

Tissue Distribution of *S*-Adenosylmethionine and *S*-Adenosylhomocysteine in the Rat

EFFECT OF AGE, SEX AND METHIONINE ADMINISTRATION ON THE METABOLISM OF *S*-ADENOSYLMETHIONINE, *S*-ADENOSYLMETHIONINE AND POLYAMINES

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The tissue distribution of *S*-adenosylmethionine, *S*-adenosylhomocysteine, methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase was explored in the rat. Also the effects of methionine administration on the accumulation of *S*-adenosylmethionine, *S*-adenosylhomocysteine and polyamines were studied in rat liver, brain and kidney. The tissue distribution of *S*-adenosylmethionine, *S*-adenosylhomocysteine, methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase was similar in both sexes, and was only slightly changed with age. The specific activity of *S*-adenosylhomocysteine hydrolase greatly exceeded that of methionine adenosyltransferase, and the concentration of *S*-adenosylmethionine was higher than that of *S*-adenosylhomocysteine in all tissues examined. However, the hepatic *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio was dependent on food supply and on the age of the animal. No correlation was noticed between the activity of methionine adenosyltransferase and the concentrations of the adenosyl compounds in different tissues. Intraperitoneal administration of methionine resulted in a profound but transient increase in the hepatic concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine. The concentration of *S*-adenosylmethionine was elevated also in the brain during the first 2 h after methionine injection. The rise of *S*-adenosylmethionine concentration after methionine treatment could be diminished by simultaneous glycine administration. The results support the view that the rate-limiting factor of *S*-adenosylmethionine synthesis is the tissue concentration of methionine. They further suggest that glycine *N*-methyltransferase may have a regulatory role in the utilization of *S*-adenosylmethionine in the liver.

S-Adenosyl-L-methionine is the sole methyl donor for all mammalian transmethylases except those involved in the biosynthesis of methionine (Cantoni, 1975; Fuller, 1976). Most of the *S*-adenosylmethionine is rapidly utilized by a variety of transmethylation reactions (Finkelstein, 1974), yielding *S*-adenosyl-L-homocysteine, a potent inhibitor of most methyl-transferring enzymes (for references see Walker & Duerre, 1975). *S*-Adenosylhomocysteine is further cleaved to adenosine and L-homocysteine by a specific hydrolase (Lombardini & Talalay, 1971), provided that the reaction products do not accumulate (de la Haba & Cantoni, 1959; Cortese *et al.*, 1974). Adenosine is effectively metabolized to uric acid (Cortese *et al.*, 1974), and homocysteine can be either remethylated or irreversibly converted via cystathionine into cysteine and other metabolites (Finkelstein, 1974). The latter reaction chain, the trans-sulphuration pathway, is also the major route for methionine catabolism in mammals (Finkelstein & Mudd, 1967; Finkelstein, 1974). In addition to these metabolic functions, *S*-adenosylmethionine acts also as the propylamine-group donor in the bio-

synthesis of polyamines (for references see Raina & Jänne, 1975).

Owing to the central role of *S*-adenosylmethionine in animal metabolism, and to the inhibitory effects of *S*-adenosylhomocysteine on many important trans-methylases, factors affecting the accumulation of these adenosyl compounds are of special interest. Tissue distribution and the effects of various hormones, drugs and other treatments on the activities of methionine adenosyltransferase (for references see Mudd, 1973) and *S*-adenosylhomocysteine hydrolase (Finkelstein & Harris, 1973) are fairly well known. However, these enzyme activities probably do not correlate with the tissue concentrations of the adenosyl compounds (cf. Eloranta *et al.*, 1976b). The contents of *S*-adenosylmethionine (Baldessarini & Kopin, 1963; Salvatore *et al.*, 1971; Lombardini & Talalay, 1973) and especially that of *S*-adenosylhomocysteine (Salvatore *et al.*, 1971) in different tissues have not been thoroughly explored. Methodological improvements (Hoffman, 1975; Eloranta *et al.*, 1976b) have significantly simplified the analysis of these compounds.

