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POLYAMINES AS TARGETS FOR THERAPEUTIC INTERVENTION

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ABSTRACT

Polyamines are ubiquitous cell components essential for normal growth. Compounds interfering with polyamine biosynthesis or function have considerable potential for use as therapeutic agents. Inhibitors of ornithine decarboxylase have been shown to be valuable for the treatment of diseases caused by parasitic protozoa, most notably African sleeping sickness. They may also be useful chemopreventive and antineoplastic agents. Inhibitors of *S*-adenosylmethionine decarboxylase also have potential as treatments of these diseases. Protocols minimizing uptake of exogenous polyamines via the polyamine-transport system will probably be needed for the full potential of the inhibitors to be realized. Polyamine analogues, notably those with ethyl or benzyl groups on the terminal nitrogen atoms, have potent antiproliferative activity and are promising agents for the treatment of cancer. These analogues are transported by the polyamine-transport system, and their therapeutic effects are less likely to be blocked by the availability of the exogenous polyamines.

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INTRODUCTION

The pharmacology of polyamines has been reviewed quite extensively in many books and articles prior to 1991 (1–9). The number of publications relating to polyamines continues to increase, however, and in this review article the majority of the citations are to papers published in the past three years. More comprehensive lists of references can be found in the earlier reviews listed above.

Polyamines are now considered essential in cell proliferation. Only two biological species have been identified thus far—*Methanobacteriales* and *Halobacteriales,* which appear to grow in the absence of measurable levels of polyamines, although the *Halobacteriales* produce agmatine (10). Bacterial, yeast, and mammalian cells that have been mutated to diminish the activity of the polyamine biosynthetic enzymes are auxotrophic for polyamines. Pharmacological intervention that inhibits polyamine biosynthetic enzymes results in diminution of growth and/or cell death in virtually every system that has been investigated. Active research over the last few years has indeed provided us with numerous inhibitors of polyamine biosynthesis that have not only helped to define the roles of the polyamines in cellular growth processes and in the regulation of their own biosynthetic enzymes but have yielded clinically useful tools, particularly for the treatment of parasitic diseases and recurrent brain tumors. Although there seems to have been a tendency to focus on failures with regard to clinical utility, in truth, the last decade has provided us with several notable successes and with the insights necessary to mount an exciting and focused program on refining the clinical use of polyamine biosynthesis inhibitors. Ongoing studies that are clinically promising involve the relationship between the induction of L-ornithine decarboxylase (ODC) leading to accumulation of the polyamines and carcinogenesis. The use of polyamine biosynthesis inhibitors in cancer chemoprevention for a variety of tumor types is now in the early phases of clinical study.

The knowledge now accruing related to polyamine transport will provide us with more ways to manipulate polyamine levels within cells and, thus, alter cell growth and viability. Organisms such as *Trypanosoma cruzi* exist that may not synthesize their own polyamines but rather obtain the polyamines necessary for growth from their environment via specific transport systems (KJ Hunter, SA LeQuesne & AH Fairlamb, personal communication). Obviously, the failure of biosynthesis inhibitors in such systems is not surprising, but it has disappointed those who believe that all organisms must synthesize their own polyamines. As our knowledge grows, we realize that effective therapeutic approaches to such organisms must include inhibition of transport and/or replacement of naturally occurring polyamines with synthetic analogues that can bind at specific and important sites while exhibiting modified function

vis-à-vis the natural polyamines that they replace. This latter approach, the synthesis of polyamine analogues, is emerging as a potent and readily applicable direction for the creation of new antitumor agents.

Although classical, rational drug development has not been possible with regard to the polyamine analogues, inasmuch as specific binding sites of the polyamines are not completely defined, a "semirational" approach has been quite useful. Structure-activity correlation studies have given us insights into the effects of several specific modifications of the polyamines that result in therapeutically useful analogues (11–14). The ability of these analogues to inhibit polyamine biosynthesis by feedback inhibition on the biosynthetic enzymes and to induce the activity of spermidine-spermine N^1-acetyltransferase (SSAT) has likewise given us insight into useful compounds. In addition, using polyamine-DNA interactions as a surrogate measurement for intracellular activity has led to the synthesis of a series of compounds of potential clinical value (15–17).

In this review, we try to provide insights into how fundamental scientific discovery can elucidate therapeutic approaches. We also try to encourage active investigators to consider the implications of polyamines in their own sphere of research. Although physical chemists are aware of the significant DNA and RNA conformational changes induced by polyamines, the fact that polyamines are fundamental to cellular growth processes must also be considered. Recent publications (18) indicate that the sites of integration of tumor viruses into DNA relate to the status of chromatin structure and that the polyamines affect integration. Thus the reality that polyamines are potent modulators of chromatin structure must become a significant focus of investigation.

OVERVIEW OF POLYAMINE SYNTHESIS AND INHIBITORS

The naturally occurring polyamines in mammalian cells are putrescine, spermidine, and spermine (1, 2, 4). Some microorganisms, including trypanosomes, contain only trace amounts of spermine. A wide variety of related amines are found in other organisms, particularly thermophilic bacteria, and may play critical roles in their physiology. The four key enzymes making up the polyamine pathway are ODC that forms putrescine from L-ornithine; *S*-adenosylmethionine decarboxylase (AdoMetDC) that forms decarboxylated *S*-adenosylmethionine (dcAdoMet), which acts as an aminopropyl donor; spermidine synthase that transfers the aminopropyl group from dcAdoMet to putrescine; and spermine synthase that transfers the aminopropyl group from dcAdoMet to spermidine (Figure 1).

Powerful, specific inhibitors of all of these enzymes are now available. Some

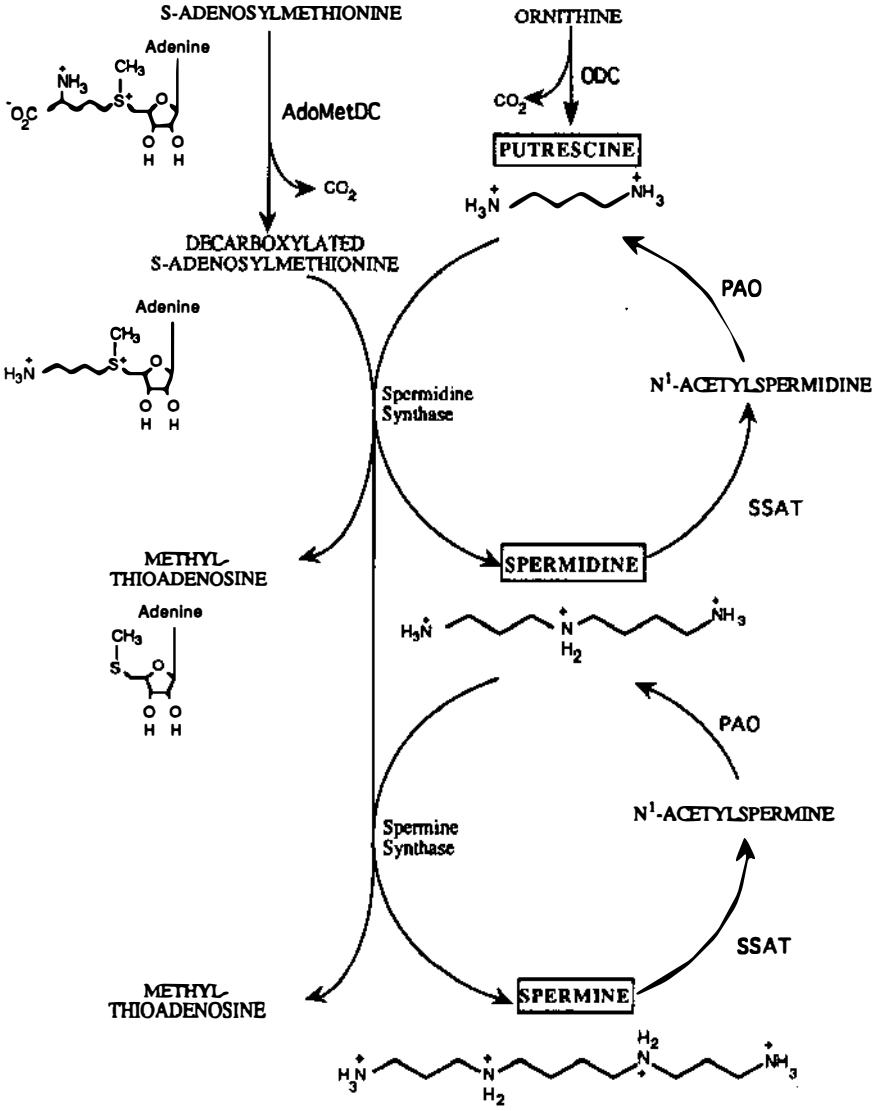


Figure 1 Polyamine biosynthetic pathway. Full details are given in References 1–5.

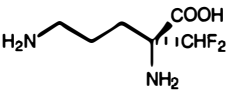
of these are summarized in Table 1 and the structures of the key compounds are shown in Figure 2. The effects of these compounds and some polyamine analogues that also affect the activity of the enzymes (shown in Figure 1) on cellular growth and viability are indicated in Table 2 and are discussed in detail in reviews and books (1, 4–9).

Table 1 Inhibitors of key enzymes involved in polyamine metabolism

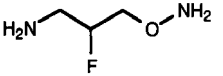
Ornithine decarboxylase
α-difluoromethylornithine (Eflornithine, Ornidyl®) (DFMO)
α-monofluoromethylornithine (MFMO)
(2R,5R)-δ-methylacetylenicputrescine] (MAP)
(E)-α-Monofluoromethyldehydroornithine (Δ-MFMO) and its methyl ester
3-aminooxy-1-propanamine
3-(aminooxy)-2-fluoro-1-propanamine
AdoMet decarboxylase
S-(5'-deoxy-5'-adenoxyl)methylthioethylhydroxylamine (AMA)
5'-deoxy-5'-[(2-aminooxyethyl)methylamino]adenosine (MAOEA)
5'-deoxy-5'-[(3-hydrazinopropyl)methylamino]adenosine (MHZPA)
5'-{[(Z)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine (AbeAdo)
S-(5'-deoxy-5'-adenosyl)-1-amino-4-methylthio-2-cyclopentene (AdoMac)
methylglyoxal bis(guanylhydrazone) (MGBG)
ethylglyoxal bis(guanylhydrazone)
diethylglyoxal bis(guanylhydrazone)
[2,2-bipyridine]-6,6'-dicarboximidamide (CGP-39937)
4-amidinoindan-1-one 2'-amidinohydrazone (CGP-48664)
Spermidine synthase
S-adenosyl-1,8-diamino-3-thiooctane (AdoDATO)
cyclohexylamine
n-butylamine
trans-4-methylcyclohexylamine (4MCHA)
Spermine synthase
S-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD)
N-(*n*-butyl)-1,3-diaminopropane (BDAP)
N-(3-aminopropyl)cyclohexylamine (APCHA)
Spermidine/spermine-N^1-acetyltransferase
No useful compounds for in vivo studies
N-[2-(S-coenzyme A)acetyl-*sym*-norspermidine amide for in vitro studies
Polyamine Oxidase
N^1,N^4-bis(2,3-butanedienyl)1,4-butanediamine (MDL 72527)
N^1-methyl-N^2-(2,3-butadienyl)-1,4-butanediamine (MDL 72521)

Inhibition of ODC leads to a major reduction in putrescine and spermidine but, usually, to only a small reduction in spermine. Growth is significantly reduced when spermidine becomes depleted but, in most cases, the effects of α-difluoromethylornithine (DFMO) and other specific ODC inhibitors are cytostatic rather than cytotoxic. The lack of cytotoxicity may be due to the residual spermine in the cells.

ODC Inhibitors

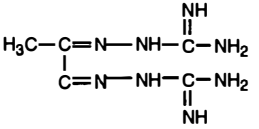


α-Difluoromethylornithine (DFMO; eflornithine)

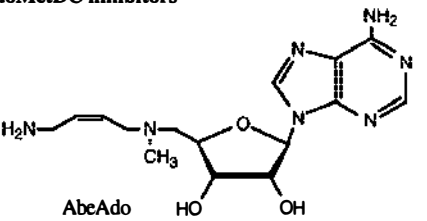


3-Aminooxy-2-fluoro-1-propanamine

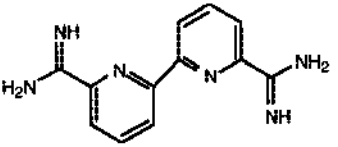
AdoMetDC inhibitors



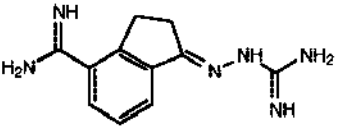
MGBG



AbeAdo



CGP-39937

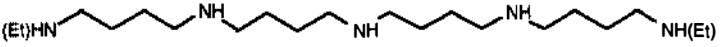


CGP-48664

Polyamine analogs

BE-3-3-3

BE-3-7-3



BE-4-4-4-4

Figure 2 Structures of key inhibitors of polyamine biosynthesis and of polyamine analogues.

