Differential stimulation of *S*-adenosylmethionine decarboxylase by difluoromethylornithine in the rat colon and small intestine

Allan G. HALLINE, Pradeep K. DUDEJA and Thomas A. BRASITUS*

Departments of Medicine, The University of Chicago and Michael Reese Hospitals,
Pritzker School of Medicine of the University of Chicago, Chicago, IL 60637, U.S.A.

The effects of chronic inhibition of ornithine decarboxylase (ODC) by the specific inhibitor difluoromethylornithine (DFMO) in the rat colon and small intestine on mucosal contents of polyamines, decarboxylated *S*-adenosylmethionine (decarboxylated AdoMet) and *S*-adenosylmethionine decarboxylase (AdoMet decarboxylase) activity were studied. Administration of 1 % DFMO in the drinking water for 10 or 15 weeks resulted in inhibition of ODC and decreases in intracellular putrescine and spermidine contents in both proximal and distal segments of small intestine and colon. At both time points DFMO administration resulted in a dramatic stimulation of AdoMet decarboxylase activity and a rise in decarboxylated AdoMet content in the proximal and distal small-intestinal segments compared with controls, which was not seen in either colonic segment of DFMO-treated animals. This differential stimulation of AdoMet decarboxylase by DFMO in the small intestine and colon could not be entirely explained on the basis of differences in polyamine contents, which are known to regulate this enzyme activity. Kinetic and inhibition studies of AdoMet decarboxylase in control small and large intestine revealed that: (1) there was no difference in $V_{\max}$ values between the tissues; (2) the K_m for AdoMet was higher in the small intestine than in the colon; and (3) the K_i for product inhibition by decarboxylated AdoMet was higher in the small intestine than in the colon. These results suggest that the differential stimulation of AdoMet decarboxylase by DFMO in the small intestine and colon may be due to different isoenzymes and could play a significant role in the regulation of polyamine contents throughout the gut.

INTRODUCTION

Polyamines are cationic compounds essential to the normal processes of cellular proliferation and differentiation (Tabor & Tabor, 1984; Pegg & McCann, 1982; Heby, 1981). Elevated contents of polyamines are associated with rapidly proliferating tissues (Jänne *et al.*, 1978), and such increases have been found in many human neoplasms, including colorectal carcinoma (Kingsnorth & Wallace, 1985; LaMuraglia *et al.*, 1986; Porter *et al.*, 1987). Increases in ornithine decarboxylase (ODC; EC 4.1.1.17), the initial and rate-limiting enzyme of polyamine biosynthesis, have also been described in human colonic adenomas and carcinomas (Rozhin *et al.*, 1984; LaMuraglia *et al.*, 1986; Herrera-Ornelas *et al.*, 1987). Furthermore, during chemically induced colon carcinogenesis in the rodent, intestinal ODC activity has been found to be increased after administration of various colonic carcinogens (Ball *et al.*, 1976; Takano *et al.*, 1981; Luk *et al.*, 1986), whereas concomitant administration of the specific ODC inhibitor difluoromethylornithine (DFMO) with these carcinogens results in a marked decrease in tumour yield (Kingsnorth *et al.*, 1983; Rozhin *et al.*, 1984).

The mechanism by which DFMO inhibits the formation of chemically induced tumours is uncertain, but appears to be due, at least in part, to the depletion of intracellular polyamines, particularly putrescine and spermidine (Kingsnorth *et al.*, 1983). In this regard, DFMO is also known to stimulate indirectly *S*-adenosyl-

methionine (AdoMet) decarboxylase (EC 4.1.1.50), another rate-limiting enzyme involved in the synthesis of spermidine and spermine (Oredsson *et al.*, 1986). This increase in DFMO-induced AdoMet decarboxylase activity, together with putrescine and spermidine depletion secondary to ODC inhibition, results in accumulation of decarboxylated AdoMet [*S*-adenosyl-(5')-deoxy-(5')-3-methylthiopropylamine], the aminopropyl donor for the conversion of putrescine into spermidine and spermidine into spermine (Mamont *et al.*, 1982). Such DFMO-induced increases in decarboxylated AdoMet, which at times may be elevated several hundred fold, can potentially affect various aspects of cellular metabolism (Zappia *et al.*, 1969; Pegg *et al.*, 1982b, 1986). From these observations, it has therefore been suggested that the increases in decarboxylated AdoMet may also be important in the anti-proliferative effect of DFMO (Pegg *et al.*, 1986). The relative importance of polyamine depletion and decarboxylated-AdoMet accumulation in DFMO-induced inhibition of tumours has not, however, been extensively investigated. Moreover, studies on the effects of DFMO on AdoMet decarboxylase activity in the colon, where the anti-tumorigenic effect of DFMO during chemically induced carcinogenesis is most pronounced, are lacking.

