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NEW PERSPECTIVES ON FOLATE CATABOLISM

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■ **Abstract** Folate catabolism has been assumed to result from the nonenzymatic oxidative degradation of labile folate cofactors. Increased rates of folate catabolism and simultaneous folate deficiency occur in several physiological states, including pregnancy, cancer, and when anticonvulsant drugs are used. These studies have introduced the possibility that folate catabolism may be a regulated cellular process that influences intracellular folate concentrations. Recent studies have demonstrated that the iron storage protein ferritin can catabolize folate in vitro and in vivo, and increased heavy-chain ferritin synthesis decreases intracellular folate concentrations independent of exogenous folate levels in cell culture models. Ferritin levels are elevated in most physiological states associated with increased folate catabolism. Therefore, folate catabolism is emerging as an important component in the regulation of intracellular folate concentrations and whole-body folate status.

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INTRODUCTION

The importance of folate-mediated one-carbon metabolism in fundamental metabolic and cellular processes, including DNA synthesis and methylation, has been recognized since the 1940s. Almost immediately following the discovery of folate as a metabolic cofactor, folate analogs or antifolates were developed that proved to be effective antimicrobial and antineoplastic agents. More recent studies have established a role for folate in disease prevention. Epidemiological, genetic, and biochemical data are revealing that impairments in folate metabolism are partially determinant in the initiation and progression of certain cancers, birth defects, and cardiovascular disease. Alterations of folate metabolism can result not only from folate deficiency but also from pharmaceutical therapies, genetic predisposition including single nucleotide polymorphisms, and certain physiological states. With respect to the latter, studies have identified individuals with adequate folate intake who nonetheless display symptomatic folate deficiency. Therefore, elucidating the biochemical mechanisms that regulate intracellular folate concentrations is critical to understanding the complex relationship between folate status, folate metabolism, and disease. This review focuses on the role of folate catabolism and turnover in regulating intracellular folate concentrations and on recent biochemical evidence that indicates folate catabolism may be a regulated, enzyme-mediated process.

PHYSIOLOGICAL FUNCTIONS OF FOLATE

Folate is present in cells as a family of structurally related derivatives comprised of 2-amino-4-hydroxypteridine linked through a methylene carbon to *p*-aminobenzoylpolyglutamate (Figure 1). Reduced tetrahydrofolates serve as cofactors that carry one-carbon units at three different oxidation levels from methanol to formate. The one-carbons are carried on the N-5 and/or N-10 of tetrahydrofolate, and these one-carbon forms can be enzymatically interconverted. While serum folates contain a single glutamate residue, intracellular folates contain a polyglutamate peptide usually consisting of 5–8 glutamate residues that are polymerized through unusual γ-linked peptide bonds (111). The polyglutamate moiety increases the affinity of folate for folate-dependent enzymes, aids in sequestering folate within the cell, and may serve as a swinging arm that permits metabolic channeling of the cofactor among folate-dependent enzymes (103, 111). Folate

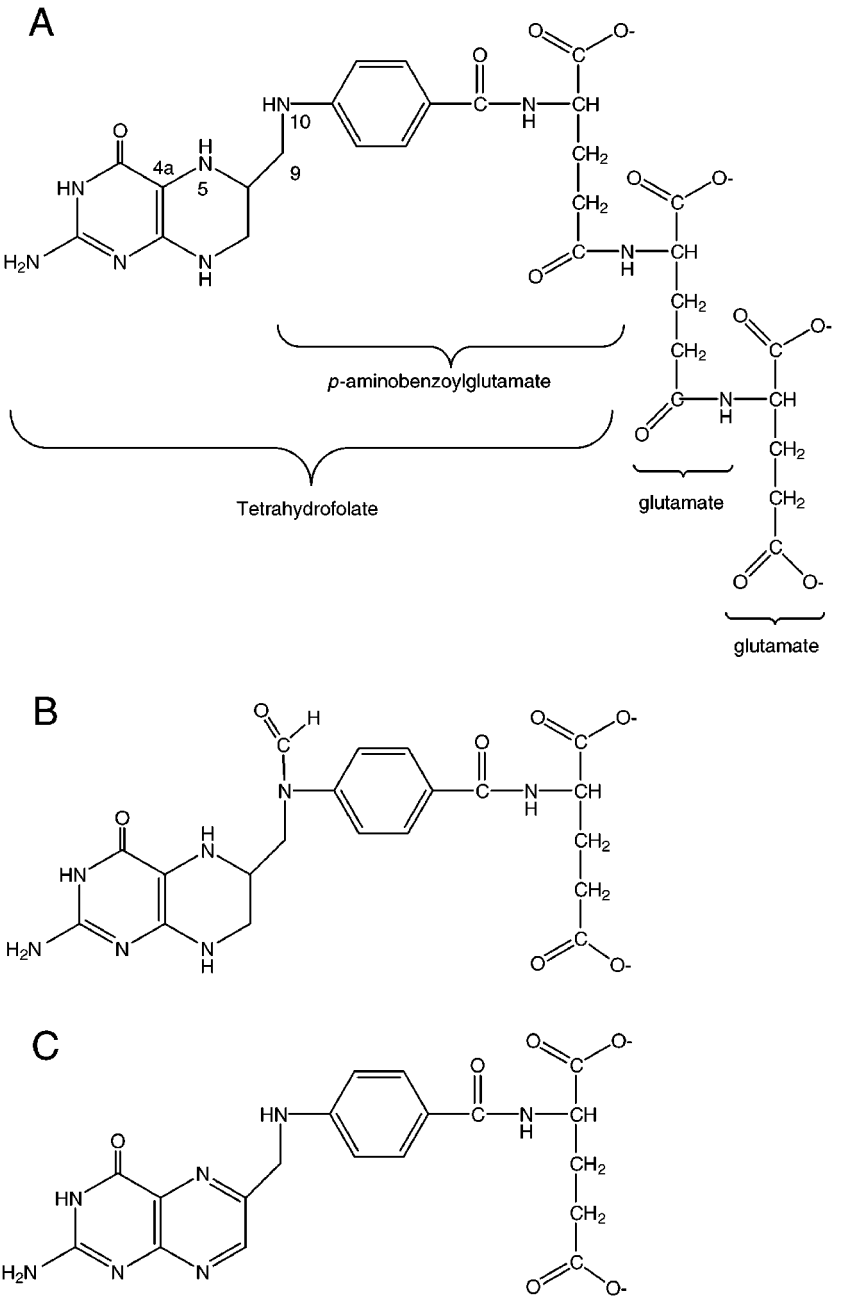

Figure 1 Structure of folates. (*A*) Structure of tetrahydrofolate triglutamate; (*B*) structure of a one-carbon substituted folate, 10-formyltetrahydrofolate monoglutamate; (*C*) structure of synthetic folic acid.

polyglutamates are coenzymes that donate or accept one-carbon units in a set of reactions characterized as one-carbon metabolism, which occurs both in the mitochondria and the cytoplasm (130). One-carbon metabolism in the cytoplasm is necessary for the de novo synthesis of purines and thymidylate, and for the remethylation of homocysteine to methionine. Methionine can be adenylated to form *S*-adenosylmethionine (SAM), which is a cofactor and one-carbon donor for numerous other methylation reactions (130). Increasing evidence suggests that the primary role of mitochondrial one-carbon metabolism is to generate glycine and formate from serine. Mitochondria-derived formate traverses to the cytoplasm, where it is a major source of one-carbon units for cytoplasmic one-carbon metabolism (4).