In the present work the metabolism of *S*-adenosylmethionine and the effect of methionine administration on it have been studied in several rat tissues. The results demonstrate great differences in the contents of *S*-adenosylmethionine and *S*-adenosylhomocysteine and in the activities of methionine adenosyltransferase (EC 2.5.1.6) and *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1) in different tissues. They further show that the accumulation of these compounds can be markedly affected by exogenous methionine and glycine.

Experimental

Chemicals

L-[*Me*-¹⁴C]Methionine (sp. radioactivity 51.2 mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. DL-[1-¹⁴C]Ornithine monohydrochloride (32.2 mCi/mmol) and DL-[1-¹⁴C]methionine (8.66 mCi/mmol) were from New England Nuclear Corp., Boston, MA, U.S.A. DL-[1-¹⁴C]Ornithine was treated with HCl (Hölttä & Raina, 1973) before use. Carboxy-¹⁴C-labelled and unlabelled *S*-adenosylmethionine were synthesized essentially as described by Pegg & Williams-Ashman (1969). *S*-[8-¹⁴C]Adenosylhomocysteine was prepared as described by Eloranta *et al.* (1976b). L-Homocysteine was prepared from L-homocysteine thiolactone as described by Duerre & Miller (1966). Other chemicals and materials have been described elsewhere (Eloranta *et al.*, 1976b).

Animals

Albino rats of the Wistar strain were used and were maintained under regularly alternating 12 h periods of light and dark and allowed to eat (a standard diet made by Hankkija Oy, Turku, Finland) and drink *ad libitum*. All the animals were killed between 08:00 and 10:00 h to minimize diurnal variation in enzyme activities.

Assay methods

The rats were decapitated under ether anaesthesia, and the tissues were rapidly removed and cut into two pieces. One piece was homogenized with an Ultra-Turrax (Janke and Kunkel KG, Staufen i. Breisgau, Germany) homogenizer in 2–6 vol. of 10% (w/v) trichloroacetic acid containing 1–3 nCi of both *S*-[8-¹⁴C]adenosyl-L-homocysteine and *S*-adenosyl-L-[*Me*-¹⁴C]methionine/ml. If not homogenized immediately the piece was rapidly frozen in liquid N₂ and stored at –80°C. After centrifugation at 10000g for 15 min at 7°C, the supernatant was used for the analysis of *S*-adenosylhomocysteine, *S*-adenosyl-

methionine and polyamines. The other piece was washed in ice-cold 0.25 M-sucrose and homogenized with a Potter–Elvehjem homogenizer in 2–6 vol. of ice-cold 0.25 M-sucrose containing 1 mM-2-mercaptoethanol, 0.1 mM-EDTA and 1 mM-dithiothreitol, and used for enzyme assays.

S-Adenosylhomocysteine and *S*-adenosylmethionine were analysed from the trichloroacetic acid supernatants (corresponding to 1–10 g of wet tissue) by the phosphocellulose-column-chromatographic method of Eloranta *et al.* (1976b). The adenosyl compounds were eluted in 5 ml fractions with 50 mM- and 0.5 M-HCl respectively. The specificity of the method for different tissues was checked electrophoretically (Eloranta *et al.*, 1976b), and was found to be acceptable for all tissues except the spleen and kidneys. The *S*-adenosylhomocysteine or *S*-adenosylmethionine preparations of these two tissues were contaminated by unknown u.v.-absorbing material, and were therefore analysed electrophoretically as previously described (Eloranta *et al.*, 1976b).

Polyamines were extracted from the trichloroacetic acid supernatants into alkaline butan-1-ol, separated electrophoretically (Raina, 1963) and determined by the ninhydrin method (Raina *et al.*, 1967).

Protein was determined from the 105000 g_{av.} supernatants by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

All the enzyme activities were assayed as a routine in the 105000 g_{av.} supernatant fractions of the homogenates (Eloranta *et al.*, 1976b). Occasionally the activities of methionine adenosyltransferase were also checked with dialysed enzyme preparations. Dialysis was performed overnight at 4°C against 400 vol. of 30 mM-potassium phosphate (pH 6.9) containing 1 mM-2-mercaptoethanol, 0.1 mM-EDTA and 0.1 mM-dithiothreitol. The activity of methionine adenosyltransferase (EC 2.5.1.6) was determined by the phosphocellulose-paper method of McKenzie & Gholson (1973), essentially as described earlier (Eloranta *et al.*, 1976a). The specific radioactivity of the substrate, 2 mM-L-[*Me*-¹⁴C]methionine, was 0.5 μCi/μmol for liver preparations and 2.0 μCi/μmol for the enzyme preparations of other tissues. The assay method was further modified so that only one phosphocellulose disc was used for each analysis and up to 40 discs were washed together with 3 × 2 litres of water. The washings were performed in a large plastic beaker by using gentle mechanical stirring for 5 min for each wash.

