Chapter 4

MODELING OF FOLATE METABOLISM[1]

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I. INTRODUCTION

Folate is a generic term for the group of structurally related pteroylglutamates and related compounds that exhibit similar vitamin activity as substrates and coenzymes involved in one carbon metabolism. Through this metabolic function, folate is involved, either directly or indirectly, in essential cellular processes, including the synthesis of nucleic acids, regeneration of methionine from homocysteine, and methylation of proteins, nucleic acids, and other compounds with *S*-adenosyl-methionine as methyl donor. Thus, many diverse cellular functions depend on an adequate supply of this vitamin and are impaired during periods of inadequate nutritional intake. Because of the role of folate in nucleic acid synthesis, the replication and maturation of various cell types (e.g., erythrocytes and intestinal mucosal epithelium) is impaired in folate deficiency. The metabolism and function also involves other nutrients, including zinc, riboflavin, vitamin B12, and vitamin B6, consequently, folate metabolism and utilization can be impaired as a secondary effect of other nutrient deficiencies.

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Folic acid (i.e., pteroylglutamic acid) is the synthetic form of the vitamin employed in food fortification and in nutritional supplements. Naturally occurring forms of folate exist mainly as tetrahydrofolate species with variable length of a polyglutamyl side chain (Fig. 1). The intestinal absorption of folic acid and reduced folates occurs in the jejunum via specific carrier mediated transport of the vitamin in monoglutamyl form. Deconjugation of dietary polyglutamyl folates is catalyzed mainly by intestinal pteroylpolyglutamate hydrolases (conjugases) and must occur prior to absorption. Most studies comparing the bioavailability of monoglutamyl versus polyglutamyl folates have indicated superior utilization of the monoglutamyl form. This provides strong evidence of the rate limiting nature of the *in vivo* action of the intestinal conjugase in folate absorption. Food composition appears to have considerable influence on the bioavailability of dietary folates. Folic acid added to various cereal grain-based foods exhibits approximately 50% bioavailability relative to folic acid administered as a supplement in water (Colman, 1982; Colman *et al.*, 1975). In addition, unidentified components of many foods have the ability to inhibit intestinal conjugase activity in vitro (Bhandari and Gregory, 1990) and, thus, may inhibit the deconjugation of dietary folates *in vivo*.

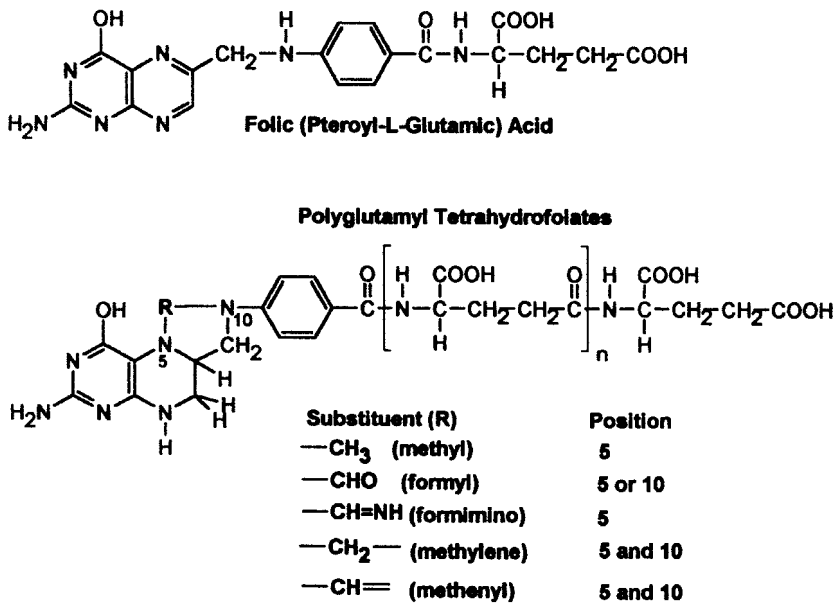


FIG. 1. Chemical structures of folic acid and tetrahydrofolates. Tetrahydrofolates are shown as the polyglutamyl form, n = 2–9. R represents the one-carbon substituent for the folates shown, and R = H for unsubstituted tetrahydrofolates.