Unlike most bacteria and yeast, mammals cannot synthesize folates de novo and, therefore, require folates in the diet. Naturally occurring dietary folates have a reduced pteridine ring and a polyglutamate polypeptide that must be hydrolyzed in the intestinal lumen to a monoglutamate form before being absorbed by the intestinal cell (40). Fully oxidized folic acid is a synthetic form of folate and is found only in food that has been fortified with it. Once the pteridine ring of folic acid is reduced in the cell to the active tetrahydrofolate form, it is considered to be folate and serves the same biological functions (111).

CELLULAR COMPARTMENTATION OF FOLATE

Most cellular folate is compartmentalized, with up to half of the folate residing in the mitochondria and the remainder in the cytoplasm (51, 72). Folate is also present in other organelles, including the nucleus, although this folate does not make significant contributions to total cellular folate concentrations. Several studies have indicated that most cellular folate is protein bound. Initial work (143) estimated that approximately 60% of cytoplasmic folate and 20% of mitochondrial folate is tightly bound to proteins in rat liver. Subsequent studies of cell culture systems indicated that the cellular concentrations of non–protein-bound or free folate cofactor are very low or negligible (80, 81, 120) when the concentrations of all folate-binding proteins, not just the tight-binding proteins, are considered. The overall concentration of folate-binding proteins in liver might exceed the concentration of folate by as much as 5- to 10-fold (60). Considering that folate polyglutamates bind to many of these enzymes with high affinity (K_d in the 100 nM range) and that the concentration of folate in liver is 25–35 μM (20, 51), it is likely that in vivo, most liver folate is protein bound. The major intracellular folate tight-binding proteins present in rat liver are the mitochondrial sarcosine and dimethylglycine dehydrogenases (136), cytoplasmic glycine N-methyltransferase (140), and 10-formyltetrahydrofolate dehydrogenase (90). These proteins are highly enriched or exclusively expressed in liver, an organ that may contain half of total body folate (42). Also consistent with the notion that most folates are protein bound, studies have demonstrated that tissue folate concentrations in rats do not increase proportionally with folate intake (21, 42, 56). Collectively, these studies indicate

that intracellular folate concentrations are saturable and independent of excess exogenous folate supply, and that the threshold for intracellular folate accumulation is limited by the folate-binding capacity of the cell.

Consequences of Impaired Folate Status

Inadequate folate status has been reported in many population groups (102). Clinically, impaired folate status is associated with gastrointestinal disorders, smoking, alcohol consumption, antiepileptic drug use. In addition, certain dietary factors can interfere with folate bioavailability (40, 102). Biochemical indices of folate status are serum and red blood cell (RBC) folate levels. Serum folate represents circulating folate, which can change quickly and is influenced by diet, whereas RBC folate levels represent intracellular folate status (40, 102). RBC folate levels are a good indicator of long-term status because cellular folate stores that accumulate during erythropoiesis are retained throughout the cell's life span and correlate strongly with hepatic concentrations (74). Therefore, in the absence of pernicious anemia, RBC folate is most representative of body stores (74).

Although cells may not accumulate excess folate relative to their binding capacity, mild folate deficiency at both the cellular and organismal levels disrupts folate-requiring anabolic pathways and leads to identifiable pathologies. Both homocysteine remethylation and thymidylate synthesis are sensitive to folate deficiency. Plasma homocysteine is considered a sensitive marker of folate status (128), but it can also be influenced by vitamin B_6 and B_{12} status as well as by age (110). Biochemically, elevated cellular homocysteine can result from impaired methionine synthesis and lead to elevations in intracellular *S*-adenosylhomocysteine, a potent inhibitor of SAM-dependent reactions (141). Therefore, SAM-dependent reactions, including DNA and protein methylation, are sensitive to impairments in homocysteine remethylation. Clinically, elevated serum homocysteine is positively correlated with neural tube defects (NTDs), cardiovascular disease (7, 35, 128), and certain types of cancer (64). The relationship between folate status and NTDs is well established, with folic acid supplementation preventing three out of four cases of human NTDs (107). Furthermore, supplemental folic acid can prevent neural tube defects in *Pax3* (32) and *CartI* (144) mouse mutants, whereas methionine, but not folic acid, can lower the frequency of NTDs in *Axd* mutants (30). An independent role for folate in cardiovascular disease has also been reported (100). Studies in humans (10) and animal models (52) have demonstrated an inverse association between folate status and uracil content in DNA, presumably because of impaired thymidylate synthesis and subsequent misincorporation of dUTP into DNA (10). For example, cancerous cells, which often display cellular folate deficiency, contain increased uracil content in DNA (64). Mice with the *Pax3* mutation display impaired thymidylate synthesis, and thymidine supplementation alone can replace folate in ameliorating NTDs in these mice, indicating that folate is critical for efficient DNA synthesis in neural tube formation (32). The effect of folate deficiency on DNA synthesis is also evidenced by its morphological presentation in megaloblastic anemia and hypersegmented polymorphonuclear neutrophils (74).

BIOCHEMICAL MECHANISMS FOR ACCUMULATION AND RETENTION OF FOLATE

Transport of Folate

Folate monoglutamates are hydrophilic, bivalent anions present in serum at nanomolar concentrations and therefore require concentrative transport systems to meet cellular demand (116). Folate transport is required not only for cellular uptake but also for intracellular compartmentation of the cofactor (3). There are three physiological mechanisms for the transport of folates into cells (Figure 2). One route is through a family of folate-binding proteins/folate receptors (FR), which are membrane-bound receptors that mediate the unidirectional transport of folate into the cell (1). There is strong evidence that membrane-bound FRs may act via receptor-mediated endocytosis (1, 3). Alternatively, folates can be transported by reduced folate carriers, mobile carrier-mediated folate transport systems that facilitate internalization of folates across membranes (116). Unlike FR-mediated transport, this process is capable of bidirectional flux. Although different carrier-mediated systems exist in different cells, they do have universal properties (116). The reduced folate carrier cDNA and gene (*RFC-1*) have been cloned, and tissue-specific alternatively spliced variants have been identified (116). There is some evidence that the reduced folate transporter may act as an anion exchanger to concentrate intracellular folate (116). Carrier-mediated systems have been documented in absorptive cells and in subcellular organelles, specifically lysosomes and mitochondria (116). Recently, the gene encoding a novel mitochondrial folate transporter, unrelated to *RFC1*, was reported (126). Although receptor- and carrier-mediated systems may occur together in some cell types, the contribution of each to net translocation is unclear and depends on the expression level of the genes involved (116). Carrier-mediated processes are known to be much more efficient than receptor-mediated transport (48, 54). Finally, passive diffusion has been proposed to occur across cell membranes at pharmacological and possibly physiological levels of folate (1, 49). For all transport mechanisms, net accumulation of folate is dependent on the activity of multidrug-resistant ATPases. These proteins facilitate the removal of folate monoglutamate species and perhaps folate degradation products from various mammalian cell types (116).

