Adaptation of Adenosylmethionine Metabolism and Methionine Recycling to Variations in Dietary Methionine in the Rat (43110)

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Abstract. **Weanling rats were fed a casein-based diet supplemented to give dietary methionine (Met) concentrations of 0.41, 0.61, and 1.50%. After 2 weeks of feeding, the rats received intraperitoneally 800 nCi of 2-^{14}C-labeled and/or *methyl*-^{3}H-labeled L-Met. The animals were killed 20 min, 1 hr, or 2 hr after the isotope injection and the specific radioactivity of adenosylmethionine (AdoMet) as well as the total acid-soluble radioactivity was analyzed in the liver and skeletal muscle.**

Met concentrations of the liver and skeletal muscle were increased 20-fold by the diet containing 1.50% of Met. In the liver, but not in skeletal muscle, accumulation of AdoMet closely followed changes in Met concentration. Within 2 hr after intraperitoneal injection, the rate of disappearance of ^{3}H label from the acid-soluble fraction was slow in both tissues; increasing in the liver and decreasing in skeletal muscle with increasing dietary Met concentration. At the same time, disappearance of ^{14}C label was slow in both tissues in the rats fed the toxic Met diet, and also in the liver of the rats fed the Met-deficient diet. Decline of the specific radioactivity of the AdoMet pool with respect to ^{3}H label was similar to that of ^{14}C label in the skeletal muscle at all dietary Met concentrations. In the liver, the rate of disappearance of ^{14}C label from the AdoMet pool was markedly increased and that of the ^{3}H label slightly decreased with increasing dietary Met supply. Met deprivation resulted in rapid disappearance of ^{3}H label from the hepatic AdoMet pool, whereas the disappearance of the ^{14}C label was very slow.
The results indicate that hepatic Met recycling is very effective with deficient or adequate dietary Met concentrations. In skeletal muscle, the capacity to catabolize extra Met is very limited and continuous flow of Met to liver takes place. Unlike in the liver, in skeletal muscle the transsulfuration route is not adaptable to changes in Met supply and plays a minor role in Met catabolism.

The approach used to determine the efficacy and adaptation of methionine salvage pathways by following simultaneously the decline of the specific radioactivities of the methyl group and the methionyl carbon chain of AdoMet following intraperitoneal injection of double-labeled Met has several advantages over that used in literature reports. It offers a reliable means of observing these metabolic pathways in whole animals without disruption of metabolite fluxes. [P.S.E.B.M. 1990, Vol 194]

Methionine (Met) is considered nutritionally essential for all mammals despite significant differences in species and age effects (1), general toxicity (2), and the ability of a variety of nonmalignant human and rodent cell lines to grow in culture conditions devoid of Met (3). The biochemical reason for the extreme sensitivity of mammals to excess dietary Met has not been resolved, but accumulation of toxic catabolites due to excessively induced transsulfuration or transamination pathways has been suggested (2) and documented (4). The quantitative importance of the two catabolic routes of Met is likely to be tissue specific and may vary a great deal in response to varied dietary Met. Very little is known about the adaptability of these pathways, however, although experiments have been conducted with isolated hepatocytes (5) and other *in*

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vitro systems (6, 7). Due to the kinetic properties of the isoenzyme forms of Met adenosyltransferase (8–10), the ability of extrahepatic tissues to increase catabolism of extra Met via the transmethylation-transsulfuration route seems unlikely. On the other hand, liver is known to be equipped with enzymes adaptable to marked variations in the catabolic needs of Met via the transsulfuration pathway (8, 9, 11–13). Furthermore, our recent studies indicate that extrahepatic tissues have very limited capacity to catabolize Met, whereas liver seems to play the major role in the overall catabolism and toxicity of extra Met in the rat (14, 15).

