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# Differential Effect of α-Difluoromethylornithine on the *in Vivo* Uptake of $^{14}$C-labeled Polyamines and Methylglyoxal Bis(guanylhydrazone) by a Rat Prostate-derived Tumor

**Warren D. W. Heston,[1,2] Dov Kadmon,[3] Douglas F. Covey,[4] and William R. Fair[2,3]**

*Urological Oncology Research Laboratory, Division of Urology, Department of Surgery [W. D. W. H., D. K., W. R. F.], and the Department of Pharmacology [D. F. C.], Washington University School of Medicine, St. Louis, Missouri 63110*

## ABSTRACT

The uptake of exogenously administered radiolabeled polyamines by a rat prostate-derived tumor line, the Dunning R3327 MAT-Lu, and various normal tissues was studied. Pretreatment of tumor cells *in vitro* with α-difluoromethylornithine (DFMO), a polyamine synthesis inhibitor, resulted in a markedly enhanced uptake of both [$^{14}$C]putrescine and [$^{14}$C]spermidine. The *in vitro* uptake of [$^{14}$C]putrescine by these cells was effectively inhibited by unlabeled spermine, spermidine, 1,8-diaminooctane, 1,7-diaminoheptane, 1,6-diaminohexane, 1,5-diaminopentane, 1,4-diaminopentane, and 1,4-diaminobutane, but less effectively by 1,4-diamino-2,3-butene and 1,4-diamino-2,3-butyne. The diamines, 1,3-diaminopropane and 1,2-diaminoethane, were ineffective in inhibiting [$^{14}$C]putrescine uptake *in vitro* into the R3327 MAT-Lu cell line. When tumor-bearing animals were pretreated with DFMO or with DFMO and 5-α-dihydrotestosterone propionate, the tumor and prostate uptake of [$^{14}$C]putrescine and [$^{14}$C]-cadaverine was enhanced but not substantially increased in other tissues. In contrast to the *in vitro* results, spermidine and spermine were not enhanced substantially by DFMO pretreatment into any tissue, and their uptake into the tumor actually decreased. Ethylenediamine, which does not utilize the polyamine transport system, did not have its uptake increased into any tissue following DFMO pretreatment. The chemotherapeutic agent, methylglyoxal bis(guanylhydrazone), which utilizes the polyamine transport system for uptake into cells, exhibited uptake behavior different from that of the polyamines. Thus, methylglyoxal bis(guanylhydrazone) uptake into the tumor was not significantly increased or decreased by DFMO or by DFMO + 5-α-dihydrotestosterone propionate pretreatment, and only the ventral, but not the dorsal-lateral, lobe of the prostate showed increased uptake of methylglyoxal bis(guanylhydrazone) following DFMO + 5-α-dihydrotestosterone propionate pretreatment.

## INTRODUCTION

The natural polyamines putrescine, spermidine, and spermine are found in high concentrations in the rat and human prostate (31, 37). They are considered important for prostate cell proliferation and for prostatic secretory function (5, 18). Although prostatic tissue has sufficient biosynthetic capability to maintain these high polyamine concentrations, Clark and Fair (3) reported that radiolabeled putrescine injected into intact rats accumulated in the prostate to a greater extent than in many other tissues (36). It has been proposed, and experimentally verified, that putrescine might be useful as an imaging agent for the prostate and prostate-derived tumors and other tumors with a positron emission transaxial tomography scanner using the positron-emitting putrescine analogue, $^{11}$C-methylated putrescine (8, 20, 36). Other laboratories have also found that tumors take up more [$^{14}$C]putrescine or [$^{11}$C]methyl putrescine than does corresponding normal tissue (35, 38).

Since these initial experiments, DFMO,[5] a compound which blocks polyamine synthesis, has become available. DFMO is an enzyme-activated irreversible inhibitor of ODC (19). Since ODC is responsible for the first and rate-limiting step in polyamine synthesis in mammalian cells, DFMO reduces the intracellular levels of polyamines (6, 7). Danzin *et al.* (7) have shown that the ODC activity in the prostate of the rodent was more sensitive to inhibition by DFMO than was the ODC of other tissues. They have shown that DFMO administration, *in vivo*, caused a marked depletion of prostatic polyamines (7). We have shown that the R3327 prostatic tumor and its derivative lines are as sensitive to ODC inhibition by DFMO as is the normal prostate (10, 11, 28).

It has been demonstrated that the inhibition of ODC activity by DFMO will markedly increase the uptake of exogenous spermine, spermidine, or putrescine (1). The uptake of putrescine, a nontoxic diamine, has been shown previously by us to be increased *in vivo* by DFMO pretreatment (10, 12, 13). Androgen supplementation with DFMO pretreatment further increased putrescine uptake into the prostate and androgen-sensitive prostatic tumors, so that DHTP + DFMO pretreatment increased putrescine uptake 3- to 10-fold over that of the unstimulated controls with minimal additional changes in uptake into nonprostatic tissues (12, 13).

