Effect on prostatic growth of 2-difluoromethylornithine, an effective inhibitor of ornithine decarboxylase

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2-Difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase, causes marked changes in the polyamine metabolism of ventral prostate when given to adult rats in drinking water (20 g/l) for 3 consecutive days. A 90% inhibition of ornithine decarboxylase activity is accompanied by approx. 80% decreases of the concentrations of putrescine and spermidine and by a 36% decrease in spermine. Concomitantly, *S*-adenosylmethionine decarboxylase activity increases 7-fold and the concentration of decarboxylated *S*-adenosylmethionine 450-fold. When DFMO is given to immature rats for 12 consecutive days the above described changes are accompanied by a marked reduction in the age-dependent increases of the wet weight and RNA and DNA contents of the ventral prostate. In adult rats DFMO decreases the weight and RNA content of the ventral prostate within 4 days by 32% and 24% respectively and maintains them constant for the next 19 days. After 23 days of treatment, the prostatic weight is 46% of that of control animals of the same age, whereas the weights of other organs are only slightly decreased. Cytological studies carried out at this time show that DFMO reduces the size of both prostatic acini and the epithelial cells lining the acini.

Large amounts of polyamines and high activities of their biosynthetic enzymes are found in rat ventral prostate (Williams-Ashman *et al.*, 1975a; Jänne *et al.*, 1978). There are only minor changes in polyamine concentrations during age-dependent growth of rat ventral prostate (Takay *et al.*, 1977). In the castrated rat, testosterone-induced prostatic regrowth is accompanied by an increase of polyamine concentrations (Pegg *et al.*, 1970). Under these conditions, increases in the prostatic content of RNA but not of DNA correlate with increases in spermidine but not in spermine concentrations (Williams-Ashman *et al.*, 1975b). However, the interpretation of these results is complicated by the presence of high concentrations of polyamines in prostatic secretions (Williams-Ashman & Lockwood, 1970), which makes it difficult to estimate the intracellular content of polyamines.

Several authors have suggested that concentrations of polyamines and activities of their biosynthetic enzymes could reflect secretory functions of the prostatic gland rather than its growth (Fuller *et al.*, 1975; Williams-Ashman *et al.*, 1975b; Jänne

et al., 1978). New approaches are required to clarify the role of polyamines in the rat ventral prostate. Specific polyamine-biosynthesis inhibitors could be particularly useful for this purpose.

DFMO (RMI 71782) is a potent enzyme-activated irreversible inhibitor, both *in vitro* (Metcalfe *et al.*, 1978) and *in vivo* (Danzin *et al.*, 1979a), of ornithine decarboxylase (EC 4.1.1.17), the enzyme responsible for the biosynthesis of putrescine. The inhibitor slowed the testosterone-induced weight gain of the ventral prostate in castrated rats (Danzin *et al.*, 1979b). This slowing was mainly due to inhibition of the restoration of prostatic secretions, whereas the testosterone-stimulated cellular hypertrophy was only marginally affected and the cellular proliferation was not affected at all.

We now report that prolonged administration of DFMO to intact rats decreases the polyamine concentrations and the volume of secretions in the ventral prostate and suppresses its normal age-dependent hypertrophic and hyperplastic growth.

Experimental

Chemicals

The following compounds were purchased: tetrasodium EDTA (Calbiochem, San Diego, CA,

Abbreviation used: DFMO, 2-difluoromethylornithine.

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U.S.A.); L-ornithine, pyridoxal phosphate, sucrose, buffer reagents, diphenylamine, 3,5-dihydroxy-toluene, haematoxylin and eosin (Merck, Darmstadt, Germany); S-adenosyl-L-methionine, putrescine dihydrochloride, dithiothreitol, bovine serum albumin, yeast RNA (type XI) and calf thymus DNA (type I) (Sigma Chemical Co., St. Louis, MO, U.S.A.); acetaldehyde (Aldrich Chemical Co., Milwaukee, WI, U.S.A.); Light Green (Prolabo, Paris, France); DL-[1-¹⁴C]ornithine (sp. radioactivity 58 Ci/mol) and S-adenosyl-L-[carboxy-¹⁴C]methionine (sp. radioactivity 60 Ci/mol) (The Radiochemical Centre, Amersham, Bucks., U.K.); scintillators (Beckman Instruments Inc., Fullerton, CA, U.S.A.). DFMO (RMI 71782) was synthesized in our laboratories (Metcalf *et al.*, 1978).

