J. theor. Biol. (2000) **204,** 521–532
doi:10.1006/jtbi.2000.2035, available online at http://www.idealibrary.com on IDEAL®

A Substrate Switch: A New Mode of Regulation in the Methionine Metabolic Pathway

Michael V. Martinov*, Victor M. Vitvitsky*, Eugene V. Mosharov†, Ruma Banerjee†‡ and Fazoil I. Ataullakhanov*‡

**National Research Center for Hematology, Moscow* 125167, *Russia and †Biochemistry Department, University of Nebraska, Lincoln, NE* 68588-0664, *U.S.A.*

(*Received on* 15 *October* 1999, *Accepted in revised form on* 1 *March* 2000)

We propose a simple mathematical model of liver *S*-adenosylmethionine (AdoMet) metabolism. Analysis of the model has shown that AdoMet metabolism can operate under two different modes. The first, with low metabolic rate and low AdoMet concentration, serves predominantly to supply the cell with AdoMet, the substrate for various cellular methylation reactions. The second, with high metabolic rate and high AdoMet concentration, provides an avenue for cleavage of excess methionine and can serve as a source of cysteine when its increased synthesis is necessary. The switch that triggers interconversion between the "low" and "high" modes is methionine concentration. Under a certain set of parameters both modes may coexist. This behavior results from the kinetic properties of (i) the two isoenzymes of AdoMet synthetase, MATI and MATIII, that catalyse AdoMet production; one is inhibited by AdoMet, whereas the other is activated by it, and (ii) glycine-*N*-methyltransferase that displays highly cooperative kinetics that is different from that of other AdoMet-dependent methyltransferases. Thus, the model provides an explanation for how different cellular needs are met by regulation of this pathway. The model also correctly identifies a critical role for glycine *N*-methyltransferase in depleting excess methionine in the high mode, thus avoiding the toxicity associated with elevated levels of this essential amino acid.

Introduction

Biological transmethylation reactions have attracted the attention of biochemists since they are important in a variety of cellular processes such as methylation of nucleic acids, proteins, phospholipids, and other molecules. Most of these reactions utilize *S*-adenosylmethionine (AdoMet) as a methyl donor. In the liver, there are two isoforms of methionine-adenosyltransferase (MATI and MATIII), that catalyse the synthesis of AdoMet from methionine and ATP (Mato *et al.*, 1997) (Fig. 1). AdoMet activates MATIII and inhibits MATI (Cabrero *et al.*, 1987; Cabrero & Alemani, 1988). There are many interesting questions that remain to be answered about methionine metabolism. For instance, what is the rationale for the coexistence of the two MAT isoforms in the liver with reciprocal responses to the product, AdoMet? This is the first question that we have sought to answer in this study by developing a mathematical model.

In liver and kidney cells, dietary methionine can be metabolized to cysteine (Womak & Rose,

‡ Authors to whom correspondence should be addressed.

0022–5193/00/120521 + 12 $35.00/0

1941; Finkelstein *et al.*, 1986, 1988). Homocysteine is an intermediate at a key junction in this pathway (Fig. 1). It can either be remethylated back to methionine or it can undergo a condensation reaction with serine to form cystathionine. The latter is the direct precursor of cysteine. This source of cysteine derived from methionine is very important in the maintenance of the intracellular glutathione pool (Beatty & Reed, 1980; Mosharov & Banerjee, 2000). The question thus arises, how do cells control flow through a single methionine metabolite, AdoMet, to provide two different consumers, i.e. AdoMet for methyl transfer reactions and glutathione for redox buffering? This is the second question that we have sought to answer with the model described in this study.

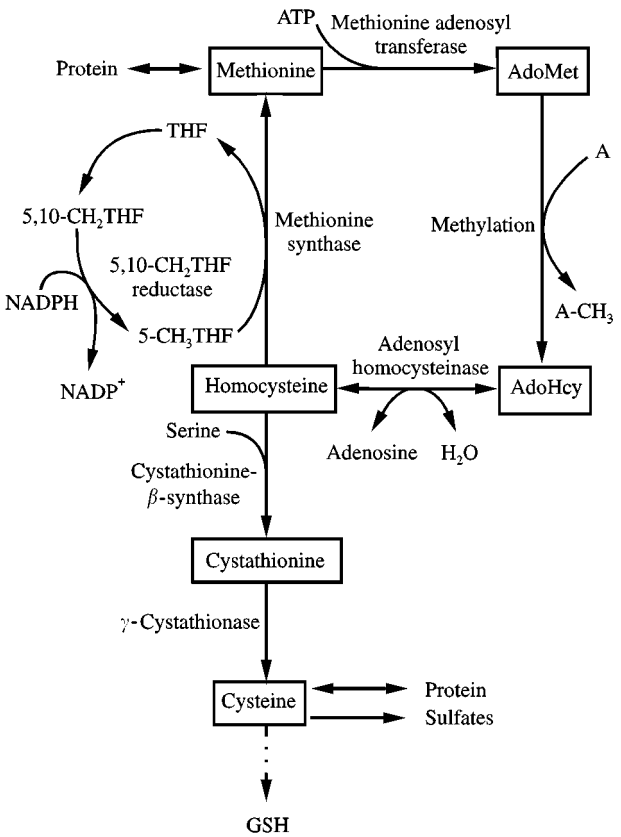

FIG. 1. Pathways for methionine metabolism in mammals. Abbreviations: AdoMet: *S*-adenosyl methionine, AdoHcy: *S*-adenosylhomocysteine, GSH: glutathione, A: methylation substrates, A-CH_3: methylated substrates, THF: tetrahydrofolate, 5,10-CH_2THF: 5,10-methylenetetrahydrofolate, 5-CH_3THF: 5-methyl-tetrahydrofolate, ATP: adenosine triphosphate, $NADP^+$: nicotinamide adenine dinucleotide phosphate, NADPH: nicotinamide adenine dinucleotide phosphate, reduced form.

Elevated levels of homocysteine are a significant and independent risk factor for cardiovascular diseases (reviewed in Refsum *et al.*, 1998) and neural tube defects (Mills *et al.*, 1995). The mechanism by which high homocysteine levels lead to disease is not known. Understanding the regulatory mechanisms operative in homocysteine metabolism is key to understanding aberrations in homocysteine homeostasis. Mathematical modeling has proven to be a very powerful tool for the study of metabolic control (Selkov, 1975; Martinov *et al.*, 1999; Ni & Savageau, 1996). A mathematical model describing the homocysteine metabolic junction could be a useful approach. It could help to integrate the existing experimental data and test their validity, provide a comprehensive overview of the regulation, and suggest testable hypotheses for further experimental work.