Considerable uncertainty and controversy exists concerning the folate requirement for humans. The review of data concerning the human folate requirement by the Food and Nutrition Board (1989) suggests that the daily maintenance requirement is 100–200 μg of available folic acid equivalents. The 1989 RDAs were reduced to 200 and 180 μg for adult men and women, respectively, from the previous RDA of 400 on the basis of such evidence (Food and Nutrition Board, 1989). Similarly, the Canadian RDA for folate was set at 3 μg/kg body wt or 210 μg for a 70-kg individual. These lower RDAs may be inadequate for certain population groups, however (Sauberlich, 1990; Bailey, 1992; McPartlin *et al.*, 1993). It is currently difficult or impossible to predict the quantitative effect on folate nutritional status of factors such as: (a) changes in folate intake, (b) differences in folate bioavailability, (c) effects of pregnancy and lactation on folate requirements, and (d) pharmaceuticals with antifolate properties. In addition, the development of mathematical models would improve our ability to evaluate methods of nutritional status assessment for this vitamin.

II. KEY ELEMENTS OF FOLATE METABOLISM RELEVANT TO MODELING

Although many studies of the *in vivo* kinetics of folate have been conducted, none has fully represented all aspects of folate metabolism in any single animal species. The documented differences in certain aspects of folate digestion and metabolism among certain animal species may further complicate modeling, as does the influence of the dietary folate intake before and during the kinetic study. However, since the basic processes of absorption, metabolic function, catabolism, and excretion are quite similar, one would expect that similar models would apply to most species. At its simplest, folate metabolism can be described as two kinetically detectable pools: (a) one small pool with rapid turnover kinetics, which is composed mainly of the monoglutamyl forms of folates in plasma, and (b) a larger, slow-turnover pool of folates functionally trapped in tissues as polyglutamyl forms, many of which are noncovalently bound to proteins. A schematic overview of whole body folate metabolism is shown in Fig. 2.

The commercial availability of [^{3}H]folic acid at high-specific activity has made feasible many studies of folate kinetics. The studies involving bolus dose administration of the labeled folate consistently have shown a biphasic curve for disappearance of folate by urinary and fecal routes. Representative of this approach is the well-designed study by Tamura and Halsted (1983) of folate metabolism in monkeys. Following a bolus dose of [^{3}H]folic acid, the isotope was excreted by fecal and urinary routes. The extent of

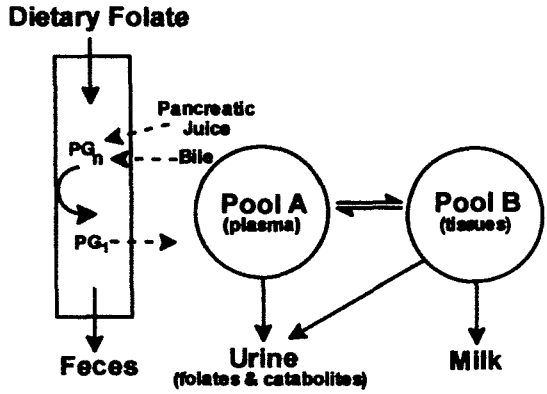


FIG. 2. Schematic diagram of whole-body folate metabolism. Dietary forms of polyglutamyl folates are deconjugated in the jejunum (vertical rectangle on left) by mucosal and, to a lesser extent, pancreatic hydrolases. Secretion of folate in bile accounts for an enterohepatic circulation that has not been incorporated into modeling. Tissues behave, at least superficially, as a large, kinetically slow, pool.

fecal loss was quite pronounced and approached 30% of daily turnover. On the basis of urinary excretion of total tritium, and assuming a two-pool model with output from the rapid turnover pool (Fig. 3), half times of 1.8 ± 0.3 and 119 ± 11 days were reported for rapid and slow phases of folate turnover, respectively. Another important finding was that chronically alcoholic monkeys exhibited substantially altered short-term kinetics; half times for folate turnover in alcoholic monkeys were 1.1 ± 0.2 and 85 ± 26 days

