Nicotinamide Methyltransferase and *S*-Adenosylmethionine:5′-Methylthioadenosine Hydrolase. Control of Transfer Ribonucleic Acid Methylation†

Kenneth R. Swiatek,* Lionel N. Simon, and Kuen-Lan Chao

ABSTRACT: Previous studies (Buch, L., Streeter, D., Halpern, R. M., Simon, L. N., Stout, M. G., and Smith, R. A. (1972), *Biochemistry 11*, 393) have demonstrated that nicotinamide inhibits tRNA methyltransferase enzymes from both human tumor cell line grown in tissue culture (KB cell) and rat liver. In the present studies, we have demonstrated that the inhibition of porcine liver tRNA methyltransferase activity by nicotinamide is due to an active methyltransferase enzyme which in the presence of *S*-adenosylmethionine and nicotinamide, produces 1-methylnicotinamide and *S*-adenosylhomocysteine, the latter a potent inhibitor of methyltransferase enzymes. Furthermore, nicotinamide inhibition of tRNA methyltransferase activity in rat liver and KB cells is also due to *S*-adenosylhomocysteine production by the nicotinamide methyltransferase system in these tissues. Finally, we have shown that porcine liver contains an *S*-adenosylmethionine-splitting enzyme which is not nicotinamide dependent but does cleave *S*-adenosylmethionine to 5′-methylthioadenosine and α-amino-γ-butyrolactone. It is postulated that nicotinamide methyltransferase and *S*-adenosylmethionine:5′-methylthioadenosine hydrolase participate in regulating tissue levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine.

The control of tRNA methylation has received considerable attention in recent years (Borek and Kerr, 1972; Kerr and Borek, 1972). Three systems appear to exert the main control: (1) enzyme systems which utilize or hydrolyze *S*-adenosylmethionine resulting in a decreased intracellular *S*-adenosylmethionine concentration, *e.g.*, glycine methyltransferase; (2) naturally occurring or synthetic inhibitors of tRNA methyltransferases have also appeared (Blumenstein and Williams, 1960; Hildesheim *et al.*, 1971, 1972; Kerr, 1972; Pegg, 1972; Heady and Kerr, 1973). Recently, the role of nicotinamide and nicotinamide analogs as inhibitors of rat liver and human KB cell tRNA methyltransferases have been reported in this regard (Chaney *et al.*, 1970; Halpern *et al.*, 1971; Murai *et al.*, 1972; Buch *et al.*, 1972; Kuchino and Endo, 1972), and finally, (3) the production of *S*-adenosylhomocysteine, a demonstrated potent inhibitor of methyltransferase enzymes (Hurwitz *et al.*, 1964; Kerr, 1972; Pegg, 1972) by virtue of the interaction of *S*-adenosylmethionine as a methyl donor in several naturally occurring systems.

We have investigated the function of nicotinamide as an inhibitor of tRNA methylation and report on the presence in porcine liver of an active methyltransferase enzyme which in the presence of *S*-adenosylmethionine and nicotinamide, produces 1-methylnicotinamide and *S*-adenosylhomocysteine. Furthermore, we will show that both rat liver and human KB cells also have this nicotinamide methyltransferase system. In addition, we have shown that the *S*-adenosylmethionine-splitting enzyme isolated by Gross and Wildenauer (1972) from rat liver is in reality not a *S*-adenosylmethionine-cleaving enzyme but rather the nicotinamide methyltransferase enzyme. Finally, we have shown that porcine and rat liver and KB cells contain a *S*-adenosylmethionine cleavage enzyme which is not nicotinamide dependent, but does cleave *S*-adenosylmethionine to 5′-methylthioadenosine and α-amino-γ-butyrolactone or its more stable product, homoserine.

Experimental Procedures

Chromatography. Initial studies to identify the reaction products obtained on incubation of *S*-adenosylmethionine-*methyl*-^{14}C with pig liver preparations were performed on precoated Eastman Kodak cellulose plates. Thin layer plates were initially developed for 11 cm according to the method of Gross and Wildenauer (1972) in solvent system A. As this method failed to give complete separation of the components of the reaction mixture, aliquots were spotted on Whatman 3MM paper and developed overnight in the following ascending systems: A, 1-butanol–acetic acid–water (60:15:25, v/v); B, ethanol–water–acetic acid (65:35:1, v/v); C, isopropyl alcohol–formic acid–water (70:10:20, v/v); isopropyl alcohol–concentrated ammonium hydroxide–water (70:10:20, v/v); and E, methanol–water–pyridine (80:20:4, v/v).