In this study, we propose a simple mathematical model for methionine metabolism in liver cells. The model qualitatively fits the experimental data concerning metabolic fluxes and intermediate concentrations under physiological conditions. Analysis of this model shows that methionine metabolism can exist in two steady states. The first corresponds to the physiological values of metabolites, whereas the second occurs at a ten-fold higher AdoMet concentration and leads to a two- to three-fold faster rate of cysteine synthesis from methionine. Under a certain range of parameters, both steady states can coexist. There are at least two factors which can switch the system from the first steady state to the second: an increase in methionine concentration or a change in the ratio of MATI/MATIII activities. Finally, our mathematical model proposes the principles of regulation of methionine metabolism and offers a plausible explanation for why the liver has MATIII and glycine-*N*-methyltransferase, enzymes that are absent in most of the other tissues.

Model Description

The pathway for methionine metabolism is shown in Fig. 1 and starts from the conversion of methionine to AdoMet. The latter is a substrate for many methylases, which represent the second step in this pathway. Generally, pathways

producing a substrate for multiple reactions are regulated by negative feedback, for instance in the synthesis of ATP. Such a strategy permits relative independence for different consumers, i.e. changes in the rate of one of the consumers leads to corresponding change in the rate of substrate production thus preventing significant deviations in substrate concentration. A similar mode of regulation operates in methionine metabolism where AdoMet is a strong inhibitor of the two MAT isoenzymes, the hepatic MATI and the extrahepatic MATII.

The third step in the pathway is catalysed by adenosylhomocysteinase. This step is the hydrolysis of *S*-adenosylhomocysteine, and formation of adenosine and homocysteine (Hcy). The activity of adenosylhomocysteinase in the liver cells is about 900 mmol $h^{-1}l^{-1}$ cells (Hoffman *et al.*, 1979) that is at least ten times higher than the activities of all other enzymes included in the model. The reaction is reversible under intracellular conditions (De la Haba & Cantoni, 1959; Finkelstein, 1990) and proceeds in the direction of hydrolysis only if the products, adenosine and homocysteine are metabolized (Hoffman *et al.*, 1980; Finkelstein, 1990). This implies that this step is not rate limiting and the metabolic flux through it is determined by the rates of AdoHcy formation and adenosine and homocysteine consumption. Usually, reversible reactions catalysed by highly active enzymes are not rate-limiting steps in metabolic cycles. Thus, we assume that the adenosylhomocysteinase reaction is at equilibrium. The pathway bifurcates at the next step since homocysteine has two metabolic destinies. It can be transformed into cystathionine in a reaction catalysed by cystathionine-β-synthase. Cystathionine is subsequently converted to cysteine, and can either be degraded to sulfate or support protein and glutathione biosyntheses. Alternatively, homocysteine can be reconverted to methionine in a reaction catalysed by methionine synthase or betaine homocysteine methyltransferase. Both branches of homocysteine metabolism are rather complex. Both the transmethylation catalysed by methionine synthase and the transsulfuration catalysed by cystathionine β-synthase are irreversible. Thus, we assume that the products of these and downstream reactions do not influence the upstream enzymes. We therefore assume that the rate of homocysteine utilization depends only on its own concentration and on the sum of the activities of the transmethylases and cystathionine-β-synthase.

In liver cells, there are two additional enzymes that contribute to the complexity of the pathway. The first, MATIII, is activated by its product, AdoMet. This positive feedback could potentially lead to instability in the pathway by accelerating ATP-dependent AdoMet synthesis. The second enzyme, glycine-*N*-methyltransferase is also activated by AdoMet (Ogawa & Fujioka, 1982). The product of this reaction is sarcosine that is either oxidized to glycine in a reaction catalysed by sarcosine dehydrogenase or its transported out of cells.

For the development of a simple mathematical model that encompasses the salient features of methionine metabolism, the pathway was simplified as shown in Fig. 2. For this analysis, we have made the following assumptions.

1. The concentrations of methionine, adenosine, ATP, glycine and substrates for methylation (A in Fig. 2) are constant.

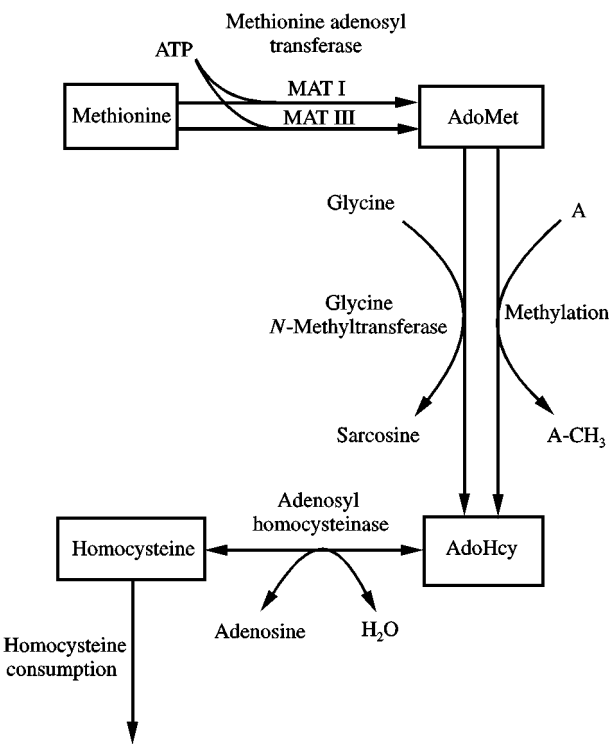

FIG. 2. Simplified pathway of methionine metabolism in liver cells employed for modeling in this study. For abbreviations see Fig. 1.

