Transmethylation, Transsulfuration, and Aminopropylation Reactions of S-Adenosyl-L-Methionine in Vivo*

Paolo Giulidori, Marzia Galli-Kienle, Emilia Catto, and Giorgio Stramentinoli

From the BioResearch Co., Research Laboratories, Liscate (Milan), Italy and the Department of Medical Chemistry and Biochemistry, University of Milan, Italy

S-Adenosylmethionine (AdoMet) is metabolized through three main pathways, i.e. (a) transfer of its methyl group to a variety of methyl acceptors, (b) decarboxylation followed by aminopropylation leading to polyamine synthesis, and (c) cleavage of the bond between the sulfur atom and carbon 4 of the amino acid chain, resulting in formation of methylthioadenosine and homoserine thiolactone.

In this study the metabolism of AdoMet through these pathways was studied after intravenous administration to rats of [1-14C], [3,4-14C], [methyl-14C], and [35S]AdoMet at various doses. The relative utilization of AdoMet and methionine was also investigated. The results show that intravenously administered AdoMet is efficiently metabolized in vivo up to the highest tested dose (250 μmol·kg⁻¹ body weight), about two-thirds of the metabolized compound being utilized via transmethylation and cleavage to methylthioadenosine and one-third via decarboxylation. The efficient incorporation of the methyl group of AdoMet into muscle creatine indicates unambiguously that the compound is taken up and metabolized by the liver. Moreover, intravenously administered AdoMet is shown to be a better precursor than methionine both in creatine formation and in the utilization of the sulfur atom in transsulfuration reactions.

Since the elucidation by Cantoni (1, 2) of the structure of AdoMet¹ and of its importance in biological transmethylation, a number of reports have been published which suggest that this sulfonium compound is able to cross mammalian cell membranes. Stekol et al. (3) demonstrated that AdoMet can be utilized efficiently by rats in vivo for intracellular methylolation reactions. These authors compared the extent of incorporation of the [methyl-14C]group of methionine and of AdoMet into tissue creatine-creatinine and choline and found that in the first 60 min following a single intraperitoneal dose of radiolabeled AdoMet, 5% of the label was excreted in the respiratory CO₂, while 10% was incorporated in creatine and 6% in choline 5 hr after intraperitoneal injection. With radiolabeled methionine and identical experimental conditions, 4.7% of the radioactivity was recovered in creatine, 30% in choline, and 12% in the respiratory CO₂. In liver perfusion experiments, Zappia et al. (4) demonstrated an uptake of AdoMet from the medium, and Stramentinoli et al. and Pezzoli et al. (5, 6) found that AdoMet was taken up both by rabbit erythrocytes and hepatocytes. However, in contrast to these data, Hoffman et al. (7) reported perfused liver to be impermeable to AdoMet. More recently, it was shown that the AdoMet methyl group is incorporated into phospholipids after incubation of isolated hepatocytes (8) with [methyl-3H] AdoMet. In this case, however, it was concluded that AdoMet does not enter the intracellular AdoMet pool, although its methyl groups may be incorporated into plasma membrane phospholipids.

The administration of AdoMet to animals and humans results in a variety of interesting and significant pharmacological effects (9-16). We thought of it to obtain some information on the in vivo utilization of AdoMet by comparing the metabolism of [1-14C], [3,4-14C], [35S]-, and [methyl-14C] AdoMet after intravenous administration of AdoMet to rats. The main AdoMet metabolic pathways are schematically reported in Fig. 1. When AdoMet is metabolized through any of them (A, B, and C in the scheme in Fig. 1), one should expect carbon dioxide to be the end product of the degradation of the carboxyl carbon atom of AdoMet. Measurement of expired 14CO₂ after treatment of rats with [1-14C]AdoMet should, therefore, reflect metabolism of the compound through decarboxylation to DecaAdoMet, transmethylation leading to AdoHcy, and cleavage giving methylthioadenosine and homoserine thiolactone. In contrast, labeled CO₂ from [3,4-14C]AdoMet should originate only from the last two pathways.

The results reported herein, already partially communicated elsewhere (17), show that AdoMet is efficiently metabolized in vivo after intravenous administration to rats of doses up to 250 μmol·kg⁻¹ body weight; about two-thirds of the metabolized compound is utilized both via transmethylation and cleavage to TMA (B and C in Fig. 1), and one-third via decarboxylation. The metabolism of [1-14C]AdoMet to CO₂ is more than twice as efficient as that of [1-14C]Met, at a similar dose. This is also true for the transsulfuration pathway, as shown by the amount of [35S]-sulfate excreted after either [35S]AdoMet or [35S]Met administration.

