***S*-Adenosylmethionine Metabolism and its Relation to Polyamine Synthesis in Rat Liver**

EFFECT OF NUTRITIONAL STATE, ADRENAL FUNCTION, SOME DRUGS
AND PARTIAL HEPATECTOMY

By TERHO O. ELORANTA and AARNE M. RAINA
*Department of Biochemistry, University of Kuopio, P.O. Box 138,
SF-70101 Kuopio 10, Finland*

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S-Adenosylmethionine metabolism and its relation to the synthesis and accumulation of polyamines was studied in rat liver under various nutritional conditions, in adrenalectomized or partially hepatectomized animals and after treatment with cortisol, thioacetamide or methylglyoxal bis(guanyldrazone) {1,1'-[(methylethanediyldine)dinitrilo]diguanidine}. Starvation for 2 days only slightly affected *S*-adenosylmethionine metabolism. The ratio of spermidine/spermine decreased markedly, but the concentration of total polyamines did not change significantly. The activity of *S*-adenosylmethionine decarboxylase initially decreased and then increased during prolonged starvation. This increase was dependent on intact adrenals. Re-feeding of starved animals caused a rapid but transient stimulation of polyamine synthesis and also increased the concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine. Similarly, cortisol treatment enhanced the synthesis of polyamines, *S*-adenosylmethionine and *S*-adenosylhomocysteine. Feeding with a methionine-deficient diet for 7–14 days profoundly increased the concentration of spermidine, whereas the concentrations of total polyamines and of *S*-adenosylmethionine showed no significant changes. The results show that nutritional state and adrenal function play a significant role in the regulation of hepatic metabolism of *S*-adenosylmethionine and polyamines. They further indicate that under a variety of physiological and experimental conditions the concentrations of *S*-adenosylmethionine and of total polyamines remain fairly constant and that changes in polyamine metabolism are not primarily connected with changes in the accumulation of *S*-adenosylmethionine or *S*-adenosylhomocysteine.

Although biological transmethylation and the polyamine-biosynthetic pathway share a common substrate, i.e. *S*-adenosylmethionine, little is known about the relationship between polyamine and *S*-adenosylmethionine metabolism. Several recent observations indicate that polyamines may play an essential role in cellular metabolism, e.g. in the synthesis of macromolecules (for references see Raina & Jänne, 1975; Tabor & Tabor, 1976). Because polyamine synthesis and biological methylations are connected via a common substrate, it seemed desirable to explore whether these two important metabolic routes have common regulatory features.

Methionine adenosyltransferase (EC 2.5.1.6) and *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1) are involved in the conversion of methionine, via *S*-adenosylmethionine and its metabolite *S*-adenosylhomocysteine, into homocysteine, which can enter the trans-sulphuration pathway, the main catabolic route for methionine in animal tissues (Finkelstein,

1974). Although some factors, such as the nutritional state of the animal and treatment with corticoids, have been reported to affect the hepatic activities of methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase (Finkelstein, 1967; Pan & Tarver, 1967; Pan *et al.*, 1968; Finkelstein & Harris, 1973), as well as the hepatic synthesis and accumulation of polyamines in the rat (Panko & Kenney, 1971; Domschke & Söling, 1973), mouse (Seiler *et al.*, 1969) and chick (Eloranta *et al.*, 1976a), the effects of nutritional state and adrenal function on the tissue concentration of *S*-adenosylmethionine and *S*-adenosylhomocysteine are poorly known at the present time. Developments in analytical techniques (Hoffman, 1975; Eloranta *et al.*, 1976b) have made it possible to explore these aspects of *S*-adenosylmethionine metabolism in greater detail.

In the present work the relationship of hepatic *S*-adenosylmethionine and polyamine metabolism has been studied under a variety of conditions expect-

ted to change the synthesis and accumulation of these compounds.

Experimental

Treatment of the animals

Female albino rats of the Wistar strain weighing 110–130 g (age 5–6 weeks) were used, unless otherwise indicated. The animals were housed at 20°C and 40% humidity, 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*. The animals were killed between 08:00 h and 10:00 h to minimize diurnal variation in enzyme activities.

