and urine. Direct evidence for a "methyl trap" has come from studies with L1210 cells cultured in a defined medium in which cell replication in methionine-supplemented media was

dependent on exogenous vitamin B_{12}. 5-Methyl-H_4PteGlu$_n$ accumulated in cobalamin-deficient cells but represented only a small proportion of folates in B_{12}-repleted cells (32).

Total endogeneous hepatic folate levels and the hepatic uptake of labeled PteGlu doses are significantly reduced, by up to 75%, in vitamin B_{12}/methionine-deficient animals (36, 92). The decreased hepatic folate levels are associated with elevated serum folate levels in the experimental animals. Short-term supplementation of the doubly deficient animals with either vitamin B_{12} or methionine partially restored to normal hepatic and serum folate levels and the decreased net hepatic uptake of labeled PteGlu. Supplementation with both vitamin B_{12} and methionine normalized these parameters. In fact, intraperitoneal administration of large doses of methionine alone to vitamin B_{12}/methionine-deficient rats resulted in supranormal net hepatic uptake of a labeled folate dose. Although vitamin B_{12} deficiency invariably increases the proportion of 5-methyl-H_4PteGlu$_n$ in the livers of experimental animals, the absolute level of methylated folate is often decreased as a result of the decreased total tissue folate levels in these animals.

Methotrexate uptake by sheep liver slices was reduced under conditions of vitamin B_{12} or methionine deficiency (35), and a decreased uptake of 5-methyl-H_4PteGlu by marrow cells from pernicious anemia patients has been reported (109). These data led to the postulate that vitamin B_{12} and methionine are involved in the membrane transport of folates. In the latter study, PteGlu uptake was also reduced although not as severely as 5-methyl-H_4PteGlu. Marrow cells from folate-deficient patients also demonstrated a decreased ability to transport 5-methyl-H_4PteGlu, an observation that is difficult to interpret in terms of vitamin B_{12}/methionine effects on folate transport. Incubation of marrow cells with vitamin B_{12} significantly increased 5-methyl-H_4-PteGlu uptake in cells from patients with pernicious anemia but had no significant effect on cells from folate-deficient or control subjects. Although these data suggest a defect in the membrane transport of folates, in most of the experiments described, net folate uptake at a single time period rather than initial rates of influx were measured. The lowered net folate uptake could be explained by decreased tissue retention of the vitamin.

Practically all the folate derivatives in tissues and blood cells are pteroylpolyglutamates. In vitamin B_{12}/methionine-deficient animals and in red blood cells from pernicious anemia patients, the large drop in endogeneous folate levels is due to a large decrease in folylpolyglutamates (76, 108). The small amounts of monoglutamate derivatives present are not significantly affected by the deficiency. Similar effects were seen on the uptake and metabolism of labeled folate doses. Labeled PteGlu was rapidly metabolized by rat liver to polyglutamate derivatives. During the first two hours after the dose, when pteroylmonoglutamates comprised the major labeled folate derivatives, no

differences in net hepatic uptake of the dose were observed between vitamin B_{12}/methionine-deficient and supplemented animals (92). At longer time periods, differences in net hepatic uptake were observed that could be correlated directly with the amount of folylpolyglutamates synthesized. The absolute rate of loss of labelled hepatic pteroylmonoglutamate was similar in both groups of animals. The lowered tissue net uptake of labeled folate, and by extension the lower level of endogeneous folate, in deficient animals could be entirely explained by a decreased ability to synthesize folylpolyglutamates, which resulted in a decreased ability to retain folates.

The absence of a vitamin B_{12} or methionine effect on the membrane transport of folates has also been demonstrated by closed system perfusion studies (14). Although the net uptake of labeled PteGlu by perfused vitamin B_{12}–deficient rat liver was reduced in the absence of methionine, much of the folate in the perfusate was in the form of 5-methyl-H_4PteGlu, which indicates that it had been taken up by the liver and then released. When the uptake of labeled PteGlu was assessed as labeled folate in liver plus reduced folate in the perfusate, no effect of methionine on uptake could be demonstrated. The possibility that 5-methyl-H_4PteGlu transport is affected by vitamin B_{12} and methionine was investigated using isolated rat hepatocytes (44). No effect of vitamin B_{12} or methionine on initial transport rates was observed. The results of an earlier report (45), suggesting lowered 5-methyl-H_4PteGlu uptake by vitamin B_{12}/methionine-deficient rat hepatocytes, were apparently due to the low yields of viable hepatocytes obtained from the deficient animals. Lowered cell viability may also explain some of the previous results suggesting decreased initial rates of folate transport in tissues derived from vitamin B_{12}/methionine-deficient animals.

