The Decarboxylation of *S*-Adenosylmethionine by Detergent-Treated Extracts of Rat Liver

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1. The production of $^{14}CO_2$ from *S*-adenosyl[*carboxyl*-^{14}C]methionine by rat liver extracts was investigated. It was found that, in addition to the well-known cytosolic putrescine-activated *S*-adenosylmethionine decarboxylase, an activity carrying out the production of $^{14}CO_2$ could be extracted from a latent, particulate or membrane-bound form by treatment with buffer containing 1 % (v/v) Triton X-100 [confirming the report of Sturman (1976) *Biochim. Biophys. Acta* **428**, 56–69]. 2. The formation of $^{14}CO_2$ by such detergent-solubilized extracts differed from that by cytosolic *S*-adenosylmethionine decarboxylase in a number of ways. The reaction by the solubilized extracts did not require putrescine and was not directly proportional to time of incubation or the amount of protein added. Instead, activity showed a distinct lag period and was much greater when high concentrations of the extracts were used. The cytosolic *S*-adenosylmethionine decarboxylase was activated by putrescine, showed strict proportionality to protein added and the reaction proceeded at a constant rate. Cytosolic activity was not inhibited by homoserine or by *S*-adenosylhomocysteine, whereas the Triton-solubilized activity was strongly inhibited. 3. By using an acetone precipitate of Triton-treated homogenates as a source of the activity, it was found that decarboxylated *S*-adenosylmethionine was not present among the products of the reaction, although 5′-methylthioadenosine and 5-methylthio-ribose were found. Such extracts were able to produce $^{14}CO_2$ when incubated with [U-^{14}C]-homoserine, and $^{14}CO_2$ production was greater when *S*-adenosyl[*carboxyl*-^{14}C]methionine that had been degraded by heating at pH6 at 100°C for 30min (a procedure known to produce mainly 5′-methylthioadenosine and homoserine lactone) was used as a substrate than when *S*-adenosyl[*carboxyl*-^{14}C]methionine was used. 4. These results indicate that the Triton-solubilized activity is not a real *S*-adenosylmethionine decarboxylase, but that $^{14}CO_2$ is produced via a series of reactions involving degradation of the *S*-adenosyl-[*carboxyl*-^{14}C]methionine. It is probable that this degradation can occur via several pathways. Our results would suggest that part of the reaction occurs via the production of *S*-adenosylhomocysteine, which can then be converted into 2-oxobutyrate via the trans-sulphuration pathway, and that part occurs via the production of homoserine by an enzyme converting *S*-adenosylmethionine into 5′-methylthioadenosine and homoserine lactone.

The *S*-adenosylmethionine decarboxylase that is stimulated by putrescine and a few related aliphatic amines has been stated to be localized almost exclusively in the soluble supernatant fraction obtained by ultracentrifugation of homogenates of a variety of mammalian tissues (Pegg & Williams-

Ashman, 1969*a*; Schmidt & Cantoni, 1973; Tabor & Tabor, 1976; Williams-Ashman *et al.*, 1976, 1977). This decarboxylase is clearly distinguishable from the soluble aminopropyltransferases that utilize the decarboxylated *S*-adenosylmethionine product for the synthesis of polyamines (Jänne *et al.*, 1971; Pegg, 1974; Raina *et al.*, 1976). Sturman (1976*a*) reported that addition of the non-ionic detergent Triton X-100 to the homogenization medium greatly increases the yield of *S*-adenosylmethionine

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decarboxylase activity in the supernatant fraction obtained after ultracentrifugation of rat liver homogenates. The additional S-adenosylmethionine decarboxylase activity extracted in soluble form by Triton X-100 was very unstable and seemed to be derived from membranous material associated with crude nuclear fractions of rat liver homogenates. Similar although less dramatic results were obtained with the livers of mouse, gerbil and ferret, but not of guinea pig, rabbit, chicken, frog, monkey and human. Moreover, although Triton X-100 greatly increased the yield of apparent soluble S-adenosyl-methionine-decarboxylating activity from rat liver, the detergent exerted no such effect with rat brain, kidney and spleen. It was also found that the additional catalysis of CO_2 liberation from S-adenosyl-methionine evoked by Triton X-100 was not greatly enhanced by putrescine (Sturman, 1976a) or inhibited by methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediylidine)-dinitrilo]diguanidine} (Sturman, 1976b).

