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# Effect of inhibition of polyamine synthesis on the content of decarboxylated *S*-adenosylmethionine

Anthony E. PEGG, Hannu PÖSÖ, Kay SHUTTLEWORTH and Richard A. BENNETT
*Department of Physiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033, U.S.A.*

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1. The content of decarboxylated *S*-adenosylmethionine (AdoMet) in transformed mouse fibroblasts (SV-3T3 cells) was increased 500-fold to about 0.4 fmol/cell when ornithine decarboxylase was inhibited by α-difluoromethylornithine. This increase was due to the absence of putrescine and spermidine, which serve as substrates for aminopropyltransferases with decarboxylated AdoMet as an aminopropyl donor, and to the enhanced activity of AdoMet decarboxylase brought about by depletion of spermidine. The increase in decarboxylated AdoMet content was abolished by addition of putrescine, but not by 1,3-diaminopropane. 2. 5'-Methylthiotubercidin also increased decarboxylated AdoMet content, presumably by direct inhibition of aminopropyltransferase activities, but the increase in its content and the decline in spermidine content were much less than those produced by α-difluoromethylornithine. 3. Decarboxylated AdoMet content of regenerating rat liver was measured in rats treated with inhibitors of ornithine decarboxylase. The content was increased by 60% 32h after partial hepatectomy in control rats, by 90% when α-difluoromethylornithine was given to the partially hepatectomized rats, and by 330% when 1,3-diaminopropane was used to inhibit putrescine and spermidine synthesis. After 48h of exposure to 1,3-diaminopropane, which completely prevented the increase in spermidine after partial hepatectomy, there was a 5-fold rise in hepatic decarboxylated AdoMet concentration. These increases were prevented by treatment with putrescine or with methylglyoxal bis(guanylhydrazone), an inhibitor of AdoMet decarboxylase. 4. These results show that changes in AdoMet metabolism result from the administration of specific inhibitors of polyamine synthesis. The possible consequences of the accumulation of decarboxylated AdoMet, which could, for example, interfere with normal cellular methylation or lead to depletion of cellular adenine nucleotides, should be considered in the interpretation of results obtained with such inhibitors.

The propylamine portions of the polyamines, spermidine and spermine, are derived from methionine in mammalian cells and in many microorganisms (Tabor & Tabor, 1976; Jänne *et al.*, 1978; Williams-Ashman & Pegg, 1981). The immediate aminopropyl donor needed by spermidine synthase and by spermine synthase is decarboxylated AdoMet. This nucleoside is produced by the action of a decarboxylase, which in many eukaryotes is putrescine-activated (Pegg & Williams-Ashman, 1969; Williams-Ashman & Pegg, 1981). Such activation provides a regulatory mechanism by which the supply of decarboxylated AdoMet is linked to the production of putrescine, which provides the other substrate for spermidine synthase. This regulation, and the fact that spermidine synthase and spermine synthase activities are present in substantial excess of that of AdoMet decarboxylase (Jänne *et al.*, 1978), suggest that the tissue content of decarboxylated AdoMet would normally be quite small, and our measurements confirm this (Hibasami *et al.*, 1980; Pegg & Hibasami, 1980; Pegg & Coward, 1981; Pegg *et al.*, 1981*a*). In rat liver the amount of decarboxylated AdoMet was about 1.5 nmol/g wet wt., and in ventral prostate, which is very active in polyamine synthesis, the content was about twice this (Hibasami *et al.*, 1980). Small but statistically significant

Abbreviation used: AdoMet, *S*-adenosylmethionine.



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increases in the content of this nucleoside were observed in the liver after partial hepatectomy (Pegg & Coward, 1981; Pegg *et al.*, 1981*a*), in the ventral prostate after treatment of castrated rats with androgens (Pegg *et al.*, 1981*a*) and in the heart undergoing hypertrophy in response to thyroxine (Pegg & Hibasami, 1980). These are all situations in which AdoMet decarboxylase activity is stimulated, but even under these conditions the ratio of the content of AdoMet to that of its decarboxylated derivative is more than 30:1. In cultured fibroblasts we were unable to measure the content of decarboxylated AdoMet, because it was below the limit of detection, but this ratio (determined by comparing the radioactivity incorporated from [$^{35}$S]-methionine into these nucleotides) was at least 100:1 (Hibasami *et al.*, 1980). Thus, under normal physiological circumstances the decarboxylated derivative of AdoMet is present at concentrations 1–2 orders of magnitude lower than the parent compound and is unlikely to have an influence on methyl-transfer reactions.