The experiments described above strongly support the postulate that vitamin B_{12} and/or methionine deficiency increases the proportion of tissue folate in the 5-methyl-H_4PteGlu$_n$ form. In addition, tissue folate levels are reduced in the vitamin B_{12}– and/or methionine-deficient animal as a result of a decreased ability to synthesize folylpolyglutamates. Two explanations have been suggested for the decreased synthesis of folylpolyglutamates. First, 5-methyl-H_4PteGlu may be a poor substrate for folylpolyglutamate synthetase, and second, methionine and vitamin B_{12} may affect the activity of folylpolyglutamate synthetase. These possibilities are discussed in a later section.

The profolate effect of methionine may be due to adenosylmethionine inhibition of methylenetetrahydrofolate reductase (55). Inhibition of the reductase would prevent the trapping of folate as 5-methyl-H_4PteGlu under conditions of cobalamin deficiency and would consequently spare folate coenzymes to be utilized in purine and thymidylate biosynthesis. Strong evidence for this role of methionine came from studies with the perfused rat liver and rat

hepatocytes. Both systems demonstrate disturbances in folate metabolism, such as defective histidine catabolism, characteristic of vitamin B_{12} deficiency irrespective of the dietary regimen of the host animal (14, 53), disturbances that result from induction of a methionine deficiency during perfusion of the liver or in the preparation of the hepatocytes. Supplementation of the perfusion medium with methionine or ethionine restores histidine catabolism to normal, decreases the proportion of methylated folate in the liver, and increases the net uptake of labeled PteGlu and its conversion to polyglutamate forms (93). Similarly, addition of methionine or ethionine to rat hepatocyte suspensions promotes the degradation of formiminoglutamate and the oxidation of formate (53). It should be stressed that the biochemical disturbances in folate metabolism in the perfused liver and in hepatocytes, which resemble the symptoms of vitamin B_{12} deficiency, were caused solely by a deficiency of methionine. Similar disturbances in hepatic folate metabolism in the whole animal were discussed above in this review. In these disturbances, methionine or vitamin B_{12} deficiency impairs folate availability, an effect exacerbated by a double deficiency of both methionine and vitamin B_{12}.

These data have been interpreted to indicate an important regulatory role of methionine in normal folate metabolism (53). When endogeneous methionine levels are high, adenosylmethionine levels build up and reduce the activity of methylenetetrahydrofolate reductase. The decreased synthesis of 5-methyl-H_4PteGlu$_n$ reduces the rate of de novo methionine synthesis from homocysteine and increases the levels of other folate coenzymes, such as H_4PteGlu$_n$ and 10-formyl-H_4PteGlu$_n$. Tissue levels of 10-formyl-H_4PteGlu$_n$ are below their K_m values for 10-formyltetrahydrofolate dehydrogenase (54, 87), and any elevation in the level of 10-formyl-H_4PteGlu$_n$ should significantly affect the rate of oxidation of the one-carbon unit to CO_2. This would serve as a mechanism by which excess one-carbon units are removed for the folate pool. Rat hepatocytes prepared from animals 24 hr after a dose of [^{3}H]PteGlu contained labeled polyglutamates, predominantly 5-methyl-H_4PteGlu$_5$. Addition of increased levels of methionine to the hepatocyte culture increased the proportion of H_4PteGlu$_5$ and 10-formyl-H_4PteGlu$_5$. As the ratio of nonmethylated folate to total folate increased, histidine oxidation increased in a cooperative fashion (11). It should be noted that any effect on folate transport was eliminated in this experiment. In the case of vitamin B_{12} deprivation, the same effect of methionine would prevent the trapping of folate as 5-methyl-H_4PteGlu. In addition, the recent observation that H_2PteGlu polyglutamates are potent inhibitors of methylenetetrahydrofolate reductase (62) suggests a mechanism by which the cell can divert the one-carbon flux away from methionine synthesis and into thymidylate synthesis under conditions of DNA synthesis; it also emphasizes the important role of methylenetetrahydrofolate reductase in the regulation of one-carbon metabolism.