Animals

Male Sprague-Dawley rats were purchased from Charles River, Saint-Aubin-les-Elbeuf, France. Animals had access to a standard diet *ad libitum* and were kept on a constant 12 h light/12 h dark schedule. Treated animals received a solution of DFMO in water (20 g/l) as the sole drinking fluid, resulting in a daily intake of approx. 2 g/kg body wt. Rats were killed by decapitation between 11:00 and 12:00 h to minimize effects due to diurnal fluctuations.

Preparation of tissue extracts

The ventral prostate and other organs were removed, freed of fat and connective tissue, weighed and frozen in liquid N₂. The organs were homogenized at 4°C in 5 ml of 5% (w/v) trichloroacetic acid and the homogenates were centrifuged at 3000 g. The supernatants were used for polyamine and amino acid determinations, and the precipitates were used for nucleic acid measurements. In parallel experiments, ventral prostate homogenates were made in 5 vol. of 30 mM-sodium phosphate buffer, pH 7.1, containing 0.1 mM-EDTA, 0.1 mM-pyridoxal phosphate and 5 mM-dithiothreitol for measurements of enzyme activities.

Polyamine and amino acid determinations

The supernatants were filtered through a Millipore membrane (pore size 0.22 µm), before measurement of their polyamine content with a Durrum D-500 amino acid analyser (Durrum Instruments Corp., Palo Alto, CA, U.S.A.) by the method of Marton *et al.* (1974) as modified by Bartos *et al.* (1977).

Ornithine and DFMO were determined by a modification of the method of Lee (1973), with a Liquimat II (Kontron, Paris, France) amino-acid analyser equipped with a 2.5 mm-internal-diameter glass column filled to a height of 30 cm with Durrum DC-4 cation-exchange resin.

S-Adenosylmethionine, S-adenosylhomocysteine and decarboxylated S-adenosylmethionine [S-adenosyl-(5')-3-methylthiopropylamine] were determined by reversed-phase ion-pair high-pressure liquid chromatography (Wagner *et al.*, 1981) with a µBondapack C₁₈ column (10 µm particle size; 3.9 mm × 30 cm). A linear gradient, leading in 30 min from an eluent consisting of a mixture of 47:3 (v/v) NaH₂PO₄ (0.1 M) and acetonitrile (pH 2.5) to one consisting of a mixture of 37:13 (v/v) NaH₂PO₄ (0.1 M) and acetonitrile (pH 3.1), was used at a flow rate of 1.5 ml/min and a temperature of 28°C. Octane sulphonic acid (8 mM) in both eluents was used as ion-pairing agent. The u.v. absorption was monitored at 254 nm. The high-pressure liquid chromatograph consisted of models 6000 A and 660 as solvent delivery system, of a WISP 710 instrument, as automatic sample injector, and of model 440 as u.v. detector, all from Waters Associates (Milford, MA, U.S.A.). The signal was recorded on an SP. 4100 digital integrator from Spectra Physics (Santa-Clara, CA, U.S.A.).

Nucleic acid determination

The procedure for measuring RNA and DNA described by Schneider (1957) was modified as follows. The trichloroacetic acid-precipitated material was resuspended in 2 ml of 0.3 M-KOH for 20 h at 37°C. Thereafter, 5 ml of 1.6 M-HClO₄ was added, and the suspension was cooled to 0°C during 1 h followed by centrifugation at 3000 g for 10 min. The supernatant was used to determine RNA by the orcinol reaction (Mejbaum, 1939) with purified yeast RNA as standard. The precipitate was resuspended in 2 ml of 0.5 M-HClO₄ and digested for 20 min at 90°C. The suspension was centrifuged at 2000 g for 10 min. The supernatant was used to determine DNA by the diphenylamine method (Burton, 1968) with calf thymus DNA as reference material.