The aim of the present study was, by following the decline of the specific radioactivities of tissue adenosylmethionine pools after a single intraperitoneal dose of radioactive Met, to estimate the quantitative significance of Met salvage pathways (3) and the transsulfuration route in response to varied dietary Met. Skeletal muscle was chosen to represent extrahepatic tissues due to its significant contribution to total body protein and its indispensable role in the growth rate of the animal. As shown in Figure 1, the transamination of Met is a reversible process but further catabolism of the oxo acid irreversibly simultaneously removes ^{3}H and ^{14}C label from the [*Me*-^{3}H]-[2-^{14}C]Met pool. In contrast, formation of adenosylmethionine (AdoMet) is an irreversible reaction and further metabolism by the transmethylation-transsulfuration pathway irreversibly segregates the metabolism of the methyl group from that of the carbon chain. From the pool of [Me-^{3}H]-[2-^{14}C] AdoMet, ^{3}H label can reenter the Met pool only as a result of the methylthioadenosine salvage pathway and ^{14}C label only via the homocysteine remethylation cycle (3). At least in rodent liver, the specific radioactivity of the AdoMet pool is likely to be identical to that of the Met pool due to the very rapid turnover rate of the AdoMet pool, as shown by studies with isolated rat hepatocytes (5) and mice (16). Our results show that the double-isotopic method used here provides a reliable means of unraveling the flow rates of methionine via catabolic and salvage pathways in the whole animal under varied Met conditions.

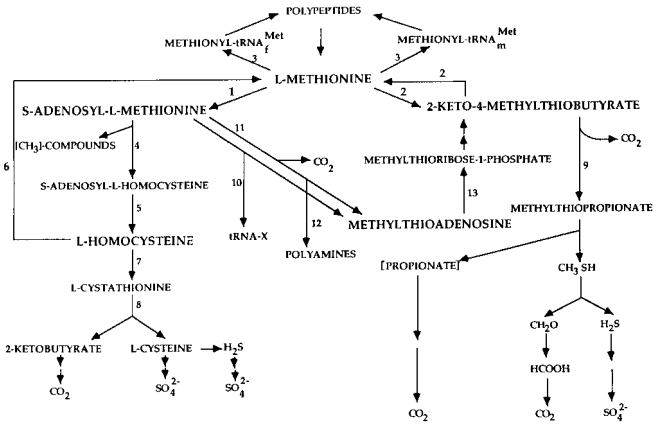

Figure 1. Fate of Met in mammalian metabolism. 1 Met adenosyltransferase (EC 2.5.1.6); 2 aminotransferases (EC 2.6.1), most notably glutamine-pyruvate aminotransferase (EC 2.6.1.15); 3 methionyl-tRNA synthetase, (EC 6.1.1.10); 4 methyltransferases (EC 2.1.1), 5 adenosylhomocysteinase (EC 3.3.1.1); 6 betaine-homocysteine methyltransferase (EC 2.1.1.5) and 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13); 7 cystathionine β-synthase (EC 4.2.1.22); 8 cystathionine γ-lyase (EC 4.4.1.1) 9 2-oxoisovalerate dehydrogenase (EC 1.2.4.4); 10 tRNA-uridine aminocarboxypropyltransferase (EC 2.5.1.25); 11 AdoMet decarboxylase (EC 4.1.1.50); 12 spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22); 13 5′-methylthioadenosine phosphorylase (EC 2.4.2.28).

Materials and Methods

Rats were housed and fed *ad libitum* with either a commercial nonpurified diet (Hankkija Oy, Turku, Finland) or a casein-based semipurified diet supplemented with Met at the expense of glutamic acid, as described previously in detail (14). Met concentrations in the isonitrogenous diets were adjusted to 0.41 (deficient), 0.61 (control), and 1.50% (toxic). Rats had free access to food until killing, unless otherwise indicated. Individual differences in food consumption during the day of experiment were not determined with variation included as experimental error. However, during the preceding 2-week feeding period, food consumption and weight gains were recorded daily. Only healthy, actively eating rats were chosen for the experiments from each dietary group. The effect of fasting control animals was also tested to confirm that individual eating habits did not influence tissue AdoMet concentrations. When fasting was studied, the duration of fasting is given and represents the interval from removal of feed until killing. This was always long enough to ensure that animals were utilizing body energy reserves.

Starting 2 hr after the end of the 10-hr dark period (11), the rats were injected intraperitoneally with L-[2-^{14}C]Met (57.6 mCi/mmol; Amersham) and L-[*methyl*-^{3}H]Met (87 Ci/mmol; Amersham) in 0.9% (w/v) NaCl. Injections were made in 2-min intervals starting with the rats having the longest treatment time and proceeding so that the animals could be killed in chronological order as to the treatment time. Control animals received the vehicle only. Isotope purity was confirmed by mixing them with unlabeled L-Met to give identical concentrations and using these mixtures as substrates for partially purified Met adenosyltransferase (17). Both isotopes were converted to AdoMet with yields exceeding 70%. This is close to the maximum attainable yield considering the equilibrium constant of the reaction and the lability of AdoMet and the product inhibition caused by this metabolite under the reaction conditions (8, 9, 17, 18).