The activity of *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1) was assayed by the electrophoretic method of Kajander *et al.* (1976), and that of *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) by that of Pegg & Williams-Ashman (1968) with minor modifications as previously described (Eloranta *et al.*, 1976a).

Table 1. Stability of endogenous S-adenosylmethionine and S-adenosylhomocysteine in liver preparations

Male rats aged 8 weeks were fed *ad libitum* until decapitation under light ether anaesthesia. Livers were rapidly removed and cut into pieces. The control pieces (Expts. I and II) were directly homogenized with an Ultra-Turrax homogenizer in 3 vol. of 10% (w/v) trichloroacetic acid containing $S\text{-}[8\text{-}^{14}\text{C}]\text{adenosylhomocysteine}$ and $S\text{-adenosyl-L-[Me-}^{14}\text{C]methionine}$ (both 3 nCi/ml) as internal standards. Portions of pooled homogenates, each obtained from two livers, containing 3 g of wet tissue, were centrifuged (at 10000g for 15 min at 4°C) and analysed as described in the Experimental section. Other pieces were treated similarly after the storage period indicated. In Expt. III livers were immediately homogenized in 3 vol. of ice-cold 0.25 M-sucrose containing 1 mM-mercaptoethanol, 0.1 mM-EDTA and 1 mM-dithiothreitol. After storage in an ice-bath for the periods indicated, portions of pooled homogenates, each derived from two livers, were thoroughly mixed with the internal standards and with an appropriate volume of 50% (w/v) trichloroacetic acid to give a final concentration of 8%. The analysis was then performed as described in the Experimental section. All the values are means of two pooled samples, each obtained from two livers.

Expt. no.	Method of storage	S-Adenosylmethionine		S-Adenosylhomocysteine (nmol/g of wet liver)	S-Adenosylmethionine + S-adenosylhomocysteine (nmol/g of wet liver)
		(nmol/g of wet liver)	Degradation (%)		
I	Control	68.6	—	19.1	87.7
	Frozen (liquid N ₂) tissue at -80°C for 5 days	67.5	1.6	20.5	88.0
II	Control	74.8	—	21.5	96.3
	Tissue in ice-cold 0.25 M-sucrose for 15 min 60 min	71.7 46.4	4.1 38.0	28.0 44.4	99.7 90.8
III	Sucrose homogenate in ice-bath for 0 min	61.2	—	36.5	97.7
	10 min	29.5	51.8	61.0	90.5
	30 min	21.6	64.7	67.8	89.4

Results

Stability of endogenous S-adenosylmethionine in tissue preparations

Endogenous *S*-adenosylmethionine was observed to be degraded very rapidly in the sucrose homogenates of liver. More than 50% was decomposed within 10 min (Table 1) and even the zero-time value (Expt. III) was clearly abnormally low (cf. Expts. I and II), suggesting significant degradation already during homogenization. The degradation occurred also in whole tissues (Expt. II), although at a significantly slower rate. When the tissue was immediately frozen in liquid N₂ no degradation was noticed during further storage at -80°C for several days. This was true also for other tissues. Thus to avoid any significant decomposition of *S*-adenosylmethionine, tissues had to be either frozen quickly or homogenized directly in acid within 5–10 min after the death of the animal.

The degradation product of *S*-adenosylmethionine was inseparable from endogenous *S*-adenosylhomocysteine by the column-chromatographic method used, by paper electrophoresis (cf. Kajander *et al.*, 1976) in 50 mM-glycine/NaOH buffer (pH 11), or by ascending paper chromatography with butan-1-ol/acetic acid/water (2:1:1, by vol.). Thus endogenous *S*-adenosylmethionine seems to be rapidly cleaved enzymically to *S*-adenosylhomocysteine, especially in liver homogenate prepared in sucrose solution. This enzyme activity was high also in the supernatant fractions of liver and kidney homogenates (results not shown).