We have confirmed and extended many of the observations of Sturman (1976a,b). Our experiments indicate, however, that the increased loss of CO_2 from S-adenosylmethionine manifested by soluble rat liver extracts prepared in the presence of Triton X-100 appears to be catalysed by an enzyme system that degrades S-adenosylmethionine before any release of CO_2 from this substrate, and which does not involve the action of putrescine-activated S-adenosylmethionine decarboxylase. A similar conclusion has also been reached by Eloranta & Raina (1978), whose findings were published after our studies had been completed.

Materials and Methods

The preparation and purification of S-adenosyl-L-[carboxyl-^{14}C]methionine and determination of its enzymic decarboxylation were by the method of Pegg & Williams-Ashman (1969a). The compositions of the reaction mixtures used for enzyme-activity assays are recorded in the appropriate legends.

S-Adenosyl[carboxyl-^{14}C]methionine   (52 mCi/mmol)   and S-adenosyl[methyl-^{14}C]methionine (45.9 mCi/mmol) were also purchased from New England Nuclear Corp., Boston, MA, U.S.A. L-[U-^{14}C]Homoserine (40 mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Decarboxylated S-adenosylmethionine was prepared as described by Pösö et al. (1976). Total protein was measured by the method of Lowry et al. (1951), with prior trichloroacetic acid precipitation (Jänne & Williams-Ashman, 1971) for removal of interfering substances when necessary. All rats were of the Sprague-Dawley strain; unless stated otherwise, elderly male rats (350–450 g) were used. Twice-

recrystallized ox liver catalase was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of the highest grade of purity. Determinations of pH were made with a glass electrode and refer to the stated temperatures.

Highly purified rat liver cytosolic putrescine-activated S-adenosylmethionine decarboxylase was obtained from livers of rats pretreated with methyl-glyoxal bis(guanylhydrazone) as described by Pegg (1977).

Standard soluble liver extracts were prepared as follows. Fresh chopped liver from exsanguinated rats was homogenized at 2°C with 4 vol. of buffer (25 mM-sodium phosphate, pH 7.2, unless stated otherwise), with or without addition of 1% (v/v) Triton X-100. After centrifugation at 100 000 g for 1 h at 2°C, the supernatant fluid was removed with a Pasteur pipette. Enzyme activities were determined within 1 h of preparation of these extracts unless stated to the contrary. Extracts of other tissues were prepared in the same fashion. Acetone-desiccated preparations of liver extracts were made by rapid addition of 5 or more vol. of acetone (−25°C) to the soluble extracts (prepared with or without Triton X-100) held in an ice bath with stirring. These soluble extracts were prepared in 25 mM-sodium 4-morpho-linepropanesulphonate, pH 7.2, instead of the phosphate buffer to prevent the precipitation of phosphate by the acetone. The mixture was filtered on a sintered-glass funnel without allowing the protein cake to crack, and the insoluble material washed three to five times with cold acetone. The insoluble material was dried in vacuo over a desiccant and stored at 2°C for not longer than 2 weeks. The powders were extracted by homogenization at 0°C with 15–30 ml of 100 mM-sodium phosphate (pH 7.2)/g of dried material. After centrifugation at 25 000 g for 30 min, the supernatant fluid was used as an enzyme source.

Analysis of the compounds generated by the action of such reconstituted acetone-dried powders on S-adenosylmethionine was carried out as follows. The volatile material released from S-adenosyl-[carboxyl-^{14}C]methionine by incubation with the extracts was confirmed to be $^{14}CO_2$. The radioactivity displaced from the assay medium on acidification was trapped in 0.25 ml of 1 M-NaOH in a small plastic well. The contents of the well were transferred to another flask closed with a rubber cap fitted with a second well containing 1 M-NaOH. The contents were then acidified by injection through the cap of 1 ml of 2 M-HCl. All of the radioactivity was displaced by this and trapped in the alkali. The alkaline solution was then treated with $BaCl_2$ and the precipitated $Ba^{14}CO_3$ washed and counted for radio-activity as described by Fiume et al. (1970). All of the volatile radioactivity present from the enzyme assay could be accounted for as $Ba^{14}CO_3$.

The non-volatile products of the reaction were investigated by substituting S-adenosyl[methyl-^{14}C]-methionine for the carboxyl-labelled material. After incubation, the reaction was freed from protein by the addition to the assay volume (0.5 ml) of 1.5 ml of ice-cold water followed by 2 ml of cold 10% (w/v) trichloroacetic acid. The precipitate was removed by centrifugation and the supernatant extracted twice with ether to remove the trichloroacetic acid and applied to a small column (0.5 cm × 4 cm) of Dowex-50 (H$^+$ form).