EXPERIMENTAL PROCEDURES

Materials—Radioactive compounds were obtained from the Radiochemical Centre (Amersham, England). S-Adenosyl-L-methionine sulfate p-toluenesulfonate (18) was from BioResearch SpA (Liscate, Italy); TMA was prepared from AdoMet as described elsewhere (19); and AdoHcy was from Sigma. Their purity was higher than 98% as demonstrated by HPLC using the conditions reported below for the purification of AdoMet. All other reagents were of analytical grade.

Purification of AdoMet by HPLC—Purity of all AdoMet specimens was checked by HPLC. To this purpose, weighed amounts of AdoMet were dissolved in 0.025 M H₂SO₄, and aliquots (50-100 μg AdoMet...
were injected into a Perkin-Elmer series 3 liquid chromatograph, equipped with a Whatman Partisil SCX 10-μm column (0.4 × 25 cm) and a UV detector LC-75 set at 254 nm. Elution was performed using a concentration gradient with aqueous ammonium formate, pH 4, kept 0.1 M for 10 min, and then linearly brought to 0.6 M in 10 min; flow rate was 3 ml/min. The retention time of AdoMet was 18 min under these conditions which allowed its complete separation from adenosine, adenine, methyIthioadenosine, homoserine, and methionine. The compound was taken as pure when area of the peak at the retention time of AdoMet was higher than 98% of the theoretical value as calculated from a reference standard. When purity was less than 98%, preparative HPLC was carried out and a fraction was collected at the AdoMet retention time. After lyophilization, the purity of the recovered compound was checked again by HPLC.

When radioactive AdoMet was purified, aliquots of the eluted fractions were counted for radioactivity. Purified labeled AdoMet was analyzed as above and taken as pure when at least 95% of the injected radioactivity was found associated with the authentic compound.

Preparation of [35S]AdoMet—AdoMet, labeled at the sulfur atom, was prepared according to the procedure described by Mudd and Cantoni (23) with minor modifications; 100 g of freeze-dried Schizosaccharomyces cerevisiae was suspended in 0.07 M K2HPO4 (350 ml) containing L-methionine (600 mg) and stirred for 4 h in a water bath at 32°C. The supernatant (190 ml) obtained by centrifugation (0°C) at 2500 rpm for 20 min (1 extract) was processed as described (23) up to precipitation with calcium phosphate gel. The precipitate was washed by centrifugation with 70 ml of 0.03 M sodium acetate buffer, pH 5.5, and suspended in 0.04 M potassium phosphate, pH 6.6 (15 ml). Gentle stirring for 1 h at 0°C and centrifugation at 18,000 x g for 3 min gave a supernatant (21 ml) which was used as the enzyme source for the preparation of [35S]AdoMet. For this purpose, 0.3 ml of L-[35S] Met (116 mCi/mmol) was incubated with the enzyme preparation (10 mg of protein) in 250 μl Tris-HCl buffer, pH 7.8, containing 0.2 M KCl, 10 mM MgCl2, 5 mM mercaptoethanol, 5 mM ATP. The final volume was 10 ml, and the incubation lasted 18,000 x g for 3 min gave a supernatant (21 ml) which was used as the enzyme source for the preparation of [35S]AdoMet. For this purpose, 0.3 ml of L-[35S] Met (116 mCi/mmol) was incubated with the enzyme preparation (10 mg of protein) in 250 μl Tris-HCl buffer, pH 7.8, containing 0.2 M KCl, 10 mM MgCl2, 5 mM mercaptoethanol, 5 mM ATP. The final volume was 10 ml, and the incubation lasted 1 h at 37°C with shaking. The reaction was stopped by addition of 10% trichloroacetic acid (7 ml) and 0.2 ml of 0.1 M HCl, and the resulting sample was centrifuged. Trichloroacetic acid was removed from the supernatant by washing three times with diethyl ether previously shaken with a 20% FeSO4 solution to eliminate peroxides. The supernatant was then added to 1 ml of concentrated formic acid and freeze-dried. Purification of [35S]AdoMet in the lyophilized material was carried out by HPLC using the conditions described above; specific radioactivity of purified [35S]AdoMet was 116 mCi/mmol.