Adrenalectomy was performed 10 days before the start of the experiment. Adrenalectomized animals received 0.9% NaCl as drinking water. Cortisol acetate, as a commercial suspension, was supplied by Lääke Oy, Turku, Finland, and administered intraperitoneally (50 mg/kg body wt.).

Methionine-deprived rats and their control animals weighing 200–210 g were fed with a synthetic diet containing (w/w): 74.2% glucose, 5% maize oil, 4% inorganic salts, 0.2% vitamins and 16.6% amino acids (with or without methionine). The composition of the salt, vitamin and amino acid mixtures is described in detail by Byington *et al.* (1972). The diet was given to the rats three times per day in 5 g batches suspended in water by a stomach tube. Before starting the feeding schedule, the animals were starved for 12 h.

Methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediyldine)dinitrilo]diguanidine} (30 mg/kg) and thioacetamide (150 mg/kg body wt.) were administered to rats by intraperitoneal injections in 0.5 ml of 0.9% NaCl. Control animals were injected with the solvent alone. The animals were starved for 12 h before death.

Partial hepatectomy was performed as described by Higgins & Anderson (1931).

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 was treated with HCl (Hölttä & Raina, 1973), and [1,4-¹⁴C]putrescine was purified on a Dowex 50 (H⁺ form) column (Raina *et al.*, 1970) before use. *carboxy*-¹⁴C-labelled and unlabelled *S*-adenosylmethionine were synthesized essentially as described by Pegg & Williams-Ashman (1969). *S*-[8-¹⁴C]-Adenosylhomocysteine (59 mCi/mmol) was synthesized and purified as previously described (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 by Eloranta *et al.* (1976a,b).

Assay methods

The tissue concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine were determined by the method of Eloranta *et al.* (1976b). Polyamines were extracted from the trichloroacetic acid supernatants into alkaline butan-1-ol, separated by paper electrophoresis and analysed by the ninhydrin method (Raina *et al.*, 1967). Nucleic acids were extracted into hot 5% (w/v) trichloroacetic acid, and DNA was then determined by the diphenylamine reaction and RNA by the orcinol method (Ashwell, 1957). Protein was determined from the 105000_{gav.} supernatants by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

The activities of L-ornithine decarboxylase (EC 4.1.1.17) and *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) were determined as previously described (Eloranta *et al.*, 1976a). The activity of methionine adenosyltransferase (EC 2.5.1.6) was assayed by the method of McKenzie & Gholson (1973) with minor modifications (Eloranta *et al.*, 1976a). The assay method for spermidine synthase (EC 2.5.1.16) has been reported by Raina & Hannonen (1971) and Jänne & Williams-Ashman (1971). The assay of *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) was performed by the electrophoretic method of Kajander *et al.* (1976).

Results

Effect of starvation and re-feeding on the metabolism of S-adenosylmethionine and polyamines in rat liver

It has been demonstrated that starvation decreases the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in rat (Domschke & Söling, 1973) and chick liver (Eloranta *et al.*, 1976a). The data in Table 1 confirmed this finding. However, Table 1 also includes a novel observation: during a prolonged starvation the activity of *S*-adenosylmethionine decarboxylase was paradoxically increased. Spermidine synthase activity was decreased only a little during the 48 h starvation period (results not shown). Starvation did not significantly change the total concentration of spermidine and spermine, but markedly decreased the ratio spermidine/spermine (Table 1). Removal of food also resulted in a slow decrease in the activities of methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase, whereas the concentration of *S*-adenosylmethionine declined only transiently and that of *S*-adenosylhomocysteine remained practically unchanged (Table 2).