Tissue distribution of S-adenosylmethionine, S-adenosylhomocysteine, methionine adenosyltransferase and S-adenosylhomocysteine hydrolase

The concentration of *S*-adenosylmethionine exceeded that of *S*-adenosylhomocysteine in all the tissues examined (Table 2). The concentrations for the adenosyl compounds were also checked electrophoretically (see the Experimental section). Contaminating u.v.-absorbing material was noticed in the *S*-adenosylmethionine preparation of the kidneys and in the *S*-adenosylhomocysteine preparation of the spleen. In epididymal fat the concentrations of the adenosyl compounds were too low to be accurately determined from up to 10 g of tissue.

The specific activity of methionine adenosyltransferase varied profoundly between different tissues (Table 2). This variation did not correlate with the concentrations of *S*-adenosylmethionine in the tissues examined. Though the activity of methionine adenosyltransferase was 40–200 times higher in the liver than in the kidneys, the prostate, the adrenals, the spleen or the heart, differences in the

Table 2. *Distribution of S-adenosylmethionine, S-adenosylhomocysteine, methionine adenosyltransferase and S-adenosylhomocysteine hydrolase in rat tissues*
Male rats aged 6 weeks and fed *ad libitum* were used for analysis. The values represent one pooled preparation or the mean (±s.d.) of three pooled preparations, each obtained from the number of organs indicated in parentheses. For details see the Experimental section.

Tissue	<i>S</i> -Adenosylmethionine (nmol/g of wet tissue)	<i>S</i> -Adenosylhomocysteine (nmol/g of wet tissue)	Methionine adenosyltransferase (pmol/min per mg of protein)	<i>S</i> -Adenosylhomocysteine hydrolase (nmol/min per mg of protein)
Adrenals (25)	51.5	16.1	86	11.7
Brain (4)	25.4±0.9	3.4±1.2	43±10	11.6±1.5
Epididymal fat (6)	<4.0	<2.0	22	7.7
Heart (17)	38.5	3.9	42	6.3
Kidneys (8)	47.2*	22.5±3.4	192±36	25.0±0.0
Liver (2)	67.5±1.1	43.8±3.2	7700±160	51.1±6.5
Lungs (4)	31.2±1.3	3.7±0.3	38±11	5.9±1.1
Pancreas (20)	39.8	11.4	558	59.9
Skeletal muscle (4)	22.7±2.1	4.7±0.7	13	9.4
Small intestine (4)	32.6	3.7	83	10.5
Spleen (14)	42.2*	6.4*	65	10.0
Testes (11)	21.3	5.9	70±6	7.0±0.9
Ventral prostate (25)	59.5	10.4	141	4.7

* Determined after electrophoretic purification.

Table 3. Effect of age on the concentrations of S-adenosylmethionine and S-adenosylhomocysteine and on the activities of methionine adenosyltransferase and S-adenosylhomocysteine hydrolase in rat brain and liver

Male rats fed *ad libitum* were used. The values for newborn animals were determined from a pooled preparation obtained from 15 organs. All the other values are means \pm S.D. of three preparations, each derived from two to four organs. For assay methods see the Experimental section.

Age (weeks)	S-Adenosylmethionine (nmol/g of wet tissue)	S-Adenosylhomocysteine (nmol/g of wet tissue)	Methionine adenosyltransferase (pmol/min per mg of protein)	S-Adenosylhomocysteine hydrolase (nmol/min per mg of protein)
Brain				
0	39.0	4.6	130	11.4
2	35.9 \pm 0.6	2.6 \pm 0.8	72 \pm 26	11.1 \pm 0.9
6	25.4 \pm 0.9	3.4 \pm 1.2	43 \pm 10	11.6 \pm 1.5
12	22.1 \pm 0.8	2.5 \pm 1.1	30 \pm 12	11.8 \pm 2.2
29	21.9 \pm 1.3	3.4 \pm 0.4	39 \pm 5	11.1 \pm 0.6
Liver				
0	69.7	16.5	6530	48.3
2	78.4 \pm 1.6	22.6 \pm 1.8	7770 \pm 320	53.8 \pm 2.0
6	67.5 \pm 1.1	43.8 \pm 3.2	7700 \pm 160	51.1 \pm 6.5
12	76.1 \pm 4.9	35.1 \pm 3.0	6910 \pm 340	68.1 \pm 2.5
29	67.0 \pm 1.7	33.4 \pm 6.9	7710 \pm 710	73.9 \pm 5.5