Potent inhibitors of polyamine production have been synthesized and attracted considerable biochemical and pharmacological interest (Mamont *et al.*, 1978; Heby & Jänne, 1981; Koch-Weser *et al.*, 1981; McCann *et al.*, 1981). Many studies have been carried out with inhibitors of ornithine decarboxylase which prevent the production of putrescine and lead to significant depletion of putrescine and spermidine. The inhibition of growth and some other pharmacological effects brought about by these agents can be reversed by putrescine or polyamines, which has been taken as evidence that the effects are due to the absence of these amines (Mamont *et al.*, 1978; McCann *et al.*, 1981; Heby & Jänne, 1981). In the present work, we have tested the effects of inhibition of ornithine decarboxylase on the content of decarboxylated AdoMet in the cell. A substantial accumulation of this nucleoside occurs, such that in mouse fibroblasts exposed to α-difluoromethylornithine the content of decarboxylated AdoMet exceeds that of AdoMet itself. The implications of this accumulation in the interpretation of results obtained with ornithine decarboxylase inhibitors and in the biochemistry of AdoMet are discussed.

## Materials and methods

### *Materials*

Decarboxylated AdoMet was prepared by use of bacterial AdoMet decarboxylase and purified as described by Pösö *et al.* (1976). 1,3-Diaminopropane and methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediylidine)-dinitrilo]diguanidine} were purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. DL-α-Difluoromethylornithine was a generous gift from Merrell Research Center, Cincinnati, OH, U.S.A. All other biochemicals, including AdoMet and polyamines, were obtained from Sigma Chemcial Co., St. Louis, MO, U.S.A.

### *Cell-culture experiments*

Simian-virus-40-transformed 3T3 mouse fibroblasts (SV-3T3 cells) were maintained in Dulbecco's modified Eagle's medium with 3% horse serum/2% foetal calf serum as previously described (Pegg *et al.*, 1981*b*). Cells were seeded at a density of $4 \times 10^5$ per 100mm-diameter dish. Cells were harvested for polyamine analysis as described by Pegg *et al.* (1981*b*). A similar procedure was used to obtain samples for the determination of decarboxylated AdoMet, except that the cells were extracted with 5% (w/v) trichloroacetic acid instead of 0.2M-$HClO_4$. The extracts in trichloroacetic acid were applied to a small column of Dowex 50W X4 resin. The resin was previously washed in 6M-HCl and then extensively in water, and the columns were prepared in Pasteur pipettes plugged with glass wool so that each column contained about 1.5ml of the packed resin. After application of the sample the column was washed with 5ml of 6M-HCl. The 6M-HCl eluate was evaporated to dryness at 40°C under reduced pressure in a rotary evaporator and the sample reconstituted with 0.25ml of water and stored frozen at −20°C until analysed by high-performance liquid chromatography. This method gave greater than 95% recovery of authentic decarboxylated AdoMet added to the initial cell extracts and was suitable for extracts from $5 \times 10^6$–$5 \times 10^8$ cells.

### *Animal experiments*

Male Sprague–Dawley rats weighing 150 ± 10g were subjected to two-thirds partial hepatectomy to induce liver regeneration. 1,3-Diaminopropane (750μmol/kg) and α-difluoromethylornithine (400mg/kg) were given every 3h by intraperitoneal injection, commencing at the time of the operation. Methylglyoxal bis(guanylhydrazone) was given as a single dose of 45mg/kg by intraperitoneal injection 5h before death. All drugs were dissolved in 0.9% (w/v) NaCl for injection. Tissue samples were prepared for polyamine analysis as described by Pösö & Pegg (1981). For decarboxylated AdoMet analysis, approx. 2g of liver was removed rapidly and homogenized in 12ml of ice-cold 5% (w/v) trichloroacetic acid in a Polytron homogenizer. The extracts in trichloroacetic acid were applied to a column (9cm × 0.7cm) of Dowex 50W X4 resin which had been washed as described above under 'Cell-culture experiments'. After application of the sample, the column was first washed with 20ml of 1.5M-HCl and then the decarboxylated AdoMet was eluted with 12ml of 6M-HCl. This eluate was evapor-

ated to dryness at 40°C and the sample dissolved in 0.25 ml of water and stored frozen at −20°C until required for analysis. Recovery of authentic decarboxylated AdoMet was more than 90% when checked by addition of a tracer amount of $^{14}C$-labelled nucleoside. In some experiments, the size of the Dowex column was decreased to 5 cm × 0.9 cm and the 6 M-HCl elution volume to 6 ml without affecting the yield of decarboxylated AdoMet.

