A mathematical model of the methionine cycle

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Abstract

Building on the work of Martinov et al. (2000), a mathematical model is developed for the methionine cycle. A large amount of information is available about the enzymes that catalyse individual reaction steps in the cycle, from methionine to \(S\)-adenosylmethionine to \(S\)-adenosylhomocysteine to homocysteine, and the removal of mass from the cycle by the conversion of homocysteine to cystathionine. Nevertheless, the behavior of the cycle is very complicated since many substrates alter the activities of the enzymes in the reactions that produce them, and some can also alter the activities of other enzymes in the cycle. The model consists of four differential equations, based on known reaction kinetics, that can be solved to give the time course of the concentrations of the four main substrates in the cycle under various circumstances. We show that the behavior of the model in response to genetic abnormalities and dietary deficiencies is similar to the changes seen in a wide variety of experimental studies. We conduct computational “experiments” that give understanding of the regulatory behavior of the methionine cycle under normal conditions and the behavior in the presence of genetic variation and dietary deficiencies.

Keywords: Folate metabolism; Genetic deficiency; Homocysteine; Mathematical model; Methionine; Methionine synthase; Methylation; Vitamin B

1. Introduction

The methionine cycle has three important functions in cellular metabolism. First, it regulates the balance between methionine and cysteine for protein synthesis; second, it provides the substrate for polyamine synthesis, and third, it provides the mechanism by which methyl groups are transferred from \(5\)-methyltetrahydrofolate to a broad variety of substrates and constitutes the primary mechanism for transmethylation reactions in mammals (Finkelstein, 1990). Normal functioning of the methionine cycle is essential for growth and development, and abnormalities in methionine metabolism and transmethylation efficiency are associated with cardiovascular disease (Refsum et al., 1998), liver disease (Finkelstein, 2003), neural tube defects (Eskes, 2001), and cancer (Duthie, 1999; Potter, 1999).

Because of its central role in cell metabolism, the operation of the methionine cycle (Fig. 1) has been the subject of numerous experimental studies (Finkelstein, 1990). This work has revealed complex responses to experimental variation in its various components. Some of this complexity arises from the fact that enzymes of the methionine cycle are activated and inhibited by several of the intermediates of the cycle. A significant part of the complexity arises from nonlinearities in the interactions among the components of the cycle that make the response to perturbation context-dependent, and therefore non-intuitive and unpredictable. Much of what is known about the properties and behavior of the pathway comes from a broad body of empirical experience, both in vivo and in vitro (e.g. Banerjee et al., 1990; Finkelstein, 1990, 2001; Finkelstein et al., 1982; Finkelstein and Martin, 1984, 1986; Hoffman et al., 1980).

A mathematical model of a portion of the methionine cycle has been developed by Martinov et al. (2000), who used it to explain the mechanism behind the sudden switch from a low to a high \(S\)-adenosylmethionine (AdoMet) concentration with a gradual increase in the
The main metabolites are shown in boxes and the enzymes in ellipses. Abbreviations: 5mTHF = 5-methyl tetrahydrofolate; AdoHcy = S-adenosylhomocysteine; AdoMet = S-adenosylmethionine; AH = S-adenosylhomocysteine hydrolase; BHMT = betaine:homocysteine methyltransferase; CBS = cystathionine β-synthase; GNMT = glycine N-methyltransferase; MAT = methionine adenosyl transferase; Metin = rate of methionine input; MS = methionine synthase; SM = S-adenosylmethionine-dependent methyltransferases.

The main metabolites are shown in boxes and the enzymes in ellipses. Abbreviations: 5mTHF = 5-methyl tetrahydrofolate; AdoHcy = S-adenosylhomocysteine; AdoMet = S-adenosylmethionine; AH = S-adenosylhomocysteine hydrolase; BHMT = betaine:homocysteine methyltransferase; CBS = cystathionine β-synthase; GNMT = glycine N-methyltransferase; MAT = methionine adenosyl transferase; Metin = rate of methionine input; MS = methionine synthase; SM = S-adenosylmethionine-dependent methyltransferases.

concentration of methionine. The elevated level of AdoMet is associated with a higher rate of methionine metabolism and provides a mechanism for dealing with excess methionine. The analysis of Martinov et al. (2000) showed that the bistability of AdoMet concentration was a consequence of the kinetic properties of two isoenzymes of methionine adenosyl transferase (MATI and MATIII), and of the cooperative kinetics of glycine-N-methyltransferase. Martinov et al., however, treated the methionine concentration as a parameter and did not consider the reactions by which homocysteine, a key metabolite in the methionine cycle, is remethylated to form methionine. Thus, they could not study the dynamics of the methionine concentration nor the role that AdoMet plays in regulating the alternative fates of homocysteine. In short, they could not study the dynamic properties of the methionine cycle as a whole.

In the present paper we extend the mathematical model of Martinov et al. (2000) by closing the cycle and taking into account the influence of S-adenosylmethionine and S-adenosylhomocysteine on the fates of homocysteine. Homocysteine can enter the transsulfuration pathway and be converted to cystathionine, or it can be methylated by either 5-methyltetrahydrofolate or betaine to form methionine, which closes the cycle, as illustrated in Fig. 1. In our model we follow the time course of the four main metabolites, methionine (Met), S-adenosylmethionine (AdoMet), S-adenosylhomocysteine (AdoHcy), and homocysteine (Hcy). We are interested not only in the steady-state behavior but in the responses of the system away from equilibrium. We want to use the model to understand effects of temporal variation in methionine and folate input on the metabolite concentrations. In addition, we want to understand the mechanisms by which dietary deficiencies (protein, vitamins B6, B12) and genetic abnormalities cause changes in metabolite concentrations and affect the operation of the cycle as a whole.