The Role of Folylpoly-γ-Glutamate Synthetase

Because newly transported folate contains a single glutamate moiety, it is inefficient as a cofactor and can efflux from the cell. The active, coenzyme forms of folate are folylpolyglutamates, which contain a glutamate polypeptide attached to the *p*-aminobenzoylglutamate (*p*ABG) ring (111). The enzyme folylpoly-γ-glutamate synthetase (FPGS) can sequentially add up to eight L-glutamic acid residues to folate monoglutamates via a γ-carboxyl peptide linkage. Polyglutamylation of folates by FPGS is necessary to sequester folates, which need to be at least

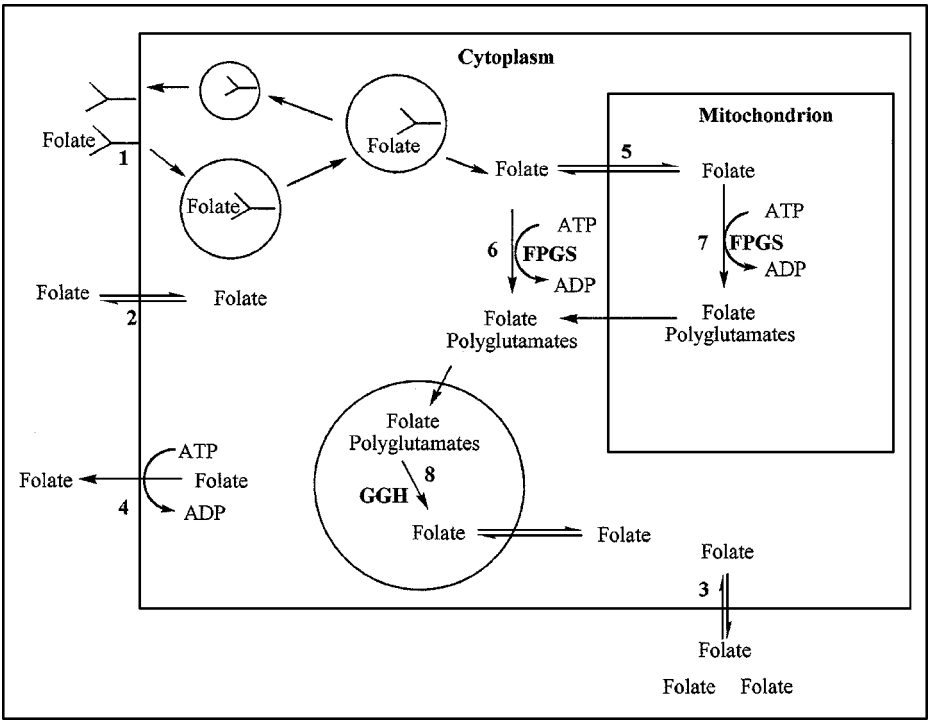

Figure 2 Cellular transport and accumulation of folates. Folate monoglutamates can be internalized by receptors (FR) on the cellular membrane and delivered into the cell through receptor-mediated endocytosis (1). Alternatively, folate monoglutamates can be transported through reduced folate carrier-mediated systems in addition to or exclusive of FRs (2). It has also been proposed that folate monoglutamates at high extracellular concentrations can enter the cell through passive diffusion (3). Intracellular folate monoglutamate concentrations are regulated in part by ATP-dependent exporters (4). Transport into mitochondria is limited to reduced folate monoglutamates (5) whereas both folate mono- and polyglutamates can exit, but the mechanism for the exit of folate polyglutamates has not been identified. Folate monoglutamates that enter the cytoplasm or the mitochondrion are converted to folate polyglutamates by the action of folylpoly-γ-glutamate synthetase (FPGS) (6, 7). Folate polyglutamates can enter the lysosome through a carrier-mediated system and be converted to folate monoglutamates by the action of γ-glutamyl hydrolase (GGH) (8). The mechanism for exit of folate monoglutamates from components of the endocytic pathway or from lysosomes has not been identified.

triglutamates to avoid efflux by folate exporters (94). FPGS isozymes function in both the cytosol and mitochondria of mammalian cells (72, 73). Cytosolic FPGS is necessary to maintain normal cytosolic folate pools whereas the mitochondrial isozyme is necessary for accumulation of folates in the mitochondria (17). Increased cellular FPGS activity lengthens folate polyglutamate chain length whereas increased import of folate monoglutamates lowers overall folate

polyglutamate chain lengths because of substrate competition (111). Additionally, mitochondrial FPGS is necessary to avoid glycine auxotrophy (73). Polyglutamates are not transported into the mitochondria (72), but they can be effluxed into the cytoplasm from the mitochondria without prior hydrolysis (61, 62, 73). FPGS activity can be detected in most mammalian tissues, although the highest activity is found in the liver (94).

The Role of Folylpoly-γ-Glutamate Hydrolase

Retention of folates is also influenced by the hydrolytic action of folylpolyglutamate hydrolase [γ-glutamyl hydrolase (GGH)]. This enzyme catalyzes the removal of the polyglutamate peptide from intracellular folate generating folate monoglutamate, which can then exit the cell. GGH is widely distributed in tissues and primarily localized in the lysosomes. Its catalytic activity is specific to the γ-glutamyl peptide bond of polyglutamates (83) and acts as either an exo-peptidase or an endo-peptidase, depending on the source from which it is derived (83). It has low specificity for the pteroyl portion of the molecule and can remove the polyglutamate polypeptide from the folate degradation product *p*-aminobenzoylypolyglutamate (83). Protein-bound polyglutamates are not hydrolyzed, which accounts for the increased half-life of cellular folate polyglutamates compared with monoglutamates (132). The role of GGH activity in regulating intracellular folate concentrations has never been directly explored.

FOLATE TURNOVER

Although folate transport and polyglutamylation are critical for delivering and sequestering folate within the cell, and disruption of these processes impairs folate accumulation, there is no evidence that these processes fully determine intracellular folate concentrations. Some studies have indicated that the expression and activity of the reduced folate carriers may respond to intracellular folate concentrations (53, 79, 120, 137), but definitive biochemical mechanisms for this regulation have not been established. Expression and localization of membrane-associated FRs may also be regulated by extracellular folate concentrations (55). Nonetheless, there is no direct experimental evidence that the regulation of transport processes alone can set or maintain intracellular folate concentrations at subsaturation levels in the presence of exogenous folate. Therefore, the role of folate catabolism and turnover in regulating intracellular folate concentrations must be considered.

