Kinetic modeling of folate metabolism through use of chronic administration of deuterium-labeled folic acid in men1–3

Tracy E Stites, Lynn B Bailey, Karen C Scott, John P Toth, Waldo P Fisher, and Jesse F Gregory III

ABSTRACT This study was conducted as an initial investigation of in vivo folate kinetics in healthy men (n = 4) and made use of a chronic-administration protocol with stable-isotope labeling. Subjects were given 0.453 μmol (200 μg) total folic acid in aqueous solution daily throughout the 18-wk study while they consumed self-selected folate-adequate diets. After a 2-wk pretrial period with unlabeled folic acid, subjects were given 0.227 μmol (100 μg) pteroyl-l-[3H]glutamic acid/d ([3H]folic acid) combined with 0.227 μmol nonlabeled folic acid or [3H]pteroylhexaglutamic acid/d for the next 8 wk; then for the next 8 wk the [3H]folic acid was withdrawn and the subjects received only nonlabeled folic acid. Little unmetabolized folic acid was excreted in urine. Isotopic enrichment of urinary folate during [3H]folic acid administration and withdrawal was consistent with a kinetic model having a rapid turnover pool and a slow turnover pool. In contrast with previous two-pool models, provisions were made for folate turnover by urinary folate excretion (as measured here) and by fecal excretion and catabolic processes. The precision of modeling will be improved in future studies by measurement of enrichment of additional pools. However, this study shows clearly the slow turnover of the whole-body folate pool (≤ 1% per day) and the feasibility of further long-term kinetic analysis. Am J Clin Nutr 1997;65:53–60.

KEY WORDS Folate, folic acid, kinetics, deuterium, in vivo study, metabolism

INTRODUCTION

Folate is an essential nutrient required for many reactions of one-carbon metabolism, including methionine regeneration and the synthesis of nucleic acids. Associations of folate deficiency with the incidence of megaloblastic anemia, neural tube defects, cardiovascular disease, and certain forms of cancer emphasize the significance of adequate folate nutrition. However, the nutritional requirement of humans for folate has been the subject of controversy and uncertainty. Estimates of the nutritional requirement for folate in humans have been based largely on assessment of folate status in controlled depletion-repletion protocols and in population-based studies (1). In vivo kinetic studies have contributed to the quantitative understanding of the requirement for certain nutrients, for example, ascorbic acid (1), but applicable kinetic information is generally lacking for folate.

Numerous investigators have determined that the excretion of folate follows a biphasic decay pattern that is consistent with a two-compartment kinetic model (2–7). Kinetic studies in animals have provided detailed information regarding folate kinetics, metabolism, and tissue distribution (2, 4, 6), but the reliability of animal models in kinetic studies is uncertain at this time. Certain features of the kinetic studies conducted to date with humans made the results of limited relevance to the estimation of nutrient requirements and long-term metabolism. The study by Von Der Porten et al (7) involved large doses of supplemental folate (1.6 mg/d), whereas the study by Russell et al (5) used a folate acid maintenance regimen of 5 mg/d, both of which would be predicted to increase the rate of folate turnover. The thorough study by Krundieck et al (3) involved long-term measurement of urinary and fecal excretion of label after several tracer doses of radiolabeled folate, although the protocol involved only one subject. Various other studies have involved folate kinetics in the context of short-term changes in plasma concentrations after therapeutic doses (8).

The objective of this study was to investigate a protocol and to obtain initial information regarding folate kinetics and metabolism in human subjects. Novel aspects of this study were the long-term application of [3H]folic acid and the use of a protocol involving the chronic administration of the labeled folate followed by a withdrawal period under conditions of a steady state of folate turnover. A major objective was to determine the feasibility of kinetic modeling based solely on the measurement of isotopic enrichment of folate in urine, which is easily collected and readily analyzed through use of

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methods devised in this laboratory (9). This long-term kinetics study was conducted concurrently with a short-term bioavailability study in which all subjects received a daily dose of a deuterium-labeled polyglutamyl folate ([2H4]PteGlu6) during the first week of the protocol. This short-term administration of [2H4]PteGlu6 had no bearing on long-term kinetics of labeled folates in vivo and thus will not be discussed in this publication.

