Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Theoretical Biology 226 (2004) 33–43
Journal of Theoretical Biology
www.elsevier.com/locate/jtbi

A mathematical model of the methionine cycle

Michael C. Reed[a,*], H. Frederik Nijhout[b], Rachel Sparks[c], Cornelia M. Ulrich[c]

[a] Department of Mathematics, Duke University, Durham, NC 27708, USA
[b] Department of Biology, Duke University, Durham, NC 27708, USA
[c] Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA

Received 7 March 2003; received in revised form 1 August 2003; accepted 4 August 2003

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.
© 2003 Elsevier Ltd. All rights reserved.

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

*Corresponding author. Tel.: +1-919-660-2821; fax: +1-919-606-2808.

E-mail addresses: reed@math.duke.edu (M.C. Reed), hfn@acpub.duke.edu (H.F. Nijhout), rsparks@fhcrc.org (R. Sparks), nulrich@fhcrc.org (C.M. Ulrich).

0022-5193/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtbi.2003.08.001

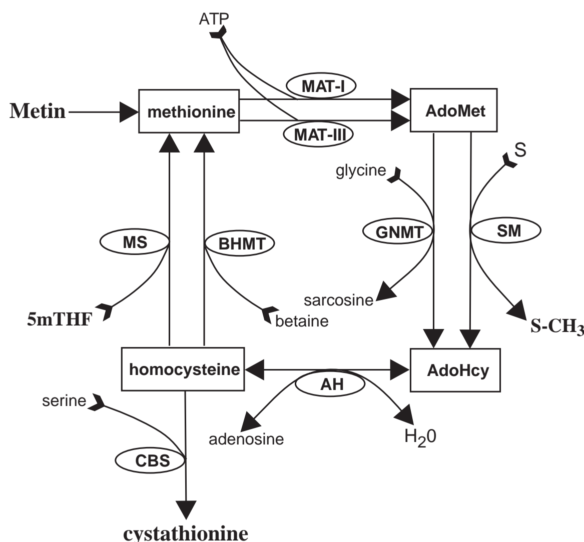

Fig. 1. Components of methionine metabolism modeled in this paper. The main metabolites are shown in boxes and the enzymes in ellipses. Abbreviations: $5mTHF$ = 5-methyl tetrahydrofolate; $AdoHcy$ = S-adenosylhomocysteine; $AdoMet$ = S-adenosylmethionine; AH = S-adenosylhomocysteine hydrolase; $BHMT$ = betaine:homocysteine methyltransferase; CBS = cystathionine β-synthase; $GNMT$ = glycine N-methyltransferase; MAT = methionine adenosyl transferase; $Metin$ = rate of metionine input; MS = methionine synthase; S = substrates for methylation; SM = S-adenosylmethionine-dependent methyltransferases.

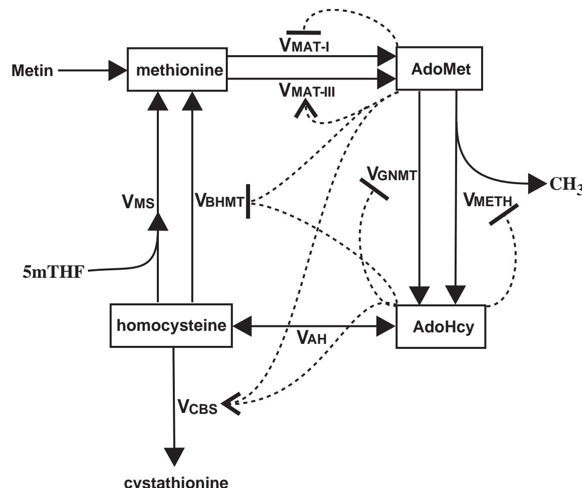

Fig. 2. The methionine cycle. For each arrow, the corresponding subscripted V denotes the current rate of the reaction. The dotted lines indicate the influence of $AdoMet$ and $AdoHcy$ on enzymes in the pathway. Arrows indicate activation; bars indicate inhibition.