Radioactivity Measurements—Radioactivity was measured in an Intertechique SL 30 liquid scintillation counter. Counting efficiency was determined using either [14C]toluene or Na235SO4 (Radiochemical Centre, Amersham, England) as internal standard. Efficiency ranged from 70 to 85% for both 14C and 35S. Counts for 35S-labeled samples were always corrected for sulfur decay.

For the detection of radioactivity in the urine, 100 μl were counted in 1 ml of water and 10 ml of Lumagel (Lumac, Basel, Switzerland). For tissues, either aliquots of homogenates or weighed amounts of slices were digested for 2 h at 50°C in 0.5 ml of Lumasolve (Lumac, Basel, Switzerland), diluted with 0.5 ml of 0.5 M HCl and counted in 10 ml of Lumagel.

Radioactivity associated with CO2 was determined in 1-ml aliquots of 2-ethoxyethanol ethanolamine used for CO2 trapping as described below. These were diluted with 5 ml of methanol and counted in 10 ml of Lipoluma (Lumac, Basel, Switzerland).

The radioactivity in fractions obtained during HPLC assay was measured in a 1-ml sample added to 10 ml of Lumagel. To evaluate the amount of excreted radioactive sulfates, 0.4 ml of urine was treated with 0.6 ml of 0.1 M Na2SO4 and 1 ml of 1 M BaCl2. The precipitate was centrifuged and washed three times by suspension in 3 ml of water followed by centrifugation. The final precipitate was suspended in a 6% Triton X-100 aqueous solution and counted in 10 ml of Lumagel. Counting time was always selected to obtain a maximum ±5% cpm S.D. Recovery of sulfates was complete as determined after addition to the urine of various amounts of Na235SO4.

Determination of Radioactivity Associated with CO2 in Expired Air—Immediately after treatment, animals were individually placed in glass metabolic cages (Bradley Inc., Sawbridgeworth, England) and kept there until sacrifice. Cages were maintained under a 2-liter/min constant air flow. Humidity was eliminated from the air by passing it through 150 ml of cold 2-ethoxyethanol. Three traps containing 100 ml of 2-ethoxyethanol:ethanolamine (3:1, v/v) allowed recovery of the expired CO2 Preliminary experiments, where known amounts of 14CO2 were produced inside the cages by acidification of an NaH14CO3 solution, showed a complete recovery between the first and the second ethanolamine-containing trap. The radioactivity was determined in aliquots of the ethanolamine solution as described above.

Determination of Radioactivity Associated with Proteins and Nucleic Acids in Liver—Livers from animals treated with [methyl-14C]AdoMet and [methyl-14C]Met and with [35S]AdoMet were frozen in liquid nitrogen and powdered in a mortar. Separation of the various fractions of compounds was carried out as follows. Aliquots of the powdered tissue (2 g) were homogenized in 4 ml of cold saline (0–4°C) using an Ultra Turrax homogenizer (type 18/10, Janke & Kunkel, Breisgau, Germany), diluted to 10 ml with saline, and treated with 1 volume of 10% cold trichloroacetic acid. The pellet obtained by centrifugation was washed by suspension twice in 10% trichloroacetic acid and once in water, followed by centrifugation. Supernatants of all the centrifugations were combined and counted for eval-
vation of the radioactivity associated with water-soluble compounds. Lipophilic compounds were extracted from the pellet with 95% ethanol containing 1.5% sodium acetate (8 ml, twice), with absolute ethanol (8 ml, three times), and chloroform:methanol (1:1, v/v; 8 ml, three times). Each extraction was followed by centrifugation, and the radioactivity in the combined supernatants represented that associated with lipophilic compounds. The pellet-containing proteins, nucleic acids, and polysaccharides were dried under vacuum and weighed. Protein content and radioactivity measurements in aliquots of this fraction permitted calculation of the radioactivity associated with macromolecules in the tissue.

Samples of methyl-labeled compounds were suspended in 10% trichloroacetic acid (4 ml) and heated up to 90 °C for 10 min to hydrolyze the nucleic acids (21). After cooling, the suspension was centrifuged and the pellet obtained was washed with 4 ml of 10% trichloroacetic acid. The washing and the first supernatant were combined and filtered. The radioactivity in this fraction was very low and probably due to protein contamination. The radioactivity in the pellet was that associated with proteins. Radioactivity and protein concentration were determined at every step of the procedure.