Adenosylmethionine is a noncompetitive inhibitor of mammalian methylenetetrahydrofolate reductase with a K_i of 2.8 μM for the pig liver enzyme (55, 111). This level of adenosylmethionine is lower than that normally found in mammalian tissues, which suggests that full expression of enzyme activity is rarely found under physiological conditions. The level of adenosylmethionine in tissues is dependent on the supply of methionine. In vitamin B_{12}/methionine-deficient rats, hepatic adenosylmethionine levels are approximately 30 μM (112). Administration of methionine (50 mg) caused an increase in adenosylmethionine levels, which peaked after 2–4 hr (approx 250 μM) and then dropped to near basal levels by 24 hr (91). The net hepatic uptake of folate 12 hr after a labeled PteGlu dose was significantly increased when the labeled dose was given within 12 hr of the methionine injection. No increase in net hepatic uptake was observed when the labeled folate dose was given 24 hr after the methionine dose. Adenosylethionine is metabolically more stable than adenosylmethionine. After a single dose of ethionine (50 mg), hepatic adenosylethionine levels in the rat reached high levels (>1.5 mM) and persisted at a high level for more than 24 hr. The net hepatic uptake of a labeled folate dose was greatly stimulated by ethionine administration, even when the labeled dose was administered 24 hr following ethionine administration (91). In these experiments, increased net hepatic uptake of labeled folate correlated well with increased adenosylmethionine or adenosylethionine levels, as did the proportion of total folate in the nonmethyl form. Formate oxidation to CO_2 was increased in rat hepatocytes incubated with agents, such as methionine or adenosylmethionine, that increased intracellular adenosylmethionine levels (8). Conversely, addition of substrates of hepatic catechol *O*-methyltransferase, such as L-dopa methyl ester, that decrease adenosylmethionine levels also decreased formate oxidation.

The methyl trap hypothesis, and the mechanism for the ameliorating effect of methionine discussed above, would only be valid if the vitamin B_{12}–dependent methionine synthetase reaction was the only significant pathway for the metabolism of 5-methyl-H_4PteGlu$_n$. Attempts at detecting a mammalian cobalamin-independent 5-methyltetrahydrofolate: homocysteine transmethylase, using mono- and polyglutamate substrates, have been unsuccessful (17). The irreversible nature of the methylenetetrahydrofolate reductase reaction under physiological conditions has been suggested on kinetic grounds (55). The irreversibility of the reaction has also been demonstrated by studies with cultured mammalian cells. The labeled one-carbon of 5-[^{14}C]methyl-H_4[^{3}H]PteGlu was metabolized rapidly by L1210 cells via methionine synthesis, while most of the tritium label remained associated with 5-methyl-H_4PteGlu. This reveals that the serine hydroxymethyltransferase-methylenetetrahydrofolate reductase-methionine synthetase cycle represented the major pathway of one-carbon flux in these cells and that the methionine synthetase reaction, although rapid, was

the rate-limiting step in this pathway (72). The absence of ^{14}C-labeled purine or thymidylate metabolites indicated the physiological irreversibility of the methylenetetrahydrofolate reductase reaction.

Methionine Synthetase

The methyl trap hypothesis implies that a deficiency of vitamin B_{12} would not itself be sufficient to disturb folate metabolism. The deficiency would have to result in lowered levels of the holoenzyme of methionine synthetase before any such disturbance would be manifest.

Vitamin B_{12} deficiency in the rat caused an 80% decrease in hepatic methionine synthetase activity without significantly affecting the levels of methylenetetrahydrofolate reductase or formiminotransferase (56). A similar vitamin B_{12}–dependent drop in methionine synthetase levels was reported for sheep liver (35). Methionine synthetase holoenzyme levels are significantly lowered in bone marrow cells from pernicious anemia patients (83, 107) and most of the enzyme was present as apoenzyme. Treatment of the patients with vitamin B_{12} converted most of the apoenzyme to holoenzyme and increased total levels of the enzyme. Similarly, cultured fibroblasts from a child with recurrent megaloblastic anemia due to defective vitamin B_{12} metabolism contained low levels of the methyltransferase, and approximately 90% was in the apoenzyme form (26). A patient with a defect involving methionine synthetase has been reported (4). Hepatic methionine synthetase levels were reduced to 30% of normal and the clinical symptoms of megaloblastic anemia and biochemical data, including elevated serum 5-methyl-H_4PteGlu levels, were consistent with the methyl trap hypothesis.