At the indicated time intervals, the rats were decapitated under diethyl ether anesthesia, and the livers and leg muscles were rapidly excised in ice-cold 0.25 *M* sucrose, washed, weighed, and immediately homogenized in three volumes of 10% (w/v) trichloroacetic

acid and treated as described previously (18). AdoMet and adenosylhomocysteine were isolated from supernatants by Cellex-P column chromatography, quantified, and counted for radioactivity (18). When necessary, the isolated compounds were concentrated into 500 μl of 1 m*M* HCl (18), 20-μl samples were subjected to high-performance liquid chromatography analysis and 400-μl samples were counted in 10 ml of ACS (Packard), as described previously (19, 20). Occasionally, high-performance liquid chromatography analysis was also used to ensure the purity of the column chromatographic preparations. Tissue amino acid concentrations were determined from the ether-extracted supernatants using norleucine (Sigma) as an internal standard (15).

Results

Since individual eating habits of the experimental animals may vary, especially when fed different diets, the effects of fasting were tested on some parameters crucial to the studies performed. As shown in Table I, fasting for 15 hr decreased liver weight to a greater extent than body weight, i.e., by 27.7% and 9.1%, respectively, but had negligible effect on the hepatic concentrations of AdoMet. Since the rats received radioactive Met on body weight basis, the doses expressed as nCi/g liver differed in the two groups; 215.9 ± 6.9 in the fasted and 171.5 ± 7.9 in the fed animals. However, the concentration of hepatic acid-soluble radioactivity (nCi/g) and the proportion and specific radioactivity (pCi/nmol) of the AdoMet pool were similar in both groups 20 min after injection. During the following hour, disappearance of acid-soluble radioactivity from the liver was significantly slower in fasted than in fed animals with the apparent half-lives being 43.7 min and 28.5 min, respectively. The share of the AdoMet pool of this radioactivity was also decreased at markedly different rates with half-lives of approximately 87 min in fasted and 440 min in fed animals. However, fasting had no apparent effect on the decline of the specific radioactivity of AdoMet during the 1-hr period from 20 to 80 min after injection with values for the half-lives being 27.5 min for fed and 28.8 min for fasted animals.

Table I. Effect of Fasting on the Distribution and Turnover of Hepatic Radioactivity after Intraperitoneal Administration of L-[2-^{14}C]Met[a]

	Rats fed *ad libitum*	Rats fasted for 15 hr
Body weight (g)	96.8 ± 5.3	88.0 ± 3.2
Liver weight (g)	4.58 ± 0.40	3.31 ± 0.17
Hepatic AdoMet (nmol/g)	72.7 ± 10.2	68.1 ± 8.6
Hepatic radioactivity (nCi/g)		
20 min after injection	5.64	5.47
40 min after injection	2.86	4.15
80 min after injection	1.31	2.11
Hepatic radioactivity as AdoMet (% of total)		
20 min after injection	27.4	28.2
40 min after injection	26.1	21.5
80 min after injection	24.9	17.4
Specific radioactivity of AdoMet (pCi/nmol)		
20 min after injection	22.7	23.8
40 min after injection	8.9	12.2
80 min after injection	5.0	5.6

[a] Female F344 rats were fed a commercial nonpurified diet (Hankkija Oy, Turku, Finland) and watered *ad libitum*, or fasted for 15 hr, and injected intraperitoneally with 769 nCi of L-[2-^{14}C]Met (specific radioactivity, 57.6 nCi/nmol) in 250 μl of 0.9% NaCl/95 g body wt at the indicated time intervals before killing. The values are means of two animals or mean ± SD of six animals.