**Determination of Creatinine in the Urine**—The urine of animals treated with either AdoMet or methionine labeled with 14C in the methyl group was daily collected in 1 ml of 1 N HCl. The volume of urine was measured, and the pH of the injected solution was brought to 4.0 with HCl, to 4.6 with HCl. After centrifugation for 10 min at 10,000 rpm, 10 μl of the supernatant were assayed by HPLC under the following conditions (22). A Whatman Partisil PXS 10/25 SCX column was used; the elution was carried out with 0.1 N ammonium phosphate acidified to pH 2.6 with 85% phosphoric acid. The retention time of creatinine with a 2 ml/min flow was 4.0 min. For the detection, a UV spectrophotometric detector set at 215 nm was used. The amount of creatinine was determined on the basis of a standard curve. When collected at the detector outlet the recovery of injected creatinine was 93 ± 2%, as determined by reinjecting part of the fraction.

To check the homogeneity of the HPLC peak of creatinine, another part of the fraction was analyzed by cellulose TLC (Merek, Darmstadt, Germany) (n-butyl alcohol:acetic acid:water, 60:15:15, v/v/v) (23). A single spot was obtained at the Rf of authentic creatinine. For the measurement of creatinine specific radioactivity, the supernatant obtained after centrifugation of 1 ml of urine in 10 ml of methyl cyanidewater (80:20, v/v) solution was treated with dichloromethane (10 ml). After shaking, the aqueous phase was collected and aliquots (100 μl) were analyzed by HPLC under the above described conditions. The peak of creatinine retention time was collected and counted for radioactivity. The recovered creatinine in the collected fraction was quantitated by analyzing some aliquots by HPLC. Creatinine specific radioactivity was calculated as the ratio between the radioactivity and the amount of compound in the fraction.

**Determination of Creatine in Muscle**—Aliquots (200 mg) of muscle were dissected from each rat, cleaned from fat and connective tissue, sliced, and counted to determine tissue radioactivity. For HPLC measurement of creatine 1-g tissue slices were homogenized in 10% trichloroacetic acid in 0.05 N HCl (4 ml). Part of the supernatant after centrifugation (2.5 ml) was washed three times with diethyl ether previously saturated with 0.05 N HCl. The aqueous phase (2 ml) was treated with 0.4 ml of 6 N HCl and heated in boiling water for 4 h to hydrolyze creatine phosphate to creatinine, essentially as described by Foster et al. (24). After cooling to room temperature, the pH was raised to 4.0 and water was added to obtain a final volume of 2.5 ml. Aliquots of this solution were analyzed by HPLC for the measurement of creatinine concentration and its specific radioactivity as described for creatinine in the urine.

**Animal Treatment**—Male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 244 ± 2 g were used. Within each experiment, animals were selected in order to have a maximum standard deviation of ±2 g from the mean body weight.

Treatments with various labeled samples of AdoMet and methionine were all carried out on animals fasted 16 h, which were then housed in individual cages till the end of the experiment. For each treatment, the animal number per group, the time intervals in urine collection, and the time of sacrifice are reported in the following sections. The treatments were made by intravenous injection either of AdoMet or of Met solutions in 0.18 M phosphate buffer (0.5 ml/kg body weight); the pH of the injected solution was 6.5. The animals were sacrificed by cervical dislocation. The urine was directly collected in 1 ml of 1 N HCl to avoid AdoMet decomposition. Tissues to be analyzed were dissected immediately after sacrifice and frozen at −20 °C till time for use.

**RESULTS**

**Radioactivity in Expired CO2 and Urine after Administration of [1-14C]AdoMet, [methyl-14C]AdoMet, [1-14C]Met, [methyl-14C]Met, or [3,4-14C]AdoMet—AdoMet radiolabeled with 14C (13 μCi: kg−1 body weight; approximately 3 μCi/rat) was given to groups of three rats. The doses of AdoMet shown in the tables and figures were obtained by dilution of the radiolabeled material with unlabeled AdoMet before administration.**

The 14CO2 formed from carboxyl carbon atom-labeled AdoMet was considered indicative of the overall metabolism of the compound, because it can arise not only from AdoMet decarboxylation (Fig. 1, pathway A), but also as the end product of degradation of the homoserine formed from AdoMet cleavage to TMA (pathway B) or from α-ketobutyrate derived from cystathionine (pathway C). The rate of [1-14C]AdoMet conversion to 14CO2 reached its maximum 1.5–2 h after treatment (Fig. 2). Comparison was made with 14CO2 excretion from [1-14C]Met; in order to minimize alterations of endogenous intracellular pools of AdoMet, ranging from 21.6 μM in skeletal muscles to 83.6 in the liver and of Met reported to be around 70 μM (25), the labeled compounds were administered undiluted (60 μCi/mmol).