The present studies were therefore undertaken to examine and compare the chronic effects of 1 % DFMO administered in the drinking water of rats for 10 and 15 weeks on small- and large-intestinal polyamines, decarboxylated AdoMet contents and ODC and AdoMet

Abbreviations used: ODC, ornithine decarboxylase; DFMO, difluoromethylornithine; AdoMet, *S*-adenosylmethionine.

*To whom correspondence, including reprint requests should be sent.

decarboxylase activities. Since previous studies (Luk *et al.*, 1987) have indicated that distal, but not proximal, chemically induced colonic tumours were inhibited by administration of DFMO, regional differences in these biochemical parameters in the large and small intestine were also evaluated. The results of these studies serve as the basis for the present paper.

MATERIALS AND METHODS

Chemicals

Spermidine, spermine and putrescine were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Decarboxylated AdoMet was generously provided by Dr. Anthony E. Pegg, Hershey, PA, U.S.A. DFMO was provided by Merrell Dow Pharmaceuticals, Cincinnati, OH, U.S.A. L-[1-¹⁴C]Ornithine (54.3 mCi/mmol) and S-adenosyl[carboxy-¹⁴C]methionine (Ado[¹⁴C]Met; 59.6 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. H.p.l.c.-grade acetonitrile and octanesulphonic acid were purchased from Alltech Associates, Arlington Heights, IL, U.S.A. All other chemicals were of reagent grade and were obtained from Sigma Chemical Co. unless otherwise stated.

Animal treatment

For all studies 32 male Sherman albino rats, weighing 200–250 g, were used. In addition to standard rat chow diet, half of the animals were given 1% DFMO in their drinking water for 10 or 15 weeks or regular tap water *ad libitum*.

Enzyme assays

All enzyme assays were performed on cytosolic fractions of mucosal homogenates taken from rats starved overnight. Mucosa from proximal and distal segments of colon and small intestine was scraped and homogenized in 50 mM-Tris/HCl buffer (pH 7.4)/5 mM-dithiothreitol in a Brinkman Polytron at setting 4 for 60 s (4 × 15 s). Homogenates were then centrifuged at 105 000 *g* for 60 min, and the supernatant were used as the enzyme source. Protein was determined by the method of Bradford (1976).

ODC was assayed by a modification of the method of Pegg & Williams-Ashman (1968) as described by Steeves & Lawson (1985). The reaction mixture consisted of 50 mM-Tris/HCl (pH 7.4), 5 mM-dithiothreitol, 0.2 mM-pyridoxal phosphate, 0.2 μ Ci of L-[1-¹⁴C]ornithine and 0.5–1.0 mg of protein in a final volume of 0.3 ml. The reaction was incubated at 37 °C in the 16 mm × 100 mm glass tube sealed with a rubber stopper supporting a centre well (Kontes, Morton Grove, IL, U.S.A.). The released ¹⁴CO₂ was trapped on a disc of Whatman GFA glass-fibre filter paper soaked with 200 μ l of NCS (Amersham, Arlington Heights, IL, U.S.A.) in the suspended centre well. The reaction was stopped at 30 min by injecting 0.5 ml of 2 M-HCl through the rubber stopper and then leaving the mixture at room temperature for 30 min to trap the released ¹⁴CO₂ completely. The filter paper was then removed and placed in a scintillation vial containing 2 ml of ethanol; 10 ml of Scintiverse E was added and radioactivity measured in a Beckman LS 6800 liquid-scintillation spectrometer.

AdoMet decarboxylase was assayed by the method of Steeves & Lawson (1985). The reaction mixture contained

50 mM-Tris/HCl, pH 7.4, 5 mM-dithiothreitol, 2.2 mM-putrescine, 0.2 μ Ci of Ado[¹⁴C]Met (59.6 mCi/mmol) and 0.5–1.0 mg of protein in a final volume of 0.3 ml. The mixture was incubated for 30 min in a shaking water bath at 37 °C, and released ¹⁴CO₂ was trapped and measured as described above. The binding constant, *K_m*, and the maximal velocity of AdoMet decarboxylase were determined by varying the concentration of AdoMet from 10 to 250 μ M and calculating the abscissa and ordinate intercepts of a Lineweaver–Burk plot. The inhibitory constant, *K_i*, for decarboxylated AdoMet was measured by addition of this compound to the assay mixture at concentrations in the range 10–70 μ M.