2. The Michaelis constants are the same for all methylases except glycine-*N*-methyltransferase.
3. Reactions catalysed by cystathionine-β-synthase and methionine synthase are not influenced by their products. The rate of homocysteine utilization depends linearly on homocysteine concentration.
4. The adenosylhomocysteinase reaction is at equilibrium because the activity of adenosylhomocysteinase is much higher than the activity of all other reactions in this pathway (Hoffman *et al.*, 1979):

$$K_{\mathrm{AHC}} = \frac{[\mathrm{Adenosine}]\ [\mathrm{Hcy}]}{[\mathrm{AdoHcy}]}, \qquad (1)$$

$$K_{\mathrm{AHC}} = 0.1\ \mu\mathrm{M}, \qquad [\mathrm{Adenosine}] = 1\ \mu\mathrm{M}.$$

With the above assumptions two differential equations are needed to describe the kinetics of AdoMet and AdoHcy concentrations:

$$\frac{\mathrm{d}[\mathrm{AdoMet}]}{\mathrm{d}t} = (V_{MATI} + V_{MATIII}) - (V_{GNMT} + V_{MET}) = V^{+} - V^{-} \qquad (2)$$

$$\frac{\mathrm{d}[\mathrm{AdoHcy}]}{\mathrm{d}t} = \frac{(V_{GNMT} + V_{MET}) - (V_{MS} + V_{CBS})}{1 + K_{AHC}/[\mathrm{Adenosine}]} = \frac{V^{-} - V_{D}}{1 + K_{AHC}/[\mathrm{Adenosine}]} \qquad (3)$$

where $V^{+} = V_{MATI} + V_{MATIII}$,

$$V^{-} = V_{GNMT} + V_{MET},$$

$$V_{D} = V_{MS} + V_{CBS}.$$

Here V_{MATI}, V_{MATIII}, V_{GNMT}, V_{MET}, V_{MS} and V_{CBS} denote the rates of methionine *S*-adenosyltransferase I, methionine *S*-adenosyltransferase III, glycine *N*-methyltransferase, total rate of methyltransferases except glycine *N*-methyltransferase, methionine synthase and cystathionine-β-synthase, respectively. K_{AHC} denotes the equilibrium constant of the adenosylhomocysteinase reaction. [AdoMet] and [AdoHcy] correspond to intracellular *S*-adenosylmethionine and *S*-adenosylhomocysteine concentrations.

Names, notations and values of the variables, parameters, and reaction rates that have been employed in the model are listed in Tables 1 and 2.

Enzymatic Reaction Rates

Notation

V_{max}^{ENZ}—Activity of enzyme
K_{m}^{ENZ}—Michaelis constant of enzyme
K_{i}^{ENZ}—Inhibition constant of enzyme

METHIONINE *S*-ADENOSYLTRANSFERASE I (MATI)

The enzyme exhibits Michaelis–Menten dependence on methionine concentration and is inhibited by AdoMet. Since the ATP concentration is kept as a constant in our model, there is no

TABLE 1

Metabolite concentrations obtained in the model using physiologically normal values of parameters. Experimental data were taken from Finkelstein & Martin (1986), *Finkelstein* (1990) *and Cabrero et al.* (1988)

		$\mu\mathrm{mol}\,\mathrm{l}^{-1}$ cells	
Designation	Description	Model	Experiment
Met	Methionine	—	45–80
AdoMet	*S*-Adenosyl-methionine	60	20–100
AdoHcy	*S*-Adenosyl-homocysteine	3	1–30
Hcy	Homocysteine	0.3	0.1–1

TABLE 2
Values of the kinetic constants and enzymatic rates employed in the model

Parameter	Description	Value		References
		Model	Experiment	
MATI				
V_{MATI} (μmol hr^{-1} l^{-1} cells)	Rate*	200	270–470	Oka *et al.* (1981), Cabrero *et al.* (1987), Pajares *et al.* (1992)
V_{max}^{MATI} (μmol hr^{-1} l^{-1} cells)	Activity	561	2000–2200	Cabrero *et al.* (1988), Hoffman (1983)
K_m^{MATI} (μM)	Michaelis constant for Met	41	41	Sullivan & Hoffman (1983)
K_i^{MATI} (μM)	Inhibition constant for AdoMet	50	300–400	Cabrero *et al.* (1987), Sullivan & Hoffman (1983), Cabrero *et al.* (1988)
MATIII				
V_{MATIII} (μmol h^{-1} l^{-1} cells)	Rate*	200	100–260	Oka *et al.* (1981), Cabrero *et al.* (1987), Pajares *et al.* (1992)
V_{max}^{MATIII} (mmol hr^{-1} l^{-1} cells)	Activity	22.87	5–9	Cabrero *et al.* (1988), Hoffman (1983)
K_{m1}^{MATIII} (mM)	—	19.1 (at 60 μM AdoMet)	4	Calculated from Sullivan & Hoffman (1983)
K_{m2}^{MATIII} (μM)	—	21.1	200	Calculated from Sullivan & Hoffman (1983)
GNMT				
V_{GNMT} (μmol hr^{-1} l^{-1} cells)	Rate*	0.3	80–300	Mudd *et al.* (1980)
V_{max}^{GNMT} (mmol hr^{-1} l^{-1} cells)	Activity	10.6	6–10	Ogawa & Fujioka (1982), Yeo & Wagner (1992)
K_m^{GNMT} (μM)	Michaelis constant for AdoMet	4500	50	Ogawa & Fujioka (1982)
K_i^{GNMT} (μM)	Inhibition constant for AdoHcy	20	35–80	Kerr & Headley (1974)
Methylation				
V_{MET} (μmol hr^{-1} l^{-1} cells)	Rate*	399.7	—	—
V_{max}^{MET} (μmol hr^{-1} l^{-1} cells)	Activity	4544	—	—
K_{m1}^{MET} (μM)	Michaelis constant for AdoMet	17 (at 3 μM AdoHcy)	4–18	Duerre *et al.* (1977) Hoffmann & Cornatzer (1978)
K_{m2}^{MET}	Michaelis constant for substrate A	$[A]/K_{m2}^{MET} = 0.1$	2.5–6 μM	Duerre *et al.* (1977) Hoffmann & Cornatzer (1978)
Homocysteine consumption ($V_{MS} + V_{CBS}$)				
V_D (μmol hr^{-1} l^{-1} cells)	Rate*	400	400–600	Mudd & Poole (1975)
α_d(hr^{-1})	Rate constant	1333	—	—

TABLE 2 (*Continued*)

Parameter	Description	Value: Model	Value: Experiment	References
Other functions used in the model				
$V^+ = V_{MET} + V_{MATIII}$ (μmol hr^{-1} l^{-1} cells)	Total rate of AdoMet synthesis	400	370–730	Oak *et al.* (1981), Cabrero *et al.* (1987), Pajares *et al.* (1992)
$V^- = V_{MATI} + V_{GNMT}$ (μmol hr^{-1} l^{-1} cells)	Total rate of AdoMet consumption	400	420–780	Mudd *et al.* (1980)

* The rates were calculated employing the metabolite concentrations shown in Table 1.

dependence on this substrate:

$$V_{MATI} = V_{max}^{MATI} \frac{1}{1 + \frac{K_m^{MATI}}{[\text{Met}]}\left(1 + \frac{[\text{AdoMet}]}{K_i^{MATI}}\right)} \tag{4}$$