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 B_6, B_{12}) 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

Table 1
The time-dependent variables

Variable	Units	Description
$[Met]$	μM	Methionine concentration
$[AdoMet]$	μM	*S*-adenosylmethionine concentration
$[AdoHcy]$	μM	*S*-adenosylhomocysteine concentration
$[Hcy]$	μM	Homocysteine concentration

equations:

$$\frac{d[Met]}{dt} = V_{MS} + V_{BHMT} + Metin - V_{MATI} - V_{MATIII}, \quad (1)$$

$$\frac{d[AdoMet]}{dt} = V_{MATI} + V_{MATIII} - V_{METH} - V_{GNMT}, \quad (2)$$

$$\frac{d[AdoHcy]}{dt} = V_{METH} + V_{GNMT} - V_{AH}, \quad (3)$$

$$\frac{d[Hcy]}{dt} = V_{AH} - V_{CBS} - V_{MS} - V_{BHMT}, \quad (4)$$

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):

$$V_{MATI} = \frac{V_{max}^{MATI}}{1 + \frac{K_m^{MATI}}{[Met]}\left(1 + \frac{[AdoMet]}{K_i^{MATI}}\right)}, \quad (5)$$

$$V_{MATIII} = \frac{V_{max}^{MATIII}}{1 + \frac{K_{m1}^{MATIII} K_{m2}^{MATIII}}{[Met]^2 + [Met]K_{m2}^{MATIII}}}, \quad (6)$$

where

$$K_{m1}^{MATIII} = \frac{20000}{1 + 5.7\left(\frac{[AdoMet]}{[AdoMet]+600}\right)^2}. \quad (7)$$

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),

$$V_{GNMT} = \frac{V_{max}^{GNMT}}{1 + \left(\frac{K_m^{GNMT}}{[AdoMet]}\right)^{2.3}} \frac{1}{1 + \frac{[AdoHcy]}{K_i^{GNMT}}}. \quad (8)$$

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

$$V_{METH} = \frac{V_{max}^{METH}}{1 + \frac{K_{m1}^{METH}}{[AdoMet]} + \frac{K_{m2}^{METH}}{[A]} + \frac{K_{m2}^{METH}}{[A]}\frac{K_{m1}^{METH}}{[AdoMet]}}, \quad (9)$$

Table 2
Rate constants and references

Enzyme	Parameter	Units	Value	References
MATI	V_{max}^{MATI}	μM/h	561	(1),(2),(3)
	K_m^{MATI}	μM	41	(1),(4)
	K_i^{MATI}	μM	50	(1),(2),(4),(5)
MATIII	V_{max}^{MATIII}	μM/h	22,870	(1),(2),(3)
	K_{m1}^{MATIII}	μM	†	(1),(4)
	K_{m2}^{MATIII}	μM	21.1	(1),(4)
GNMT	V_{max}^{GNMT}	μM/h	10,600	(1),(6),(7)
	K_m^{GNMT}	μM	4500	(1),(6)
	K_i^{GNMT}	μM	20	(1),(8)
Methylation	V_{max}^{METH}	μM/h	4521	(1)
	K_{m1}^{METH}	μM	†	(1),(9),(10)
	$K_{m2}^{METH}/[A]$	—	10	∗,(1),(9),(10)
AH	V_{AH}	μM/h	†	∗, (11)
	α_1	1/h	100	∗
	α_2	None	10	∗
CBS	V_{CBS}	μM/h	†	∗, (12),(13),(14),(15),(16),(17)
	β_1	1/μM h	1.7	∗
	β_2	$(h)^{-1}$	30	∗
MS	V_{max}^{MS}	μM/h	500	∗, (18),(19)
	$K_{m,Hcy}^{MS}$	μM	0.1	(20)
	$K_{m,5mTHF}^{MS}$	μM	25	(20)
	K_d^{MS}	μM	1	(21)
BHMT	V_{max}^{BHMT}	μM/h	2500	∗, (12), (22)
	K_m^{BHMT}	μM	12	∗, (23)

∗ 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 Fujioka (1982); (7) Yeo and Wagner (1992); (8) Kerr and Headly (1974); (9) Dueree 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

$$K_{m1}^{METH} = 1.0\left(1 + \frac{[AdoHcy]}{4}\right). \quad (10)$$

We take $K_{m2}^{METH}/[A] = 10$. 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:

$$V_{AH} = \alpha_1([AdoHcy] - \alpha_2[Hcy]). \quad (11)$$

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

Homocysteine has two fates. It can be converted to cystathionine via an irreversible transsulfuration reaction, V_{CBS}, catalysed by cystathionine β-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 β-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 $V_{CBS} = 1.7([Adomet] + [AdoHcy]) - 30$. 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 μM. Therefore, we take the following as a good approximation to the rate V_{CBS} at physiological concentrations of $[Hcy]$:

$$V_{CBS} = (\beta_1([Adomet] + [AdoHcy]) - \beta_2)[Hcy], \quad (12)$$

where $\beta_1 = 1.7\ (\mu\mathrm{M})^{-1}(\mathrm{h})^{-1}$ and $\beta_2 = 30\ (\mathrm{h})^{-1}$.