*Polyamine analysis*

Polyamines were separated and quantified by using a Dionex D-500 amino acid analyser fitted with fluoresence detection and with an 11.5 cm (1.75 mm internal diam.) stainless-steel column packed with sulphonated polystyrene cation-exchange resin as previously described (Pegg *et al.*, 1981*b*).

*Assay of ornithine decarboxylase and AdoMet decarboxylase*

These enzymes were assayed in liver extracts as described by Pegg *et al.* (1970) and in fibroblast extracts as described by Bethell & Pegg (1979). Results were expressed as pmol of $^{14}CO_2$ released/30 min per mg of protein. Protein was determined by the method of Bradford (1976).

*Determination of decarboxylated AdoMet*

Samples were separated by high-performance liquid chromatography on a column of Partisil-10SCX (Whatman, Clifton, NJ, U.S.A.) eluted at 50°C with 0.5 M-ammonium formate (pH 4.0). This method is essentially the same as that reported by Zappia *et al.* (1980). They reported elution of decarboxylated AdoMet after 12.5 min at ambient temperature with a flow rate of 3 ml/min and a column of 25 cm × 4.6 mm. In our experiments, it was necessary to raise the temperature to achieve elution of decarboxylated AdoMet in a reasonably short time, which at 50°C was 7 min. This provided excellent resolution of decarboxylated AdoMet from all other u.v.-absorbing materials and was used for some samples, but the procedure generated high pressures in the detector unit, and several times this cracked the glass in the cell. Therefore a shorter column (10 cm × 4.6 mm) was employed and eluted at a flow rate of 1 ml/min at 50°C. Decarboxylated AdoMet was eluted at 11 min in this system, and all other nucleosides were eluted before 8 min. Chromatography was performed with a Micromeritics Instrument Corp. (Norcross, GA, U.S.A.) model 750 solvent-delivery system, model 730 universal injector and model 731 column oven. Sample components were detected by monitoring the $A_{254}$ of the column effluent with a Waters Associates (Milford, MA, U.S.A.) model 440 absorbance monitor. Decarboxylated AdoMet was quantified by comparing the peak heights observed for this compound in samples with those obtained for injections of known quantities of authentic standard. A standard curve was constructed and the amount was proportional to the peak height up to at least 3 nmol. The limit of detection was about 50 pmol with the short column and about 80 pmol with the longer column.

## Results

The determination of decarboxylated AdoMet as described above was considerably more sensitive than the chromatographic method described previously (Hibasami *et al.*, 1980), and was almost as sensitive as the much more laborious enzymic assay using spermidine synthase (Hibasami *et al.*, 1980). As shown in Table 1, the present method gave results for control rat liver and for rat liver regenerating after partial hepatectomy identical with those with the enzymic assay (Hibasami *et al.*, 1980; Pegg *et al.*, 1981*a*). Sham operations did not lead to a significant change in hepatic decarboxylated AdoMet content (Pegg *et al.*, 1981*a*). Therefore comparisons were made with unoperated controls in the present work. By 32 h after partial hepatectomy, spermidine content and AdoMet decarboxylase activity were about twice the control value, and there was a small (60%) increase in decarboxylated AdoMet content. By 48 h, the spermidine content was increased 2.5-fold and decarboxylated AdoMet content almost 2-fold. However, this nucleoside was still present at concentrations much lower than that of putrescine and up to three orders of magnitude less than those of spermidine and spermine. These results are consistent with the postulated rate-limiting role of this nucleoside in polyamine production (Pegg & Hibasami, 1979).