The rate of 14CO2 excretion from [1-14C]Met was much lower than that from [1-14C]AdoMet at a similar dose and at all tested times.

The rate of 14CO2 excretion from [1-14C]AdoMet increased with the dose (Fig. 3); the percentage of [1-14C]AdoMet converted to 14CO2 was 68% at the dose of 25 μmol·kg−1 body weight and decreased to 28% at 250 μmol·kg−1 body weight.

The radioactivity expired as CO2 from [3,4-14C]AdoMet differed from that originating from [1-14C]AdoMet (Fig. 4).

**Fig. 2. Rate of 14CO2 production in rats treated intravenously with (a) [1-14C]AdoMet at doses of 25 (○), 125 (△), and 250 (■) μmol·kg−1 body weight or with (b) [1-14C]AdoMet (○) or [1-14C]Met (■) (0.22 μmol·kg−1 body weight).**
AdoMet methyl group is incorporated back into methionine have been suggested (26-28). Our results showing that conversion to CO$_2$ of the carboxyl carbon atom of methionine and of methyl carbon atom (data not shown) is lower than that of the same carbon atom of AdoMet indicate that at least part of intravenously administered AdoMet is metabolized without previous re-conversion to methionine. The recovery of radioactivity in urine, as per cent of the administered dose, increased by raising the dose of AdoMet (Table I). No significant difference was observed between radioactivity from [1-14C]AdoMet and [3,4-14C]AdoMet with any of the doses employed, thus confirming that the difference found in the conversion of these two labeled compounds to CO$_2$ is likely to reflect the incorporation of a fraction of AdoMet moiety containing carbons 3 and 4 into a stable pool. As expected from the excretion of methylated compounds, the radioactivity in urine from [methyl-14C]AdoMet was significantly higher than that from [1-14C]AdoMet and [3,4-14C]AdoMet at the lowest dose and at 25 μmol·kg$^{-1}$ body weight. At the highest dose, no difference was observed among the three differently labeled AdoMet molecules, because at this dose the amount of unmodified AdoMet excreted in urine accounted for most of the urinary radioactivity (Table I). Excretion of radioactivity from [1-14C]Met and [methyl-14C]Met was lower than that from the corresponding labeled AdoMet molecules in agreement with the expected incorporation of Met but not AdoMet into proteins.

**Utilization of AdoMet for Creatine Synthesis**—From the results reported by Mudd and Poole (29) it can be calculated that more than 80% of the methionine methyl group utilized for methylation is involved in the synthesis of creatine. These authors reported results from which it can be calculated that creatine synthesis accounts, on average, for 12.9 out of a total outflow of 15.6 mEq of labile methyl groups. It was of interest, therefore, to confirm the findings of Stekol et al. (3) and compare the relative incorporation of the methyl group of AdoMet and methionine into creatine/creatinine. Accordingly, rats were treated with [methyl-14C]AdoMet or [methyl-14C]Met, as indicated in Tables II and III. These tables show incorporation of label into muscle creatine, urinary creatinine, and total radioactivity for the 24-h period that follows administration of the radioactive compounds. In muscle, when AdoMet was used as the precursor, about 76% of the total radioactivity was found in creatine; the fraction of the methyl group of methionine found in creatine was smaller, probably because label derived from methionine but not from AdoMet is also incorporated into muscle proteins. Excretion of creatinine in the 24-h urine collected after the administration was not modified by any of the treatments with AdoMet or methionine.

**TABLE I**

| Treatment | Dose (μmol·kg$^{-1}$ body weight) | Total radioactivity (% of the dose) | Radioactivity as unmodified AdoMet (% of the dose) |
|-----------|----------------------------------|-----------------------------------|-----------------------------------------------|
| AdoMet    | 0.22                             | 9 ± 1                             | 28 ± 3*                                       |
| AdoMet    | 25                               | 12 ± 1                            | 16 ± 1                                        |
| AdoMet    | 125                              | 44 ± 4                            | 53 ± 3*                                       |
| AdoMet    | 250                              | 53 ± 7                            | 59 ± 6                                        |
| Met       | 0.22                             | 1.8 ± 0.1                         | 6.3 ± 0.2*                                    |
A 6-h urine sample contained inorganic sulfate in addition to AdoMet excreted as such. In a cumulative 7-day treatment of [Y3]methionine. Urine was collected for the first 6 h after intravenous administration of [Y3]AdoMet or with [methyl-14C]Met. Each value represents the mean ± S.E. of results obtained in three animals. Formulation of creatinine from AdoMet or Met was calculated from the radioactivity found associated with creatinine and the specific radioactivity of the administered compound.