Counsell *et al.* (4) showed that spermidine and spermine were taken up in higher concentrations into the rat prostate than was putrescine. In a nonprostatic cell line, Alhonen-Hongisto *et al.* (1) showed that DFMO pretreatment increased *in vitro* methyl-GAG uptake as well as polyamine uptake. In this study, we examined the ability of DFMO pretreatment to enhance the uptake *in vivo* of spermidine, spermine, and methyl-GAG into the prostate and prostatic tumors.

Currently, we have established the prostate-derived R3327 Mat-Lu line both *in vitro* as well as *in vivo* (10, 17). This tumor is a hormone-independent, anaplastic, metastatic derivative of the Dunning R3327 tumor (17). As the androgen-sensitive Dunning R3327 tumor and the normal prostatic tissue were similar in their uptake of putrescine, we included normal prostatic tissue as

[1] To whom requests for reprints should be addressed.

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[5] The abbreviations used are: DFMO, α-difluoromethylornithine; DHTP, 5-α-dihydrotestosterone propionate; methyl-GAG, methylglyoxal bis(guanylhydrazone); ODC, L-ornithine decarboxylase (EC 4.1.1.17).

representative of the uptake of androgen-sensitive prostate for comparison with the androgen-insensitive R3327 MAT-Lu tumor. Ethylenediamine, a nonphysiological diamine, does not use the polyamine transport system for uptake into this tumor and was included as a control for nonspecific effects of DFMO on polyamine uptake.

We continue these efforts in the hope that polyamines may eventually be used in lymphoscintography to noninvasively determine whether or not prostatic cancer has metastasized beyond the prostate to the lymph nodes. We wish also to determine which polyamine analogues would be appropriate for prostate tumors in the development of chemotherapeutic agents for use in conjunction with DFMO.

## MATERIALS AND METHODS

### Animals and Tumors

The animals used were mature (400 to 450 g), male, COP × F344 $F_1$ hybrids as described previously (28). The R3327MAT-Lu tumor was maintained and transplanted as described previously (17). In some experiments, the animals used were obtained through the courtesy of the National Prostatic Cancer Project at Roswell Park Memorial Institute, Buffalo, NY, from Dr. Norman H. Altman of the Papanicolaou Cancer Research Institute, Miami, FL. DFMO and DHTP pretreatment protocols were the same as those described previously (12, 13).

### Chemicals

DFMO was the generous gift of Merrell Dow Pharmaceuticals, Inc., Cincinnati, OH, through the courtesy of Dr. Peter McCann and Dr. W. J. Hudak.

$^{14}$C-labeled amines putrescine (113 mCi/mM), spermine (109 mCi/mmol), and ethylenediamine (25 mCi/mmol) as well as [$^{14}$C]methyl-GAG (12.5 mCi/mmol) were obtained from Amersham, Arlington Heights, IL, and cadaverine (108 mCi/mmol) and spermine (81 mCi/mmol) were obtained from New England Nuclear, Boston, MA. NCS tissue solubilizer was obtained from Amersham. ScintiVerse I and ScintiLene scintillation cocktails were obtained from Fisher Scientific Co., Fair Lawn, NJ.

Most of the unlabeled amines were obtained from Aldrich Chemical Co., Milwaukee, WI. 1,4-Diamino-2,3-butyne was obtained from Calbiochem-Behring, San Diego, CA. 1,4-Diamino-2,3-butene was obtained from Alfa Products, Danvers, MA. 1,4-Diaminopentane was synthesized by the method of Vita and Bucher (34). 4-Xylylenediamine was obtained from Columbia Organic Chemicals Co., Columbia, SC. Histamine, serotonin, ornithine, lysine, arginine, and standard laboratory reagents were obtained from Sigma Chemical Co., St. Louis, MO.

### Tissue Culture Supplies

RPMI-1640, Hanks' balanced salt solution, fetal calf serum, sterile tissue culture flasks, etc. were obtained from KC Biologicals, Lenexa, KS. Male rat serum was obtained by cardiac puncture from mature male $F_1$ rats. All serum was heat inactivated at 50° for 30 min.

### Polyamine Uptake

***In Vitro.*** R3327 MAT-Lu cells were grown and maintained *in vitro* as described previously (10). For the *in vitro* uptake experiments, $1 \times 10^5$ R3327MAT-Lu cells were plated per well of a Costar 12-well plate in 1 ml of RPMI-1640 medium with 10% rat serum. Twenty-four hr following plating, the medium was replaced with fresh plating medium or plating medium containing 1 mM DFMO. Twenty-four hr later, $^{14}$C-labeled polyamine at 3 μM concentration with or without excess unlabeled amine was added to the cells and incubated for 30 min at 37° in a humidified incubator at 7.5% $CO_2$ and saturation humidity. One ml of ice-cold stop solution, Hanks' balanced salt solution containing 1 mg of bovine serum albumin and 1 mM unlabeled putrescine, was added in order to terminate polyamine uptake. The supernatant was aspirated, and the cells were rinsed twice with stop solution. Following the last rinse, the radioactivity was extracted with 1 ml of 0.1 N sodium hydroxide, and a 0.5-ml aliquot was taken for scintillation counting, using a Packard Tri-Carb 300 scintillation spectrophotometer.