The behavior of the methionine cycle is surprisingly complex. Several steps in the pathway are catalysed by multiple enzymes, many of which have rather complicated kinetics. Furthermore, two of the metabolites, AdoMet and AdoHcy, not only influence the enzymes that catalyse their own synthesis, but also activate and inhibit several other enzymes in the pathway (see Fig. 2). As we will see, these interactions are critical for the remarkable regulatory properties of the network.

### 2. Description of the mathematical model

We wish to follow the time course (away from equilibrium) as well as the steady states of the variables: methionine (Met); S-adenosylmethionine (AdoMet); S-adenosylhomocysteine (AdoHcy); homocysteine ([Hcy]) (Table 1).

We regard the rate of methionine input (Metin) and the concentration of 5-methyltetrahydrofolate (5mTHF) as given functions of time and wish to compute the variables Met, AdoMet, AdoHcy, and Hcy as functions of time. These variables satisfy the four differential
The time-dependent variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Met]</td>
<td>μM</td>
<td>Methionine concentration</td>
</tr>
<tr>
<td>[AdoMet]</td>
<td>μM</td>
<td>S-adenosylmethionine concentration</td>
</tr>
<tr>
<td>[AdoHcy]</td>
<td>μM</td>
<td>S-adenosylhomocysteine concentration</td>
</tr>
<tr>
<td>[Hcy]</td>
<td>μM</td>
<td>Homocysteine concentration</td>
</tr>
</tbody>
</table>

equations:

\[
\frac{d[\text{Met}]}{dt} = V_{MS} + V_{BHMT} + \text{Metin} - V_{\text{MATI}},
\]

\[
\frac{d[\text{AdoMet}]}{dt} = V_{\text{MATI}} + V_{\text{MATIII}} - V_{\text{METH}} - V_{\text{GNMT}},
\]

\[
\frac{d[\text{AdoHcy}]}{dt} = V_{\text{METH}} + V_{\text{GNMT}} - V_{\text{AH}},
\]

\[
\frac{d[Hcy]}{dt} = V_{\text{AH}} - V_{\text{CBS}} - V_{\text{MS}} - V_{\text{BHMT}},
\]

where the terms on the right-hand sides are the rates of the reactions shown in Fig. 2. These rates depend, of course, on the current values of one or more of the variables. It is here that the fundamental kinetic information comes into play. We begin by restating the rates used by Martinov et al. (2000), for the creation of AdoMet from Met and then work our way around the diagram in Fig. 2 in clockwise fashion.

In the liver there are two isoforms of methionine adenosyl transferase, MATI and MATIII. The first, MATI, is inhibited by its product, AdoMet, whereas, the second, MATIII, is activated by AdoMet. The various ways in which the metabolites AdoMet and AdoHcy interact with various enzymes in the methionine cycle are illustrated in Fig. 2. We adopt the kinetic schemes and rate constants for the MATI and MATIII reactions used by Martinov et al. (2000):

\[
V_{\text{MATI}} = \frac{V_{\text{MATI}}^\text{max}}{1 + \frac{K_{\text{MATI}}}{[\text{Met}]} (1 + \frac{\text{AdoMet}}{K_{\text{GNMT}}})},
\]

\[
V_{\text{MATIII}} = \frac{V_{\text{MATIII}}^\text{max}}{1 + \frac{K_{\text{MATIII}}}{[\text{Met}]} \cdot \frac{1 + \text{AdoMet}}{K_{0,\text{MATIII}}}},
\]

where

\[
K_{\text{MATI}} = \frac{20000}{1 + 5.7 \left( \frac{\text{AdoMet}}{[\text{AdoMet}] + 600} \right)^2}.
\]

The next step in the pathway is the demethylation of AdoMet to AdoHcy. There are two separate reactions for this demethylation. The first is catalysed by glycine N-methyltrasferase (GNMT), which methylates glycine to become sarcosine. Following Martinov et al. (2000),

\[
V_{\text{GNMT}} = \frac{V_{\text{GNMT}}^\text{max}}{1 + \left( \frac{K_{\text{GNMT}}}{\text{AdoMet}} \right)^2 + \frac{1}{V_{\text{GNMT}}^\text{max}}}
\]

Note that the rate of this reaction is sigmoidally dependent on AdoMet and is inhibited by the product, AdoHcy. The second lumps together several methyl transfer reactions that use AdoMet as a methyl donor. The overall rate of these methyl transfer reactions is given by

\[
V_{\text{METH}} = \frac{V_{\text{METH}}^\text{max}}{1 + \frac{K_{\text{METH}}}{[\text{AdoMet}]} + \frac{K_{\text{METH}}}{[\text{AdoMet}]} + \frac{K_{\text{METH}}}{[\text{AdoMet}]}},
\]

\[
V_{\text{GNMT}} = \frac{V_{\text{GNMT}}^\text{max}}{1 + \left( \frac{K_{\text{GNMT}}}{\text{AdoMet}} \right)^2 + \frac{1}{V_{\text{GNMT}}^\text{max}}}
\]

Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATI</td>
<td>( \gamma_{\text{MATI}} )</td>
<td>μM/h</td>
<td>561</td>
<td>(1),(2),(3)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{MATI}}} )</td>
<td>μM</td>
<td>41</td>
<td>(1),(4)</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{MATI}} )</td>
<td>μM/h</td>
<td>50</td>
<td>(1),(2),(4),(5)</td>
</tr>
<tr>
<td>MATIII</td>
<td>( \gamma_{\text{MATIII}} )</td>
<td>μM/h</td>
<td>22,870</td>
<td>(1),(2),(3)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{MATIII}}} )</td>
<td>μM</td>
<td>1</td>
<td>(1),(4)</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{MATIII}} )</td>
<td>μM</td>
<td>21.1</td>
<td>(1),(4)</td>
</tr>
<tr>
<td>GNMT</td>
<td>( \gamma_{\text{GNMT}} )</td>
<td>μM/h</td>
<td>10,600</td>
<td>(1),(6),(7)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{GNMT}}} )</td>
<td>μM</td>
<td>4500</td>
<td>(1),(6)</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{GNMT}} )</td>
<td>μM</td>
<td>20</td>
<td>(1),(8)</td>
</tr>
<tr>
<td>Methylation</td>
<td>( \gamma_{\text{METH}} )</td>
<td>μM/h</td>
<td>4521</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{METH}}} )</td>
<td>μM</td>
<td>1</td>
<td>(1),(9),(10)</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{METH}} )</td>
<td>μM</td>
<td>10</td>
<td>(1),(9),(10)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{METH}}} )</td>
<td>μM</td>
<td>1</td>
<td>(1),(9),(10)</td>
</tr>
<tr>
<td>AH</td>
<td>( V_{\text{AH}} )</td>
<td>μM/h</td>
<td>100</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>( \beta_1 )</td>
<td>μM/h</td>
<td>1.7</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>( \beta_2 )</td>
<td>μM/h</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td>CBS</td>
<td>( V_{\text{CBS}} )</td>
<td>μM/h</td>
<td>100</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{CBS}} )</td>
<td>μM</td>
<td>0.1</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{CBS}}} )</td>
<td>μM</td>
<td>25</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>( K_{\text{CBS}} )</td>
<td>μM</td>
<td>1</td>
<td>(21)</td>
</tr>
<tr>
<td>BHMT</td>
<td>( V_{\text{BHMT}} )</td>
<td>μM/h</td>
<td>2500</td>
<td>*(12),(22)</td>
</tr>
<tr>
<td></td>
<td>( K_{\gamma_{\text{BHMT}}} )</td>
<td>μM</td>
<td>12</td>
<td>*(23)</td>
</tr>
</tbody>
</table>

* See text; † depends on variables; (1) Martinov et al. (2000); (2) Cabrero and Alemany (1988); (3) Hoffman (1983); (4) Sullivan and Hoffmann (1983); (5) Cabrero et al. (1987); (6) Ogawa and Fujikawa (1982); (7) Yeo and Wagner (1992); (8) Kerr and Headly (1974); (9) Duerece et al. (1977); (10) Hoffman and Cornatzer (1978); (11) Hoffman et al. (1979); (12) Finkelstein and Martin (1984); (13) Finkelstein and Martin (1986); (14) Nakagawa and Kimura (1968); (15) Kashiwamata and Greenberg (1970); (16) Brown and Gordon (1971); (17) Borcsok and Abeles (1982); (18) Finkelstein and Martin (1986); (19) Banerjee et al. (1997); (20) Banerjee et al. (1997); (21) Banerjee et al. (1990); (22) Finkelstein (1990); (23) Finkelstein et al. (1972).
where \( A \) represents the substrates for methylation and
\[
K_{m1}^{\text{METH}} = 1.0 \left( 1 + \frac{[\text{AdoHcy}]}{4} \right).
\]

We take \( K_{m1}^{\text{METH}} / [A] = 10 \). All of the preceding kinetics and the values of constants (some of which are given in Table 2) are taken from Martinov et al. (2000), and we refer the reader to that paper for detailed justifications.

The next step in the pathway is the synthesis of Hcy from AdoHcy, catalysed by adenosyl homocysteine hydrolase (Prigge and Chiang, 2001). This reaction is reversible and the enzyme has a much higher activity than other enzymes in the pathway (Hoffman et al., 1979; Martinov et al., 2000). Therefore [Hcy] will rapidly adjust to variation in [AdoHcy] and flux through this portion of the pathway will rapidly adjust to variation in flux in the overall pathway. We take the following simple form for the kinetics of this reaction:
\[
V_{AH} = \alpha_1([\text{AdoHcy}] - \alpha_2[Hcy]).
\]

Since typical concentrations measured for AdoHcy and Hcy have a ratio of about 10, we take \( \alpha_2 = 10 \) (unitless) and choose \( \alpha_1 = 100 \) h\(^{-1}\) to ensure rapid adjustment.