Assays of ornithine decarboxylase and S-adenosylmethionine decarboxylase activities

The homogenates prepared as described above were centrifuged at 105 000 g. Ornithine decarboxylase activity was measured in the dialysed supernatant, essentially as described by Ono *et al.* (1972). S-Adenosylmethionine decarboxylase activity was measured in the same supernatant, in the presence of 2.5 mM-putrescine, by the method of Jänne & Williams-Ashman (1971). One unit of either ornithine decarboxylase or S-adenosylmethionine decarboxylase activity is defined as the amount of enzyme catalysing the formation of 1 nmol of CO₂/30 min. Proteins were measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Staining of prostate gland sections

Ventral prostates were fixed for 20h in Carnoy's medium and sections were stained with the modified Gomori's trichrome (Haematoxylin/Light Green/Eosin) in a similar way to the procedure described by Mangan *et al.* (1973).

Results

Effects of 3 days of DFMO administration on the polyamine metabolism of rat ventral prostate

DFMO given over 3 days to rats in their drinking water (20g/l) produced a significant (24%) decrease of wet weight of the ventral prostate (Table 1). Ornithine decarboxylase activity was inhibited by 90% and there were marked decreases in putrescine and spermidine concentrations and a lesser decrease in spermine concentration. *S*-Adenosylmethionine decarboxylase activity increased 7-fold and the concentration of decarboxylated *S*-adenosylmethionine 450-fold. The concentrations of ornithine and of *S*-adenosylmethionine increased slightly, whereas the concentration of *S*-adenosylhomocysteine was not significantly affected (Table 1).

Effects of chronic administration of DFMO on the growth of rat ventral prostate

Immature rats (80g, 4 weeks old) were given DFMO (20g/litre, in water) as the only drinking fluid for 12 days. The concentrations of putrescine, spermidine and spermine in the ventral prostate were decreased to 30, 6 and 76% respectively of the values from matched controls (Table 2). This decrease in polyamine concentration was accompanied by a 400-fold increase in the concentration of decarboxylated *S*-adenosylmethionine.

The concentration of *S*-adenosylmethionine was not modified, whereas the concentrations of *S*-adenosylhomocysteine and ornithine were slightly higher than in the same tissue from control animals.

The DFMO-induced metabolic perturbations were accompanied by a marked reduction in the growth of the ventral prostate (Table 2). In treated animals, the age-dependent weight increase of this gland was only 27% of that of control animals. The corresponding increase in RNA content was similarly affected, whereas the increase in DNA content was completely suppressed during DFMO treatment.

In adult rats (300g; 10 weeks old), polyamine concentrations, nucleic acid contents and growth of the ventral prostate were measured at various times during DFMO treatment. Within 4 days of treatment with DFMO, the concentrations of putrescine, spermidine and spermine fell to 2, 13 and 65% respectively of the control values (Table 3) and continued to decrease slowly thereafter. After 23 days of treatment, they reached 1, 5 and 49% respectively of the values for control animals of the same age. The wet weight and RNA content of the ventral prostate decreased within 4 days below the starting values and remained constant thereafter, whereas these parameters steadily increased in the control animals. At the end of treatment, the wet weight of ventral prostate was only 46% of that from control animals of the same age and the RNA content of this gland was 60% of that of controls. In adult rats, the DNA content of the ventral prostate changes only slightly with increasing age (Takayi & Thomas, 1977; Heyns *et al.*, 1978); DFMO had no significant effect on it.