Folylpolyglutamate Synthesis

The decreased synthesis of folylpolyglutamates in the tissues of vitamin B_{12}–deficient animals and human blood cells, with the consequent decreased retention of folate has led to the postulates that either vitamin B_{12} and/or methionine directly affect the expression of folylpolyglutamate synthetase activity (76), or that the redistribution of folate coenzymes that occurs under conditions of vitamin B_{12}/methionine deficiency leads to the accumulation of folate derivatives, e.g. 5-methyl-H_4PteGlu$_n$, that are poor substrates for folylpolyglutamate synthetase (92).

The levels of folylpolyglutamate synthetase in Chinese hamster ovary cells were unaffected by culturing the cells in the presence or absence of vitamin B_{12} or by varying the concentration of methionine in the medium (105). Additions of products of one-carbon metabolism, such as adenosine, thymidine, and glycine, and varying the medium folate concentration were also without significant effect. Addition of cyanocobalamin, 5-deoxyadenosylcobalamin, or methylcobalamin to the in vitro synthetase assay mixture did not affect the

activity of folylpolyglutamate synthetase. Consequently, there is no experimental evidence to support the contention that impaired folylpolyglutamate synthesis is due to a direct effect of methionine or vitamin B_{12} on the enzyme.

Direct evidence that 5-methyl-H_4PteGlu is a poorer substrate than H_4PteGlu for mammalian folylpolyglutamate synthetase came from recent studies with partially purified folylpolyglutamate synthetase from Chinese hamster ovary cells (105) and rat liver (67). These studies found that 5-methyl-H_4PteGlu is 10–20% as active as H_4PteGlu when low substrate concentrations are used. The purified hog liver enzyme preferentially utilizes H_4PteGlu and 10-formyl-H_4PteGlu as substrates (relative $V_{max}/K_m \simeq 100$ and 95, respectively) and 5-methyl-H_4PteGlu is a poor substrate (relative $V_{max}/K_m = 6$) with a decreased affinity and V_{max} for the enzyme (20). The only effective polyglutamate derivatives are $H_4PteGlu_n$ forms of the vitamin; 5-methyl-$H_4PteGlu_2$ is almost inactive as a substrate. In in vitro metabolism experiments with the purified enzyme, H_4PteGlu was converted to hexa- and heptaglutamate derivatives (20). Under the same conditions, 5-methyl-H_4PteGlu was converted primarily to the diglutamate derivative while 10-formyl-H_4PteGlu was converted to tri- and tetraglutamate derivatives (20).

The decreased synthesis of folylpolyglutamates in the tissue and blood cells of vitamin B_{12}–deficient animals can be considered to be a direct consequence of the primary defect in these tissues, the decreased activity of methionine synthetase. Tissue levels of H_4PteGlu, which are present at levels below the K_m for folylpolyglutamate synthetase, drop and this leads to a decreased synthesis of polyglutamates. Although the tissue levels of 5-methyl-H_4PteGlu initially increase, this compound is a very poor substrate for folylpolyglutamate synthetase and is metabolized only to short-chain-length polyglutamate derivatives that can leak out of the cell. The decreased ability to elongate the glutamate chain length of the folate molecule results in a decreased retention of folate by the tissue.

Thymidylate Synthesis in Bone Marrow

Labeled thymidine incorporation into the DNA of bone marrow cells is reduced in the presence of deoxyuridine due to the conversion of dUMP to dTMP and the subsequent competition between labeled and unlabeled dTTP. The extent of the competition has been used as a measure of the one-carbon flux through the thymidylate synthetase reaction (49). Under conditions of low cellular folate, the conversion of dUMP to dTMP is reduced, and the suppression by deoxyuridine of labeled thymidine incorporation into DNA is reduced. Similar effects have been noted for marrow cells from pernicious anemia patients and various studies have demonstrated that the impaired suppression of thymidine incorporation is due to a functional folate deficiency in the marrow cells. Decreased suppression of [^{3}H]thymidine incorporation in marrow cells from vitamin B_{12}–