Homocysteine has two fates. It can be converted to cystathionine via an irreversible transulfuration reaction, \( V_{CBS} \), catalysed by cystathionine \( \beta \)-synthase, which removes it from the methionine cycle, or it can acquire a methyl group, either from 5mTHF or from betaine, and be reconverted into Met by the reactions \( V_{MS} \) or \( V_{BHMT} \) utilizing the enzymes methionine synthase and betaine-homocysteine methyltransferase, respectively.

The first of these reactions is catalysed by cystathionine \( \beta \)-synthase. As shown in Fig. 2, this enzyme is activated by AdoMet and AdoHcy (Finkelstein and Martin, 1984). They measured the rate \( V_{CBS} \) as well as the AdoMet and AdoHcy concentrations in chow-fed rats with diets containing different percentages of Met. A simple regression on the data in Table 3 of Finkelstein and Martin (1984) shows that a good approximation for the dependence of \( V_{CBS} \) on [AdoMet] and [AdoHcy] is \( V_{CBS} = 1.7([\text{AdoMet}] + [\text{AdoHcy}]) - 30 \). Of course, the rate \( V_{CBS} \) must also depend on [Hcy]. Since the \( K_m \) for Hcy is exceptionally high (in the range 5700 to 20,000: Nakagawa and Kimura, 1968; Kashiwamata and Greenberg, 1970; Brown and Gordon, 1971; Borcsok and Abeles, 1982), the rate must be approximately linear in [Hcy] at physiological concentrations, which are of the order of magnitude of 1 \( \mu \)M. Therefore, we take the following as a good approximation to the rate \( V_{CBS} \) at physiological concentrations of [Hcy]:
\[
V_{CBS} = (\beta_1([\text{AdoMet}] + [\text{AdoHcy}]) - \beta_2[Hcy]),
\]
where \( \beta_1 = 1.7 \) (\( \mu \)M\(^{-1}\) h\(^{-1}\)) and \( \beta_2 = 30 \) (h\(^{-1}\)).

The detailed kinetics of the reaction in which methionine synthase transfers a methyl group from 5mTHF to Hcy to make Met has been investigated in a series of studies by Ruma Banerjee, Rowena Matthews, and others (Banerjee et al., 1990, 1997). They conclude that the reaction is most likely a sequential bi–bi reaction. Since the reaction is extremely fast (ms) on our time scale, and is not reversible, we take the simple dependence of the initial rate of a sequential bi–bi reaction on the substrates (Segel, 1975, p. 564) as our formula for \( V_{MS} \):
\[
V_{MS} = \frac{V_{\text{max}}^{\text{MS}}[5\text{mTHF}][\text{Hcy}]}{K_{d_{MS}}^{\text{MS}}K_{m,\text{Hcy}}^{\text{MS}} + K_{m,\text{Hcy}}^{\text{MS}}[5\text{mTHF}] + K_{m,5\text{mTHF}}^{\text{MS}}[\text{Hcy}] + [5\text{mTHF}][\text{Hcy}]}.
\]

We take \( K_{m,\text{Hcy}}^{\text{MS}} = 0.1 \) \( \mu \)M and \( K_{m,5\text{mTHF}}^{\text{MS}} = 25 \) \( \mu \)M as found by Banerjee et al. (1997). A number of different values have been found for \( V_{\text{max}}^{\text{MS}} \) ranging from 524 \( \mu \)M/h/kg (Finkelstein and Martin, 1986) to 1380 \( \mu \)M/h/kg (Banerjee et al., 1997). We take \( V_{\text{max}}^{\text{MS}} = 500 \) \( \mu \)M/h/kg. In Banerjee et al. (1990), \( K_{d_{MS}}^{\text{MS}} \) is taken to be 1 \( \mu \)M and they comment that the simulation results are quite insensitive to this choice (as one can see from Eq. (13) above since the first term in the denominator is quite small compared to the third term at physiological concentrations). Thus, we also choose \( K_{d_{MS}}^{\text{MS}} = 1 \) \( \mu \)M.

The second reaction by which Hcy is remethylated to become Met, with rate \( V_{BHMT} \), utilizes betaine as a substrate and is catalysed by betaine:homocysteine methyltransferase. In our model we assume that the concentration of betaine remains constant and is thus absorbed into \( V_{\text{BHMT}}^{\text{max}} \). It is known (Finkelstein, 1990) that betaine:homocysteine methyltransferase is inhibited by [AdoMet] and by [AdoHcy] (see Fig. 2). We take the following simple form for \( V_{BHMT} \):
\[
V_{BHMT} = (0.7 - 0.025([\text{AdoMet}] + [\text{AdoHcy}] - 150)) \times \frac{V_{\text{max}}^{\text{BHMT}}[\text{Hcy}]}{K_{m}^{\text{BHMT}} + [\text{Hcy}]}.
\]

The value \( K_{m}^{\text{BHMT}} = 12 \) \( \mu \)M for Hcy was determined in Finkelstein et al. (1972), and we take \( V_{\text{max}}^{\text{BHMT}} = 2500 \). If Metin = 200, then under steady-state conditions (see Section 3) we have [AdoMet] + [AdoHcy] \approx 150 and [Hcy] = 0.88 \( \mu \)M. Thus, the rate \( V_{BHMT} \) will be (0.7)(2500) \( \frac{0.88}{12+0.88} \approx 120 \) \( \mu \)M/h, consistent with the range seen in Finkelstein and Martin (1984).