In starved animals, highest amounts of radioactivity were recovered in the liver within 5 min after intraperitoneal injection with [2-^{14}C]Met. This was also true for the specific radioactivity of the AdoMet pool, whereas the share of AdoMet of the acid-soluble radioactivity remained unchanged from 5 to 15 min after injection, suggesting continued flow of radioactive Met to the liver, and only then started to decrease (data not shown). As shown in Figure 2, the decline of both the acid-soluble radioactivity and the specific radioactivity of AdoMet closely obeyed the logarithmic equations given, indicating that the disappearance of radioactivity from both pools slowed continually (but at different rates) following injection. The average half-lives, calculated from the experimental values and not from the theoretical fits (Fig. 2), of the acid-soluble radioactivity (nCi/g or percentage of the total dose recovered in liver), the specific radioactivity of the AdoMet pool (pCi/nmol) and the share of AdoMet of the acid-soluble radioactivity (percentage) from 30 to 60 min after injection were 48 min, 25 min, and 65 min, respectively. Only the last parameter decayed linearily with time during the first hour after injection and then slowly increased (data not shown). Values for the above parameters from 60 to 120 min after injection were 120 min, 74 min, and 294 min, respectively. Three separate experiments gave similar results indicating that any (but exactly identical) time period between 20 and 120 min after injection could be chosen for comparative studies of the effects on Met recycling via the hepatic AdoMet pool.

To check that the AdoMet fraction was not contaminated to any significant extent by other radioactive metabolites of methionine, e.g., polyamines, fed animals were killed 20 and 120 min after intraperitoneal injection with 1 μCi of L-[2-^{14}C]Met, a dose that corresponded to 133 ± 4.4 nCi/g of liver, and the specific radioactivities of both AdoMet and adenosylhomocysteine were determined. The values for AdoMet were 12.1 ± 0.47 (n = 5), and 2.6 ± 0.77 (n = 4) pCi/nmol and for adenosylhomocysteine 11.1 ± 1.1 (n = 5) and

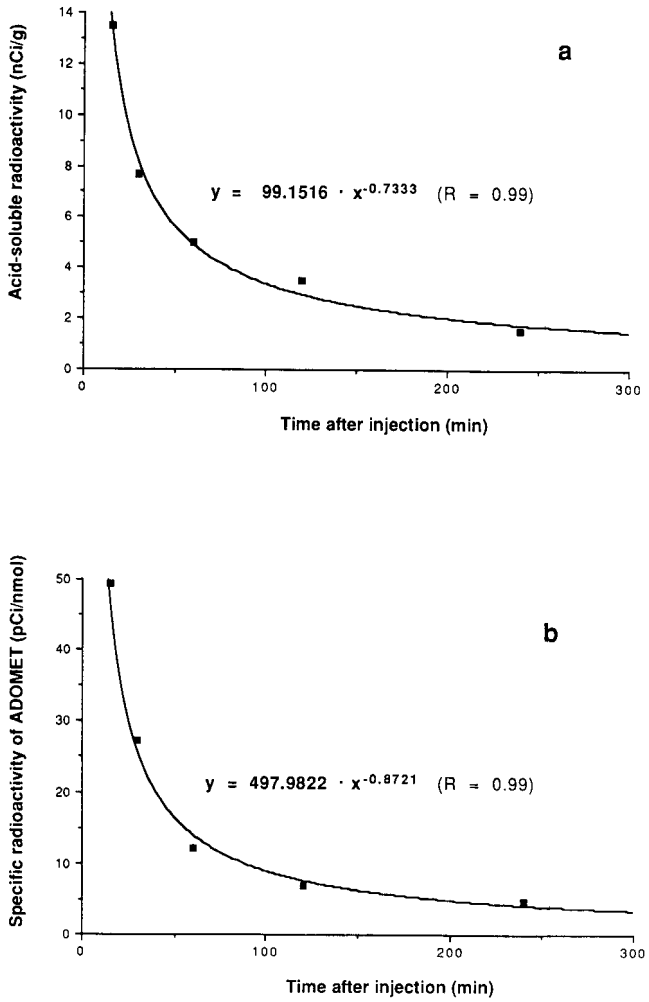

Figure 2. Decay of the acid-soluble radioactivity (a) and the specific radioactivity of AdoMet (b) in rat liver after intraperitoneal injection with 926 nCi of L-[2-^{14}C]Met (57.6 nCi/nmol) in 250 μl of 0.9% NaCl/90 g body wt. Female F344 rats were fasted for 17 hr before killing. Rat weight was 85.3 ± 1.6 g, liver weight 3.22 ± 0.15 g, injected dose 273 ± 10 nCi/g of liver, and hepatic AdoMet concentration 73.3 ± 6.5 nmol/g (n = 10). Each point represents the mean of two animals.

2.4 ± 0.64 (n = 6) pCi/nmol at 20- and 120-min time points, respectively. Thus, there was no indication of any accumulation of radioactive impurities in either one of the metabolite fractions studied.

