Metabolic Stability of α-Methylated Polyamine Derivatives and Their Use as Substitutes for the Natural Polyamines*

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Metabolically stable polyamine derivatives may serve as useful surrogates for the natural polyamines in studies aimed to elucidate the functions of individual polyamines. Here we studied the metabolic stability of α-methylspermidine, α-methylspermine, and bis-α-methyl spermine, which all have been reported to fulfill many of the putative physiological functions of the natural polyamines. In vivo studies were performed with the transgenic rats overexpressing spermidine/spermine N\(^2\)-acetyltransferase. α-Methylspermidine effectively accumulated in the liver and did not appear to undergo any further metabolism. On the other hand, α-methylspermine was readily converted to α-methylspermidine and spermidine; similarly, bis-α-methyl spermine was converted to α-methylspermidine to some extent, both conversions being inhibited by the polyamine oxidase inhibitor N\(^1\),N\(^2\)-bis(2,3-butadienyl)-1,4-butanediamine. Furthermore, we used recombinant polyamine oxidase, spermidine/spermine N\(^1\)-acetyltransferase, and the recently discovered spermine oxidase in the kinetic studies. In vitro studies confirmed that methylation did not protect spermine analogs from degradation, whereas the spermidine analog was stable. Both α-methylspermidine and bis-α-methylspermine overcame the proliferative block of early liver regeneration in transgenic rats and reversed the cytostasis induced by an inhibition of ornithine decarboxylase in cultured fetal fibroblasts.

Although the requirement of the natural polyamines spermidine, spermine, and their precursor putrescine for the growth of mammalian cells is extremely well documented, their specific functions in proliferative processes are largely unknown (1). Some of the published data appear to assign a central role to spermidine, whereas putrescine is supposed to serve as its precursor and spermine as a storage pool convertible back to spermidine. For the elucidation of the physiological roles of individual polyamines, metabolically stable derivatives of polyamines fulfilling their specific cellular functions would be extremely valuable. Methyl derivatives of spermidine and spermine have been used as substitutes for the natural polyamines both in vitro and in vivo. α-Methylspermidine (MeSpd),\(^1\) α-methylspermine (MeSpm), and bis-α-methylspermine (1,12-dimethyl spermine, Me\(_2\)Spm) are equally effective as the natural polyamines in inducing the conversion of right-handed B-DNA to left-handed Z-DNA (2). In addition to spermidine and spermine, cytostasis that resulted from the inhibition of the S-adenosylmethionine decarboxylase can be reversed by MeSpd but not by Me\(_2\)Spm (3). Spermidine, spermine (because of its conversion to spermidine), and MeSpd serve as substrates for the synthesis of deoxyhypusine (an integral part of eukaryotic initiation factor 5A), whereas Me\(_2\)Spm does not (3). Interestingly, all of the mentioned methylated derivatives of spermidine and spermine have been reported to reverse the cytostasis induced by difluoromethylornithine, a specific inhibitor of mammalian ornithine decarboxylase (4). MeSpd appears to undergo slow conversion to MeSpm. Me\(_2\)Spm has not been reported to be metabolized. MeSpd is not a substrate for spermidine/spermine N\(^1\)-acetyltransferase (SSAT) (4). In line with the above studies, we found that MeSpd prevents zinc-induced pancreatitis and restores liver regeneration in transgenic rats overexpressing SSAT under the control of the metallothionein promoter (5). Under these conditions where polyamine catabolism was intensely activated, the natural polyamines could not be used as they were rapidly acetylated and degraded with no net tissue accumulation (5).

Here we studied the metabolic stability of the three methylated polyamine derivatives, namely MeSpd, MeSpm, and Me\(_2\)Spm. Transgenic rats with activated polyamine catabolism because of overexpression of SSAT were used for the experiments in vitro, immortalized fibroblasts derived from the same animals and liver extracts from normal rats were used for experiments in vitro. The metabolisms of these polyamine analogs were also studied by using purified recombinant human polyamine oxidase, human spermine oxidase, and mouse SSAT. We found no evidence indicating that MeSpd would be further metabolized in vivo and the compound was also a poor substrate for the studied enzymes in vitro. Surprisingly, both MeSpd and Me\(_2\)Spm were catabolized at the methylated ends both in vivo and in vitro although Me\(_2\)Spm was far more stable. Furthermore, MeSpd and Me\(_2\)Spm were competitive inhibitors of SSAT.