We note that we have chosen simple factors, linear in [AdoMet] + [AdoHcy], in Eqs. (12) and (14) to model the activation of cystathionine \( \beta \)-synthase and the inhibition
of betaine:homocysteine methyltransferase by AdoMet and AdoHcy. The detailed kinetics of the activation of cystathionine synthase are not known, so, as noted above, the linear factor in \( V_{CBS} \) is derived directly from the experimental data of Finkelstein and Martin (1984). Likewise the inhibition kinetics of betaine:homocysteine methyltransferase are not well understood, so we also chose a simple linear factor that expresses this inhibition. We have found that the relative slopes of these two linear relationships play an important role in the regulatory behavior of the system. For instance, under steady-state conditions with \( \text{Met} = 200 \, \mu\text{M/h} \) and \([\text{Hcy}] \approx 1 \, \mu\text{M}\), the slope of \( V_{CBS} \) with respect to AdoMet is approximately 1.7. Suppose that Metin rises, driving up AdoMet, and \( V_{BHM T} \) does not change. Then one unit increase in Metin will cause approximately 1.7 units of mass to be removed from the system by transsulfuration. In order to conserve mass, \([\text{Hcy}]\) would have to decline, which is not consistent with physiological observation. If, however, the increase in AdoMet strongly inhibits \( V_{BHM T} \), then less Hcy will be recirculated, causing its concentration to rise, which drives the excess mass removal in the transsulfuration pathway. In Eq. (14), with \([\text{Hcy}] \approx 1 \, \mu\text{M}\), the slope of \( V_{BHM T} \) with respect to AdoMet is approximately \(-5\), and the resulting kinetics reproduce the observed transsulfuration fraction and correct pattern of changes in \([\text{Hcy}]\). We have found through simulation that these results are robust to at least 20% variation in parameter values. Of course, we cannot expect the real kinetics of the interactions of AdoMet, AdoHcy, and Hcy with cystathionine synthase and betaine:homocysteine methyltransferase to be linear over a very wide range of concentrations. Thus, we expect our model to accurately simulate the real biochemistry only over moderate physiological ranges of AdoMet and AdoHcy. As more detailed information on the kinetics becomes available, it can be readily incorporated into the model. 

The differential equations (1)–(4) were solved using a simple forward Euler method with one exception. The reaction between AdoHcy and Hcy equilibrates extremely rapidly. Therefore, given the current concentrations of Adomet and AdoHcy, the current concentration of Hcy was found by setting the right-hand side of Eq. (4) to zero and solving for \([\text{Hcy}]\) by using Newton’s method. All calculations were carried out by MatLab.

### 3. Results

We begin (Part A) by analysing how the concentrations of the reactants (Met, AdoMet, AdoHcy, and Hcy) depend on rate of input of methionine (Metin). Next we examine the dynamic, non-steady-state, behavior of the system with special attention to regulatory control and its biological consequences (Part B). Finally, we compare the model predictions to a large variety of experimental results (Part C).

**A. Steady-state behavior.** At steady state, the rate of production of cystathionine, \( V_{CBS} \), must equal the rate of input of methionine, Metin. However, the steady-state values of the reactants in the system depend on Metin; see Fig. 3 where Metin is varied from 50 to 300 \( \mu\text{M/h} \). Perhaps the most striking result is the insensitivity of Met to Metin. Despite a 6-fold variation in Metin, the Met concentration varies between 49.7 and 53.6 \( \mu\text{M}\). Evidently, this system is able to tightly control the concentration of Met in the face of extreme variation of methionine input. One mechanism by which this control is achieved depends on the activities of MATI and MATIII that ensure rapid conversion of Met to Adomet. As can be seen from Fig. 3, the steady-state concentration of AdoMet increases dramatically as the rate Metin increases.

One would expect that the rate of methylation (\( V_{METH} \)) would depend strongly on the concentration of AdoMet, the methyl donor. However, as can be seen from Fig. 4, below, \( V_{METH} \) is almost entirely independent of Metin and [AdoMet]. The fundamental reason for this stability is that, at physiological concentrations of AdoMet, the \( V_{METH} \) reaction is running at near saturation. An important consequence of the stability of \( V_{METH} \) is the relative stability of its downstream metabolites, AdoHcy and Hcy. The steady-state concentrations of AdoHcy and Hcy do increase with Metin as observed by Finkelstein and Martin (1984), but the increase is moderate compared to the increase in Metin. Neither the methionine input to mammalian hepatocytes nor the portion that enters the methionine cycle are well known quantitatively and both may vary in time. One can deduce an estimate of Metin from the following considerations. The flux through the methionine pathway from Met to AdoMet to AdoHcy to Hcy is in the range of 400 \( \mu\text{M/h} \) (see references in Martinov et al., 2000). About 50% of Hcy is transsulfurated under

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**Fig. 3.** The effect of methionine input (Metin) on the steady-state levels of the four metabolites of the methionine cycle.
normal conditions (Finkelstein and Martin, 1986; Finkelstein, 1990), so about 200 μM/h leaves the methionine cycle. At steady state, the input of methionine must match the output so the input is probably, on average, about 200 μM/h.

