Review

Methionine metabolism in mammals

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Introduction

The availability of methionine is necessary for the normal growth and development of mammals. This essential role derives from the participation of this amino acid, or its derivatives, in several fundamental biologic processes, including protein synthesis; the numerous S-adenosylmethionine (AdoMet)-dependent transmethylation reactions; the formation of the polyamines, spermidine and spermine; the synthesis of cystathionine, cysteine, and other metabolites of the transsulfuration pathway; and the provision of homocysteine, which is necessary for both the metabolism of intracellular folates and the catabolism of choline.

During the last two decades, work in several laboratories has defined the potential pathways for methionine metabolism. Concurrently, other studies have established the existence of regulatory processes and have provided some insight into the individual mechanisms which may contribute to this metabolic modulation. In this review, I will summarize the current state of our knowledge.

The metabolic pathway

In all likelihood, the transmethylation-transsulfuration sequence (*Figure 1*) is the major pathway for methionine metabolism in mammalian liver. The pathway may be incomplete in other tissues (see below).

Methionine adenosyltransferase (EC 2.5.1.6; *Figure 1*, reaction 1), which catalyzes the synthesis of S-adenosylmethionine, is the first reaction in the pathway. There are at least three isoenzymic forms of the mammalian enzyme.[1-5] The liver-specific forms have been designated as the "high K_m" isoenzyme (β form, MAT-III) and the "intermediate K_m" isoenzyme (α form, MAT-I). The former appears to be derived from the latter by posttranslational modification.[6] Dimethylsulfoxide is a potent activator of MAT-III and converts the kinetics from a nonlinear, sigmoidal type to a classic, hyperbolic form. Under these conditions, the apparent K_m for methionine declines from 1.3 mM to 33 μM.[7] Extrahepatic tissues and fetal liver contain the "low K_m" isoenzyme (γ form, MAT-II). S-adenosylmethionine activates MAT-III but inhibits both MAT-I and MAT-II. Consequently, liver, which alone contains MAT-III, may be unique in the ability to adapt immediately to high levels of methionine.[8] Adaptation in other tissues may require the synthesis of additional enzyme.

In mammals, the tissue content of S-adenosylmethionine is a function of both the specific organ and the availability of methionine. Concentrations in the livers of rats fed a standard chow range from 50 to 100 nmol/g, but may increase to 650 nmol/g when the diet contains excessive methionine.[9] Several studies indicate the existence of at least two intracellular pools of adenosylmethionine, a rapidly exchanging pool in the cytosol and a less active pool in the mitochondria.[10] It is likely that adenosylmethionine is retained within the cell of its origin[11] and that, at physiologic concentrations, uptake from the extracellular space is limited to binding to membrane phosphatidylethanolamine methyltransferases.[12,13] In contrast, cellular uptake may occur in the presence of high, pharmacologic concentrations of the compound.[14]

In his initial, classic description of S-adenosylmethionine, Cantoni suggested that the compound should be able to transfer any of the moieties attached to the sulfonium center.[15] The transfer of adenosine remains to be demonstrated; however, both the methyl and aliphatic groups are "donated" in essential biologic reactions.

Pegg has summarized the properties and regulation of S-adenosylmethionine decarboxylase (EC 4.1.1.50; *Figure 1*, reaction 13).[16] This is the rate-limiting reaction in the synthesis of polyamines. Decarboxylated adenosylmethionine can transfer its propylamine moiety to either putrescine or spermidine to yield either spermidine or spermine. Despite the biologic importance, the synthesis of polyamines may use only a small fraction of the available S-adenosylmethionine.[17] Furthermore, the recent work of Backlund et al. establishes the presence of a cycle which allows the resynthesis of methionine from 5′-methylthioadeno-