Table 2 Effects of inhibitors of polyamine synthesis and polyamine analogues on polyamine content and cell growth

Inhibitor or analogue[a]	Target	Putrescine	Spermidine	Effect on spermine	Total polyamines	dcAdoMet	Growth
DFMO	ODC	⇓	⇓	NC[b]	⇓	⇑⇑	⇓⇓
AbeAdo or CGP48664	AdoMetDC	⇑⇑	⇓	⇓	⇑	⇓	⇓[c]
AdoDATO or 4MCHA	Spermidine synthase	⇑	⇓	⇑	NC	⇑	NC
BDAP or AdoDATAD	Spermine synthase	⇓	⇑	⇓	NC	⇑	NC
BE-3-3-3, BE-3-4-3, or BE-3-7-3	ODC and AdoMetDC	⇓	⇓	⇓	⇓⇓	⇓	⇓⇓⇓[d]
BE-4-4-4-4	Not fully defined	⇓	⇓	⇓	⇓⇓	NR[e]	⇓⇓⇓[d]

[a] See Table 1 for definitions of abbreviations and Figure 2 for the structures of some of these compounds.
[b] NC, little or no change
[c] After prolonged exposure, treatment with AbeAdo becomes cytotoxic.
[d] Cytotoxic
[e] NR, not reported

Inhibition of AdoMetDC leads to a large increase in putrescine and a decline in spermidine and spermine. The total polyamines actually increase because putrescine content rises by more than the fall in the higher polyamines. Application of these inhibitors is initially less growth inhibitory than exposure to DFMO, probably because of the increased putrescine (19–21), but prolonged exposure to a potent and stable AdoMetDC inhibitor such as 5′-{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine [AbeAdo] leads to cytotoxicity (21, 22). Byers et al (21, 22) suggested that this toxicity is caused by the depletion of the hypusinated form of eIF-5A in the treated cells. Hypusine is formed in a posttranslational modification of this protein in a reaction that uses spermidine as substrate. eIF-5A that contains hypusine is essential for cell viability in yeast and may be involved in cell-cycle regulation, as well as act as an initiation factor in protein synthesis (23, 24). Application of spermidine synthase inhibitors has little effect on cell growth in short-term experiments, but spermidine depletion is not complete, and there are parallel increases in putrescine and spermine (25, 26). Similarly, spermine synthase inhibitors have little effect on cell growth in the short-term, but there is a compensatory increase in spermidine (26, 27). Also, 1-methylspermidine can support the long-term growth of AbeAdo-treated L1210 cells and is not converted into a spermine derivative under these conditions (28). These experiments question the function of spermine in mammalian cells. However, a separate spermine synthase would probably not have evolved and been maintained in mammalian cells if there was no need for spermine. Spermine may be more active than spermidine in at least one function, such as the stimulation of mitochondrial transport processes, and thus spermine may be formed solely for this purpose. Another possibility is that the critical function of spermine may be needed only in certain cell types and not in cultured tumor cells used for the published experiments with inhibitors of spermine synthase. Finally, spermine may also act as a storage form for the other polyamines. Because of its four positive charges, spermine is predominantly bound to cellular components, and its free concentration in the cell is very low. However, as described below, it can be converted into spermidine and putrescine and can be used to maintain a critical cellular level of these compounds.

A significant problem with the use of inhibitors of polyamine biosynthesis is that the key regulatory enzymes in the biosynthesis—ODC and AdoMetDC—are strongly repressed by polyamines (4, 5). Thus, when polyamines are depleted there is a large compensatory increase in the activities of these enzymes. This increase makes it more difficult to obtain complete inhibition at the step being affected. It also leads to extensive changes in some of the metabolites involved. A several-hundredfold increase in the content of dcAdoMet occurs when ODC is blocked because the amount of AdoMetDC activity is increased at a time when putrescine and spermidine, which are needed in

the aminopropyltransferase reactions to use up the dcAdoMet, are depleted. Similarly, there is a vast increase in putrescine in cells treated with AbeAdo inhibitors because ODC is de-repressed, and there is not any dcAdoMet available to convert it into spermidine.

The aminopropyltransferase reactions that form spermidine and spermine are essentially irreversible but the retroconversion of spermine into putrescine can be accomplished by the sequential action of two enzymes, polyamine oxidase (PAO) and SSAT (4, 29, 30). PAO is an FAD-dependent enzyme that cleaves spermidine or spermine internally, forming putrescine or spermidine, respectively. This reaction is very slow with parent polyamines; the preferred substrates for PAO are their N^1-acetyl derivatives. These are formed by SSAT, which is a highly inducible enzyme and is the rate-limiting step in the conversion pathway. Although under normal physiological circumstances the SSAT-PAO pathway plays a role in maintaining polyamine levels by converting spermine into spermidine and putrescine as needed, it becomes particularly important in preventing polyamine levels from getting too high after excess synthesis or uptake. Induction of SSAT leads to a conversion of cellular polyamines to putrescine and N^1-acetylspermidine, which are readily excreted from cells. Putrescine may also be degraded by diamine oxidase.

Increased SSAT leading to polyamine degradation and excretion occurs when polyamines, such as spermine, are exogenously added to cells, but a variety of polyamine analogues—such as N^1,N^{12}-bis(ethyl)spermine (BE-3-4-3), N^1,N^{11}-bis(ethyl)norspermine (BE-3-3-3) (30, 31), and two related unsymetrically substituted polyamine analogues {N^1-ethyl-N^{11}-propargyl-4,8-diazaundecane and N^1-ethyl-N^{11}-[(cyclopropyl)methyl]-4,8-diazaundecane} (32)—are even more powerful inducers of SSAT. Such induction leads to a striking loss of polyamines from the treated cells, and these analogues also mimic the effects of natural polyamines by repressing ODC and AdoMetDC. Synthesis is therefore reduced at a time when degradation and excretion are enhanced and a dramatic decrease in the content of all cellular polyamines occurs. This may be further facilitated by the ability of these compounds to displace polyamines from bound sites inside the cell and to act as competitive inhibitors of polyamine uptake. Therefore, application of BE-3-4-3 or BE-3-3-3 to cells leads to a virtually complete loss of normal polyamines and to significant cytotoxicity.

Recent work on inhibitors of ODC and AdoMetDC and on the effects of polyamine analogues are discussed in more detail below, since all of these classes of compounds have clear potential for therapeutic usage. The aminopropyltransferase inhibitors may also be useful drugs, but at present, their use is clearly restricted to research; therefore, they are not further discussed in this review.

ODC AS A PUTATIVE ONCOGENE

Two separate studies suggest that overexpression of ODC activity may cause cell transformation (33, 34). In these experiments, plasmid vectors containing inserts that coded for ODC under the expression of a strong promoter were used to transfect NIH 3T3 cells. Clones expressing high levels of ODC from these transfections showed a transformed phenotype and anchorage-independent growth in soft agar and produced tumors in nude mice. A third study did not find transformation when ODC was expressed alone, but the construct used contained non-ODC sequences that may have attenuated the expressed ODC activity. However, even with this construct, coexpression of ODC with c-Ha-*ras* produced a transformed phenotype (35).

NIH 3T3 cells can be malignantly transformed by the overexpression of eIF-4E, a factor that is known to facilitate the translation of mRNAs containing excessive secondary structure in their 5′ noncoding regions (5′-UTR) (36, 37). ODC has such a 5′-UTR, and the level of ODC protein in the cells producing large amounts of eIF-4E was increased by 30- to 100-fold without any change in the ODC mRNA content (38). The elevation of ODC in these cells was particularly apparent at later times of cell culture, since the decline in activity that normally occurs as cells become quiescent did not materialize. These results suggest that the up-regulation of ODC translation may be a critical factor in the transformation by eIF-4E.

The mechanism by which malignant transformation is brought about by expression of ODC remains obscure. There are no known functions for ODC protein except for the production of putrescine. A control experiment in which an ODC gene that contains mutations and therefore greatly reduces activity in putrescine production, such as K69A/C360A (39, 40), was overexpressed would be useful to totally rule out the possibility of a second function for ODC protein that leads to transformation. However, the simplest interpretation of most experiments is that the abnormal formation of polyamines leads to malignant growth. This is supported by the finding that DFMO reverses the transformed phenotype of 3T3 cells transformed by overexpression of ODC (41) or eIF-4E (38). Addition of exogenous putrescine does not seem to transform cells, but it is possible that unregulated expression of ODC is a more efficient way of increasing intracellular levels than the transport via the uptake system, which is highly regulated. Transgenic mice expressing the human ODC gene in addition to their endogenous ODC have a much higher level of putrescine in some tissues (42) and showed an enhanced production of papillomas in response to dimethylbenzanthracene followed by phorbol esters (43). Another way in which ODC can be linked to oncogenes is indicated by the recent findings that transcription of the ODC gene is increased by c-*myc* (44) and by TGFβ in response to H-*ras* (45).

As mentioned above, there is also a wide body of evidence that ODC activity is increased at an early stage of carcinogenesis and that a variety of tumor promoters cause elevations in ODC (46–50). These studies strengthen the case for the use of ODC inhibitors as antitumor or chemopreventive agents. Even though ODC itself is ubiquitous, the reduction of excessive ODC levels may have a useful therapeutic effect. This may be brought about by application of specific ODC inhibitors (see below) or by dietary manipulations that reduce ODC induction (48, 49).

INHIBITION OF ORNITHINE DECARBOXYLASE

Although many substances inhibiting ODC have been described, the only compounds that have been demonstrated unequivocally to have useful pharmacological and experimental potential are the enzyme-activated irreversible inactivators, of which DFMO is the prototype (reviewed in 51). Other potent inhibitors have also been made based on the synthesis of aminoxy derivatives of putrescine and shown to be effective in cultured cells (52–54). The first of these compounds, 3-aminooxy-1-propanamine was shown to inhibit ODC and affect polyamine content and cell growth in cultured tumor cells (52, 53). More recently, a series of related compounds have been synthesized and 3-(aminooxy)-2-fluoro-1-propanamine was found to be about four times more active against ODC and the growth of bladder carcinoma cells in vitro (54). These compounds are very potent and apparently specific inactivators of ODC that appear to function by binding very tightly to the active site (55). In cell culture experiments they have effects very similar to DFMO but at lower concentrations (52, 53, 55). Moreover, 3-(aminooxy)-2-fluoropropanamine was active against the T24 tumor carried in nude mice when given on a daily treatment scheme (55). However, the long-term stability in vivo and the suitability for therapeutics of these aminooxy derivatives have not yet been fully evaluated.

Enzyme-Activated Irreversible Inhibitors of ODC

In agreement with the original hypothesis underlying the work leading to its synthesis, DFMO is accepted as a substrate by ODC, and its decarboxylation leads to the formation of a reactive species that forms a covalent adduct with the protein (56, 57). Inactivation is achieved with a stoichiometric attachment of material from DFMO into the protein. About 90% of this material is attached at cysteine-360 as the cyclic imine *S*-{[2-(1-pyrroline)]methyl}cysteine and the remaining 10% combines with lysine-69 (57). Both of these amino acids are important in the catalytic mechanism of ODC. Mutation of lysine-69, which is the residue that forms a Schiff base with the pyridoxal-5′-phosphate cofactor, reduces activity by >99%, and conversion of cysteine-360 to alanine reduces activity by 98% (39, 40). The C360A mutant ODC is still inactivated by

DFMO, albeit with somewhat different kinetics, and in this case, the entire inactivating adduct is bound to lysine-69 (39).

Because of the presence of two distinct sites for attachment of DFMO, both of which are critical for enzyme activity (39, 40), resistance to DFMO is unlikely to arise through alterations in the protein that render the drug ineffective. This inaction appears to be the case. In protozoa, the predominant means of resistance appears to be through a reduced uptake of DFMO (58), although overproduction of the enzyme due to gene amplification has been reported in a resistant strain of *Leishmania donovani* (59). In mammalian cells, the major method of resistance appears to be amplification of the ODC gene (reviewed in 5, 8, 60, 61). Significant amplification occurs quite readily in neoplastic cells exposed to DFMO, and this can lead to increases of 100-fold or more in ODC production. These cells are highly resistant to DFMO. A significant but more modest resistance can also occur by virtue of changes in ODC structure leading to an increase in its stability (61–64). Such changes including deletion of portions of the carboxyl-terminal domain (63) or the point mutation C441W (64). The increased half-life leads to a higher steady state of the enzyme protein that imparts some resistance to DFMO.

The inactivation of ODC by DFMO is not particularly rapid, and the half-life of the enzyme in the presence of a saturating dose of DFMO is 3.1 min. This duration is a significant fraction of the normal half-life of the enzyme, which is about 20 min, and therefore, a fraction of the ODC pool is not irreversibly inactivated. DFMO is also a competitive inhibitor of putrescine production, so the remaining ODC is not fully active; however, a residual ODC activity clearly occurs even in cells treated with large doses of DFMO. This residual activity accounts for the continued production and maintenance of near-normal levels of spermine described above.

Many other enzyme-activated irreversible inactivators of ODC have been prepared based on DFMO as a lead compound (51, 56). Some of these compounds have properties that suggest that they may be preferable to DFMO in terms of potency, cellular uptake, or pharmacokinetics. Notable are the methyl ester of (E)-α-monofluoromethyldehydroornithine (Δ-MFMO) and (R)-α-ethynyl-(R)-δ-methylputrescine, which is also called (2R,5R)-δ-methylacetylenicputrescine (MAP). Δ-MFMO methyl ester was designed so that the ester would facilitate entry into the cell and would then be cleaved by intracellular esterases to generate Δ-MFMO, which has a tenfold lower K_i for ODC than for DFMO (56). Δ-MFMO methyl ester was much more potent than DFMO against an animal trypanosomiasis model (65) and as an antitumor agent against L1210 leukemia, Lewis lung carcinoma, and B16F1 melanoma (66, 67). MAP has both a lower K_i and a higher rate constant for inactivation than DFMO (56) and affects tumor cell polyamines and growth at much lower concentrations than DFMO. However, its toxicity is higher, causing renal

damage that is not found with DFMO, and the C360A mutant ODC was completely refractory to MAP (39). Nevertheless, further clinical tests of these ODC inhibitors are clearly warranted. Unfortunately, such testing and, indeed, the further development of DFMO itself and of the enzyme-activated inhibitors of AdoMetDC described below have been hampered by the high costs of drug development and the absence of perceived profitable markets, despite the seriousness of the conditions that could be treated.