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 (h), 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}:

$$V_{MS} = \frac{V_{MS}^{max}[5mTHF][Hcy]}{K_d^{MS}K_{m,Hcy}^{MS} + K_{m,Hcy}^{MS}[5mTHF] + K_{m,5mTHF}^{MS}[Hcy] + [5mTHF][Hcy]}. \quad (13)$$

We take $K_{m,Hcy}^{MS} = 0.1\ \mu\mathrm{M}$ and $K_{m,5mTHF}^{MS} = 25\ \mu\mathrm{M}$ as found by Banerjee et al. (1997). A number of different values have been found for V_{MS}^{max} ranging from 524 μM/h/kg (Finkelstein and Martin, 1986) to 1380 μM/h/kg (Banerjee et al., 1997). We take $V_{MS}^{max} = 500$ μM/h/kg. In Banerjee et al. (1990), K_d^{MS} is taken to be 1 μM 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 $K_d^{MS} = 1\ \mu\mathrm{M}$.

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_{BHMT}^{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}:

$$V_{BHMT} = (0.7 - (.025)([AdoMet] + [AdoHcy] - 150)) \times \frac{V_{BHMT}^{max}[Hcy]}{K_m^{BHMT} + [Hcy]}. \quad (14)$$

The value $K_m^{BHMT} = 12\ \mu\mathrm{M}$ for Hcy was determined in Finkelstein et al. (1972), and we take $V_{BHMT}^{max} = 2500$. If $Metin = 200$, then under steady-state conditions (see Section 3) we have $[AdoMet] + [AdoHcy] \approx 150$ and $[Hcy] = 0.88\ \mu\mathrm{M}$. Thus, the rate V_{BHMT} will be $(0.7)(2500)\frac{0.88}{12+0.88} \approx 120$ μM/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 β-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 $Metin = 200\ \mu M/h$ and $[Hcy] \approx 1\ \mu M$, the slope of V_{CBS} with respect to *AdoMet* is approximately 1.7. Suppose that *Metin* rises, driving up *AdoMet*, and V_{BHMT} 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, [*Hcy*] would have to decline, which is not consistent with physiological observation. If, however, the increase in *AdoMet* strongly inhibits V_{BHMT}, 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 $[Hcy] \approx 1\ \mu M$, the slope of V_{BHMT} with respect to *AdoMet* is approximately -5, and the resulting kinetics reproduce the observed transsulfuration fraction and correct pattern of changes in [*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 [*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).

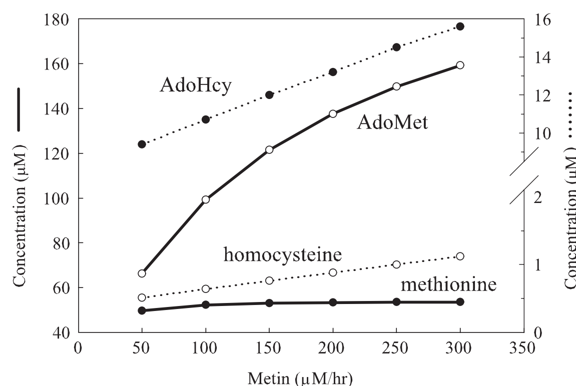

Fig. 3. The effect of methionine input (*Metin*) on the steady-state levels of the four metabolites of the methionine cycle.

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 $300\ \mu M/h$. 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 μM. 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 hepatocyctes 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 μM/h (see references in Martinov et al., 2000). About 50% of *Hcy* is transsulfurated under