Radioactive creatinine accounted for a small percentage of total urinary radioactivity (Table III) both with methionine and AdoMet, since a large fraction of radioactivity is associated with other methyalted metabolites and with unchanged compounds and also because urinary creatinine is derived from the nonenzymatic conversion of creatine phosphate, a conversion that proceeds at the rate of about 2% of total creatine phosphate/day.

Formation of labeled creatinine increased with the dose of AdoMet (Tables II and III).

**Table II.** Recovery of total radioactivity and labeled creatinine in muscles of rats treated intravenously with [methyl-35S]AdoMet or with [methyl-14C]Met.

| Treatment | Dose (nmol·kg⁻¹ body weight) | Total radioactivity in muscle (% of the dose) | Per cent of the total radioactivity | Formation from AdoMet or Met (nmol·kg⁻¹ body weight) |
|-----------|-----------------------------|-----------------------------------------------|------------------------------------|---------------------------------------------------|
| [methyl-35S]AdoMet | 0.72 | 29 ± 1 | 75.9 | 0.16 |
| [methyl-14C]AdoMet | 125 | 10 ± 1 | 61.0 | 7.7 |
| [methyl-38S]Met | 0.72 | 33 ± 2 | 27.2 | 0.08* |

*p < 0.001 versus AdoMet (0.72 nmol).

**Table III.** Recovery of total radioactivity and labeled creatinine in rat urine during the 24 h following intravenous treatment with [methyl-14C]AdoMet or with [methyl-14C]Met.

| Treatment | Dose (nmol·kg⁻¹ body weight) | Total radioactivity in urine (% of the dose) | Per cent of total radioactivity | Formation from AdoMet or Met (nmol·kg⁻¹ body weight) |
|-----------|-----------------------------|-----------------------------------------------|--------------------------------|---------------------------------------------------|
| [methyl-14C]AdoMet | 0.72 | 32 ± 2 | 5.2 | 0.01 |
| [methyl-14C]AdoMet | 125 | 57 ± 12 | 5.4 | 3.9 |
| [methyl-14C]Met | 0.72 | 6 ± 1 | 7.2 | 0.003 |

*p < 0.001 versus AdoMet at the same dose (Student’s t test).

**Table IV.** Urinary recovery of total radioactivity and [35S]sulfate after intravenous administration of [35S]AdoMet or of [35S]Met.

| Treatment | Dose (nmol·kg⁻¹ body weight) | Total radioactivity in urine (% of the dose) | Per cent of total radioactivity | Formation from AdoMet or Met (nmol·kg⁻¹ body weight) |
|-----------|-----------------------------|-----------------------------------------------|--------------------------------|---------------------------------------------------|
| [35S]AdoMet | 0.62 | 25 ± 3 | 65.5 | 0.1 ± 0.01 |
| [35S]AdoMet | 125 | 49 ± 2 | 20.6 | 13 ± 1 |
| [35S]Met | 0.62 | 8.7 ± 0.7 | 67.4 | 0.04 ± 0.004 |

**Table V.** Radioactivity in liver fractions of rats treated intravenously with [35S]AdoMet.

| Treatment | Dose (nmol·kg⁻¹ body weight) | Radioactivity in liver (% of the dose) | Per cent of total radioactivity in liver fractions |
|-----------|-----------------------------|-------------------------------------|-----------------------------------------------|
| [35S]AdoMet | 0.62 | 0.03± ± 0.002 | 4.0 | ND | 96.0 |
| [35S]AdoMet | 125 | 0.08 ± 0.0004 | 4.3 | ND | 95.7 |

an increase of the [35S]SO₄ originating from AdoMet (Table IV).

The analysis of liver fractions showed that most of the radioactivity was associated with the macromolecular fraction (Table V), presumably as protein cysteine and/or methionine ([35S]methionine could be formed from [35S]AdoMet via TMA and its conversion to methionine (28)). The [35S]cysteine/[35S]methionine ratio was not determined. Since conversion of the sulfur atom of AdoMet to inorganic sulfate can occur only via pathway C in Fig. 1, the results here discussed...
demonstrate, in conclusion, that intravenously administered AdoMet is utilized \textit{in vivo} for biological methylations.