Re-feeding after a starvation period of 16–20 h rapidly stimulated polyamine synthesis, as shown by marked increases in the activities of both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, and in the rise of the concentration of sper-

Table 1. *Effect of starvation and re-feeding on the synthesis and accumulation of polyamines in rat liver*

During the starvation period, the animals were allowed to drink *ad libitum*. Before the start of the experiment and during the re-feeding periods the animals had free access to food. All the values are means $\pm$ s.d. for three pooled preparations, each obtained from two livers. The activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase are expressed as pmol of $^{14}\text{CO}_2$ formed/30min per mg of soluble protein. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, for the significance of the differences (based on Student's *t* test) as compared with the control group or (in the case of re-feeding) the group starved for 24h.

Time of starvation (h)	Time of re-feeding (h)	Liver wt. (g)	DNA phosphate ($\mu\text{mol/g}$ wet wt. of liver)		Ornithine decarboxylase	S-Adenosyl-methionine decarboxylase	Spermidine (nmol/g wet wt. of liver)	Spermine (nmol/g wet wt. of liver)	Ratio of spermidine/spermine
0		5.7 $\pm$ 0.3	5.5 $\pm$ 0.4		13.1 $\pm$ 5.8	144 $\pm$ 10	885 $\pm$ 105	777 $\pm$ 37	1.14 $\pm$ 0.20
16		4.0 $\pm$ 0.6	8.8 $\pm$ 0.8		8.2 $\pm$ 4.1	108 $\pm$ 19*	686 $\pm$ 32*	997 $\pm$ 81*	0.69 $\pm$ 0.06*
24		3.8 $\pm$ 0.4	9.0 $\pm$ 0.1		4.9 $\pm$ 1.4	153 $\pm$ 15	678 $\pm$ 26*	1018 $\pm$ 59**	0.67 $\pm$ 0.06*
32		3.4 $\pm$ 0.2	8.9 $\pm$ 0.6		2.4 $\pm$ 0.4*	194 $\pm$ 27*	723 $\pm$ 113	865 $\pm$ 61	0.83 $\pm$ 0.04
48		3.6 $\pm$ 0.0	8.4 $\pm$ 0.2		4.3 $\pm$ 1.2	199 $\pm$ 28*	678 $\pm$ 20*	933 $\pm$ 61*	0.73 $\pm$ 0.05*
20	4	4.7 $\pm$ 0.2	6.7 $\pm$ 0.2		52.0 $\pm$ 28.8*	272 $\pm$ 21**	765 $\pm$ 95	763 $\pm$ 84*	1.00 $\pm$ 0.06**
16	8	5.6 $\pm$ 0.4	5.5 $\pm$ 0.2		20.1 $\pm$ 1.0***	171 $\pm$ 59	834 $\pm$ 43**	716 $\pm$ 25**	1.17 $\pm$ 0.09**

Table 2. *Effect of starvation and re-feeding on the synthesis and accumulation of S-adenosylmethionine and S-adenosylhomocysteine*

All the values are means $\pm$ s.d. for three pooled preparations, each obtained from two livers. The enzyme activities are expressed as nmol of product formed/min per mg of soluble protein. For other details see the legend for Table 1.

Time of starvation (h)	Time of re-feeding (h)	S-Adenosylmethionine (nmol/g wet wt. of liver)	S-Adenosylhomocysteine (nmol/g wet wt. of liver)	Methionine adenosyl-transferase	S-Adenosylhomocysteine hydrolase
0		84.3 $\pm$ 4.1	31.8 $\pm$ 1.9	6.17 $\pm$ 0.11	47.2 $\pm$ 0.4
16		72.4 $\pm$ 7.6	28.8 $\pm$ 4.5	5.34 $\pm$ 0.30*	44.8 $\pm$ 3.4
24		69.6 $\pm$ 2.3**	29.2 $\pm$ 2.3	5.41 $\pm$ 0.27**	42.9 $\pm$ 2.0*
32		78.4 $\pm$ 0.6	29.3 $\pm$ 1.7	5.63 $\pm$ 0.11**	39.8 $\pm$ 2.3**
48		76.0 $\pm$ 6.0	32.7 $\pm$ 1.4	6.18 $\pm$ 0.26	37.9 $\pm$ 3.9*
20	4	107.4 $\pm$ 6.5***	39.6 $\pm$ 3.7*	5.97 $\pm$ 0.11*	45.0 $\pm$ 0.7
16	8	83.2 $\pm$ 1.2	35.2 $\pm$ 6.9	6.29 $\pm$ 0.34*	46.6 $\pm$ 2.8