* The abbreviations used are: MeSpd, α-methyl spermidine; SSAT, spermidine/spermine N\(^1\)-acetyltransferase; PAO, polyamine oxidase; SMO, spermine oxidase; MeSpm, α-methyl spermine; Me\(_2\)Spm, bis-α-methyl spermine.

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6595
Both MeSpd and MeSpm restored early liver regeneration in transgenic rats with activated polyamine catabolism, in which a profound spermidine and spermine depletion developed after partial hepatectomy. Although MeSpm was metabolized to MeSpd to some extent, the restoration of the proliferative activity was attributable to MeSpd and not to the low contents of MeSpd.

MATERIALS AND METHODS

Chemicals—α-Methylated polyamine analogs were synthesized as described in Refs. 6 and 7 and administered in saline. The PAO inhibitor MDL72527 (N',N'-bis(2,3-butadienyl)-1,4-butanediamine) was a generous gift from Hoechst-Roussel. All other chemicals were purchased from Sigma and Fluka. [6-3H]Thymidine (specific radioactivity 18 Ci/mmol) was obtained from PerkinElmer Life Sciences.

Transgenic Rats and the Studies of α-Methylated Spermine Analogs—The production of transgenic rats has been described in detail earlier (8). Partial hepatectomy of the transgenic rats was carried out essentially as described in Ref. 9. Treatments before partial hepatectomy and the determination of DNA synthesis were carried out as described in the legend to Ref. 5. Transgenic 10-week-old male rats were injected twice with MDL72527 (50 mg/kg intraperitoneal) at 16-h intervals to inactive PAO according to Bolkenius et al. (10) and further with MeSpd, MeSpm, or Me2Spm twice (25 mg/kg intraperitoneal) 2 and 8 h after the second MDL72527 treatment. Animals not treated with the PAO inhibitor were injected with α-methylated spermine analogs or MeSpd at the same time points as MDL72527-pretreated animals. The animals were sacrificed 24 h after the second MDL72527 injection; liver pieces were frozen in liquid nitrogen and homogenized in the standard buffer (25 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 1 mM dithiothreitol) using a Polytron (Brinkman Instruments). PAO, however, could not be concentrated nor washed in a similar way as it bound to the filter membrane very tightly. Desalting of the PAO elutes after affinity chromatography were performed with NAP-10™ gel filtration columns according to the manufacturer's instructions (Amersham Biosciences).

Production of Recombinant SSAT—To obtain the SSAT cDNA (exons only) the pool of the first strand cDNA was PCR amplified using primers 5'-TAGTGCAGCAAGTGGAGACCCAGC-3' and 5'-CTAGCCGGCGACGTGTGCTAGTCA-3'. The resultant PCR product was gel-purified, restriction enzyme digested, and cloned. For protein expression the coding sequence of SSAT was amplified by PCR using the cloned SSAT cDNA as a template. The primers used are: 5'-TATGCGATACATATGATGTCATTGCTACTAC-3' and 5'-CTACTGCAAGCTTTCCTGCTG-3'. The upstream primer contained His8 and an enterokinase cleavage site. The NdeI/XhoI-digested PCR products were cloned into the PET 30a vector (Novagen, Inc., Madison, WI) and sequenced. For the protein production Escherichia coli strain BL21 (DE3) was used as the host and the recombinant protein was purified under native conditions using nickel-nitriotropic acid His Bind resin (Novagen) according to the manufacturer's instructions.

All purified proteins were analyzed by SDS-PAGE, and the protein concentrations were measured using the Bio-Rad Protein assay (Bio-Rad). The kinetic studies of the oxidizing enzymes were performed in duplicates with 4 to 6 different 10 to 1000 μM substrate concentrations. The SMO and PAO reactions were carried out in a total volume of 180 μl of 10 mM Tris buffer as described in Ref. 5. The extract reaction mixture was added to proceed for 10 to 30 min at 37 °C before addition of 20 μl of 100 μM dianboxylase acid. The kinetic study of SSAT was performed with 100, 400, 700, and 1000 μM 2-oxo-spermidine or 2-oxo-spermine as a substrate and 100, 400, 700, and 1000 μM MeSpd or MeSpm as a competitive inhibitor.