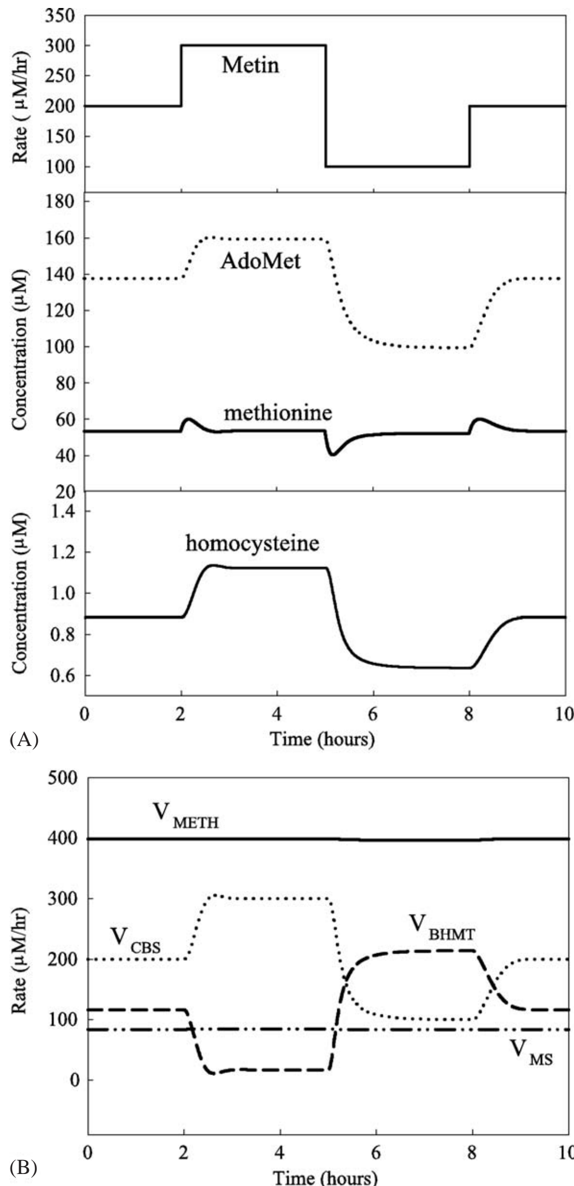

Fig. 4. The effects of dynamic changes in *Metin* on the metabolites (Panel A) and the rates of methylation and the reactions that use homocysteine as a substrate (Panel B).

normal conditions (Finkelstein and Martin, 1986; Finkelstein, 1990), so about 200 μM/h leaves the methione 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: $[Met] = 53.5$ μM, $[AdoMet] = 137.6$ μM, $[AdoHcy] = 13.2$ μM, $[Hcy] = 0.88$ μM. 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, $V_{METH} = 398.5$ μM/h and $V_{GNMT} = 2.1$ μM/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_{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 regulative 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_{CBS}, and remethylation of homocsyteine via the betaine reaction, V_{BHMT}. This redistribution of homocysteine between competing pathways is due to complementary changes in the activities of betaine:homocysteine methyltrasferase and cystathionine β-synthase controlled by *AdoMet*. Between hours 2 and 5, as [*AdoMet*] rises, the activity of cystathionine β-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 β-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 β-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_{METH}, change very little. Since

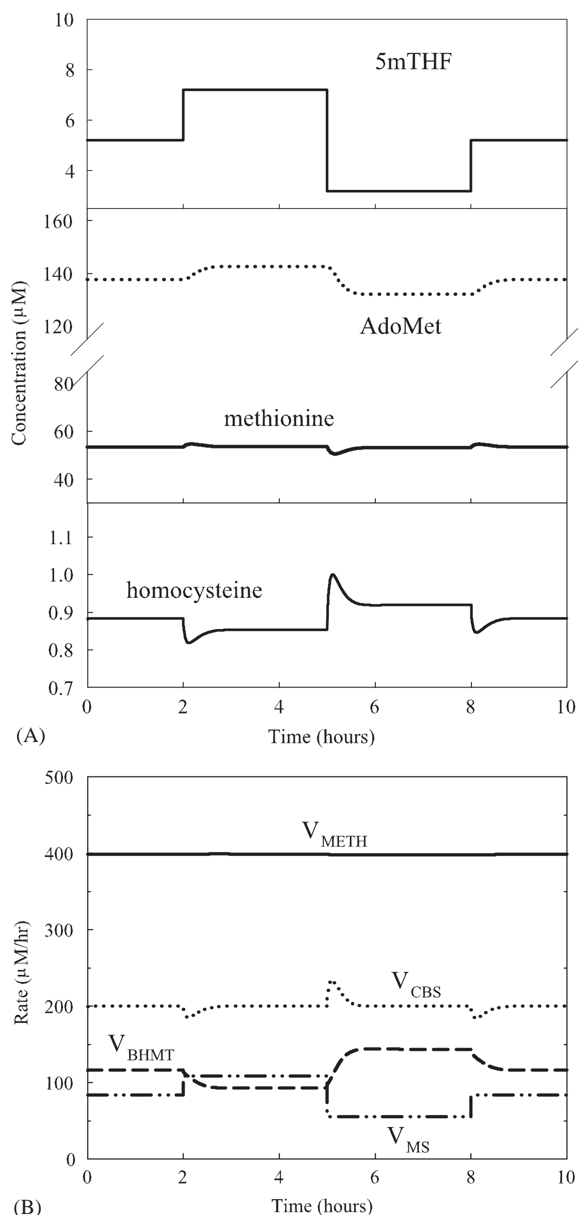

Fig. 5. The effects of dynamic changes in *5mTHF* on the metabolites (Panel A) and the rates of methylation and the reactions that use homocysteine as a substrate (Panel B).