The column was washed with 5 ml portions of water, 0.5 M-HCl and then 4 M-HCl. Each fraction was evaporated to dryness under reduced pressure at 45°C and analysed by high-voltage paper electrophoresis in 0.05 M-citrate buffer (adjusted to pH 3.6 with 1 M-NaOH) for 2 h and by paper chromatography. Decarboxylated S-adenosylmethionine and S-adenosylmethionine are both quantitatively recovered in the 4 M-HCl wash and can be separated easily by paper electrophoresis (Pösö et al., 1976). 5-Methylthioribose was present in the water and 0.5 M-HCl washes; 5′-methylthioadenosine was present both in the 0.5 M-HCl wash and in the 4 M-HCl eluate; an unidentified material migrating rapidly toward the cathode in the electrophoresis was present in the 4 M-HCl eluate. Further identification of 5-methylthioribose and 5′-methylthioadenosine was carried out by paper chromatography by using the solvents described by Shapiro & Mather (1958) and Mudd (1959).

Results

Initial studies confirmed the findings of Sturman (1976a,b) that inclusion of Triton X-100 in the homogenization medium enhanced the rate of adenosylmethionine decarboxylation catalysed by ultracentrifuged supernatant fractions of rat liver homogenates. However, many of the properties of such detergent-treated extracts were strikingly different from those of liver cytosol preparations obtained without Triton X-100 treatment. The representative experiment summarized in Table 1 shows that the results depended critically on the relative amounts of the two types of liver extract added per unit volume of reaction mixture. When the amount (250 μl) of extract added per 500 μl of total assay-system volume was equivalent to 50 mg of fresh liver tissue (Table 1b), the extracts prepared with 1% (v/v) Triton X-100 in the homogenization medium were much more active in catalysing release of CO_2 from the carboxy group of 0.2 mM-adenosylmethionine than were the equivalent quantities of cytosol preparations isolated without the detergent. With these relatively high concentrations of Triton X-100-treated extracts, there was no inhibition by 10 μM-methylglyoxal bis(guanylhydrazone) in the presence of putrescine concentrations (2.5 mM) that saturate the amine-activated adenosylmethionine decarboxylase of cytosolic extracts prepared without detergent. The decarboxylation reaction by Triton-treated extracts was only slightly enhanced by exogenous putrescine. By contrast, putrescine activated the reaction and methylglyoxal bis(guanylhydrazone) was inhibitory with equivalent amounts of ultracentrifuged liver extracts prepared without Triton X-100. Furthermore, when exogenous putrescine was added, 1 mM-S-adenosylhomocysteine inhibited CO_2 formation by 54% with the detergent-treated preparations, but was not inhibitory with the normal cytosolic extracts. The results were strikingly different when 75 μl of extract (equivalent to 15 mg of fresh tissue) was added (Table 1a). In this case, there was little difference in the rate of decarboxylation with either the normal or the detergent-

Table 1. *Inhibitors of S-adenosylmethionine decarboxylation by normal and detergent-treated soluble liver extracts*
The reaction mixture contained 100 mM-sodium phosphate, pH 7.2, 0.2 mM-S-adenosyl[carboxyl-^{14}C]methionine, 2.5 mM-putrescine, 5 mM-dithiothreitol and inhibitors at the designated concentrations. The total volume of the reaction mixture was 500 μl and was incubated at 37°C for 60 min. The formation of CO_2 with two different amounts of liver extract (expressed as μl of extract per reaction mixture) is shown. Abbreviation: MGBG, methylglyoxal bis(guanylhydrazone).

		CO$_2$ released (pmol/60 min)			
		Normal extract		Triton X-100 extract	
Liver extract added	Inhibitors	Minus putrescine	Plus putrescine	Minus putrescine	Plus putrescine
(a) 75 μl	None	9.5	262	168	278
	MGBG (10 μM)	0	55	183	184
	S-Adenosylhomocysteine (1 mM)	0	271	146	316
(b) 250 μl	None	289	959	3411	4368
	MGBG (10 μM)	37	131	2563	4535
	S-Adenosylhomocysteine (1 mM)	120	1027	2361	2002