Determination of Ornithine Decarboxylase, SSAT, PAO Activities, and Polyamines—The activity of ornithine decarboxylase was assayed as described previously (13), and SSAT activity was assayed as described in Ref. 14. The PAO activity was assayed essentially as described by Kumazawa et al. (15) using radioactive N',N'-diacetylepiperidine, instead of N'-acetylspermine, as the substrate. High performance liquid chromatography was used to determine the concentrations of the polyamines and their methylated analogs essentially as described by Hyvönen et al. (16).

RESULTS

Purity of α-Methylated Polyamine Analogs: NMR and High Performance Liquid Chromatography Studies—H and 13C spectra were recorded on an Avance DRX spectrometer operating at 500.13 MHz. NMR showed >99.5% purity for all studied polyamine analogs. MeSpd, MeSpm, and Me2Spm
were further tested with high performance liquid chromatography for typical orthophtaldehyde reactive impurities. In 10,000 pmol of each sample, the amount of orthophtaldehyde reactive impurities were less than 50 pmol (results not shown).

Stability of the Analogs in the Liver of SSAT Transgenic Rats—Fig. 1 displays the typical polyamine pattern in the liver of SSAT overexpressing rats. Putrescine pool was greatly increased, whereas the spermine level was decreased when compared with wild-type rat liver where putrescine remains almost undetectable (8). An exposure of the rats to the polyamine oxidase inhibitor MDL72527 expectedly greatly reduced putrescine content and expanded the pool of N₁-acetylspermidine (Fig. 1A). All the analogs accumulated in the liver of the transgenic rats, MeSpd showing the highest tissue concentrations (Fig. 1B, C, D). The latter analog likewise appeared to be metabolically stable and, in contrast to an earlier report (4), we found no evidence that MeSpm was converted to MeSpd (Fig. 1B). As indicated in Fig. 1C, MeSpm was effectively converted to MeSpd. The latter conversion was markedly inhibited by the MDL72527 compound (Fig. 1C). Fig. 1D shows that also Me₂Spm was converted to MeSpd, but to a much lesser extent than MeSpm. The conversion of MeSpm to MeSpd was totally prevented by MDL72527 (Fig. 1D).

Effect of the Analogs on SSAT and PAO Activities in the Liver of SSAT Transgenic Rats—The methylated polyamine analogs did not appear to be very effective inducers of SSAT activity. In fact, only Me₂Spm significantly increased the hepatic SSAT activity, which was further enhanced by combining the latter analog with MDL72527 (Fig. 2A). However, the modest induction of the SSAT activity in response to the methylated analogs may be tissue-specific as the analogs much more effectively induced SSAT activity in the pancreas of the transgenic rats (results not shown). The analogs had little effect on PAO activity, whereas the used doses of MDL72527 alone or in combination with the analogs virtually completely inhibited PAO activity (Fig. 2B). All the analogs stimulated ornithine decarboxylase activity to some extent (results not shown).

Studies with Liver Extracts Obtained from Wild-type Rats—We used crude liver extracts to study the stability of
metabolism of spermine and its methylated derivatives was fully or partially blocked by MDL72527 (Table I). Exogenous spermine was converted to spermidine even in the absence of benzaldehyde but the conversion was greatly enhanced by the latter compound. Inclusion of 1 mM MeSpd yielded substantial amounts of spermidine and MeSpd already without benzaldehyde, which further enhanced the conversions by a factor of about 3 (Table I). The only product derived from 1 mM MeSpd was MeSpd, the formation of which was stimulated nearly 5-fold by benzaldehyde. It is interesting to note that MeSpd was even more effectively converted to MeSpd in the presence of benzaldehyde than was MeSpd. The metabolism of spermine and its methylated derivatives was fully or partially blocked by MDL72527 (Table I).

Polymamines and Their Analogs as Substrates for Recombinant SSAT, PAO, and SMO—We also produced purified recombinant mouse SSAT, human PAO, and human SMO to study the substrate specificities of these enzymes. SSAT and SMO purifications yielded very pure enzymes according to SDS-PAGE, whereas purification of PAO resulted in about 80% pure enzyme. However, the contaminating bacterial protein did not have any PAO-like activity (data not shown).