Chemicals. Nicotinamide, 1-methylnicotinamide, and homoserine were obtained from Sigma Chemical Co. *S*-Adenosylmethionine-*methyl*-^{14}C was obtained from International Chemical Nuclear Corp. and had a specific activity of 40–55 Ci/mol. *S*-Adenosylmethionine-*carboxyl*-^{14}C was obtained from Amersham/Searle Corp. and had a specific activity of 61 Ci/mol. 5′-Methylthioadenosine and α-amino-γ-butyrolactone were prepared by acid hydrolysis of *S*-adenosylmethionine and purified by paper chromatography prior to their use (Stekol, 1963).

Protein was determined by the method of Lowry *et al.* (1951).

The position of homoserine and α-amino-γ-butyrolactone on paper chromatograms was located by ninhydrin spray.

Enzyme Preparation. Several different fractions were prepared from porcine liver. The liver, obtained from adult pigs anesthetized with sodium amytal (10 mg/100 g body wt), was minced and homogenized in a Douce homogenizer with five volumes of ice-cold 0.25 M sucrose–0.01 M $MgCl_2$ buffer. The homogenate was centrifuged first at 1000*g* for 10 min and

† From the Research Department, Illinois State Pediatric Institute, Chicago, Illinois 60608 (K. R. S. and K.-L. C.), and ICN Nucleic Acid Research Institute, Irvine, California 92664 (L. N. S.). *Received April 9, 1973.*

TABLE I: Identification and R_F Values of Reaction Products from Incubation of Pig Liver S-2 with *S*-Adenosylmethionine-*methyl*-^{14}C and Nicotinamide.[a]

R_F	Solvent Systems				
	A	B	C	D	E
Peak I	0.05	0.21	0.06	0.09	0.10
S-Adenosylmethionine-*methyl*-^{14}C	0.07	0.25	0.07	0.10	0.09
Peak II	0.35	0.68	0.45	0.27	0.12
1-Methylnicotinamide	0.36	0.70	0.52	0.27	0.13
Peak III	0.68	0.80	0.71	0.76	0.74
5′-Methylthioadenosine	0.70	0.78	0.68	0.73	0.76

[a] Incubation mixture as indicated in Methods. Incubation was in the presence of 18×10^{-6} M *S*-adenosylmethionine-*methyl*-^{14}C and 6.6×10^{-3} M nicotinamide.

TABLE II: Identification and R_F Values of Reaction Products from Incubation of Pig Liver S-2 with *S*-Adenosylmethionine-*carboxyl*-^{14}C and Nicotinamide.[a]

R_F	Solvent Systems				
	A	B	C	D	E
Peak I	0.06	0.20	0.07	0.10	0.10
S-Adenosylmethionine-*carboxyl*-^{14}C	0.07	0.27	0.07	0.10	0.09
Peak II	0.12	0.40	0.20	0.22	0.12
S-Adenosylhomocysteine	0.14	0.38	0.19	0.20	0.13
Peak III	0.29	0.63	0.61	0.42	0.50
Homoserine	0.30	0.63	0.62	0.38	0.50
Peak IV	0.45	0.79	0.67		0.80
α-Amino-γ-butyrolactone	0.43	0.78	0.64		0.79

[a] Incubation mixture as indicated in Methods. Incubation was in the presence of 18×10^{-6} M *S*-adenosylmethionine-*carboxyl*-^{14}C and 6.6×10^{-3} M nicotinamide.

then at 105,000*g* for 60 min. The high-speed supernatant (S-100) when treated with solid ammonium sulfate (40% final concentration) is referred to as supernatant 1 (S-1), or it was fractionated according to the method of Kerr (1971) to give a reneutralized pH 5 supernatant and a pH 5 enzyme preparation. This reneutralized supernatant was treated with solid arnmonium sulfate (40% final concentration) and is referred to as supernatant 2 (S-2). The enzyme preparation from KB cells was a 100,000*g* fraction (S-100) obtained from a human tumor cell line grown in tissue culture (Buch *et al.*, 1972). A liver S-2 preparation was prepared from adult Sprague-Dawley rats in a manner identical with that for porcine liver. In order to prepare enzyme fractions free of nicotinamide, the appropriate preparations were passed through a Sephadex G-10 column previously equilibrated with 0.005 M Tris-HCl–0.005 M 2-mercaptoethanol buffer (pH 8.0). The protein fraction was dialyzed against this same buffer overnight. The protein was then precipitated with solid ammonium sulfate (40% final concentration), collected by centrifugation, and resuspended in 0.005 M Tris-HCl buffer.