treated preparations with exogenous 2.5mM-putrescine (although without added putrescine, the Triton-treated extracts were relatively much more active). And with both types of preparations, methylglyoxal bis(guanylhydrazone) was inhibitory with putrescine present, whereas there was no depression by S-adenosylhomocysteine. Thus, with added putrescine and with 75μl of either type of enzyme preparation, the properties of the S-adenosyl-methionine decarboxylase reaction were very similar to those previously found with cytosolic extracts of liver and other rat tissues (Williams-Ashman *et al.*, 1972, 1977). By contrast, with higher amounts of enzyme extract, the Triton-treated preparations were insensitive to methylglyoxal bis(guanylhydrazone) and inhibited by S-adenosylhomocysteine, and exhibited a much greater rate of CO_2 release in the controls than would be expected from an increase in extract concentration of only a little above 3-fold. It should be emphasized that considerably more protein was present in the Triton-treated ultra-centrifuged extracts. Also, addition of Triton X-100 to the incubation mixtures containing the extracts prepared without the detergent, and in quantities equivalent to those present in appropriate reaction mixtures containing the extracts prepared with Triton, had no effect on the decarboxylation rates or their response to either methylglyoxal bis(guanyl-hydrazone) or S-adenosylhomocysteine.

Two explanations for the foregoing results were considered. The first was that at high concentrations of cytosolic preparations isolated in the presence of the detergent, there was present a novel form of adenosylmethionine decarboxylase that was liberated from particulate matter in the homogenate by the action of Triton X-100. The second possible explanation was that the detergent liberated in soluble form an enzyme system that degraded adenosyl-methionine to products from which CO_2 was subsequently formed enzymically from the carbon atom originally present in the carboxy group of adenosyl-methionine. The second hypothesis seemed more probable on the grounds that all attempts to fractionate [by treatment with $(NH_4)_2SO_4$ and other methods] the Triton-liberated decarboxylase activity were unsuccessful, and that this activity was both extremely unstable on storage at 2°C and readily lost after dialysis against the homogenization medium without Triton X-100. The activity lost after storage or dialysis could not be restored by addition of pyridoxal phosphate (50μM), $MgCl_2$ (5mM), putrescine (2.5mM) or spermidine (5mM).

Under the conditions of experiment (*b*) in Table 1, addition of 1mM-adenosine inhibited the decarboxyl-ations catalysed by the Triton-treated extracts by about 50% and had a lesser effect on the values obtained with cytosol prepared in the absence of detergent; however, the effects of adenosine were variable, perhaps because the compound underwent enzymic deamination under these conditions. With either the normal or Triton-treated cytosol extracts added at 250μl per reaction mixture, the rates of CO_2 release were maximal when the S-adenosylmethionine concentration was 0.4mM in both instances, and roughly 80% of the maximal reaction rates was observed when the substrate concentration was 0.2mM. The optimal pH for CO_2 formation by the normal and Triton-treated preparations was pH 7.2 and 7.6, respectively. CO_2 formation with the higher concentration of Triton-treated preparations was inhibited by 80% or more by pyruvate and oxalo-acetate, and by about 65% by 2-oxoglutarate when the concentration of added keto acids was 5mM; by contrast, these keto acids did not significantly affect S-adenosylmethionine decarboxylation in the absence or presence of 2.5mM-putrescine by the normal cytosol extracts. Similar amounts of activity were found to be released by treatment with Triton X-100 when male and female rats were compared. The Triton-solubilized activity was not inhibited by addition of catalase to the assays.

The dependence of $^{14}CO_2$ production from S-adenosyl[*carboxyl*-^{14}C]methionine by Triton-extracted preparations both on times of incubation and on the amount of protein added also supported the concept that a multiple enzyme system catalysing degradation of the S-adenosylmethionine might be involved. As shown in Fig. 1, there was a distinct lag period for CO_2 release by the Triton-extracted preparations, but not by the normal cytosol extracts, regardless of whether or not putrescine was added to the assay system. The variation of activity with the amount of protein added is shown in Fig. 2. The Triton-solubilized preparations showed a complex dependence on extract concentration, with relatively little activity at low concentrations. In contrast, the cytosolic enzyme assayed in the presence of putrescine showed activity proportional to the amount of protein added.