***In Vivo.*** DFMO was administered p.o. as a 2% solution in the drinking water for 3 days. The androgen DHTP (2.0 mg/0.2 ml) was injected s.c. daily for 3 days. Placebo injections included 0.2 ml of sesame oil s.c. Following the respective drug regimens, rats were given injections i.v. via the dorsal vein of the penis with 114 nM $^{14}$C-labeled amine/kg of body weight. Three hr later, the animals were killed by ether inhalation. The tumors and various tissues were removed, freed of fat and connective tissue, and weighed to the nearest mg. A 150- to 300-mg aliquot of tissue or tumor was taken, placed in a scintillation vial, and digested overnight with 1.5 ml of NCS tissue solubilizer at 50°. The following day, 10 ml of Scintilene scintillation cocktail were added to the digested samples, the radioactivity of which was then counted in a liquid scintillation counter, and the dpm/g of tissue (wet weight) were calculated as described previously (13). Differences between control and treatment groups were analyzed for significance by the Student *t* test.

## RESULTS

### *In Vitro* Polyamine Uptake

At a concentration of 3 μM of putrescine or spermidine, the R3327MAT-Lu cellular uptake showed a linear correlation with the number of cells plated and with time for up to 1 hr. Serum stimulation increased the amount of uptake (Table 1). Uptake was further increased 3-fold by previous incubation of the cells with DFMO. DFMO therefore stimulated the cellular uptake of exogenous polyamines into the R3327 MAT-Lu cell line in a similar fashion as that seen for other cell lines (1).

Various polyamines were examined for their ability to inhibit uptake of [$^{14}$C]putrescine (Table 2). The 2- and 3-carbon diamines did not inhibit [$^{14}$C]putrescine uptake. The unsaturated putrescine analogues 1,4-diamino-2,3-butene or -2,3-butyne were able to block putrescine uptake but less efficiently so with increasing unsaturation. The longer chain diamines as well as spermidine and spermine inhibited putrescine uptake. The chemotherapeutic agent, methyl-GAG, reportedly utilizes the polyamine transport system for entry into cells and was also an effective inhibitor of putrescine uptake. The other amines, histamine, serotonin, (*o*,*m*,*p*)-phenylenediamine, and (*o*,*m*,*p*)-xylylenediamine, did not block putrescine uptake to any considerable

Table 1

*Effect of DFMO pretreatment on the uptake of [$^{14}$C]putrescine or [$^{14}$C]spermidine into R3327MAT-Lu cells in vitro*

| Conditions[a] | Putrescine | Spermidine |
|---|---|---|
| Medium not changed[b] | 13,358 ± 1,650[c] | 18,776 ± 3,282 |
| Medium changed | 40,513 ± 2,660 | 69,748 ± 11,381 |
| Medium changed plus 1 mM DFMO | 131,082 ± 5,805 | 206,681 ± 10,205 |

[a] Cells were incubated at $10^5$ cells/well in a 24-well Costar plate in 1 ml RPMI medium with 10% heat-inactivated male rat serum. Two days later, cultures were either fed by exchanging 1 ml of old medium with an equal volume of fresh medium with or without 1 mM DFMO, or the medium was not changed.

[b] Three days after plating, either [$^{14}$C]putrescine or [$^{14}$C]spermidine at 3 μM final concentration was added, and the cells were incubated at 37° for 1 hr. The medium was then aspirated, and the cells were washed 3 times with stop solution. The cells were then solubilized in hydroxide, and the radioactivity was determined by scintillation counting.

[c] Mean of S.D. of cpm/$10^5$ cells for a triplicate assay. No binding was observed in wells without cells.

extent. The basic amino acids, lysine, arginine, and ornithine, likewise, did not inhibit putrescine uptake into these cells.

### *In Vivo* Polyamine Uptake

**Tissue Uptake.** The effect of DHTP + DFMO pretreatment on the uptake of ethylenediamine, putrescine, and spermidine by the R3327MAT-Lu tumor and by various tissues is shown in Chart 1. In experiments in which just the androgen DHTP was administered, the uptake of the various polyamines was not significantly different from those of the non-androgen-treated control. However, when the androgen was added to the DFMO treatment, an increase significantly greater than that of DFMO only was observed for the androgen-sensitive, male, sex accessory tissues, the ventral and dorsal lateral prostate. In the non-sex accessory tissue, the addition of DHTP to DFMO resulted in no significant difference in polyamine uptake from that seen with DFMO alone. Therefore, these figures compare the results for the combination of DHTP + DFMO treatment with those of solvent-treated controls.