**DISCUSSION**

Both the synthesis and the metabolism of AdoMet are ubiquitous reactions, and enzymes that catalyze some of these reactions are found in practically all cells. In mammals, AdoMet is metabolized through three main pathways: 1) transfer of its methyl group to a variety of methyl acceptor compounds with concomitant formation of AdoHcy in stoichiometric amounts (31); 2) decarboxylation with formation of DecaAdoMet (32); 3) cleavage of the bond between the sulfur atom and carbon 4 of the amino acid chain resulting in formation of TMA and homoserine lactone (33).

In the past, the metabolism of AdoMet has been studied almost exclusively \textit{in vitro}. Therefore, the relative quantitative importance of the different metabolic pathways is as yet incompletely understood. In an earlier study, Stekol \textit{et al.} (3) found that in the intact rat the methyl group of AdoMet was rapidly incorporated into creatine and choline. No further results were, however, reported concerning the other two pathways of AdoMet catabolism: decarboxylation, the first stage of the process involving AdoMet in polyamine synthesis, and the splitting of the molecule into TMA and homoserine. The results of Stekol \textit{et al.} (3), moreover, cannot be used to quantitate the metabolism of endogenous AdoMet, because the administered dose could in theory have modified the endogenous pool. It is possible that in the experiments of Stekol \textit{et al.} an unsaturated pathway was preferred over others which were saturated.

The experiments described above confirm and extend the observations of Stekol \textit{et al.}; a more meaningful evaluation of the relative utilization of AdoMet and methionine was obtained by use of radiolabeled compounds in doses sufficiently small so as to eliminate, or at least minimize, alterations in the endogenous pools. The incorporation of the methyl group of intravenously administered AdoMet into muscle creatine indicates unambiguously that, after intravenous injection, AdoMet is taken up and metabolized by the liver. Equally clear is the interpretation of the experiments with $^{[35}S]$ AdoMet; these show that the homocysteinylo moiety of AdoMet is utilized in transsulfuration reactions and is a better precursor of urinary sulfate than is methionine.

The excretion of CO$_2$ after administration of [1-$^{14}$C] AdoMet, that we considered to be an index of the overall \textit{in vivo} metabolism of the compound, was shown to be dose-dependent, and it was not saturated up to the highest dose used. Previous studies of AdoMet kinetics in plasma after administration of 25 $\mu$mol\textperiodcentered kg$^{-1}$ body weight to the rat (34) revealed a 30-min half-life of the compound. In addition, the low recovery of AdoMet in urine suggested a fast metabolism of the compound. This is now confirmed by the observation that the curve of AdoMet decay in plasma fits well the curve of $^{14}$CO$_2$ excretion from [1-$^{14}$C]AdoMet (Fig. 5). Urinary excretion of the unaltered compound also increased with the dose. Previous results obtained by Placidi \textit{et al.} (35) showed that after intravenous administration of 25 $\mu$mol\textperiodcentered kg$^{-1}$ body weight of [methyl-$^{14}$C]AdoMet to the rat, a significant fraction of radioactivity was found in the kidney. It is, therefore, probable that the increase of the dose results in a saturation of the reabsorption of the compound, part of which is then excreted in the urine.

Since transmethylation processes such as phospholipid methylations and protein carboxymethylation are catalyzed by membrane enzymes, the \textit{in vivo} observed metabolism of AdoMet can be hypothesized to occur outside the cell. As a matter of fact, Van Phi and Söling (8) have recently found that the methyl group of exogenous AdoMet could be utilized by isolated hepatocytes for the methylation of phospholipids without entering the intracellular AdoMet pool. The interpretation proposed by these authors is that exogenous AdoMet equilibrates rapidly with a small sucrose inaccessible compartment on the outer side of plasma membranes and that it can be utilized for cellular enzyme reactions occurring on the outer side of these membranes. While it is easy to visualize phospholipid methylation to be localized in plasma membranes, it is more difficult to consider creatine synthesis as taking place on the cell surface, especially since guanidinoacetic acid methyltransferase behaves as a cytoplasmic enzyme (36). Moreover, AdoHcy that originates via transmethylation processes has been demonstrated not to be taken up by liver cells (37), and AdoHcy was shown to be actively metabolized \textit{in vivo} to S-adenosyl-$\gamma$-thio-$\alpha$-ketobutyrate (38); the latter compound is recovered in urine. As this compound preserves the carboxyl carbon of AdoMet and the data reported indicate that at least 85% of radioactivity of this carbon atom is found either as CO$_2$ or as unmodified AdoMet at all tested doses, it can be concluded that intravenously administered AdoMet, similarly to the endogenous compound, is mainly catabolized within the cell.

