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S-adenosyl-L-methionine: hepatic levels in rats during various stress situations

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S-Adenosyl-L-methionine (SAMe) plays a key role in many cell reactions; the proceedings of a symposium on its biochemistry were published recently [1]. Since this compound is now available in stable form there is increasing interest in studies on it [2–5].

This paper summarizes the effects of various experimental conditions such as fasting, exsanguination and tumor on SAMe levels in the liver, which contains the major part of the compound present in the body [6].

Chemicals. Labelled *S*-methyl-[^{14}C] adenosyl-L-methionine (58 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England. Stable di-*p*-toluendisulphonate salt of unlabelled SAMe was supplied by BioResearch Co.

Animals. Male Sprague–Dawley rats (CD-COBS, Charles River, Italy) weighing 220 ± 10 g were maintained on water and standard laboratory diet (Altromin MT, Rieper, Italy) *ad lib.* or fasted for 24 or 48 hr.

Walker carcinosarcoma 256 was transplanted subcutaneously into the right flank of animals under slight ether anesthesia.

Exsanguination experiments: (1) Rats were slightly anesthetized with ether and blood (4 ml) was taken by cardiac puncture. The animals were then killed after various periods of time and SAMe was determined in liver.

(2) Rats were anesthetized (sodium phenobarbital 50 mg/kg + chloralose 60 mg/kg i.v.) the carotid artery was cannulated and blood samples were then withdrawn at various intervals (up to 30 per cent of total blood volume).

SAMe determination. Liver tissue was frozen immediately with liquid nitrogen and the tissue was then pulverized in a mortar [7]. The powder was weighed and homogenized in cold (4°) trichloracetic acid (0.62 M in 0.05 N HCl - 1:10 wt/v). Blood samples were diluted with trichloracetic acid (1:1 v/v). Clear supernatant obtained after centrifugation (4000 *g*, 15 min, 4°) was used for SAMe determination according to Baldessarini and Kopin [6].

The statistical significance of the results was analyzed by Dunnett's test [8].

The effect of fasting on SAMe levels in rat liver is summarized in Table 1. Rats fed the standard diet or fasted for 24–48 hr were killed by rapid exsanguination from carotid arteries. Livers were quickly excised and frozen in liquid nitrogen. Fasting significantly decreased both SAMe concentration per g of liver tissue and the total quantity in the organ. No difference could be found between 24 and 48 hr fasted animals. Refeeding restored control SAMe levels in the liver.

Table 1. Effect of fasting on SAMe levels in rat liver

Condition	Liver wt g (mean ± S.E.)	%	SAMe μg/g liver (mean ± S.E.)	%	Total SAMe μg/liver (mean ± S.E.)	%	No. rats
Controls fed	10.06 ± 0.27	100	36.13 ± 1.42	100	372.6 ± 22.1	100	20
24 hr fasted	6.90 ± 0.29*	68	25.78 ± 0.95*	71	176 ± 5.5*	47	10
24 hr fasted + 6 hr refeeding	8.34 ± 0.29	83	36.81 ± 1.50	101	306.0 ± 11.5	82	5
24 hr fasted + 24 hr refeeding	10.90 ± 1.05	108	31.28 ± 0.91	86	342.1 ± 35.3	92	5
48 hr fasted	6.55 ± 0.19*	65	24.98 ± 1.00*	69	163.2 ± 42.6*	44	5

*P < 0.01 vs controls
Statistical significance was calculated by Dunnett's test [8].