Table 2

*Ability of various polyamines to inhibit the uptake of [$^{14}$C]putrescine into DFMO-pretreated R3327MAT-Lu cells in vitro*

| Unlabeled competitor[a] | [$^{14}$C]Putrescine uptake[b] (% of control) |
|---|---|
| Amine (100 μM) | |
| None (3 μM [$^{14}$C]putrescine only) | 100 |
| 1,2-Diaminoethane (ethylenediamine) | 117 |
| 1,3-Diaminopropane | 80 |
| 1,4-Diaminobutane (putrescine) | 6 |
| 1,4-Diamino-2,3-butene | 24 |
| 1,4-Diamino-2,3-butyne | 53 |
| 1,4-Diaminopentane | 15 |
| 1,5-Diaminopentane (cadaverine) | 13 |
| 1,6-Diaminohexane | 10 |
| 1,7-Diaminoheptane | 7 |
| 1,8-Diaminooctane | 7 |
| Spermidine | 5 |
| Spermine | 5 |
| methyl-GAG | 4 |

[a] The amines histamine, serotonin, (*o,m,p*)-phenylenediamine, (*o,m,p*)-xylylenediamine, and the basic amino acids lysine, arginine, and ornithine did not inhibit putrescine uptake into these cells.

[b] R3327MAT-Lu cells were incubated for 48 hr with 1 mM DFMO in RPMI medium with 10% rat serum at 37° and 7% $CO_2$ at saturation humidity. To this medium was added [$^{14}$C]putrescine at 3 μM concentration with or without 100 μM unlabeled amine. Following a 0.5-hr 37° incubation, the medium was aspirated, and the cells were washed 3 times with stop solution. The cells were then solubilized in hydroxide, and the radioactivity was counted with a scintillation spectrophotometer. The mean number of counts from the triplicate assay in the nonsupplemented group is considered the 100% uptake control, and the mean number of counts from the various amine-supplemented groups divided by the mean number of counts of the control group represents the relative putrescine uptake (× 100) as a percentage of control.

**Ethylenediamine.** Treatment with DHTP + DFMO (or DFMO only) resulted in a decreased uptake of ethylenediamine into the liver that was significant at $p < 0.05$. The decrease in the uptake of ethylenediamine into the dorsal-lateral prostate was not significant. The kidney, liver, and dorsal-lateral prostate displayed the greatest uptake among the tissues studied, while skeletal muscle, tumor, and ventral prostate exhibited the least uptake. DHTP + DFMO as with DFMO pretreatment did not enhance the uptake of [$^{14}$C]ethylenediamine into any tissue. DHTP by itself, as emphasized previously, had no effect by itself on uptake into any tissue.

**Putrescine.** [$^{14}$C]Putrescine showed the greatest uptake in the intestine and the least in the skeletal muscle in the nontreated group. Following DFMO + DHTP pretreatment, the prostate, tumor, and intestine showed the highest uptake. The liver and kidney had an intermediate uptake, while skeletal muscle uptake was the lowest. DFMO + DHTP pretreatment significantly increased uptake into the tumor, as well as into both prostatic lobes, and intestine, but not the liver, kidney, or skeletal muscle. A 2-fold or higher increased uptake of putrescine was displayed only by the tumor and the prostate lobes. None of the other tissues examined increased their uptake by 2-fold or greater.