Determination of polyamines and decarboxylated AdoMet

The cellular contents of putrescine, spermidine, spermine and decarboxylated AdoMet were determined by reversed-phase h.p.l.c. by the method of Wagner *et al.* (1984).

Sample preparation of colonic and small-intestinal mucosa was accomplished by rapid tissue removal, rinsing with iced 0.9% NaCl, dividing into proximal and distal portions, and scraping into 0.2 M-HClO₄ (approx. 1 g tissue wet wt. per 5–10 ml of acid). Samples were homogenized as described above, centrifuged at 10 000 *g* for 30 min to remove precipitated proteins and then filtered through a 0.22 μ m-pore-size membrane (Millex-GV; Millipore, Bedford, MA, U.S.A.). Samples (25–100 μ l) were then directly injected on to the h.p.l.c. column for separation and analysis. Sample components were stable when kept at 4 °C for weeks or –70 °C for several months.

The h.p.l.c. system consisted of two pumps, model 6000A and M45, controlled by a model 680 automated programmer, a WISP 710A autosampler and a model 481 spectrophotometer from Waters (Milford, MA, U.S.A.). Post-u.v.-detector derivative formation of polyamines with phthalaldehyde was accomplished with a P-3 peristaltic pump (Pharmacia, Uppsala, Sweden), and derivatives were analysed in a model 2070 fluorescence spectrofluorimeter from Varian (Sugarland, TX, U.S.A.), with excitation and emission wavelengths of 340 nm and 455 nm respectively. Detector outputs were integrated on model 3393 integrators (Hewlett–Packard, Naperville, IL, U.S.A.).

RESULTS

Effects of DFMO on polyamines and ODC activity

In agreement with the previous results of Hosomi *et al.* (1986), the contents of putrescine and spermidine in control animals were significantly lower in both segments of the colon compared with values in the proximal and distal small intestine (Table 1). Such differences were not seen for spermine, and thus the ratios of putrescine/spermine and spermidine/spermine were higher in the small intestine than in the colon. ODC activities were also higher in the small intestine than in the colon of control animals (Table 1), a finding previously noted by several laboratories (Luk *et al.*, 1982; Rozhin *et al.*, 1984; Tabata & Johnson, 1986).

As shown in Table 1, administration of 1% DFMO in the drinking water of animals for 10 or 15 weeks resulted in inhibition of ODC activity in all intestinal segments compared with their control counterparts. Although the

Table 1. Polyamine content and ODC activity in proximal and distal segments of small intestine (SI) and colon of control and DFMO-treated rats at 10 and 15 weeks

DFMO was administered as a 1% solution in the drinking water. Results are given as means $\pm$ S.E.M. for at least eight determinations on four separate preparations in each group. * $P < 0.05$ compared with control counterpart; ** $P < 0.001$ compared with either segment of control SI; † $P < 0.01$ compared with spermidine content in distal SI of DFMO-treated rats at 15 weeks.