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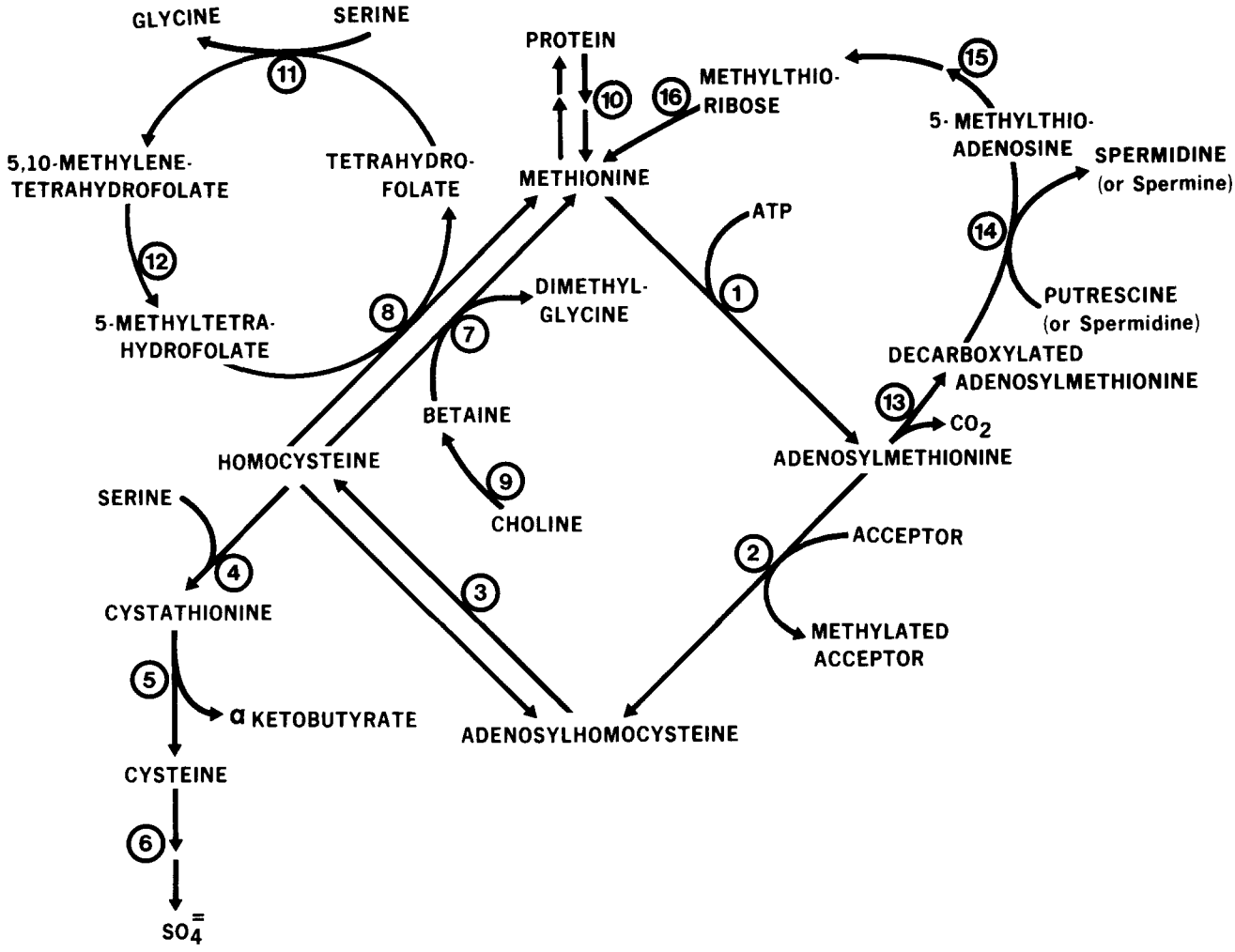

Figure 1 Metabolic pathways for methionine metabolism. The numbers represent the following enzymes or sequences: 1, methionine adenosyltransferase; 2, S-adenosylmethionine-dependent transmethylation; 3, adenosylhomocysteinase; 4, cystathionine-β-synthase; 5, cystathionase; 6, multiple sequences resulting in sulfate formation; 7, betaine-homocysteine methyltransferase; 8, methylfolate-homocysteine methyltransferase; 9, choline dehydrogenase plus betaine aldehyde dehydrogenase; 10, equilibrium between free methionine and protein methionine; 11, serine hydroxymethylase; 12, methylenetetrahydrofolate reductase; 13, adenosylmethionine decarboxylase; 14, spermidine and spermine synthases; 15, methylthioadenosine phosphorylase; and 16, conversion of methylthiorbose-1-phosphate to methionine.

sine via methylthioribose-1-phosphate and 2-keto-4-methylthiobutyrate (*Figure 1*, reactions 15 and 16).[18,19] As a result, there may be no net loss of methionine even during augmented polyamine synthesis.

S-adenosylmethionine is the primary methyl group donor in virtually all mammalian systems. Despite the large number of specific methyltransferases, most share the common property of inhibition by the product, S-adenosylhomocysteine. Consequently, changes in the ratio of S-adenosylmethionine to S-adenosylhomocysteine may regulate many physiologically significant reactions.[20,21] For this reason, the removal of S-adenosylhomocysteine is essential. However, the adenosylhomocysteinase reaction (EC 3.3.1.1; *Figure 1*, reaction 3) is reversible. Indeed, the thermodynamics favor synthesis of S-adenosylhomocysteine.[22] In vivo, the reaction proceeds in the direction of hydrolysis only if the products, adenosine and homocysteine, are metabolized. The administration of either homocysteine or adenosine[11] and the inhibition of adenosine deaminase result in the accumulation of S-adenosylhomocysteine.[23] Intracellular S-adenosylhomocysteine exists both in the free state and in a protein-bound form.[24] The latter appears to involve specific, saturable sites, largely in the microsomal fraction. With the accumulation of high concentrations of intracellular S-adenosylhomocysteine, export to the extracellular compartment occurs.[11,25] This may provide an additional mechanism for detoxification since the metabolite is not taken up by cells and administered S-adenosylhomocysteine is excreted as the keto-derivative in the urine.[26]