SUBJECTS AND METHODS

Folate compounds

Pteroyl-L-[β,γ,γ,γ-2H4]glutamic acid ([2H4]folic acid) was prepared according to the procedure of Gregory and Toth (9). In short, the synthetic procedure involved the conversion of pteric acid to 10N-trifluoroacetylpteroic acid, which was then coupled to [2H4]-L-glutamate methyl ester. This product was deuterified by incubation in 0.1 mol NaO2H/L at 100 °C for 60 min, then purified by chromatography on a column packed with CF-11 cellulose (Whatman Inc, Clifton, NJ). Proton nuclear magnetic resonance spectroscopy analysis revealed complete labeling of the glutamyl β and γ positions of the [2H4]folic acid molecule (10). The identity and purity of the product was also confirmed by HPLC (9) and ultraviolet absorption spectrophotometry.

[2H4]PteGlu6 ([3',5'-2H2]Pteroylhexaglutamatic acid) was used in a second aspect of this study for a short-term comparison of bioavailability, as described below. This compound was synthesized by coupling 10N-trifluoroacetyl-[3',5'-2H2]pteroyl acid to resin-bound hexaglutamic acid (11, 12), essentially according to the method of Krumdieck and Baugh (13). The purity and identity of the compound were established with HPLC and ultraviolet absorption spectroscopy.

Human subjects and protocol

Subject selection

Four white male subjects (aged 20–30 y) were selected to participate in this study (Table 1). The subjects were free of apparent disease, had no previous gastrointestinal surgery or digestion problems, were not taking medication or vitamin supplements, were nonsmokers, and had normal blood chemistry and hematologic indexes. They also had normal serum and erythrocyte folate concentrations as determined by Lactobacillus casei assay (14). The protocol, as described below, was approved by the University of Florida Institutional Review Board, and informed consent was obtained from each subject.

Pretrial period

The protocol consisted of three major phases regarding the use of isotopically labeled folate: a pretrial period, an administration period, and a withdrawal period. Throughout the study, all subjects were allowed to consume a self-selected diet. For the first 2 wk, designated the pretrial period, subjects consumed a daily supplement of 0.453 μmol (200 μg) nonlabeled folic acid as a commercial tablet (Baxter Healthcare Corp, Pharmaceuticals Division, Deerfield, IL) for 7 d, then as an aqueous solution in 180 mL distilled water for the next 7 d. The subjects collected all their urine during the 24 h of the last day of the pretrial period to provide baseline data. For this and all other urine collections, urine was collected into 2-L brown plastic containers containing 3 g sodium ascorbate.

Administration of deuterated folate

On the next morning after the pretrial period (designated protocol day 15), before food consumption an initial venous blood sample was taken from each subject. Total urine volume from the previous 24-h collection was measured and five 100-mL samples were kept and frozen at −20 °C until analyzed. Whole blood and serum samples were stored at −20 °C in 50 mmol ascorbate/L until analyzed.

During days 15–22 of the protocol, all subjects received 0.227 μmol (100 μg) [2H4]folic acid/d and 0.227 μmol [2H4]PteGlu6/d in 180 mL water before food consumption each morning. During this week of the protocol, 24-h urine samples were collected every other day. From protocol days 22–70, urine was collected weekly. All the subjects continued consumption of 0.227 μmol (100 μg) [2H4]folic acid/d for a total of 8 wk of [2H4]folic acid administration. On protocol day 22, the [2H4]PteGlu6 was discontinued and was replaced with 0.227 μmol (100 μg) nonlabeled folic acid daily through day 70.

Withdrawal of deuterated folate

On protocol day 70, [2H4]folic acid administration was stopped and replaced with unlabeled folic acid. From day 71 to 126 the subjects consumed a preprandial supplement each morning of 0.453 μmol (200 μg) folic acid/d in tablet form. Twenty-four-hour urine samples were collected every other day from day 70 to 77, then every other week for the duration

<p>| TABLE 1 |
| Physical characteristics of human subjects and their estimated dietary intakes of selected nutrients |</p>
<table>
<thead>
<tr>
<th>Subject</th>
<th>Height (cm)</th>
<th>Body mass (kg)</th>
<th>Energy (MJ/d)</th>
<th>Folate (μg/d)</th>
<th>Vitamin B-6 (mg/d)</th>
<th>Vitamin B-12 (μg/d)</th>
<th>Riboflavin (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A²</td>
<td>183</td>
<td>80</td>
<td>17.1</td>
<td>423</td>
<td>2.52</td>
<td>4.04</td>
<td>2.93</td>
</tr>
<tr>
<td>B²</td>
<td>173</td>
<td>66</td>
<td>15.1</td>
<td>584</td>
<td>4.06</td>
<td>8.52</td>
<td>5.13</td>
</tr>
<tr>
<td>C</td>
<td>188</td>
<td>86</td>
<td>8.62</td>
<td>286</td>
<td>1.36</td>
<td>1.93</td>
<td>1.48</td>
</tr>
<tr>
<td>D</td>
<td>191</td>
<td>100</td>
<td>14.6</td>
<td>480</td>
<td>1.48</td>
<td>1.81</td>
<td>2.59</td>
</tr>
<tr>
<td>x ± SEM</td>
<td>184 ± 4</td>
<td>83 ± 7</td>
<td>13.9 ± 1.8</td>
<td>443 ± 62</td>
<td>2.36 ± 0.63</td>
<td>4.08 ± 1.57</td>
<td>3.03 ± 0.76</td>
</tr>
</tbody>
</table>