THERAPEUTIC USES OF DFMO

Antiparasitic

The pioneering observations of Bacchi, McCann, Sjoerdsma, and colleagues that showed that DFMO could cure acute infections of *Trypanosma brucei brucei* in mice (reviewed in 6, 51, 68) were soon followed by the demonstration that DFMO alone is an extremely active agent against human African sleeping sickness caused by *T. brucei gambiense* (69–72). DFMO has now been approved by the US Food and Drug Administration and the World Health Organization for this purpose, which makes it the first new drug in 40 years for the treatment of sleeping sickness (51, 71). The therapy with DFMO is extremely effective and the toxic side effects are much less than with other less-effective agents (70, 73). The major problems are the expense of the drug and the fact that the recommended route of administration involves hospitalization for intravenous doses given every 6 hours for 14 days (70, 72). The costs of treatment are therefore a serious problem in the impoverished African countries in which sleeping sickness is endemic.

The reasons for the sensitivity of *T. brucei* to DFMO are still not fully understood, although multiple factors may contribute (74). The *T. brucei* enzyme is actually slightly less sensitive to DFMO than the host enzyme (51), and the effects of DFMO on trypanosomes in culture are cytostatic. Although the trypanosome ODC differs from the mammalian enzyme in not turning over rapidly (60), this does not seem to be a critical factor. Possible reasons for the potent antitrypanosomal effect are (*a*) blockage of the synthesis of trypanothione [N^1,N^8-bis(L-γ-glutamyl-L-hemicystinyl-glycyl) spermidine], which is needed as a substrate for trypanothione reductase in order for the parasites to resist oxidative stress and is not present in mammalian cells (75); (*b*) perturbation of *S*-adenosylmethionine (AdoMet) metabolism, since the parasite AdoMet synthase is not regulated in the same way as its mammalian counterpart, and inhibition of polyamine synthesis leads to a massive accumulation of AdoMet and related compounds (58, 65, 76, 77); and (*c*) the ability of the immune system to deal with trypanosomes whose growth and development are slowed by the lack of polyamines (68).

Unfortunately, the sleeping sickness caused by many strains of *T. brucei rhodesiense* is significantly less sensitive to DFMO than that caused by *T. brucei gambiense* (58, 72, 74, 77). The insensitivity of *T. brucei rhodesiense* may be due to a very low rate of AdoMet synthesis and AdoMet decarboxylation in these strains, which prevents the huge overaccumulation of AdoMet and its decarboxylated product (58, 74, 77). Combination of DFMO with other drugs, such as suramin or melarsen oxide, is a very effective way to treat clinical isolates of resistant strains of *T. brucei rhodesiense* in acute laboratory model infections (77a) and may provide a means for effective clinical treatment of these strains.

DFMO has also shown clinical utility against the pneumonia caused by *Pneumocystis carinii* and is particularly valuable for the treatment of patients who have failed therapy with pentamidine or trimethoprim-sulfamethoxazole (51, 78). The gastric disease caused by the opportunistic infection with *Cryptosporidium,* a coccidian protozoan parasite, has also been shown to respond to DFMO in a significant number of patients (51). Thus, DFMO may help control some of the critical diseases affecting AIDS patients and others who have a compromised immune system. Laboratory experiments have also revealed activity of DFMO against a variety of other parasites, including *Acanthamoeba, Leishmania, Giardia, Plasmodia* and *Eimeria* (6, 59, 79, 80). Whether these observations can be exploited therapeutically remains to be seen, but they are certainly worthy of further investigation.

Antitumor

DFMO was quite effective in some animal tumor models, but when used as a single agent or in combination with other agents, including interferon in Phase I and II trials, it showed little activity in most cases (3, 5, 8, 9, 51). An important exception was demonstrated in patients with recurrent glial tumors, where treatment with DFMO alone or combined with bis(chloroethyl)nitrosourea (BCNU) or methylglyoxal bis(guanylhydrazone) (MGBG) produced a significant response (81–83). The combination of MGBG and DFMO has been discontinued temporarily until further animal studies can be conducted, owing to the possibility of unpredictable severe liver toxicity (83). However, the response to DFMO alone was as good as or better than that traditionally obtained with other more toxic agents; thus further study of DFMO as a therapy for brain tumors is clearly warranted.

There are several possible reasons for the lack of response to DFMO in these clinical trials. A major factor may be the availability of polyamines from other sources. Polyamines can be obtained from the diet, from the products of intestinal microbial flora, and via the SSAT-PAO retroconversion pathway from other cells in the body having significant spermine stores (84–86). Much stronger antitumor effects of DFMO have been obtained in animal models

when the drug is combined with regimes designed to minimize the availability of polyamines from these sources. These regimes include the use of a polyamine-deficient chow, antibiotics to reduce the contribution of polyamines from intestinal microorganisms, and a PAO inhibitor to prevent recycling (9, 85–87). The extent to which such procedures can be duplicated with human cancer patients and the degree of improvement of the therapeutic effect that might result remains to be determined. However, preliminary studies with four patients with prostatic adenocarcinoma who were treated with DFMO, a polyamine-depleted diet, and intestinal antibiotics showed some promising results (J-P Moulinoux, personal communication). Although deprivation of polyamines by such means clearly affects the response of tumors to DFMO, the absence of polyamines stimulates natural killer-cell activity, and this may also have an effect on tumor development by stimulating an immune response (88).

Chemoprevention

DFMO administered well after the initiating agent greatly reduces tumor development in rodents treated with a wide variety of chemical carcinogens and tumor promoters. The production of tumors of the skin, bladder, stomach, intestine, colon, oral cavity, and mammary gland has been blocked by DFMO treatment in animal models (89). As described above, ODC activity is enhanced by a variety of tumor promoters, and ODC may act like an oncogene if its expression can be elevated to high enough levels with carcinogen treatment. Several other chemopreventive agents, such as retinoids and piroxicam, are also known to reduce ODC via indirect mechanisms affecting the level of ODC protein. These results provide a strong rationale for the possible use of DFMO as a chemopreventive agent, either alone or in combination with other chemopreventive agents, in populations at high risk for the development of neoplasms (51, 89, 90). Several initial trials to determine levels of DFMO that will reduce ODC and polyamines significantly without toxicity have been reported (89, 91–93). The results of long-term studies in which efficacy will be evaluated are eagerly awaited.

Other Possible Uses for DFMO

DFMO might play a role in the treatment of a wide variety of other diseases that usually involve abnormal cellular proliferation. Possible targets include psoriasis; autoimmune diseases, including lupus and arthritis; and pathophysiological damage to the nervous system (reviewed in 8, 9, 51). Although some of these suggestions have been supported by preliminary laboratory data on experimental animals, much needs to be done before clinical efficacy is established. DFMO is a potent contragestational agent if administered at the time of embryonic implantation, with no effects on subsequent pregnancies (94). This effect is not unexpected in view of the well-documented rise in uterine

ODC activity during placenta formation. However, no attempts have been made to develop this approach for fertility control.

Toxicity of DFMO

DFMO is remarkably nontoxic, a finding that is perhaps explained by the availability of exogenous polyamines and the difficulties in maintaining a complete blockage of ODC activity described above. Thrombocytopenia occurs in a significant number of patients receiving large doses of DFMO over a prolonged period, as does anemia, leucopenia, and gastrointestinal effects—including a high incidence of diarrhea after oral treatments (8, 69, 73, 83). These effects are consistent with an adverse effect on cells having a high rate of turnover. A reversible but distressing loss of hearing occurs in a significant number of patients treated with DFMO (reviewed in 51). Animal studies have now shown that cochlear damage is caused by the inhibitor. Several groups have shown that there is a high level of ODC in the cochlear nerves and that DFMO reduces polyamines in the cochlea (reviewed in 51, 95). These observations suggest a key role for polyamines in some aspect of the physiology of the cochlea. This function remains obscure, but spermidine may be involved in cochlear neurotransmission. Such an effect is consistent with the extensive literature on the modulation of the *N*-methyl-D-aspartate (NMDA) receptor by polyamines (96, 97). All of the toxic effects of DFMO are readily reversed when the drug is discontinued.

Although DFMO itself is quite nontoxic, its combination with other agents can produce significant synergistic toxicity. A critical example of this is the combination with agents such as MGBG that are accumulated in cells by means of the polyamine-transport system. Since this system is activated by polyamine depletion, a significantly greater accumulation of the drug can occur with potentially devastating effects (8). The antiproliferative action of DFMO is also likely to retard the healing of lesions caused by other more toxic agents (6). This factor should be taken into account in the chemoprevention trials, when DFMO may be given over a very long period.

INHIBITION OF ADOMETDC

Although AdoMetDC has received less attention than ODC as a therapeutic target since DFMO became widely available, there has been a long history of interest in inhibitors of AdoMetDC, ever since it was discovered more than 20 years ago that MGBG, a known antitumor agent, was a powerful competitive inhibitor (98,99). More recent studies have shown that MGBG is by no means specific for AdoMetDC, and the extent to which inactivation of this enzyme contributes to the pharmacological and toxic effects of MGBG is still unclear. These doubts are emphasized by observations showing that MGBG stabilizes

AdoMetDC and leads to a 10- to 30-fold increase in the amount of the enzyme protein. It also appears very probable that the general toxicity of MGBG, which may be related to its accumulation to very high levels via the polyamine-transport system and consequent antimitochondrial actions, is not linked to the inhibition of AdoMetDC (1, 6, 8, 98, 99). More specific and potent inhibitors of AdoMetDC are now available and can be placed into three classes: active site–directed irreversible inhibitors, enzyme-activated irreversible inhibitors, and MGBG analogues, which are competitive inhibitors (1, 98, 99).

Active Site–Directed Irreversible Inhibitors

These compounds, which are designed to bind to the active site of the enzyme and then form a covalent bond with the pyruvate group, contain an adenosyl moiety attached to a positively charged group (either sulfonium or a tertiary nitrogen) and a side chain ending in a carbonyl-reactive group. The most-studied of these compounds, *S*-(5′-deoxy-5′-adenosyl)methylthioethylhydroxylamine (AMA), 5′-deoxy-5′-[(2-aminooxyethyl)methylamino]adenosine (MAOEA), and 5′-deoxy-5′-[(3-hydrazinopropyl)methylamino]adenosine (MHZPA) have an aminooxy or hydrazino group and are potent and specific AdoMetDC inhibitors that can be shown to be active-site directed (100, 101). After inactivation of human AdoMetDC with MHZPA, there was a covalent attachment of the inhibitor to the protein. Following digestion with the protease Lys-C, this attachment was found to be at the peptide SMFVSK, which represents the amino terminus of the α subunit. Analysis of this derivatized peptide by mass spectroscopy was consistent with it being the hydrazone formed by MHZPA and the pyruvate group that is located at the amino terminus of the α subunit (101).

There were some interesting differences in the responses of the AdoMetDCs from different species to inactivation by a series of these active site–directed irreversible inhibitors, which are related to MHZPA; the trypanosome AdoMetDC showed a greater interaction than the human AdoMetDC with an inhibitor containing one less carbon atom in the side chain. This difference suggests that the active site of the trypanosome may be more sterically hindered and that it may be possible to make species-specific inactivators of the parasite enzyme (102). The recent cloning of the cDNAs for the AdoMetDCs from *T. brucei* and *L. donovani* (B Ullman, personal communication) should aid in these efforts.

Inhibitors such as AMA and MHZPA produce substantial depletion of spermidine and spermine when added to tumor cell cultures (19, 103, 104). However, in short-term experiments, they had little effect on cell growth, perhaps because of the major rise in putrescine. During longer exposures, the effects of these compounds may be attenuated because of their reactive nature, which leads to a loss of inhibitor through a slow reaction with a variety of cellular and medium components including pyruvate. These considerations

may limit the effectiveness of these agents as therapeutic agents or, at the very least, substantially increase the dose and frequency of treatment with them. For these reasons, the second class of inhibitors, which are stable compounds that act as enzyme-activated irreversible inhibitors, are likely to be preferable.

Enzyme-Activated Irreversible Inhibitors

The best enzyme-activated irreversible inhibitor is AbeAdo, which has a K_i of about 0.6 μM for the rat AdoMetDC (105). Another compound that apparently acts as a suicide substrate for AdoMetDC is *S*-(5′-deoxy-5′-adenosyl)-1-amino-4-methylthio-2-cyclopentene (AdoMac), but this has a significantly higher K_i of 18 μM (106). The rationale for the synthesis of AbeAdo as an AdoMetDC inhibitor involves the formation of a Schiff base between the drug and the pyruvate prosthetic group of AdoMetDC followed by an enzyme-mediated abstraction of a proton forming a conjugated imine, which could then react with a nucleophilic residue of the enzyme (107). However, subsequent experiments have indicated that the enzyme is, in fact, inactivated by a transamination of the pyruvate prosthetic group (101). Such transamination occurs with the normal AdoMet substrate by incorrect protonation of the enolate intermediate, but the rate with AbeAdo is much greater.