	Period of treatment (weeks)	Proximal SI		Distal SI		Proximal colon		Distal colon	
		Control	DFMO	Control	DFMO	Control	DFMO	Control	DFMO
Putrescine (nmol/g)	10	125.1 $\pm$ 7.0	11.2 $\pm$ 1.0 ^a	98.8 $\pm$ 9.8	13.2 $\pm$ 1.0 ^a	21.8 $\pm$ 1.6 ^b	6.7 $\pm$ 0.7 ^a	16.0 $\pm$ 0.7 ^b	8.9 $\pm$ 0.1 ^a
	15	115.4 $\pm$ 12.1	8.8 $\pm$ 1.6 ^a	178.0 $\pm$ 10.0	21.9 $\pm$ 0.7 ^a	16.0 $\pm$ 1.8 ^b	6.5 $\pm$ 0.2 ^a	14.9 $\pm$ 1.8 ^b	6.7 $\pm$ 2.9 ^a
Spermidine (nmol/g)	10	1198 $\pm$ 41	494 $\pm$ 18 ^a	1073 $\pm$ 49	449 $\pm$ 7 ^{a,c}	644 $\pm$ 17 ^b	458 $\pm$ 11 ^a	580 $\pm$ 49 ^b	462 $\pm$ 82
	15	1074 $\pm$ 30	452 $\pm$ 20 ^a	1347 $\pm$ 18	542 $\pm$ 4 ^a	530 $\pm$ 41 ^b	398 $\pm$ 13 ^a	556 $\pm$ 11 ^b	324 $\pm$ 11 ^a
Spermine (nmol/g)	10	699 $\pm$ 14	982 $\pm$ 32 ^a	676 $\pm$ 23	815 $\pm$ 31 ^a	789 $\pm$ 17	850 $\pm$ 11 ^a	767 $\pm$ 9	855 $\pm$ 25 ^a
	15	732 $\pm$ 33	1001 $\pm$ 29 ^a	905 $\pm$ 25	985 $\pm$ 25	667 $\pm$ 36	775 $\pm$ 21 ^a	671 $\pm$ 26	738 $\pm$ 46
ODC (pmol/30 min per mg of protein)	10	49.4 $\pm$ 0.8	1.02 $\pm$ 0.04 ^b	49.5 $\pm$ 3.7	0.31 $\pm$ 0.07 ^a	7.97 $\pm$ 0.32	0.58 $\pm$ 0.28 ^a	2.22 $\pm$ 0.06	0.02 $\pm$ 0.01 ^a
	15	20.4 $\pm$ 11.1	2.63 $\pm$ 1.40	41.7 $\pm$ 3.2	2.65 $\pm$ 0.66 ^a	3.40 $\pm$ 1.16	0.99 $\pm$ 0.15	0.95 $\pm$ 0.18	0.39 $\pm$ 0.17

subsequent fall in colonic putrescine and spermidine contents in response to DFMO treatment was significant ($P < 0.05$), the percentage decrease in these polyamines was not as great as that found in the small intestine. The putrescine contents in the small intestine fell to approx. 10% of control values, whereas colonic contents fell to about 40% of their control values. Spermidine, which showed a 60% decrease in the small intestine in response to DFMO, showed only a 20–40% fall in the proximal and distal colon at 10 and 15 weeks. This apparent resistance to DFMO and depletion of polyamines in the colon may in part be due to bacterial production of polyamines within the lumen and subsequent uptake by colonocytes (Tabata & Johnson, 1986). Spermine, however, was found to increase in response to ODC inhibition by DFMO in both the small intestine and the colon at these time periods. This latter phenomenon has previously been reported in other organs, and may be due to incomplete inhibition of ODC by DFMO, with enhanced synthesis of spermine owing to an excess of the amino-propyl donor, decarboxylated AdoMet (Pegg, 1988). In addition, decreased inhibition of spermine synthase activity secondary to depletion of putrescine, a known inhibitor of this enzyme (Oredsson *et al.*, 1980), may also be important.

Effects of DFMO on decarboxylated-AdoMet contents and AdoMet decarboxylase activity

In control animals, the AdoMet decarboxylase activities and the tissue contents of its products, decarboxylated AdoMet, were comparable in proximal and distal segments of the small intestine and colon at the 10- and 15-week periods (Table 2). There was, however, a marked difference in the stimulation of AdoMet decarboxylase activity in response to DFMO in the small intestine compared with the colon at these times. In the small intestine, AdoMet decarboxylase increased 8–16-fold in the proximal and distal segments, respectively, in response to DFMO administration, whereas the contents of decarboxylated AdoMet increased approx. 50–100-fold at 10 and 15 weeks. This DFMO-induced stimulation of AdoMet decarboxylase activity has been well described in other tissues and cell systems (Alhonen-Hongisto, 1980; Pegg, 1984; Shirahata & Pegg, 1985) and is believed to be due to depletion of intracellular spermidine, the major negative inhibitor of this enzyme (Mamont & Danzin, 1981). In contrast with these findings, DFMO administration for 10 and 15 weeks caused only a slight rise in the AdoMet decarboxylase activities and a modest increase in decarboxylated AdoMet in the proximal and distal colonic segments (Table 2). Although the DFMO-induced stimulation of AdoMet decarboxylase activity was greater at 10 weeks compared with 15 weeks in both proximal and distal segments of small and large intestine, there was no significant difference in the decarboxylated AdoMet contents when respective segments were compared at 10 and 15 weeks (Table 2).