1 Recommended dietary allowances for the selected nutrients are as follows: folate, 200 μg/d; vitamin B-6, 2.0 mg/d; vitamin B-12, 2.0 μg/d; and riboflavin, 1.7 mg/d. Individual values for energy and vitamins are daily means for each subject. All subjects consumed an additional 200 μg supplemental folate per day throughout the study.

2 These subjects had a high level of physical activity from bicycling (A) and weightlifting (B).
of the study (days 78–126). The protocol is summarized in Figure 1. A key feature of this protocol was that the subjects were maintained with approximately constant intake of total folate throughout the study. The subjects consumed the same total dose of supplemental folate (0.453 μmol/d), in addition to their self-selected dietary folate intake, for the entire protocol.

**Food records**

To estimate nutrient intake, a 3-d dietary record was collected during the first week of the experimental period (protocol days 15–22). Two 24-h dietary records were collected during the week and one 24-h record was collected on the weekend. Dietary records were analyzed with THE FOOD PROCESSOR II software (version 3.0; ESHA Research, Salem, OR), which uses a database that contains values for 30 nutrients in 2400 foods, along with a fast food database. For all foods evaluated in this computerized dietary assessment, the database contained a tabulated value for folate that had been determined by direct analysis.

**Analytical methods**

The folate concentration of erythrocytes and serum was determined by a microtitr plate adaptation of the microbiological assay with *L. casei* (14). As determined in this laboratory, the interassay and intraassay CVs were 8% and 7%, respectively.

Urinary folate was isolated for analysis by HPLC and gas chromatography–mass spectrometry (GCMS) by affinity chromatography (9) by using a modification of the method of Selhub et al (15). The affinity matrix, bovine whey folate-binding protein coupled to a commercially activated agarose derivative (Affigel 10; BioRad Laboratories, Richmond, CA), was packed in columns of 2-mL bed volume. Urine (adjusted to pH 7.0) was applied to the column and extraneous materials were removed by washing with 5 mL of 0.025 mol potassium phosphate/L (pH 7.0) containing 1.0 mol sodium chloride/L, followed by 5 mL of 0.025 mol potassium phosphate/L (pH 7.0). The specifically bound folate was then eluted with 0.1 mol HCl/L (5 mL). HPLC analysis of urinary folate in a portion (100 mL) of the folate fraction eluted from affinity columns was conducted with a solvent delivery system (Rabbit HP; Rainin Instrument Co, Woburn, MA) in isocratic mode with a mobile phase of 0.033 mol phosphoric acid/L containing 100 mL acetonitrile/L and an octadecylsilyl column (UltraspHERE IP; 5 mm particle size, 4.6 mm inside diameter by 25 cm length; Beckman Instrument Co, Fullerton, CA). An ultraviolet photodiode array detector (LC 235 Diode Array Detector; Perkin Elmer, Oak Brook, IL) was used at 280 nm in series with a fluorescence detector (FD-300 dual monochromator fluorescence detector; GTI/Spectrovision, Concord, MA) with excitation and emission wavelengths of 288 and 353 nm, respectively. Urinary folate consisted primarily of 5-methyltetrahydrofolate, with only small quantities (0–5% of total) of folic acid.

Before GCMS analysis, the urinary folates isolated by affinity chromatography were subjected to reductive cleavage to yield *p*-aminobenzoylglutamate (*p*ABG) and a perin, followed by isolation and derivatization of the *p*ABG (9). *p*ABG produced in these reductive cleavage reactions was isolated by preparative HPLC and evaporated to dryness under nitrogen gas. Trifluoroacetic anhydride (400 μL) and trifluoroethanol (200 μL) were added to the dry residue and the mixture was heated at 90 °C for 1 h. Excess reagents were then evaporated to dryness under a stream of nitrogen gas, and the residue dissolved in 25 μL dry ethyl acetate, capped tightly, and stored at −4 °C until GCMS analysis (9, 16).