Effect of dietary Met supply on the turnover rates of the methyl group in comparison to that of the carbon chain of Met in the liver and skeletal muscle was examined with the aid of a double isotope technique. As shown in Table II, radioactivity originating from either moiety of Met molecules was more concentrated in the liver than in skeletal muscle at all dietary Met levels. In muscle, radioactivity from the methyl moiety remained in the acid-soluble pool much longer than that from the carbon chain, but the accumulation of radioactivity from both moieties was increased with increasing dietary Met. In the liver, ^{3}H disappeared more rapidly when more dietary Met was fed, whereas the rate of disappearance of ^{14}C label fluctuated independently of the ^{3}H label.

The rates of disappearance of both the ^{3}H label and the ^{14}C label in the AdoMet pool of skeletal muscle were fairly similar and independent of the Met supply (Table II). In the liver, increasing dietary Met clearly resulted in markedly accelerated disappearance of the ^{14}C label and slightly retarded disappearance of the ^{3}H label from the AdoMet pool.

Considering that the ratio of ^{3}H:^{14}C in the injected Met was 5.3:1, it can be calculated (Table II) that this ratio was retained in the AdoMet pool of skeletal muscle at all dietary Met concentrations. This further confirms the identical purity of the Met isotopes used since only the unoxidized form of Met can be used for the synthesis of the sulfonium-centered structure of AdoMet. In the acid-soluble fraction of both liver and skeletal muscle, this ratio was retained only in the case of the Met toxic diet. At normal or deficient Met levels, the ^{3}H:^{14}C ratio was increased in both tissues. In the hepatic AdoMet pool, however, the ^{3}H:^{14}C ratio was markedly decreased. This decreased more rapidly when less Met was fed.

During the time period from 20 to 60 min after intraperitoneal injection with the double-labeled Met, half-life of the ^{14}C label was 32 ± 3 (n = 4) min and that the ^{3}H label was 77 ± 11 (n = 4) min in the acid-soluble fraction of both liver and skeletal muscle at adequate or deficient Met levels. When these levels were fed, the half-life of both labels in the hepatic AdoMet pool was 19 ± 4 (n = 4) min. These results indicate that during the first hour after injection, the disappearance of the ^{14}C label from the acid-soluble and AdoMet pools closely followed the logarithmic equations described in Figure 2, revealing no significant differences between the two tissues and the two diets. They further indicate that the disappearance of the ^{3}H label from the acid-soluble pool was identical in both tissues and with both diets, but did not obey the logarithmic equation characteristic to the disappearance of the ^{14}C label (Fig. 2a) from the same pool. When excess dietary Met was fed, more than 20 min was required for the injected Met to equilibrate with the tissue Met pools (data not shown).

Table III is included to show the tight metabolic interrelationship between Met and some other amino acids. As seen, hepatic concentrations of glycine and serine are inversely related to, whereas that of threonine is proportional to Met supply and hepatic concentration.

Discussion

The highest concentration of radioactivity ever found in liver was 30 nCi/g at 5 min after intraperitoneal injection. If it were totally Met, it would correspond to an extra Met load of 0.52 nmol/g, which is

Table II. Effect of Dietary Met on the Distribution and Turnover of Radioactivity in Rat Liver and Skeletal Muscle after Intraperitoneal Administration of L-[2-^{14}C]-[*Me*-^{3}H]Met[a]