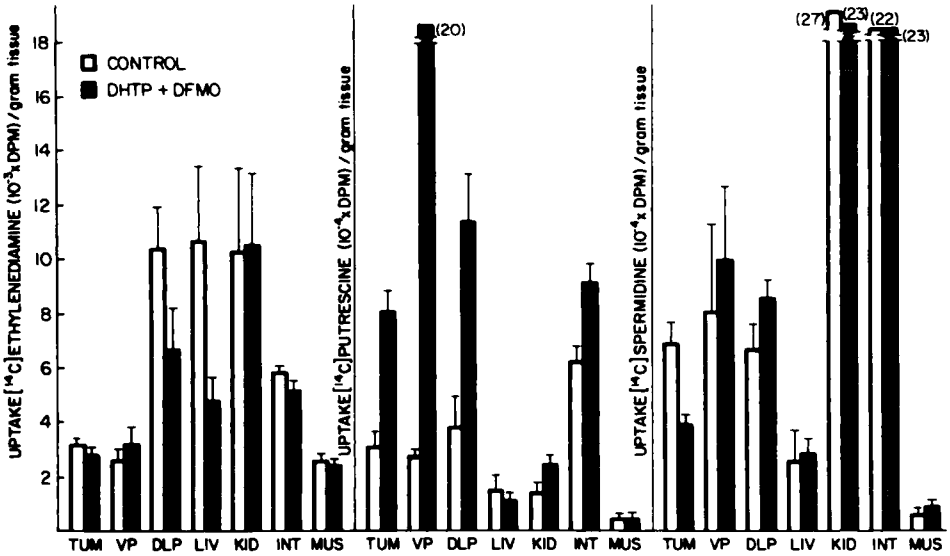


Chart 1. Effect of DFMO and DHTP pretreatment on the uptake of ethylenediamine, putrescine, and spermidine. Control animals received plain tap water and daily s.c. injection of 0.2 ml of sesame oil. DHTP + DFMO-pretreated animals received 2% DFMO in the drinking water and 0.2 ml of sesame oil containing DHTP, 10 mg/ml. Three days after the initiation of the treatments, the animals were given 114 nmol of either ethylenediamine, putrescine, or spermidine per kg i.v. via the dorsal vein of the penis. Three hr later, the various tissues were removed, freed of fat, weighed to the nearest mg, and digested overnight at 50° with the aid of NCS tissue solubilizer. The following day, scintillation cocktail was added, the samples were counted in a scintillation counter, and the dpm/g of tissue were determined. The abbreviations used for the tissues are: *TUM*, R3327MAT-Lu tumor; *VP*, ventral prostate; *DLP*, dorsal-lateral prostate; *LIV*, liver; *KID*, kidney; *INT*, intestine; and *MUS*, abdominal wall muscle. *Columns*, mean level of uptake; *bars*, S.E. Statistically significant differences ($p < 0.05$) were found between the tissues of the control and DFMO + DHTP-treated groups for the various polyamines as follows: ethylenediamine, liver; putrescine, tumor, ventral prostate, dorsal-lateral prostate, and intestine; and spermidine, tumor. There were 5 animals/group, each animal bearing 2 R3327MAT-Lu tumors, one on the right and one on the left flank. Therefore, there were 10 tumors and 5 tissues for each group.

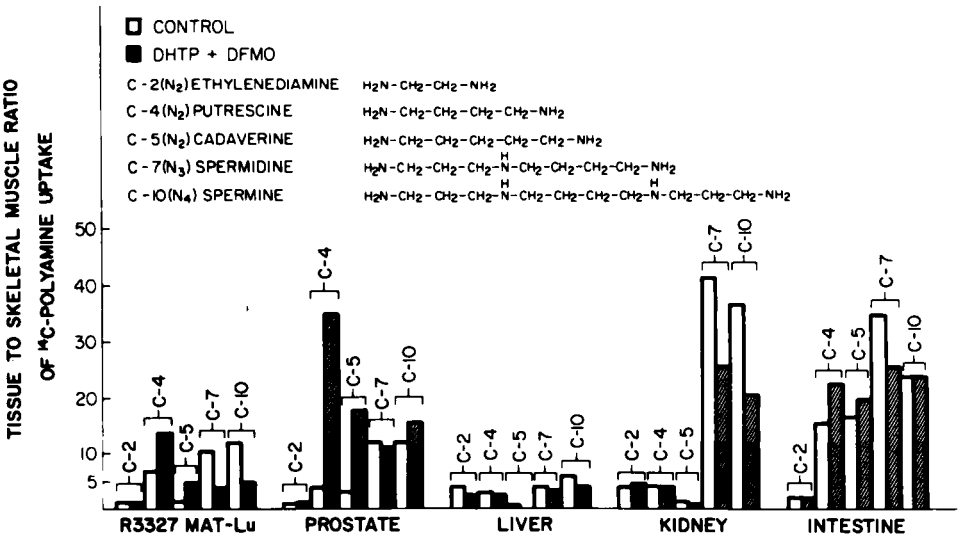


Chart 2. Effect of DFMO + DHTP pretreatment on the uptake of a series of polyamines in tissue relative to skeletal muscle. Control animals received plain tap water and 0.2 ml of sesame oil s.c. once daily. DHTP + DFMO-pretreated animals received 2% DFMO in their drinking water and 0.2 ml of sesame oil containing DHTP (10 mg/ml) s.c. daily. Three days after the initiation of treatment, the animals were given 114 nmol of radiolabeled polyamine per kg i.v. Three hr later, the tissues were removed, weighed, digested, and counted in a scintillation spectrophotometer, and the dpm/g of tissue were computed. The mean dpm/g of tissue were divided by the mean dpm/g of muscle to obtain the ratio of tissue to skeletal muscle. The prostatic lobe illustrated here is the ventral lobe of the prostate.

**Spermidine.** With [$^{14}$C]spermidine, highest uptake was found in the kidney and intestine in the nontreated, control animals. The liver and the muscle exhibited the lowest uptake. Pretreatment with DFMO + DHTP significantly suppressed uptake of spermidine by the tumor but did not significantly affect uptake in the other tissues.

### Polyamine Uptake Ratio of Tissue to Muscle

**Tumor.** The ratio of tissue to skeletal muscle uptake for the different polyamines in various tissues is shown in Chart 2. In nontreated animals, putrescine, spermidine, and spermine exhibited the highest uptake ratio of tumor to muscle. With DFMO + DHTP pretreatment, the tumor uptake ratio of putrescine and cadaverine was enhanced 2-fold, but the uptake ratio of spermidine and spermine was decreased.