METHIONINE *S*-ADENOSYLTRANSFERASE III (MATIII)

This enzyme exhibits sigmoidal dependence on methionine concentration (Kunz *et al.*, 1980). AdoMet activates the enzyme by decreasing K_m for methionine. The expression for this reaction rate was fitted to the experimental data described previously (Duerre *et al.*, 1977):

$$V_{MATIII} = V_{max}^{MATIII} \frac{1}{1 + \frac{K_{m1}^{MATIII} K_{m2}^{MATIII}}{[\text{Met}]^2 + [\text{Met}] K_{m2}^{MATIII}}} \tag{5}$$

$$K_{m1}^{MATIII} = \frac{20000}{1 + 5.7\left(\frac{[\text{AdoMet}]}{[\text{AdoMet}] + 600}\right)^2} \tag{6}$$

METHYLATION

Many methyltransferases exist that utilize AdoMet as a methyl donor. All of them are inhibited by AdoHcy and exhibit low Michaelis constant for AdoMet (except glycine *N*-methyltransferase) (Duerre *et al.*, 1977; Hoffman & Cornatzer, 1983). Equation (7) describes the dependence of the rate of methyl transfer reactions (V_{MET}) on AdoMet, AdoHcy, and substrates for methylation (A) concentrations:

$$V_{MET} = V_{max}^{MET} \frac{1}{1 + \frac{K_{m1}^{MET}}{[\text{AdoMet}]} + \frac{K_{m2}^{MET}}{[\text{A}]} + \frac{K_{m2}^{MET}}{[\text{A}]} \frac{K_{m1}^{MET}}{[\text{AdoMet}]}} \tag{7}$$

$$K_{m1}^{MET} = 10\left(1 + \frac{[\text{AdoHcy}]}{4}\right) \tag{8}$$

GLYCINE *N*-METHYLTRANSFERASE (GNMT)

Glycine *N*-methyltransferase exhibits kinetic parameters that are quite different from other methyl transfer reactions, i.e. sigmoidal dependence on AdoMet. The enzyme is also inhibited by AdoHcy, which is a feature common to most methyltransferases:

$$V_{GNMT} = V_{max}^{GNMT} \frac{1}{1 + \left(\frac{K_m^{GNMT}}{[\text{AdoMet}]}\right)^{2.3}} \cdot \frac{1}{1 + \frac{[\text{AdoHcy}]}{K_i^{GNMT}}}. \tag{9}$$

HOMOCYSTEINE CONSUMPTION (METHIONINE SYNTHASE AND CYSTATHIONINE β-SYNTHASE)

The description of homocysteine (Hcy) cleavage is simplified in the present model [eqn (10)]. The rate of Hcy degradation (V_D) depends linearly on substrate concentration and is not

affected by AdoMet. This rate also does not depend on methyltetrahydrofolate and serine levels, the second substrates for methionine synthase and cystathionine-β-synthase, respectively (Fig. 1)

$$V_D = \alpha_d \quad [\text{Hcy}],$$

$$[\text{Hcy}] = [\text{AdoHcy}]\frac{K_{AHC}}{[\text{Adenosine}]}. \qquad (10)$$

Results

Since the model describes the kinetic behavior with two variables, a phase diagram is the most convenient way to analyse the results. The phase diagram for eqns (2) and (3) is shown in Fig. 3. The solid lines are the null-clines $\mathrm{d}[\text{AdoMet}]/\mathrm{d}t = 0$ and $\mathrm{d}[\text{AdoHcy}]/\mathrm{d}t = 0$. Phase trajectories are shown by dashed lines. They are parallel to the axis of AdoHcy until they meet the null-cline $\mathrm{d}[\text{AdoHcy}]/\mathrm{d}t = 0$. The main conclusion from these data is that AdoHcy is the fast variable (i.e. time constant is in the seconds time regime), whereas AdoMet is the slow variable (i.e. time constant is about tens of minutes). Thus, following initiation of the reactions, AdoHcy quickly reaches its quasi-steady state within seconds (dashed lines), whereas AdoMet changes more slowly in tens of minutes according to eqns (11) and (12).

$$\frac{\mathrm{d}(\text{AdoMet})}{\mathrm{d}t} = V^+ - V^- \qquad (11)$$

$$\frac{\mathrm{d}[\text{AdoHcy}]}{\mathrm{d}t} = 0 \qquad (12)$$

According to eqn (11) the interplay between the overall rates of AdoMet production (V^+) and consumption (V^-) and their dependence on methionine and AdoMet concentrations determines the kinetic behavior of the whole metabolic system. Figure 4 shows the dependence of V^+ (solid lines) and V^- (dashed line) on the concentration of AdoMet. A steady state can be achieved only if the rate of AdoMet production is equal to the rate of its cleavage. The intersections of V^+ and V^- yield the concentrations of AdoMet at this steady state. As follows from eqns (4)–(9), changes in methionine concentration affect V^+ only without altering V^-. Curves 1–3 in Fig. 4 represent V^+ under three different concentrations of methionine.

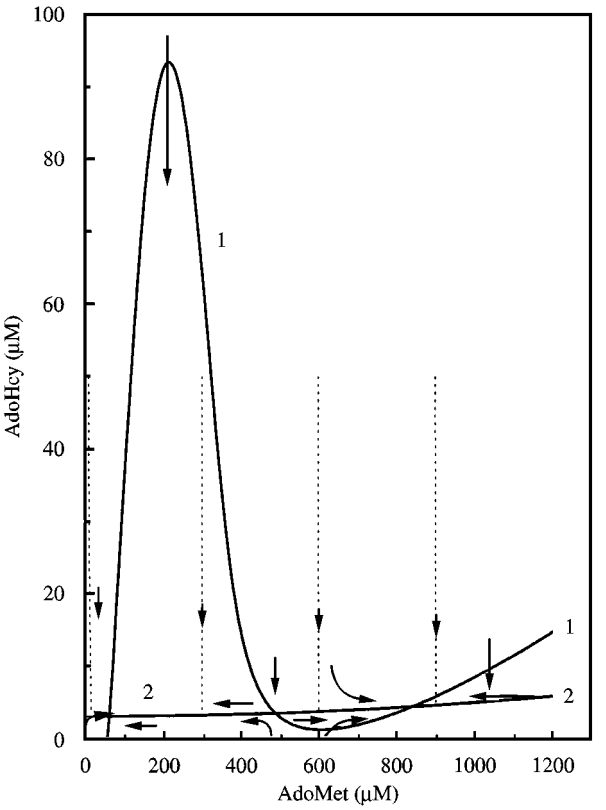

FIG. 3. Phase diagram for eqns (2) and (3) at physiological values of parameters. Phase trajectories are shown as dashed lines. Arrows indicate the direction of phase trajectories. 1—null-cline ($\mathrm{d}[\text{AdoMet}]/\mathrm{d}t$) = 0; 2—null-cline ($\mathrm{d}[\text{AdoHcy}]/\mathrm{d}t$) = 0.