AbeAdo is a very effective and specific inhibitor of polyamine synthesis in protozoal parasites, in cultured tumor cells, and in vivo (21, 22, 28, 108–110). Its uptake into trypanosomes occurs via the purine-nucleoside-transport system (109). The mechanism of uptake into mammalian cells is unclear and may not be active transport. However, some L1210 cells resistant to AbeAdo have been derived by prolonged exposure to the drug. These cells have a reduced accumulation of the drug that could be due to the induction of an efflux mechanism (22). Other mechanisms of resistance are also known. Resistance to AbeAdo and to ethylglyoxal bis(guanylhydrazone) (EGBG) was obtained in mouse FM3A cells by treatment with a mutagen, which led to an increased production of AdoMetDC mRNA and protein, but the mechanism underlying the elevated mRNA level is obscure (111).

Competitive Inhibitors

The third approach to the production of AdoMetDC inhibitors has been taken by Regenass and colleagues (112–115), who have used the traditional approach of making a large number of compounds related to MGBG, a known competitive inhibitor of the enzyme (99), and then screening these compounds for those with good activity against AdoMetDC and less activity towards both diamine oxidase and the ability to cause mitochondrial damage, which are two additional well-known sites of MGBG action. Several compounds that are strong and apparently specific AdoMetDC inhibitors have emerged from these trials, including [2,2-bipyridine]-6,6′-dicarboximidamide (CGP-39937) (112,

114) and 4-amidinoindan-1-one 2′-amidinohydrazone (CGP-48664) (113, 115). These compounds are such powerful inhibitors of AdoMetDC in tumor cell cultures that a major depletion of spermidine and spermine is produced. The binding of these inhibitors, like that of MGBG, stabilizes AdoMetDC, which leads to an increase in the total protein. However, the inhibitors' potency is sufficiently great that AdoMetDC activity remains reduced, even in the face of this increase (112). This contrasts with MGBG itself, which produces only a modest decrease in dcAdoMet concentrations in treated cells, owing to the increased content of AdoMetDC. It remains to be seen whether these newer and more complex derivatives of MGBG are actually more useful and specific inhibitors of AdoMetDC than other MGBG congeners, including EGBG and diethylglyoxal bis(guanylhydrazone), which have been used in laboratory experiments but have not proceeded into clinical trials (1, 98, 99, 116). However, CGP-48664 differs from MGBG in that it does not rely on the polyamine-transport system for uptake and does not have strong antimitochondrial activity. Furthermore, resistance to CGP-48664 appears to be due to amplification of the AdoMetDC gene (113). These results suggest that AdoMetDC is indeed the major target of this drug.

POTENTIAL OF ADOMETDC INHIBITORS FOR THERAPEUTIC APPLICATIONS

Antiparasitic

AbeAdo was at least 100 times more potent than DFMO in curing *T. brucei brucei* infections in mice and was much more effective against *T. brucei rhodesiense*, including isolates producing infections that could not be cured by DFMO (58, 74, 117, 118). The combination of DFMO and AbeAdo was also very effective against this parasite. These results are very exciting in suggesting that a treatment can be found for the sleeping sickness caused by *T. brucei rhodesiense* if funding can be provided for the development of AbeAdo. Work with AbeAdo also supports the idea that derangement of adenosine-nucleoside metabolism may be the key site of action of these drugs. The killing of trypanosomes by AbeAdo correlated much more closely with rapid increases in AdoMet levels rather than with the subsequent reductions noted in putrescine and spermidine content (58, 74, 117, 118). A much greater rise occurred in AdoMet content in the parasites than in the host cells when exposed to AbeAdo. Another point of specificity may be that AbeAdo is a substrate for the active purine-nucleoside-transport system of these organisms and is accumulated very rapidly to much higher levels than in the host cells (109).

AbeAdo was effective in blocking polyamine synthesis and the growth of

the human malarial parasite *Plasmodium falciparum* in erythrocytes, but disappointingly, it had no effect on the parasitemia in mice infected with *P. berghei* (108). Preliminary studies have suggested that AbeAdo may be effective in blocking the ability of *T. cruzi* to infect and to multiply within rat heart myoblasts (119).

Antitumor

Although it now appears that the antitumor effects of MGBG may not be caused by its effect on AdoMetDC, the more specific derivatives do posses significant potential as antitumor agents (112–115). In particular, CGP 48664 showed potent antitumor activity against a spectrum of human tumor xenografts (113). These results suggest that AdoMetDC inhibition may indeed be a useful antitumor therapy, but there may be another site of action of the MGBG analogues, since AbeAdo, which is as good or better an AdoMetDC inhibitor, was not very active as an antitumor agent against Lewis lung carcinoma or L1210 leukemia when given alone (20, 98). The interaction of the MGBG analogues, such as CGP 48664, with this putative site may, however, be facilitated by the depletion of cellular polyamines. There is clearly significant potential for combining AdoMetDC inhibitors with compounds interfering with polyamine synthesis at other steps.

IMPORTANCE OF POLYAMINE TRANSPORT IN RESPONSE TO INHIBITORS AND ANALOGUES

Addressing the question of uptake of exogenous polyamines will probably be necessary in order to maximize the effects of inhibitors of ODC and AdoMetDC. Exogenous polyamines are provided both by release from other cells and via external polyamine sources. Polyamines are present in virtually all foods and are produced and excreted by intestinal microorganisms. The extent to which such polyamine sources are taken up from the gut, avoid oxidation, and are actually available to tissues is not entirely clear, but there are convincing examples that, under some circumstances, they can be utilized by cells (see 85, 120). Furthermore, all cells contain an energy-dependent, carrier-mediated, and saturable transport system for the uptake of exogenous polyamines (5, 121–123). As expected from their charged structure, the membrane is quite impermeable to polyamines in the absence of this uptake system. There are multiple components of this system, with overlapping specificities for the respective polyamines (124, 125). At least one of these systems is Na^+ dependent. The physiological significance of this system was not fully appreciated at first, because all mammalian cells also contain the biosynthetic enzymes for polyamine formation and are thus able to produce their own polyamines. Furthermore, extracellular polyamine levels are very low due to the presence

of plasma oxidases that degrade them. However, there is now convincing evidence from three separate approaches that uptake is important in maintaining normal cellular polyamine levels and that it plays a particularly important role when biosynthesis is impaired by the absence of precursors or the application of biosynthesis inhibitors.

Firstly, the transport system is tightly regulated by intracellular polyamines and transport is increased when cellular polyamine levels fall. Thus, in cells treated with DFMO or AbeAdo, substantial increase occurs in the uptake of exogenous polyamines (104, 121, 122, 126, 127). The importance of this uptake is shown clearly by the comparison of the effects of DFMO on tumor cells lacking the transport system with cells possessing this system. Mice inoculated with the former are much more effectively treated with DFMO (128, 129). Secondly, feeding a diet deficient in polyamines (including antibiotics to reduce polyamine production from intestinal flora) and using inhibitors of polyamine salvage pathways enhances the antitumor effects of inhibitors of polyamine biosynthesis (84, 85). Finally, mutant cells in culture lacking the polyamine-transport system (see below) show a greater reduction in intracellular polyamines in response to inhibitors, such as DFMO or AbeAdo, than controls (126, 127). Since the culture medium used contained very little, if any, polyamines, this phenomenon is probably due to the more efficient re-uptake of polyamines (particularly putrescine) lost from the cell. Indeed, the recapture from the local microenvironment of polyamines lost from the cell may be the primary role of the transport system under normal physiological circumstances.

The transport system for polyamines is quite distinct from systems transporting other normal cellular components, such as amino acids (5, 121, 122). However, it is not specific and is able to facilitate the accumulation of a number of other amines. These include paraquat, MGBG and related compounds, and polyamine analogues such as BE-3-4-3, BE-3-3-3, and 1,19-bis(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4). Mutant cells lacking the transport system have been selected and these are strikingly refractory to the cytotoxic effects of these agents (130). The possibility that such mutations will arise in tumors in vivo and cause resistance appears to be quite likely. However, the mutant cells are much more sensitive to inhibitors of polyamine synthesis such as DFMO or AbeAdo because of their inability to take up circulating polyamines. Transport-related resistance could therefore be dealt with by treating the resistant cells with appropriate inhibitors of biosynthesis.

The well-established up-regulation of the polyamine-transport system in response to polyamine depletion is now beginning to become understood at the molecular level. A protein with a very short half-life is involved in shutting off transport when cellular polyamines are high (62, 127). This protein is likely to be antizyme (131), which is better known for its ability both to inhibit and

to enhance the degradation of ODC (132). Disruption of this regulatory mechanism such that transport is not down-regulated allows the accumulation of polyamines or analogues to very high levels, which lead to cell death (127, 133–135).

Several cells have been identified in which polyamine transport is enhanced due to a lack of feedback inhibition of the polyamine-transport system. These include D-R cells, an L1210 cell line selected for resistance to DFMO that greatly overexpresses ODC as a result of gene amplification (134; R Hu & AE Pegg, unpublished observations), and DH23b cells, a rat hepatoma cell line that also overproduces ODC (133). High levels of ODC in these cells might bind all the available antizyme; indeed, the fact that numerous stimuli increase both ODC and transport might be explained by such a link. The fact that cells overexpressing ODC may also have an elevated polyamine-transport system is not yet widely appreciated and has important connotations for the interpretation of experiments with such cells and for the appearance of a transformed phenotype in them.

Polyamine transport is increased by a variety of factors including hormones and growth factors that increase ODC activity. There are several reports of transformed cells having activated polyamine transport (121), and transport was also reported to increase in Rat-1 cells transformed by N-*myc* (136). Phorbol esters increase transport of polyamines, possibly via effects on Na^+ transport (137), and protein kinase C inhibitors block spermidine uptake (138). The increased uptake of polyamines in cells treated with phorbol esters may augment the increases owing to elevated ODC levels and contribute to tumor promotion.

The use of pretreatment with DFMO to enhance the uptake of drugs carried by this transport system has been suggested and tried for several agents, including MGBG, aziridinylputrescine, and bis(alkyl)polyamines (reviewed in 3, 5, 121). Although MGBG uptake is increased in this way, this combination should be viewed with great caution because MGBG may accumulate to toxic levels even in the absence of DFMO (139).

The lack of specificity of the polyamine-transport system and the ability to stimulate this system in tumor cells with inhibitors of biosynthesis, such as DFMO, raises the possibility that the addition of a polyamine function to cytotoxic agents could be used to enhance their uptake and effectiveness (3, 5, 121, 123). Promising results have been obtained with nitroimidazole-polyamine conjugates (140), chlorambucil-spermidine conjugates (141), monaziridinylputrescine (123), N^1- and N^8-aziridinyl analogues of spermidine (142), and Diam-3 (122), but no effect was obtained in another study in which derivatives of *N*-acetyldopamine and chlorambucil were tested (143).

Clearly, the ability to manipulate polyamine transport would be a valuable tool in the most efficient therapeutic use of polyamine analogues, derivatives,

and biosynthesis inhibitors. Although the bacterial polyamine transporters have been cloned and expressed, attempts to clone the mammalian system have not yet been successful, despite development of strategies for isolation and expression of these genes (124). Other factors regulating transport may include Ca^{2+}-calmodulin, since a variety of Ca^{2+} ionophores and calmodulin antagonists have been shown to inhibit transport (121, 123, 138, 144).

POLYAMINES AND ANALOGUE EFFECTS ON DNA

It is well known that the association of cationic polyamines with negatively charged DNA induces significant structural changes in DNA in cell-free systems (145). Spermidine and spermine can cause DNA to condense and aggregate (146) and induce both B-to-Z (147) and B-to-A (148) transitions in certain DNA sequences. Computer modeling and physical-chemical studies (145) support the existence of specific interactions between spermine and DNA; the induction of bends in DNA may have important physiological implications. The hypothesis that structural transitions and condensation in specific DNA sequences caused by polyamines may be related to nucleosome formation and the condensation of DNA into chromatin is gaining experimental support. Several examples of reports that describe the effects of spermidine and spermine on the condensation of chromatin in cell-free systems are presented here. Hewish & Burgoyne (149) used electron microscopy to show that the nucleosomal structure of rat liver chromatin is better preserved when polyamines are added to the isolation buffer. Electric dichroic studies show that spermidine and spermine induce specific compact DNA structures in chromatin before aggregation takes place (150), and now there is evidence that indicates that polyamine-induced stabilization of nucleosomal DNA differs from stabilization that is induced by magnesium or hexamine cobalt (151).

Experimental evidence from cellular systems also exists. Hougaard et al (152) found that polyamines are intimately associated with highly condensed chromatin within the cell, and Snyder (153) used sucrose-density gradient centrifugation and nick-translation procedures to show that nuclei isolated from HeLa cells after polyamine depletion with DFMO are more susceptible to digestion by DNase I, DNase II, and micrococcal nuclease, thus indicating that modifications in nucleosomal structure are caused by polyamine depletion. Studies of human brain tumor cells treated with the cytotoxic polyamine analogue BE-4-4-4 indicate that they are also more susceptible than untreated control cells to digestion by micrococcal nuclease and DNase I (154). In contrast, cells that are treated with a nontoxic analogue, *sym*-norspermine (3-3-3), do not show any significant difference between treated and untreated cells with regard to micrococcal nuclease digestion (154). These observations suggest that the ability of an analogue to modify certain specific characteristics within the cell may correlate with the ability of an analogue to kill tumor cells.