tRNA Methyltransferase. The assay medium routinely contained (in micromoles) in a final volume of 0.20 ml: Tris-HCl buffer, pH 8.5, 40; magnesium acetate, 5; 2-mercaptoethanol, 18; *Escherichia coli* B tRNA, 80 μg; *S*-adenosylmethionine-*methyl*-^{14}C or -*carboxyl*-^{14}C as indicated; and the various enzyme fractions. Control reactions, without tRNA, were treated identically to determine background methylation levels. Where indicated, nicotinamide and/or nicotinamide methyltransferase enzyme (S-2) were also added. After incubation at 37°, for 30 min, 0.05-ml aliquots were removed and spotted on Whatman 3MM filter disks and air-dried. Filters were placed in ice-cold 6% trichloroacetic acid for 20 min, then washed seven times with cold 6% trichloroacetic acid, twice in ethanol–ether (2:1, v/v), twice in diethyl ether and air-dried. Filters were transferred to vials containing dioxane scintillation solution and counted in a Packard liquid scintillation counter.

Routine Nicotinamide Methyltransferase Assay. Extracts from pig and rat liver and KB cells were assayed for enzyme activity in a system which was identical with that described for tRNA methyltransferase except that tRNA was omitted from the reaction and nicotinamide (2 μmol) was used in a total volume of 0.2 ml. Control reactions, with boiled enzyme, were treated identically to determine background methylation levels. When kinetic constants were being determined, nicotinamide concentration was varied from 1×10^{-1} to 1×10^{-4} M at a *S*-adenosylmethionine concentration of 48×10^{-6} M; similarly, *S*-adenosylmethionine was varied from 6×10^{-6} to 96×10^{-6} M at a nicotinamide concentration of 1×10^{-2} M. When *S*-adenosylhomocysteine was studied as an inhibitor of nicotinamide methyltransferase, its concentration was varied from 1×10^{-5} to 5×10^{-5} M at *S*-adenosylmethionine concentrations of 24×10^{-6} and 48×10^{-6} M and nicotinamide at 1×10^{-2} M. At the end of 30-min incubations, 0.01-ml aliquots were removed and spotted onto Whatman 3MM paper and the paper developed in the appropriate solvent systems. The chromatograms were cut into 1-cm sections

TABLE III: Stoichiometry of Nicotinamide Methyltransferase and *S*-Adenosylmethionine: 5′-Methylthioadenosine Hydrolase in Porcine Liver.[a]

	−Nicotinamide	+Nicotinamide
Experiment 1		
S-Adenosylmethionine-*methyl*-^{14}C	−1560	−1735
1-Methylnicotinamide	+1053	+1295
5′-Methylthioadenosine	+402	+433
Experiment 2		
S-Adenosylmethionine-*carboxyl*-^{14}C	−1560	−2028
S-Adenosylhomocysteine	+1287	+1310
Homoserine + lactone	+332	+452

[a] The activity is expressed as picomoles of product produced (+) or consumed (−) in 30 min. The reaction mixtures were identical except for the position of the label in the *S*-adenosylmethionine. The *S*-adenosylmethionine concentration was 18×10^{-6} M, and where added, nicotinamide was 6.6×10^{-3} M. The reactions were started by the addition of porcine S-2 enzyme (3.9 mg of protein). At the end of 30 min, 0.01-ml aliquots were removed and spotted onto Whatman 3MM paper and developed overnight in solvents B and C. The chromatograms were then cut into 1-cm strips and the radioactivity was determined.

TABLE IV: Nicotinamide Methyltransferase and *S*-Adenosylmethionine: 5′-Methylthioadenosine Hydrolase Activity in G-10-Treated Porcine S-2 Preparation.[a]

Products	No Additions	Nicotinamide	Isonicotinamide	Isonicotinamide Hydrazide
S-Adenosylmethionine-*methyl*-^{14}C	−456	−1580	−827	−507
1-Methylnicotinamide	+211	+1404	+593	+191
5′-Methylthioadenosine	+308	+117	+144	+211

[a] The activity is expressed as picomoles of product formed (+) or lost (−) in 30 min. The *S*-adenosylmethionine concentration was 18×10^{-6} M and the nicotinamide and derivatives were 6.6×10^{-3} M. The reactions were started by the addition of G-10-treated porcine S-2 enzyme preparation (3.9 mg of protein) and the radioactivity was determined as indicated in Table III.

which were placed in counting vials and the radioactivity incorporated into 1-methylnicotinamide was determined.