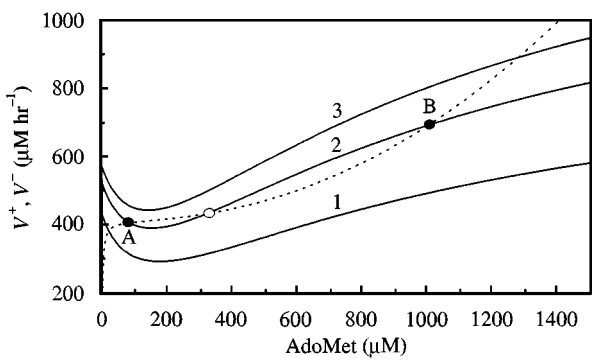

FIG. 4. The total rates of AdoMet production (V^+, ——) and consumption (V^-, - - -) as a function of AdoMet concentration. V^+ is shown at three different methionine concentrations: 1 is at 42.5 μM, 2 is at 52.5 μM, and 3 is at 57.5 μM. (●) indicates stable steady states with low (A) and high (B) AdoMet concentrations; (○) indicates an unstable steady state.

One steady state exists if methionine concentration is lower than 50 μM (Fig. 4, curve 1). This steady state is stable. This represents the "low" mode of methionine metabolism, at a rather low AdoMet concentration and low metabolic rate. If the concentration of methionine is higher than 54 μM (Fig. 4, curve 3), the system achieves a second stable steady state in which the concentration of AdoMet and the metabolic flux are much higher. This represents the "high" mode for methionine metabolism.

When the methionine concentration lies within a narrow range between 50 and 54 μM, unusual kinetic behavior in this pathway is predicted (Fig. 4, curve 2). There are three points of intersection, i.e. three steady states. The middle point is a saddle and is unstable. The other two represent the steady states at low and high concentrations of AdoMet. These data imply that for the same concentration of methionine, the rates of AdoMet degradation and synthesis can either be low (Fig. 4, curve 2, steady state A) or high (Fig. 4, curve 2, steady state B). Thus, the system is bi-stable.

How would the system behave, for instance, if the methionine concentration changed 20% from 45 to 55 μM? Figure 5(a) shows a time course for incremental changes in methionine concentration from 45 to 52 μM, then up to 55 μM followed by a reversal. Figure 5(b) shows the kinetics for the corresponding changes in AdoMet concentration. If the methionine concentration was initially low and increased to 52 μM, then the AdoMet concentration would increase slightly [Fig. 5(b)]. In contrast, if the methionine concentration decreased from 55 to 52 μM the AdoMet concentration would also decrease but would stabilize at a much higher level than before, since the system remains in the "high" steady state. Thus, for the same concentration of methionine, the AdoMet level can be either low or high [see concentrations at 15 min vs. 75 min in Fig. 5(b)]. The kinetics for changes in AdoHcy concentrations are very similar to AdoMet [Fig. 5(b)].

Figure 6 shows the steady-state concentration of AdoMet as a function of methionine concentration. The system stays in the "low" steady state if methionine is below 54 μM. In this range of methionine concentrations, AdoMet levels increase slowly with increasing methionine but do not exceed 100 μM. When the methionine concentration exceeds 54 μM the concentration of AdoMet increases sharply rising ~10-fold (Fig. 6, right arrow). The most notable feature in this behavior is the jump in the AdoMet concentration. Thus, the change in substrate concentration in this range triggers a switch in this metabolic pathway from one mode to another. In contrast, a decrease in methionine concentration from a "high" level to 54 μM is not accompanied by a corresponding jump to the "low" steady state. Instead, this transition occurs only when the methionine concentration decreases to 50 μM (Fig. 6, left arrow). This represents a typical hysteresis curve. With increasing methionine concentration there is one dependence and with decreasing methionine concentration there is another.

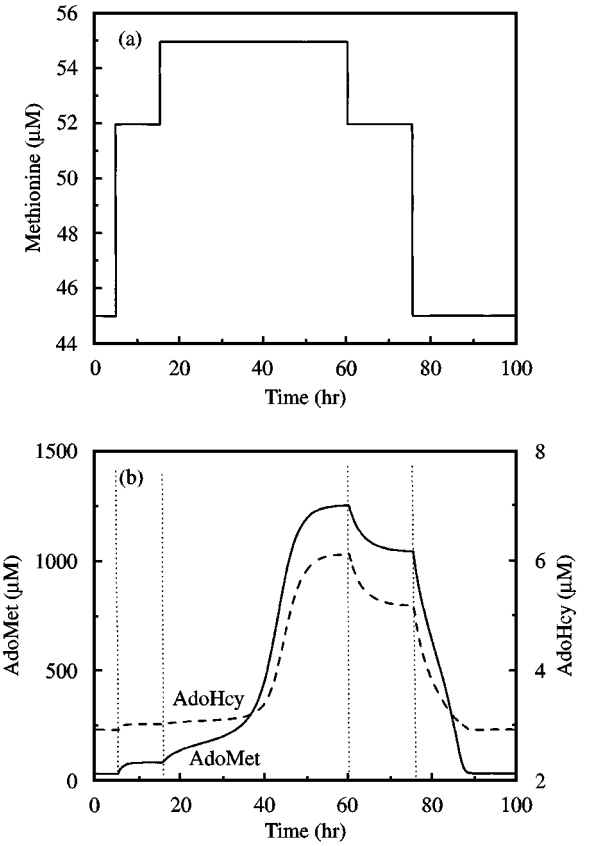

FIG. 5. Kinetics of AdoMet and AdoHcy concentration changes triggered by a change in methionine concentration: (a) time course for changes in methionine concentration, (b) kinetics of AdoMet and AdoHcy concentration changes corresponding to changes in methionine concentration shown in (a).