The present results also show that part of the intravenously administered AdoMet is metabolized without previous conversion to methionine. In fact the rate of $^{14}$CO$_2$ excretion decreased rapidly between the first and the third hour (Fig. 26), then very slowly from hour 6 to 24. A mean rate of 2.7 $\pm$ 0.9 and 1.2 $\pm$ 0.2 $\mu$mol\textperiodcentered kg$^{-1}$ body weight$^{-1}$ could be calculated for breakdown of AdoMet and Met, respectively, in the 6- to 24-h time interval. The slow phase of $^{14}$CO$_2$ excretion suggested that part of the administered radioactivity is incorporated into pools of compounds with long half-lives in the body as, for example, proteins or any other compound containing the carbon atom of AdoMet or Met carboxy group. Because recovery of radioactivity as the sum of that found as CO$_2$ (Fig. 4) and that excreted in the urine (Table I) reached 85% of the dose for AdoMet but only 35% for Met after administration of the 1-$^{14}$C-labeled compounds, it appears that the fraction of intravenously administered methionine,
which is incorporated into stable pools, is much higher than that of intravenously administered AdoMet. It may be, therefore, concluded that only part of the intravenously administered methionine is converted to AdoMet. Radioactivity recovered in creatinine derived from [methyl-"C]AdoMet and in sulfates derived from [35S]Met, which is about three times lower when compared to that recovered from the corresponding labeled AdoMet molecules, confirmed this conclusion.

The excretion of 14CO2 from [3,4,4']AdoMet during the 24 h following administration (Fig. 4) corresponded to 70% of that obtained from [1,14C]AdoMet, while recovery of radioactivity in the urine was equal for both the labeled compounds. As the carbon atoms 3 and 4 are incorporated into polyamines (Fig. 1), our data indicate that about 25% of intravenously administered AdoMet is utilized for the aminopropylation pathway following decarboxylation. Increasing the dose does not substantially modify this distribution. On the basis of data reported by Mudd and Poole (29), it was calculated (36) that creatine synthesis accounts for about 80% of methylations. Since 24 h after administration of the low dose of [methyl-14C]AdoMet, 22% of the radioactivity was recovered as creatinine from the muscle and 1.7% from the urine, we can assume that transmethylation accounts for about one-third of AdoMet metabolism. Incorporation of AdoMet into stable pools accounts for 17% of the administered dose, as it can be calculated from 83% recovery of [1,14C]AdoMet radioactivity in CO2 and urine during the first day after administration. Therefore, AdoMet involved in aminopropylation (25%) and transmethylation (30%) pathways and that incorporated into stable pools (17%) represented 72% of the administered dose. Chemical decomposition of AdoMet to TMA was excluded because in rat plasma at 37°C the compound showed a 5.4-h half-life, much longer than that observed for the in vivo data (0.5 h).

The above calculated balance for AdoMet metabolism via the different pathways was derived from results obtained when the amounts of AdoMet and Met administered to animals were low enough to ensure that the endogenous pools were unaltered. One could, therefore, assume that the behaviors of endogenous AdoMet and Met are those observed here for the intravenously administered compounds. However, it cannot be assumed that intravenously administered AdoMet or Met will mix with all the in vivo pools where the described metabolic processes occur.

The distribution of AdoMet (administered intravenously) through the various metabolic pathways seems not to be altered by an increase of the administered dose. This finding may be of metabolic importance with regard to recent results which have shown a deficiency of transsulfurating activity in cirrhoses due to a decreased activity of the enzyme catalyzing AdoMet synthesis from methionine (39).

In conclusion, the present results show that intravenously administered AdoMet is efficiently metabolized in vivo within the cell. The metabolism occurs, at least partially, without previous reconversion of AdoMet to methionine, and it is almost equally distributed among the three main metabolic pathways, decarboxylation, transmethylation, and splitting of the molecule into TMA and homoserine.

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P Giulidori, M Galli-Kienle, E Catto and G Stramentinoli

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