5mTHF is a substrate for the conversion of *Hcy* to *Met* via methionine synthase, the rate V_{MS} follows the abrupt changes in *5mTHF*. The sharp increase in V_{MS} at $t = 2$ h rapidly draws down [*Hcy*] which in turn decreases V_{CBS}. The increase V_{MS} also increases [*Met*] which drives up [*AdoMet*]. The increase in [*AdoMet*] increases the activity of cystathionine β-synthase. Consequently, as V_{CBS} 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 methyltrasferase 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 *Metin* 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

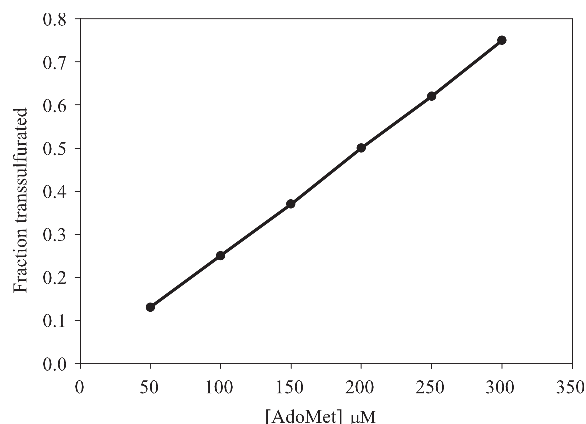

Fig. 6. The relationship between [*AdoMet*] and the fraction of homocysteine transsulfurated.

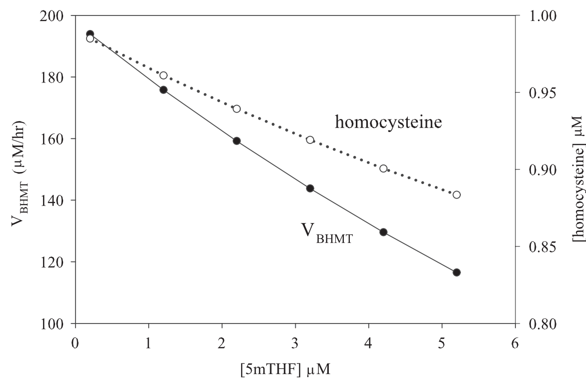

Fig. 7. The effect of [$5mTHF$] on the steady-state [Hcy] and V_{BHMT}.

V_{BHMT}) 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 $5mTHF$. Fig. 7 shows that the steady-state level of [Hcy] and the steady-state rate V_{BHMT} increase significantly with a decline in $5mTHF$. 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 $5mTHF$. The increase of 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 [$AdoMet$] (see Fig. 4). Thus, the flux around the pathway will be only a little less than 400 μM/h. Since $Metin = 200$ μM/h, we must have $V_{CBS} = 200$ μM/h at steady state. Thus the remethylation flux from Hcy to Met must be a little less than 200 μM/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 [Hcy] or by lowering [$AdoMet$], which removes inhibition from V_{BHMT}. In the model, and in experiments, both occur.

Cystathionine β-synthase activity. Genetic deficiency in cystathionine β-synthase causes an accumulation of Hcy and 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, 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 Met but only at reduced