We therefore take Metin = 200 μM/h as our “normal” methionine input. At steady state, this yields the following concentrations of the metabolites: \[ [\text{Met}] = 53.5 \, \mu\text{M}, \quad [\text{AdoMet}] = 137.6 \, \mu\text{M}, \quad [\text{AdoHcy}] = 13.2 \, \mu\text{M}, \quad [\text{Hcy}] = 0.88 \, \mu\text{M}. \] These metabolite concentrations are in the normal physiological range (see references in Martinov et al., 2000). Unless otherwise specified, we take [5mTHF] = 5.2 μM, consistent with the data in Cook (2001). At this steady state, \( V_{\text{METH}} = 398.5 \, \mu\text{M/h} \) and \( V_{\text{GNMT}} = 2.1 \, \mu\text{M/h} \), so that at each step from Met to AdoMet to AdoHcy to Hcy the net flux is approximately 400 μM/h. Because at steady state the input into the pathway (Metin = 200 μM/h) must equal the output, the flux out of the pathway, \( V_{\text{CBS}} \), is also 200 μM/h at steady state. Thus, in this normal mode, 50% of the flux around the cycle is removed by transsulfuration to cystathionine. It may seem that the steady-state concentration of Hcy is low. However, we note that, in life, 90% of the Hcy may be bound to protein (Gregory et al., 1998), and in our model the concentration refers to the free metabolite.

B. Dynamics and control. As emphasized by Finkelstein, the methionine cycle exhibits remarkable regulatory properties (Finkelstein and Martin, 1984, 1986; Finkelstein, 1990, 2001.) This can be observed if one examines the effect of temporal variation in Metin. Fig. 4 shows the effects of a sudden change of the methionine input from 200 to 300 μM/h for 3 h followed by a decrease to 100 μM/h for 3 h after which the input returns to 200 μM/h.

As shown in Fig. 4 (Part A), the Met concentration varies very little. Most of the fluctuation of Metin is absorbed as a fluctuation in [AdoMet]. Throughout, the methylation rate remains virtually unaltered. The major changes in the system are in the rates of transsulfuration of homocysteine, \( V_{\text{CBS}} \), and remethylation of homocysteine via the betaine reaction, \( V_{\text{BHMT}} \). This redistribution of homocysteine between competing pathways is due to complementary changes in the activities of betaine:homocysteine methyltransferase and cystathionine \( \beta \)-synthase controlled by AdoMet. Between hours 2 and 5, as [AdoMet] rises, the activity of cystathionine \( \beta \)-synthase is increased and that of betaine:homocysteine methyltransferase is decreased (see Fig. 4B). Then when Metin drops to 100 μM/h between hours 5 and 8, the activity of cystathionine \( \beta \)-synthase is decreased and that of betaine:homocysteine methyltransferase is increased (Fig. 4B). The biological significance of this regulation is that the increase in transsulfuration between hours 2 and 5 is accomplished without a major increase in the concentration of homocysteine. In fact, as can be seen in Fig. 4, the steady-state level of Hcy increases only moderately as Metin rises from 200 to 300 μM/h. If the activity of cystathionine \( \beta \)-synthase were not sensitive to Adomet, the increased rate of transsulfuration and removal of excess methionine could only be accomplished by a dramatic rise in the concentration of homocysteine.

The effect of temporal variation in folate availability is shown in Fig. 5. We varied the concentration of 5mTHF from 5.2 to 7.2 μM for 3 h followed by a decrease to 3.2 μM for 3 h after which the level returns to 5.2 μM. As one can see, [Met], [AdoMet], and the methylation rate, \( V_{\text{METH}} \), change very little. Since
5mTHF is a substrate for the conversion of Hcy to Met via methionine synthase, the rate VMS follows the abrupt changes in 5mTHF: The sharp increase in VMS at t = 2 h rapidly draws down [Hcy] which in turn decreases VCBS. The increase VMS also increases [Met] which drives up [AdoMet]. The increase in [AdoMet] increases the activity of cystathionine β-synthase. Consequently, as VCBS returns to its original steady-state level, [Hcy] can rise to its new (somewhat lower) steady-state by t = 3 h. The moderate drop in [Hcy], combined with the inhibition of betaine:homocysteine methyltransferase by the increase in [AdoMet] reduces V_BHMT.

When 5mTHF drops sharply to 3.2 μM at t = 5 h, [Hcy] rises sharply instigating a similar sequence of events to those just described, except that all concentrations change in the opposite direction. Finkelstein and Martin (1984) describe experimental results in which removal of 5mTHF resulted in an increase in betaine:homocysteine methyltransferase activity, and an “unexplained reduction in cystathionine synthase” activity. As can be seen from Fig. 5, the reduction in [5mTHF] results in a moderate increase in [Hcy] and a reduction in [AdoMet]. The rate V_CBS quickly returns to 200 μM/h. This is due to a decrease in the activity of cystathionine β-synthase (as seen by Finkelstein and Martin, 1984) caused by the drop in [AdoMet]. The drop in activity is balanced by the increase in [Hcy].

The main effect of the regulation of the activities of cystathionine synthase and betaine:homocysteine methyltransferase by AdoMet is that, under variation in 5mTHF, the new equilibrium can be achieved with only moderate changes in [Hcy]. Throughout all these changes in metabolite concentrations and reaction velocities, the methylation rate, V_METH, remains in the range 397.8–399.1 μM/h.