If the ultracentrifuged extracts from both normal and Triton-treated homogenates were treated with acetone, desiccated, and then extracted with phosphate buffer as described in the Materials and Methods section, results similar to those observed with the fresh cytosol extracts were obtained. Provided that sufficiently large amounts of the extracts of acetone-dried material were added, the Triton-treated preparations showed an unusual dependence on extract concentration (cf. Fig. 2; Triton extracts), and were neither stimulated by exogenous putrescine nor inhibited by methylglyoxal bis(guanylhydrazone). These acetone-dried preparations provided a relatively stable solubilized form of the Triton-extracted activity, which could be assayed in the absence of the detergent. By using such preparations as an enzyme source and incu-
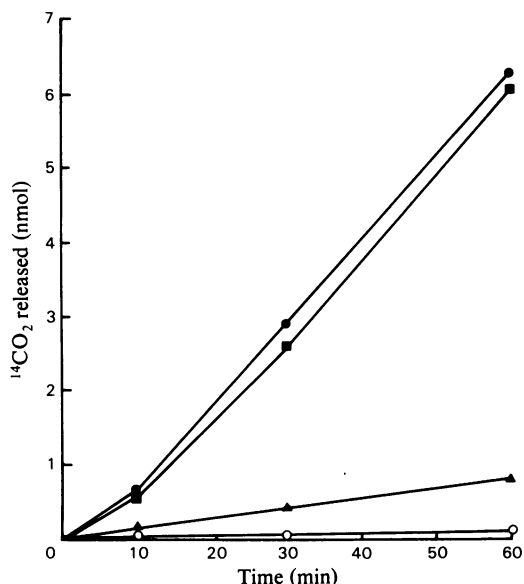
Fig. 1. *Time course of* $^{14}CO_2$ *production catalysed by liver extracts*

The assays were conducted as described in Table 1 with incubation at 37°C for the time shown. Results are shown for extracts prepared in buffer containing 1% (v/v) Triton X-100 assayed in the absence of putrescine (■) or in the presence of putrescine (●) and for extracts prepared in the standard buffer lacking detergent and assayed in the absence (○) or presence (▲) of putrescine. For each determination 0.2 ml of extract was used as an enzyme source.
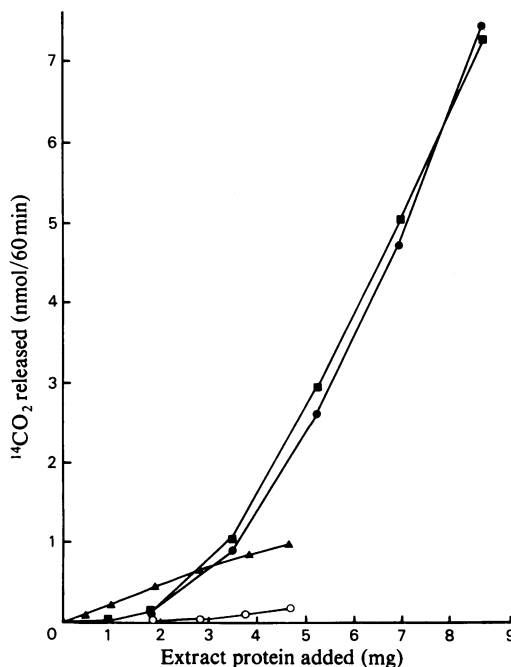

Fig. 2. *Effect of protein concentration on* $^{14}CO_2$ *production*

The assays were carried out by incubations for 1 h at 37°C as described in Table 1. Results are shown for extracts prepared in buffer containing 1% (v/v) Triton X-100 assayed in the absence (■) or presence (●) of putrescine and for extracts prepared in the standard buffer lacking detergent and assayed in the absence (○) or presence (▲) of putrescine.

bation for 60 min at 37°C, the products of the reaction were investigated with *S*-adenosyl[*methyl-*^{14}C]methionine as a substrate.

The products were analysed by ion-exchange chromatography and by high-voltage paper electrophoresis. There was no trace of labelled decarboxylated *S*-adenosylmethionine among the products of the reaction. A number of radioactive compounds were present in addition to unchanged *S*-adenosylmethionine. Two of these products were identified as 5'-methylthioadenosine and 5-methylthioribose by their mobility in the electrophoresis and on paper chromatography as described in the Materials and Methods section. Together, these accounted for 50% of the radioactivity not present in *S*-adenosylmethionine. Another product, which moved rapidly towards the cathode under the electrophoretic conditions used, was not identified, but accounted for a further 30% of the products and is likely to have resulted from a transmethylation reaction. The possibility that decarboxylated *S*-adenosylmethionine was formed in the reaction, but was then further metabolized to other products, was

ruled out as follows. An amount of decarboxylated *S*-adenosyl[*methyl-*^{14}C]methionine equivalent to the amount of $^{14}CO_2$ produced in a 1 h incubation was added to the standard assay mixture, replacing the labelled *S*-adenosylmethionine substrate. After incubation at 37°C for 1 h, the products were analysed as described above and more than 90% of the added decarboxylated *S*-adenosylmethionine was recovered unchanged.