	Met concentration in the diet					
	0.41% (deficient)		0.61% (adequate)		1.50% (toxic)	
Body weight (g)	159 ± 12.3		172 ± 16.4		132 ± 8.6	
Liver weight (g)	6.89 ± 0.84		7.81 ± 0.97		5.88 ± 1.29	
Hepatic AdoMet (nmol/g)	56.8 ± 3.41		85.7 ± 32.17		509.9 ± 71.03	
Muscle AdoMet (nmol/g)	18.9 ± 6.69		18.7 ± 4.90		21.6 ± 2.75	
Acid-soluble radioactivity (nCi/g)	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H
In liver						
60 min after injection	2.31 ± 0.42	25.29 ± 3.76	3.09 ± 0.42	29.83 ± 1.01	9.30 ± 0.41	52.54 ± 1.80
120 min after injection	2.13 ± 0.50	20.93 ± 3.57	1.76 ± 0.31	23.98 ± 4.14	8.34 ± 1.63	39.22 ± 7.33
Half-life (min)	513	220	74	191	381	142
In skeletal muscle						
60 min after injection	1.35 ± 0.16	9.51 ± 0.31	1.58 ± 0.04	12.29 ± 0.77	6.99 ± 0.01	24.68 ± 3.02
120 min after injection	0.68 ± 0.05	11.90 ± 0.84	0.91 ± 0.06	11.28 ± 0.50	5.92 ± 0.77	23.99 ± 2.06
Half-life (min)	60	320	75	485	250	1460
Specific radioactivity of AdoMet (pCi/nmol)						
In liver						
60 min after injection	5.60 ± 1.95	4.36 ± 0.12	5.79 ± 0.71	5.20 ± 1.17	4.58 ± 1.26	4.91 ± 1.09
120 min after injection	5.09 ± 0.39	1.42 ± 0.13	4.33 ± 0.77	3.13 ± 0.55	2.13 ± 0.48	3.24 ± 0.66
Half-life (min)	436	37	143	82	54	100
Mass turnover rate (nmol/min × g)	0.065	0.768	0.300	0.523	4.72	2.55
In skeletal muscle						
60 min after injection	14.97 ± 0.45	43.79 ± 8.21	14.19 ± 1.21	69.07 ± 37.85	6.98 ± 3.43	36.55 ± 5.80
120 min after injection	7.32 ± 1.51	29.06 ± 6.84	6.20 ± 1.30	41.72 ± 9.57	3.66 ± 0.67	21.17 ± 4.70
Half-life (min)	58	101	50	83	64	76
Mass turnover rate (nmol/min × g)	0.163	0.094	0.187	0.113	0.169	0.142

[a] Male Wistar rats were fed the indicated diets for 2 weeks before injecting intraperitoneally with 5.54 nCi of L-[2-^{14}C]Met (specific radioactivity, 57.6 nCi/nmol) and 29.3 nCi of L-[*Me*-^{3}H]Met (specific radioactivity, 87 μCi/nmol) in 0.9% NaCl/g body wt. The rats were allowed to eat and drink *ad libitum* until killed. The radioactivity values (nCi/g and pCi/nmol AdoMet) are mean ± SD of three animals. All of the other values are mean ± SD of the whole dietary group including seven rats. Mass turnover rate was calculated by dividing half of the tissue AdoMet pool by the half-life. Hepatic amino acid concentrations for these rats are given in Table III.

less than 2% of the hepatic Met concentration. Thus, the treatment itself did not change tissue Met levels.

The turnover rate of AdoMet via transmethylation reactions in mouse liver has been determined by preventing further breakdown of adenosylhomocysteine with the aid of *in vivo* inactivation of adenosylhomocysteine hydrolase, and found to be at least 20 nmol/min/g of liver, indicating a half-life of only a few minutes (16). Half-life values of 1–2 min have also been estimated in rat hepatocytes (5). Thus, the significantly longer half-life of the specific radioactivity of the hepatic AdoMet pool found in the present study suggests that the carbon chain of Met is reutilized several times in the liver of rats fed a commercial nonpurified diet (Table I). Fasting for up to 15 hr seemed to have very little effect on the reutilization, indicating that both Met and AdoMet pool sizes remained unchanged and that the activity of the homocysteine remethylation cycle was not affected. However, the share of AdoMet of the total hepatic radioactivity declined five times faster in the fasted than in the fed animals, suggesting that accumulation of Met catabolites in the liver is increased during fasting.

If Met concentration is the rate-limiting factor in AdoMet synthesis, as seems likely in liver since the hepatic synthetic enzyme is normally unsaturated (8) and responds to dietary Met supply (14, 15), then the specific activities of both metabolite pools should be the same at all times following radioactive Met administration. In the case of liver, this assumption is based on the rapid turnover rate of the AdoMet pool (5, 16), the 2- to 3-fold difference in metabolite pool size (14, 15), and the marked and rapid response of the AdoMet pool to intraperitoneally injected Met (21) as well as to variation in dietary Met (14, 15). There is, however, indirect evidence suggesting the existence of intracellular Met pools that are unavailable for AdoMet synthesis (5). Most of these studies have been conducted using isolated rat hepatocytes with varied extracellular concentrations of [^{14}C]Met or [^{14}C]AdoMet as tracers