After 23 days of DFMO administration, several

Table 1. *Effects of a 3-day treatment with DFMO on polyamine metabolism in ventral prostate of adult rats*

The treated animals received DFMO dissolved in drinking water (20g/l) for 3 consecutive days. Enzyme assays and amine and amino acid determinations are described in the Experimental section. Each value is the mean $\pm$ S.E.M. for five animals. The significance of the differences between treated and control animals was calculated by Student's *t* test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Control	DFMO treatment
Body wt. (g)	323 $\pm$ 7	313 $\pm$ 2
Ventral prostate (g wet wt.)	0.57 $\pm$ 0.03	0.43 $\pm$ 0.03**
Ornithine decarboxylase activity (units/mg of protein)	2.5 $\pm$ 0.2	0.23 $\pm$ 0.02***
<i>S</i> -Adenosylmethionine decarboxylase activity (units/mg of protein)	2.6 $\pm$ 0.2	18 $\pm$ 1***
Putrescine (nmol/g wet wt.)	270 $\pm$ 20	40 $\pm$ 10***
Spermidine (nmol/g wet wt.)	6600 $\pm$ 400	1400 $\pm$ 200***
Spermine (nmol/g wet wt.)	4200 $\pm$ 300	2700 $\pm$ 300**
<i>S</i> -Adenosylmethionine (nmol/g wet wt.)	50 $\pm$ 6	80 $\pm$ 8*
Decarboxylated <i>S</i> -adenosylmethionine (nmol/g wet wt.)	2 $\pm$ 0.2	900 $\pm$ 100***
<i>S</i> -Adenosylhomocysteine (nmol/g wet wt.)	11 $\pm$ 3	10 $\pm$ 1
Ornithine (nmol/g wet wt.)	66 $\pm$ 10	190 $\pm$ 20***
DFMO (nmol/g wet wt.)	—	340 $\pm$ 50

Table 2. *Effects of chronic administration of DFMO on wet weight, DNA and RNA contents and concentrations of polyamines and their precursors in ventral prostate of immature rats*

The treated animals received DFMO dissolved in drinking water (20 g/l) for 12 consecutive days. Methods of DNA, RNA, amine and amino acid determination are described in the Experimental section. Each value is the mean $\pm$ s.e.m. for five animals. The significance of the differences between treated and control animals of the same age was calculated by Student's *t* test. **P* < 0.01; ***P* < 0.001.

	Day	Control	DFMO treatment
Body wt. (g)	0	86 $\pm$ 4	—
	12	188 $\pm$ 3	138 $\pm$ 2**
Ventral prostate (g wet wt.)	0	0.06 $\pm$ 0.01	—
	12	0.17 $\pm$ 0.01	0.09 $\pm$ 0.01**
DNA (μ g/ventral prostate)	0	300 $\pm$ 20	—
	12	410 $\pm$ 20	270 $\pm$ 20*
RNA (μ g/ventral prostate)	0	520 $\pm$ 40	—
	12	1500 $\pm$ 100	770 $\pm$ 90*
Putrescine (nmol/g wet wt.)	0	470 $\pm$ 40	—
	12	460 $\pm$ 50	140 $\pm$ 30**
Spermidine (nmol/g wet wt.)	0	9000 $\pm$ 300	—
	12	6200 $\pm$ 600	400 $\pm$ 40**
Spermine (nmol/g wet wt.)	0	3000 $\pm$ 500	—
	12	2900 $\pm$ 80	2200 $\pm$ 100*
S-Adenosylmethionine (nmol/g wet wt.)	0	61 $\pm$ 12	—
	12	76 $\pm$ 8	85 $\pm$ 8
Decarboxylated S-adenosylmethionine (nmol/g wet wt.)	0	4 $\pm$ 1	—
	12	5 $\pm$ 1	2100 $\pm$ 200**
S-Adenosylhomocysteine (nmol/g wet wt.)	0	3.1 $\pm$ 0.6	—
	12	4.6 $\pm$ 0.5	11 $\pm$ 1**
Ornithine (nmol/g wet wt.)	0	48 $\pm$ 7	—
	12	55 $\pm$ 1	101 $\pm$ 12*
DFMO (nmol/g wet wt.)	12	—	230 $\pm$ 60