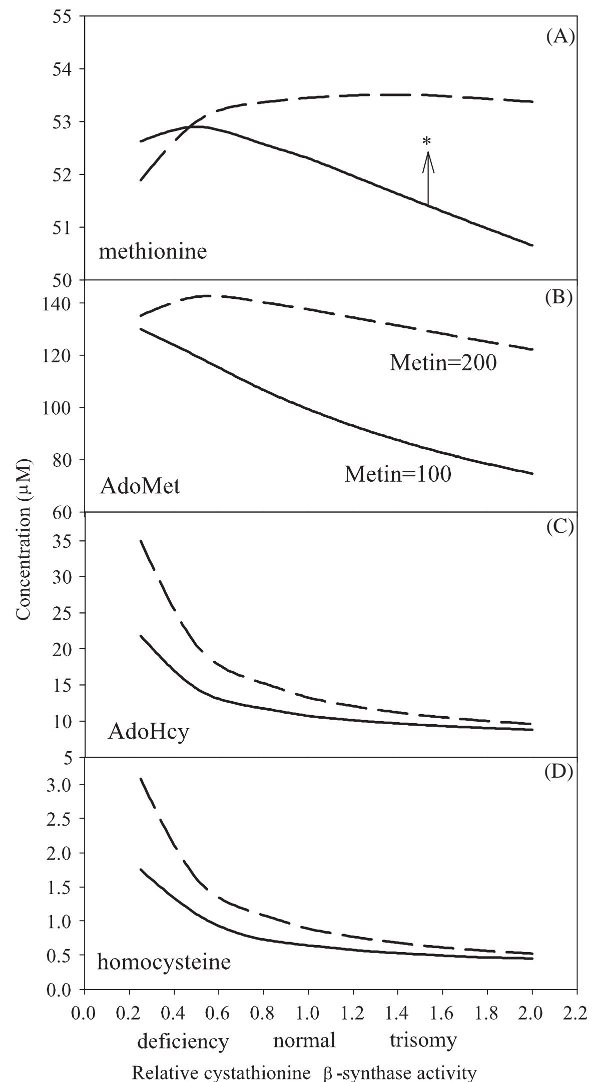

Fig. 8. The effects of variation in cystathionine β-synthase activity on the metabolites of the methionine cycle at steady-state. Each panel shows two curves: $Metin = 100$ μM/h (solid lines) and $Metin = 200$ μM/h (dashed lines). Normal cystathionine β-synthase activity is designated as 1.0 on the abscissa. The activity of cystathionine β-synthase given a triple genetic dosage (as in trisomy 21) is indicated by 1.5. The star indicates the level to which the steady-state concentration of methionine rises when V_{max}^{BHMT} is increased from 2500 to 3500 μM/h (see text).

levels of $Metin$ (see $Metin = 100$ in Panel A of Fig. 8). The flux V_{CBS} must remain equal to $Metin$ at steady state. This could be accomplished in two ways: first, [Hcy] can increase, and second, [$AdoMet$] and [$AdoHcy$] can increase enhancing cystathionine β-synthase activity. Both methods occur in practice, both in the model and in experiments.

Excess cystathionine β-synthase activity, as occurs in chromosome 21 trisomy, causes a reduction in the levels of 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 $Metin$ 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_{max}^{BHMT}. If we raise V_{max}^{BHMT} 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_{max}^{MATI} and V_{max}^{MATIII} 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 $V_{max}^{BHMT} = 0$. Fig. 9 shows the time course of V_{CBS} after removal of betaine. Starting from the steady-state rate of 200 μM/h, V_{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_{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_{CBS}.

But why does V_{CBS} have this transient behavior? When betaine is removed, [*Hcy*] rises rapidly driving the increase of V_{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

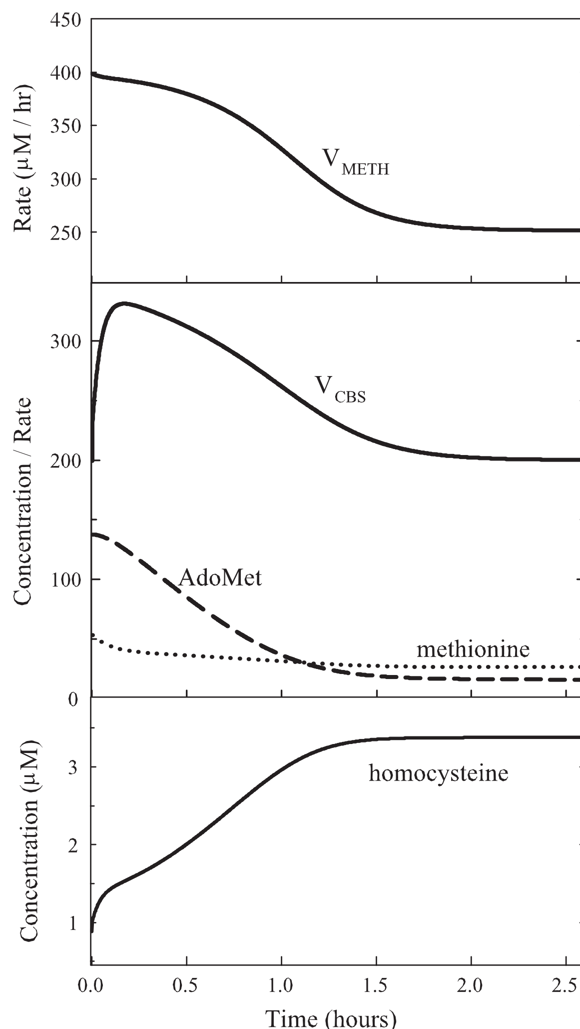

Fig. 9. The behavior of the system after removal of betaine at time = 0. The transsulfuration rate of *Hcy* (V_{CBS}) rises transiently and then returns to its original steady state. [*Hcy*] rises dramatically and the methylation rate (V_{METH}) drops.

V_{CBS} to return to 200 μM/h even though [*Hcy*] remains greatly elevated. We remark that, in contrast to the stability of V_{METH} under variations in *Metin* and [*5mTHF*] (see Figs. 4 and 5), removal of betaine causes a dramatic drop in methylation rate (Fig. 9).