Table 2. Effect of hypovolemic shock on SAMe levels in liver

Condition	Liver wt.g (mean ± S.E.)	SAMe μg/g liver (mean ± S.E.)	%	Total SAMe μg/liver (mean ± S.E.)	%	No. rats
Control rats	9.91 ± 0.43	33.62 ± 1.10	100	335.4 ± 22.0	100	10
Exsanguination 0 min	10.12 ± 0.37	31.30 ± 1.51	93	317.2 ± 20.5	94	4
15 min	10.12 ± 0.33	25.07 ± 2.08*	74	252.9 ± 18.2*	75	4
30 min	9.54 ± 0.35	22.80 ± 2.04*	68	214.5 ± 16.9*	64	10
45 min	8.47 ± 0.44	29.97 ± 2.22	89	253.6 ± 21.4*	75	4
60 min	9.09 ± 0.16	30.13 ± 1.27*	89	274.8 ± 14.5*	82	10
120 min	9.46 ± 0.38	35.60 ± 1.78	106	335.8 ± 18.1	100	5
360 min	9.04 ± 0.32	33.08 ± 1.01	98	298.2 ± 7.6	89	5
24 hr	8.24 ± 0.46*	30.55 ± 0.80	91	251.8 ± 15.6*	75	5

*P < 0.01 vs control rats (Dunnett's test [8]).

In experiments to observe the effects of hypovolemic shock on hepatic SAMe content, about 30 per cent of blood—this volume is considered the survival limit—was removed by cardiac puncture and hepatic SAMe levels were measured after various intervals (see Table 2). Both the concentration and the total amount of SAMe decreased, reaching the lowest level—about 64 per cent of control values—30 min after blood withdrawal. The decrease was statistically significant up to 60 min after hypovolemic shock. A low total quantity due to diminished weight of the liver was noted 24 hr after beginning the experiments.

In another experiment blood levels of SAMe were followed after gradual withdrawal of 30 per cent of blood from the cannulated carotid artery (see Table 3). The values found were compared with the concentration in blood obtained by total exsanguination (which is often considered normal). Initial concentrations of SAMe were about 30 per cent higher than those obtained in total exsanguination, decreasing thereafter.

Table 3. Effect of hypovolemic shock on blood levels of SAMe

Condition	SAMe μg/ml blood (Mean ± S.E.)	%
Total exsanguination	2.33 ± 0.09	100
Partial exsanguination*		
0 min	3.16 ± 0.20†	135†
5 min	3.03 ± 0.17†	130†
10 min	3.07 ± 0.20†	132†
15 min	2.85 ± 0.27	122
30 min	2.87 ± 0.18	120
60 min	2.79 ± 0.18	120

*Loss of 30 per cent of blood volume during 60 min.
†$P < 0.02$ vs controls (Dunnett's test [8]).

The last stress situation model considered in this paper is the extrahepatic Walker carcinosarcoma. Levels of SAMe decreased in the liver 4 days after tumor transplantation when the amount of neoplastic tissue was low (see Table 4) and remained at nearly the same value till the end of the experiment (15th day). Mean survival time of the tumor bearing animals was 16 days. The proliferative part of the tumor taken on the 11th day after transplantation contained 24.22 ± 1.62 μg SAMe/g tissue. The presence of Walker carcinosarcoma did not affect blood levels of SAMe which remained similar to controls, 2.24 ± 0.13 μg/ml after total exsanguination.

It is interesting that the lowest concentrations of SAMe found in the liver in different stress situations (fasting, hypovolemic shock, liver perfusion, tumor) are equal. The experiments reported in this paper suggest that there is a lower limit concentration of SAMe (about 22–24 μg/g liver tissue) which is maintained even in extreme experimental situations.

On the other hand refeeding of fasted animals or recovery from hypovolemic shock demonstrated that normal SAMe levels can be regained in healthy animals.

Table 4. Effect of extrahepatic Walker 256 carcinosarcoma on SAMe levels in rat liver

Condition	Tumor wt g (Mean ± S.E.)	SAMe μg/g liver (Mean ± S.E.)	%
Controls		35.30 ± 1.19	100
Tumor rats			
4th day	0.67 ± 0.14	24.40 ± 0.32*	69*
8th day	10.31 ± 1.19	21.40 ± 0.49*	60*
11th day	21.26 ± 1.44	23.40 ± 1.57*	66*
15th day	28.34 ± 1.64	22.70 ± 1.94*	64*

*$P < 0.005$ vs. controls (Dunnett's test [8]).

SAMe levels in blood are relatively stable even in the presence of a tumor. This stability may be due to SAMe released by various organs, but very little is known about the transport and exchange of SAMe between tissues and blood.

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