Table 3. *Effects of chronic administration of DFMO on wet weight, DNA and RNA contents and concentration of polyamines in the ventral prostate of adult rats*

The treated animals received DFMO dissolved in drinking water (20 g/l) for various times up to 23 consecutive days. DNA, RNA and polyamine determinations are described in the Experimental section. Each value is the mean $\pm$ s.e.m. for five animals. The significance of the differences between treated and control animals of the same age was calculated by Student's *t* test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

	Day of treatment	0	4	9	16	23
	DFMO					
Body wt. (g)	—	295 $\pm$ 3	316 $\pm$ 6	330 $\pm$ 4	374 $\pm$ 8	417 $\pm$ 6
	+		314 $\pm$ 10	326 $\pm$ 10	331 $\pm$ 8**	364 $\pm$ 11**
Ventral prostate (g wet wt.)	—	0.44 $\pm$ 0.01	0.50 $\pm$ 0.04	0.51 $\pm$ 0.04	0.61 $\pm$ 0.04	0.71 $\pm$ 0.07
	+		0.34 $\pm$ 0.02**	0.31 $\pm$ 0.03**	0.30 $\pm$ 0.03**	0.33 $\pm$ 0.03**
Putrescine (nmol/g wet wt.)	—	170 $\pm$ 10	190 $\pm$ 20	170 $\pm$ 40	200 $\pm$ 20	210 $\pm$ 40
	+		4 $\pm$ 1***	4 $\pm$ 1***	4 $\pm$ 1***	2 $\pm$ 1***
Spermidine (nmol/g wet wt.)	—	4700 $\pm$ 500	5700 $\pm$ 500	4800 $\pm$ 200	5000 $\pm$ 500	5600 $\pm$ 600
	+		760 $\pm$ 180***	400 $\pm$ 100***	220 $\pm$ 30***	270 $\pm$ 20***
Spermine (nmol/g wet wt.)	—	3200 $\pm$ 200	3400 $\pm$ 400	3000 $\pm$ 100	2400 $\pm$ 200	3900 $\pm$ 200
	+		2200 $\pm$ 300*	2000 $\pm$ 200**	1900 $\pm$ 100*	1900 $\pm$ 500**
DNA (μ g/ventral prostate)	—	830 $\pm$ 80	710 $\pm$ 90	700 $\pm$ 50	840 $\pm$ 60	730 $\pm$ 60
	+		780 $\pm$ 90	720 $\pm$ 60	750 $\pm$ 120	680 $\pm$ 180
RNA (μ g/ventral prostate)	—	3900 $\pm$ 400	4200 $\pm$ 400	4300 $\pm$ 300	5300 $\pm$ 300	5500 $\pm$ 1200
	+		3200 $\pm$ 300	3000 $\pm$ 500**	3000 $\pm$ 700**	3300 $\pm$ 300**

organs were removed from control and treated rats and weighed. The absolute wet weights of male accessory sex organs were significantly decreased in

the DFMO-treated animals (Table 4). However, the organ weight/body weight ratio was significantly decreased only for the ventral prostate.

Table 4. *Effects of chronic administration of DFMO on absolute and relative organ weights of adult rats*

The wet weights of various organs were measured in rats treated for 23 days and in control rats (see Table 3). For each organ, the first line indicates the control value and the second line the value obtained from treated animals. Each value is the mean $\pm$ S.E.M. of five animals. The significance of the differences between the two groups of animals was calculated by Student's *t* test.