S-Adenosylmethionine: 5′-Methylthioadenosine Hydrolase. Activity of this enzyme was routinely determined using the buffer system described for tRNA methylase and the production of 5′-methylthioadenosine measured using the paper chromatographic system described for nicotinamide methyltransferase. When kinetic constants for this enzyme were being determined, *S*-adenosylmethionine and S-adenosylhomocysteine concentrations used were identical with those used for the nicotinamide methyltransferase studies.

Results

Isolation and Characterization of Reaction Products using S-Adenosylmethionine-methyl-^{14}C. Preliminary experiments using thin-layer chromatography of the reaction mixture containing *S*-adenosylmethionine-*methyl*-^{14}C, nicotinamide, and enzyme revealed that there was not a clean separation of the reaction products. When the thin-layer plates were developed for a longer time (solvent front traveled 20 cm), three main areas of radioactivity could be found. These peaks were isolated, eluated from the cellulose plates with water, concentrated, and rechromatographed on Whatman 3MM paper in solvent systems A–E along with appropriate standards and control incubations. The results of these experiments are summarized in Table I. The radioactive spots were located, eluted from the paper, and cochromatographed with 1-methylnicotinamide or 5′-methylthioadenosine for positive identification. In all five systems the radioactivity corresponded with the uv region of the pure reference standard.

Isolation and Characterization of Reaction Products Using S-Adenosylmethionine-carboxyl-^{14}C. In a similar set of experiments, *S*-adenosylmethionine labeled in the carboxyl position was used. The results of these experiments are summarized in Table II. It appears from the major products produced in the complete system containing nicotinamide that at least two separate enzymatic reactions must be occurring. First, the methylation of nicotinamide to yield 1-methylnicotinamide and *S*-adenosylhomocysteine and secondly, the direct cleavage of *S*-adenosylmethionine to produce 5′-methylthioadenosine and α-amino-γ-butyrolactone or the more stable product, homoserine. Both of these reactions were found to be enzyme dependent.

Stoichiometry of the Reaction. The results presented in Table III demonstrate the stoichiometry of the reaction that occurred using pig liver S-2 enzyme in the presence of nicotinamide. As can be seen, the major products are 1-methylnicotinamide and *S*-adenosylhomocysteine. It is also apparent from these results that the cleavage of *S*-adenosylmethionine to 5′-methylthioadenosine was not dependent nor stimulated by nicotinamide. In addition, no stimulation of nicotinamide

TABLE V: Activity of Nicotinamide Methyltransferase and *S*-Adenosylmethionine: 5′-Methylthioadenosine Hydrolase in KB Cell S-100 Extract and Rat Liver S-2 Preparation.[a]

Additions	1-Methylnicotinamide	5′-Methylthioadenosine
None		
KB cell S-100	0	15
Rat liver S-2	1130	54
Nicotinamide (6.6×10^{-3} M)		
KB cell S-100	1050	17
Rat liver S-2	1420	30

[a] Values represent picomoles of product formed in 30 min. Reactions were run at a *S*-adenosylmethionine concentration of 18×10^{-6} M and started by the addition of 0.5 mg of protein of KB cell S-100 and 3 mg of protein of rat liver S-2 preparation.

TABLE VI: Effect of Nicotinamide and Nicotinamide Methyltransferase on Porcine Liver tRNA Methyltransferase.

Additions	tRNA Methyltransferase Act.[a] Enzyme Source: S-100	S-1	pH 5 Enzyme
1. None	22.8	33.1	138
2. Nicotinamide	8.6	11.6	127
3. Nicotinamide methyltransferase (S-2)	24.4	37.0	130
4. 2 + 3	7.4	2.5	49

[a] Activity is expressed as picomoles of $^{14}CH_3$ transferred to tRNA per milligram of tRNA methyltransferase protein. Each reaction mixture of 0.2 ml was as indicated in Methods and *S*-adenosylmethionine-*methyl*-^{14}C (0.0048 μmol) and where added, nicotinamide (2 μmol). The amount of protein added to each reaction was: S-100, 0.69 mg; S-1, 0.49 mg; and pH 5 enzyme, 0.26 mg. The amount of nicotinamide methyltransferase (S-2) where added, was 0.48 mg. Incorporation of $^{14}CH_3$ into tRNA was as indicated in Methods.