and folate-deficient patients was corrected by addition of PteGlu to the culture medium (69). Addition of vitamin B_{12} partially corrected the abnormal suppression in cells from vitamin B_{12}–deficient patients but had no effect on cells from folate-deficient patients. 5-Methyl-H_4PteGlu restored to normal the defective suppression in cells from folate-deficient subjects but failed to correct the abnormal suppression in cells from vitamin B_{12}–deficient patients, unless vitamin B_{12} was also added to the medium. In addition, the most effective form of vitamin B_{12} in the marrow cell cultures was methylcobalamin, the cofactor of methionine synthetase (110). 5-Formyl-H_4PteGlu is more effective than H_4PteGlu or PteGlu in correcting the abnormal deoxyuridine suppression (104). This may reflect the increased capacity of mammalian cells to transport reduced folates and possibly the lability of H_4PteGlu. Essentially identical abnormalities in DNA synthesis, which strongly support the methyl trap hypothesis, have been described in phytohemagglutinin (PHA)-stimulated lymphocytes from vitamin B_{12}– and folate-deficient patients (23).

The deoxyuridine suppression test has been criticized on the grounds that abnormal results do not necessarily reflect impaired flux through the thymidylate synthetase reaction; they can also result from disturbances in the activity of nucleoside kinases and in other enzymatic steps responsible for the incorporation of dTMP into DNA (75). However, other abnormalities in DNA precurser metabolism in marrow cells from pernicious anemia subjects, such as lowered incorporation of deoxyuridine into DNA and increased dUMP pools, are also corrected by the same manipulations that correct the abnormal deoxyuridine suppression test (104). Therefore this test, although indirect, provides a good measure of the thymidylate synthetase reaction flux in this condition. Thymidylate synthetase (82) and DNA polymerase alpha levels (46) are elevated in bone marrow from pernicious anemia patients. Thus, the impaired functioning of the thymidylate synthetase pathway, as measured by the deoxyuridine suppression test, is not due to low enzyme levels but results from decreased folate cofactor availability.

Marrow cells from vitamin B_{12}–deficient rats do not demonstrate abnormalities in folate metabolism, as judged by the deoxyuridine suppression test. This finding is consistent with the failure to observe megaloblastic anemia in the vitamin B_{12}–deficient rat (18). Although methionine exerts a profolate effect on hepatic folate metabolism in B_{12}-deficient experimental animals and humans, methylation of dUMP, as measured by the deoxyuridine suppression test, was impaired by methionine in human and rat bone marrow culture (18, 115). Recent studies indicated that this impairment was due to the high levels of methionine used, which caused product inhibition of methionine synthetase. When lower levels of methionine were used, a profolate effect was noted that was consistant with adenosylmethionine inhibition of methylenetetrahydrofolate reductase (97).

EFFECT OF NITROUS OXIDE

Exposure of patients to nitrous oxide can lead to a megaloblastic anemia typical of that observed in vitamin B_{12} deficiency (1, 57). The Co(I) form of vitamin B_{12} is susceptible to oxidation by nitrous oxide, and in vitro inactivation of purified vitamin B_{12}–dependent enzymes has been demonstrated with this agent (10). Because of this effect, nitrous oxide exposure of experimental animals proved to be a very useful model for vitamin B_{12}–deficiency effects.

Rats exposed to nitrous oxide exhibit large decreases in methionine synthetase activity in liver and brain (24, 44). Nitrous oxide is thought to inhibit this enzyme irreversibly by oxidizing the enzyme-B_{12s} (Co^{+}) complex that arises during catalysis to give a presumptively inactive enzyme-B_{12r} (Co^{2+}) complex. Hepatic methionine synthetase levels are depressed as much as 90% and return to normal 2–4 days following cessation of nitrous oxide administration (52). In general, the disturbances in folate metabolism that arise as a result of nitrous oxide inhalation are consistent with those predicted by the methyl trap hypothesis. Net hepatic uptake of labeled PteGlu by the mouse and rat is significantly depressed and the in vivo synthesis of folylpolyglutamates is impaired, with a considerable portion of the total labeled folate remaining as monoglutamate (58, 65, 85). The decreased net hepatic uptake of labeled folate is corrected by methionine administration (64) and does not result from impaired membrane transport of folates (44). The decreased synthesis of folylpolyglutamates can be explained by the increased proportion of methylfolates under these conditions (65). Labeled folate doses are excreted in elevated amounts and 5-[^{14}C]methyl-H_4PteGlu is excreted primarily as 5-[^{14}C]methyl-H_2PteGlu, indicating that removal of the methyl group does not take place to any significant extent in the methionine synthetase-inactivated animal (85). Lowered hepatic adenosylmethionine levels and formate oxidation are also corrected by methionine (28). As methionine synthetase remains inactive under these conditions, it is clear that the prevention of a methyl trap is not due to adenosylmethionine stimulation of methionine synthetase.