Table 3. *Effect of starvation on the activity of S-adenosylmethionine decarboxylase and the concentrations of polyamines in the livers of adrenalectomized rats*

Adrenalectomies were performed 10 days before the starvation period. The values for S-adenosylmethionine decarboxylase activity represent means $\pm$ s.d. for six livers and those for polyamine concentrations means $\pm$ s.d. of three pooled preparations, each obtained from two livers. The average liver weights after starvation for 0, 16 or 48 h were 7.47, 4.77 and 4.25 g in the intact and 7.12, 4.63 and 3.90 g in the adrenalectomized groups respectively. For other details see the Experimental section. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, for the significance of the differences (based on Student's *t* test) compared with the corresponding unstarved group.

Time of starvation (h)	S-Adenosylmethionine decarboxylase (pmol of $^{14}\text{CO}_2$ /30min per mg of protein)		Spermidine (nmol/g wet wt. of liver)		Spermine (nmol/g wet wt. of liver)	
	Intact	Adrenalectomized	Intact	Adrenalectomized	Intact	Adrenalectomized
0	129 $\pm$ 21	99 $\pm$ 9	749 $\pm$ 109	970 $\pm$ 123	988 $\pm$ 44	977 $\pm$ 3
16	64 $\pm$ 18***	56 $\pm$ 22**	934 $\pm$ 136	981 $\pm$ 34	1256 $\pm$ 118*	1195 $\pm$ 86*
48	121 $\pm$ 34	24 $\pm$ 9***	1076 $\pm$ 136*	1270 $\pm$ 83*	940 $\pm$ 72	983 $\pm$ 40

Fig. 1. Effect of cortisol on the synthesis and accumulation of polyamines

Cortisol acetate (50mg/kg body wt.) was given as a single intraperitoneal injection. Control animals were injected with 0.25ml of 0.9% NaCl 3h before being killed. Food was removed 12h before death. The values for ornithine decarboxylase (○) and S-adenosylmethionine decarboxylase (●) represent means $\pm$ S.E.M. for six livers, and those for spermidine (□) and spermine (■) are means $\pm$ S.E.M. for three pooled preparations, each obtained from two livers. Where not shown, the S.E.M. bars lie within the symbols. The average liver weights at 0 (control group), 3, 6, 12 and 24h after the hormone injection were 4.16, 4.31, 4.31, 4.59 and 4.88 g respectively.

midine and the ratio of spermidine/spermine (Table 1). Re-feeding also profoundly but transiently increased the concentrations of liver S-adenosylmethionine and S-adenosylhomocysteine, although methionine adenosyltransferase activity was stimulated only slightly (Table 2).

Effect of starvation on polyamine synthesis in adrenalectomized animals

The paradoxical increase in liver S-adenosylmethionine decarboxylase activity during prolonged starvation of normal rats suggested the involvement of the adrenals in the regulation of this enzyme activity. As shown in Table 3, the rise in S-adenosylmethionine decarboxylase activity seen in normal animals after 16h starvation did not occur in the livers of adrenalectomized rats, but the enzyme activity continued to decrease during the 48h observation period. The changes in ornithine decarboxylase activity during starvation (cf. Table 1) were quite similar in both groups (results not shown). Interestingly, the concentration of total polyamines in adrenalectomized animals after 48h starvation was not significantly different from that of the control group despite the very low activity of S-adenosylmethionine decarboxylase (Table 3).

Adrenalectomy had no significant effect on the concentrations of S-adenosylmethionine and S-adenosylhomocysteine or on the activities of methionine adenosyltransferase and S-adenosylhomocysteine hydrolase in fed or starved animals (results not shown).