C. Comparison of model performance with experimental results.

Transulfuration fraction. Finkelstein and Martin (1984) demonstrated in rat-liver homogenates that the fraction of Hcy that is utilized for cystathionine synthesis is a function of the concentration of AdoMet. Fig. 6 shows that in our model, at steady-state, the fraction of Hcy that is transsulfurated to cystathionine varies from 13% to 75% as [AdoMet] varies from 66 to 159 μM. This explains the observation of Finkelstein and Martin (1986) that an increase in dietary methionine causes an increase in cystathionine synthesis. The range of the fraction is also similar to that seen by Finkelstein and Martin (1984). An increase in Met causes an increase in the steady-state value of [AdoMet] (see Fig. 3). As we saw above, an increase in [AdoMet] inhibits betaine:homocysteine S-methyltransferase (lowers
and activates cystathionine synthase (raises $V_{CBS}$) thus increasing the fraction transsulfurated.

**Folate deficiency.** Many physiological studies have been done that relate the behavior of methionine metabolism to variation in folate levels. In our model, folate enters via 5$\text{mTHF}$: Fig. 7 shows that the steady-state level of $\text{Hcy}$ and the steady-state rate $V_{BHMT}$ increase significantly with a decline in 5$\text{mTHF}$. These results are in close agreement with the findings of Finkelstein and Martin (1984) who demonstrated an increase in the activity of betaine:homocysteine $S$-methyltransferase upon removal of 5$\text{mTHF}$. The increase of $\text{Hcy}$ is in accord with the widespread observation that folate insufficiency is associated with elevated levels of homocysteine (see, for example, Carmel, 2001). Consistent with a number of studies (e.g. Jacob et al., 1998), a reduction of the folate level in our model, resulted in a slight decrease in methylation rate (not shown).

The explanation for these changes in the presence of folate deficiency is as follows. At steady state, the methylation rate declines only slightly because it is very insensitive to $\text{AdoMet}/\text{C138}$ (see Fig. 4). Thus, the flux around the pathway will be only a little less than 400 $\mu$M/h. Since $\text{Met}_{in} = 200$ $\mu$M/h at steady state. Thus the remethylation flux from $\text{Hcy}$ to $\text{Met}$ must be a little less than 200 $\mu$M/h. However, the rate $V_{MS}$ has declined because of the folate deficiency. Thus the rate $V_{BHMT}$ must rise. This could be accomplished in two ways, by increasing $\text{Hcy}$ or by lowering $\text{AdoMet}$, which removes inhibition from $V_{BHMT}$. In the model, and in experiments, both occur.

**Cystathionine $\beta$-synthase activity.** Genetic deficiency in cystathionine $\beta$-synthase causes an accumulation of $\text{Hcy}$ and $\text{Met}$, Finkelstein (1990), as does a deficiency in vitamin $B_6$, which is a required cofactor for this enzyme. As one can see in Fig. 8, $\text{Hcy}$ accumulates to significantly higher levels in the model when we decrease cystathionine synthase activity. The model also produces an increase in the level of $\text{Met}$ but only at reduced levels of $\text{Met}_{in}$ (see $\text{Met}_{in} = 100$ in Panel A of Fig. 8). The flux $V_{CBS}$ must remain equal to $\text{Met}_{in}$ at steady state. This could be accomplished in two ways: first, $\text{Hcy}$ can increase, and second, [AdoMet] and [AdoHcy] can increase enhancing cystathionine $\beta$-synthase activity. Both methods occur in practice, both in the model and in experiments.

Excess cystathionine $\beta$-synthase activity, as occurs in chromosome 21 trisomy, causes a reduction in the levels of $\text{Met}$, AdoMet, AdoHcy, and Hcy (Pogribna et al., 2001). These results are also produced by the model (see Panels A and D). As above, the flux $V_{CBS}$ must remain equal to $\text{Met}_{in}$ at steady state. Our simulations show
that this is accomplished by lowering [Hcy], and by lowering [AdoMet] and [AdoHcy], which decreases cystathionine β-synthase activity. Our simulations show that the effect of trisomy on [Met] and [AdoMet] is greatest under moderate Metin (100 μM/h) but that increasing Metin can reverse each of these effects. As can be seen from Fig. 8, the effects of trisomy on [AdoHcy] and [Hcy] can also be reversed by increasing Metin. In clinical practice, it is preferable to increase dietary betaine (it tastes better). As noted above, the effect of betaine in our model is incorporated in $V_{\text{BHMT}}$. If we raise $V_{\text{BHMT}}^{\max}$ from 2500 to 3500 μM/h in our model, the normal level of Met is restored (see the star in Panel A) but the levels of the other metabolites are largely unaffected.

Genetic deficiency in MATI and MATIII. The enzymes MATI and MATIII are encoded by the same gene. MATI is a tetramer and MATIII is a dimer of the same polypeptide. Interestingly, these two isozymes have very different kinetic properties as can be seen from Eqs. (5)–(7). We modeled genetic deficiency in this gene by reducing $V_{\text{MATI}}^{\max}$ and $V_{\text{MATIII}}^{\max}$ to half their normal values. This resulted in a 70% increase in Met concentration. These results are consistent with the observations reported by Finkelstein (1990) and Mato et al. (2001), and the reason is straightforward. Deficiencies in MATI and MATIII cause a slowing of the rate at which Met is turned into AdoMet thus causing the input, Metin, to accumulate as Met. The steady-state concentrations of the other metabolites in the methionine cycle were completely unaffected.