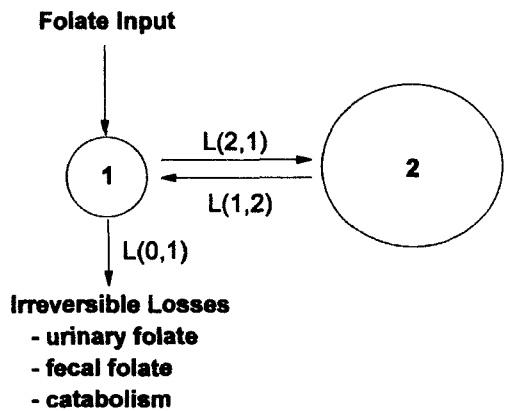


FIG. 3. Two-pool model of folate metabolism with output only from the rapid turnover pool. Output represents the sum of urinary, fecal, and catabolic losses.

for rapid and slow phases, respectively. Whether this difference reflected the nutritional status of the monkeys, effects of alcohol on folate catabolism, or other metabolic factors was not determined. The cumulative excretion curves showed markedly reduced *in vivo* retention of the isotope in alcoholic animals, with increased excretion by both urinary and fecal routes.

The excretion of folate and its metabolites by both urinary and fecal routes was demonstrated in a study with [2-^{14}C]folic acid administered to a single adult woman as several daily doses followed by periodic urine and fecal collection over several months (Krumdieck *et al.*, 1978). Fecal excretion accounted for nearly half of the isotope elimination. From excretion of urinary ^{14}C, two pools were readily apparent with half times of ~31.5 hr and ~100 days. Periodic administration of diphenylhydantoin to this subject (a Hodgkin's disease patient in remission) did not notably alter the turnover kinetics.

A similar study with radiotracers was conducted in our laboratory to examine the short-term kinetics of folate metabolism in rats. Three different folates were separately employed to assess any differences in bioavailability among these forms of the vitamin (Bhandari and Gregory, 1992). Similar to the study with monkeys, cumulative excretion via the fecal route comprised approximately one-third of the folate turnover, with the remainder as urinary excretion of intact folates and catabolic products. There were no detectable differences among these forms of the vitamin with respect to excretion kinetics by urinary or fecal routes and only minor short-term differences in tissue distribution in relation to the form of folate administered. Assuming a two-pool model with one output, half times of ~0.1 and 13–16 days were calculated. Further kinetic studies in rats are in progress using controlled dietary folate intake and longer collection periods to permit long-term modeling (Scott and Gregory, 1994). Other previous studies with radiolabeled folates in rats also have shown biphasic patterns of urinary excretion (Murphy and Scott, 1979).

Many short-term kinetic studies have been reported on the basis of plasma and serum folate levels in humans. Typical protocols involve the administration of a folate tracer dose to folate-saturated subjects or else a large folate dose given to subjects of more normal folate status (Reich and Gonczy, 1979; Bunni *et al.*, 1989; Wolfrom *et al.*, 1990; Priest *et al.*, 1991; von der Porten *et al.*, 1992). Although these studies have clinical relevance with respect to the short-term kinetics of acute doses of the vitamin (e.g., postchemotherapy 5-formyl-tetrahydrofolate), they often provide little or no information about the major body folate pool that exhibits much slower kinetics.

A key aspect of folate metabolism is the catabolism. Folate catabolism occurs through oxidative cleavage of tetrahydrofolates at the C9–N10 bond

to yield a pterin (e.g., pterin-6-carboxylic acid or pterin-6-carboxaldehyde) and *para*-aminobenzoylglutamate (pABG), or its polyglutamate form. This presumably occurs within tissue folate pools through nonspecific oxidation or interaction with metabolically generated free radicals (Shaw *et al.*, 1989). Following enzymatic hydrolysis of polyglutamyl forms of pABG to monoglutamyl pABG, this catabolite is acetylated in the liver to *para*-acetamidobenzoylglutamate (apABG), which is rapidly excreted in urine (Murphy *et al.*, 1976; Murphy and Scott, 1979; McPartlin *et al.*, 1992, 1993). Although folate catabolism has been known for many years (Dinning *et al.*, 1957), its quantitative significance has only recently been recognized. McPartlin and associates (1986) have shown that apABG excretion is much greater than urinary folate excretion in adult humans. Significantly, folate catabolism increases during the second trimester of pregnancy and accounts for much of the increased folate requirement (McPartlin *et al.*, 1993). The excretion of total pABG (free and acetamido) in rats and humans far exceeds urinary folate (Murphy *et al.*, 1976; Murphy and Scott, 1979; McNulty *et al.*, 1993; Kownacki-Brown *et al.*, 1993; Wang *et al.*, 1994).