These results strongly support the possibility that $^{14}CO_2$ generation from *S*-adenosyl[*carboxyl-*^{14}C]methionine by the Triton-solubilized extracts involves a complex series of reactions resulting in degradation of the molecule rather than a one-step decarboxylation. Such a reaction series could be commenced by a methyl-transfer reaction yielding *S*-adenosylhomocysteine, and the inhibition of $^{14}CO_2$ production by addition of unlabelled *S*-adenosylhomocysteine (Table 1) supports the contention that this pathway could be responsible for part of the reaction. However, a second possible mechanism would involve the conversion of *S*-adenosylmethionine into 5'-methylthioadenosine and homoserine lactone. Further

metabolism of the homoserine could then lead to CO_2 production. Several results support this pathway. Firstly, as described above, 5'-methylthioadenosine and 5-methylthioribose, which can arise from the former nucleoside by action of a widely distributed phosphate-dependent nucleosidase (Pegg & Williams-Ashman, 1969*b*; Garbers, 1977; Toohey, 1978), were present among the reaction products. Secondly, as shown in Table 2, unlabelled homoserine was a strong inhibitor of $^{14}CO_2$ production by the Triton-solubilized extracts, but did not affect the reaction of highly purified cytosolic putrescine-activated *S*-adenosylmethionine decarboxylase even when present at a concentration of 40 mM.

Finally, the production of $^{14}CO_2$ from *S*-adenosyl-[*carboxyl*-^{14}C]methionine by the Triton-solubilized extracts was not diminished when the substrate was first degraded by heating at pH 6 for 30 min at 100°C. This procedure converts *S*-adenosylmethionine into

5'-methylthioadenosine and homoserine (or homoserine lactone) (Parks & Schlenk, 1958). Such degraded *S*-adenosylmethionine preparations were, in fact, 5 times more active as a substrate for $^{14}CO_2$ production by the detergent-solubilized extracts, but were virtually inactive as substrates for authentic *S*-adenosylmethionine decarboxylase (Table 3). Furthermore, when [^{14}C]homoserine was substituted for the labelled *S*-adenosylmethionine, the cytosolic *S*-adenosylmethionine decarboxylase did not produce a significant release of $^{14}CO_2$, but the Triton-solubilized extracts were highly active (Table 3).

Discussion

The present findings and those published by Eloranta & Raina (1978) confirm the original report of Sturman (1976*a*) that an activity converting *S*-adenosyl[*carboxyl*-^{14}C]methionine into $^{14}CO_2$ is present in rat liver in a particulate form and can be solubilized by treatment with Triton X-100. Our results provide an explanation for the failure of Symonds & Brosnan (1977) to detect this activity. As shown in Table 1 and Fig. 2, activity is not directly proportional to protein added to the assay mixtures, and at low protein concentrations little activity was observed. It is therefore possible that the failure of Symonds & Brosnan (1977) to repeat the original finding was due to insufficient extract being added to the assays. Sturman (1976*a*) reported that the particulate *S*-adenosylmethionine decarboxylase activity in the rat appeared to be limited to the liver and was not present in brain, kidney or spleen. We have also found that this activity was not present in the rat ventral prostate or bulbourethral glands, which are active sources of the cytosolic putrescine-activated enzyme. The difference in properties between the particulate activity, which is not activated by putrescine or inhibited by methylglyoxal bis-(guanylhydrazone), and the cytosolic enzyme suggests that these are likely to result from entirely different proteins. This conclusion is supported by the observation that a specific antiserum prepared by immunization of rabbits with highly purified rat liver

Table 2. *Effect of homoserine on production of $^{14}CO_2$ from S-adenosyl[carboxyl-^{14}C]methionine by Triton-solubilized liver extracts and by purified cytosolic putrescine-activated S-adenosylmethionine decarboxylase*

The reaction was assayed as described in Table 1 with the additions of homoserine indicated. The equivalent of 250 μl of liver extract as a reconstituted acetone-dried powder of the Triton-treated homogenates was used for assays which were carried out in the absence of putrescine. Purified cytosolic enzyme was assayed in the presence of 2.5 mM-putrescine.