Rates of Folate Turnover

Consistent with the complexity of biochemical pathways that regulate folate import and accumulation in mammalian cells, analyses of human whole-body folate turnover have revealed the presence of several kinetically distinguishable turnover pools (69, 101, 118, 129). Biochemically, the fast-turnover pools have a half-life

of hours and probably reflect newly absorbed folate that escapes cellular import or extensive polyglutamylation, whereas the slow-turnover pools reflect turnover of folate polyglutamate cofactors (69, 129). Recent kinetic modeling of folate turnover in normal human males supplemented with physiologic levels of folate revealed that the long-lived folate pools have a mean residence time of greater than or equal to 100 days (118). This study made provisions for, without directly measuring, fecal folate excretion and catabolites, which may account for nearly 50% of folate turnover (69, 118). Fecal excretion of endogenous folate cannot be measured easily because of the presence of folate from bacteria residing in the gut (118). However, fecal excretion of folate and its degradation products has been found to be an important excretory process in humans and rats (9, 69, 109).

Chemical Stability of Folate In Vitro

The reduced forms of folate are unstable in vitro and readily undergo oxidative degradation. At pH 7.0, tetrahydrofolate has a solution half-life of about 40 min (99) but can be stabilized in vitro by the addition of reduced thiols or antioxidants, including ascorbate. Oxidation has been proposed to occur by at least two distinct irreversible mechanisms (Figure 3). The pteridine ring can be sequentially oxidized to dihydrofolate and then to folic acid through a quininoid dihydrofolate intermediate (18, 99). This mechanism is similar to that elucidated for tetrahydropterin oxidation (5, 92), which proceeds through a 4a-carbinolamine intermediate. Reduced thiols protect reduced folates from oxidation (142), presumably by forming transient 4a adducts. Alternatively, reduced folates can undergo oxidative scission at the C9-N10 bond, after which folate is no longer a viable metabolic cofactor. It has been proposed that electron loss at N10 results in the formation of an N10 nitrenium ion intermediate that rapidly converts to a more stable C9-N10 Schiff base (99). However, there is no evidence for oxidation occurring directly at N10. Hydrolysis of the Schiff base yields the scission products 6-formyltetrahydropterin (or 6-formyldihydropterin in the case of dihydrofolate catabolism) and *p*ABG (99). One-carbon substitution at the N5 or N10 position can alter the sensitivity of reduced folates to oxidative degradation (71, 78). 5-Formyltetrahydrofolate is the most stable derivative of tetrahydrofolate, in part due to steric protection of the C4a oxidation site.

In Vivo Catabolism of Folate

Intracellular folate turnover can occur by at least three mechanisms. Newly transported folate monoglutamate species that escape polyglutamylation can efflux from the cell. Folate polyglutamates can undergo hydrolysis of the polyglutamate peptide by GGH, resulting in their conversion to folate monoglutamate species, which then efflux from the cell. Lastly, folates can be catabolized to yield inactive degradation products. Folates are stabilized when they are protein bound. During folate deficiency in rats, free folate is depleted to a greater extent than is

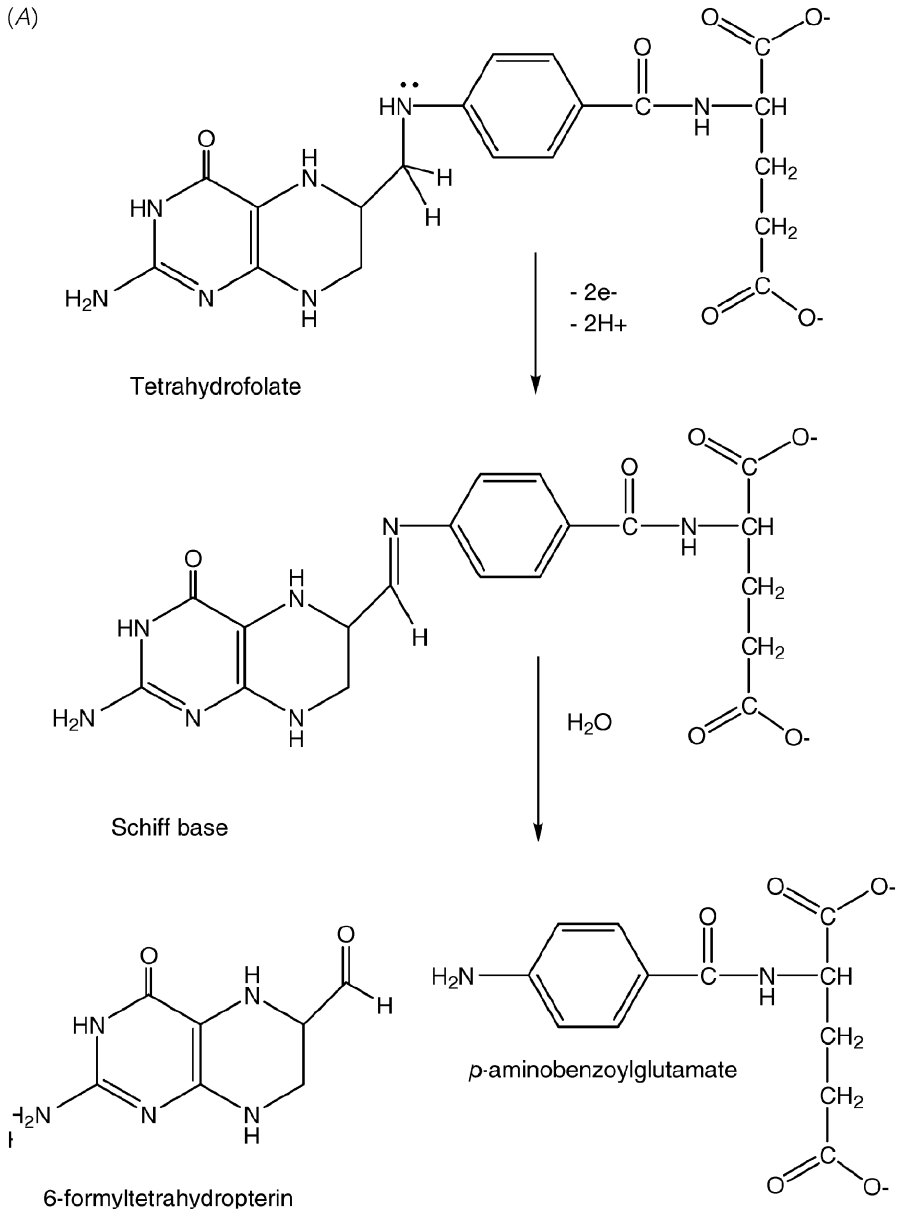

Figure 3 Oxidation of tetrahydrofolate in vitro. Reduced folate cofactors are susceptible to oxidation by two independent pathways. (*A*) Oxidation can result in the formation of a C9-N10 Schiff base that undergoes hydrolysis to yield inactive degradation products. (*B*) The reduced pterin ring can be oxidized to form dihydrofolate, which can undergo further oxidation to produce scission products.