Betaine deficiency. Finkelstein and Martin (1984), have reported that, in liver homogenates, the absence of betaine causes an increase in cystathionine β-synthase activity. As we will see, this seemingly paradoxical effect is, in fact, a dynamic phenomenon that can easily be understood by using the mathematical model. In our model we simulate the absence of betaine by setting $V_{\text{BHMT}}^{\max} = 0$. Fig. 9 shows the time course of $V_{\text{CBS}}$ after removal of betaine. Starting from the steady-state rate of 200 μM/h, $V_{\text{CBS}}$ rises rapidly to about 300 μM/h and then returns to its steady-state level after about 2 h (indeed, the steady-state level of $V_{\text{CBS}}$ must match Metin). Finkelstein and Martin (1984), measured cystathionine β-synthase activity by measuring the rate of transsulfuration 10 min after the initiation of the reaction, so what they observed was probably this transient rise in $V_{\text{CBS}}$.

But why does $V_{\text{CBS}}$ have this transient behavior? When betaine is removed, [Hcy] rises rapidly driving the increase of $V_{\text{CBS}}$ during the first hour. Because of the drop in remethylation of Hcy to Met, the Met concentration drops considerably (to 38 μM) causing a gradual, even more dramatic, drop in [AdoMet] to 20 μM. This, drop in [AdoMet] greatly reduces the activity of cystathionine β-synthase causing the rate $V_{\text{CBS}}$ to return to 200 μM/h even though [Hcy] remains greatly elevated. We remark that, in contrast to the stability of $V_{\text{MET}}$ under variations in Metin and $\text{[5mTHF]}$ (see Figs. 4 and 5), removal of betaine causes a dramatic drop in methylation rate ($V_{\text{MET}}$).

Methionine synthase deficiency. It has been known for a long time that humans with impaired methionine synthase activity have hypomethionemia and homocysteinuria (Finkelstein, 1974; Rosenblatt, 2001). In our model we expressed the impairment of methionine synthase activity by reducing $V_{\text{MS}}^{\max}$. This models a mutational deficiency in the enzyme as well as a vitamin B12 deficiency. Our results show a slight decrease in [Met], a moderate decrease in Adomet, and a substantial increase in homocysteine, see Fig. 10. This makes sense. The increase in [Hcy] drives both the rates $V_{\text{BHMT}}$ and $V_{\text{MS}}$ up and the decrease in Adomet removes inhibition on $V_{\text{BHMT}}$ driving it up further. These combined effects
compensate for the reduction in methionine synthase activity. Our model also shows that the increase in homocysteine (but not the decrease in Met) can be reversed by decreasing Met (Fig. 10).

4. Discussion

We have shown that our model of the methionine cycle is able to simulate many of the key regulatory features of this interesting and complex mechanism. The model is relatively simple, but is based on known kinetics or approximations to kinetics. By computational experiments in which inputs and kinetic parameters were varied, we were able to determine the causal chains of events by which many of the experimentally observed effects arise. This quantitative approach to the analysis of the methionine cycle has allowed us to verify and understand the marvelous regulatory properties of this system discovered and emphasized by James Finkelstein.

Of course, we recognize that this model is incomplete. First, the kinetic parameters that we have used were obtained from a wide variety of experiments on many different animal models. Therefore the parameters may not closely resemble those of any specific in vivo system. Secondly, we have used simple, linear approximations to the potentially complex kinetics by which AdoMet and AdoHcy alter the activities of cystathionine β-synthase and betaine:homocysteine methyltransferase. Thirdly, the methionine cycle does not operate in isolation. We have ignored the fact that AdoMet contributes to the polyamine cycle and inhibits the synthesis of 5mTHF. Likewise we have not included the interconversion of homocysteine and homocysteine thiolactone, nor the effects of the protein pool on the concentrations of Met and cysteine. Fourthly, we have not considered the sequestration of components of this system in different subcellular compartments, nor have we considered the partitioning of substrates and metabolites between the cell and the circulatory system (Storch et al., 1988; Gregory and Scott, 1996; MacCoss et al., 2001; Gregory and Quinlivan, 2002). Finally, we have not considered how nutrient status may affect the levels of expression and therefore the activity of enzymes in the pathway. A full quantitative account of the role that the methionine cycle plays in whole-body physiology, will have to await the incorporation of many of these features.

In spite of these limitations, the model gives a remarkably good simulation of a broad diversity of experimental results and clinical observations. Moreover, the computational experiments have allowed us to understand how the regulatory properties arise from the complex interactions among the metabolites and enzymes of the system. The stability of [Met], despite fluctuations in Metin and 5mTHF, arises from the kinetics properties of MATI and MATIII that insure rapid conversion of Met to AdoMet. The fact that the methylation reactions run at near saturation at physiological concentrations explains the stability of methylation rate in the presence of large fluctuations in AdoMet. The ability of AdoMet and AdoHcy to regulate the activities of cystathionine β-synthase and betaine:homocysteine methyltransferase enables the system to change the fraction of Hcy transsulfurated without significant changes in [Hcy]. Finally, the model allows us to understand why a variety of dietary deficiencies and genetic abnormalities cause [Hcy] to rise.

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