Table 4. Effect of methionine on the hepatic synthesis and accumulation of S-adenosylmethionine and S-adenosylhomocysteine

Unstarved female rats (age 6 weeks, weight 120 g) were injected intraperitoneally with 100 mg of L-methionine/kg body wt. in 0.5 ml of 0.9% (w/v) NaCl 0.5–12 h before being killed. The animals treated for 72 h received 50 mg of L-methionine/kg body wt. twice per day, the last injection being given 30 min before death. The control animals received the solvent 30 min before being killed. All the values are means \pm S.D. of three or four preparations, each obtained from two livers. For the significance of the differences (based on Student's *t* test) compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Time of treatment (h)	S-Adenosylmethionine (nmol/g of wet liver)	S-Adenosylhomocysteine (nmol/g of wet liver)	Methionine adenosyltransferase (nmol/min per mg of protein)	S-Adenosylhomocysteine hydrolase (nmol/min per mg of protein)
Control	65.5 \pm 1.2	45.8 \pm 3.9	5.4 \pm 0.4	45.6 \pm 3.2
0.5	364.4 \pm 6.6***	114.5 \pm 23.8**	4.6 \pm 0.3*	42.9 \pm 5.1
2	78.0 \pm 14.5	57.4 \pm 2.8**	5.0 \pm 0.2	42.7 \pm 1.2
12	70.5 \pm 7.9	50.2 \pm 8.6	5.2 \pm 0.2	42.0 \pm 6.0
72	150.6 \pm 30.3**	82.5 \pm 3.0**	5.4 \pm 0.1	38.8 \pm 5.6

corresponding concentrations of *S*-adenosylmethionine were less than twofold for these tissues.

Differences in the activities of *S*-adenosylhomocysteine hydrolase in different tissues were less than 20-fold. Further, the specific activity of the hydrolase was much higher than that of methionine adenosyltransferase in all tissues.

Effect of age and sex

The tissue distributions of the enzymes and the concentrations of the adenosyl compounds concerned were similar in both sexes (results not shown). In the brain the concentration of *S*-adenosylmethionine and the activity of methionine adenosyltransferase decreased with age (Table 3). In the liver they remained fairly unchanged, but the concentration of *S*-adenosylhomocysteine and the activity of *S*-adenosylhomocysteine hydrolase tended to increase (Table 3). The changes in the activities of both enzymes were rather small in the kidneys and in skeletal muscle, though somewhat higher values were observed in newborn than in old animals (results not shown).

Effect of administration of methionine and glycine

A single injection of methionine markedly increased the accumulation of *S*-adenosylmethionine in the liver (Table 4). The rise in *S*-adenosylhomocysteine concentration was smaller. The activities of methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase remained fairly unchanged (Table 4). The same was true for *S*-adenosylmethionine decarboxylase (results not shown). The increase in the contents of the adenosyl compounds was very sharp (Fig. 1) and dependent on the methionine dose (results not shown). When 0.7 mmol of L-methionine/kg body wt. was administered, the concentrations of the adenosyl compounds reached their maximum value within 20 min (Fig. 1) and decreased almost to the control value within 2 h after the treatment (Table 4). The concentration of *S*-adenosylmethionine, but not that of *S*-adenosylhomocysteine, was increased by about 50% also in the brain within 30 min after methionine injection, but neither compound showed any significant change in the kidneys within 2 h after the treatment (results not shown). Methionine adenosyltransferase, *S*-adenosylhomocysteine hydrolase and *S*-adenosylmethionine decarboxylase activities and the concentrations of the polyamines spermidine and spermine remained fairly constant in these three tissues (liver, brain and kidney). The apparent small decrease in the hepatic activity of methionine adenosyltransferase at 30 min after methionine injection (Table 4) is probably due to a greatly elevated liver methionine concentration, diluting the radioactive substrate used in the assay. When dialysed enzyme preparations were used, no difference was noticed in the activities of methionine

adenosyltransferase between the control group and the groups treated with methionine (results not shown).