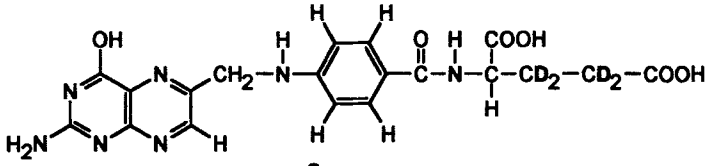
It must be recognized in modeling that folate catabolism, which occurs by mainly oxidative cleavage of tetrahydrofolates by free radicals and various oxidants (primarily in liver and other tissues), represents a major irretrievable loss from the body. Most previous studies of the *in vivo* kinetics of folate have employed a two-pool model with output only from the rapid turnover pool representing the sum of urinary, fecal, and catabolic losses (Fig. 3). Our current working hypothesis is that kinetic models, for greatest physiological accuracy, should include a provision for irretrievable losses from the tissue pool as well as from the rapid turnover (i.e., plasma) pool.

III. STABLE-ISOTOPIC STUDIES IN HUMANS

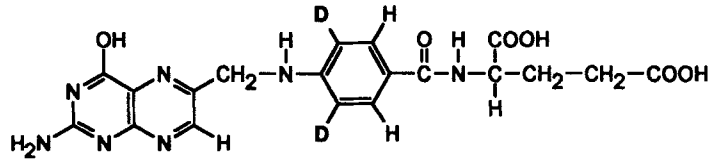
Stable-isotopic labeling methods permit the evaluation of micronutrient kinetics with the high specificity of isotopic procedures but with no radiation exposure to the subjects. Another advantage is the ability to conduct studies in which two or more labeled forms are administered simultaneously (e.g., monoglutamyl and polyglutamyl folates, oral and iv routes, etc.). The synthesis, use, and mass spectral analysis of folates labeled with deuterium or other stable isotopes has been reviewed (Gregory and Toth, 1990; Gregory, 1989). Folates labeled with deuterium in either the 3′,5′-positions of the central benzoyl moiety ($^2H_2$-folate or d2-folate; Gregory and Toth, 1988a; Gregory, 1990) or the $\beta,\beta,\gamma,\gamma$-positions of the glutamate moiety ($^2H_4$-folate or d4-folate; Gregory and Toth, 1988b) may be readily prepared and are

fully suitable for *in vivo* use (Fig. 4). Although initially developed for use in the study of folate bioavailability, these labeled folates permit the study of long-term folate kinetics through protocols that involve chronic administration for extended periods of time. These protocols and modeling procedures are in their early stages of development at this time. Studies conducted to date have been based primarily on measurement of changes in the isotopic enrichment of urinary folate (isolated by affinity chromatography).

Von der Porten *et al.* (1992) conducted a study of the changes in folate nutritional status during chronic (4 week) supplementation with a relatively large (1.6 mg/day) dose of folic acid. The use of d2-folic acid permitted initial observations of *in vivo* labeling, although the protocol was not designed for isotopic kinetics. In addition, the protocol of 4-week supplementation followed by total withdrawal of the supplement was, by design, not a steady-state situation in terms of folate status of the subjects and, presumably, the size of body folate pools. After only 7 days of supplementation and continuing over the 28-day period, mean enrichment of urinary folate was 69%, in comparison to the fact that the isotopic enrichment of *total* ingested folate (dietary + supplemental) was approximately 89%. Thus initial labeling of body folate occurred rapidly, but isotopic equilibrium was not obtained even after 28 day with an intake of ~9 times the RDA value. Analysis of erythrocyte folate over the course of supplementation indicated a maximum isotopic enrichment of only ~10%. This is consistent with the fact that folate is deposited in the erythrocyte primarily at the time of erythropoiesis;



[Glu-$^2H_4$]Folic Acid



[3',5'-$^2H_2$]Folic Acid

FIG. 4. Structure of deuterium-labeled forms of folic acid.

thus, labeling of erythrocyte folate lags behind other body pools as a result of the long life time of preexisting unlabeled erythrocytes.