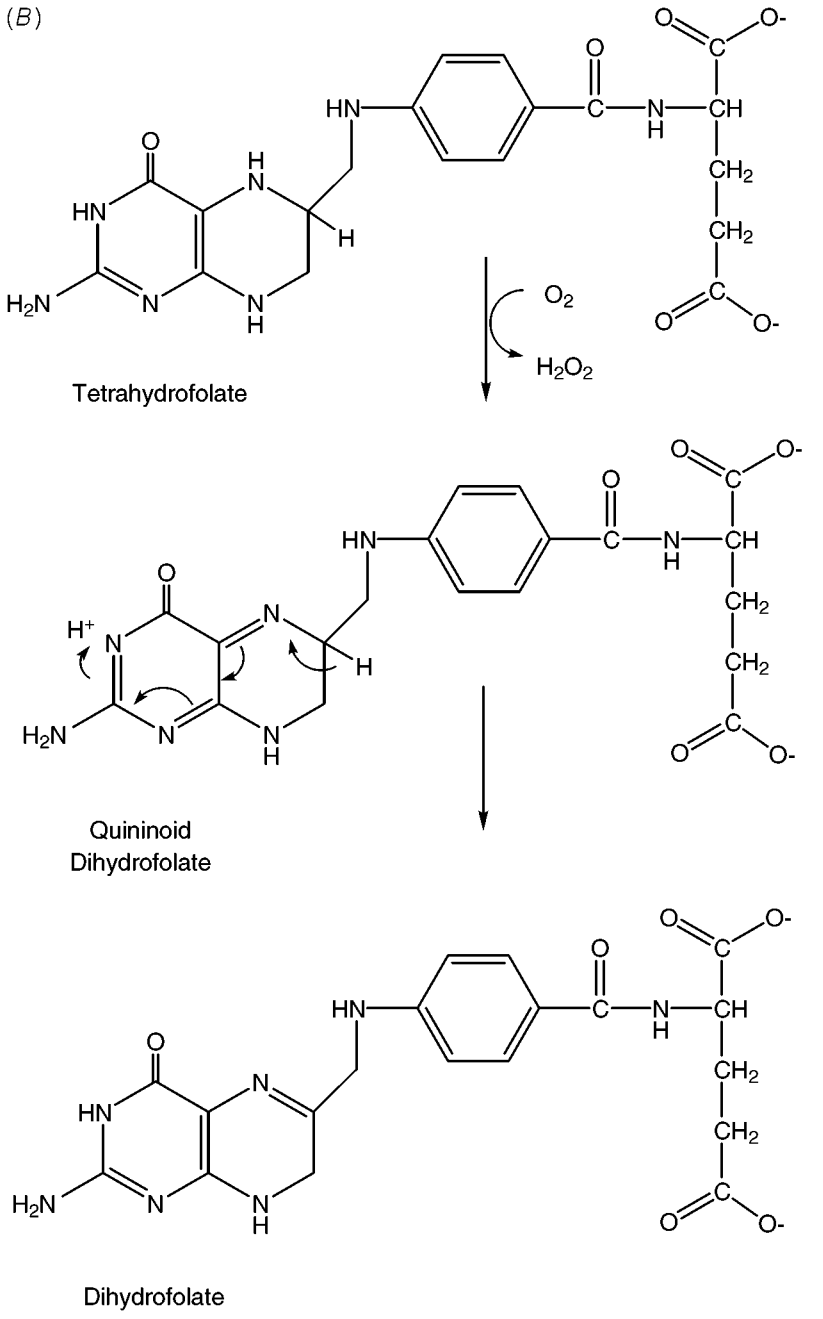

Figure 3 (*Continued*)

protein-bound folate (142). In humans, early observations indicated that the amount of folate in the diet is at least 10-fold greater than that found excreted intact in urine (12, 24). This discrepancy is not accounted for by stool folate (11). Dinning et al (26) were the first to observe in vivo cleavage of folate resulting in the formation of primary aromatic, diazotizable amines in rat urine following administration of both 5-formyltetrahydrofolate and folic acid. The formation of these catabolites was determined to be a major excretory process associated with folate turnover. These amines were later identified as *p*ABG and its acetylated derivative, acetamidobenzoylglutamate (A*p*ABG) (68). Similarly, these folate catabolites were also identified in urine of human subjects (68, 69).

The products of folate catabolism undergo different fates. Following folate cleavage, the export of *p*ABG polyglutamates is facilitated by removal of the polyglutamate polypeptide by GGH, and *p*ABG is likely acetylated in the cytosol by arylamine *N*-acetyltransferases to produce A*p*ABG (91), which represents >80% of total p*A*BG excretion in humans (42, 86). A*p*ABG does not accumulate in cells and is rapidly excreted in the urine (95). Geoghegan et al (34) suggest that the presence of free *p*ABG in urine is not due to incomplete acetylation in vivo but rather to nonspecific extracellular degradation of intact labile folates in the circulation or bladder, or to degradation during urine collection. Pterin aldehydes generated by folate catabolism through cleavage at the C9-N10 bond are further metabolized, retained in the liver, and slowly released (69, 98).

Mechanisms of folate turnover have been validated in rodent models. Labeled folic acid becomes fully equilibrated into tissue folate polyglutamate pools within 2–3 days in rats (59). Following equilibration, one study found only cleaved folates in the urine (59) whereas another found low levels of reduced folate monoglutamates (34, 96, 109) in addition to cleaved products in urine up to 12 days postdose. It is interesting to note that, in this animal model, folate turnover rates displayed tissue-specific variations. Turnover rates of folate in the liver and kidney were very slow relative to heart, spleen, testes, and hind leg muscle at 32 days following a single administration of labeled folic acid (109). The increased stability of folates in the liver may be accounted for by its high concentration of folate tight-binding proteins.

Mechanism of Folate Catabolism

In vivo folate catabolism has been assumed to result from random, nonregulated, nonenzymatic degradation of labile folate cofactors, in particular dihydrofolate and 10-formyltetrahydrofolate (77, 85). Both are particularly sensitive to oxidative degradation, and cellular accumulation of these folate derivatives is associated with decreases in cellular folate concentrations. The enzyme 10-formyltetrahydrofolate dehydrogenase (FDH) regulates 10-formyltetrahydrofolate levels by catalyzing the oxidative cleavage of the N10 formyl group of 10-formyltetrahydrofolate, resulting in the formation of CO_2 and tetrahydrofolate (15). Homozygous deletion of the gene encoding FDH in mice results in the accumulation of hepatic

10-formyltetrahydrofolate and a 30% decrease in intracellular folate concentrations when the animals are maintained on a standard chow diet (15). Similarly, intracellular folate concentrations and FDH levels positively correlate in regions of rat brain, which supports the hypothesis that 10-formyltetrahydrofolate accumulation impairs folate accumulation (77). However, there is no direct evidence that cellular accumulation of 10-formyltetrahydrofolate results in the catabolism of this folate derivative by either enzymatic or nonenzymatic mechanisms.