Acutely ill or traumatized patients are more susceptible to development of megaloblastic anemia on exposure to nitrous oxide than other patients (1, 2). The abnormal deoxyuridine suppression test produced by nitrous oxide was more severe and recovery slower than the abnormality seen in patients undergoing cardiac-bypass surgery. Among the patients receiving intensive care as little as one hour of exposure to nitrous oxide produced abnormalities in deoxyuridine suppression, and two to four hours produced megaloblastic bone marrow changes. In cardiac-bypass patients, these changes occurred with 12- to 24-hour exposure (1). Administration of large doses (30 mg) of 5-formyl-H_4PteGlu before anaesthesia minimized the deoxyuridine suppression test abnormalities (3).

These clinical results are similar to in vitro studies in which it was found that the deoxyuridine suppression test abnormalities observed after exposure to nitrous oxide could be partially corrected by PteGlu and more completely by 5-formyl-H_4PteGlu (25, 74). This is consistent with the observation that polyglutamate formation in the rat is inhibited when PteGlu is injected but is normal when 5-formyl-H_4PteGlu is given (77). In the latter study and several other investigations, it was noted that the in vivo synthesis of hepatic folylpolyglutamates in the nitrous-oxide-treated animal was impaired after H_4PteGlu injection but was normal after 5-formyl-H_4PteGlu injection. Abnormal polyglutamate formation could be corrected to some extent by methionine, and 5-methylthioadenosine was even more effective. These data were interpreted to mean that formylfolates are the natural substrates for folylpolyglutamate synthetase and that H_4PteGlu and 5-methyl-H_4PteGlu are inactive as substrates. Although it has been claimed that these experiments refute the methyl trap hypothesis, trapping of H_4PteGlu as 5-methyl-H_4PteGlu could explain the data. In addition, studies with purified mammalian folylpolyglutamate synthetase, discussed previously, indicate that the H_4PteGlu$_n$ derivatives are the only effective polyglutamate substrates for the enzyme.

Prolonged exposure of mice to nitrous oxide does not result in megaloblastosis (85). Nitrous oxide treatment of human lymphocytes results in an abnormal deoxyuridine suppression test, similar to that observed under conditions of cobalamin deficiency, and also inhibits the incorporation of thymidine into DNA; i.e. nitrous oxide directly affects DNA synthesis (79). Although nutritional vitamin B_{12} deficiency does not affect methionine synthetase levels in marrow cells from rodents and consequently does not result in an abnormal deoxyuridine suppression test (18), this test does demonstrate abnormal suppression in nitrous-oxide-treated rodents, presumably because methionine synthetase is inactivated (24, 25, 85). The test is corrected by the addition of PteGlu or 5-formyl-H_4PteGlu to the marrow cell culture medium (85). As would be expected for a methyl trap, 5-methyl-H_4PteGlu was ineffective. However, different results were reported by Deacon et al (24, 25), who found that H_4PteGlu was less effective than formylfolates in correcting abnormal results with the deoxyuridine suppression test, and that PteGlu and 5-methyl-H_4PteGlu were ineffective. These results were also interpreted as an indication that formylfolates are the natural substrates for folylpolyglutamate synthetase and that 5-methyl-H_4PteGlu can escape the methyl trap via its oxidation to 5,10-methylene-H_4PteGlu (25). However, the effectiveness of formylfolate may reflect its stability and the increased capacity of mammalian cells to transport this compound.

Monkeys receiving nitrous oxide developed subacute combined degeneration of the spinal cord, which could be prevented by methionine administration (84, 86). This strongly suggests that the neurological symptoms of vitamin B_{12}

deficiency also result from impairment of the methionine synthetase reaction, and may reflect a methylation defect.