Although α-difluoromethylornithine is a very potent irreversible inhibitor of ornithine decarboxylase *in vitro* and in cell-culture systems (Mamont *et al.*, 1978; Heby & Jänne, 1981) and substantially inhibits hepatic ornithine decarboxylase, it is not particularly effective in preventing the increase in spermidine seen in regenerating liver (Table 1). Although putrescine was decreased below the limit of detection, the increase in spermidine was inhibited by only about 50% after repeated injections of α-difluoromethylornithine. Such treatment produced a small rise in decarboxylated AdoMet content of the liver. 1,3-Diaminopropane decreases ornithine decarboxylase activity (Pösö & Jänne, 1976) via an indirect mechanism which is not fully understood (Jänne *et al.*, 1978; Heby & Jänne, 1981). This drug was more effective in decreasing the accumulation of spermidine in the liver and produced a greater enhancement of the decarboxylated AdoMet content, which was more than 3 times the control value

Table 1. *Content of decarboxylated AdoMet and polyamines, and activities of ornithine decarboxylase and AdoMet decarboxylase, in regenerating rat liver*

Time refers to time after operation. Methylglyoxal bis(guanylhydrazone) (MGBG) was given 5h before death. The other drugs were given from the time of operation every 3h. Results are given as means ± S.D. for four or five rats in each group. All values for decarboxylated AdoMet are significantly different ($P < 0.01$) from the value for partial hepatectomy at 32h, except for the group treated with putrescine.

| Treatment | Time (h) | Decarboxylated AdoMet (nmol/g) | Putrescine (nmol/g) | Spermidine (μmol/g) | Spermine (μmol/g) | 1,3-Diaminopropane (μmol/g) | AdoMet decarboxylase (pmol/30min per mg) | Ornithine decarboxylase (pmol/30min per mg) |
|---|---|---|---|---|---|---|---|---|
| Unoperated control | 32 | 1.51 ± 0.11 | 10 ± 8 | 1.38 ± 0.19 | 0.98 ± 0.07 | — | 136 ± 46 | 75 ± 30 |
| Partial hepatectomy alone | 32 | 2.37 ± 0.23 | 42 ± 5 | 2.51 ± 0.36 | 1.03 ± 0.14 | — | 285 ± 18 | 358 ± 60 |
| Partial hepatectomy + α-difluoromethylornithine | 32 | 2.90 ± 0.20 | <2 | 1.80 ± 0.08 | 0.92 ± 0.19 | — | 344 ± 66 | 28 ± 6 |
| Partial hepatectomy + 1,3-diaminopropane | 32 | 4.96 ± 0.53 | <2 | 1.46 ± 0.15 | 0.90 ± 0.02 | 3.30 ± 0.65 | 289 ± 16 | 15 ± 3 |
| Partial hepatectomy + α-difluoromethylornithine + putrescine | 32 | 2.40 ± 0.26 | 49 ± 7 | 2.57 ± 0.34 | 0.93 ± 0.04 | — | 146 ± 30 | 37 ± 14 |
| Partial hepatectomy + 1,3-diaminopropane + MGBG | 32 | 1.56 ± 0.15 | 146 ± 30 | 1.40 ± 0.08 | 0.65 ± 0.06 | 3.24 ± 0.85 | 612 ± 48* | 146 ± 30 |
| Partial hepatectomy + MGBG | 48 | 0.56 ± 0.16 | 203 ± 38 | 2.63 ± 0.28 | 1.13 ± 0.18 | — | 716 ± 88* | 217 ± 70 |
| Partial hepatectomy alone | 48 | 2.88 ± 0.41 | 90 ± 25 | 3.37 ± 0.26 | 1.15 ± 0.22 | — | 281 ± 50 | 623 ± 126 |
| Partial hepatectomy + 1,3-diaminopropane | 48 | 7.76 ± 0.86 | <2 | 1.06 ± 0.12 | 0.98 ± 0.14 | 5.26 ± 1.61 | 221 ± 86 | 10 ± 4 |

* The activity of AdoMet decarboxylase in the liver of rats treated with MGBG was measured after removal of the inhibitor by dialysis. The decarboxylase activities in the other conditions were not influenced by dialysis.

by 32h and 5 times by 48h. The greater ability of 1,3-diaminopropane to increase decarboxylated AdoMet content may also be related to its capacity to inhibit aminopropyltransferases directly (Hibasami & Pegg, 1978) and to replace putrescine as an activator of AdoMet decarboxylase (Williams-Ashman & Pegg, 1981; Heby & Jänne, 1981). Table 1 also shows that the increase in decarboxylated AdoMet produced by α-difluoromethylornithine could, as expected, be prevented by putrescine. The drug methylglyoxal bis(guanylhydrazone), which is a competitive inhibitor of AdoMet decarboxylase (Williams-Ashman & Pegg, 1981), completely inhibited the rise in decarboxylated AdoMet concentration brought about by partial hepatectomy and 1,3-diaminopropane. [AdoMet decarboxylase activity is enhanced by this drug when measured in extracts freed from the inhibitor by dialysis, as shown in Table 1, because the degradation of the enzyme is decreased when it is present (Williams-Ashman & Pegg, 1981). This limits the effectiveness of the inhibitor *in vivo* and probably accounts for the residual decarboxylated AdoMet found in this experiment].