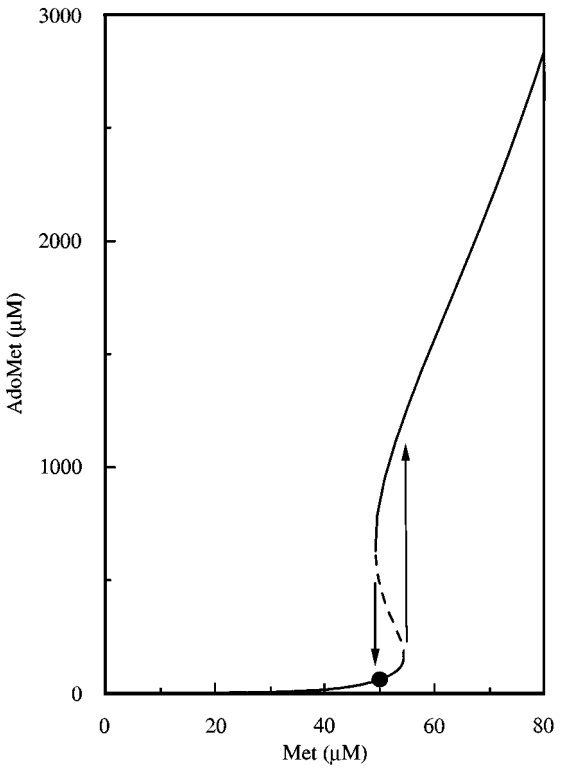

FIG. 6. Steady-state concentration of AdoMet as a function of methionine concentration. (- - -) indicates unstable stationary points, (→) shows points at which a jump in the AdoMet concentration is observed and (●) indicates the physiological state in the "low" mode.

The dependence of the rates of individual enzymes as a function of methionine concentration is shown in Fig. 7. At low methionine concentrations (i.e. at the "low" steady state) the total rate of AdoMet synthesis [Fig. 7(a), curve 1] is largely determined by MATI [Fig. 7(a), curve 2] while the flux through MATIII is small [Fig. 7(a), curve 3]. The rate of AdoMet synthesis is balanced by its consumption as determined by the total methylase activity [Fig. 7(b), curves 1 and 2, respectively]. Under these conditions, the flux through glycine *N*-methyltransferase is negligible [Fig. 7(b), curve 3]. When the system is in the "high" steady state, the rate of AdoMet production is governed mostly by MATIII [Fig. 7(a), curve 3]. Under these conditions, the AdoMet production exceeds its consumption by the methyltransferases and could render the system unstable [Fig. 7(b), curve 2]. However, this is averted by the compensatory increase in the flux of AdoMet through glycine-*N*-methyltransferase.

The critical methionine concentration (i.e. at the bifurcation point), where metabolism is triggered to a "high" mode depends strongly on the kinetic parameters of the isoforms MATI and MATIII. For example, Fig. 8 shows the AdoMet concentration as a function of methionine

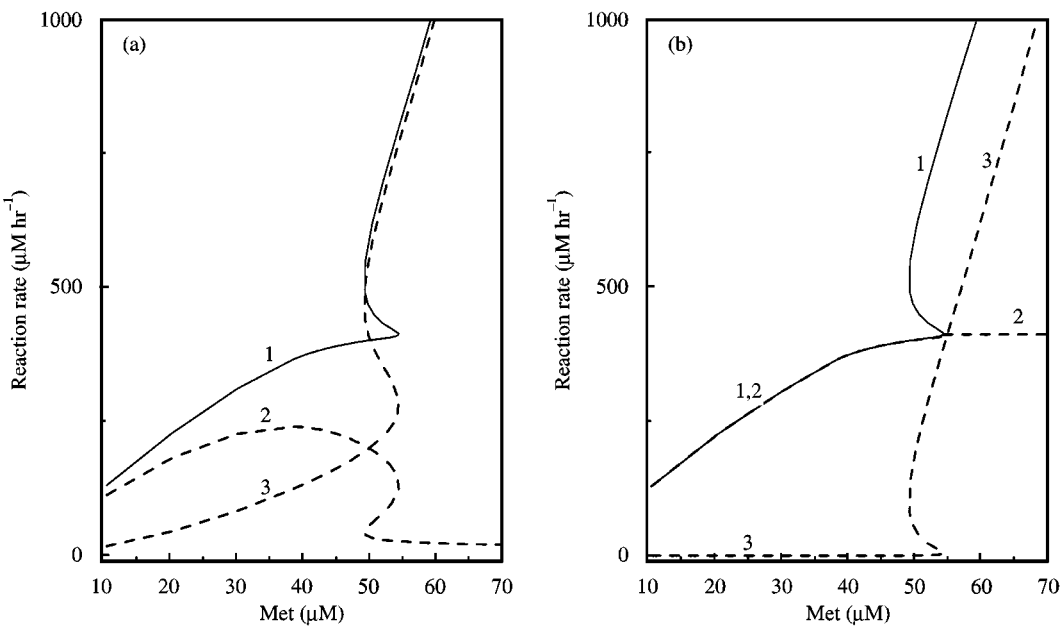

FIG. 7. The dependence of steady-state rates of AdoMet production and AdoMet consumption on methionine concentration. (a) Dependence of AdoMet production (1) and the activities of MATI (2) and MATIII (3) on methionine concentration. (b) Kinetics of AdoMet consumption (1) and of its components, total methylation (2) and glycine-*N*-methyltransferase (3) on methionine concentration.

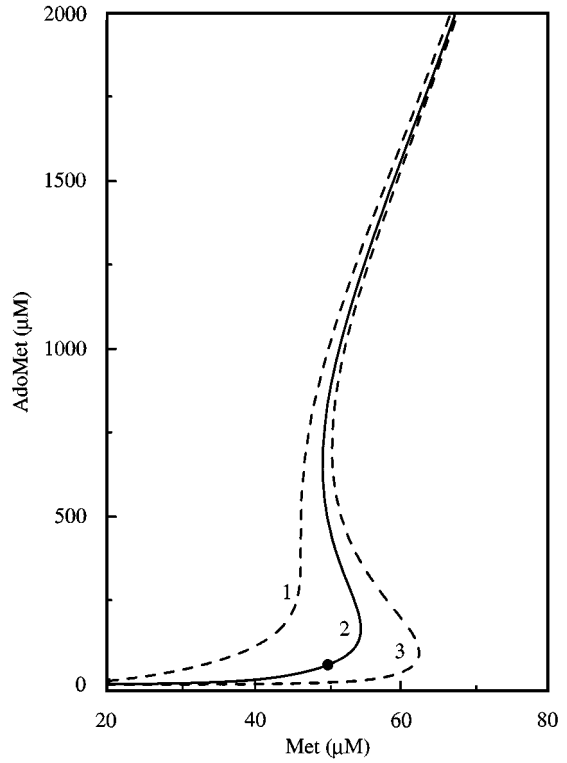

FIG. 8. The steady-state concentration of AdoMet as a function of methionine concentration at three different values of MATI activity (% of normal value) where 1 is at 200%, 2 is 100%, and 3 is at 50%. (●) indicates the physiological state in the "low" mode.