Three enzymes use homocysteine. Both the betaine-homocysteine methyltransferase (EC 2.1.1.5; *Figure 1*, reaction 7) and the 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13; *Figure 1*, reaction 8) reactions yield methionine. By these means, the homocysteine moiety is conserved and the

Table 1 Enzymes of methionine metabolism in rat tissues[a]

	MAT	AHCy Enz	Cyst. Syn.	BHMT	MFMT	C-ase	CH_2-THF Red.
Liver	100	100	100	100	100	100	100
Small intestine	6	6	17	0	0[b]	8	34
Pancreas	24	134	90	<1	128	52	176
Adipose	4	8	21	<1	92	2	172
Brain	10	21	20	0	118	<1	30
Kidney	29	37	45	1	405	37	415
Spleen	7	5	2	<1	77	<1	37
Adrenal	12	33	<1	1	256	1	24
Lung	5	8	<1	0	33	1	22
Testes	10	15	<1	<1	190	<1	218
Heart	3	3	<1	0	108	0	49

Abbreviations: MAT, methionine adenosyltransferase; AHCy Enz, adenosylhomocysteinase; Cyst. Syn., cystathionine synthase; BHMT, betaine-homocysteine methyltransferase; MFMT, methylfolate-homocysteine methyltransferase; C-ase, cystathionase; CH_2-THF Red., methylenetetrahydrofolate reductase.

[a] Previously published results[33-36] for the specific activities of the enzymes are expressed as a percentage of the value for liver. Adult rats were fed a standard laboratory diet.

[b] Enzyme activity detected by Keating et al.[37]

methionine cycle is completed. The reactions are equally important for the metabolism of the cosubstrates. Betaine-homocysteine methyltransferase is the only means for the degradation of betaine which is an obligatory intermediate in the catabolism of choline. Similarly, the methylfolate methyltransferase is the only enzyme which uses 5-methyltetrahydrofolate. Impairment of this reaction, for example, by the absence of its coenzyme, methylcobalamin, may limit the capacity to regenerate tetrahydrofolate. The resultant functional deficiency is central to the "folate trap" hypothesis which attempts to explain why deficiencies of folate and vitamin B_{12} may have similar pathologic results.[27]

Cystathionine-β-synthase (EC 4.2.1.22; *Figure 1*, reaction 4) requires pyridoxal phosphate as a cofactor.[28,29] Under most conditions, the reaction is irreversible in vivo and the homocysteine moiety is committed to the transsulfuration pathway. Conversely, the irreversibility of the cystathionine synthase reaction explains the inability of cysteine to serve as a precursor of methionine. Cystathionase (cystathionine-γ-lyase; EC 4.4.1.1.; *Figure 1*, reaction 5), another pyridoxal phosphate enzyme, catalyzes the last reaction in the transsulfuration sequence—the formation of cysteine and α-ketobutyrate. In their initial description, Matsuo and Greenberg identified both the reversibility of the reaction and the wide range of potential substrates.[30,31] Cysteine is the precursor of glutathione, taurine, and other metabolites of biologic significance. These considerations are beyond the scope of this review.

Despite the apparent complexity, *Figure 1* contains several simplifications. Choline can be synthesized by the methylation of phosphatidylethanolamine. Consequently, there is another methyl group cycle comprised of methionine-adenosylmethionine-choline-betaine-methionine. Similarly, *Figure 1* does not indicate that the sequential demethylation of dimethylglycine and sarcosine involves folate enzymes. Both dimethylglycine dehydrogenase (EC 1.5.99.2) and sarcosine dehydrogenase (EC 1.5.99.1) are flavoproteins which tightly bind tetrahydrofolate pentaglutamate. The latter is converted to the methylenetetrahydrofolate form during the reactions, which therefore represent an intersection between choline and folate metabolism.[32] Finally, the potential significance of methionine transamination, of the direct cleavage of S-adenosylmethionine, and of the oxidation of both homocysteine and cystathionine will be discussed in a later section.