Much more striking increases were produced in decarboxylated AdoMet content when cultured fibroblasts were exposed to α-difluoromethylornithine. Decarboxylated AdoMet was present in control SV-3T3 cells in very low amounts. When samples derived from 1–2 million cells were analysed, no peak corresponding to this nucleoside was seen (Fig. 1*b*), but when cells treated with α-difluoromethylornithine were examined a significant peak was observed (Fig. 1*a*). This peak was not decreased when 1,3-diaminopropane was added in addition to α-difluoromethylornithine (Fig. 1*c*), but was abolished entirely when putrescine was added (Fig. 1*d*).

Quantitative results are shown in Table 2, which also gives values for the polyamine contents of the cells and the activities of the decarboxylases. The amount of decarboxylated AdoMet (0.8 amol/cell) present in control cells was measured by processing samples from $10^8$ cells and should be regarded as an approximation, because only a small peak on the shoulder of the earlier, unidentified, peak seen at about 8 min in Fig. 1 was used for the measurement. However, it is most likely an overestimate, and in

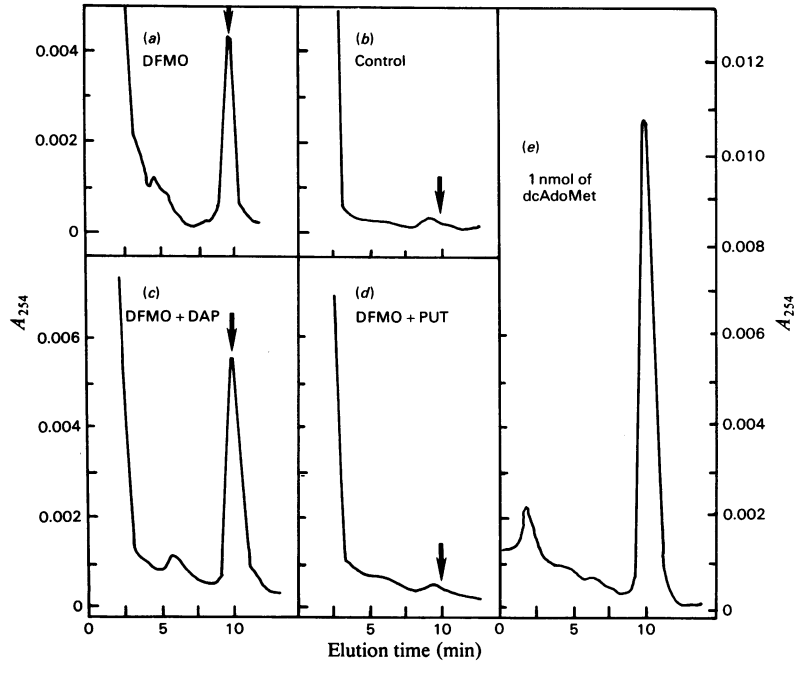


Fig. 1. *High-performance liquid-chromatographic analysis of decarboxylated AdoMet in SV-3T3 cells*
Samples were obtained from $1.5 \times 10^6$ cells and chromatographed as described in the Materials and methods section. Panel (*e*) shows the peak obtained with 1 nmol of standard decarboxylated AdoMet (dcAdoMet). Panel (*a*) shows results from cells cultured for 7 days in the presence of 5 mM-α-difluoromethylornithine (DFMO), panel (*b*) shows results for control cells grown for 3 days, panel (*c*) shows results for cells grown for 3 days in the presence of 5 mM-DFMO plus 0.5 mM-1,3-diaminopropane (DAP) and panel (*d*) shows results for cells grown for 3 days in the presence of 5 mM-DFMO plus 50 μM-putrescine (PUT).