When equimolar amounts of glycine and methionine were injected simultaneously, the increase in *S*-adenosylmethionine concentration in liver and in brain was significantly lower than with methionine alone (results not shown). This effect became more obvious when a greater amount of glycine was used (Fig. 1). The concentration of *S*-adenosylhomocysteine was not significantly increased, however (cf. injection of methionine alone; Fig. 1). Glycine alone had no significant effect on the concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine in the liver (Fig. 1). The uptake of methionine into the liver was not affected by glycine, as shown by intraperitoneal administration of L-[Me-¹⁴C]methionine (100 mg/kg body wt.) with or without glycine (100 mg/kg body wt.). Both the total and acid-soluble radioactivities in liver homogenates were similar in both groups of animals at 20 or 40 min after the treatment (results not shown).

The activities of methionine adenosyltransferase, *S*-adenosylhomocysteine hydrolase and *S*-adenosylmethionine decarboxylase remained unchanged in the liver, in the brain and in the kidneys after a simultaneous injection of methionine and glycine (results not shown).

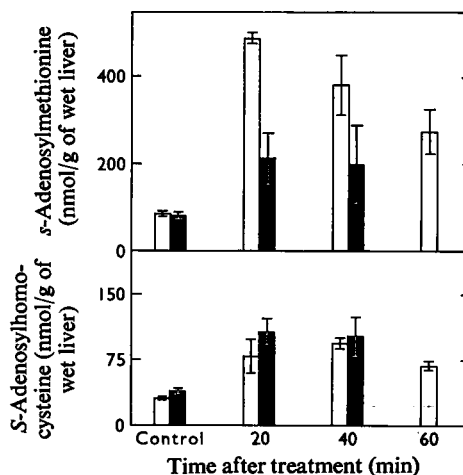


Fig. 1. Effect of glycine on the accumulation of *S*-adenosylmethionine and *S*-adenosylhomocysteine in the livers of methionine-treated rats

Unstarved female rats (age 10 weeks, weight 160 g) were injected intraperitoneally with 100 mg of L-methionine with (black columns) or without (white columns) 100 mg of glycine/kg body wt. in 0.5 ml of 0.9% (w/v) NaCl 20–60 min before death. One control group received the solvent alone (white column) and the other 100 mg of glycine/kg body wt. (black column) 20 min before death. The columns indicate means \pm s.d. of four livers.

To determine whether increased tissue concentrations of methionine, glycine, *S*-adenosylmethionine or *S*-adenosylhomocysteine had any effect on these enzyme-activity determinations, some assays were made *in vitro*. Neither methionine nor glycine up to 1 mM concentration had any effect on the hepatic activity of methionine adenosyltransferase, *S*-adenosylhomocysteine hydrolase and *S*-adenosylmethionine decarboxylase. The last-mentioned enzyme activity was not affected by either 1 mM-*S*-adenosylmethionine or 1 mM-*S*-adenosylhomocysteine. Neither was the activity of methionine adenosyltransferase affected by 1 mM-*S*-adenosylhomocysteine, whereas 1 mM-*S*-adenosylmethionine produced about 15% inhibition. However, even the highest *S*-adenosylmethionine concentrations (500 nmol/g) measured in this work were diluted to less than 0.05 mM in the enzyme assays. Since 0.1 mM-*S*-adenosylmethionine was not inhibitory *in vitro*, it is obvious that even the increased concentration of endogenous *S*-adenosylmethionine could not interfere with the methionine adenosyltransferase assay.

About 50% inhibition of hepatic *S*-adenosylhomocysteine hydrolase activity was produced by 1 mM-*S*-adenosylhomocysteine and about 40% inhibition by 1 mM-*S*-adenosylmethionine, whereas 0.1 mM concentrations of the adenosyl compounds produced less than 20% inhibitions *in vitro*. Since in the routine assays the concentrations of endogenous *S*-adenosylmethionine and *S*-adenosylhomocysteine always remained less than 1 μ M, these could not significantly interfere with the assay.