GCMS analysis was performed with a gas chromatograph–mass spectrometer data system (model 5989; Hewlett-Packard, Palo Alto, CA) in the negative chemical ionization mode with methane as the reagent gas. Analyses were performed in the selected-ion monitoring mode at mass-to-charge ratios (m/z) of 426, 428, and 430 (9, 16). Working response curves relating the ratio of observed peak areas in GCMS analysis to actual molar ratios were generated with standards of *p*ABG derivatives of known ratios of [2H₄] to unlabeled and [2H₃] to unlabeled forms. Derivatized samples and standards were analyzed in duplicate or triplicate, and the ratio of the labeled *p*ABG derivative to unlabeled *p*ABG derivative was determined based on the experimentally determined working curve.

**Kinetic modeling**

All kinetic modeling was done with CONSAM31, the conversational version of the Simulation, Analysis and Modeling Program, version 31 (provided by Loren A Zech, Laboratory of Mathematical Biology, National Institutes of Health, Bethesda, MD) (17, 18), which was run on a 386-based personal computer.

The time course of appearance (protocol days 15–70) and disappearance (protocol days 71–126) of [2H₄]folate in urine was initially assessed by compartmental analysis with a two-compartment model with a single output (2, 7). This initial model involved folate uptake into compartment 1, interchange with the main tissue pool (compartment 2), and loss from compartment 1 (Figure 2).

Because we recognized that the initial model was an oversimplification of folate metabolism, the model was first mod-

![FIGURE 1. Timeline showing the various phases of the protocol.](https://academic.oup.com/ajcn/article-abstract/65/1/53/4655405/7)
The exchange rate constants were expressed as \(L(I, J)\), and represent the fraction of folate in compartment \(J[C(I)]\) transferred to compartment \(I[C(J)]\) per unit time. For example, the rate constant \(L(2, 1)\) indicates the fractional flow to compartment 2 from compartment 1 per unit time. The masses of folate transferred per unit time are defined as \(R(I, J)\), and represent \(M(J) \times L(I, J)\), where \(M(J)\) is the mass of folate in compartment \(J\). A constant bioavailability of 67% for total dietary and supplemental folate was assumed in modeling. This was implemented by multiplying the input of folate (labeled and nonlabeled) into compartment 5 (intestine) by a factor of 67%. The intake from dietary sources was estimated from the 3-d dietary records for each subject (Table 1). A value of 1.0 d\(^{-1}\) for \(L(1, 5)\), the first-order rate constant for transfer from intestine to pool 1, was assumed in modeling; however, variation of \(L(1, 5)\) by more than two orders of magnitude had no effect on model solutions.

Twenty-four-hour urine samples were collected on designated days and the urinary folate data were entered into compartments 3 and 4 during modeling. It should be recognized that the use of both of these compartments was a modeling tool not related to the physiology of the system. Compartment 3 represents cumulative urinary excretion throughout the study. In contrast, the content of urinary folate in compartment 4 is reset to zero each day to allow a representation of the daily excretion on days of urine collection. Rate constants for transfers to pools 3 and 4 were equivalent [ie, \(L(3, 1) = L(4, 1)\) and \(L(3, 6) = L(4, 6)\)]. Because of the existence of these two pools representing urinary folate, the model also included pathways with negative rate constants \([-L(3, 6)\) and \(-L(3, 1))\] to avoid an overestimation of the actual rate of urinary excretion and to keep the model in balance (not shown on Figure 3). Note also that compartment 2 has no physiologic equivalent but is merely a sink into which all losses of folate that are not currently measured are directed.

In modeling it was assumed that the subjects were in a steady state with respect to folate status and that deuterium-labeled and nonlabeled folates exhibited equivalent behavior, with the rate constants \([L(I, J)]\) of the tracer and tracer set to be equivalent. In this model, compartments 1 and 6 constitute two kinetically identifiable pools of folate exhibiting rapid and slow turnover, respectively. These compartments undergo interchange of folate as reflected by \(L(6, 1)\) and \(L(1, 6)\). Losses of folate from the compartments (ie, 1 and 6) occur either by excretion of intact (ie, noncatabolized) folate molecules in urine [described by \(L(3, 6), L(4, 6), L(3, 1),\) and \(L(4, 1))\] and as fecal folate, presumably from bile and sloughed mucosal cells. Another irreversible loss of folate occurs by excretion of catabolic products (pterins, \(pABG\), and \(N\)-acetyl-\(pABG\)) via urinary and fecal routes. Because the excretion of endogenous fecal folate cannot readily be measured and differentiated from the products of bacterial folate synthesis, fecal folate was not determined in this study. All irreversible losses of folate by processes other than urinary excretion were designated as collective pool 2, described by rate constants \(L(2, 1)\) and \(L(2, 6)\).