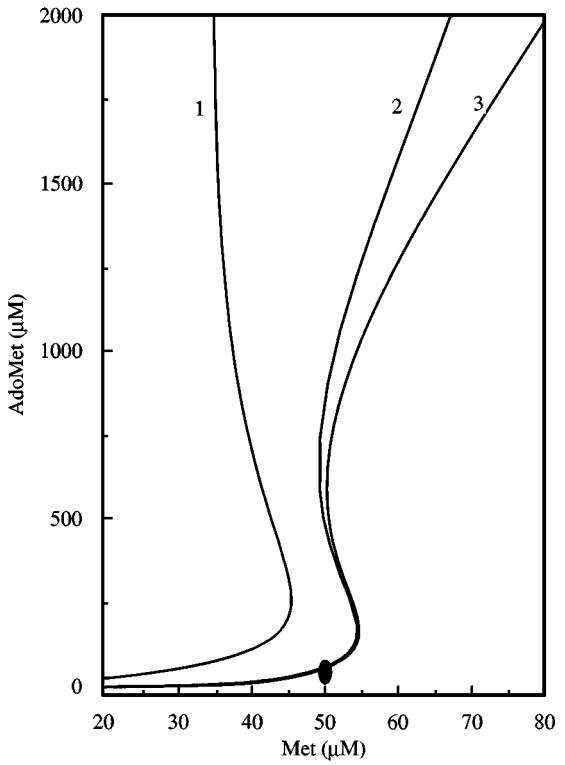

FIG. 9. The steady-state concentration of AdoMet as a function of methionine concentration at three different rates of homocysteine degradation: 1—$\alpha_d = 0.01\,\alpha_{d,0}$; 2—$\alpha_d = \alpha_{d0}$; 3—$\alpha_d = 100\,\alpha_{d0}$. The rate of AdoHcy degradation is determined by the parameter α_d: $V_D = \alpha_d \cdot \mathrm{Hcy}$. (●) indicates the physiological state in the "low" mode.

concentration for three different MATI activities. The curve shifts from the right to the left, when the enzyme activity is increased which triggers metabolism to the "high" mode at a fixed methionine concentration. An analogous shift can be achieved by decreasing the rate of AdoHcy degradation as shown in Fig. 9. However, the influence of α_d [see eqn (10)] is much weaker than that of MATI. The rate of AdoHcy degradation would have to be decreased 100-fold to switch the system to the "high" mode.

Discussion

The methionine metabolic pathway plays at least two major roles in liver cells: (1) production of methyl groups to support a variety of methyl transfer reactions and (2) the conversion of methionine to cysteine. According to the model developed in this study, the pathway can operate in two modes to fulfil these physiological functions. The first mode represents a low metabolic rate, occurs at low methionine concentrations, results in low AdoMet concentrations and serves to maintain the cells supply of AdoMet which is the major substrate for methylation. The second mode represents a high metabolic rate, occurs at high methionine concentrations, results in high AdoMet concentrations, serves to enhance cysteine synthesis if it is necessary, and provides a degradative pathway for excess methionine.

In the "low" mode (Fig. 7), the system functions predominantly to produce the methylation substrate, AdoMet. The regulatory mechanism under these conditions is the same as that seen in many metabolic pathways where the product, AdoMet in this instance, inhibits its own production. This strategy permits relatively independent regulation of AdoMet levels by its various consumers, the methylases. Thus, changes in the rate of one of the consumers lead to corresponding changes in the rate of substrate production thus preventing significant deviations in steady-state

AdoMet levels. In this steady state, both the AdoMet concentration and the flux through this metabolic pathway are low. In the "high" mode (Fig. 7), AdoMet levels and the rates of its synthesis and degradation are greatly increased. The flow of methionine through AdoMet under these conditions greatly exceeds the demand of the methylases [Fig. 7(b)]. Under these conditions, the AdoMet levels are critically dependent on the methionine concentration (Fig. 5). The behavior predicted by this model is supported by experimental studies in which the concentrations of AdoMet and AdoHcy and the activity of glycine *N*-methyltransferase were found to increase during adaptation to excess dietary methionine (Finkelstein & Martin, 1986; London *et al*., 1987).

The kinetic behavior of the system described above is determined mostly by the equilibrium between production and consumption of AdoMet. The synthesis of AdoMet is controlled by the activities of the two isozymes, MATI and MATIII, which display differential responses to their product, AdoMet. MATIII is activated by its product whereas MATI is inhibited. This kinetic property of MATIII is the reason that the dual steady states are possible in this pathway (Fig. 4). However, this feature also renders the system unstable since high AdoMet concentrations accelerate the rate of its synthesis and therefore of ATP consumption. Glycine-*N*-methyltransferase counters this instability under conditions of increased AdoMet synthesis since AdoMet also activates this enzyme and the extent of this activation is higher than for MATIII (Fig. 4). This unlimited increase in AdoMet concentration is therefore averted and a second steady state is stabilized. From the biochemical point of view, glycine-*N*-methyltransferase may be viewed as performing a futile task since it converts glycine to sarcosine which is not a useful metabolite. Sarcosine can either be converted back to glycine by sarcosine dehydrogenase or transported out of the cell. Our mathematical model suggests a rationale for the existence of this enzyme. It endows the cell with the ability to greatly increase the flow from methionine to homocysteine under conditions of excess methionine. Thus, as has been noted previously, the liver which alone contains MATIII, may be unique in its ability to adapt quickly to high levels of methionine (Finkelstein, 1990).

Our model also describes the transitions between the two steady states in methionine metabolism. The first factor affecting this system is methionine. At physiological concentrations of methionine (Finkelstein & Martin, 1986), the metabolic system remains in the "low" steady state. Increase in methionine levels triggers the transition to the "high" steady state. Only a 10% increase in methionine concentrations leads to a two-fold higher rate in its conversion to homocysteine and a ten-fold increase in the AdoMet levels. The concentration of methionine thus serves as a key toggle switch between the two modes. This is consistent with the observation that the concentration of methionine in rat liver is relatively insensitive to changes in dietary protein intake, in contrast to the hepatic concentrations of AdoMet and AdoHcy which increase with augmented dietary protein (Finkelstein *et al*., 1982).