Effect of cortisol treatment

A single injection of cortisol sharply increased the specific activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase, followed by a small but significant rise in the concentration of spermidine (Fig. 1). These changes were temporary

Table 4. Effect of cortisol on the synthesis and accumulation of S-adenosylmethionine and S-adenosylhomocysteine
For details of the treatment of animals see the legend for Fig. 1. The enzyme activities are expressed as nmol of product formed/min per mg of soluble protein, and represent means $\pm$ S.D. for six enzyme preparations. Other values are means $\pm$ S.D. for three pooled samples, each obtained from two livers. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, for the significance of the differences (based on Student's *t* test) as compared with the control group.

Time of cortisol treatment before killing (h)	S-Adenosylmethionine (nmol/g wet wt. of liver)	S-Adenosylhomocysteine (nmol/g wet wt. of liver)	Methionine adenosyltransferase	S-Adenosylhomocysteine hydrolase
Control	82.6 $\pm$ 9.8	36.9 $\pm$ 1.4	6.7 $\pm$ 0.3	37.0 $\pm$ 2.8
3	87.3 $\pm$ 3.0	46.0 $\pm$ 4.1*	6.8 $\pm$ 0.4	38.8 $\pm$ 2.6
6	90.7 $\pm$ 4.6	46.1 $\pm$ 3.4*	8.3 $\pm$ 0.6***	45.5 $\pm$ 4.5**
12	75.5 $\pm$ 3.3	44.9 $\pm$ 11.0	10.6 $\pm$ 1.1***	53.0 $\pm$ 6.5***
24	83.4 $\pm$ 3.5	37.7 $\pm$ 7.2	8.7 $\pm$ 0.3***	48.0 $\pm$ 4.3***

Table 5. *Effect of methionine deprivation on the synthesis and accumulation of polyamines and S-adenosylmethionine in the liver*
 Female albino rats weighing 200–210 g were used. All the animals had free access to drinking water and were starved for 10 h before death. The control group was fed *ad libitum* with a standard diet. The values for S-adenosylmethionine concentrations are means $\pm$ s.d. for three pooled preparations, each obtained from two livers. Other values are means $\pm$ s.d. for six livers. The activities of S-adenosylmethionine decarboxylase and methionine adenosyltransferase are expressed as pmol of $^{14}\text{CO}_2$ formed/30 min per mg of protein and nmol of S-adenosylmethionine formed/min per mg of protein respectively. For details see the Experimental section. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, for the significance of the differences (based on Student's *t* test) compared with the corresponding methionine-supplemented group.

Group	Liver wt. (g)	S-Adenosyl- methionine decarboxylase	Spermidine (nmol/g wet wt. of liver)	Spermine (nmol/g wet wt. of liver)	Ratio of spermidine/ spermine	S-Adenosyl- methionine (nmol/g wet wt. of liver)	Methionine adenosyl- transferase
Control	5.40 $\pm$ 0.42	68 $\pm$ 24	720 $\pm$ 90	1390 $\pm$ 81	0.52 $\pm$ 0.05	84.0 $\pm$ 7.0	7.33 $\pm$ 0.26
7-day diet							
Methionine-deficient	6.66 $\pm$ 0.29	419 $\pm$ 67**	1250 $\pm$ 142***	849 $\pm$ 94**	1.47 $\pm$ 0.08***	74.3 $\pm$ 11.3	6.46 $\pm$ 0.48
Methionine-supplemented	6.78 $\pm$ 0.40	277 $\pm$ 56	908 $\pm$ 87	1068 $\pm$ 79	0.87 $\pm$ 0.12	67.5 $\pm$ 5.5	6.86 $\pm$ 0.49
14-day diet							
Methionine-deficient	6.47 $\pm$ 0.41	237 $\pm$ 64	1520 $\pm$ 94***	706 $\pm$ 48***	2.15 $\pm$ 0.20***	99.9 $\pm$ 1.3*	7.56 $\pm$ 0.88
Methionine-supplemented	6.59 $\pm$ 0.43	286 $\pm$ 22	971 $\pm$ 49	1020 $\pm$ 118	0.95 $\pm$ 0.03	73.7 $\pm$ 10.0	6.91 $\pm$ 0.63

and the control value was reached again within 12 h.