Recently, it has been shown that HeLa cells treated with the polyamine analogue BE-4-4-4 display an altered DNA-nuclear-matrix association as measured by a nucleoid halo assay (155). Thus, not only the conformation of the DNA itself is affected by the polyamines, but the attachment of the DNA to the nuclear matrix and, possibly, replication and transcription are also affected. As alluded to in the introduction, the ability of murine leukemia virus to integrate into SV40 minichromosomes has been shown to be affected by nucleosome formation and chromatin condensation (156). More recently, it has been demonstrated that spermidine added to cell-free integration systems affects the pattern of viral integration (18). The association of polyamines with DNA is thus implicated.

The possible utility of using specific measurements of polyamine-DNA interactions as a means of determining which modifications of polyamine structure might yield therapeutically active compounds has been studied for some time. It makes sense from a theoretical perspective that analogues that are able to bind strongly to nucleic acid, while displaying some modified function relative to the naturally occurring polyamines, are most likely to be successful agents in terms of therapeutic intervention. Although DNA interactions are used as "surrogate" measurements, since other anionic sites in the cell bind polyamines and since we are not yet certain that DNA is the site of primary importance for a cytotoxic effect, this approach has been promising. The characteristics that seem to relate best in this "semi-rational" approach to drug development are affinity for DNA and diminished ability to aggregate DNA versus the parent polyamine. Using this approach, a series of polyamine analogues were theoretically constructed, synthesized, and subsequently shown to have cytotoxic effects (15–17). Most notable of the compounds developed thus far using this approach, with regard to subsequent study and outcome, is the pentamine BE-4-4-4-4. This compound has been studied extensively in human brain-tumor lines in cell culture (17), and in nude mouse xenografts both for human brain tumors (158; CJ Bergeron, LJ Marton, K Lamborn, HS Basu, A Shirahata, K Samejima & BG Feuerstein, unpublished data) and for a variety of other tumors (158). Interestingly, pentamines were first identified in the thermophile *Thermus thermophilus* grown at high temperature (159), and while 4-4-4-4, the parent compound, is not a naturally occurring pentamine, studies of the interactions of pentamines with DNA (160) were important to the development of BE-4-4-4-4.

More recently, three novel polyamine analogues were designed on the basis of computer modeling and physical-chemical studies of polyamine-DNA interactions that differ from the natural polyamines and from one another with regard to the charge distribution on the surface of their aliphatic backbones (161). Addition of two methyl groups to a central nitrogen of a 4-4-4 backbone, creating a quaternary amine, resulted in a compound with a greater degree of

cell-killing ability than a compound created by the addition of a single methyl group to the same backbone. Further modification by bis-ethylation of the terminal nitrogens of the quarternary amine compound appeared to add additional advantage. Bergeron et al (11, 12) have showed that modification by bis-ethylation is one of the more effective modifications with regard to cell killing that can be made to a polyamine analogue.

In addition to the studies of polyamine-DNA interactions and the modification of those interactions as a basis for the development of the new analogues already discussed, structure-activity correlations between modifications of polyamine chain length and/or alkyl additions to the primary amines of the polyamines have been investigated, as previously mentioned. Many polyamine analogues are able to diminish both ODC and AdoMetDC activities through feedback inhibition resulting in treated cells having lowered levels of their endogenous polyamines. In addition, the induction of SSAT, in those systems where such induction takes place, further reduces intracellular levels. The activity of analogues must thus be considered not only in light of their potential ability to function as binding agents to specific intracellular sites, but in terms of their ability to lower intracellular polyamine levels as well. An attempt to clarify whether certain polyamine analogues might function even in the presence of intracellular polyamines utilized an ornithine decarboxylase negative CHO cell mutant that had been transfected either with mammalian ODC or with trypanosomal ODC, which does not exhibit feedback inhibition as does the mammalian ODC (162). This study indicated that certain polyamine analogues do require that polyamine depletion take place in order for them to be effective, while others have the capability of functioning even in the presence of significant levels of intracellular polyamines. For some of the active polyamine analogues, the very presence of the analogue and its ability to bind at specific and important sites within the cell, thus replacing the naturally occurring polyamines at those sites, might constitute a significant portion of their activity.

ODC is clearly not the critical target for BE-3-4-3, since cells overexpressing very high levels of ODC are still sensitive to the drug (162, 163). Although the synthesis of mitochondrial DNA is blocked by BE-3-4-3 and related analogues (11, 164), the mitochondrial DNA is also probably not a critical target, since mutant cells lacking mitochondrial DNA are very sensitive (163). The best correlation with cytotoxicity is with the total accumulation of the analogue (163, 165). The depletion of normal polyamines, however, probably permits the binding of the analogues to critical intracellular sites.

ANTITUMOR POTENTIAL OF POLYAMINE ANALOGUES

While a large series of polyamine derivatives—including analogues of putrescine, spermidine, and spermine—have been synthesized and studied in tissue

culture and in some animal systems, over the past several years three compounds have emerged that have entered or are soon approaching clinical trial. As such, we review each of these in greater detail as examples of the potential use of polyamine analogues for therapeutic intervention in cancer. Further studies of analogues in parasitic and other diseases such as the use of *N,N'*-bis(benzyl)-substituted analogues for therapy of *T. cruzi* (166), *L. donovani* (80), and *P. falciparum* (167) are presently ongoing and results are anxiously awaited.

The polyamine analog, *N,N'*-bis[3-(ethylamino)-propyl]-1,7-heptanediamine (BE-3-7-3), synthesized by Edwards et al (13) at Marion Merrell Dow, has been studied extensively in HeLa cells in culture and in L1210 leukemia cells in culture and in mice (168). Interestingly, in these studies, although the compound was cytotoxic, it had only marginal effects on modifying intracellular polyamine concentrations. However, in both cell lines the incorporation of radioactive precursors into DNA, RNA, and protein was significantly reduced by treatment with BE-3-7-3. The authors of the study speculated that BE-3-7-3 "causes growth inhibition by antagonizing some of the natural polyamines at the nucleic acid level" (168). They further noted that mice innoculated with L1210 cells followed by administration of BE-3-7-3 showed significantly prolonged survival time and that when these same mice were treated, not only with BE-3-7-3 but with an inhibitor of polyamine oxidase, a 100% cure rate was achieved. The synergism between BE-3-7-3 and the polyamine oxidase inhibitors suggests that the drug may be metabolized by PAO, which is known to act on a variety of α,ω-polyamine derivatives (169, 170). Later studies have shown that not only BE-3-7-3 but also BE-3-3-3 and bis(benzyl)-3-7-3 are dealkylated by PAO (AE Pegg, R Wechter & R Hu, unpublished observations).

Interestingly, when the combination of BE-3-7-3 and a PAO inhibitor was used, the cured animals were resistant to subsequent challenge with L1210 cells (168). In a subsequent study (171), although animals successfully cured of L1210 tumors were immune to subsequent challenge with L1210 tumor cells, these same mice did not reject P-388 leukemic cells. Further, they showed that coculturing lymphocytes from cured mice with L1210 cells generated a potent tumor-specific cytolytic response against L1210 target cells, whereas lymphocytes from naive mice did not generate any significant cytolytic activity. Both the tissue culture and in vitro activities were completely eliminated by pretreating the splenic lymphocyte population with anti-Thy-1.2 monoclonal antibodies and complement, indicating that T-cells were the effector population. Further studies with BE-3-7-3 have now been conducted by Marion Merrell Dow. Phase I clinical trials have also begun.

A second compound of particular interest is the polyamine analogue BE-3-3-3, which was synthesized by Bergeron et al (12) and has been studied

extensively by Porter, Bergeron, and their colleagues (31, 172; RJ Bernacki, EJ Oberman, RJ Bergeron & CW Porter, personal communication). As indicated elsewhere in this paper, BE-3-3-3 induces high levels of activity of SSAT, and there is an apparent correlation between induction of SSAT and the ability of this compound to be an effective therapeutic agent in a variety of melanoma and lung tumor cell lines. While it is unclear, even for BE-3-3-3, whether the correlation holds true in all systems or whether the induction of this enzyme is responsible in some measure for its activity, what is striking is the response to this compound of a variety of melanomas and other tumors grown in nude mouse systems. With appropriate dose and administration protocols, BE-3-3-3 produces a remarkable inhibition of the growth of the MALME-3M, PANUT-2, and SH-I melanomas; A549 lung adenocarcinoma; and A121 ovarian carcinoma (RJ Bernacki, EJ Oberman, RJ Bergeron & CW Porter, personal communication). Studies by Chang et al (174) indicate activity for this compound in pancreatic tumors as well. Phase I trials of BE-3-3-3 are now underway.

The last compound slated for clinical trial is BE-4-4-4-4, which was discussed above. This compound shows significant activity against a variety of human brain tumors in tissue culture (17) and in nude mouse xenografts as well (158; CJ Bergeron, LJ Marton, K Lamborn, HS Basu, A Shirahata, K Samejima & BG Feuerstein, unpublished data). Of interest is the fact that in the SF767 brain-tumor cell line grown in the xenograft, three of eight animals were cured after two cycles of therapy. On the other hand, the U-87MG cell line in the xenograft was not as responsive to this compound. This compound was also extremely effective against the A549 lung carcinoma (158). Thus, at least two of the analogues, BE-4-4-4-4 and BE-3-3-3, are effective against this same line. Histopathologic examination of representative tumors from A549 cells in control versus mice treated with BE-4-4-4-4 showed that mitotic indices were approximately 18 times lower in the treated group than in the control group (158). Thus, these compounds may not only kill cells but modify growth rate and/or differentiation in some fashion. BE-4-4-4-4 also showed activity against the HCT116 colon cell line when grown in the xenograft, although it was less effective against tumors formed by the HT29 colon cell line (158). BE-4-4-4-4 is presently being studied by the National Cancer Institute with regard to preclinical toxicology, and it is anticipated that it will enter Phase I clinical trial in the near future.

Both BE-4-4-4-4 and BE-3-3-3 show activity for tumor types not normally amenable to therapy, such as melanomas; pancreatic, brain, lung, and colon tumors; and perhaps others. Although BE-3-3-3 induces SSAT enormously, BE-4-4-4-4, at best, minimally induces this enzyme in the models studied (158). As such, their modes of action might be similar in some fashions but different in others.

Although the compounds discussed above show promise, there is an almost unlimited potential for the synthesis of these type of polyamine analogues. The unsymmetrical-substituted analogues, the bis(benzyl)-substituted analogues, and the quarternary amines previously mentioned are examples. Clearly, even more effective compounds might yet be synthesized. The information gleaned from the preclinical and clinical studies underway, taken along with continued fundamental studies, assures significant additional activity in this area.

COMBINATIONS OF POLYAMINE ANALOGUES WITH OTHER THERAPEUTIC MODALITIES

Although the utility of polyamine analogues as single agents seems fairly promising for a variety of tumor types, the ability to combine such agents with other therapeutic modalities is also highly important. Previous studies have shown that polyamine depletion produced by DFMO, MGBG, or other inhibitors of polyamine biosynthesis could modify the activity of other DNA-directed chemotherapeutic agents, such as the chloroethylnitrosoureas; cis-platinum (175); and topoisomerase-related agents such as 4′-(9-acridinylamino) methanesulfon-M-anisidide (m-AMSA) (176, 177). Although the exact mechanism of interaction has not been clearly defined, a variety of possibilities ranging from modification of DNA structure by polyamine depletion to modifications of intracellular enzymatic activities to modification of cell cycle have all been considered and explored to some degree (175). Similarly, a number of the analogues have been investigated with regard to their ability to potentiate the activities of other therapeutic modalities. Specifically, BE-4-4-4-4 has been shown to significantly potentiate bis(chloroethyl)- nitrosourea (BCNU) in the SF767 human brain tumor line in tissue culture (M Pellarin, HS Basu, BG Feuerstein, LJ Marton & DF Deen, unpublished results) and BE-3-3-3 and BE-4-4-4-4 have been shown to potentiate cis-platinum activity in U251MG and SF188 human brain tumor cells in culture, respectively (HS Basu, M Pellarin, BG Feuerstein, DF Deen & LJ Marton, unpublished results). Preliminary studies of the combination of BE-4-4-4-4 and BCNU in xenograft models indicate the potential for such a combination (158). The possibility of potentiating x-irradiation with the use of polyamine inhibition or analogues has also been studied (178), and there are indications that such interactions are worthy of further study. Also of interest are the effects of inhibition of polyamine biosynthesis on ^{125}I-induced injury to normal brain. DFMO treatment strikingly reduced the volume of edema, necrosis, and contrast enhancement when compared to untreated controls (179).

FUTURE DIRECTIONS

Historically there has been much overlap and interchange between the basic and clinical aspects of polyamine research. This has served us well in that compounds devised for clinical use have found utility in the basic science laboratory and vice-versa. Clearly, the initial failures in terms of transition to the clinic have only been because of our lack of understanding of how to correctly apply the drugs that were developed. Our current knowledge now makes the possibility of clinical utility very real. As we gain further understanding in the areas of polyamine transport, the mechanisms that relate ODC overexpression to cellular transformation, and the interactions between polyamines and nucleic acids, we will not only further our knowledge of cell regulation but develop new tools for clinical utility.

Although drug development in the polyamine field has been satisfying from an intellectual point of view and in terms of outcome, we must remember that with compounds as fundamental to cellular growth processes as the polyamines, we must always be cautious of unexpected effects. The relationship of polyamines to central nervous system receptors, as exemplified by the potent effects of polyamines on the NDMA receptor (96, 97), clearly opens up an exciting new area for research; however, the potential that CNS effects might be invoked by interfering with polyamine biosynthesis or by therapy with analogues must obviously be kept in mind. Nevertheless, the future for polyamine research is bright and its rewards are now being recognized.