Cellular accumulation of dihydrofolate does result in increased folate catabolism in prokaryotes (97). Bacteria exposed to the dihydrofolate reductase inhibitor methotrexate display rapid accumulation of both dihydrofolate and *p*ABG, with subsequent folate deficiency. In mammals, increased rates of folate catabolism are associated with increased rates of cell division, presumably resulting from robust thymidylate synthesis and therefore dihydrofolate formation (85). Nonenzymatic catabolism of dihydrofolate is the assumed, but not proven, biochemical mechanism for turnover (85).

Recently, a protein was purified from rat tissue that generated *p*ABG from 5-formyltetrahydrofolate (121). The purified protein was identified as ferritin, the major iron-storage protein in the body [as reviewed by Harrison & Arosio (47)]. Ferritins are 24mers that form a hollow protein shell with a $M_r \approx 500{,}000$ and can store up to 4500 atoms of Fe^{3+} (33, 47). In vertebrates, ferritin is primarily localized to the cytoplasm and is comprised of varying proportions of heavy-chain (HCF) and light-chain (LCF) subunits (47). Both cell type and iron status determine the relative proportions of HCF and LCF.

Ferritin-mediated folate catabolism exhibits less than a single turnover per ferritin monomeric subunit and therefore is not a catalytic event in vitro (121). The K_m of 5-formyltetrahydrofolate for ferritin is in the millimolar range; the affinity for other forms of folate was not determined in this study, including the more-labile reduced folates (121). If the more oxidatively labile folates are the best substrates for ferritin-mediated catabolism, their accumulation may result in low cellular folate concentrations. Previous attempts to purify an enzyme from mammalian tissue that catalyzed the oxidative cleavage of folates to *p*ABG had not been successful (106). These attempts utilized the more-labile forms of reduced folates and, therefore, suffered from high background rates of nonenzymatic degradation, or utilized oxidized synthetic folic acid, which is not a substrate for ferritin-mediated catabolism.

Expression of rat HCF two- to fourfold over endogenous concentrations in Chinese hamster ovary cells increases rates of folate turnover nearly proportionally and reduces intracellular folate concentrations by 15% relative to control cells when cultured in the presence of pharmacological concentrations of folic acid (121). The same cells cultured in the absence of folate for 48 h display up to a 40% decrease in intracellular folate concentrations. Folates were shown to be less susceptible to ferritin-mediated catabolism with increased residency time in the cell, indicating that ferritin preferentially scavenges non–protein-bound folates that lack longer glutamate polypeptides (121). Therefore, folate turnover

can regulate intracellular folate concentrations independent of exogenous folic acid concentrations, and folate catabolism may play a key role in regulating intracellular folate concentrations.

PHYSIOLOGICAL STATES ASSOCIATED WITH ALTERED FOLATE STATUS AND INCREASED RATES OF FOLATE CATABOLISM

Folate Nutriture and Status

The presence of A*p*ABG in urine is indicative of folate catabolism, and its concentration in urine is considered a reliable indicator of folate status (131) and turnover (34) in rats. Total urinary *p*ABG excretion exceeded that of folate excretion in rats fed diets containing a range of folic acid from deficient to adequate (131). In this study, urinary *p*ABG and hepatic folate increased linearly with increases in dietary folate (131). Total urinary A*p*ABG excretion also greatly exceeded that of intact folates (most of which was 5-methyltetrahydrofolate) in nonpregnant women under conditions of controlled dietary intake (41). However, in this study, urinary folate, but not A*p*ABG, increased with increasing folic acid intake (41), a finding that was attributed to small sample size. Larger, related studies from the same laboratory did observe increased urinary A*p*ABG, *p*ABG, as well as intact folate as a function of folate intake. Therefore, folate intake likely influences the rate of folate turnover and catabolism in humans (14).

Folate supplementation at pharmacological levels also influences the urinary excretion of folate and total *p*ABG in humans. One study found that folic acid supplementation caused large increases in urinary folate concentration but only small ones in total *p*ABG (68). Both reduced folates and oxidized folic acid were found in urine, indicating that not all the administered folic acid was processed to reduced cellular polyglutamate cofactors. Similarly, McPartlin et al (88) did not observe any significant change in total *p*ABG with supplementation of folic acid at levels more than 10 times the recommended daily allowance over the course of 3 days, but this may have been due to the shorter supplementation period (68). In conclusion, the increases in total *p*ABG associated with increased folate intake and status are consistent with the idea that folate catabolism is necessary to maintain low intracellular folate concentrations during periods of increased folate intake.

Pregnancy and Neonatal Growth

Pregnancy imposes stress on folate stores (84) due to increased needs for growth of the fetus, the placenta, and maternal tissues (2). Increased folate is also required for uterine enlargement and expansion of blood volume, which necessitates increased red cell production (84). This increased need for folate does not come at the expense of the fetus, as newborns have significantly higher plasma and RBC folate levels

compared with maternal values (14, 28, 36). Inadequate folate status is common in pregnancy (6) and is associated with increased incidence of maternal megaloblastic anemia, low-birth-weight babies (102), and preterm delivery (105).

In pregnant women, the need for folate exceeds that of the calculated total fetal and placental folate content at term, indicating that increased folate turnover may occur during pregnancy (87). Studies of rat models have demonstrated not only increased need for folate cofactors and increased one-carbon metabolism during pregnancy but also elevated rates of folate catabolism. Urinary A*p*ABG production was shown to be higher in pregnant dams relative to nonpregnant control dams when free-fed or pair-fed on a purified, folate-free diet (84). Although some of the increase in A*p*ABG excretion was accounted for by weight gain and therefore was independent of pregnancy, there was an authentic effect of pregnancy on folate turnover. In this study, urinary A*p*ABG excretion increased from day 2 of gestation and peaked at day 18 to levels of up to three times that of nonpregnant rats and then fell off significantly despite increasing weight gain until day 21, the day of parturition (84). The authors of this study noted that within the rat placenta, DNA content does not change after day 17 of gestation, and growth becomes hypertrophic whereas fetal growth is almost entirely hyperplastic. Therefore, elevated A*p*ABG excretion may represent the increasing needs for folate for DNA synthesis by the fetal and placental tissues, whereas the decrease in excretion corresponds to decreased needs as the placental tissue switches to hypertrophic growth (84). Elevated rates of folate catabolism continue during growth of weanling rats (85). Using a catch-up growth model, urinary A*p*ABG excretion was shown to increase with hyperplastic growth. This suggests that the rate of folate catabolism is related to folate utilization in cell division, presumably resulting from increased generation of oxidatively labile dihydrofolate during DNA synthesis (85). These studies indicate a paradox: Folate catabolism increases during periods of increased physiological needs for folate in rats.