We found that there were many possible solutions to the model, many of which were inconsistent with the physiology of folate metabolism. Accordingly, we applied the following constraints to the model: 1) Modeling solutions in which the mass of the major tissue pool (pool 6) did not greatly exceed the mass of pool 1 were excluded because of the known large difference in mass and concentration of folate in plasma (typically 5–10 \(\mu g\)/L) and tissues (0.1–10 \(\mu g\)/g), 2) Other solutions
were excluded in which the apparent mass of total-body folate exceeded physiologic relevance (ie, mass > 100 mg), and 3) Modeling was constrained such that urinary folate excretion was a minor component of total folate turnover. Within these physiologic constraints, the various rate constants \([U(I, J)]\) were adjusted for each subject until a best fit was established by minimizing the sum of squares of the residuals.

**Statistical analysis**

Statistical analysis with one-way repeated-measures analysis of variance was performed on all data regarding serum and erythrocyte folate concentrations (20). Multiple comparisons were done with the Student-Newman-Keuls method at \(P < 0.05\). These analyses were performed to determine the significance of differences between individuals and to assess the significance of changes over the course of the study. All data were expressed as means ± SEMs. The statistical analyses were performed with SIGMA-STAT for Windows (version 1.0; Jandel Corporation, San Rafael, CA).

**RESULTS**

**Nutrient intake and folate status**

Nutrient intakes estimated from food records indicated adequate dietary intake of nutrients. The estimated energy, folate, vitamin B-6, vitamin B-12, and riboflavin intakes for all subjects are shown in Table 1. Estimated mean (± SEM) dietary folate intake was 443 ± 62 μg/d, in addition to the 200 μg/d (0.453 nmol/d) of administered supplemental folate consumed by each subject. Intakes of two of the subjects (A and B) exceeded that of subjects C and D in part because of high levels of physical activity. Snacking with fortified breakfast cereals also contributed to the high intake of all vitamins by subject B.

Analysis of variance of serum folate concentrations indicated that these values did not change significantly over the course of the study (Table 2), and urinary folate excretion also did not change significantly during the study (data not shown). Subjects did not differ significantly with respect to serum folate concentration (Table 3), although the between-subject difference in urinary folate excretion was small but significant \((P < 0.05)\); Table 3). All the subjects in this study had serum folate concentrations that would be classified as moderately low (6.8–13.6 nmol/L), although erythrocyte folate concentrations were in the normal range (> 362 nmol/L). Consistent with this apparently adequate folate nutritional status was the fact that the mean intake of dietary plus supplemental folate was 643 ± 62 μg/d, which was well above the requirement for this vita-

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Serum and erythrocyte folate concentrations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of protocol</td>
<td>Serum folate</td>
</tr>
<tr>
<td></td>
<td>nmol/L</td>
</tr>
<tr>
<td>Start of prestudy period</td>
<td>10.9 ± 1.86</td>
</tr>
<tr>
<td>Protocol day 21</td>
<td>12.2 ± 1.63</td>
</tr>
<tr>
<td>Protocol day 71</td>
<td>9.43 ± 1.97</td>
</tr>
<tr>
<td>Protocol day 126</td>
<td>12.4 ± 2.13</td>
</tr>
</tbody>
</table>

† \(\bar{x} \pm \text{SEM}; \ n = 4\), ND, not determined.

‡ Significantly different from erythrocyte folate concentrations on protocol days 21 and 71, \(P < 0.05\).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Mean serum and erythrocyte folate concentrations and urinary folate excretion for individual subjects‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>Serum folate</td>
</tr>
<tr>
<td></td>
<td>nmol/L</td>
</tr>
<tr>
<td>A</td>
<td>12.1 ± 1.44</td>
</tr>
<tr>
<td>B</td>
<td>13.5 ± 2.24</td>
</tr>
<tr>
<td>C</td>
<td>9.98 ± 0.96</td>
</tr>
<tr>
<td>D</td>
<td>9.39 ± 2.23</td>
</tr>
</tbody>
</table>

† Values with different superscript letters are significantly different, \(P < 0.05\).

‡ \(\bar{x} \pm \text{SEM}\) for blood samples taken on days 7, 57, and 112.

³ \(\bar{x} \pm \text{SEM}\) of all urine samples analyzed for each subject \(n = 19–21\).

min. Erythrocyte folate concentration exhibited a small but significant increase over the course of this study \((P < 0.05)\) (Table 3). Both urine excretion and serum folate concentration reflect a composite of long-term folate status as well as recent folate intake, whereas erythrocyte folate concentration is believed to reflect more accurately long-term folate status and whole-body folate stores (21).