The complete pathway shown in *Figure 1* does not occur in all mammalian cells. A survey of rat tissues (*Table 1*) demonstrated that all contained methionine adenosyltransferase, adenosylhomocysteinase, methylenetetrahydrofolate reductase, and methyltetrahydrofolate-homocysteine methyltransferase (Keating et al. have explained the initial failure to detect this enzyme in intestinal mucosa.[37]) Only liver contained betaine-homocysteine methyltransferase, and five organs lacked cystathionine synthase. Thus, all tissues possess the capacity to synthesize adenosylmethionine, to degrade adenosylhomocysteine derived from the many ubiquitous transmethylation reactions, and to metabolize homocysteine by remethylation if not by cystathionine synthesis. Cysteine may be an essential nutrient for cells which lack the transsulfuration enzymes. The accumulation of cystathionine in brain[38] may result from the presence of cystathionine synthase in the relative absence of cystathionase. Finally, it should be noted that different patterns occur in other species. Human kidney and brain contain betaine-homocysteine methyltransferase.[39,40]

Metabolic regulation

Evidence for existence

The essential biologic functions of methionine were identified in the first paragraph. Any regulatory mechanism must allow the organism to satisfy these com-

peting requirements even as they change in response to diverse stimuli. Furthermore, the mechanism should be apparent in the ability to adapt to perturbations in the dietary availability of methionine, cyst(e)ine, other sulfur-containing nutrients, choline, folic acid, and vitamin B_{12}. Consequently, the first suggestions of regulatory processes occur in nutritional studies such as those of duVigneaud and Rachele.[41] However, it was the observation of Womack and Rose[42] that dietary cystine reduced the requirement for methionine which, in retrospect, established the presence of a regulatory mechanism. We know now that due to the irreversibility of the cystathionine synthase reaction, cystine is not a precursor of methionine. Cystine can replace methionine in only one metabolic function: the synthesis of cysteine and its derivatives. Methionine sparing by dietary cystine must derive from increased conservation and reutilization of methionine. In 1967, 25 years following the original observation, the enzyme changes associated with this adaptation were defined.[43] Those results led to the suggestion that methionine metabolism was a cycle with a unidirectional outlet formed by the cystathionine synthase reaction and that the distribution of homocysteine between remethylation and cystathionine synthesis provided a major regulatory site.[43,44] The competition between protein synthesis and the formation of S-adenosylmethionine for available methionine provides a second potential regulatory site.[44,45]

Research in several laboratories has supported and extended the hypothesis that the disposition of homocysteine constitutes a regulatory mechanism. Homocysteine remethylation has been demonstrated in vivo in rat liver and brain[46] and cat kidney[47] as well as in the isolated perfused rat liver[48] and liver slices.[49] Direct evidence for regulation derived from studies involving the perfusion with either [^{14}C-methyl] methionine or [^{3}H-methyl] ^{35}S-methionine.[50] Together, the data allowed an estimation of the half-life of the methyl group of methionine and the percentage of homocysteine converted to cystathionine during each cycle. In livers from chow-fed rats, the values were 5.5 minutes and 45%. A comparison of the metabolic patterns in livers from rats fed either a 3.5% casein diet or a 55% casein diet demonstrated that a decrease in the methyl group half-life from 9.3 to 4.8 minutes and an increase in the percentage transsulfuration from 10% to 70% characterized the high protein group. More rapid cycling with augmented cystathionine synthesis was the apparent response to the increased availability of protein and methionine.

Krebs et al. also used the isolated perfused rat liver to demonstrate the inverse relationship between methionine availability and the rate of catabolism of one-carbon units.[51]

Mudd and Poole, in a notable series of balance studies with normal human subjects, estimated that 53% of the available homocysteine was converted to cystathionine during each cycle in males fed a basal diet.[17] The value declined to approximately 20% following the restriction in the diet content of methionine and choline. These conclusions were extended by studies of two patients with sarcosinemia.[52] Again, the percentage of homocysteine which was transsulfurated related directly to the dietary content of methyl groups (from methionine and choline). Furthermore, the estimated half-life of hepatic S-adenosylmethionine was 2.4 to 4.9 minutes when the subject ate a normal diet.

Components of the regulatory mechanism

In this pathway, as in others, regulation may be achieved either by changes in the content of the relevant enzymes or by the inherent kinetic properties of the enzymes as affected by changes in the concentrations of both substrates and effectors. Both factors appear to be significant in the regulation of methionine metabolism in mammalian liver. The kinetic properties of the enzymes provide a mechanism for immediate response to metabolic perturbations while changes in the tissue content of the enzymes may become significant in the adaptation to more sustained stimuli.