These differences in DFMO-induced stimulation of AdoMet decarboxylase activity between the small intestine and colon were most likely due to the greater percentage fall in baseline contents of spermidine in the small intestine compared with the colon (Table 1). It has been previously shown, however, that isoenzymes of AdoMet decarboxylase exist, which differ in their

Table 2. Decarboxylated-AdoMet content and AdoMet decarboxylase activity in proximal and distal segments of small intestine (SI) and colon of control and DFMO-treated rats at 10 and 15 weeks

DFMO was administered as a 1% solution in the drinking water. Results are given as means $\pm$ S.E.M. for eight or more determinations on four separate preparations: ^a $P < 0.001$ compared with control counterpart; ^b $P < 0.05$ compared with control counterpart; ^c $P < 0.01$ compared with 15-week DFMO-treated counterpart.

	Period of treatment (weeks)	Proximal SI		Distal SI		Proximal colon		Distal colon	
		Control	DFMO	Control	DFMO	Control	DFMO	Control	DFMO
Decarboxylated AdoMet (nmol/g)	10	0.59 $\pm$ 0.07	80.0 $\pm$ 6.4 ^a	0.90 $\pm$ 0.22	105 $\pm$ 11 ^a	2.39 $\pm$ 0.25	4.67 $\pm$ 0.73 ^b	1.51 $\pm$ 0.11	1.74 $\pm$ 0.27
	15	1.79 $\pm$ 0.93	74.9 $\pm$ 10.0 ^a	2.01 $\pm$ 0.18	85.5 $\pm$ 29.2 ^b	1.68 $\pm$ 0.22	4.88 $\pm$ 2.57	1.35 $\pm$ 0.12	2.12 $\pm$ 0.75
AdoMet decarboxylase (pmol/30 min per mg of protein)	10	30.1 $\pm$ 5.6	273.5 $\pm$ 15.7 ^{ac}	34.7 $\pm$ 5.4	536.7 $\pm$ 39.5 ^{ac}	32.6 $\pm$ 4.6	58.3 $\pm$ 1.6 ^b	41.1 $\pm$ 2.3	150 $\pm$ 66
	15	14.1 $\pm$ 2.1	172.9 $\pm$ 23.0 ^a	15.2 $\pm$ 2.1	132.2 $\pm$ 23.7 ^b	15.8 $\pm$ 0.8	36.6 $\pm$ 5.4 ^b	15.9 $\pm$ 2.2	20.8 $\pm$ 0.6

Table 3. Kinetics of AdoMet decarboxylase from whole small intestine and colon of normal rats

Results were calculated from the abscissa and ordinate intercepts of Lineweaver-Burk plots, based on eight determinations: ^a95% confidence intervals were 84.1–127 in the small intestine and 44.1–59.3 in the colon.

	Small intestine	Colon
V_{max} (pmol/30 min per mg)	216	202
K_m (μ M) ^a	102	51
K_i for decarboxylated AdoMet (μ M)	29	14

sensitivity to inhibition *in vivo* in response to spermidine administration (Pösö & Pegg, 1981).

In order to explore the possibility that isoenzymes of AdoMet decarboxylase might contribute to the differential stimulation of this enzyme in response to DFMO, the kinetic parameters V_{max} and K_m for AdoMet decarboxylase in the small intestine and colon of normal rats were measured. In addition, the kinetics of inhibition by the product, decarboxylated AdoMet, were studied to determine whether this factor could also play a role in the differential stimulation.

Studies of AdoMet decarboxylase kinetics

Table 3 shows the results of studies of AdoMet decarboxylase kinetics in whole small intestine and colon of control animals. There was no difference in V_{max} values for AdoMet decarboxylase between the small intestine and colon. The small-intestinal AdoMet decarboxylase, however, had a K_m value for AdoMet of 102 μ M, compared with a value of 51 μ M in the colon. These differences in binding constants between the small intestine and colon suggest that different AdoMet decarboxylase isoenzymes may exist in the two organs. It is noteworthy that other investigators have found differences in K_m values for AdoMet decarboxylase in other tissues and that our values in the small intestine and colon agree with those reported for striated muscle (100 μ M) and liver (50 μ M) (Pegg *et al.*, 1982a).