$K_m$ and $V_{max}$ values of spermidine and spermine as substrates for SSAT were 52 $\mu$M and 2.4 nmol/min/$\mu$g; 33 $\mu$M and 0.43 nmol/min/$\mu$g, respectively. The $K_m$ value of MeSpd with spermidine as substrate was 144 $\mu$M, $K_m$ values of Me$_2$Spd with spermidine and spermine as substrates were 30 $\mu$M and 34 $\mu$M, respectively. MeSpd was not tested as it could serve as a substrate for SSAT at the unmethylated end. The kinetic values for different analogs are listed in Table II in respect to both recombinant oxidases. PAO strongly preferred $N^1$-acetylspermidine; furthermore, PAO was able to oxidize methylated spermine derivatives, but not MeSpd, to spermidine and MeSpd. In the presence of benzaldehyde, the $K_m$ values for PAO decreased and the enzyme readily used spermine and its analogs but not very effectively spermidine or MeSpd, probably because of the inhibitory $N^8$ Schiff base. Spermidine, $N^1$-acetyl-spermidine, and MeSpd did not serve as substrates for SMO, whereas spermine and its mono- and dimethyl derivatives did. Expectedly, neither oxidase produced spermidine from 1-acetylspermidine (Table I). Inclusion of 1 mM MeSpd appeared to yield some putrescine in the presence of benzaldehyde, but this putrescine was in all likelihood derived from endogenous spermine (Table I). Exogenous spermine was converted to spermidine even in the absence of benzaldehyde but the conversion was greatly enhanced by the latter compound. Inclusion of 1 mM MeSpd yielded substantial amounts of spermidine and MeSpd already without benzaldehyde, which further enhanced the conversions by a factor of about 3 (Table I). The only product derived from 1 mM Me$_2$Spd was MeSpd, the formation of which was stimulated nearly 5-fold by benzaldehyde. It is interesting to note that Me$_2$Spd was even more effectively converted to MeSpd in the presence of benzaldehyde than was MeSpd. The metabolism of spermine and its methylated derivatives was fully or partially blocked by MDL72527 (Table I).

Restoration of Early Liver Regeneration in Transgenic Rats Overexpressing SSAT—We have earlier found that partial hep-
The liver extracts were prepared as described under "Materials and Methods" and incubated for 60 min at +37 °C with the indicated additions. Reactions with MDL72527 were preincubated for 10 min before the drug addition. The concentration of added polyamine was 1 mM, benzaldehyde 5 mM, and MDL72527 250 μM. The abbreviations used are: Put, putrescine; N²-AcSpd, N²-acetylspersimidine; Spd, spermidine; Spm, spermine; BA, benzaldehyde.

| Treatment | Polyamine or analog | pmol/mg protein |
|-----------|---------------------|-----------------|
| Liver extracts | Put | Spd | MeSpd | Spm |
| without 1 h + 37 °C incubation | ND | 796 ± 27 | 1,490 ± 190 |
| after 1 h + 37 °C incubation | 70 ± 15 | 824 ± 68 | 1,270 ± 88 |
| + 5 mM BA | 635 ± 22 | 825 ± 110 | 20 ± 34 |
| + 250 μM MDL72527 | 51 ± 24 | 739 ± 110 | 1,430 ± 120 |
| + 5 mM BA + 250 μM MDL72527 | 4 ± 5 | 835 ± 49 | 1,270 ± 110 |
| 1 mM N²-AcSpd | 18,900 ± 940 | 1,120 ± 100 | 1,520 ± 310 |
| + 5 mM BA | 15,100 ± 1100 | 1,080 ± 210 | 1,190 ± 67 |
| + 250 μM MDL72527 | ND | 1,210 ± 140 | 1,300 ± 74 |
| + 5 mM BA + 250 μM MDL72527 | ND | 899 ± 150 | 1,600 ± 93 |
| 1 mM Spd | 186 ± 24 | — b | 905 ± 78 |
| + 5 mM BA | 3330 ± 290 | — b | 310 ± 83 |
| + 250 μM MDL72527 | 44 ± 30 | — b | 876 ± 78 |
| + 5 mM BA + 250 μM MDL72527 | 9 ± 10 | — b | 784 ± 51 |
| 1 mM MeSpd | ND | 394 ± 10 | — b | 839 ± 130 |
| + 5 mM BA | 1030 ± 55 | 620 ± 74 | — b | 210 ± 72 |
| + 250 μM MDL72527 | ND | 387 ± 9 | — b | 734 ± 55 |
| + 5 mM BA + 250 μM MDL72527 | ND | 344 ± 12 | — b | 789 ± 120 |
| 1 mM Spm | ND | 3,140 ± 390 | — b | 4,350 ± 343 |
| + 5 mM BA | ND | 21,000 ± 1700 | — b | 4,440 ± 353 |
| + 250 μM MDL72527 | ND | 397 ± 64 | — b | 735 ± 18 |
| + 5 mM BA + 250 μM MDL72527 | ND | 659 ± 39 | — b | 389 ± 33 |
| 1 mM MeSpm | ND | 1,830 ± 170 | 3,100 ± 390 | 441 ± 36 |
| + 5 mM BA | ND | 6,240 ± 110 | 9,220 ± 270 | 343 ± 25 |
| + 250 μM MDL72527 | ND | 735 ± 99 | ND | 375 ± 18 |
| + 5 mM BA + 250 μM MDL72527 | ND | 568 ± 140 | ND | 389 ± 33 |
| 1 mM Me₂Spm | ND | 744 ± 89 | 4,390 ± 680 | 1,110 ± 140 |
| + 5 mM BA | ND | 999 ± 66 | 20,400 ± 1,300 | 640 ± 85 |
| + 250 μM MDL72527 | ND | 697 ± 81 | 617 ± 32 | 968 ± 120 |
| + 5 mM BA + 250 μM MDL72527 | ND | 746 ± 42 | 602 ± 28 | 1,050 ± 61 |