Many studies have demonstrated that dietary factors (particularly the content of protein or methionine), hormone administration, and aging can affect the hepatic levels of the enzymes of methionine metabolism.[44,50] The patterns of response are variable and there are few examples of coordinate changes. Both homocysteine methyltransferases together with methionine adenosyltransferase are decreased while cystathionine synthase and cystathionase are increased in the livers of older rats. These findings suggest a transition from methionine conservation to methionine catabolism. Conversely, methionine supplementation of an adequate diet results in a decrease in methyltetrahydrofolate-homocysteine methyltransferase and increases in the transsulfuration enzymes.[9,43] The hepatic content of betaine-homocysteine methyltransferase increases. This finding, together with the observation that increased dietary choline (or betaine) also resulted in increased hepatic enzyme, led to the suggestion that betaine-homocysteine methyltransferase functioned primarily in the catabolism of choline and homocysteine.[53] Recent studies have reexamined this question. Hepatic levels of betaine-homocysteine methyltransferase are increased by either methionine restriction or by methionine supplementation.[54] The mechanism of this unusual, biphasic response remains undefined; however, it is apparent that betaine-homocysteine methyltransferase may have multiple roles which are determined by the nutritional state of the animal. The enzyme may serve to metabolize excessive betaine or homocysteine. Alternatively, with restriction of dietary methyl groups, betaine-homocysteine methyltransferase may function to conserve methionine.[55]

Table 2 summarizes the kinetic properties of the enzymes of methionine metabolism. Examination of the Michaelis constants for the sulfur-containing substrates suggests the presence of an intrinsic regulatory mechanism. The values for the transsulfuration enzymes, cystathionine synthase and cystathionase, are

Table 2 Kinetic properties of enzymes of methionine metabolism

Enzyme	K_s (mM)	Effect of Metabolites			Source
		Met	AdoMet	AdoHcy	
Methionine adenosyltransferase					
I-α	.003–.041	S	P,I		1–5
II-γ	.004–.023	S	P,I		
III-β	.03–1.3	S	P,A		
S-adenosylmethionine decarboxylase	.05–.08		S		16
Adenosylhomocysteinase					
Hydrolysis	.008–.06			S	35, 56
Synthesis	.16			P,I	
Betaine homocysteine methyltransferase	.002	P,I	I	I	57–59
Methylfolate homocysteine methyltransferase	.06	P,I	CoS	I	60, 61
Cystathionine synthase	1–25		A	A	28,29,62–64
Cystathionase	3				30,31,65
Methylenetetrahydrofolate reductase	—		I	A	66

Abbreviations: K_s, Michaelis constant for S-containing substrate; Met, methionine; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; S, substrate; P, product; I, inhibitor or inactivator; CoS, cosubstrate; A, activator.

two orders of magnitude greater than those for the homocysteine methyltransferases. At low concentrations of substrate, remethylation would be at a kinetic advantage. *Table 2* also highlights the effector properties of S-adenosylmethionine which allow this metabolite to serve as a "switch," regulating the flow of homocysteine between the remethylation and transsulfuration sequences. Increased concentrations of S-adenosylmethionine, usually the result of an increase in the availability of methionine, would favor transsulfuration. S-adenosylmethionine inhibits the two lower-Km isoenzymes of MAT and inactivates betaine-homocysteine methyltransferase.[2–5,59] Simultaneously, both MAT-III and cystathionine synthase would be activated.[2–5,62] The effect of S-adenosylmethionine on methyltetrahydrofolate-homocysteine methyltransferase appears anomolous since the compound is a cofactor for the enzyme in vitro. However, S-adenosylmethionine inhibits methylenetetrahydrofolate reductase[66] and may reduce the availability of methyltetrahydrofolate. By this means, S-adenosylmethionine accumulation may limit the methyltetrahydrofolate-homocysteine methyltransferase reaction in vivo.

Table 2 indicates the potential regulatory roles of methionine and S-adenosylhomocysteine. The former is the product inhibitor of both homocysteine methyltransferases. S-adenosylhomocysteine inhibits the negative effects of S-adenosylmethionine on betaine-homocysteine methyltransferase[59] and methylenetetrahydrofolate reductase,[66] but the two metabolites are synergistic in the activation of cystathionine synthase.[62,64]

Taken together, the kinetic properties and the responses to dietary methionine allow the definition of two enzymes groups. The methionine-catabolyzing enzymes have K_m values greater than 1.0 mM, they may be activated by S-adenosylmethionine, and they tend to increase in response to increases in dietary methionine. MAT-III, cystathionine synthase, and cystathionase fulfill these criteria. In contrast, the methionine-conserving enzymes are characterized by K_m values les than 0.1mM, inactivation or inhibition by S-adenosylmethionine, and the tendency of the liver content to decrease or remain unchanged with increased availability of methionine. None of the other enzymes fit perfectly into this group. Hepatic levels of MAT-I increase with increasing dietary methionine[67] and hepatic levels of betaine-homocysteine methyltransferase, as noted previously, increase in response to both methionine deprivation and methionine supplementation. Inhibition by S-adenosylmethionine of the synthesis of methyltetrahydrofolate, rather than of methyltetrahydrofolate-homocysteine methyltransferase itself, permits the inclusion of that enzyme in the methionine-conserving group. Adenosylhomocysteinase combines characteristics of both groups, the low K_m in the physiologic, hydrolytic direction together with induction of the hepatic enzyme by dietary protein.[35]

Since most of the S-adenosylmethionine-dependent transmethylases have K_m values less than 0.1mM, the methionine-conserving enzymes constitute the methionine cycle depicted in *Figure 1*. Similarly, MAT-III, cystathionine synthase, and cystathionase, together with a higher K_m adenosylmethionine-dependent transmethylase (for example, glycine methyltransferase which shows positive cooperativity with S-adenosylmethionine[68]), may comprise the transsulfuration sequence.