Table III. Effect of Dietary Met Supply on Hepatic Amino Acid Concentrations[a]

Amino acid	Dietary Met concentrations		
	0.41% (deficient)	0.61% (adequate)	1.50% (toxic)
Ala	5870 ± 1088	3950 ± 2040	2790 ± 1790
Asp	538 ± 46	724 ± 248	584 ± 137
Gly	1300 ± 448[b]	857 ± 126	336 ± 49[c]
Lys	492 ± 76	442 ± 53	366 ± 43[b]
Met	15 ± 5[b]	37 ± 20	591 ± 369[d]
Orn	230 ± 29[c]	153 ± 26	126 ± 34
Ser	1100 ± 195[c]	308 ± 131	128 ± 35[d]
Thr	612 ± 57[d]	427 ± 88	1450 ± 740[d]
Val	216 ± 24	200 ± 36	131 ± 16[d]

[a] Values indicate nmol/g wet wt of the liver and are mean ± SD of five to seven rats. Significant differences from control (the group fed adequate Met diet) values were detected by Student's *t* test. For other details, see footnote *a* for Table II.
[b] $P < 0.05$.
[c] $P < 0.001$.
[d] $P < 0.01$.

of the corresponding intracellular pools. The results of such studies are generally contradictory because of flaws in experimental design including the oxidation of Met to biologically inactive forms (sulfoxide and sulfone), chemical lability of AdoMet (22), and the capacity of cells to convert degradation products back to AdoMet and to prevent intracellular accumulation of AdoMet catabolites by excreting them into the surrounding medium (23–27). More carefully controlled studies are necessary to demonstrate that free Met and AdoMet pools function independently in cytoplasm.

The kinetics properties of muscle Met adenosyltransferase differ from those of the hepatic enzyme (8) and the rate of AdoMet synthesis cannot be increased by increasing Met availability (14, 15). If the turnover rate of the muscle AdoMet pool is not much slower than that seen in liver, the specific activity of the AdoMet pool should be closely linked to that of the Met pool. Since no estimates of the turnover rate of the muscle AdoMet pool are available, it is possible that the changes in the specific activity of muscle AdoMet following intraperitoneal injection of the radioactive tracer lag behind those of Met. It is very unlikely, however, that the specific activity of the muscle Met pool would still be increasing 60 min after injection. The present approach therefore also yields valid estimates of Met recycling in muscle.

Although absorption of Met from the peritoneal cavity was probably almost completed within 15 min, a continued flow of Met or its metabolites to liver from extrahepatic tissues over a period of 4 hr seems likely (Fig. 1) and may significantly affect the half-life of the specific activity of the AdoMet pool (Fig. 2). In any case, the linear decline of the proportion of AdoMet in the acid-soluble fraction during the first hour after injection is indicative of the irreversible catabolism of Met at a constant rate with a maximal half-life of 66 min. This again supports the view that only a small fraction of Met is catabolized to excretable products as compared with the fraction that is recycled.

Although some details of the pathway converting the ribose moiety of methylthioadenosine to Met still need to be clarified, evidence of the activity and significant role of this salvage route in mammalian cells is convincing (23–27). However, the quantitative importance of this pathway varies greatly depending on the cell type (27). In the tissues of adult animals, the importance of this salvage pathway is likely to be much less than that of the L-homocysteine remethylation cycle. In rat liver, more than 99% of AdoMet is consumed in transmethylation reactions, with the rest being directed to the reactions producing methylthioadenosine (22). Thus, the fairly slow disappearance of the tritium label from the methyl group of hepatic AdoMet, as compared with the expected turnover rate of the AdoMet pool (16, 28), probably indicates a continuous flow of either Met from extrahepatic tissues or methyl groups from hepatic sources rather than hepatic salvage of the methyl groups via methylthioadenosine. Apparently, skeletal muscle has a fairly limited and unadaptable transmethylation ability (14, 15). Slower disappearance of radioactivity from the methyl moiety than from the carbon chain of Met in the acid-soluble fraction (Table II) indicates that the transamination pathway (Fig. 1) is unlikely to play any major role in Met catabolism in muscle. Reversible conversion to 2-keto-4-methylthiobutyrate may, however, occur and thus form a potential store of Met. Apparently, some methylated products formed in or transported to muscle are further catabolized more slowly than homocysteine. Thus, methyl groups may also recycle several times from muscle to liver and vice versa. The half-lives of ^{3}H and ^{14}C labels in the AdoMet pool (Table II) may be significantly longer than the true rate of AdoMet consumption due to significant recycling of both homocysteine and methyl groups at identical rates. This seems unlikely, however, and the data indicating constant mass turnover rate of AdoMet (Table II) in spite of markedly elevated Met levels (15), strongly support the view that Met in the skeletal muscle is mostly catabolized via an unadaptable transmethylation pathway.