TABLE VII: Specific Activities of tRNA Methyltransferase, Nicotinamide Methyltransferase, and *S*-Adenosylmethionine : 5′-Methylthioadenosine Hydrolase in Various Porcine Liver Preparations.

Enzyme Prepn	Enzyme Act. (pmol/mg of Protein)			
	tRNA Methylase[a]	Nicotinamide Methylase[b]	*S*-Adenosylmethionine: 5′-Methylthioadenosine Hydrolase[c]	Nicotinamide Methylase/tRNA Methylase
S-100	23	2430	61	107
S-1	33	3370	99	102
pH 5 enzyme	138	1760	108	14
S-2	0	3180	117	

[a] Activity is expressed as pmoles of $^{14}CH_3$ incorporated into tRNA per mg of protein. [b] Activity is expressed as pmoles of 1-methylnicotinamide formed per mg of protein. [c] Activity is expressed as pmoles of 5′-methylthioadenosine formed per mg of protein. Each reaction mixture of 0.2 ml contained: *S*-adenosylmethionine-*methyl*-^{14}C, 0.0048 μmol and enzyme protein, S-100 (0.69 mg); S-1 (0.49 mg); pH 5 enzyme (0.26); S-2 (0.48 mg); and where needed, *E. coli* B tRNA (80 μg) or nicotinamide (2 μmol). The mixtures were incubated at 37° for 30 min, and the enzyme activities were determined as indicated in Methods.

methylation by increasing levels of nicotinamide could be demonstrated. This could be explained if the enzyme preparation contained tightly bound nicotinamide. That this might be the case was substantiated by using a S-2 enzyme preparation which had been previously passed through a G-10 column as described under Methods to remove bound nicotinamide. As seen in Table IV, only one-fifth the amount of 1-methylnicotinamide was formed using this preparation as compared to the S-2 enzyme preparation used previously (Table III). Approximately the same amount of 5′-methylthioadenosine was formed using the G-10 enzyme as compared to the S-2 fraction. The addition of nicotinamide to this system restored the rate of nicotinamide methylation to that observed previously (Table III), but appeared to actually inhibit rather than stimulate the *S*-adenosylmethionine : 5′-methylthioadenosine hydrolase enzyme.

Isonicotinamide was a poor substrate for the enzyme and isonicotinamide hydrazide was completely inactive. The methylated product formed using isonicotinamide as a substrate was not characterized. It had an R_F value in solvent system C identical with 1-methylnicotinamide. Two explanations appear possible: (1) isonicotinamide displaces residually bound nicotinamide making it available for methylation, or (2) it is possible that 1-methylisonicotinamide was formed and had an R_F identical with 1-methylnicotinamide in solvent system C.

Nicotinamide Methyltransferase and S-Adenosylmethionine : 5′-Methylthioadenosine Hydrolase Activity in KB Cells and Rat Liver. Both nicotinamide methyltransferase and *S*-adenosylmethionine : 5′-methylthioadenosine hydrolase activity were demonstrated in KB cells and rat liver by the same techniques employed for porcine liver and the activities are summarized in Table V. The inability of KB cells to produce 1-methylnicotinamide in the absence of added nicotinamide as opposed to porcine and rat liver indicates that human tumor tissue may lack nicotinamide while normal tissue contains sufficient amounts to have an active system participating in the overall regulation of cellular concentration of *S*-adenosylmethionine and *S*-adenosylhomocysteine.