Several studies have been conducted to examine *in vivo* kinetics of folate under conditions approximating more normal intake levels. We have recently conducted a study in which a single adult female was maintained on a protocol with controlled diet for 10 weeks with a total folate intake of approximately 202 μg/day, composed of ~12 μg/day from a low-folate diet and 190 μg/day from synthetic folic acid in apple juice (Gregory *et al.*, 1994). The first 2 weeks of the study were an equilibration period in which the 190 μg/day folic acid was not isotopically labeled, while for the next 8-week period the subject consumed d2-folic acid. This protocol maintained the subject in steady state as judged by serum and urinary folate concentration. As shown in Fig. 5, enrichment of urinary folate increased progressively over the experimental period, although the final enrichment (~10%) was far less than that calculated for total ingested folate (~28.4%). Fitting these data to the two-pool model with output only from pool 1 (e.g., Fig. 4), a fractional catabolic rate (FCR) for total body folate was estimated to be ~0.0055 day^{-1}, corresponding to a system mean residence time (MRT) of ~182 days. Preliminary analysis of these data with an expanded model to incorporate catabolic losses primarily from the tissue pool (Fig. 6) yielded a similar fit to that shown in Fig. 5. The estimated FCR was 0.00342

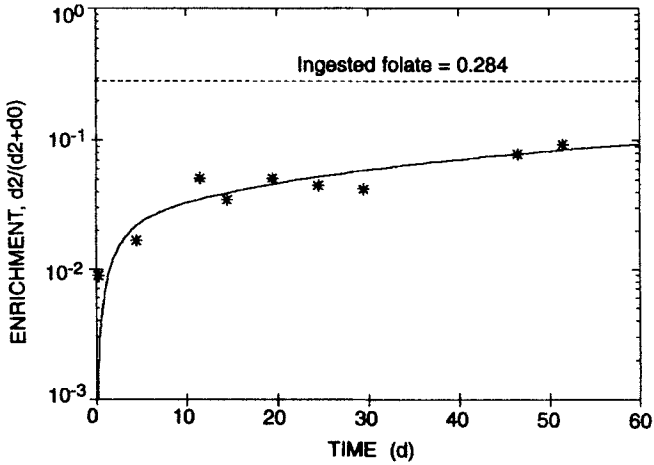


FIG. 5. Isotopic enrichment of urinary folate in an adult female subject during chronic ingestion of a low-folate diet supplemented with d2-folic acid. The value of 0.284 enrichment represents the labeling (i.e., isotopic enrichment) of ingested folate from dietary and supplemental sources. In this figure, the data were fit to the two-pool, one-output model depicted in Fig. 3, which yielded the solid regression line (Gregory *et al.*, 1994).

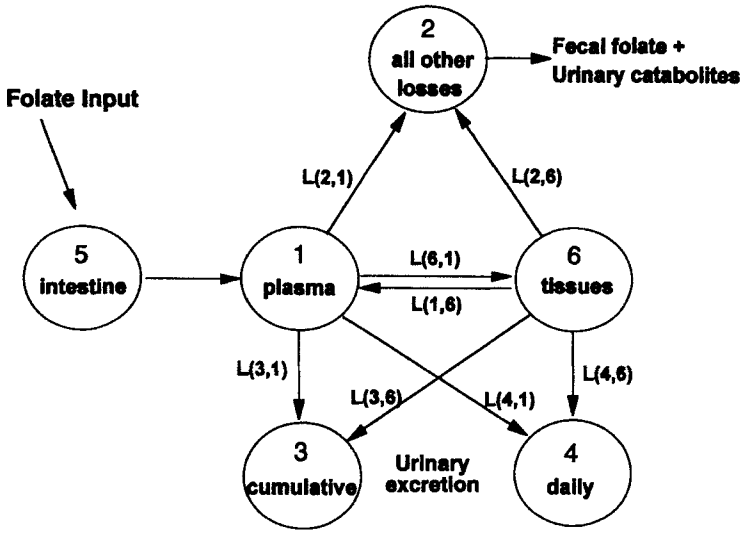


FIG. 6. Expanded model of *in vivo* folate metabolism. The pools are defined as follows: 1, rapid turnover folate; 6, slow turnover folate (tissues); 2, irretrievable losses by fecal excretion and catabolism; 3, cumulative excretion of urinary folate; 4, fractional (daily) excretion of urinary folate. Analysis was performed with parallel models for labeled and nonlabeled folate.