Results from studies of human folate catabolism during pregnancy are conflicting. An initial pilot study of six healthy nonpregnant women and six age-matched pregnant women demonstrated that urinary excretion of *p*ABG and A*p*ABG increased during the second (∼200%) and third (∼150%) trimesters in pregnant women compared with total *p*ABG excretion of pregnant women during the first trimester and compared with nonpregnant controls (87). A larger subsequent study by the same laboratory, including 24 healthy gravid women and 25 nonpregnant controls, supported the pilot study, as evidenced by increased urinary A*p*ABG during pregnancy, with maximal rates of folate catabolism occurring during the third trimester (50). At this time, rates of folate catabolism were elevated by more than twofold relative to nonpregnant controls. From this study, it was estimated that folate requirements during pregnancy are increased by 200–300 μg per day to offset losses from catabolism. In contrast, compared with nonpregnant controls, Caudill et al (14) did not find increased catabolism in the second trimester for pregnant women in either week 14 or week 26 of gestation. In contradiction with other studies, results from this study indicated that folate catabolic rates actually decrease

during pregnancy. Excretion of A*p*ABG at week 26 of gestation was significantly lower than in nonpregnant controls when women were given 450 μg of folate per day, although this effect was not seen with a folate intake of 850 μg per day. The disparate results between these two studies have been attributed to different analytical procedures used to measure A*p*ABG and various aspects of the patient treatment protocol, including the duration of controlled dietary intake (14, 87).

Pregnancy is also associated with elevated ferritin expression. Recently, HCF message levels were found to be markedly elevated during the implantation stage of pregnancy in rats (145). Compared with minimal levels of HCF mRNA normally found in the uteri of nonpregnant (estrous cycle) rats, HCF mRNA increased 8- to 10-fold in pregnant rats. The HCF mRNA and protein expression profiles parallel progesterone concentrations, which are low in the estrous cycle, surge after fertilization and remain high until parturition, when they drop sharply. HCF mRNA also declines rapidly after parturition (145). Increased HCF expression during pregnancy was localized primarily in the cytoplasm of endometrial stromal cells whereas LCF mRNA expression did not change appreciably. Progesterone alone can induce HCF message levels and antiprogestins abolish elevations in HCF protein and mRNA when administered in early pregnancy. Likewise, progesterone treatment of ovariectomized animals results in a 25-fold increase in HCF message in the uterus. Estrogen priming can enhance the effect of progesterone in ovariectomized rats, whereas estrogen, glucocorticoid, or androgen treatment alone cannot influence expression of HCF (145).

During pregnancy, fetal iron requirements are greatest in the third trimester (70). In general, maternal serum ferritin levels decline with gestation as a result of haemodilution occurring in early pregnancy and as a result of heavy demands for iron (38, 96). Maternal serum ferritin concentrations are lowest during the third trimester of pregnancy (104), with mean levels approximating those of iron-deficiency anemia, and they decline despite the use of iron supplements (96). Failure of serum ferritin levels to decline in the third trimester is associated with iron-deficiency anemia, anemia, and lower levels of serum and RBC folate during pregnancy. High maternal serum ferritin is also a marker for maternal infection (123). Furthermore, in the third trimester, both high ferritin levels and low RBC folate are associated with preterm and very preterm delivery. High ferritin levels in the second trimester are also a predictor for preterm birth (38, 123). It is possible that low maternal ferritin levels during periods of high fetal iron requirements may occur in order to spare folate by decreasing catabolism. Although serum ferritin levels are not considered reliable indicators of tissue ferritin levels, well-designed animal studies investigating the effects of elevated maternal ferritin on folate catabolism, folate status, and pregnancy outcome are clearly warranted.

Oral Contraceptive Use

The use of oral contraceptive agents (OCA) has been associated with low serum and RBC folate levels in women 20–44 years of age (74). Long-term use has also

been associated with folate deficiency (16). The relationship between OCA use and folate status was first described in 1968 (115), but whether or not OCA use has a detrimental effect on folate status is still controversial (39). In a rat model, OCA treatment led to decreased plasma and RBC folate associated with moderate hyperhomocysteinemia (27). In addition, women who reported using OCAs regularly before pregnancy had lower RBC folate in the first trimester than did nonusers (127). Women's mean serum and RBC folate concentrations were significantly lower in users than nonusers (114), but serum and RBC folate levels were similar to those of controls in adolescent females using OCAs (39). Although some studies did not detect systemic folate deficiency, they did show localized megaloblastic changes in Papanicolaou smears from uterine cervix cells in patients taking OCAs. Cervical cells reverted to normal after folic acid therapy (75). Although several mechanisms have been proposed to explain the possible influence of OCAs on blood folate levels, two of direct relevance to this review are (*a*) the increased serum clearance and urinary excretion of folate (114) and (*b*) the increased hepatic catabolism of folates (82). The latter may be accounted for, in part, by increased ferritin synthesis associated with hormonal contraceptive use (124).

Cancer

Rapidly proliferating cancer cells have accelerated DNA synthesis and therefore an increased requirement for folate (64). Cancerous tumor cells exhibit increased rates of folate uptake (31). Large doses of folate in well-established cancers lead to accelerated proliferation whereas folate deprivation leads to reduced or delayed growth (64). Tumor cells also display increased rates of folate catabolism. The presence of ascitic tumors in mice results in a 50% increased rate of folate catabolism, as measured by the urinary catabolites, [^{3}H]*p*ABG and [^{3}H]*N*-A*p*ABG (58). Cancer patients with active, untreated, or metastatic malignancies exhibit folate deficiency without evidence of malnourishment, malabsorption, or increased intact folate excretion (43). In the absence of significantly low serum or RBC folate concentrations, localized tissue deficiency has been observed in patients with colorectal adenoma whose normal mucosa have significantly lower folate levels in comparison to patients with nonneoplastic polyps (63). The folate content of neoplastic cells is significantly lower than the adjacent normal cells, and folate levels in normal cells from patients and controls were not significantly different (89).

Increased folate catabolism in cancerous tissue may be at least partly due to altered ferritin levels. Ferritin is increased in both cancerous tissue and in serum of afflicted patients. Because elevated serum ferritin is associated with malignant disorders (66), liver disease (23), infection, total body iron stores, and inflammation (45, 47), tissue expression is likely a better marker for carcinogenic processes. Increased generation of intracellular ferritin has been detected in leukemic cells (135), kidney cancer (65), and mammary carcinomas (76) relative to normal tissue. Patients with malignant breast carcinoma showed a sevenfold increase in tissue

cytosol ferritin compared with those with benign breast disease. These differences in tissue ferritin were observed without detectable differences in serum ferritin levels (45). In a related study, ferritin concentration was elevated sixfold in malignant versus benign mammary tissue, with high cytosolic concentrations correlating with poor prognosis and dedifferentiation (134).

Increased ferritin levels in cancer cells may be influenced by expression of the oncogene c-*myc*. Using a subtraction-enhanced display technique, both HCF and c-myc were identified as mRNAs that are overexpressed in chemically induced hepatocellular carcinoma in rats. In this same model, the expression of HCF increased 10-fold as the tumor progressed (138). The relationship between c-myc and ferritin expression was validated in cell culture. Transfection of nontumorigenic clones with the c-myc cDNA increased expression of HCF mRNA in clones that acquired a tumorigenic phenotype (93). Wu et al (139) found increased HCF mRNA expression in patients with hepatocellular carcinoma, but not in patients with benign tumors (adenomas) or cirrhosis, and not in healthy patients. HCF mRNA levels were 2- to 12-fold higher in tumors compared with adjacent nontumor tissues in 70% of patients and were highly correlated with c-myc mRNA levels in most of the patients (139). There have been no studies relating ferritin levels and folate concentrations in cancer cells.