Values for the half-life of ^{14}C in the AdoMet pool (Table II) clearly demonstrate the existence of a very efficient and greatly adaptable homocysteine remethylation pathway in the liver. The slight increase in the half-life of the ^{3}H label in the hepatic AdoMet pool with increasing dietary Met supply probably reflects a continuous flow of Met or 2-keto-4-methylthiobutyrate from extrahepatic tissues into liver. The apparent half-lives of ^{3}H and ^{14}C labels in the AdoMet pool are much

longer than the reported rate of AdoMet consumption (5, 16), and thus indicate the ability of the rat to conserve methyl groups and homocysteine, respectively. With aid of the values given in Table II, it can be calculated that the ^{3}H:^{14}C ratios of the hepatic AdoMet pools of animals fed deficient, adequate, and toxic amounts of methionine were 0.8, 0.9, and 1.1 one hr after injection and 0.3, 0.7, and 1.5 two hr after injection, respectively. The corresponding ratios in the acid-soluble metabolite pools, corrected for the shares of AdoMet, were 12.6, 11.3, and 7.2 one hr after injection and 11.3, 17.1, and 5.2 two hr after injection, respectively. The ^{3}H:^{14}C ratios of the hepatic metabolite pools of animals fed the deficient, adequate, and toxic amounts of methionine were, therefore 16, 13, and 7 times greater than the corresponding ratios for the AdoMet pools 1 hr after injection and 38, 24, and 3 times greater 2 hr after injection. These values indicate that the hepatic salvage of homocysteine to AdoMet is markedly adaptable to Met supply and may repeatedly recycle the carbon chain of Met also when Met is present in excess of requirements. It can similarly be calculated that the ^{3}H:^{14}C ratios of the muscle metabolite pools of animals fed deficient, adequate, and toxic amounts of Met were three, two, and one times greater than the corresponding values for AdoMet 1 hr after injection and five, two, and one times greater 2 hr after injection. The capacity of muscle to conserve Met by homocysteine remethylation is therefore very modest compared with that of liver.

The importance of liver in the overall catabolism or salvage of Met is demonstrated in Table III. Although the dietary manipulation with Met was done at the expense of glutamic acid only, marked changes in the hepatic concentrations of glycine, serine, and threonine were seen. These changes can be explained as an indication of the adaptation to extra Met through increased hepatic glycine methylation due to an unresolved mechanism such as altered threonine aldolase activity (15). In general, the present data are in good accordance with earlier results of the response of homocysteine utilizing pathways to variations in Met supply (6).

The present work demonstrates a practical way to simultaneously study the flow rates and salvage efficiencies of different moieties of the Met molecule in the whole animal. When used in combination with dietary studies, feeding with a stomach tube is recommended to avoid the effects of individual eating habits. Due to a continuous tissue to tissue flow of metabolites involved in Met metabolism, the data should be interpreted with caution, especially when only a single label is used in the Met molecule (7, 29). The earlier attempt to investigate adaptation to Met excess through the use of a simulated *in vitro* regulatory locus model (7) was very complicated and technically difficult and the resulting data were subject to errors of interpretation. Although the authors found inverse correlations between hepatic serine and dietary Met concentrations, agreeing with the present results, their observations regarding the effects of dietary Met on hepatic concentrations of Met, AdoMet, and adenosylhomocysteine (7) differ significantly from our previous findings (14, 15). Increased accumulation of adenosylhomocysteine before any visible changes in AdoMet or Met concentrations (7) suggest that post-mortem reactions (21) and fasting have biased the data used for construction of the *in vitro* model system. Moreover, the *in vitro* system does not include substrates of the quantitatively most important methyltransferases (21) and is devoid of the membrane-bound enzymes needed for the oxidation of homocysteine catabolites (30). These complications are avoided by the use of whole animals in the present method. This allows monitoring of metabolic pathways without disruption of metabolite fluxes.

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