Further evidence for different AdoMet decarboxylase isoenzymes in the colon and small intestine comes from inhibition studies using decarboxylated AdoMet. This product inhibition of AdoMet decarboxylase has been described previously in other tissues (Jänne *et al.*, 1971; Yamanoha & Samejima, 1980). As shown in Table 3, the K_i value for decarboxylated AdoMet obtained in the small intestine was approximately twice that found in the colon.

In this regard it should be noted that, because the assay procedure (Steeves & Lawson, 1985) was run at less than V_{max} conditions, the higher small-intestinal K_m would tend to cause underestimation of enzyme activity in the small intestine compared with the colon. Also, because the enzyme samples of DFMO-treated rats were not dialysed, contamination of the assay with decarboxylated AdoMet might yield lower inhibition of the small-intestinal AdoMet decarboxylase activity compared with the colon activity, owing to its higher K_i , and in part could account for the greater stimulation seen in the small intestine.

DISCUSSION

The present experiments demonstrate for the first time that there is a marked difference in decarboxylated-AdoMet accumulation between the small and large intestine in response to chronic inhibition of polyamine synthesis. This differential accumulation of decarboxylated AdoMet during chronic administration of DFMO can, for the most part, be explained on the basis of differences in the stimulation of AdoMet decarboxylase activity in these two tissues. In both proximal and distal small intestine, the oral administration of DFMO resulted in a marked increase in AdoMet decarboxylase activity, with over a 100-fold increase in decarboxylated AdoMet at 10 weeks and a 40-fold increase at 15 weeks. In both proximal and distal colon, however, there was only a slight rise in AdoMet decarboxylase activity and a minimal accumulation of decarboxylated AdoMet in the DFMO-treated groups at these time periods.

The regulation of AdoMet decarboxylase by intracellular polyamines and the effects of ODC inhibition on this enzyme have been well characterized in a number of cell systems (Mamont *et al.*, 1978; Alhonen-Hongisto, 1980; Pegg, 1984). Spermidine and spermine are inhibitors of AdoMet decarboxylase, and their intracellular depletion results in increases in intracellular AdoMet decarboxylase enzyme protein (Mamont & Danzin, 1981; Pegg, 1988). Thus the greater stimulation of AdoMet decarboxylase in the proximal and distal small intestine may in part be explained by a greater percentage fall in the spermidine content in this tissue compared with either segment of colon. This would not appear to be the entire explanation, however, since in the DFMO-treated rats the final intracellular content of spermidine was comparable in all segments of the colon and small intestine. Differences in the content of spermine, which inversely correlate with rates of translation of AdoMet decarboxylase mRNA (Pegg *et al.*, 1987), also cannot be invoked as an explanation, since spermine contents actually tended to be higher in both the proximal and distal small intestine of DFMO-treated rats compared with the colon. Finally, putrescine, which is a known stimulator of AdoMet decarboxylase activity *in vivo* (Kameji & Pegg, 1987), may play some role in the differential stimulation, as intracellular contents tended to be higher in the small intestine compared with the colon of DFMO-treated animals. It seems unlikely, however, that the slight differences in putrescine contents between the colon and small intestine could account for the large difference in stimulation of AdoMet decarboxylase activity in these tissues.

To study further this differential stimulation of AdoMet decarboxylase by DFMO, kinetic analyses of this enzyme were performed in the small intestine and colon of control rats. The results of these experiments, which demonstrated differences in the K_m values of AdoMet decarboxylase, suggested that different isoenzymes may be present in these organs. Further support for this contention was derived from inhibition studies, which demonstrated a higher K_i for decarboxylated AdoMet in the small intestine compared with the colon. This finding of lower product inhibition in the small intestine may also explain, in part, why there was greater accumulation of decarboxylated AdoMet in this tissue, especially since the intracellular concentration of this

compound during ODC inhibition may approach or slightly exceed these inhibitory constants.

In this regard, other investigators have now demonstrated AdoMet decarboxylase activity in the liver and psoas muscle, which differ in their K_m values, activation by putrescine and sensitivity to inhibition by exogenously administered spermidine (Pöso & Pegg, 1981; Pegg *et al.*, 1982a), which may play an important role in regulation of polyamine metabolism. These results, taken together with the present data, therefore suggest that the higher contents of spermidine found in the small-intestinal segments compared with their colonic counterparts may be due to differential sensitivity of AdoMet decarboxylase in these tissues to inhibition by spermidine.