Homoserine (mM)	$^{14}CO_2$ released (pmol/60 min)	
	Triton-solubilized extract	Purified cytosolic enzyme
0	6209	3691
0.6	3050	3654
1.2	2226	3805
2.4	1199	3808
4.8	696	*
9.6	408	3791
40	173	3826

* Not measured.

Table 3. *Production of $^{14}CO_2$ from S-adenosyl[carboxyl-^{14}C]methionine and from [^{14}C]homoserine by Triton-solubilized liver extracts and purified cytosolic S-adenosylmethionine decarboxylase*

The sources of enzyme were as described in Table 2. Assays of $^{14}CO_2$ production were carried out by incubations for 30 min at 37°C. The labelled substrate was either authentic *S*-adenosyl[*carboxyl*-^{14}C]methionine or a preparation degraded by heating at pH 6 at 100°C for 30 min or [^{14}C]homoserine as indicated.

Substrate	$^{14}CO_2$ released (pmol/30 min)	
	Triton-solubilized extract	Purified cytosolic extract
0.2 mM-*S*-adenosyl[*carboxyl*-^{14}C]methionine	1357	1610
0.2 mM-*S*-adenosyl[*carboxyl*-^{14}C]methionine heated at pH 6 at 100°C for 30 min	7817	69
0.2 mM-[^{14}C]homoserine	8150	7

cytosolic putrescine-activated *S*-adenosylmethionine decarboxylase had no effect on the Triton-solubilized activity, but completely inactivated and precipitated the cytosolic enzyme (Pegg, 1979).

Our results and those of Eloranta & Raina (1978) are in agreement in concluding that $^{14}CO_2$ is produced from *S*-adenosyl[*carboxyl*-^{14}C]methionine by Triton-solubilized extracts via a series of reactions involving degradation of the substrate in a manner not producing decarboxylated *S*-adenosylmethionine. The finding that decarboxylated *S*-adenosylmethionine is not produced is in accord with the observations that treatment of rats with methylglyoxal bis(guanylhydrazone) produces a transient, but complete, block of spermidine synthesis (Pegg, 1973): this indicates that the supply of decarboxylated *S*-adenosylmethionine, the propylamine donor essential for spermidine formation, must derive entirely from reactions sensitive to this drug.

Eloranta & Raina (1978) have suggested that the detergent-solubilized activity involves the demethylation–trans-sulphuration pathway and that the $^{14}CO_2$ production results from the oxidation of 2-oxobutyrate derived from *S*-adenosylmethionine via *S*-adenosylhomocysteine, which is then converted into homocysteine and thence into cystathionine and 2-oxobutyrate (Mudd *et al.*, 1965; Finkelstein, 1974). This pathway may well be responsible for some of the $^{14}CO_2$ production, but our data also support the possibility that $^{14}CO_2$ may be derived by oxidative metabolism of homoserine. Homoserine lactone can be formed from *S*-adenosylmethionine by a cleavage enzyme, which also gives rise to 5′-methylthioadenosine. This enzyme is present in bacteria (Shapiro & Mather, 1958), yeast (Mudd, 1959) and pig liver (Swiatek *et al.*, 1973), and there is indirect evidence that it may occur in the rat (Edwards *et al.*, 1977). Although we have not characterized the enzyme, our finding of 5′-methylthioadenosine among the reaction products is consistent with the presence of this activity in rat liver. The results of Table 3 show that the extracts can produce $^{14}CO_2$ from labelled homoserine at a faster rate than that from the same concentration of *S*-adenosylmethionine. The relative extents to which the degradation of homoserine and of cystathionine contribute (via 2-oxobutyrate) to the observed reaction may well depend on the concentration of *S*-adenosylmethionine, the amount of extract added and the time of incubation. Both pathways may require the presence of low-molecular-weight molecules in the tissue extracts for the reaction to proceed, but this might be more critical for the demethylation–trans-sulphuration mechanism. This could account for the fact that Eloranta & Raina's (1978) results favour this route, whereas ours favour the homoserine pathway, since their studies used fresh tissue extracts and the experiments described

in Tables 2 and 3 used reconstituted acetone-dried powders, which are less likely to contain low-molecular-weight metabolites.