Table 2. *Content of decarboxylated AdoMet and polyamines and activities of ornithine decarboxylase and AdoMet decarboxylase in SV-3T3 cells*

N.D., not determined. Results are given as means ± S.D. for at least four determinations or a single number for the mean of three separate determinations.

| Treatment | Time in culture (days) | $10^{-6}$ × Cell number/dish | Decarboxylated AdoMet (amol/cell) | Putrescine (fmol/cell) | Spermidine (fmol/cell) | Spermine (fmol/cell) | 1,3-Diaminopropane (fmol/cell) | AdoMet decarboxylase (nmol/30min per mg) | Ornithine decarboxylase (nmol/30min per mg) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 3 | 10.6 | 0.8 ± 0.4 | 0.28 ± 0.09 | 3.18 ± 0.22 | 1.17 ± 0.11 | — | 0.28 ± 0.06 | 3.1 ± 0.4 |
| 5 mM-α-difluoromethylornithine | 3 | 1.2 | 426 ± 46 | <0.02 | 0.10 ± 0.05 | 0.87 ± 0.12 | — | 3.03 ± 0.29 | <0.05 |
| 5 mM-α-difluoromethylornithine | 7 | 1.5 | 275 ± 30 | <0.02 | 0.02 ± 0.01 | 0.58 ± 0.05 | — | 1.85 ± 0.34 | <0.05 |
| 5 mM-α-difluoromethylornithine + 50 μM-putrescine | 3 | 11.6 | 0.5 ± 0.3 | 0.61 ± 0.12 | 3.36 ± 0.26 | 1.33 ± 0.20 | — | 0.36 ± 0.10 | <0.05 |
| 5 mM-α-difluoromethylornithine + 50 μM-1,3-diaminopropane | 3 | 4.1 | 316 ± 20 | <0.02 | 0.08 ± 0.05 | 0.46 ± 0.08 | 1.94 ± 0.21 | 3.15 ± 0.41 | <0.05 |
| 100 μM-5′-methylthiotubercidin | 3 | 5.5 | 5 ± 2 | 0.52 | 0.92 | 0.96 | — | N.D. | N.D. |

these transformed fibroblasts this nucleoside is present in amounts more than 3 orders of magnitude less than those of the polyamines. Exposure of these cells to α-difluoromethylornithine for 3 days produced an almost complete loss of putrescine and spermidine, as previously reported (Pegg *et al.*, 1981*b*), and a substantial enhancement of AdoMet decarboxylase activity (Table 2), in agreement with reports on other cells by Mamont *et al.* (1981) and Alhonen-Hongisto (1980). Decarboxylated AdoMet content rose by 500-fold in these cells. This rise was completely prevented by the presence of putrescine, which reversed the effects of the drug on polyamine concentrations and on AdoMet decarboxylase (Table 2). 1,3-Diaminopropane led to only a small decrease in decarboxylated AdoMet in cells exposed to α-difluoromethylornithine, which is consistent with its very limited conversion into norspermidine and norspermine by these cells (Pegg *et al.*, 1981*c*). Part of this decline may also be related to the increase in cell number brought about by 1,3-diaminopropane, which increases the growth rate of cells inhibited in growth by α-difluoromethylornithine (Table 2; Pegg *et al.*, 1981*c*). Table 2 also shows that decarboxylated AdoMet content declined slightly when cells were maintained for a further 4 days in the presence of α-difluoromethylornithine. This may be explained by the decrease in AdoMet decarboxylase activity, but other explanations, such as the loss of decarboxylated AdoMet from the cells or the further metabolism of the nucleoside, cannot be ruled out.

5′-Methylthiotubercidin is an inhibitor of spermidine synthase and spermine synthase (Pegg & Coward, 1981; Pegg *et al.*, 1981*b*), and this drug also produced a significant increase in decarboxylated AdoMet content. This increase was much less than that produced by α-difluoromethylornithine, but, as shown in Table 2, the content of spermidine was decreased only by 70% by 5′-methylthiotubercidin.