Inhibition of Porcine Liver tRNA Methyltransferases by Nicotinamide. Nicotinamide has been found to inhibit crude tRNA methyltransferase preparations (Chaney *et al.*, 1970; Gross and Wildenauer, 1972) and not to inhibit more purified systems (Gross and Wildenauer, 1972). When tRNA methyltransferase preparations of varying purity were used, nicotinamide was effective as an inhibitor only against the cruder preparations (S-100 and S-1). The liver pH 5 enzyme fraction required the presence of both nicotinamide and nicotinamide methyltransferase to restore inhibition (Table VI). In Table VII are summarized the specific activities of tRNA methyltransferase, nicotinamide methyltransferase, and the *S*-adenosylmethionine : 5′-methylthioadenosine hydrolase in the various porcine liver preparations. It is interesting that although the pH 5 enzyme fraction also contains a significant amount of nicotinamide methyltransferase activity, nicotinamide by itself was relatively ineffective as an inhibitor (Table VI). This may be explained by noting the ratio of nicotinamide methyltransferase to tRNA methyltransferase in these fractions (Table VII). When this ratio is increased from 14 in the pH 5 enzyme fraction to approximately 50 by the addition of nicotinamide methyltransferase to this same preparation, the inhibition of tRNA methyltransferase is restored as indicated in Table VI. These results indicate that the inhibition of tRNA methyltransferase in this system is due primarily to the formation of *S*-adenosylhomocysteine and not simply competition for *S*-adenosylmethionine by these two methylating enzymes as concluded by Kuchino and Endo (1972). Since the tRNA methyltransferase enzymes are more sensitive to *S*-adenosylhomocysteine than is the nicotinamide methyltransferase enzyme as judged by their K_i values of 3.6×10^{-6} and 7.0×10^{-5} M, respectively, it is possible to postulate a regulatory function of nicotinamide methyltransferase on tRNA methylation similar to that proposed by Kerr (1972) for glycine methyltransferase.

Properties of Porcine Liver Nicotinamide Methyltransferase. The enzyme displayed normal Michaelis–Menten kinetics and the K_m's for nicotinamide and *S*-adenosylmethionine were calculated to be 1.1×10^{-3} and 5×10^{-5} M, respectively. From a Dixon plot (Dixon and Webb, 1964) in which inhibitor concentration was plotted against the reciprocal velocity, *S*-adenosylhomocysteine was a competitive inhibitor of *S*-adenosylmethionine and a K_i value of 7.0×10^{-5} M was determined.

Properties of Porcine Liver S-Adenosylmethionine : 5′-Methylthioadenosine Hydrolase. The enzyme displayed Michaelis–Menten Kinetics and the K_m for *S*-adenosylmethionine

was calculated to be 5×10^{-5} M. From a Dixon plot, *S*-adenosylhomocysteine was found to be a competitive inhibition of *S*-adenosylmethionine and K_i value was calculated to be 1.0×10^{-4} M.

Discussion

The effect of nicotinamide on tRNA methyltransferase enzymes appears to be due to the activation of nicotinamide methyltransferase and the production of *S*-adenosylhomocysteine and its subsequent inhibition of the tRNA methyltransferases. Enzyme preparations with a low ratio of nicotinamide methyltransferase to tRNA methyltransferase are only slightly inhibited by nicotinamide (Table VI). We therefore agree with the overall view of Gross and Wildenauer (1972) that nicotinamide itself does not inhibit the tRNA methyltransferase enzymes directly. In addition, we believe that the nicotinamide inhibition is primarily due to the sensitivity of the tRNA methyltransferase enzymes to *S*-adenosylhomocysteine and not simply to competetion for *S*-adenosylmethionine as concluded by Kuchino and Endo (1972). We have shown that the proposed "nicotinamide-dependent *S*-adenosylmethionine-splitting enzyme" in rat liver described by Gross and Wildenauer (1972) is in reality the nicotinamide methyltransferase enzyme identified by Kuchino and Endo (1972). Our results are also consistent with those of Murai *et al.* (1972) which demonstrated a nondialyzable inhibitor in rat liver that was active as an inhibitor of tRNA methylation in the presence of nicotinamide.

It is apparent that both nicotinamide methyltransferase and *S*-adenosylmethionine: 5′-methylthioadenosine hydrolase are but two of several enzymes involved in the regulation of cellular concentration of *S*-adenosylmethionine as Kerr (1971, 1972) has recently reported that rabbit liver, kidney, and pancreas also contain an inhibitory system which generates *S*-adenosylhomocysteine *via* the methylation of glycine to sarcosine. The specific role that the enzyme *S*-adenosylmethionine: 5′-methylthioadenosine hydrolase has in mammalian tissue remains to be elucidated. Enzymes that cleave *S*-adenosylmethionine to 5′-methylthioadenosine have been previously reported in cell-free extracts of *Aerobacter aerogenes* (Shapiro and Mather, 1958) and in *E. coli* B cells after T_3 phage infection (Gefter *et al.*, 1966).

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