The existence of two states in a system is a common phenomenon in biology. The switching mechanism in many cases is dependent on a signal transduction pathway and involves covalent modification, viz. phosphorylation/dephosphorylation reactions. Analysis of the methionine metabolic pathway reveals the existence of a different type of metabolic switch, one which is accomplished by changes in the concentration of the substrate.

This work was supported in part by a grant from the National Institutes of Health (HL58984). R.B. is an Established Investigator of the American Heart Association.

REFERENCES

Beatty, P. W. & Reed, D. J. (1980). Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. *Arch. Biochem. Biophys.* **204,** 80–87.

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

Cabrero, C. & Alemany, S. (1988). Conversion of rat liver *S*-adenosyl-L-methionine synthetase from high-Mr form to low-Mr form by LiBr. *Biochim. Biophys. Acta* **952,** 277–281.

Cabrero, C., Duce, A. M., Oritz, P., Alemany, A. & Mato, J. M. (1988). Specific loss of the high-molecular-weight form of *S*-adenosyl-L-methionine synthetase in human liver cirrhosis. *Hepatology* **8,** 1530–1534.

De la Haba, G. & Cantoni, G. L. (1959). The enzymatic synthesis of *S*-adenosyl-L-homocysteine from adenosine and homocysteine. *J. Biol. Chem.* **234,** 603–608.

Duerre, J. A., Wallwork, J. C., Quick, D. P. & Ford, K. M. (1977). *In vitro* studies on the methylation of histones in rat brain nuclei. *J. Biol. Chem.* **252,** 6981–6985.

Finkelstein, J. D., Kyle, W. E., Harris, B. J. & Martin, J. J. (1982). Methionine metabolism in mammals: concentration of metabolites in rat tissues. *J. Nutr.* **112,** 1011–1018.

Finkelstein, J. D. & Martin, J. J. (1986). Methionine metabolism in mammals. Adaptation to methionine excess. *J. Biol. Chem.* **261,** 1582–1587.

Finkelstein, J. D., Martin, J. J. & Harris, B. J. (1986). Effect of dietary cystine on methionine metabolism in rat liver. *J. Nutr.* **116,** 985–990.

Finkelstein, J. D., Martin, J. J. & Harris, B. J. (1988). Methionine metabolism in mammals. The methionine-sparing effect of cystine. *J. Biol. Chem.* **263,** 11 750–11 754.

Finkelstein, J. D. (1990). Methionine metabolism in mammals. *J. Nutr. Biochem.* **1,** 228–237.

Hoffmann, D. R. & Cornatzer, W. E. (1978). Transmethylation reactions in brain and liver. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1833–1838.

Hoffmann, D. R., Cornatzer, W. E. & Duerre, J. A. (1979). Relationship between tissue levels of A-adenosylmethionine, S-adenosylhomocysteine, and transmethylation reactions. *Can. J. Biochem.* **57,** 56–65.

Hoffmann, D. R., Marion, D. W., Cornatzer, W. E. & Duerre, J. A. (1980). S-adenosylmethionine and S-adenosylhomocysteine metabolism in isolated rat liver. *J. Biol. Chem.* **255,** 10 822–10 827.

Hoffmann, D. R. & Cornatzer, W. E. (1983). Transmethylation reactions in brain and liver. *Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1833–1838.

Hoffmann, J. L. (1983). Fractionation of methionine adenosyltransferase isozymes (rat liver). *Methods Enzymol.* **94,** 223–228.

Kerr, S. J. & Headly, J. E. (1974). Modulation of tRNA methyltransferase activity by competing enzyme systems. *Adv. Enzyme Regul.* **12,** 4248–4252.

Kunz, G. L., Hoffman, J. L., Cia, C.-S. & Stremel, B. (1980). Separation of rat liver methionine adenosyltransferase isozymes by hydrophobic chromatography. *Arch. Biochem. Biophys.* **202,** 565–572.

London, R. E., Gabel, S. A. & Funk, A. (1987). Metabolism of excess methionine in the liver of intact rat: an *in vivo* 2H NMR study. *Biochemistry* **26,** 7166–7172.

Martinov, M. V., Vitvitsky, V. M. & Ataullakhanov, F. I. (1999). Volume stabilization in human erythrocytes: combined effect of $Ca(^{2+})$-dependent potassium channels and adenylate metabolism. *Biophys. Chem.* **80,** 199–215.

Mato, J. M., Alvarez, L., Ortiz, P. & Pajares, M. A. (1997). S-adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol. Theor.* **73,** 265–280.

Mills, J. L., McPartlin, J. M., Kirke, P. N., Lee, Y. J., Conle, M. R. & Weir, D. G. (1995). Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* **345,** 149–151.

Mosharov, E. & Banerjee, R. (2000). Redox sensitivity of glutathione synthesis from homocysteine, *submitted for publication.*

Mudd, S. H. & Poole, J. R. (1975). Labile methyl balances for normal humans on various dietary regimens. *Metabolism* **24,** 721–735.

Mudd, S. H., Ebert, M. H. & Scriver, C. R. (1980). Labile methyl group balances in the human: the role of sarcosine. *Metabolism* **29,** 707–720.

Ni, T. C. & Savageau, M. A. (1996). Application of biochemical systems theory to metabolism in human red blood cells. Signal propagation and accuracy of representation. *J. Biol. Chem.* **271,** 7927–7941.

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

Oka, G., Teraoka, H. & Tsukada, K. (1981). Multiple species of mammalian S-adenosylmethionine synthetase. Partial purification and characterization. *Biochemistry* **20,** 934–920.

Pajares, M. A., Duran, C., Corrales, F., Pliego, M. & Mato, J. (1992). Modulation of a rat liver S-adenosylmethionine synthetase activity by glutathione. *J. Biol. Chem.* **267,** 17 598–17 605.

Refsum, H., Ueland, P. M., Nygard, O. & Vollset, S. E. (1998). Homocysteine and cardiovascular disease. *Annu. Rev. Med.* **49,** 31–62.

Sel'kov, E. E. (1975). Stabilization of energy charge, oscillations and multiplicity of stationary states as a result of purely stoichiometric regulation. *Eur. J. Biochem.* **59,** 151–157.

Sullivan, D. M. & Hoffman, J. L. (1983). Fractionation and kinetic properties of rat liver and kidney methionine adenosyltransferase isozymes. *Biochemistry* **22,** 1636–1641.

Womak, M. & Rose, W. C. (1941). The partial replacement of dietary methionine by cysteine for purposes of growth. *J. Biol. Chem.* **141,** 375–379.

Yeo, E.-J. & Wagner, C. (1992). Purification and properties of pancreatic glycine *N*-methyltransferase. *J. Biol. Chem.* **267,** 24 669–24 674.