day^{-1}, equivalent to an MRT of ~292 for total body folate. Estimated masses for pool 1 (rapidly exchanging folate) and pool 6 (slow-turnover tissue folates) were 10.9 and 76.8 mg, respectively. These preliminary results indicate the very slow turnover of body folate and the experimental impracticality of attempting to achieve isotopic steady state with this protocol.

An unanswered question in this type of protocol is the bioavailability of the ingested folate. If one assumes a fractional absorption of 0.67 for total ingested folate (i.e., labeled + dietary), this model yields estimates for the masses of pools 1 and 6 of 2.95 and 36.5 mg, respectively. Thus, the bioavailability of ingested folate is a critically important factor in controlling the *in vivo* pool sizes and, thus, the nutritional status of the individual.

As an alternative to the long-term controlled dietary protocol, a similar study was conducted with free-living men ($n = 4$) consuming self-selected diets (Stites *et al.*, 1994). After a 2-week equilibration period with 200 μg/day unlabeled folate supplement, the subjects consumed 200 μg/day of a 1:1 mixture of d4-folic acid and unlabeled folic acid for an 8-week period, followed by a switch back to 200 μg/day of unlabeled folate. Dietary folate intake was estimated from food records using a computerized data base. Typical results (Fig. 7) indicate slow labeling of body folate, as discussed above. Analysis of these data using the expanded model (Fig. 6) yielded

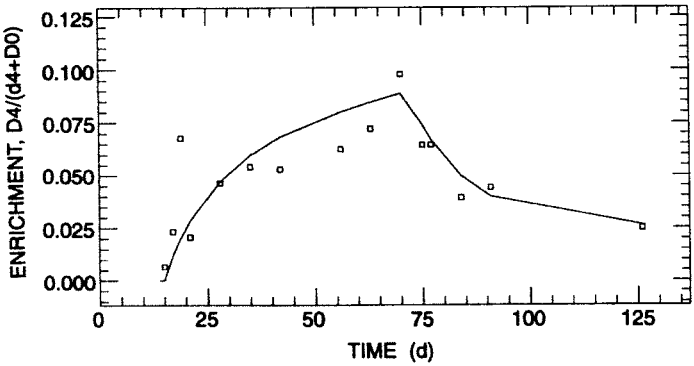


FIG. 7. Isotopic enrichment of urinary folate in an adult male subject. In addition to dietary folate, the subjects received the following daily supplement: Days 15–75, 100 μg d4-folic acid + 100 μg nonlabeled folic acid; Days 76–126, 200 μg nonlabeled folic acid (no d4-folic acid) (Stites *et al.*, 1994). The solid line represents enrichment as predicted using the model shown in Fig. 6.

FCR and MRT values of approximately 0.0088 day^{-1} and 114 days, respectively. The mean masses of folate in pool 1 (rapidly exchanging folate) and pool 6 (slow turnover tissue folates) were 10.9 and 76.8 mg, respectively. These masses were greater than those estimated from the previous study with one female subject, which may reflect the larger body mass of the male subjects as well as their greater folate intake in this study.

On the basis of analysis of these results, it has become apparent that reliance on measurements of enrichment of urinary folate leaves excessive uncertainty in all aspects of modeling and kinetic calculations. Since urinary folate accounts for only several percentages of the intake, the fate of the remaining >98% cannot be determined with certainty solely from the enrichment of urinary folate. It is unlikely that determination of the enrichment of fecal folate would provide useful information for modeling because colonic bacteria can both synthesize and utilize the endogenous folate that bypasses reabsorption in the enterohepatic circulation. Catabolism to form pABG and its acetylated derivative, which are primarily excreted in urine, may account for half or more of folate turnover from the body. Thus, measurement of isotopic enrichment of these catabolites will be included in further kinetic protocols to provide a more complete accounting of the labeled folate.