Our findings emphasize that caution should be exercised in attributing to the activity of a discrete *S*-adenosylmethionine decarboxylase any release of labelled CO_2 from *S*-adenosyl[*carboxyl*-^{14}C]-methionine when this substrate is incubated with crude tissue extracts. The results obtained with Triton-treated rat liver extracts indicate that the additional putative *S*-adenosylmethionine decarboxylase activity extracted with the detergent is, in fact, an artifact due to an enzyme system that degrades *S*-adenosylmethionine to products from which CO_2 is subsequently liberated enzymically. Furthermore, other reactions are known that can masquerade as *S*-adenosylmethionine decarboxylase activity. One example is the non-enzymic decarboxylation of the substrate by Mn^{2+} and pyridoxal phosphate, which is enhanced by peroxidases (Coppoc *et al.*, 1971). Spurious *S*-adenosylmethionine decarboxylase activity can also be due to contaminating enzymes that produce H_2O_2, which then non-enzymically releases CO_2 from *S*-adenosylmethionine: Suresh & Adiga (1977) have demonstrated that this occurs when putrescine is oxidized by diamine oxidases in crude plant extracts.

References

Coppoc, G. L., Kallio, P. & Williams-Ashman, H. G. (1971) *Int. J. Biochem.* **2**, 673–681

Edwards, C. H., Wade, W. D., Freeburne, M. M., Jones, E. G., Stacey, R. E., Sherman, L., Seo, C.-W. & Edwards, G. A. (1977) *J. Nutr.* **107**, 1927–1936

Eloranta, T. O. & Raina, A. M. (1978) *Biochem. Biophys. Res. Commun.* **84**, 23–30

Finkelstein, J. D. (1974) *Metab. Clin. Exp.* **23**, 387–398

Fiume, L., Campadelli-Fiume, G., Magee, P. N. & Holsman, J. (1970) *Biochem. J.* **120**, 601–605

Garbers, D. L. (1977) *Biochim. Biophys. Acta* **523**, 82–93

Jänne, J. & Williams-Ashman, H. G. (1971) *J. Biol. Chem.* **246**, 1725–1732

Jänne, J., Williams-Ashman, H. G. & Schenone, A. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1362–1368

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mudd, S. H. (1959) *J. Biol. Chem.* **234**, 87–92

Mudd, S. H., Finkelstein, J. D., Irreverre, F. & Laster, L. (1965) *J. Biol. Chem.* **240**, 4382–4392

Parks, L. W. & Schlenk, F. (1958) *J. Biol. Chem.* **230**, 295–305

Pegg, A. E. (1973) *Biochem. J.* **132**, 537–540

Pegg, A. E. (1974) *Biochem. J.* **141**, 581–583

Pegg, A. E. (1977) *FEBS Lett.* **84**, 33–36

Pegg, A. E. (1979) *J. Biol. Chem.* in the press

Pegg, A. E. & Williams-Ashman, H. G. (1969a) *J. Biol. Chem.* **244**, 682–693

Pegg, A. E. & Williams-Ashman, H. G. (1969b) *Biochem. J.* **115**, 241–247

Pösö, H., Hannonen, P. & Jänne, J. (1976) *Acta Chem. Scand. Ser. B* **30**, 807–811

Raina, A., Pajula, R.-L. & Eloranta, T. (1976) *FEBS Lett.* **67**, 252–255

Schmidt, G. L. & Cantoni, G. L. (1973) *J. Neurochem.* **20**, 1373–1385

Shapiro, S. K. & Mather, A. N. (1958) *J. Biol. Chem.* **233**, 631–633

Sturman, J. A. (1976a) *Biochim. Biophys. Acta* **428**, 56–69

Sturman, J. A. (1976b) *Life Sci.* **18**, 879–886

Suresh, M. R. & Adiga, P. R. (1977) *Eur. J. Biochem.* **79**, 511–518

Swiatek, K. R., Simon, L. N. & Chao, K.-L. (1973) *Biochemistry* **12**, 4670–4674

Symonds, G. W. & Brosnan, M. E. (1977) *FEBS Lett.* **84**, 385–387

Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306

Toohey, J. I. (1978) *Biochem. Biophys. Res. Commun.* **83**, 27–35

Williams-Ashman, H. G., Jänne, J., Coppoc, G. L., Geroch, M. E. & Schenone, A. (1972) *Adv. Enzyme Regul.* **10**, 225–245

Williams-Ashman, H. G., Corti, A. & Tadolini, B. (1976) *Ital. J. Biochem.* **25**, 5–32

Williams-Ashman, H. G., Corti, A. & Coppoc, G. L. (1977) in *The Biochemistry of Adenosylmethionine* (Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G. & Schlenk, F., eds.), pp. 493–509, Columbia University Press, New York