**Kinetic modeling**

Kinetic modeling involved fitting the exponential equations for the isotopic enrichment of urinary folate to the experimentally derived data through use of CONSAM31 software. We found that these data could be readily fit by a two-pool (ie, biexponential) model with only one output, as performed in several previous studies (2–7). However, this approach was discontinued because the two-pool, one-output model is inconsistent with current knowledge of folate metabolism because it neglects the large contribution of fecal excretion and in vivo catabolism to folate turnover. Even when allowing for output of all types from pool 1, as shown in Figure 2, this model is unsatisfactory because the predicted mass of the tissue folate pool was excessive. The expanded model (Figure 3) yielded an equivalent fit to the data (Figure 4) and was more physiologically realistic. The isotopic enrichment of urinary folates exhibited considerable scatter, which may have been due to the fact that dietary intake was not controlled in this protocol. Urinary excretion of folate for all subjects ranged from ~0.5% to 2% of total folate intake (Table 3). This value was based on the individual subjects’ 3-d dietary food records and HPLC data of total folate excretion in urine.

A limitation of the experimental design became apparent when we attempted to analyze the data by compartmental modeling. The only kinetic data available in this initial set of experiments was urinary excretion of folate, and quantitatively this constituted < 3% of ingested folate. Accordingly, it was not surprising that the rate constants could not be determined conclusively solely on the basis of these data. On the basis of the currently available data, there were many possible solutions to the model, depending on the combination of rate constants selected. With these constraints, it was possible to develop some tentative estimates of kinetic parameters for folate metabolism (Table 4).
DISCUSSION

Although the free-living subjects of this study consumed self-selected diets, their dietary folate intakes appear to have been fully adequate and consistent with previous studies of young adult males. The estimated intake of 443 ± 62 µg/d in this study is reasonably consistent with the Second National Health and Nutrition Examination Survey (NHANES II) estimate of 304 ± 7.4 µg/d (mean ± SEM) for white males between the ages of 19 and 34 y (22). When folate intake was expressed as an energy-density value (µg folate/4184 kJ, or /1000 kcal), the observed value in this study was 135 ± 12 µg/4184 kJ compared with 116 µg/4184 kJ in the NHANES II data. The lack of dietary control of our protocol is balanced against the cost and inconvenience of conducting a long-term study of a kinetically sluggish vitamin such as folate. It is also recognized that folic acid, as used in supplements in this study, is not the primary form of dietary folate for most humans. The near absence of folic acid in urine and the far greater urinary excretion of 5-methyl-tetrahydrofolate indicate the effective metabolic utilization of the labeled folate and the supplement administered.

Caution must be taken in interpreting the quantitative results of this kinetic model, which is based solely on isotopic enrichment of urinary folate excretion of folate. Assuming a steady state for folate nutrition, the subjects in this study excreted ∼1-2% of their dietary intake of folate in the urine as intact folate. The other 98-99% of dietary folate was presumably excreted in the feces as a mixture of catabolites and intact folates or as catabolites excreted in urine. Fecal excretion of radiolabeled folates has been shown to account for ∼30-40% of folate turnover in rats (2) and ∼50% of folate turnover in a single human subject (3). Catabolic processes that yield pABG and N-acetyl-pABG were shown by McPartlin et al. (19) to account for the turnover of ∼100 µg body folate/d in nonpregnant women, with even greater catabolism during middle and late pregnancy. We observed a similar extent of catabolism in nonpregnant women (JF Gregory, J Williamson, LB Bailey, JP Toth, unpublished data, 1996).

In the absence of experimental confirmation, the relation between isotopic enrichment of urinary folate and the enrichment of other folate pools cannot be assessed. The precision of model-derived estimates will undoubtedly be improved when studies are conducted that include isotopic analysis of additional body pools. Existing GCMS and HPLC methods permit determination of isotopic enrichment of plasma folate and urinary pABG and N-acetyl-pABG, in addition to urinary folate, in future protocols. In this regard, modeling aids in the development of better experimental protocols. The small percentage of turnover measured in this study (0.5-2%), when used as the sole modeling criterion, is a limitation of the present protocol. However, this study shows the feasibility of modeling with a protocol involving chronic intake of labeled and nonlabeled folates to assess the kinetics of folate metabolism in humans. We recently conducted a controlled dietary
protocol that involved chronic administration of deuterated folate (23). Similar rates of turnover of the kinetically slow pool were observed from the isotopic enrichment of urinary \(N\)-acetyl-pABG and urinary folate in kinetic analysis (JF Gregory, J Williamson, LB Bailey, JP Toth, unpublished data, 1996). \(N\)-Acetyl-pABG is believed to be the principal product of the cellular catabolism of folate (24) and we believe that conclusions based on kinetics of urinary \(N\)-acetyl-pABG would reflect the kinetics of tissue folate pools. Thus, this observation appears to support the conclusion of the current study regarding the slow turnover of the major in vivo folate pool.