Cortisol treatment also stimulated the activities of methionine adenosyltransferase and S-adenosylhomocysteine hydrolase, although the rise was slower and of longer duration (Table 4) than that in polyamine-synthesizing enzymes. The maximum values were reached at about 12 h, but the activities still significantly exceeded the control value 24 h after a single injection of the hormone. Hormone treatment also resulted in an increase in the concentration of S-adenosylhomocysteine 3–12 h after the administration, whereas the concentration of S-adenosylmethionine remained practically unchanged (Table 4).

Effect of methionine deprivation

To test whether changes in the dietary supply of methionine could affect the synthesis and accumulation of polyamines and S-adenosylmethionine, rats were fed on a methionine-deficient diet for 1 and 2 weeks. During this period, feeding with a methionine-deficient diet had no significant effect on the weight of the rat or the total content of hepatic RNA, whereas the hepatic contents of DNA and soluble protein were decreased by 15–20% (results not shown).

Somewhat unexpectedly, methionine deprivation did not significantly change the concentration of total polyamines, and the concentration of S-adenosylmethionine was even increased after a feeding period of 2 weeks (Table 5). However, the concentration of liver spermidine was markedly increased and that of spermine decreased compared with the methionine-supplemented group. Accordingly, the ratio of spermidine/spermine was increased more than 2-fold. The activity of S-adenosylmethionine decarboxylase was also increased after a feeding period of 1 week. Methionine adenosyltransferase activity was not significantly changed by methionine deprivation for 1 or 2 weeks.

Effect of methylglyoxal bis(guanylhydrazone), thioacetamide and partial hepatectomy

Partial hepatectomy or the treatment of the rat with thioacetamide or with methylglyoxal bis(guanylhydrazone), an inhibitor of S-adenosylmethionine decarboxylase, are known to produce marked changes in the synthesis and accumulation of hepatic polyamines (for references see Raina & Jänne, 1975). Intraperitoneal administration of 30 mg of methylglyoxal bis(guanylhydrazone)/kg body wt. had no effect on the concentration of S-adenosylmethionine during 32 h, but it rapidly decreased the concentration of spermidine and the activity of S-adenosylmethionine decarboxylase (results not shown). Also, in regenerating liver, the concentrations of S-adenosylmethionine and S-adenosylhomocysteine remained fairly constant during the observation

period of 72h. Administration of 150mg of thioacetamide/kg body wt., which markedly stimulated the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase and increased the concentration of hepatic spermidine, again had no significant effect on the concentrations of liver *S*-adenosylmethionine and *S*-adenosylhomocysteine or on the activity of methionine adenosyltransferase during the observation period of 3 days (results not shown).

Discussion

As shown above, the activities of methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase decreased during starvation, but the concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine remained fairly constant. This result agrees with the notion that the synthesis of *S*-adenosylmethionine is largely controlled by the concentration of free methionine (Eloranta *et al.*, 1976b; T. O. Eloranta, unpublished work), which does not change appreciably during starvation (Thompson *et al.*, 1950; Adibi, 1971). Although no information is available on the effect of re-feeding on the hepatic methionine pool, it is plausible that the rise in *S*-adenosylmethionine concentration on re-feeding is due to an increased methionine supply. The rise in the concentration of *S*-adenosylhomocysteine, also noticed in mouse liver (Hoffman, 1975), is probably due to increased utilization of *S*-adenosylmethionine in various transmethylation reactions.