EFFECT OF THYROID FUNCTION

The feeding of thyroid powder has long been known to produce a growth depression that could be partially corrected by feeding vitamin B_{12} (81). This growth depression is accompanied by an increase in the excretion of formiminoglutamate that can be prevented by the administration of methionine (6, 118). This thus resembles vitamin B_{12} deficiency, in which there is an increased formiminoglutamate excretion that can be normalized by the addition of methionine in the absence of vitamin B_{12}.

The addition of thyroid powder to a soy protein diet low in vitamin B_{12} and methionine also increases the excretion of methylmalonate, which is metabolized by a B_{12}-dependent enzyme, and whose excretion is elevated in B_{12} deficiency. Since feed consumption is increased by feeding thyroid powder, and since it is known that increased protein intake accentuates B_{12} deficiency (37), it is possible that the increased intake of protein and branched chain amino acids would produce the observed increased excretion of methylmalonate and that the increased intake of histidine would cause the increased excretion of formiminoglutamate. This was studied by feeding different levels of soy protein so that an animal receiving no thyroid powder would have the same protein intake per day as an animal receiving thyroid powder but with a lower percentage of protein in the diet. This study showed that the elevated excretion of formiminoglutamate was not due to an increased histidine intake, but that the elevated methylmalonate excretion was largely the result of increased protein intake (6).

Thyroidectomy, or the feeding of a thyroid inhibitor such as thiouracil, reduces formiminoglutamate excretion and increases oxidation of [2-^{14}C]-histidine to $^{14}CO_2$ (16, 102). The oxidation rate of histidine to CO_2 may be taken as a direct measure of folate functioning efficiency. Addition of methionine to a low-methionine diet containing vitamin B_{12} produces an increase in histidine oxidation. Thyroidectomy produces a greater increase in histidine oxidation than does the feeding of methionine, while hyperthyroidism, produced by feeding thyroid powder, decreases histidine oxidation. Thyroidectomy also increases the proportion of H_4PteGlu to 5-methyl-H_4PteGlu similar to that produced by addition of methionine. Addition of thyroid powder decreases the proportion of H_4PteGlu. Hypothyroidism, as produced by feeding thiouracil, increases liver folate levels (103) similar to those produced by feeding methionine (36). Thus, the effect of hypothyroidism in increasing histidine oxidation, increasing the proportion of H_4PteGlu, and increasing liver folate levels, is similar to that produced by methionine.

Thyroidectomy decreases the level of hepatic methylenetetrahydrofolate reductase; feeding of thyroid powder increases it (16). This is consistent with the observed effects of methionine, which, after conversion to adenosylmethionine, would inhibit methylenetetrahydrofolate reductase by its allosteric effect (55) and the effect of methionine in increasing liver folate levels (36). Administration of thiouracil also increases hepatic levels of methionine synthetase and formiminotransferase (102). The direction of these changes in enzyme levels is consistent with a higher rate of histidine oxidation, but the main effect of hypothyroidism apparently lies in its effect on methylenetetrahydrofolate reductase. This is supported by the fact that while thiouracil increases methionine synthetase and formiminotransferase levels, the feeding of thyroid powder, which decreases histidine oxidation, does not decrease these enzymes to subnormal levels. Hyperthyroidism does, however, increase methylenetetrahydrofolate reductase to supranormal levels.

The fact that hypothyroidism, which reduces methylenetetrahydrofolate reductase levels, has the same effect on histidine oxidation, the ratio of H_4PteGlu to 5-methyl-H_4PteGlu, and liver folate levels as methionine points toward the inhibitory action of adenosylmethionine on methylenetetrahydrofolate reductase as being the main regulatory site in folic acid regulation, rather than methionine synthetase, which is stimulated by adenosylmethionine.

METHIONINE FOLATE RELATIONSHIPS IN DRUG METABOLISM

The role of methionine in regulating folate metabolism is also manifested in its activity in decreasing the toxicity of methanol. Methanol is oxidized to formate and then to carbon dioxide (68) principally by the folate-dependent formyltetrahydrofolate dehydrogenase (54). It should be noted here that formate can also be oxidized by the catalase peroxidation route in rats (but not in man or monkeys). However, this route appears to be of minor importance as shown by the small decrease in formate oxidation produced upon administration of aminotriazol, which inhibits catalase function (68). However, in systems where formate oxidation to carbon dioxide is reduced, such as in nitrous-oxide-treated rats, aminotriazol administration does produce a small (20%) decrease in formate oxidation (9).