Finally, the question arises as to what is the physiological significance of decarboxylated-AdoMet accumulation, and could this play any role in the anti-tumorigenic effect of DFMO during chemically induced colon carcinogenesis? Intracellular concentrations of decarboxylated AdoMet, which are normally maintained low, may rise several hundred-fold in the tissues of DFMO-treated animals. Such accumulations approach the concentrations of decarboxylated AdoMet which have been shown *in vitro* to inhibit various AdoMet-dependent methylation reactions (Zappia *et al.*, 1969), including DNA methylation (Heby *et al.*, 1988), which in turn could potentially have an effect on gene expression (Hoffman, 1984). Pegg *et al.* (1986) have shown *in vivo* in SV-3T3 cells that administration of DFMO resulted in accumulation of decarboxylated AdoMet, which then acted as a substrate for histone acetylase, leading to a decrease in acetylation of histones. In view of the fact that alterations in histone acetylation can alter gene expression (Ruiz-Carrillo *et al.*, 1975), it has been suggested that accumulation of decarboxylated AdoMet may also play an important role in the effects of DFMO treatment on cell proliferation (Pegg *et al.*, 1986), and therefore perhaps also on the malignant transformation process induced in the colon by various chemical carcinogens.

It is therefore of interest that significant increases in decarboxylated AdoMet were not found in the colonic segments of DFMO-treated rats. *A priori*, if decarboxylated AdoMet were intimately involved in the anti-tumorigenic effect of DFMO, its content should have been increased in the colon, particularly the distal segment, where this effect of DFMO is most pronounced (Luk *et al.*, 1987). Previous studies have also shown a dissociation between accumulation of decarboxylated AdoMet and inhibition of cell growth (Mamont *et al.*, 1982; Pegg, 1984; Pegg *et al.*, 1988). Thus, in DFMO-treated rat hepatoma cells, 1,3,6-triaminohexane reversed the anti-proliferative effects of ODC inhibition, but did not affect the elevated AdoMet decarboxylase activity or decarboxylated AdoMet contents (Mamont *et al.*, 1982). In DFMO-treated SV-3T3 cells, the elevated decarboxylated-AdoMet content was significantly decreased by addition of *sym*-norspermidine, which had little effect on reversal of growth inhibition (Pegg, 1984). Additionally, Pegg *et al.* (1988) have demonstrated that inhibitors of AdoMet decarboxylase actually blocked growth in L1210 cells. Taken together, these observations along with the present data would strongly suggest that the anti-tumorigenic effects of DFMO previously noted in various experimental models of colonic carcinogenesis

are not related to accumulation of decarboxylated AdoMet, but rather to polyamine depletion.

We thank Lynn Nelson for her excellent secretarial assistance and Alan Klitzke for his technical help in this work. These investigations were supported by U.S. Public Health Service Grant Nos. CA 36745 and CA 08266 awarded by the National Cancer Institute (N.C.I.), Department of Health and Human Services. T.A.B. is the recipient of a Merit Award from the N.C.I./National Institutes of Health.