## Discussion

The striking accumulation of decarboxylated AdoMet when putrescine production is inhibited is probably a general phenomenon, and not limited to fibroblasts, since Mamont and colleagues have seen similar results in hepatoma cells (Mamont *et al.*, 1982). This build-up is primarily due to the absence of putrescine and spermidine, which act as acceptors of the aminopropyl group. Polyamine synthesis therefore appears to be the major, if not the only, route for the further metabolism of decarboxylated AdoMet. If any other reactions involving this nucleoside are available, they are clearly not able to use up the extra material generated when the aminopropyltransferases cannot operate. A second-

ary phenomenon which contributes to the accumulation of decarboxylated AdoMet is the increase in AdoMet decarboxylase seen in spermidine-depleted cells (Table 2; Alhonen-Hongisto, 1980; Mamont *et al.*, 1981). This enzyme is repressed by spermidine (Mamont *et al.*, 1981; Pösö & Pegg, 1981), and the substantial rise in enzyme activity that occurs when spermidine cannot be made compensates for the lack of the activator, putrescine, which is also greatly decreased on treatment with α-difluoromethylornithine.

One factor that may limit the accumulation of decarboxylated AdoMet even when putrescine production is prevented is that AdoMet decarboxylase is quite strongly inhibited by its product (Pegg & Williams-Ashman, 1969; Pösö *et al.*, 1975). Unless the decarboxylated AdoMet is compartmentalized in some way, it is unlikely that a ratio of decarboxylated AdoMet to the parent compound of more than about 5 : 1 could be maintained. In the SV-3T3 fibroblasts, this ratio is approached in the presence of α-difluoromethylornithine, since the AdoMet content was about 90 amol/cell in these cells (A. E. Pegg, H. Pösö & R. A. Bennett, unpublished work).

The high concentration of decarboxylated AdoMet could have important effects on cellular metabolism. It could lead to inhibition of methyltransferase activities. Such inhibition was observed for histamine *N*-methyltransferase, acetylserotonin methyltransferase, homocysteine methyltransferase and noradrenaline *N*-methyltransferase *in vitro* (Zappia *et al.*, 1969; Borchardt *et al.*, 1976), although the inhibition was quite weak. Methyltransferases responsible for the methylation of proteins and nucleic acids appear not to have been tested in such systems *in vitro*, but inhibition of these reactions could contribute to the effects of α-difluoromethylornithine on cellular growth and differentiation (Heby & Jänne, 1981; Heby, 1981). Our results provide renewed emphasis of the close relationship between polyamine metabolism and AdoMet metabolism. In addition to the possible effect on methyltransferases mentioned above, the inhibition of putrescine production could have other effects relating to lack of aminopropyl-transfer reactions. For example, the accumulation of decarboxylated AdoMet, which is strongly basic, might help to compensate for the lack of spermidine. Secondly, decarboxylated AdoMet is likely to be a chemical methylating agent (albeit very weakly reactive). Methylation by this agent could contribute to the synergism between α-difluoromethylornithine and the nitrosoureas in toxicity towards brain-tumour cells (Hung *et al.*, 1981), since the nitrosoureas are known alkylating agents. Finally, the inhibition of aminopropyl transfer from decarboxylated AdoMet leads to decreased production of 5'-methylthioadenosine. This nucleoside is rapidly degraded by a phosphorylase, yielding adenine and 5-methylthioribose 1-phosphate, which is then converted back into methionine (Williams-Ashman & Pegg, 1981; Backlund & Smith, 1981). In this way both the purine and the thiomethyl group of methionine are conserved for re-utilization by the cell. Such re-utilization would not be possible in the absence of polyamine synthesis, and the accumulated decarboxylated AdoMet would represent a loss to the cell of both adenine and methionine.

Direct examination of the possible toxicity or growth-inhibitory activity of decarboxylated AdoMet may be hampered by lack of uptake of the nucleoside, although it has been claimed that AdoMet is taken up by a transport system in mammalian cells (Zappia *et al.*, 1978), and certain synthetic adenosylsulphonium salts are able to affect polyamine metabolism when added to cultured fibroblasts (A. E. Pegg & J. K. Coward, unpublished work). However, it is interesting that a closely related compound, *S*-adenosyl-3-thiopropylamine, was highly toxic to several lines of cultured cells (Kawase *et al.*, 1979).

The potential adverse effects of the accumulation of decarboxylated AdoMet may only have relevance to the cultured fibroblasts, in which the accumulation was many-fold, but the changes in organs of animals treated with the inhibitors of polyamine synthesis should not be ignored. Liver cells are particularly refractory to inhibition of polyamine production and, even in this system, significant accumulation of decarboxylated AdoMet took place in a relatively short time. It is likely that larger effects would be produced by long-term treatment with such inhibitors.