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_{max}^{MS}. This models a mutational deficiency in the enzyme as well as a vitamin B_{12} 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_{BHMT} and V_{MS} up and the decrease in *Adomet* removes inhibition on V_{BHMT} driving it up further. These combined effects

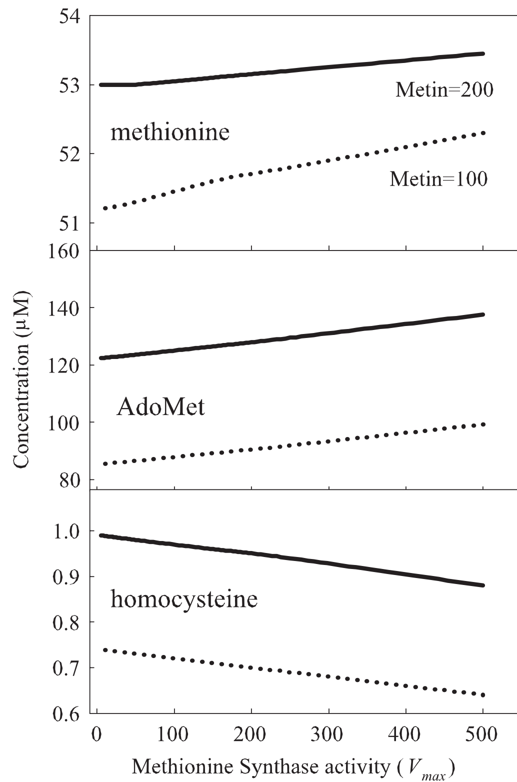

Fig. 10. The effect of variation of methionine synthase activity on the steady-state levels of three metabolites of the methionine cycle. Each panel shows two curves for $Metin = 100$ μM/h (dotted lines) and $Metin = 200$ μM/h (solid lines).

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 *Metin* (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.

Acknowledgements

The authors are grateful to Professor Ruma Banerjee for helpful discussions of the paper of Martinov et al. (2000), and to Professors J. Gregory and S. Jill James who pointed out many important experimental results. We thank Paula Budu for help in preparation of the figures. This research was supported by NSF grants #DMS-0109872 and #IBN-9975168.

References

Banerjee, R., Frasca, V., Ballou, D., Matthews, R., 1990. Participation of Cob(I)alamin in the reaction catalysed by methionine synthase from *Escherichia coli*: a steady state and rapid reaction kinetic analysis. Biochem. 29, 11101–11109.

Banerjee, R., Chen, Z., Gulati, S., 1997. Methionine synthase from pig liver. Methods Enzymol. 281, 189–197.

Borcsok, E., Abeles, R., 1982. Mechanism of action of cystathionine synthase. Arch. Biochem. Biophys. 213, 695–707.

Brown, C., Gordon, P., 1971. Cystathionine synthase from rat liver: partial purification and properties. Can. J. Biochem. 49, 484–491.

Cabrero, C., Alemany, S., 1988. Purification and conversion of rat liver S-adenosyl-L-methionine synthetase from high-Mr low-Mr form by LiBr. Biochem. Biophys. Acta 952, 277–281.

Cabrero, C., Puerta, J., Alemany, S., 1987. Purification and comparison of two different forms of S-adenosyl-L-methionine synthase from rat liver. Eur. J. Biochem. 170, 299–304.

Carmel, R., 2001. Folate deficiency. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 271–288.

Cook, R.J., 2001. Folate metabolism. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 113–134.

Dueree, J., Wallwork, J., Quick, D., Ford, K., 1977. In vitro studies on the methylation of histones in rat brain nuclei. J. Biol. Chem. 252, 6981–6985.

Duthie, S.J., 1999. Folic acid deficiency and cancer: mechanisms of DNA instability. Brit. Med. Bull. 55, 578–592.

Eskes, T.K.A.B., 2001. Homocysteine in human reproduction. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 451–465.

Finkelstein, J., 1990. Methionine metabolism in mammals. J. Nutr. Biochem. 1, 228–237.

Finkelstein, J., 2001. Regulation of homocysteine metabolism. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 92–99.

Finkelstein, J., 2003. Methionine metabolism in liver disease. Am. J. Clin. Nutr. 77, 1094–1095.

Finkelstein, J., Martin, J., 1984. Methionine metabolism in mammals: distribution of homocysteine between competing pathways. J. Biol. Chem. 259, 9508–9513.

Finkelstein, J., Martin, J., 1986. Methionine metabolism in mammals: adaptation to methionine excess. J. Biol. Chem. 261, 1582–1587.

Finkelstein, J.D., Harris, B.J., Kyle, W.E., 1972. Methionine metabolism in mammals: kinetic study of betaine:homocysteine methyltransferase. Arch. Biochem. Biophys. 153, 320–324.

Finkelstein, J.D., Harris, B.J., Martin, J.J., 1982. Methionine metabolism in mammals: concentrations of metabolites in rat tissues. Kinetic study of betaine:homocysteine methyltransferase. J. Nutr. 112, 1011–1018.