A major objective in modeling of this type is the estimation of the mass of total-body folate, the masses of the respective pools, and their turnover rates. There have been no direct calculations of the total folate pool based on complete tissue analysis. Herbert (25) estimated on the basis of unpublished data that tissue stores of folate were in the range of 7.5 ± 2.5 mg. Whitehead (26) determined that liver folate concentrations in human subjects ranged from 4.5 to 10 \(\mu\)g. Using these values and an average liver weight of 1400 g, total liver folate would range from 6.3 to 14 mg. If hepatic folate constituted 50% of body stores, then the total folate would range, on the basis of the data of Herbert (25) and Whitehead (26), from 12.6 to 28.0 mg (28.6-63.5 \(\mu\)mol). Hopper and Lampi (27) determined liver folate concentrations in 560 human livers at autopsy. The average liver folate concentration in 32 subjects aged between 21 and 30 y was 8.0 ± 2.8 \(\mu\)g/liver with a range of 3.6–14.8 \(\mu\)g/liver. Using the same calculation as above, the total liver folate content would be ≈11.2 mg (25.4 \(\mu\)mol), and the total-body folate pool would be ≈22 mg (50.8 \(\mu\)mol).

In the current form of the model, the predicted mass of the body folate pools is a linear function of the quantity of folate ingested and its bioavailability. We have assumed a net folate bioavailability of 67% in this study. The mean model-estimated masses of total folate in pools 1 (rapid) and 6 (slow) were 6.9 (15.6 \(\mu\)mol) and 52 mg (118 \(\mu\)mol), respectively, with an estimate of total-body folate of 59 mg (134 \(\mu\)mol) (Table 4). The bioavailability of folate in humans is not fully understood. Sauberlich et al (28) estimated that naturally occurring dietary folate underwent no more than 50% utilization by human subjects, whereas Colman et al (29) observed that folic acid added to various cereal-grain-based foods exhibited 30–60% bioavailability. In contrast, supplemental folate administered as an intragastric solution before meal consumption underwent nearly complete absorption in rats (2).

On the basis of these pertinent observations, we feel that an assumed 67% bioavailability of total ingested folate (both dietary and supplemental) is reasonable. The extent to which tissue folate concentration and the total in vivo pool size varies as a function of folate intake in humans has not been determined; however, the comparatively high folate intake used in this protocol may be responsible in part for the larger masses estimated in this study compared with those previously predicted. In view of the fact that the subjects consumed ample quantities of dietary folate and were given an additional supplement throughout the study, these estimates of in vivo masses agree reasonably with the previously reported estimates and appear to be physiologically reasonable. It should be noted that the estimated mass of pool 1 (6.9 mg, 15.6 \(\mu\)mol) exceeds the actual plasma folate mass of 17.5 \(\mu\)g (calculated assuming 3.5 L plasma volume). This large discrepancy may indicate that the kinetically rapid pool 1 is composed partially of tissue folates in addition to folates circulating in plasma. Additional modeling involving direct analysis of plasma will address this question.

The current protocol is based on a steady state assumption; that is, it is assumed that folate intake is constant throughout the study and that the masses of in vivo pools remain constant. Whereas the diets of the subjects were not controlled, it is likely that dietary folate intake remained reasonably constant over the course of the study. Control of dietary folate intake would minimize this source of uncertainty. It is unclear whether the 200 \(\mu\)g/d supplement used in this protocol yielded a significant increase in body folate during this study. The significant increase in erythrocyte folate concentration suggests that some degree of change in tissue stores may have occurred. The magnitude of this change apparently was minor as judged by the small change in erythrocyte folate concentration while serum folate concentration and urinary folate excretion remained constant. We conducted a simulation with this model to evaluate whether a change in total mass of body folate would occur if the protocol were extended for an additional 450 d at this rate of folate intake. No change was found, although such