REFERENCES

- Alhonen-Hongisto, L. (1980) *Biochem. J.* **190**, 747–754
- Ball, W. J., Salser, J. S. & Balis, M. E. (1976) *Cancer Res.* **36**, 2686–2689
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- Heby, O. (1981) *Differentiation* **19**, 1–20
- Heby, O., Persson, L. & Smith, S. S. (1988) International Symposium on Polyamines in Biochemical and Clinical Research, University of Naples, Sorrento, L25 (Abstr.)
- Herrera-Ornelas, L., Porter, C., Pera, P., Greco, W., Petrelli, N. J. & Mittelman, A. (1987) *J. Surg. Res.* **42**, 56–60
- Hoffman, R. M. (1984) *Biochim. Biophys. Acta* **738**, 49–87
- Hosomi, M., Smith, S. M., Murphy, G. M. & Dowling, R. H. (1986) *J. Chromatogr.* **375**, 267–275
- Jänne, J., Schenone, A. & Williams-Ashman, H. G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 758–764
- Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
- Kameji, T. & Pegg, A. E. (1987) *Biochem. J.* **243**, 285–288
- Kingsnorth, A. N. & Wallace, H. M. (1985) in *Recent Progress in Polyamine Research* (Selmeci, L., Brosnan, M. E. & Seiler, N., eds.), pp. 441–448, Akademiai Kiado, Budapest
- Kingsnorth, A. N., King, W. W. K., Diekema, K. A., McCann, P. P., Ross, J. S. & Malt, R. A. (1983) *Cancer Res.* **43**, 2545–2549
- LaMuraglia, G. M., Lacaine, F. & Malt, R. A. (1986) *Ann. Surg.* **204**, 89–93
- Luk, G. D., Hamilton, S. R., O'Ceallaigh, D., Smith, J., Natuzzi, E. & Baylin, S. B. (1982) *Gastroenterology* **82**, 1121
- Luk, G. D., Hamilton, S. R., Yang, P., Smith, J. A., O'Ceallaigh, D., McAvinchey, D. & Hyland, J. (1986) *Cancer Res.* **46**, 4449–4452
- Luk, G. D., Zhang, S. Z., Smith, J. A. & Hamilton, S. R. (1987) *Gastroenterology* **87**, 1151
- Mamont, P. S. & Danzin, C. (1981) *Adv. Polyamine Res.* **3**, 123–135
- Mamont, P. S., Duchesne, M. C., Grove, J. & Tardif, C. (1978) *Exp. Cell Res.* **115**, 387–393
- Mamont, P. S., Danzin, C., Wagner, J., Siat, M., Joder-Ohlenbusch, A. & Claverie, N. (1982) *Eur. J. Biochem.* **123**, 499–504
- Oredsson, S., Anehus, S. & Heby, O. (1980) *Biochem. Biophys. Res. Commun.* **94**, 151–158
- Oredsson, S. M., Kange, M., Mamont, P. S., Wagner, J. & Heby, O. (1986) *Mol. Cell. Biochem.* **70**, 89–96
- Pegg, A. E. (1984) *Biochem. J.* **224**, 29–38
- Pegg, A. E. (1988) *Cancer Res.* **48**, 759–774
- Pegg, A. E. & McCann, P. P. (1982) *Am. J. Physiol.* **243**, C212–C221
- Pegg, A. E. & Williams-Ashman, H. G. (1968) *Biochem. J.* **108**, 533–539
- Pegg, A. E., Pösö, H. & Bennett, R. A. (1982a) in *Biochemistry of S-Adenosylmethionine and Related Compounds* (Usdin, E., Borchardt, R. T. & Creveling, C. R., eds.), pp. 547–555, MacMillan Press, London
- Pegg, A. E., Pösö, H., Shuttleworth, K. & Bennett, R. A. (1982b) *Biochem. J.* **202**, 519–526
- Pegg, A. E., Wechter, R. S., Clark, R. S., Wiest, L. & Erwin, B. G. (1986) *Biochemistry* **25**, 379–384
- Pegg, A. E., Wechter, R. & Pajunen, A. (1987) *Biochem. J.* **244**, 49–54
- Pegg, A. E., Jones, D. B. & Secrist, J. A. (1988) *Biochemistry* **27**, 1408–1415
- Porter, C. W., Herrera-Ornelas, L., Pera, P., Petrelli, N. F. & Mittelman, A. (1987) *Cancer* **60**, 1275–1281
- Pösö, H. & Pegg, A. E. (1981) *Biochem. J.* **200**, 629–637
- Rozhin, J., Wilson, P. S., Bull, A. W. & Nigro, N. D. (1984) *Cancer Res.* **44**, 3226–3230
- Ruiz-Carrillo, A., Wangh, L. J. & Allfrey, V. (1975) *Science* **190**, 117–128
- Shirahata, A. & Pegg, A. E. (1985) *J. Biol. Chem.* **260**, 9583–9588
- Steeves, R. M. & Lawson, D. E. M. (1985) *Biochim. Biophys. Acta* **841**, 292–298
- Tabata, K. & Johnson, L. R. (1986) *Am. J. Physiol.* **251**, G270–G274
- Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790
- Takano, S., Matsushina, M., Erturk, E. & Bryan, G. T. (1981) *Cancer Res.* **41**, 624–628
- Wagner, J., Claverie, N. & Danzin, C. (1984) *Anal. Biochem.* **140**, 108–116
- Yamanoha, B. & Samejima, K. (1980) *Chem. Pharm. Bull.* **28**, 2232–2234
- Zappia, V., Zydek-Cwick, C. R. & Schlenk, F. (1969) *J. Biol. Chem.* **244**, 4499–4509

Received 25 August 1988/13 December 1988; accepted 21 December 1988