Organ	Absolute weight (g)	Relative weight (% of body wt.)
Ventral prostate	0.71 $\pm$ 0.07 0.33 $\pm$ 0.03*	0.17 $\pm$ 0.02 0.09 $\pm$ 0.01*
Seminal vesicle ^a (+ secretions)	0.56 $\pm$ 0.03 0.40 $\pm$ 0.01*	0.13 $\pm$ 0.01 0.11 $\pm$ 0.01
Seminal vesicle ^a (– secretions)	0.20 $\pm$ 0.01 0.15 $\pm$ 0.01*	0.048 $\pm$ 0.002 0.044 $\pm$ 0.003
Coagulating gland ^a	0.092 $\pm$ 0.004 0.073 $\pm$ 0.003*	0.022 $\pm$ 0.001 0.020 $\pm$ 0.001
Testis	1.48 $\pm$ 0.02 1.49 $\pm$ 0.02	0.36 $\pm$ 0.01 0.41 $\pm$ 0.01*
Kidney	1.38 $\pm$ 0.03 1.32 $\pm$ 0.02	0.33 $\pm$ 0.01 0.36 $\pm$ 0.01
Liver	12.5 $\pm$ 0.6 11.5 $\pm$ 0.7	3.0 $\pm$ 0.1 3.1 $\pm$ 0.1

^a Weight of one of the two lobes from the corresponding organ.

* $P < 0.01$.

Table 5. *Effects on prostatic morphology of chronic administration of DFMO in adult rats*

The treated animals received DFMO dissolved in drinking water (20 g/l). Histological studies were performed on ventral prostates of four treated and four control animals. The tissues were sectioned at 4 μ m and stained as described in the Experimental section. Acini diameters (measured from the length and width of the theoretical rectangles that would enclose these areas) and heights of epithelial cells lining the acini were measured with an ocular micrometer in 140 to 170 acini/prostate, located in five different regions. Values in the Table are means $\pm$ S.E.M. for the pooled measurements. The significance of the differences between treated and control animals was calculated by Student's *t* test.

	Control animals	Treated animals
Epithelium height (μ m)	25.3 $\pm$ 0.2	19.5 $\pm$ 1.7*
Acini diameter (μ m)		
Maximum	430 $\pm$ 17	327 $\pm$ 37*
Minimum	222 $\pm$ 10	172 $\pm$ 15*
Acini number/microscopic field ^a	8.0 $\pm$ 0.8	12.8 $\pm$ 1.1*
Acini number/section	100 $\pm$ 7	106 $\pm$ 9

^a Magnification 125 $\times$.

* $P < 0.05$.

Morphological changes of the ventral prostate induced by DFMO

In an additional study we compared the morphological structure of the ventral prostate in adult rats treated for 23 days with DFMO with that of control rats of the same age (Table 5). The microscopic examination indicated that DFMO induced a reduction in size of all prostatic structures. In particular, the height of the epithelial cells was

significantly reduced (77% of control). Although the number of prostatic acini was not changed, their diameters were significantly reduced, suggesting a reduced content of secretions.

Discussion

DFMO given to immature or adult rats in drinking water (20 g/litre) causes a marked decrease

of polyamine concentrations in the ventral prostate, accompanied by a dramatic increase of decarboxylated *S*-adenosylmethionine. The age-dependent increases in RNA and DNA contents and in the wet weight of the ventral prostate observed in control animals are markedly reduced or totally suppressed. These findings may support a causal inter-relationship between the concentration of polyamines and the synthesis of nucleic acids in the ventral prostate. Such an inter-relationship has already been reported for several cell-culture lines (Jänne *et al.*, 1978), including prostatic adenoma cells (Mamont *et al.*, 1978), and has been suggested for experimental tumours of animals treated with DFMO (Koch-Weser *et al.*, 1981).