**Prostate.** In the prostate, the highest uptake ratio was found with spermidine and spermine in nontreated animals. Pretreatment with DFMO + DHTP dramatically increased the tissue:skeletal muscle ratios of putrescine and cadaverine, while not affecting the uptake of ethylenediamine, spermidine, or spermine.

**Liver.** In the liver, the uptake ratio of ethylenediamine tended to be slightly higher, and the uptake of the other polyamines lower, especially spermidine and spermine. Pretreatment with DFMO + DHTP did not enhance the uptake of any polyamine by the liver, nor did it decrease such uptake.

**Kidney.** In the kidney of nonpretreated animals, cadaverine had the lowest uptake ratio. Highest ratios were observed with spermidine and spermine. In the kidneys of animals pretreated with DFMO + DHTP, the only change was exhibited by spermidine and spermine, the uptake ratios of which were significantly decreased.

**Intestine.** In the intestine, ethylenediamine yielded lower uptake ratios than did any of the other polyamines. The highest ratio was found with spermidine. DFMO + DHTP pretreatment did not affect the uptake ratio of either ethylenediamine or spermine. It increased slightly the uptake ratio of putrescine and cadaverine, while decreasing the uptake of spermidine.

### Tissue Uptake of methyl-GAG

**Tumor.** [$^{14}$C]methyl-GAG, which utilizes the polyamine transport system for uptake, did not have its uptake into the tumor significantly altered by DFMO, DHTP, or DFMO + DHTP pretreatment (Chart 3).

**Prostate.** The uptake of [$^{14}$C]methyl-GAG into the dorsal-lateral prostate was not significantly affected by DFMO, DHTP, or DFMO + DHTP pretreatment. In the ventral lobe of the prostate, only the DFMO + DHTP pretreatment group significantly differed from that of control as well as from the DFMO-only and DHTP-only pretreatment groups. The DFMO-only and DHTP-only pretreatment groups did not significantly differ in their uptake of [$^{14}$C]methyl GAG from each other or from the control group.

**Liver.** In the liver, both DFMO and DFMO + DHTP pretreatment groups significantly decreased their uptake of [$^{14}$C]methyl GAG when compared with either the control or DHTP-only groups.

**Kidney.** In the kidney, the result was just the opposite of that seen in the liver. The DFMO and DFMO + DHTP pretreatment groups each significantly increased their uptake of [$^{14}$C]methyl GAG relative to the control and DHTP groups. The uptake into DFMO and DFMO + DHTP pretreatment groups did not differ significantly from each other nor did the uptake into the control and DHTP groups significantly differ from each other.

**Intestine.** The uptake of [$^{14}$C]methyl GAG did not differ significantly between any of the various groups.

**Muscle.** The uptake of [$^{14}$C]methyl GAG was significantly less in the DFMO + DHTP group, when compared with either the control or DHTP group, but not significantly less than the DFMO-only group. The control, DFMO-only, and DHTP-only groups did not differ significantly from each other.

## DISCUSSION

Radiolabeled putrescine, injected into mature male rats, has been found previously to accumulate in the prostate to a greater extent than into many other tissues (3, 36). The high uptake of putrescine by the prostate was enhanced when the ability of the animals to synthesize endogenous polyamines was blocked by DFMO pretreatment and even further enhanced when DFMO pretreatment was combined with androgen stimulation in the form of DHTP injections (12, 13). The Dunning R3327 tumor, an androgen-sensitive rat prostate-derived tumor, closely resembles