Discussion

The tissue distribution of *S*-adenosylmethionine and especially that of *S*-adenosylhomocysteine have not been extensively studied previously, although the concentration range of these compounds is known for many tissues (Baldessarini & Kopin, 1963; Salvatore *et al.*, 1971). The concentrations of *S*-adenosylmethionine reported by Salvatore *et al.* (1971) agree well with the present results, whereas those reported by Baldessarini & Kopin (1963) are probably somewhat too high, owing to an inaccurate molar extinction coefficient (Baldessarini, 1975). This might be true also for the concentrations reported by Lombardini & Talalay (1973), although differences in the strains of rats used might be of relevance in explaining why the values are clearly higher than the present ones. The concentrations of *S*-adenosylhomocysteine have been reported only for a few rat tissues (Salvatore *et al.*, 1971), and the values measured significantly exceed those reported in the present work. In the liver, the concentration of *S*-adenosylhomocysteine is dependent on age. Also the nutritional status of the rat might have effects

on the hepatic concentration ratio *S*-adenosylmethionine/*S*-adenosylhomocysteine, as reported in the mouse (Hoffman, 1975). Since the total capacity of methyltransferases exceeds that of methionine adenosyltransferase (Kerr, 1974) and since many nutrients are metabolized by methyl-transfer reactions in the liver (Hoffman, 1975), the acceleration of hepatic *S*-adenosylmethionine utilization in fed animals could result in lower *S*-adenosylmethionine and higher *S*-adenosylhomocysteine concentrations. On the other hand, an increase in the methionine supply through the diet may increase the synthesis of *S*-adenosylmethionine. In general, the tissue concentrations of *S*-adenosylhomocysteine are lower than those of *S*-adenosylmethionine.

Except for liver and pancreas, the specific activities of methionine adenosyltransferase determined in the present work are in good accord with those reported by Mudd *et al.* (1965). Since the activity resides totally in the soluble cytosol fraction (Sheid & Bilik, 1968), the lower activities in liver and pancreas reported by Mudd *et al.* (1965) might be due to the presence of non-cytosolic proteins in their enzyme preparations. Although the hepatic activity of methionine adenosyltransferase has been reported to be lower in males than in females, probably owing to androgenic action (Natori, 1963), the present work revealed no marked sex difference in any tissues of fed young rats. Also age had only a small effect on the enzyme activity in several tissues, which disagrees with the report of Sheid & Bilik (1968) but agrees well with that of Finkelstein (1967).

S-Adenosylhomocysteine, a potent transmethylase inhibitor, is probably catabolized very rapidly in tissues by *S*-adenosylhomocysteine hydrolase [for references see Finkelstein (1974) and Eloranta *et al.* (1976b)]. The inhibitory effect of adenosine and homocysteine on the hydrolytic reaction *in vitro* (Finkelstein & Harris, 1975) is probably not significant *in vivo*, since both these degradation products are catabolized further, and do not accumulate under physiological conditions [for references see Finkelstein (1974) and Eloranta *et al.* (1976b)]. The inhibition of the synthetic reaction by *S*-adenosylmethionine, reported above to occur *in vitro*, might be due to a significant conversion of *S*-adenosylmethionine into *S*-adenosylhomocysteine under these conditions, since liver enzyme preparations have ample transmethylase activity (Kerr, 1974). It is noteworthy that the activity of *S*-adenosylhomocysteine hydrolase exceeded that of methionine adenosyltransferase in all the tissues examined. As the enzymes of both the trans-sulphuration pathway (Finkelstein, 1967) and the adenosine degradation route (Cortese *et al.*, 1974) reveal still higher activities than *S*-adenosylhomocysteine hydrolase, tissues seem to be provided with a sufficient capacity to prevent great changes in the accumulation of

S-adenosylhomocysteine. Although in some rat tissues, e.g. lung, heart and testes, the activities of the enzymes of the trans-sulphuration pathway are low or absent (Finkelstein, 1974), these tissues might have effective alternative routes for the consumption of homocysteine (Finkelstein, 1974) or *S*-adenosylhomocysteine (Schlenk & Zydek, 1968; Duerre *et al.*, 1969).

The tissue distribution of *S*-adenosylhomocysteine hydrolase reported in this work closely agrees with the results of Finkelstein & Harris (1973, 1975), but disagrees with those of Walker & Duerre (1975). The possible reason for this inconsistency is discussed elsewhere (Kajander *et al.*, 1976).