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Literature Cited

1. Pegg AE, McCann PP. 1982. Polyamine metabolism and function. *Am. J. Physiol.* 243:C212–21
2. Tabor CW, Tabor H. 1984. Polyamines. *Annu. Rev. Biochem.* 53:749–90
3. Porter CW, Sufrin JR. 1986. Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. *Anticancer Res.* 6:525–42
4. Pegg AE. 1986. Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem. J.* 234:249–62
5. Pegg AE. 1988. Polyamine metabolism and its importance in neoplastic growth

and as a target for chemotherapy. *Cancer Res.* 48:759–74
6. McCann PP, Pegg AE, Sjoerdsma A, eds. 1987. *Inhibition Of Polyamine Metabolism: Biological Significance And Basis For New Therapies.* Orlando, FL: Academic. 371 pp.
7. Bachrach U, Heimer YM. 1989. *The Physiology of Polyamines.* Boca Raton, FL: CRC. 355 pp.
8. Jänne J, Alhonen L, Leinonen P. 1991. Polyamines: from molecular biology to clinical applications. *Ann. Med.* 23:241–59
9. Seiler N. 1991. Pharmacological properties of the natural polyamines and their depletion by biosynthesis inhibitors as a therapeutic approach. In *Progress in Drug Research,* pp. 107–159. Basel: Birkhäuser Verlag
10. Hamana K, Matsuzaki S. 1992. Polyamines as a chemotaxonomic marker in bacterial systematics. *Crit. Rev. Microbiol.* 18:261–83
11. Bergeron RJ, Neims AH, McManis JS, Hawthorne TR, Vinson JRT, et al. 1988. Synthetic polyamine analogues as antineoplastics. *J. Med. Chem.* 31:1183–90
12. Bergeron RJ, Hawthorne TR, Vinson JRT, Beck DE, Ingeno MJ. 1989. The role of the methylene backbone in the antiproliferative activity of polyamine analogues. *Cancer Res.* 49:2959–64
13. Edwards ML, Prakash NJ, Stemerick DM, Sunkara SP, Bitonti AJ, et al. 1990. Polyamine analogues with antitumor activity. *J. Med. Chem.* 33:1369–75
14. Lakanen JR, Coward JK, Pegg AE. 1992. α-Methylpolyamines: metabolically stable spermidine and spermine mimics capable of supporting growth in cells depleted of polyamines. *J. Med. Chem.* 35:724–34
15. Basu HS, Feuerstein BG, Deen DF, Lubich WP, Bergeron RJ, et al. 1989. Correlation between the effects of polyamine analogues on DNA conformation and cell growth. *Cancer Res.* 49:5591–97
16. Basu HS, Schwietert HCA, Feuerstein BG, Marton LJ. 1990. Effects of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. *Biochem. J.* 269:329–34
17. Basu HS, Pellarin M, Feuerstein BG, Shirahata A, Samejima K, et al. 1993. Interaction of a polyamine analogue 1,19-bis-(Ethylamino)-5,10,15-triazanoadecane (BE-4-4-4-4), with DNA and effect on growth, survival, and polyamine levels in seven human brain tumor cell lines. *Cancer Res.* 53:3948–55
18. Pryciak PM, Müller HP, Varmus HE. 1992. Simian virus 40 minichromosomes as targets for retroviral integration in vivo. *Proc. Natl. Acad. Sci. USA* 89: 9237–41
19. Kramer DL, Khomutov RM, Bukin YV, Khomutov AR, Porter CW. 1989. Cellular characterization of a new irreversible inhibitor of *S*-adenosylmethionine decarboxylase and its use in determining the relative abilities of individual polyamines to sustain growth and viability of L1210 cells. *Biochem. J.* 259:325–31
20. Seiler N, Sarhan S, Mamont P, Casara P, Danzin C. 1991. Some biological consequences of *S*-adenosylmethionine decarboxylase inhibition by MDL. 73811. *Life Chem. Rep.* 9:151–62
21. Byers TL, Ganem B, Pegg AE. 1992. Cytostasis induced in L1210 murine leukemia cells by the *S*-adenosyl-L-methionine decarboxylase inhibitor 5′-{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine may be due to the hypusine depletion. *Biochem. J.* 287:717–24
22. Byers TL, Wiest L, Wechter RS, Pegg AE. 1993. Effects of chronic 5′-{[(Z)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine (AbeAdo) treatment on polyamine and eIF-5A metabolism in AbeAdo sensitive and resistant L1210 murine leukemia cells. *Biochem. J.* 290: 115–21
23. Park MH, Wolff EC, Folk JE. 1993. Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential role in cellular regulation. *BioFactors* 4:95–104
24. Kang HA, Hershey JWB. 1994. Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae. J. Biol. Chem.* 269:3934–40
25. Coward JK, Pegg AE. 1987. Specific multisubstrate adduct inhibitors of aminopropyltransferases and their effect on polyamine biosynthesis in cultured cells. *Adv. Enzyme Regul.* 26:107–13
26. Shirahata A, Takahashi N, Beppu T, Hosoda H, Samejima K. 1993. Effects of inhibitors of spermidine synthase and spermine synthase on polyamine synthesis in rat tissues. *Biochem. Pharmacol.* 45:1897–903
27. Pegg AE, Coward JK. 1993. Effect of *N*-(n-butyl)-1,3-diaminopropane on polyamine metabolism, cell growth and sensitivity to chloroethylating agents. *Biochem. Pharmacol.* 46:717–24
28. Byers TL, Lakanen J, Coward JK, Pegg AE. 1994. The role of hypusine depletion in cytostasis induced by *S*-adenosylmethionine decarboxylase inhibi-

tion: new evidence provided by 1-methylspermidine and 1, 12-dimethylspermine. *Biochem. J.* 303:363–68
29. Seiler N. 1987. Functions of polyamine acetylation. *Can. J. Physiol. Pharmacol.* 65:2024–35
30. Casero RA, Pegg AE. 1993. Spermidine/spermine N^1-acetyltransferase: the turning point in polyamine metabolism. *FASEB J.* 7:653–61
31. Porter CW, Bernacki RJ, Miller J, Bergeron RJ. 1993. Antitumor activity of N^1,N^{11}-bis(ethyl)norspermine against human melanoma xenografts and possible biochemical correlates of drug action. *Cancer Res.* 53:581–86
32. Saab NH, West EE, Bieszk NC, Preuss CV, Mank AR, et al. 1993. Synthesis and evaluation of unsymmetrically substituted polyamine analogues as modulators of human spermidine/spermine-N^1-acetyltransferase (SSAT) and as potential antitumor agents. *J. Med. Chem.* 36:2998–3004
33. Auvinen M, Paasinen A, Andersson LA, Hölttä E. 1992. Ornithine decarboxylase activity is critical for cell transformation. *Nature* 360:355–58
34. Moshier JS, Dosescu J, Skunca M, Luk GD. 1993. Transformation of NIH/3T3 cells by ornithine decarboxylase overexpression. *Cancer Res.* 53:2618–22
35. Hibshoosh H, Johnson M, Weinstein IB. 1991. Effects of overexpression of ornithine decarboxylase (ODC) on growth control and oncogene-induced cell transformation. *Oncogene* 6:739–43
36. Lazaris-Karatzas A, Montine KS, Sonenberg N. 1990. Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5′ cap. *Nature* 345:544–47
37. Koromilas AE, Lazaris-Karatzas A, Sonenberg N. 1992. mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO J.* 11:4153–58
38. Shantz LM, Pegg AE. 1994. Overproduction of ornithine decarboxylase caused by relief of translational repression is associated with neoplastic transformation. *Cancer Res.* 54:2313–16
39. Coleman CS, Stanley BA, Pegg AE. 1993. Effect of mutations at active site residues on the activity of ornithine decarboxylase and its inhibition by active site-directed irreversible inhibitors. *J. Biol. Chem.* 268:24,572–79
40. Coleman CS, Stanley BA, Viswanath R, Pegg AE. 1994. Rapid exchange of subunits of mammalian ornithine decarboxylase. *J. Biol. Chem.* 269:3155–58
41. Hölttä E, Auvinen M, Andersson LC. 1993. Polyamines are essential for cell transformation for pp60v-src: delineation of molecular events relevant for the transformed phenotype. *J. Cell Biol.* 122:903–14
42. Halmekytö M, Alhonen L, Alakuijala L, Jänne J. 1993. Transgenic mice overproducing putrescine in their tissues do not convert the diamine into higher polyamines. *Biochem. J.* 291:505–8
43. Halmekytö M, Syrjänen K, Jänne J, Alhonen L. 1992. Enhanced papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene. *Biochem. Biophys. Res. Commun.* 187:493–97
44. Bello-Fernandez C, Packham G, Cleveland JL. 1993. The ornithine decarboxylase gene is a transcriptional target of c-Myc. *Proc. Natl. Acad. Sci. USA* 90: 7804–8
45. Hurta RAR, Greenberg AH, Wright JA. 1993. Transforming growth factor B_1 selectively regulates ornithine decarboxylase gene expression in malignant H-*ras* transformed fibrosarcoma cell lines. *J. Cell. Physiol.* 156:272–79
46. DiGiovanni J. 1992. Multistage carcinogenesis in mouse skin. *Pharmacol. Ther.* 54:63–128
47. Gilmour SK, Robertson FM, Megosh L, O'Connell SM, Mitchell J, O'Brien TG. 1992. Induction of ornithine decarboxylase in specific subpopulations of murine epidermal cells following multiple exposures to 12-*O*-tetradecanoylphorbol-13-acetate, merzerein and ethyl phenylpropriolate. *Carcinogenesis* 13: 51–56
48. Rao CV, Reddy BS. 1993. Modulating effect of amount and types of dietary fat on ornithine decarboxylase, tyrosine protein kinase and prostaglandins production during colon carcinogenesis in male F344 rats. *Carcinogenesis* 14: 1327–33
49. Phillips RW, Kikendall JW, Luk GD, Willis SM, Murphy JR, et al. 1993. β-Carotene inhibits rectal mucosal ornithine decarboxylase activity in colon cancer patients. *Cancer Res.* 53:3723–25
50. Kim YJ, Tseng CP, Pan H, Verma AK. 1994. Tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate responsive sequences in human ornithine decarboxylase gene. *Mol. Carcinog.* In press
51. McCann PP, Pegg AE. 1992. Ornithine decarboxylase as an enzyme target for therapy. *Pharmacol. Ther.* 54:195–215
52. Hyvönen T, Alakuijala L, Andersson L, Khomutov AR, Khomutov RM, Eloranta

TO. 1988. 1-Aminooxy-3-aminopropane reversibly prevents the proliferation of cultured baby hamster kidney cells by interfering with polyamine biosynthesis. *J. Biol. Chem.* 263:11,138–44
53. Poulin R, Secrist JA III, Pegg AE. 1989. Effect of 1-amino-oxy-3-aminopropane on polyamine metabolism and growth of L1210 cells. *Biochem. J.* 263:215–21
54. Stanek J, Frei J, Mett H, Schnieder P, Regenass U. 1992. 2-Substituted 3-(aminooxy) propanamines as inhibitors of ornithine decarboxylase: synthesis and biological activity. *J. Med. Chem.* 35:1339–44
55. Mett H, Stanek J, Lopez-Ballester JA, Jänne J, Alhonen L, et al. 1993. Pharmacological properties of the ornithine decarboxylase inhibitor 3-aminooxy-1-propanamine and several structural analogues. *Cancer Chemother. Pharmacol.* 32:39–45
56. Bey P, Danzin C, Jung M. 1987. Inhibition of basic amino acid decarboxylases involved in polyamine biosynthesis. See Ref. 6, pp. 1–32
57. Poulin R, Lu L, Ackermann B, Bey P, Pegg AE. 1992. Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine. *J. Biol. Chem.* 267:150–58
58. Bacchi CJ. 1993. Resistance to clinical drugs in African trypanosomes. *Parasitol. Today* 9:190–93
59. Hanson S, Beverley SM, Wagner W, Ullman B. 1992. Unstable amplification of two extrachromosomal elements in α-difluoromethylornithine-resistant *Leishmania donovani*. *Mol. Cell. Biol.* 12:5499–5507
60. Coffino P. 1989. Molecular biology of ornithine decarboxylase. In *Ornithine Decarboxylase: Biology, Enzymology and Molecular Genetics*. ed. S Hayashi, pp. 135–44. New York: Pergamon
61. Kameji T, Hayashi S, Hoshino K, Kakinuma Y, Igarashi K. 1993. Multiple regulation of ornithine decarboxylase in enzyme-overproducing cells. *Biochem. J.* 289:581–86
62. Mitchell JLA, Dively RR, Bareyal-Leyser A. 1992. Feedback repression of polyamine uptake into mammalian cells requires active protein synthesis. *Biochem. Biophys. Res. Commun.* 186: 81–88
63. Ghoda L, Sidney D, Macrae M, Coffino P. 1992. Structural elements of ornithine decarboxylase required for intracellular degradation and polyamine-dependent regulation. *Mol. Cell. Biol.* 12:2178–85
64. Miyazaki Y, Matsufuji S, Murakami Y, Hayashi S. 1993. Single amino acid replacement is responsible for the stabilization of ornithine decarboxylase in HMO_A cells. *Eur. J. Biochem.* 214:837–44
65. Byers TL, Bush TL, McCann PP, Bitonti AJ. 1991. Antitrypanosomal effects of polyamine biosynthesis inhibitors correlate with increases in *Trypanosoma brucei brucei S*-adenosyl-L-methionine. *Biochem. J.* 274:527–33
66. Claverie N, Mamont PS. 1989. Comparative antitumor properties in rodents of irreversible inhibitors of L-ornithine decarboxylase, used as such or as prodrugs. *Cancer Res.* 49:4466–71
67. Bowlin TL, Hoeper BJ, Rosenberger AL, Davis GF, Sunkara PS. 1990. Effects of three irreversible inhibitors of ornithine decarboxylase on macrophage-mediated tumoricidal activity and antitumor activity in B16F1 tumor-bearing mice. *Cancer Res.* 50:4510–14
68. Bacchi CJ, McCann PP. 1987. Parasitic protozoa and polyamines. See Ref. 6, pp. 317–44
69. Schechter PJ, Sjoerdsma A. 1989. Therapeutic utility of selected enzyme-activated irreversible inhibitors. In *Enzymes as Targets for Drug Design*, ed. MG Palfreyman, PP McCann, W Lovenberg, JG Temple, A Sjoerdsma, pp. 201–10. San Diego: Academic
70. Milord F, Loko L, Éthier L, Mpia B, Pépin J. 1993. Eflornithine concentration in serum and cerebrospinal fluid of 63 patients treated for *Trypanosoma brucei gambiense* sleeping sickness. *Trans. R. Soc. Trop. Med. Hyg.* 87:473–77
71. Milord F, Pépin J, Loko L, Éthier L, Mpia B. 1992. Efficacy and toxicity of eflornithine for treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Lancet* 340:652–55
72. Kuzoe FAS. 1993. Current situation of African trypanosomiasis. *Acta Trop.* 54: 153–62
73. Doua F, Yapo FB. 1993. Human trypanosomiasis in the Ivory Coast: therapy and problems. *Acta Trop.* 54:163–68
74. Bacchi CJ, Yarlett N. 1993. Effects of antagonists of polyamine metabolism on African trypanosomes. *Acta Trop.* 54: 225–36
75. Bailey S, Smith K, Fairlamb AH, Hunter WN. 1993. Substrate interactions between trypanothione reductase and N^1-glutathionylspermidine disulphide at 0.28-nm resolution. *Eur. J. Biochem.* 213:67–75
76. Yarlett N, Garofalo J, Goldberg B, Ciminelli MA, Ruggiero V, et al. 1993. *S*-adenosylmethionine synthetase in