Relative contributions to regulation

In the previous sections, I have presented evidence for the existence of a mechanism for the regulation of methionine metabolism as well as some possible components of this system. The remaining questions are whether and to what extent these individual factors contribute to regulation. Recent studies have attempted to answer these questions by simulating in

vitro the metabolic site formed by the enzymes which metabolize homocysteine.[68] This experimental system is based on the determination of the simultaneous product formation by methyltetrahydrofolate-homocysteine methyltransferase, betaine-homocysteine methyltransferase, and cystathionine synthase. It uses final concentrations of both enzymes and metabolites determined to occur in vivo. Under these conditions, competition between the enzymes for limited substrate is an important determinant of the rates of the individual reactions.

The simulation of homocysteine metabolism in the livers of chow-fed rats indicated an equal contribution of both homocysteine methyltransferases to methionine resynthesis. Cystathionine synthase accounted for 46% of total product formation.[69] This value agrees remarkably with that obtained in the isolated perfused rat liver.

The simulation of the regulatory locus was used in a study of the metabolic consequences of methionine excess.[9] The parenteral injection of methionine results in a rapid increase in the hepatic concentrations of S-adenosylmethionine (from 60 μM to 300 μM) and of S-adenosylhomocysteine (from 10 μM to 50 μM).[70] These changes in metabolite concentrations, without any alteration in the content of the individual enzymes, resulted in a 43% reduction in betaine-homocysteine methyltransferase, a 625% increase in cystathionine synthesis, and a 270% increase in total product formation. Thus, the simulation indicated the potential for an immediate adaptation to methionine excess based on an increase in transsulfuration from 46% to 82% together with a marked increase in total flow through the regulatory site.

In contrast, the adaptation to sustained increases in dietary methionine, from 0.3% through 3.0%, involved changes in enzyme content as well as alterations in the concentrations of the effector metabolites (*Table 3*).[9] Product formation by each of the three enzymes was a summation of the effects of the multiple changes. Increased dietary methionine resulted in decreased hepatic levels of methyltetrahydrofolate-homocysteine methyltransferase and increases in both cystathionine synthase and betaine-homocysteine methyltransferase. Each reaction was stimulated by the progressive increase in the concentration of S-adenosylhomocysteine which provided greater availability of homocysteine; however, as anticipated from the K_m values (*Table 2*), the positive effect on cystathionine synthase was proportionately greater. Both betaine-homocysteine methyltransferase and cystathionine synthase were impaired by the progressive decrease in the concentration of the cosubstrates, serine and betaine. (Although not tested, the same is likely to be true for methyltetrahydrofolate-homocysteine methyltransferase since the administration of methionine decreases the hepatic content of 5-methyltetrahydrofolate.[71]) The net effect of these changes was that adaptation from a diet containing 0.3% methionine to one containing 1.5% appears to be due solely to a marked increase (190%) in transsulfuration. Product formation by both of the homocysteine methylases is unchanged. This is not the case with the comparison of the simulations of metabolism in livers of rats fed either 0.3% or 3.0% methionine diets in which flow through all three reactions increased significantly. However, the 12-fold increase in cystathionine synthesis was the dominant change. This capacity for a marked increase in flow through the transsulfuration pathway could provide an effective means for the catabolism of large excesses of methionine. A "benign," high-capacity transmethylation reaction would be essential, and glycine methyltransferase (EC 2.1.1.20) is a likely candidate. The enzyme uses a nonessential substrate to produce a nontoxic product (sarcosine) which can be oxidized to regenerate the glycine. In addition, hepatic levels of glycine methyltransferase increase with increased dietary methionine[9,72] and S-adenosylmethionine is a positive effector of the enzyme.[68] This proposed mechanism for the catabolism of excessive methionine (and S-adenosylmethionine) is consistent with the ability of glycine to alleviate methionine toxicity,[73] with recent direct in vivo studies of rat liver with nuclear magnetic resonance techniques,[74] and with the correlation between methionine intake and sarcosine excretion in a patient with sarcosinemia.[52] The inhibition of glycine methyltransferase by 5-methyltetrahydrofolate[75] is also consistent with the hypothesis that the enzyme functions primarily to degrade surplus S-adenosylmethionine. Decreases in the latter metabolite result in increased synthesis of the folate derivative.[66,71] In turn, this limits the utilization of S-adenosylmethionine in the "nonproductive" synthesis of sarcosine.