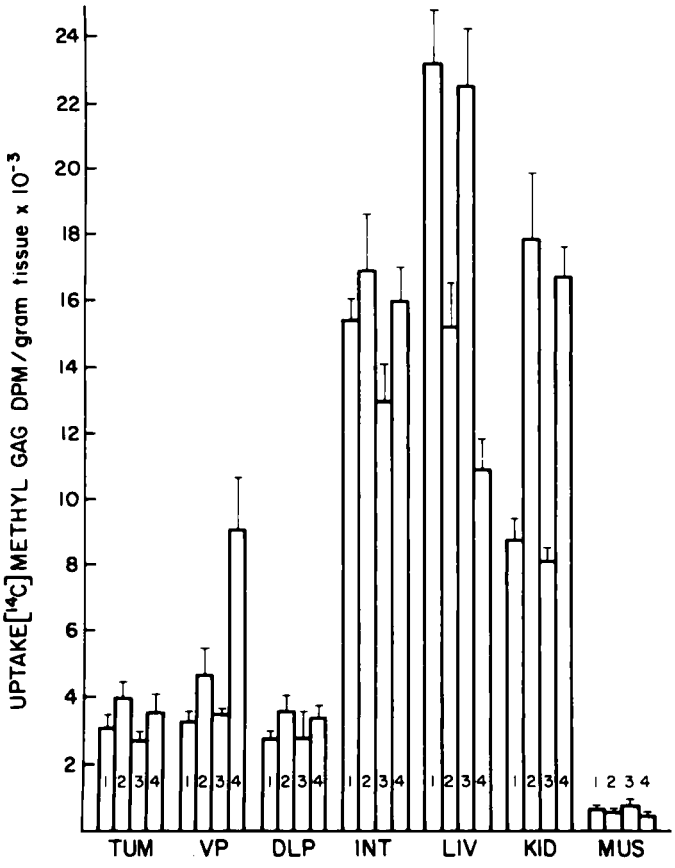


Chart 3. Effect of DFMO, DHTP, or DFMO + DHTP pretreatment on the uptake of methyl-GAG. Control animals (Group 1) received plain tap water and 0.2 ml of sesame oil daily. DFMO-pretreated animals (Group 2) received 2% DFMO in their drinking water daily. DHTP-pretreated animals (Group 3) received 0.2 ml of sesame oil containing DHTP (10 mg/ml) s.c. once daily. The combined treatment group (Group 4) received both DHTP and DFMO. Three days after the initiation of treatment, the animals were given i.v. 114 nmol of [$^{14}$C]methyl-GAG/kg. Three hr later, the tissues were removed, weighed, digested, and counted in a scintillation spectrophotometer, and the dpm/g calculated. The abbreviations used for the tissues are: *TUM*, R3327MAT-Lu tumor; *VP*, ventral prostate; *DLP*, dorsal-lateral prostate; *LIV*, liver; *KID*, kidney; *INT*, intestine; and *MUS*, abdominal wall muscle. *Columns*, mean level of uptake; *bars*, S.E. Statistically significant differences ($p < 0.05$) were found between groups as follows: tumor, no differences; ventral prostate, 1 *versus* 4, 2 *versus* 4, 3 *versus* 4; dorsal-lateral prostate, no differences; liver, 1 *versus* 2, 1 *versus* 4, 2 *versus* 3, 2 *versus* 4, 3 *versus* 4; kidney, 1 *versus* 2, 1 *versus* 4, 2 *versus* 3, 3 *versus* 4; intestine, no differences; muscle, 1 *versus* 4, 3 *versus* 4.

its prostatic tissue of origin and has been shown to have a high natural uptake of [$^{14}$C]putrescine which can be enhanced by DFMO or DFMO + DHTP pretreatment (12). Since our previous observations on the Dunning R3327 tumor, Chaney *et al.* (2) have demonstrated DFMO-enhanced uptake of [$^{14}$C]putrescine into the murine, breast carcinoma-derived, EMT-6 tumor.

In the current study, we used the R3327 MAT-Lu tumor. This is a fast-growing, anaplastic, hormone-independent derivative of the Dunning R3327 tumor. When these tumor cells were incubated *in vitro* with DFMO, they showed the expected, severalfold increase in the uptake of both [$^{14}$C]putrescine and [$^{14}$C]spermidine. We therefore considered this tumor suitable for additional studies of polyamine uptake both *in vitro* and *in vivo*. Spermidine, spermine, cadaverine, and the longer chain diamines effectively inhibited the uptake of [$^{14}$C]putrescine by these tumor cells *in vitro*. The chemotherapeutic agent, methyl-GAG, was, likewise, an effective inhibitor of putrescine uptake. This finding suggests that these cells are similar to other cells where methyl-GAG utilizes the polyamine transport system for entry into cells (1, 9, 14). Ethylenediamine, however, did not inhibit putrescine uptake and is probably incapable of utilizing the polyamine transport system for cellular uptake. Danzin *et al.* (5) have suggested that reduction of the basicity of nitrogen diminishes the ability of putrescine analogues to gain entry into cells. This could be the explanation to our finding that xylxylenediamines and phenylenediamines did not inhibit putrescine uptake *in vitro*. The unsaturated putrescine analogue, 1,4-diamino-2,3-butyne, was only moderately effective in blocking putrescine uptake. This might be due to the molecule being more linear and more rigid than the other diamines tested.

We report our *in vivo* uptake results as ratios of tumor or tissue to muscle. This particular ratio and the tissue blood uptake ratio are important factors when one considers the possibility of organ imaging by radionuclear scanning devices (8, 13). In the case of polyamines, the ratio of skeletal muscle uptake to blood level remains very close to one (3, 36). Ratios of tissue to muscle will therefore be very similar to those of tissue to blood.