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## References

Alhonen-Hongisto, L. (1980) *Biochem. J.* **190**, 747–754

Backlund, P. S. & Smith, R. A. (1981) *J. Biol. Chem.* **256**, 1533–1535

Bethell, D. R. & Pegg, A. E. (1979) *Biochem. J.* **180**, 87–94

Borchardt, R. T., Wu, Y. S., Huber, J. A. & Wycpalek, A. F. (1976) *J. Med. Chem.* **19**, 1104–1110

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
Heby, O. (1981) *Differentiation* **19**, 1–20
Heby, O. & Jänne, J. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 243–310, Marcel Dekker, New York
Hibasami, H. & Pegg, A. E. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1398–1405
Hibasami, H., Hoffman, J. L. & Pegg, A. E. (1980) *J. Biol. Chem.* **255**, 6675–6678
Hung, D. T., Deen, D. F., Seidenfeld, J. & Marton, L. J. (1981) *Cancer Res.* **41**, 2783–2785
Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
Kawase, M., Samejima, K., Okada, M. & Shibuta, H. (1979) *J. Pharmacobio-Dyn.* **2**, 12–18
Koch-Weser, J., Schechter, P., Bey, P., Danzin, C., Fozard, J. R., Jung, J., Mamont, P. S. & Seiler, N. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 437–453, Marcel Dekker, New York
Mamont, P. S., Duchesne, M.-C., Joder-Ohlenbusch, A.-M. & Grove, J. (1978) in *Enzyme-Activated Irreversible Inhibitors* (Seiler, N., Jung, M. J. & Koch-Weser, J., eds.), pp. 43–54, Elsevier/North-Holland Biomedical Press, New York
Mamont, P. S., Joder-Ohlenbusch, A.-M., Nussli, M. & Grove, J. (1981) *Biochem. J.* **196**, 411–422
Mamont, P. S., Danzin, C. & Seiler, N. (1982) in *Transmethylation* (Usdin, E., Borchardt, R. T. & Creveling, C. R., eds.), MacMillan, New York, in the press
McCann, P. P., Bacchi, C. J., Hanson, W. L., Cain, G. D., Nathan, H.-C., Hutner, S. H. & Sjoerdsma, A. (1981) *Adv. Polyamine Res.* **3**, 97–110
Pegg, A. E. & Coward, J. K. (1981) *Adv. Polyamine Res.* **3**, 153–161
Pegg, A. E. & Hibasami, H. (1979) in *Transmethylation* (Usdin, E., Borchardt, R. T. & Creveling, C. R., eds.), pp. 105–116, Elsevier/North-Holland, New York
Pegg, A. E. & Hibasami, H. (1980) *Am. J. Physiol.* **233**, E372–378
Pegg, A. E. & Williams-Ashman, H. G. (1969) *J. Biol. Chem.* **244**, 682–693
Pegg, A. E., Lockwood, D. H. & Williams-Ashman, H. G. (1970) *Biochem. J.* **117**, 17–31
Pegg, A. E., Hibasami, H., Matsui, I. & Bethell, D. R. (1981*a*) *Adv. Enzyme Regul.* **19**, 427–451
Pegg, A. E., Borchardt, R. T. & Coward, J. K. (1981*b*) *Biochem. J.* **194**, 79–89
Pegg, A. E., Shuttleworth, K. & Hibasami, H. (1981*c*) *Biochem. J.* **197**, 315–320
Pösö, H. & Jänne, J. (1976) *Biochem. Biophys. Res. Commun.* **69**, 885–892
Pösö, H. & Pegg, A. E. (1981) *Biochem. J.* **200**, 629–637
Pösö, H., Sinervirta, R., Himberg, J.-J. & Jänne, J. (1975) *Acta Chem. Scand.* **B29**, 932–936
Pösö, H., Hannonen, P. & Jänne, J. (1976) *Acta Chem. Scand.* **B30**, 807–811
Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306
Williams-Ashman, H. G. & Pegg, A. E. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 43–72, Marcel Dekker, New York
Zappia, V., Zydek-Cwick, C. R. & Schlenk, F. (1969) *J. Biol. Chem.* **244**, 4499–4509
Zappia, V., Galletti, P., Porcelli, M., Ruggiero, G. & Andreana, A. (1978) *FEBS Lett.* **90**, 331–335
Zappia, V., Galletti, P., Porcelli, M., Manna, C. & Ragione, F. D. (1980) *J. Chromatogr.* **189**, 399–405