bloodstream *Trypanosoma brucei*. *Biochim. Biophys. Acta* 1181:68–76

77. Bacchi CJ, Garofalo J, Ciminelli M, Rattendi D, Goldberg B, et al. 1993. Resistance to DL-α-difluoromethylornithine by clinical isolates of *Trypanosoma brucei rhodesiense*. Role of *S*-adenosylmethionine. *Biochem. Pharmacol.* 46: 471–81

77a. Bacchi CJ, Nathan HC, Yarlet N, Goldberg B, McCann PP, et al. 1994. Combination chemotherapy of drug-resistant *Trypanosoma brucei rhodesiense* infections in mice using DL-α-difluoromethylornithine and standard trypanocides. *Antimicrob. Agents Chemother.* 38:563–69

78. Paulson YJ, Gilman TM, Heseltine PNR, Sharma OP, Boylen CT. 1992. Eflornithine treatment of refractory *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. *Chest* 101:67–74

79. Kim BG, McCann PP, Byers TJ. 1987. Inhibition of multiplication in *Acanthamoeba castellani* by specific inhibitors of ornithine decarboxylase. *J. Parasitol.* 34:264–66

80. Mukhopadhyay R, Madhubala R. 1993. Effect of a bis(benzyl)polyamine analogue, and DL-α-difluoromethylornithine on parasite suppression and cellular polyamine levels in golden hamster during *Leishmania donovani* infection. *Pharmacol. Res.* 28:359–65

81. Prados M, Rodriguez L, Chamberlain M, Silver P, Levin V. 1989. Treatment of recurrent gliomas with 1,3-bis(2-chloroethyl)-1-nitrosourea and α-difluoromethylornithine. *Neurosurgery* 24: 806–9

82. Levin VA, Chamberlain MC, Prados MD, Choucair AK, Berger MS, et al. 1987. Phase I-II study of eflornithine and mitoguazone combined in the treatment of recurrent brain tumors. *Cancer Treat. Rep.* 71:459–64

83. Levin VA, Prados MD, Yung WKA, Gleason MJ, Ictech S, Malec M. 1992. Treatment of recurrent gliomas with eflornithine. *J. Natl. Cancer Inst.* 84: 1432–37

84. Sarhan S, Knödgen B, Seiler N. 1992. Polyamine deprivation, malnutrition and tumor growth. *Anticancer Res.* 12:457–66

85. Sarhan S, Knödgen B, Seiler N. 1989. The gastrointestinal tract as polyamine source for tumor growth. *Anticancer Res.* 9:215–24

86. Seiler N, Sarhan S, Grauffel C, Jones R, Knödgen B, Moulinoux JP. 1990. Endogenous and exogenous polyamines in support of tumor growth. *Cancer Res.* 50:5077–83

87. Moulinoux J-P, Quemener V, Cipolla B, Guillé F, Havouis R, et al. 1991. The growth of MAT-LyLu rat prostatic adenocarcinoma can be prevented *in vivo* by polyamine deprivation. *J. Urol.* 146: 1408–12

88. Chamaillard L, Quemener V, Havouis R, Moulinoux J. 1993. Polyamine deprivation stimulates natural killer cell activity in cancerous mice. *Anticancer Res.* 13:1027–34

89. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC. 1994. Chemopreventive drug development: perspectives and progress. *Cancer Epidemiol. Biomarkers Prev.* 3:85–89

90. Kulkarni N, Zang E, Kelloff G, Reddy BS. 1992. Effect of the chemopreventive agents piroxicam and DL-α-difluoromethylornithine on intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* 13:995–1000

91. Love RR, Carbone PP, Verma AJ, Gilmore D, Carey P, et al. G. 1993. Randomized phase I chemoprevention dose-seeking study of α-difluoromethylornithine. *J. Natl. Cancer Inst.* 85:732–37

92. Creaven PJ, Pendyala L, Petrelli NJ. 1993. Evaluation of α-difluoromethylornithine as a potential chemopreventive agent: tolerance to daily oral administration in humans. *Cancer Epidemiol. Biomarkers Prev.* 2:243–47

93. Loprinzi CL, Messing EM. 1992. A prospective clinical trial of difluoromethylornithine (DFMO) in patients with resected superficial bladder cancer. *J. Cell. Biochem. Suppl.* 161: 153– 55

94. Fozard JR. 1987. The contragestational effects of ornithine decarboxylase inhibition. See Ref. 6, pp. 187–202

95. Brock M, Henley CM. 1994. Postnatal changes in cochlear polyamine metabolism in the rat. *Hear. Res.* 72:37–43

96. Williams K, Romano C, Dichter MA, Molinoff PB. 1991. Modulation of the NMDA receptor by polyamines. *Life Sci.* 48:469–98

97. Reynolds IJ. 1994. [^{3}H]CGP 39653 binding to the agonist site of the *N*-methyl-D-aspartate receptor is modulated by Mg^{2+} and polyamines independently of the arcaine-sensitive polyamine site. *J. Neurochem.* 63:54–62

98. Pegg AE, McCann PP. 1992. *S*-Adenosylmethionine decarboxylase as an enzyme target for therapy. *Pharmacol. Ther.* 56:359–77

99. Pegg AE. 1983. Inhibitors of *S*-adeno-

sylmethionine decarboxylase. *Methods Enzymol.* 94:239–47

100. Weitkamp ELC, Dixon HBF, Khomutov AR, Khomutov RM. 1991. Effect of magnesium ions on the inhibition of *S*-adenosylmethionine decarboxylase from *Escherichia coli* by [2-(aminooxy)ethyl](5′-deoxyadenosin-5′-yl)(methyl) sulphonium. *Biochem. J.* 277:643–45
101. Shantz LM, Stanley BA, Secrist JA, Pegg AE. 1992. Purification of human *S*-adenosylmethionine decarboxylase expressed in *Escherichia coli* and use of this protein to investigate the mechanism of inhibition by the irreversible inhibitors, 5′-deoxy-5′- [(3-hydrazinopropyl)methylamino]adenosine and 5′{[(Z)-4- amino-2-butenyl]methylamino}-5′ deoxyadenosine. *Biochemistry* 31:6848–55
102. Tekwani BL, Bacchi CJ, Secrist JA, Pegg AE. 1992. Irreversible inhibition of *S*-adenosylmethionine decarboxylase of *Trypanosoma brucei brucei* by *S*-adenosylmethionine analogues. *Biochem. Pharmacol.* 44:905–11
103. Pegg AE, Jones DB, Secrist JA III. 1988. Effect of inhibitors of *S*-adenosylmethionine decarboxylase on polyamine content and growth of L1210 cells. *Biochemistry* 27:1408–15
104. Kramer DL, Miller JT, Bergeron RJ, Khomutov R, Khomutov A, Porter CW. 1993. Regulation of polyamine transport by polyamines and polyamine analogs. *J. Cell. Physiol.* 155:399–407
105. Danzin C, Marchal P, Casara P. 1990. Irreversible inhibition of rat *S*-adenosylmethionine decarboxylase by 5′-{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine. *Biochem. Pharmacol.* 40:1499–1503
106. Wu Y, Woster PM. 1992. *S*-(5′-deoxy-5′adenosyl)-1-ammonio-4- (methylsulfonio)-2-cyclopentene: a potent enzyme-activated irreversible inhibitor of *S*-adenosylmethionine decarboxylase. *J. Med. Chem.* 35:3196–3201
107. Danzin C, Marchal P, Casara P. 1991. Enzyme-activated irreversible inhibitors of *S*-adenosylmethionine decarboxylase, a pyruvoyl enzyme. In *Enzymes Dependent on Pyridoxal Phosphate and Other Carbonyl Compounds as Cofactors,* ed. T Fukui, H Kagamiyama, K Soda, H Wada, pp. 445–47. Oxford: Pergamon
108. Wright PS, Byers TL, Cross-Doersen DE, McCann PP, Bitonti AJ. 1991. Irreversible inhibition of *S*-adenosylmethionine decarboxylase in *Plasmodium falciparum*-infected erythrocytes: growth inhibition in vitro. *Biochem. Pharmacol.* 41:1713–18
109. Byers TL, Casara P, Bitonti AJ. 1992. Uptake of the antitrypanosomal drug 5′{[(Z)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine (MDL 73811) by the purine transport system of *Trypanosoma brucei brucei. Biochem. J.* 283: 755–58
110. Stjernborg L, Heby O, Mamont P, Persson L. 1993. Polyamine-mediated regulation of *S*-adenosylmethionine decarboxylase expression in mammalian cells. Studies using 5′{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine, a suicide inhibitor of the enzyme. *Eur. J. Biochem.* 214:671–76
111. Suzuki T, Sadakata Y, Kashiwagi K, Hoshino K, Kakinuma Y, et al. 1993. Overproduction of *S*-adenosylmethionine decarboxylase in ethylglyoxal-bis(guanylhydrazone)-resistant mouse FM3A cells. *Eur. J. Biochem.* 215:247–53
112. Regenass U, Caravatti G, Mett H, Stanek J, Schneider P, et al. 1992. New *S*-adenosylmethionine decarboxylase inhibitors with potent antitumor activity. *Cancer Res.* 52:4712–18
113. Regenass U, Mett H, Stanek J, Mueller M, Kramer D, Porter CW. 1994. CGP48664, a new *S*-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. *Cancer Res.* 54:3210–17
114. Stanek J, Caravatti G, Caprano H, Furet P, Mett H, et al. 1993. *S*-adenosylmethionine decarboxylase inhibitors: new aryl and heteroaryl analogues of methylgloxal bis(guanylhydrazone). *J. Med. Chem.* 36:46–54
115. Stanek J, Caravatti G, Frei J, Furet P, Mett H, et al. 1993. 4-Amidinoindan-1-one 2′-amidinohydrazone: a new potent and selective inhibitor of *S*-adenosylmethionine decarboxylase. *J. Med. Chem.* 36:2168–71
116. Svensson F, Kockum I, Persson L. 1993. Diethylglyoxal bis(guanylhydrazone), a potent inhibitor of mammalian *S*-adenosylmethionine decarboxylase. Effects on cell proliferation and polyamine metabolism in L1210 leukemia cells. *Mol. Cell. Biochem.* 124:141–47
117. Bitonti AJ, Byers TL, Bush TL, Casara PJ, Bacchi CJ, et al. 1990. Cure of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* infections in mice with an irreversible inhibitor of *S*-adenosylmethionine decarboxylase. *Antimicrobial Agents Chemother.* 34: 1485–90
118. Bacchi CJ, Nathan HC, Yarlett N, Goldberg B, McCann PP, et al. 1992. Cure of murine *Trypanosoma brucei rhodesiense* infections with an *S*-adeno-

sylmethionine decarboxylase inhibitor. *Antimicrob. Agents Chemother.* 36: 2736–40