Studies of the simulation of the metabolic site formed by the enzymes which use homocysteine have

Table 3 Effect of dietary methionine on hepatic concentrations of metabolites and enzymes and on homocysteine metabolism by locus models

Dietary methionine (%)	0.3	1.5	3.0
Hepatic metabolites			
Methionine (nmol/g)	45	54	82
S-adenosylmethionine (nmol/g)	141	155	673
S-adenosylhomocysteine (nmol/g)	20	61	427
Betaine (μmol/g)	7.3	0.3	0.2
Serine (μmol/g)	1.8	0.4	0.1
Hepatic enzymes (U/g)			
Methionine adenosyltransferase	67	93	102
Methylfolate methyltransferase	8.8	5.1	4.0
Betaine methyltransferase	107	173	243
Cystathionine synthase	140	208	285
In vitro model system: product formation (nmol/min)			
Methylfolate methyltransferase	0.26	0.30	0.84
Betaine methyltransferase	2.77	2.55	6.86
Cystathionine synthase	1.82	5.27	23.59
Total product	4.86	8.12	31.29
Transsulfuration (%)	37	65	75

Isocaloric diets which contained the specified amount of methionine, 0.5% cystine, and 0.3% choline were fed for 7 days. Extracts prepared from the livers of rats in each group were the enzyme sources for the in vitro model systems.
Data from Finkelstein and Martin.[9]

also extended our understanding of the methionine-sparing effect of cystine.[55] As anticipated by earlier experiments,[43] a marked diminution in cystathionine synthesis is the basis for adaptation from an adequate diet containing only methionine to one in which cystine replaces 70% of the methionine. Since the rate of homocysteine remethylation remains unchanged, a larger proportion of the homocysteine is retained within the methionine cycle. The betaine-homocysteine methyltransferase reaction appears essential for this conservation of homocysteine.

Alternate pathways for methionine metabolism

Methionine transamination

Based on an extended series of studies, Benevenga and Livesey have suggested that the direct transamination of methionine contributes significantly to the metabolism of the amino acid.[76,77] They propose the sequential conversions to 2-keto-4-methylthiobutyrate, 3-methylthiopropionate, and methanethiol. In more recent experiments,[78] Engstrom and Benevenga addressed a major criticism of the hypothesis, that demonstration of the pathway requires concentrations of methionine that are unlikely to occur in vivo. Nevertheless, the question of the quantitative importance of this pathway remains unresolved, and many contrary observations must be reconciled. The in vivo direction of the glutamine transaminase reaction is crucial. Cooper emphasizes that both the high concentrations of glutamine and the rapid use of its keto derivative (2-oxoglutaramate) make it likely that transamination will be in the direction of methionine synthesis.[79] Furthermore, a direct nuclear magnetic resonance study provided support for the importance of glycine methylation, not transamination, in the catabolism of excessive methionine in rat liver.[74] The results of human studies also tend to minimize the significance of methionine transamination. Following the ingestion of L-methionine by normal subjects, the excretion of the metabolites is limited.[80,81] Patients with cystathionine synthase deficiency do not convert methionine to sulfate[82] and do not excrete transamination metabolites despite a marked accumulation of methionine.[83] Indeed, the excretion of these compounds may account for only 20% of the ingested methionine in a patient with hypermethioninemia due to methionine adenosyltransferase (MAT-III) deficiency.[84]

Adenosylmethionine cleavage

S-adenosylmethionine hydrolase (EC 3.3.1.2) has been described in yeast,[85] porcine liver,[86] and phage-infected bacteria.[87] More recent studies failed to demonstrate this enzyme in rat liver.[9,88] Since the product, 5-methylthioadenosine, can be recycled to methionine, this reaction is likely to have little quantitative importance to the total metabolism of the amino acid. It could, however, serve to reduce the concentration of S-adenosylmethionine in specific, small, subcellular compartments.

Homocysteine and cystathionine oxidation

Both cystathionine and homocysteine are substrates for L-amino acid oxidase, and homocysteine is a substrate for glutamate dehydrogenase and glutamine transaminase.[89-91] While there is no reason to believe that these reactions contribute significantly to methionine metabolism in vivo, the keto acids derived are of interest. These can be transformed by a large number of enzymatic and chemical reactions into numerous unusual metabolites, several of which have been isolated from the urine of patients with deficiencies of either cystathionine synthase or cystathionase. The suggestion that these compounds may have pathophysiologic consequences is worthy of considerable attention.[91]

Inborn errors of methionine metabolism in humans

During the last 25 years, patients have been identified with deficiencies of methionine adenosyltransferase, cystathionine synthase, cystathionase, methylenetetrahydrofolate reductase, cobalamin metabolism with imparied synthesis of methylcobalamin, and adenosine deaminase. Studies of these individuals have provided substantial insight into normal metabolic pathways and have validated earlier extrapolations from animal experiments. I have used some of the observations in other sections and will only summarize the additional findings.