There is evidence that the high toxicity of methanol for man and monkeys compared to the rat is related to a lower capacity for formate oxidation, which results in formate acidosis (68). The oxidation of methanol to carbon dioxide by rat hepatocytes is increased by addition of methionine mainly because of its action in increasing formate oxidation (7). In the whole animal, addition of dietary methionine decreases the toxicity of methanol (29). When rats are given

methanol and exposed to nitrous oxide, which inactivates methionine synthetase, the blood formate levels increase markedly and approach blood formate levels of monkeys given methanol (27). Nitrous-oxide-treated rats exhibited a marked decrease in the rate of oxidation of formate to carbon dioxide; this is consistent with the observed decrease in nonmethylated tetrahydrofolate forms and the increase in 5-methyl-H_4PteGlu (9, 27).

Similar effects of methionine have been observed in the oxidation of (dimethylamino-^{14}C) aminopyrine by the perfused rat liver (116). In the absence of added methionine in the perfusion fluid, formate production (as measured in a nonrecirculating perfusion system) is twice that of carbon dioxide production. In the presence of 0.2-M methionine, carbon dioxide becomes the major product. The effect of methionine is very rapid. When methionine is added to the perfusion system, $^{14}CO_2$ production doubled in two minutes and reached maximum in six minutes. This shows that methionine can rapidly change the distribution of folate coenzymes, and this can influence the oxidation of formate. Similar effects of methionine on aminopyrine metabolism were observed in the intact rat (117).

MOLECULAR BASIS OF MEGALOBLASTOSIS

Megaloblastosis is the morphological expression of deranged DNA synthesis. The megaloblastosis that is characteristic of vitamin B_{12} or folate deficiency in man results from a decreased supply of folate coenzymes, presumably those involved in the biosynthesis of purines and/or thymidylate. Defective DNA synthesis in the megaloblastic state has been reviewed (43). The modal distribution of DNA in megaloblastic cells is primarily diploid with some DNA intermediate between diploid and tetraploid (23). DNA from PHA-stimulated lymphocytes from patients with megaloblastic anemia is more fragile than DNA from lymphocytes of control subjects and contains short fragments originally thought to be Ogasaki fragments (43). Mammalian cells exposed to antifolate agents exhibit depleted dTTP pools and elevated dUTP pools, and they misincorporate dUTP into DNA (38, 88). Removal of uracil by uracil-DNA glycosylase was proposed as a mechanism for the single-stranded breaks that occur in the DNA under these conditions and in the megaloblastic state. Single- and double-stranded DNA breaks occur in thymidylate-synthetase-negative mammalian cell mutants (5). However, in this case only small amounts of uracil were detected in DNA, an indication that uracil removal was probably not the major cause of the strand breaks. Although the actual mechanism responsible for the strand breaks remains controversial, it is clear that experimental thymidylate deprivation results in similar defects in DNA to those observed in the megaloblastic state.

SUMMARY

The studies discussed in this review support the view that biochemical and clinical symptoms common to both folate and vitamin B_{12} deficiency are due to the induction of a functional folate deficiency, which in turn is induced by cobalamin deprivation. The interrelationship between these two vitamins is best explained by the methyl trap hypothesis stating that vitamin B_{12} deficiency can lead to lowered levels of methionine synthetase, which results in a functional folate deficiency by trapping an increased proportion of folate as the 5-methyl derivative. In addition, as 5-methyl-H_4PteGlu is a poor substrate for folylpolyglutamate synthetase, there is a decreased synthesis of folylpolyglutamates and consequently a decreased retention of folates by tissues. The real folate deficiency that ensues because of decreased tissue folate levels is probably as important physiologically as the functional deficiency caused by the methyl trap. The sparing effect of methionine can be explained by adenosylmethionine inhibition of methylenetetrahydrofolate reductase, which would prevent the buildup of 5-methyl-H_4PteGlu$_n$. A deficiency in vitamin B_{12} would not, in itself, be sufficient to cause a disturbance in folate metabolism. The deficiency would have to result in lowered methyltransferase levels before any such disturbance would be manifest.

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