119. Yakubu MA, Majumder S, Kierszenbaum F. 1993. Inhibition of *S*-adenosyl-L-methionine (AdoMet) decarboxylase by the decarboxylated AdoMet analog 5′-{[(Z)-4-amino-2-butenyl] methyl amino}-5′-deoxyadenosine (MDL73811) decreases the capacities of *Trypanosoma cruzi* to infect and multiply within a mammalian host cell. *J. Parasitol.* 79: 525–32
120. Dowling RH, Fölsch UR, Löser C, eds. 1992. *Polyamines in the Gastrointestinal Tract.* Dordrecht: Kluwer. 538 pp.
121. Seiler N, Dezeure F. 1990. Polyamine transport in mammalian cells. *Int. J. Biochem.* 22:211–18
122. Khan NA, Quemener V, Moulinoux J. 1991. Polyamine membrane transport regulation. *Cell Biol. Int. Rep.* 15:9–24
123. Heston WDW. 1991. Prostatic polyamines and polyamine targeting as a new approach to therapy of prostatic cancer. *Cancer Surv.* 11:217–38
124. Byers TL, Wechter R, Nuttall M, Pegg AE. 1989. Expression of a human gene for polyamine transport in Chinese hamster ovary cells. *Biochem. J.* 263:745–52
125. Minchin R, Raso A, Martin RL, Ilett KF. 1991. Evidence for the existence of distinct transporters for the polyamines putrescine and spermidine in B16 melanoma cells. *Eur. J. Biochem.* 200:457–62
126. Byers TL, Pegg AE. 1990. Regulation of polyamine transport in Chinese Hamster Ovary cells. *J. Cell. Physiol.* 143: 460–67
127. Byers TL, Wechter R, Hu R, Pegg AE. 1994. Effects of the *S*-adenosylmethionine decarboxylase inhibitor, 5′-{[(Z)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine, on cell growth and polyamine metabolism and transport in Chinese hamster ovary cell cultures. *Biochem. J.* 303:89–96
128. Persson L, Holm I, Ask A, Heby O. 1988. Curative effect of DL-2-difluoromethylornithine on mice bearing mutant L1210 leukemia cells deficient in polyamine uptake. *Cancer Res.* 48:4807–11
129. Ask A, Persson L, Heby O. 1993. Increased survival of L1210 leukemic mice by prevention of the utilization of extracellular polyamines. Studies using a polyamine-uptake mutant, antibiotics and a polyamine-deficient diet. *Cancer Lett.* 66:29–34
130. Byers TL, Pegg AE. 1989. Investigation of the properties and function of the polyamine transport system using Chinese hamster ovary cells. *Am. J. Physiol.* 256:C545–53
131. Mitchell JLA, Judd GG, Bareyal-Leyser A, Ling SY. 1994. Feedback repression of polyamine transport is mediated by antizyme in mammalian tissue culture cells. *Biochem. J.* 299:19–22
132. Murakami Y, Tanaka K, Matsufuji S, Miyazaki Y, Hayashi S. 1992. Antizyme, a protein induced by polyamines, accelerates the degradation of ornithine decarboxylase in Chinese-hamster ovary-cell extracts. *Biochem. J.* 283: 661–64
133. Mitchell JLA, Dively RR, Bareyal-Leyser A, Mitchell JL. 1992. Abnormal accumulation and toxicity of polyamines in a difluoromethylornithine-resistant HTC cell variant. *Biochim. Biophys. Acta* 1136:136–42
134. Poulin R, Lakanen JR, Coward JK, Pegg AE. 1993. Enhancement of the spermidine uptake system and lethal effects of spermidine overaccumulation in ornithine decarboxylase-overproducing L1210 cells under hypoosmotic stress. *J. Biol. Chem.* 268:4690–98
135. Tome ME, Fiser SM, Gerner EW. 1994. Consequences of aberrant ornithine decarboxylase regulation in rat hepatoma cells. *J. Cell. Physiol.* 158:237–44
136. Chang BK, Libby PR, Bergeron RJ, Porter CW. 1988. Modulation of polyamine biosynthesis and transport by oncogene transfection. *Biochem. Biophys. Res. Commun.* 157:264–70
137. Khan NA, Quemener V, Moulinoux JP. 1992. Phorbol esters augment polyamine transport by influencing Na^+-K^+ pump in murine leukemia cells. *Exp. Cell Res.* 199:378–82
138. Khan NA, Sezan A, Quemener V, Moulinoux JP. 1993. Polyamine transport regulation by calcium and calmodulin: role of Ca^{2+}-ATPase. *J. Cell. Physiol.* 157:493–501
139. Porter CW, Jänne J. 1987. Modulation of antineoplastic drug action by inhibitors of polyamine biosynthesis. See Ref. 6, pp. 203–48
140. Holley J, Mather A, Cullis P, Symons MR, Wardman P, et al. 1992. Uptake and cytotoxicity of novel nitroimidazole-polyamine conjugates in Ehrlich ascites tumour cells. *Biochem. Pharmacol.* 43:763–69
141. Holley JL, Mather A, Wheelhouse RT, Cullis PM, Hartley JA, et al. 1992. Targeting of tumor cells and DNA by a chlorambucil-spermidine conjugate. *Cancer Res.* 52:4190–95
142. Yuan Z, Egorin NJ, Rosen M, Simon MA, Callery PS. 1994. Cellular phar-

macology of N^1- and N^8-aziridinyl analogues of spermidine. *Cancer Res.* 54: 742–48
143. Stark PA, Thrall BD, Meadows GG, Abdel-Monem MM. 1993. Synthesis and evaluation of novel spermidine derivatives as targeted cancer chemotherapeutic agents. *J. Med. Chem.* 35:4264–69
144. Groblewski GE, Hargattai PT, Seidel ER. 1992. Ca^{2+}/calmodulin regulation of putrescine uptake in cultured gastrointestinal epithelial cells. *Am. J. Physiol.* 262:C1356–63
145. Feuerstein BG, Williams LD, Basu HS, Marton LJ. 1991. Implications and concepts of polyamine-nucleic acid interactions. *J. Cell. Biochem.* 46:37–47
146. Gosule LC, Schellman JA. 1978. DNA condensation with polyamines. *J. Mol. Biol.* 121:311–26
147. Behe M, Felsenfeld G. 1981. Effects of methylation on a synthetic polynucleotide: the B-Z transition in poly(dG-m^5dC)•poly(dG-m^5dC). *Proc. Natl. Acad. Sci. USA* 78:1619–23
148. Jain S, Zon G, Sundarlingam M. 1989. Hexagonal crystal structure of A-DNA octamer d(GTGTACAC) and its comparison with the tetragonal structure, correlated variations in helical parameters. *Biochemistry* 28:2360–64
149. Hewish DR, Burgoyne LA. 1973. Chromatin sub-structure: the digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. *Biochem. Biophys. Res. Commun.* 52: 504–10
150. Sen D, Crothers DM. 1986. Condensation of chromatin: role of multivalent cations. *Biochemistry* 25:1495–1503
151. Morgan JE, Blankenship JW, Mathews HR. 1987. Polyamines and acetyl polyamines increase the stability and alter the conformation of nucleosome core particles. *Biochemistry* 26:3643–49
152. Hougaard DM, DelCastillo AM, Larsson LI. 1988. Endogenous polyamines associate with DNA during its condensation in mammalian tissue. A fluorescence cytochemical and immunocytochemical study of polyamines in rat liver. *Eur. J. Cell Biol.* 45:311–14
153. Snyder RD. 1989. Polyamine depletion is associated with altered chromatin structure in HeLa cells. *Biochem. J.* 260:697–704
154. Basu HS, Sturkenboom MCJ, Delcros JG, Csokan PP, Szollosi J, et al. 1992. Effect of polyamine depletion on chromatin structure in U-87 MG human brain tumour cells. *Biochem. J.* 282: 723–27
155. Basu HS, Wright WD, Deen DF, Roti-Roti J, Marton LJ. 1993. Treatment with a polyamine analog alters DNA-matrix association in HeLa cell nuclei: a nucleoid halo assay. *Biochemistry* 32:4073–76
156. Müller HP, Pryciak PM, Varmus HE. 1994. Retroviral integration machinery as a probe for DNA structure and associated proteins. In press
157. Deleted in proof
158. Dolan ME, Fleig MJ, Feuerstein BG, Basu HS, Luk GD, et al. 1994. Effect of 1,19-bis-(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4) on human tumor xenografts. *Cancer Res.* 54:4698–4702
159. Oshima T, Kawahata S. 1983. Homocaldopentamine: a new naturally occurring pentamine. *J. Biochem.* 93: 1455–56
160. Basu HS, Marton LJ. 1987. The interaction of spermine and pentamines with DNA. *Biochem. J.* 244:243–46
161. Basu HS, Marton LJ, Pellarin M, Deen DF, Bergeron RJ, Feuerstein BG. 1994. Design and testing of novel cytotoxic polyamine analogs. *Cancer Res.* In press
162. Ghoda L, Basu HS, Porter CW, Marton LJ, Coffino P. 1992. Role of ornithine decarboxylase suppression and polyamine depletion in the antiproliferative activity of polyamine analogs. *Mol. Pharmacol.* 42:302–6
163. Albanese L, Bergeron R, Pegg AE. 1993. Investigations of the mechanism by which mammalian cell growth is inhibited by N^1,N^{12}-bis(ethyl)spermine. *Biochem. J.* 291:131–37
164. Vertino PM, Beerman TA, Kelly EJ, Bergeron RJ, Porter CW. 1991. Selective cellular depletion of mitochondrial DNA by the polyamine analog N^1,N^{12}-bis(ethyl)spermine and its relationship to polyamine structure and function. *Mol. Pharmacol.* 39:487–94
165. Fukuchi J, Kashiwagi K, Kusama-Eguchi K, Terao K, Shirahata A, Igartashi K. 1992. Mechanism of the inhibition of cell growth by N^1-,N^{12}-bis(ethyl)spermine. *Eur. J. Biochem.* 209:689–96
166. Majudmer S, Kierszenbaum F. 1993. Inhibition of host cell invasion and intracellular replication of *Trypanosoma cruzi* by *N,N'*-bis(benzyl)-substituted polyamine analogs. *Antimicrob. Agents Chemother.* 37:2235–38
167. Bitonti AJ, Dumont JA, Bush TL, Edwards ML, Stemerick DM, et al. 1989. Bis(benzyl)polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (*Plasmodium falciparum*) in vitro and in combination with α-difluoromethylornithine cure

murine malaria. *Proc. Natl. Acad. Sci. USA* 86:651–55
168. Prakash NJ, Bowlin TL, Edwards ML, Sunkara PS, Sjoerdsma A. 1990. Antitumor activity of a novel synthetic polyamine analogue, *N,N'*-Bis-[3- (ethylamino)-propyl]-1–7-heptane diamine: potentiation by polyamine oxidase inhibitors. *Anticancer Res.* 10:1281–88
169. Bolkenius FN, Seiler N. 1989. New substrates of polyamine oxidase. *Biol. Chem. Hoppe-Seyler* 370:525–31
170. Bitonti AJ, Dumont JA, Bush TL, Stemerick DM, Edwards ML, McCann PP. 1990. Bis(benzyl)polyamine analogs as novel substrates for polyamine oxidase. *J. Biol. Chem.* 265:382–88
171. Bowlin TL, Prakash NJ, Edwards ML, Sjoerdsma A. 1991. Participation of T-lymphocytes in the curative effect of a novel synthetic polyamine analogue, *N,N'*-bis [3-(ethylamino) propyl]-1,7-heptanediamine, against L1210 leukemia *in vivo*. *Cancer Res.* 51:62–66
172. Bernacki RJ, Bergeron RJ, Porter CW. 1992. Antitumor activity of *N,N'*-bis-(ethyl)spermine homologues against human MALME-3 melanoma xenografts. *Cancer Res.* 52:2424–30
173. Deleted in proof
174. Chang BK, Bergeron RJ, Porter CW, Liang V. 1992. Antitumor effects of *N*-alkylated polyamine analogues in human pancreatic adenocarcinoma models. *Cancer Chemother. Pharmacol.* 30: 179–82
175. Marton LJ. 1988. Modification of the cytotoxicity of DNA-directed chemotherapeutic agents by polyamine depletion. In *Mechanisms of Drug Resistance in Neoplastic Cells*, ed. PV Wooley, KD Tew, pp. 183–94. Orlando, FL: Academic
176. Bakic M, Chan D, Freireich EJ, Marton LJ, Zwelling LA. 1987. The effect of polyamine depletion by α-difluoromethylornithine (DFMO) or (2R,5R)-6-heptyne-2,5-diamine (R,R-MAP) on drug-induced topoisomerase II-mediated DNA cleavage and cytotoxicity in human and murine leukemia cells. *Cancer Res.* 47:6437–43
177. Denstman SC, Ervin SJ, Casero RA. 1987. Comparison of the effects of treatment with the polyamine analogue N^1,N^8 bis(ethyl)spermidine (BESpd) or difluoromethylornithine (DFMO) on the topoisomerase II mediated formation of 4'-(9-acridinylamino) methanesulfon-M-anisidide (m-AMSA) induced cleavable complex in the human lung carcinoma line NCIH157. *Biochem. Biophys. Res. Commun.* 149:194–202
178. Snyder RD, Schroeder KK. 1994. Radiosensitivity of polyamine-depeleted HeLa cells and modulation by the aminothiol WR-1065. *Radiat. Res.* 137: 67–75
179. Fike JR, Gobbel GT, Marton LJ, Seilhan TM. 1994. Radiation brain injury is reduced by the polyamine inhibitor α-difluoromethylornithine. *Radiat. Res.* 138: 99–106