a ND, not detected.
b The beginning of the reaction polyamine equaled 84,700 pmol/mg protein.

Metabolic Stability of α-Methylated Polyamine Derivatives

The enzyme activities were measured as described under “Materials and Methods.” Methylspermine can be catabolized from either end of the spermine backbone. The abbreviations used are: SMO, spermineoxidase; BA, benzaldehyde; N²-AcSpd, N²-acetylspersimidine; Spd, spermidine; Spm, spermine.

| Substrate | Kₘ (μM) | Vₘₐₓ (pmol/mg protein/min) |
|-----------|---------|----------------------------|
| PAO | PAO + 5 mM BA | SMO | PAO | PAO + 5 mM BA | SMO |
| N²-AcSpd | 14 | 28 | 5900 | 4000 |
| Spd | 15 | 16 | 140 |
| MeSpd | 16 | 16 | 140 |
| Spm | 47 | 9.3 | 20 | 340 | 4600 | 12000 |
| MeSpd | 19 b | 12 b | 67 | 530 a | 4000 a | 2300 a |
| Me₂Spd | 17 b | 11 b | 34 b | 340 b | 1500 b | 1100 b |

a Kinetic values for the unmethylated end.
b Kinetic values for the methylated end.

The liver extracts were prepared as described under “Materials and Methods” and incubated for 60 min at +37 °C with the indicated additions. Reactions with MDL72527 were preincubated for 10 min before the drug addition. The concentration of added polyamine was 1 mM, benzaldehyde 5 mM, and MDL72527 250 μM. The abbreviations used are: Put, putrescine; N²-AcSpd, N²-acetylspersimidine; Spd, spermidine; Spm, spermine; BA, benzaldehyde.

Liver regeneration could be restored by a prior administration of MeSpd (5). Table III shows thymidine incorporation in livers of transgenic rats before and 24 h after partial hepatectomy. As shown, thymidine incorporation remained at the preoperative level at 24 h in untreated rats, whereas small doses (5 mg/kg) of MeSpd only insignificantly increased DNA synthesis. Higher doses (25 mg/kg) of both MeSpd and Me₂Spd resulted in about a 10-fold stimulation of DNA synthesis. Table III also lists the hepatic pools of poly-
amines without being converted to MeSpd.

levels remained unaffected in all animal groups at about 100
Bis-
of 20
the observed stimulation of DNA synthesis after Me2Spm was
late thymidine incorporation (Table III). It thus appears that
achieved with the smaller dose of MeSpd that failed to stimu-
compound remained clearly below (about half) the level
was converted to MeSpd, the hepatic concentration of the latter