### Table 4

<table>
<thead>
<tr>
<th>Calculated value</th>
<th>Subject A</th>
<th>Subject B</th>
<th>Subject C</th>
<th>Subject D</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant (d⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L(6,1))</td>
<td>0.019</td>
<td>0.019</td>
<td>0.074</td>
<td>0.019</td>
<td>0.032 ± 0.014</td>
</tr>
<tr>
<td>(L(1,6))</td>
<td>0.0010</td>
<td>0.0010</td>
<td>0.0093</td>
<td>0.0010</td>
<td>0.0031 ± 0.0021</td>
</tr>
<tr>
<td>(L(2,6))</td>
<td>0.0020</td>
<td>0.0014</td>
<td>0.0035</td>
<td>0.0010</td>
<td>0.0020 ± 0.00055</td>
</tr>
<tr>
<td>(L(3,1))</td>
<td>0.00000001</td>
<td>0.00000001</td>
<td>0.000001</td>
<td>0.00000001</td>
<td>0.00000032 ± 0.00000022</td>
</tr>
<tr>
<td>(L(3,6))</td>
<td>0.000000041</td>
<td>0.000000041</td>
<td>0.000000040</td>
<td>0.000000030</td>
<td>0.000000038 ± 0.000000003</td>
</tr>
<tr>
<td>(L(2,1))</td>
<td>0.064</td>
<td>0.064</td>
<td>0.040</td>
<td>0.040</td>
<td>0.052 ± 0.007</td>
</tr>
<tr>
<td>Fractional catabolic rate (d⁻¹)</td>
<td>0.011</td>
<td>0.0086</td>
<td>0.0085</td>
<td>0.0047</td>
<td>0.0081 ± 0.0012</td>
</tr>
<tr>
<td>Mass of pool 1 (mg)</td>
<td>5.5</td>
<td>7.2</td>
<td>5.6</td>
<td>9.3</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>Mass of pool 6 (mg)</td>
<td>34</td>
<td>56</td>
<td>32</td>
<td>87</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>Total in vivo folate mass (mg)</td>
<td>39</td>
<td>63</td>
<td>38</td>
<td>96</td>
<td>59 ± 14</td>
</tr>
</tbody>
</table>

¹ Values are based on modeling under the constraints described in the text and thus should be viewed as initial estimates subject to further refinement. Sum of squares values for subjects A through D, respectively, were 0.000902, 0.00123, 0.00154, and 0.00225, which are inversely proportional to the overall goodness of fit of the model.
simulation is highly tenuous given the current uncertainty of the model.

The major folates in tissue pools are polyglutamyl forms of various tetrahydrofolates. Tetrahydrofolate and 10-formyl-tetrahydrofolate, two major forms in tissues, are highly susceptible to oxidative degradation; 5-methyl-tetrahydrofolate is also susceptible to oxidation. An interesting observation of this kinetic study was the slow turnover and apparently high degree of stability of the total-body folate pool. Despite the high degree of uncertainty in the calculated kinetic terms under the conditions of this study, visual inspection of the data provides evidence of the slow rise in enrichment and the slow decline after cessation of administration of the labeled folate. If the overall fractional catabolic rate (FCR) of folate in humans was \( \approx 0.01 \text{ d}^{-1} \) (ie, \( \approx 1\% \text{ d}^{-1} \)) or less, this would correspond to a mean residence time \( (1/\text{FCR}) \) for in vivo folate molecules of \( \geq 100 \text{ d} \). This apparent stability in the presence of dissolved oxygen and other oxidants may be evidence of the effectiveness of in vivo antioxidative systems and possibly of protection by folate-binding proteins.

Krumdieck et al (3) observed an apparent half-time for excretion of radiohefolate from a human adult female of \( \approx 100 \text{ d} \) for the major tissue folate pool, which corresponds to a mean residence time of 144 d. This value is in good agreement with the findings of the present study. Similar findings were also observed for radiohefolated folates in monkeys (6). In contrast, folate turnover in rats occurs at a much greater rate, with a mean residence time for the major folate pool of \( \approx 20 \text{ d} \) (2). Despite these apparent differences among species, these kinetic studies provide consistent evidence of high in vivo stability and slow turnover of folates in the tissues of humans and animals.

In summary, this study provides initial kinetic data and a physiologically accurate model of folate metabolism. The results of this study indicate the feasibility of long-term examination of folate kinetics in humans using chronic administration of stable-isotope-labeled forms of this vitamin and illustrate improvements needed in protocols to achieve precise kinetic modeling.

The authors extend thanks to Loren A Zech for his contributions in the initial development of the expanded model of folate metabolism and for his helpful discussions. We also thank David L Hachey, USDA Children's Nutrition Research Center, Baylor College of Medicine, Houston, for his assistance in conducting initial GCMS analyses.

REFERENCES