The use of animal models will be of help in this area as various tissues may be sampled over time to determine folate stores and turnover. This information can then be extrapolated to a human kinetic model based on known similarities and differences in metabolism between the species.

IV. SUMMARY AND CONCLUSIONS

It can be concluded that developing, then improving, a model of folate metabolism is a long-term project. Not only must one include the multiple routes of excretion of folic acid and its catabolites (pABG, apABG, pterins), but the interconversion of the various forms of folate must also be considered, including polyglutamylation within tissues.

Many factors affect folate metabolism, including dietary folate level, nutritional status of vitamins B6, B12, and riboflavin, zinc status, alcoholism, and physical states such as pregnancy and lactation. In many cases, the effects of these factors are seen in altered excretion rates of intact folates and metabolites, but the effects on tissue levels of the various folates and transfer rates between tissues are not well understood. Preliminary human and animal kinetic models are being developed in our laboratory based on studies conducted under controlled dietary conditions. These models will provide a base from which to study the effects of altered folate nutriture as well as the influence of other factors such as pregnancy and aging on folate metabolism.

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REFERENCES

Bailey, L. B. (1992). Folate—Evaluation of new recommended dietary allowance. *J. Am. Diet. Assoc.* **92,** 463.

Bhandari, S. D., and Gregory, J. F. (1990). Inhibition by selected food components of human and porcine intestinal pteroylpolyglutamate hydrolase activity. *Am. J. Clin. Nutr.* **51,** 87–94.

Bhandari, S. D., and Gregory, J. F. (1992). Folic acid, 5-methyl-tetrahydrofolate and 5-formyl-tetrahydrofolate exhibit equivalent intestinal absorption, metabolism and in vivo kinetics in rats. *J. Nutr.* **122,** 1847–1854.

Bunni, M. A., Rembiesa, B. M., Priest, D. G., Sahović, E., and Stuart, R. (1989). Accumulation of tetrahydrofolates in human plasma after leucovorin administration. *Cancer Chemother. Pharmacol.* **23,** 353–357.

Colman, N. (1982). Addition of folic acid to staple foods as a selective nutrition intervention strategy. *Nutr. Rev.* **40,** 225–233.

Colman, N., Green, R., and Metz, J. (1975). Prevention of folate deficiency by food fortification. II. Absorption of folic acid from fortified staple foods. *Am. J. Clin. Nutr.* **28,** 459–464.

Dinning, J. S., Sime, J. T., Work, P. S., Allen, B., and Day, P. L. (1957). The metabolic conversion of folic acid and citrovorum factor to a diazotizable amine. *Arch. Biochem. Biophys.* **66,** 114–119.

Food and Nutrition Board (1989). "Recommended Dietary Allowances," 10th ed. National Research Council, National Academy of Sciences, Washington, DC.

Gregory, J. F. (1989). Chemical and nutritional aspects of folate research: Analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. *Adv. Food Nutr. Res.* **33,** 1–101.

Gregory, J. F. (1990). Improved synthesis of [3′,5′-$^{2}H_{2}$]folic acid: Extent and specificity of deuterium labeling. *J. Agric. Food Chem.* **38,** 1073–1076.

Gregory, J. F., and Toth, J. P. (1988a). Chemical synthesis of deuterated folate monoglutamate and in vivo assessment of urinary excretion of deuterated folates in man. *Anal. Biochem.* **170,** 94–104.

Gregory, J. F., and Toth, J. P. (1988b). Preparation of folic acid specifically labelled with deuterium at the 3′,5′-positions. *J. Labelled Compd. Radiopharm.* **25,** 1349–1359.

Gregory, J. F., and Toth, J. P. (1990). Stable-isotopic methods for in vivo investigation of folate absorption and metabolism. *In* "*Folic Acid Metabolism in Health and Disease*" (M. F. Picciano, E. L. R. Stokstad, and J. F. Gregory, eds.), pp. 151–170. Wiley-Liss, New York.

Gregory, J. F., Bailey, L. B., Thomas, E. A., Toth, J. P., Cerda, J. J., and Fisher, W.R. (1994). Stable-isotopic study of long-term folate metabolism in a human subject. *FASEB J.* **8,** A920.