Accumulation of polyamines, their analogs, and thymidine incorporation in regenerating liver

| Time of regeneration/treatment | Polyamines | Thymidine |
|-------------------------------|------------|-----------|
|                               | Put        | Spd       | MeSpd     | Spm       | Me2Spm    | pmol/mg tissue | cpm/mg liver |
| 0 h                           | 1030 ± 95  | 1170 ± 140| 158 ± 45  | 5640 ± 800|
| 24 h                          | 4600 ± 710 | 405 ± 91  | 45 ± 31   | 6210 ± 600|
| 24 h + MeSpd (2 × 5 mg)       | 4540 ± 690 | 183 ± 47  | 462 ± 41  | 14600 ± 4800|
| 24 h + MeSpd (2 × 25 mg)      | 3280 ± 600 | 278 ± 130 | 1070 ± 230| 60100 ± 22000|
| 24 h + MeSpd (2 × 25 mg)      | 4300 ± 250 | 241 ± 28  | 213 ± 98  | 422 ± 110 |

\(a \ p < 0.001.
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depletions of the natural polyamines spermidine and spermine
at 24 h after the operation in the transgenic rats, whereas the
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DISCUSSION

The natural polyamines spermidine and spermine are ultimately
converted to putrescine via the concerted action of
SSAT and PAO. As PAO strongly prefers acetylated poly-
amines to the unmodified spermidine and spermine, SSAT is
generally considered as the rate-controlling enzyme of the
back-conversion pathway. However, when working with SSAT-
deficient mouse embryonic stem cells we found that SSAT is
essentially necessary for the conversion of spermidine to
putrescine, but not for the degradation of spermine to spermidine
(19). In fact, the targeted cells appeared to convert spermine to
spermidine much more efficiently than did their wild-type
counterparts (19). The conversion of spermine to spermidine in
the absence of SSAT activity is obviously attributable to a
recently discovered oxidase, which, when first cloned, was
thought to be PAO (20), but was later identified as a novel
flavin-containing spermine oxidase (21). Unlike PAO, SMO
strongly prefers spermine to its acetylated derivatives. Sper-
midine is not degraded at all, but monoethylspermine is as a
good substrate for SMO as is spermine (21, 22). SMO, although
inhibited to some extent, is much more resistant to the PAO
inhibitor MDL72527 (21).

The present results have revealed that both MeSpd and
Me2Spm serve as substrates for both PAO and SMO in vitro and
in vivo. MeSpd yielded both spermidine and MeSpd, whereas
Me2Spm was converted only to MeSpd. Like in the case of
MeSpd (4), it is highly unlikely that doubly methylated spermi-
ne would serve as a substrate for SSAT. This view is supported
by the findings that in the presence of benzaldehyde in vitro
(Table I) Me2Spm was converted to MeSpd much more effect-
ively than MeSpd, whereas in vivo (Fig. 1C and D), the latter
compound yielded many times more MeSpd than its bis-meth-
ylated counterpart.

The results obtained with crude liver extracts and transgenic
animals were largely confirmed with the use of purified recom-
binant PAO, SMO, and SSAT. Whereas PAO strongly preferred
N1-acetyl spermidine as a substrate, SMO most effectively
degraded spermine, but did not use spermidine or any of its tested
analogs as substrates. The methyl derivatives of spermine were
readily metabolized by SMO and PAO (in the presence of benza-
aldehyde) (Table II). PAO did not use MeSpd as a substrate and
it was only poorly metabolized when supplemented with benzaldehyde (Table II). The recombinant SSAT studies confirmed that MeSpd and Me₂Spm were not acetylated. However, they were competitive inhibitors of SSAT.

Our earlier studies with transgenic rats overexpressing SSAT have indicated that partial hepatectomy of these animals resulted in an induction of SSAT and a profound depletion of the hepatic spermidine pool that was associated with failure to initiate liver regeneration (5, 18). Liver regeneration could be fully restored by prior administration of MeSpd (5). Based on these studies and earlier work indicating that partial hepatectomy rapidly elevates the hepatic spermidine, but not spermine, pool, we assigned a critical role to spermidine in liver regeneration (5, 18). However, the present results indicated that liver regeneration, as judged by thymidine incorporation, appeared to be slow yielding insufficient levels of MeSpd for the hepatic spermidine pool that was associated with failure to initiate liver regeneration (5, 18). However, the present results indicated that liver regeneration, as judged by thymidine incorporation, was equally well restored by Me₂Spm, even at lower concentrations than required for MeSpd (Table III), indicating that liver regeneration by Me₂Spm as directly attributable to a polyamine (spermine) function, as Me₂Spm cannot be converted to deoxyhypusine (3). Our present study shows the necessity of combining in vivo and in vitro methods to indisputably confirm the conclusions drawn in modern biochemical experiments and clearly shows the value of the analogs in the polyamine metabolism studies.

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