The most informative studies of methionine adenosyltransferase deficiency involve the patients without either hepatic dysfunction or other metabolic abnormalities. It is likely that a total deficiency of MAT would be inconsistent with survival. In contrast, impairment of MAT-III appears to result in asymptomatic hypermethioninemia.[92,93] This finding, in one well-evaluated 31-year-old man, suggests that the distribution of MAT isoenzymes in humans is similar to that in the rat, and that the low-K_m forms are sufficient for the synthesis of the essential amounts of S-adenosylmethionine. The accumulation of methionine without demonstrable pathology indicates that the synthesis of S-adenosylmethionine is central to both methionine catabolism and methionine toxicity. Finally, the inappropriately high rate of homocysteine methylation is consistent with a central, regulatory role for S-adenosylmethionine.[84]

Studies of patients with cystathionine synthase deficiency have been of particular value in the definition of normal metabolism.[94] The characteristic biochemical abnormalities and the accumulation of both homocyst(e)ine and methionine are consistent with the pathway defined in *Figure 1*. Clearly, homocysteine which cannot be converted to cystathionine is remethylated to methionine. The apparent increase in the dietary requirements for both betaine and folate supports this conclusion. In addition, the accumulation of S-adenosylhomocysteine confirms the reversibility of the adenosylhomocysteinase reaction when homocysteine is

not removed. Finally, the inability of these patients to convert methionine to sulfate establishes the transsulfuration pathway as the primary route for that conversion.

Cystathionase deficiency appears to be a benign defect which results only in increased tissue cystathionine with cystathioninuria.[94] This is predictable, based on the irreversibility of the cystathionine synthase reaction. It is possible that careful nutritional experiments would reveal that cysteine and/or other sources of organic sulfur are essential nutrients consequent to the disrupted transsulfuration pathway.

The failure to synthesize methylcobalamin, the coenzyme for methyltetrahydrofolate-homocysteine methyltransferase, impairs that reaction. Deficiency of methylenetetrahydrofolate reductase, by limiting the availability of substrate, has the same result. In both situations, the metabolic consequence is homocyst(e)ine accumulation with low or normal levels of methionine. We may conclude that, in humans, methyltetrahydrofolate-homocysteine methyltransferase is essential both for the maintenance of normal levels of methionine and for the clearing of homocysteine. The observation that supplemental betaine may alleviate the biochemical abnormalities can be explained by the demonstrated inverse relationship between availability of homocysteine and the hepatic concentration of betaine.[9]

The increase of S-adenosylhomocysteine in tissues deficient in adenosine deaminase[23] is additional proof that adenosylhomocysteinase is reversed in vivo by the accumulation of either of its products.

All of the above conditions represent the consequences of well-defined, discrete, and inherited defects of specific enzymes. In addition, there are an increasing number of situations in which abnormalities of methionine metabolism have been detected in association with significant clinical pathology. The link between homocysteine accumulation and arteriosclerosis, which began with observations in patients with cystathionine synthase deficiency,[94] is being extended to broader populations.[95,96] Similarly, Hoffman's finding that many tumor cell lines in culture are auxotrophic for methionine,[97] together with the continued interest in the relationship between DNA hypomethylation and neoplasia, establishes the relevance to oncology. Based primarily on clinical observations, including the possible therapeutic efficacy of S-adenosylmethionine in affective disorders, the relationship between abnormalities of methionine and methyl group metabolism and neuropsychiatric syndromes merits further attention.[98,99] Lastly, both the role of methyl group deficiency in the etiology of alcoholic liver disease and abnormalities of transsulfuration in patients with cirrhosis remain areas for continued investigation.[100,101]

These issues, which are of immediate relevance to human disease, will no doubt attract substantial attention. However, there are major deficiencies in our more basic information. I would emphasize these gaps by suggesting that our last 20 years have been devoted primarily to the hepatic cytosol. We now require information concerning other tissues which differ notably in the content of enzymes. Similarly, our focus on the soluble compartment tends to obscure the reality that important events occur at other intracellular sites. Both the movement of metabolites and the transfer of information within the cell are areas for exploration. Finally, it is clear that the metabolism of methionine interfaces significantly with that of other compounds. We must extend our knowledge of the regulation of the metabolism of choline, serine, folate, and vitamin B_{12}. A delightful aspect is that this cursory summary of many decades of productive investigations highlights the numerous areas which require continued study.

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