Kownacki-Brown, P. A., Wang, C., Bailey, L. B., Toth, J. P., and Gregory, J. F. (1993). Urinary excretion of deuterium-labeled folate and the metabolite p-aminobenzoylglutamate in humans. *J. Nutr.* **123,** 1101–1108.

Krumdieck, C. L., Fukushima, K., Fukushima, T., Shiota, T., and Butterworth, C. E. (1978). A long-term study of the excretion of folate and pterins in a human subject after ingestion of ^{14}C folic acid, with observations on the effect of diphenylhydantoin administration. *Am. J. Clin. Nutr.* **31,** 88–93.

McNulty, H., McPartlin, J. M., Weir, D. G., and Scott, J. M. (1993). Reversed-phase high performance liquid chromatographic method for the quantitation of endogenous folate catabolites in rat urine. *J. Chromatogr.* **614,** 59–66.

McPartlin, J. M., Weir, D. G., Courtney, G., McNulty, H., and Scott, J. M. (1986). The level of folate catabolism in normal human populations. *In* "Pteridines and Folic Acid Derivatives" (B. Cooper and V. Whitehead, eds.), pp. 513–516. de Gruyter, New York.

McPartlin, J. M., Courtney, G., McNulty, H., Weir, D. G., and Scott, J. M. (1992). The quantitative analysis of endogenous folate catabolites in human urine. *Anal. Biochem.* **206,** 256–261.

McPartlin, J. M., Halligan, A., Scott, J. M., Darling, M., and Weir, D. G. (1993). Accelerated folate breakdown during pregnancy. *Lancet* **341,** 148–149.

Murphy, M. and Scott, J. M. (1979). The turnover, catabolism, and excretion of folate administered at physiological concentrations in the rat. *Biochim. Biophys. Acta* **583,** 535–539.

Murphy, M., Keating, M., Boyle, P., Weir, D. G., and Scott, J. M. (1976). The elucidation of the mechanism of folate catabolism in the rat. *Biochem. Biophys. Res. Commun.* **71,** 1017–1024.

Priest, D. G., Schmitz, J. C., Bunni, M. A., and Stuart, R. K. (1991). Pharmacokinetics of leucovorin metabolites in human plasma as a function of dose administered orally and intravenously. *J. Natl. Cancer Inst.* **83,** 1806–1812.

Reich, S. D., and Gonczy, C. (1979). Mathematical modeling—guide to high-dose methotrexate infusion therapy. *Cancer Chemother. Pharmacol.* **3,** 25–31.

Sauberlich, H. E. (1990). Evaluation of folate nutrition in population groups. *In* "Folic Acid Metabolism in Health and Disease" (M. F. Picciano, E. L. R. Stokstad, and J. F. Gregory, eds.), pp. 211–236, Wiley-Liss, New York.

Scott, K. C., and Gregory, J. F. (1994). Long term folate metabolism in the growing rat. *FASEB J.* **8,** A704.

Shaw, S., Jayatilleke, E., Herbert, V., and Colman, N. (1989). Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem. J.* **257,** 277–280.

Stites, T., Gregory, J. F., Bailey, L., Fisher, W., and Toth, J. (1994). Kinetic modeling of deuterium labeled folate in human subjects. *FASEB J.* **8,** A704.

Tamura, T., and Halsted, C. H. (1983). Folate turnover in chronically alcoholic monkeys. *J. Lab. Clin. Med.* **101,** 623–628.

von der Porten, A. E., Gregory, J. F., Toth, J. P., Cerda, J. J., Curry, S. H., and Bailey, L. B. (1992). In vivo folate kinetics during chronic supplementation of human subjects with deuterium-labeled folic acid. *J. Nutr.* **122,** 1293–1299.

Wang, C., Song, S., Bailey, L. B., and Gregory, J. F. (1994). Relationship between urinary excretion of p-aminobenzoylglutamate and folate status of growing rats. *Nutr. Res.* **14,** 75–884.

Wolfrom, C., Hepp, R., Hartmann, R., Breithaupt, H., and Henze, G. (1990). Pharmacokinetic study of methotrexate, folinic acid and their serum metabolites in children treated with high-dose methotrexate and leucovorin rescue. *Eur. J. Clin. Pharmacol.* **39,** 377–383.