We have demonstrated here that an anaplastic, hormone-independent derivative of the original Dunning R3327 tumor can still increase its uptake of [$^{14}$C]putrescine when exposed to DFMO pretreatment. This occurred both *in vitro* and *in vivo*, although the absolute uptake *in vivo* was not as great as into the normal prostate or into the androgen-sensitive tumor (12, 13). However, when we expanded our *in vivo* uptake experiments to include spermidine, spermine, cadaverine, and ethylenediamine, we have observed unexpected differences between the diamines putrescine and cadaverine, and the other polyamines. It appears that *in vivo* exogenously administered putrescine has a different distribution pattern than do the tri- or tetraamines, spermidine and spermine. Tissues such as kidney and intestine took up substantially more spermidine and spermine than did putrescine. Also, the uptake of spermidine and spermine was higher than that of putrescine in the prostate and tumor. The higher uptake of [$^{14}$C]spermidine and spermine relative to putrescine into the prostate is similar to what was reported by Counsell *et al.* (4).

Further differences in uptake were noted between putrescine and the other polyamines following DFMO pretreatment. Although the R3327 MAT-Lu cells *in vitro* took up increased amounts of both putrescine and spermidine following DFMO + DHTP pretreatment, the uptake *in vivo* of putrescine was increased by the tumor and by the prostate, while there was no increase in the uptake of spermidine and spermine. Actually, the uptake of spermidine and spermine by the tumor was significantly decreased by DFMO + DHTP pretreatment. The kidney and intestine, tissues which normally exhibit high uptake of spermidine and spermine, did not display enhanced uptake of these substances following DFMO + DHTP pretreatment. There is evidence to suggest that the difference in the *in vivo* uptake of putrescine and the larger polyamines, spermidine and spermine, is not just a dose phenomenon, since concentrations of [$^{14}$C]-putrescine up to 100-fold greater still showed a similar distribution pattern.[6]

[6] W. D. W. Heston, D. Kadmon, D. F. Covey, and W. R. Fair, unpublished observations.

The chemotherapeutic agent methyl-GAG, which has been reported to use the polyamine transport system for entry into cells, did not have its uptake into the R3327 MAT-Lu tumor increased or decreased by DFMO or DFMO + DHTP pretreatment. The highest tissue uptake of methyl-GAG was observed by the liver. This high uptake in the liver was not seen with the other polyamines. DFMO and DFMO + DHTP pretreatment significantly suppressed methyl-GAG uptake into the liver and significantly increased the uptake by the kidney, again quite different from the response observed with the polyamines. Methyl-GAG had a high uptake into the intestine, and this uptake was not significantly altered by DFMO or DFMO + DHTP pretreatment. In the prostate, only the ventral lobe responded with a significantly increased uptake of methyl-GAG and then only following the combined effect of both DHTP + DFMO. No such difference was observed in the dorsal lateral lobe, where neither DFMO nor DHTP + DFMO exhibited an increased uptake of methyl-GAG. Thus. the distribution of methyl-GAG with or without polyamine-depleting treatment differed significantly from that seen with polyamines.

These differences in *in vivo* distribution and response to DFMO may be related to the different roles of these compounds in physiological processes (15, 16, 21, 22, 27, 30, 32). Alternatively, the differences might reflect different binding and affinity of the various polyamines to intracellular substances (33). It is also possible that tissues vary in their ability to metabolize the different polyamines. Finally, although it is assumed that the polyamines share a common transport system for entry into cells, this might not be the case. Different tissues may have polyamine transport systems that vary in their utilization of diamines, such as putrescine, *versus* tri- and tetraamines, like spermidine and spermine, *versus* methyl-GAG, respectively.

Porter and coworkers (23, 24) have demonstrated *in vitro* that a range of permissible structural analogues of putrescine and spermidine which maintain functional growth of L1210 cells is taken up by these cells. They also identified analogues which effectively utilized this uptake system but would not support tumor growth in their attempts to develop a polyamine analogue with better chemotherapeutic activity than methyl-GAG. Still, the L1210 tumor is a bone marrow-derived tumor, and the bone marrow is an area of high polyamine uptake (4). Kallio *et al.* (14) have utilized the high uptake of polyamines into the bone marrow in an attempt to rescue normal tissue from the toxicity of methyl-GAG by administering spermidine or putrescine p.o. Although the polyamines were given p.o., the intestine retained methyl-GAG more avidly than did the bone marrow (14). This finding is consistent with the possibility of differing polyamine transport mechanisms in different tissues.

Our results suggest that the radiolabeled tri- and tetraamines and methyl-GAG may have a different *in vivo* distribution from that of the diamines ethylenediamine, putrescine, and cadaverine. Even though it is hypothesized that they all share the polyamine transport system for uptake, with the exception of ethylenediamine, only the diamines putrescine and cadaverine had their uptake enhanced into both the R3327 MAT-Lu tumor or normal prostate tissue following DFMO + DHTP pretreatment. This observation needs to be considered in the future development of polyamine analogues as cytotoxins or for imaging prostatic tumors. Tumors other than the prostate also exhibit increased uptake of polyamines. DFMO enhancement of this uptake of polyamines may be useful with appropriately selected polyamine analogues for either tumor imaging or chemotherapy in management of these nonprostatic tumors as well (2, 24, 25, 26, 29, 35).

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