Although methionine concentrations have not been measured in the present experiments, it is probable that the accumulation of *S*-adenosylmethionine follows changes in tissue methionine concentrations. The observed rapid dose-dependent accumulation of *S*-adenosylmethionine in the liver and its fairly rapid decrease to the control value after methionine injection, support this conclusion. The less-prominent effect of methionine administration on the accumulation of *S*-adenosylmethionine in tissues other than the liver (see also Lombardini & Talalay, 1971) might be due to differences in methionine uptake (Tudball & Griffiths, 1976). Owing to the excretion in urine and the uptake into liver cells, circulating methionine concentrations are probably not increased sufficiently to provide other tissues with a methionine load such as in liver.

The ability of glycine to diminish the hepatic accumulation of *S*-adenosylmethionine in methionine-treated animals cannot be explained by decreased uptake of methionine into liver cells (see above). Thus glycine appears to affect the metabolism of *S*-adenosylmethionine by increasing the consumption of this compound. This would support the view that glycine *N*-methyltransferase, and possibly also other competing methyl-transfer systems, e.g. nicotinamide *N*-methyltransferase (for references see Kerr, 1974), might have a regulatory role in the utilization of *S*-adenosylmethionine, at least in the liver. Although glycine *N*-methyltransferase represents only one of numerous methyl-transferring enzymes, its capacity to utilize *S*-adenosylmethionine in liver is approximately the same as the synthesizing capacity of methionine adenosyltransferase (Kerr, 1974). It is noteworthy that the concentrations of glycine (1 mM) and *S*-adenosylmethionine (50 μ M) in the liver correspond to the respective K_m values of glycine *N*-methyltransferase (Kerr, 1972, 1974), whereas the methionine concentration (40–80 μ M) is significantly lower than the corresponding K_m value of methionine adenosyltransferase (for references see Eloranta *et al.*, 1976b). Thus it is obvious that when the concentration of methionine is increased, the rate of *S*-adenosylmethionine synthesis in liver is increased more than

its utilization by glycine *N*-methyltransferase. If the latter enzyme or other competing enzyme systems are required in maintaining tissue *S*-adenosylmethionine concentrations within narrow ranges, markedly increased methionine concentrations could be expected to increase the accumulation of *S*-adenosylmethionine to a greater extent than that of *S*-adenosylhomocysteine, since the latter can be metabolized more efficiently by *S*-adenosylhomocysteine hydrolase (Kerr, 1974). This is just what seems to happen after methionine injection. Also the increased activity of glycine *N*-methyltransferase in old animals (Kerr, 1974) is in line with the present finding showing that *S*-adenosylhomocysteine concentration increases with age. However, the possible changes in the degradation rates of adenosine and homocysteine must also be taken into consideration, though at least the trans-sulphuration pathway seems to become even more active with age (Finkelstein, 1967).

Although methionine administration caused a sharp increase in liver *S*-adenosylmethionine concentration, not even a prolonged methionine deprivation had any significant effect on it (T. O. Eloranta & A. M. Raina, unpublished work). This might indicate that the remethylation of homocysteine (Finkelstein, 1974) is able to maintain the liver methionine concentration fairly constant under these circumstances. In fact, the K_m values of the enzymes responsible for homocysteine degradation or remethylation favour remethylation when tissue homocysteine formation is decreased (Finkelstein, 1974).

In conclusion, the increased accumulation of both *S*-adenosylmethionine and *S*-adenosylhomocysteine seems primarily to be dependent on the methionine supply. Normally this supply is probably maintained sufficiently high by an effective homocysteine remethylation to provide fairly constant *S*-adenosylmethionine concentrations. When tissues are overloaded with exogenous methionine, the rate of *S*-adenosylmethionine synthesis can exceed the rate of its utilization. Competing methyltransferases may be involved in the control of its utilization. At least in the liver, glycine *N*-methyltransferase is such an enzyme, owing to its high activity and fairly good resistance to the inhibitory effect of *S*-adenosylhomocysteine (Kerr, 1972). The accumulation of *S*-adenosylmethionine does not seem to cause any significant changes in the synthesis and accumulation of polyamines. It is probable that tissue *S*-adenosylmethionine concentration plays no primary role in the regulation of polyamine metabolism.

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