Anticonvulsant Drug Use

The effects of anticonvulsants on folate status have been recently reviewed (40). Although only 1% of patients treated with anticonvulsants manifest megaloblastic anemia, a greater proportion manifest macrocytosis as well as reduced serum and RBC folate, which may indicate an increased turnover or catabolism of folate (16). There is a negative dose response between carbamazepine and RBC folate levels in patients, with RBC folate levels declining further in patients taking more than one drug (37). Compared with phenobarbital, primidone, carbamazepine, and valproate, phenytoin has the greatest impact on tissue folate concentrations in rats. Chronic phenytoin treatment reduces total liver and brain folate concentrations by 66% and 25%, respectively, in rats without affecting plasma folate concentrations (13, 44). Rats treated with diphenylhydantoin had increased excretion of total *p*ABG by 80%–100% compared with control and phenobarbitone-treated rats (59), indicating a role for enhanced folate catabolism in the lowering of tissue folate by diphenylhydantoin. Both the mean daily excretion and the cumulative excretion of total *p*ABG increased as a function of increased phenytoin administration (59). This result is in accordance with clinical observations, which suggest that folate deficiency is more highly associated with phenytoin administration than with phenobarbitone. In addition to increasing folate catabolism, anticonvulsants also can directly alter folate-dependent reactions (13). For instance, valproic acid can inhibit mitochondrial folate metabolism and is also associated with increased incidence of NTDs (133). The effects of anticonvulsant drugs on ferritin levels are unknown.

Thyroid Hormone

Purported relationships between thyroid hormone status and folate status have been conflicting, and there have been no direct studies on the effects of thyroid hormone on folate catabolism. Some studies demonstrate that hyperthyroid patients have low serum folate levels and abnormally rapid clearance of folic acid from plasma (25, 74). In contrast, other studies have indicated that serum and RBC folate levels are significantly higher in hyperthyroidic patients compared to when these same patients became euthyroidic, although these changes occurred within the reference range (33). The differences in these studies may be accounted for by lack of control for dietary intake and by the severity of the disease. Thyroid-stimulating hormone increases HCF mRNA levels in rat thyroid cells, possibly by increasing HCF transcription (22). Additionally, erythropoietin, thyrotropin-releasing hormone, and thyroid hormone all regulate ferritin translational rates (125). In rats, hyperthyroidism also decreases hepatic FDH by 65%. Both increased ferritin and decreased FDH activity would be expected to lower cellular folate concentrations.

Alcohol

It is well-known that chronic alcoholism is associated with impaired folate status and that excess ethanol is often associated with folate deficiency (46). Ethanol-related folate deficiency can develop because of dietary inadequacy, intestinal malabsorption, altered hepatobiliary metabolism, increased renal excretion, and possibly increased folate catabolism (46). It has been proposed that ethanol may directly enhance oxidation of folate. Acetaldehyde, a metabolite of ethanol via alcohol dehydrogenase, and aldehyde oxidase may produce reactive oxygen species in the presence of free iron (12). Incubation of folate (5-methyltetrahydrofolate, 5-formyltetrahydrofolate, and folic acid) with acetaldehyde, xanthine oxidase, and iron results in *p*ABG formation (13). Cleavage of 5-methyltetrahydrofolate by ethanol was inhibited by superoxide dismutase, 4-methylpyrazole (inhibitor of alcohol dehydrogenase), and desferrioxamine (tight chelator of iron) and enhanced by the addition of ferritin and free Fe^{2+} (13). Another similar in vitro study suggested free radical oxidant species target oxidation at the pteridine ring (122). However, an in vivo animal study did not find any differences in the rate of urinary folate catabolite excretion between control animals and mice after either a chronic period of or acute alcohol ingestion (57), indicating that the effects of ethanol on folate status are independent of catabolism.

CONCLUSIONS

The studies outlined in this review suggest that folate catabolism is a regulated, enzyme-mediated event that may play a central role in regulating intracellular folate concentrations. Although the act of destroying a valuable and required

nutrient seems counterintuitive, catabolism-mediated maintenance of intracellular folate at low or perhaps subsaturation levels may be necessary for effective one-carbon metabolism and may facilitate several regulatory mechanisms associated with folate metabolism. First, numerous studies have indicated that folate coenzymes are directly channeled among folate-dependent enzymes (103). Direct transfer of a folate cofactor between a donor and acceptor enzyme necessitates that the acceptor enzyme be unliganded. Therefore, metabolic channeling of folate cofactors can only occur under subsaturation conditions, and loss of channeling resulting from excess folate cofactors has been demonstrated in an in vitro reconstitution metabolic system (119). Secondly, maintenance of subsaturation levels of folate permits autotranslational regulation of thymidylate synthase and dihydrofolate reductase expression. Both dihydrofolate reductase and thymidylate synthase can bind to their respective transcripts and may inhibit translation (19, 29, 67). Thymidylate synthase can also bind to other mRNA species, including c-myc, and p53. These enzymes competitively bind mRNA and folates at the same site on the protein. Therefore, autotranslational inhibition of dihydrofolate reductase and thymidylate synthase synthesis can only function when the enzymes are unliganded. Finally, low intracellular folate permits competition between folate-dependent metabolic pathways (108). It has been proposed that purine biosynthesis, thymidylate biosynthesis, and homocysteine remethylation vie for a limited pool of folate-activated one-carbon units (108). This competition between nucleotide biosynthesis and methionine synthesis may be a regulatory mechanism that permits increased SAM-dependent methylation when synthesis of nucleotides is depressed.

A definitive role for folate degradation products in cellular function has yet to be established. There is some evidence that *p*ABG may compete with folic acid for folate-binding proteins (8) and may assist the binding of folate to 5,10-methylenetetrahydrofolate dehydrogenase (106). In vitro studies demonstrated that pterin aldehyde acts as a substrate for xanthine oxidase, and the product, pterin-6-carboxylic acid, can act as a weak inhibitor of this enzyme (117). However, there is no evidence that pterin species generated from folate catabolism function as biological cofactors.

Collectively, the studies outlined in this review strongly implicate an important role for folate catabolism in determining intracellular folate concentrations. Additionally, the identification of physiological states associated with increased folate catabolism indicate that folate catabolism is regulated. Although the role of ferritin in facilitating folate catabolism requires further validation, initial studies indicate a complex relationship among iron status, ferritin, and folate catabolism and status. Catabolism-mediated regulation of intracellular folate concentrations may be largely independent of serum folate availability and may impact folate-mediated one-carbon metabolism much more effectively than does regulation of folate transport. Therefore, folate catabolism is implicated not only in influencing DNA synthesis and cellular homocysteine concentrations, but also in the metabolic regulation of DNA methylation and in the subsequent

effects on gene expression. Elucidating the biochemical mechanisms underlying the regulation of folate catabolism and its effects on intracellular folate concentrations will be required to understand the relationships between folate status and its associated pathologies.

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