The changes in the concentrations of polyamines, i.e. a decrease in spermidine and an increase in spermine, are unlikely to be due to changes in the *S*-adenosylmethionine pool. As shown here, starvation decreased the liver ornithine decarboxylase activity to a very low value, whereas *S*-adenosylmethionine decarboxylase activity was affected much less in intact animals. The decrease in liver putrescine concentration during starvation (Domschke & Söling, 1973) probably favours the synthesis of spermine instead of spermidine (cf. Raina & Jänne, 1975). On re-feeding, the marked increase in ornithine decarboxylase activity, leading to the accumulation of putrescine, again favours the synthesis of spermidine and may inhibit spermine synthesis (cf. Raina & Jänne, 1975). However, the increase in *S*-adenosylmethionine decarboxylase activity and in *S*-adenosylmethionine concentration may also contribute to the accumulation of spermidine on re-feeding.

The absence of the paradoxical increase in *S*-adenosylmethionine decarboxylase activity during prolonged starvation of adrenalectomized animals suggests that the rise in the enzyme activity is due to stimulation of corticosteroid secretion by starvation and that *S*-adenosylmethionine decarboxylase activity is normally partly regulated by adrenal-cortical

function. The same may be true of the synthesis of *S*-adenosylmethionine. This view is supported by the observed stimulation of *S*-adenosylmethionine decarboxylase activity and the accumulation of *S*-adenosylmethionine in livers of intact animals treated with cortisol. However, it is difficult to explain why ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, both enzymes having a very short half-life (Raina & Jänne, 1975) and responding to cortisol treatment in a similar manner, behaved differently during starvation.

Cortisol treatment, which profoundly stimulated the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase and also increased liver spermidine concentration, did not significantly change the concentration of *S*-adenosylmethionine in the liver. Thus the stimulation of *S*-adenosylmethionine synthesis (cf. Table 4) did not appreciably contribute to the accumulation of spermidine, provided that all the *S*-adenosylmethionine is available for polyamine synthesis.

The failure of even a prolonged methionine deprivation to decrease the concentration of liver *S*-adenosylmethionine indicates that either the utilization of *S*-adenosylmethionine is diminished or that the free methionine pool remains fairly constant to maintain the normal rate of *S*-adenosylmethionine synthesis. The former possibility seems less plausible, because the methionine-deficient animals grew quite normally. The results suggest a very efficient re-methylation of homocysteine, since even an increased protein turnover would not be sufficient to supply enough methionine during a prolonged deficiency. According to Finkelstein *et al.* (1974), of the two homocysteine-methylating enzymes in liver, the 5-methyltetrahydrofolate – homocysteine methyltransferase is mainly responsible for the maintenance of methionine concentration. Feeding the rats with a low-methionine diet has been suggested to increase the activity of the folate enzyme and to decrease the flow of homocysteine into the trans-sulphuration pathway by decreasing the activity of cystathionine β -synthase (EC 4.2.1.22) (Finkelstein, 1974). The effects of methionine deprivation on the concentrations of spermidine and spermine resemble those observed during regenerative processes, e.g. in regenerating rat liver (cf. Raina & Jänne, 1975). The increase in the ratio of spermidine/spermine might be at least partly due to enhanced conversion of spermine into spermidine. A more detailed mechanism of this phenomenon remains to be determined.

Treatment of the rat with methylglyoxal bis-(guanyldrazone) and thioacetamide, or partial hepatectomy, did not significantly affect the synthesis and accumulation of *S*-adenosylmethionine and *S*-adenosylhomocysteine. On the other hand, it is expected that even marked changes in the rate of polyamine synthesis alone would not cause significant

changes in the concentration of S-adenosylmethionine, since the turnover rate of S-adenosylmethionine is very rapid (Baldessarini, 1975) and only a small portion of it is utilized in polyamine synthesis.

Finally, it must be emphasized that looking only at the changes in the total concentrations of polyamines may not give a true picture. At neutral pH these compounds are strong cations, much of which, especially of spermine, is probably bound non-covalently to anionic groups, e.g. nucleic acids and ribosomes (Raina & Jänne, 1975). Thus, under conditions such as methionine deprivation, the decrease in spermine and the increase in spermidine (less basic) may change the cellular pool of free polyamines even more markedly than is revealed by the changes in the total concentrations.

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