Gregory, J.F., Quinlivan, E.P., 2002. In vivo kinetics of folate metabolism. Ann. Rev. Nutr. 22, 199–220.

Gregory, J.F., Scott, K.C., 1996. Modeling folate metabolism. Adv. Food Nutr. Res. 40, 81–93.

Gregory, III.J.F., Williamson, J., Liao, J.-F., Bailey, L.B., Toth, J.P., 1998. Kinetic model of folate metabolism in nonpregnant women consuming [$^2H_2$] folic acid: isotopic labeling of urinary folate and the catabolite para-acetamidobenzoylglutamate indicates slow, intake-dependent, turnover of folate pools. J. Nutr. 128, 1896–1906.

Hoffman, J., 1983. Fractionation of methionine adonosyltransferase isozymes (rat liver). Methods Enzymol. 94, 223–228.

Hoffman, J., Cornatzer, W., 1978. Fractionation of methionine adonosyltransferase isozymes (rat liver). Methods Enzymol. 94, 223–228.

Hoffman, J., Cornatzer, W., Duerre, J., 1979. Relationship between tissue levels of A-adenosylmethionine, S-adenosylhomocysteine and transmethylation reactions. Can. J. Biochem. 57, 56–65.

Hoffman, J., Marion, D.W., Cornatzer, W., Duerre, J., 1980. S-adenosylmethionine and S-adenosylhomocysteine metabolism in isolated rat liver. Effects of L-methionine, L-homocysteine, and adenosine. J. Biol. Chem. 255, 10822–10827.

Jacob, R., Gretz, D., Taylor, P., James, S., Pogribny, I., Miller, B., Henning, S., Swendseid, M., 1998. Moderate folate depletion increases plasma homocysteine and decreases DNA methylation in post-menopausal women. J. Nutr. 128, 1204–1212.

Kashiwamata, S., Greenberg, D., 1970. Studies on cystathionine synthase of rat liver: properties of the highly purified enzyme. Biochim. Biophys. Acta 212, 488–500.

Kerr, S., Headly, J., 1974. Modulation of tRNA methyltrasferase activity by competing enzyme systems. Adv. Enzyme Regul. 12, 4248–4252.

MacCoss, M.J., Fukagawa, N.K., Matthews, D.E., 2001. Measurement of intracellular sulfur amino acid metabolism in humans. Adv. Enzyme Regul. 280, E947–E955.

Martinov, M., Vitvitsky, V., Mosharov, E., Banerjee, R., Ataullakhanov, F., 2000. A substrate switch: a new mode of regulation in the methionine metabolic pathway. J. Theor. Biol. 204, 521–532.

Mato, J., Matias, A., Corrales, F., 2001. Biosynthesis of S-adenosylmethionine. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 47–62.

Nakagawa, H., Kimura, H., 1968. Purification and properties of cystathionine synthetase from rat liver: separation of cystathionine synthetase from serine dehydratase. Biochem. Biophys. Res. Commun. 32, 208–214.

Ogawa, H., Fujioka, M., 1982. Purification and properties of glycine N-methyltransferase from rat liver. J. Biol. Chem. 257, 3447–3452.

Pogribna, M., Melnyk, S., Pogrybny, I., Chango, A., Yi, P., James, J., 2001. Homocysteine metabolism in children with Down Syndrome: in vitro modulation. Am. J. Hum. Genet. 69, 88–95.

Potter, J.D., 1999. Colorectal cancer: molecules and populations. J. Nat. Cancer Inst. 91, 916–932.

Prigge, S.T., Chiang, P.K., 2001. S-Adenosylhomocysteine hydrolase. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 79–91.

Refsum, H., Ueland, P.M., Nygard, O., Vollset, S.E., 1998. Homocysteine and cardiovascular disease. Ann. Rev. Med. 49, 31–62.

Rosenblatt, D., 2001. Inborn errors of folate and cobalamin metabolism. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 244–258.

Segel, I.H., 1975. Enzyme Kinetics. Wiley, New York.

Storch, K.J., Wagner, D.A., Burke, J.F., Young, V.R., 1988. Study in vivo of methionine cycle in humans using [methyl-2H3]- and [1-13] methionine. Am. J. Physiol. 255, E322–E331.

Sullivan, D., Hoffmann, J., 1983. Fractionation and kinetic properties of rat liver and kidney methionine adonosyltransferase isozymes. Biochemistry 22, 1636–1641.

Yeo, E.-J., Wagner, C., 1992. Purification and properties of pancreatic glycine N-methyltransferase. J. Biol. Chem. 267, 24669–24674.