The 450-fold increase in decarboxylated *S*-adenosylmethionine concentration that accompanies the decrease in polyamine concentrations in treated animals also deserves attention. Spermidine has a negative regulatory role on *S*-adenosylmethionine decarboxylase activity *in vivo*, i.e. the depletion of spermidine concentration due to inhibition of ornithine decarboxylase is followed by an increase in *S*-adenosylmethionine decarboxylase activity in cultured cells (Alhonen-Hongisto, 1980; Mamont & Danzin, 1981; Mamont *et al.*, 1981). Spermidine depletion seems to have a similar effect in the ventral prostate, even though resting *S*-adenosylmethionine decarboxylase activity is already much higher in the ventral prostate than in other tissues (Raina *et al.*, 1976). The increase in *S*-adenosylmethionine decarboxylase activity is accompanied by a 450-fold increase in the concentration of decarboxylated *S*-adenosylmethionine. It is noteworthy that under similar conditions, decarboxylated *S*-adenosylmethionine concentration is not affected in the pancreas and is increased only 4-fold in testis and 40-fold in the thymus (Wagner *et al.*, 1981).

The marked accumulation of decarboxylated *S*-adenosylmethionine in the prostate is presumably due both to the observed increase in *S*-adenosylmethionine decarboxylase activity and to the reduction in the biosynthesis of putrescine and spermidine. The role of decarboxylated *S*-adenosylmethionine as donor of the aminopropyl moiety in the biosynthesis of spermidine and spermine is well established (Jänne *et al.*, 1978), but nothing is known about its catabolism. It does not usually accumulate in mammalian cells (Hibasami *et al.*, 1980). Its enormous accumulation in ventral prostate during treatment with DFMO indicates slow catabolism and excretion. An important metabolic consequence of the accumulation of decarboxylated *S*-adenosylmethionine is trapping of adenine. In normal cells, the decarboxylated *S*-adenosylmethionine is transformed into 5'-methylthioadenosine and spermidine or spermine by spermidine

synthase and spermine synthase (Jänne *et al.*, 1978). In the prostate, a high activity of methylthioadenosine phosphorylase, which cleaves methylthioadenosine into methylthioribose 1-phosphate and adenine, was reported by Pegg & Williams-Ashman (1969) and by Seidenfeld *et al.* (1980). The suggested function of the methylthioadenosine phosphorylase is to allow metabolic recycling of adenine derived from decarboxylated *S*-adenosylmethionine (Williams-Ashman, 1981). Decarboxylated *S*-adenosylmethionine cannot be transformed into methylthioadenosine in the absence of putrescine and thus adenine is not recycled. These conditions could be similar to those observed after ethionine administration to rats, when the resulting accumulation of *S*-adenosylethionine leads to the depletion of hepatic ATP due to trapping of adenine (Shull *et al.*, 1966). Therefore, the suppression of nucleic acid accumulation within the ventral prostate in DFMO-treated rats may be partly explained by failure to synthesize adenine nucleotides *de novo* at a sufficiently high rate to compensate for the trapping of adenine by decarboxylated *S*-adenosylmethionine.

Another factor, not directly related to polyamine depletion in the ventral prostate, may have influenced prostatic growth. Chronic treatment of rats with DFMO causes a slight but significant slowing of body weight gain compared with control rats, presumably by diminishing food intake. Underfeeding tends to affect growth and secretory activity of the male accessory organs, probably due to a decreased testosterone production by the testes (Mann, 1974). However, this mechanism seems to be relatively unimportant, since the absolute and relative weights of the coagulating gland, of the seminal vesicle with or without secretions and of the testis were only slightly or not at all affected.

In a previous report (Danzin *et al.*, 1979b) we have shown that in castrated rats, DFMO did not affect the testosterone-induced hypertrophy and hyperplasia of epithelial cells. It retarded RNA accumulation only to a slight extent, but markedly slowed restoration of the prostatic secretions. However, in the present study DFMO affected the age-dependent increase of DNA and RNA contents and the height of epithelial cells. The discrepancy between the two studies might be due to the use of superphysiological doses of testosterone in castrated rats. Thus, polyamines may have only a contributory role in the action of steroid androgens on the biosynthesis of nucleic acids. Such a contributory role has already been suggested for the stimulation of casein synthesis by glucocorticoids in the mammary gland of rabbit (Houdebine *et al.*, 1978) and rat (Bolander & Topper, 1979).

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