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# Catabolism of polyamines

*Review Article*

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**Summary.** Owing to the establishment of cells and transgenic animals which either lack or over-express acetylCoA:spermidine $N^1$-acetyltransferase a major progress was made in our understanding of the role of polyamine acetylation. Cloning of polyamine oxidases of mammalian cell origin revealed the existence of several enzymes with different substrate and molecular properties. One appears to be identical with the polyamine oxidase that was postulated to catalyse the conversion of spermidine to putrescine within the interconversion cycle. The other oxidases are presumably spermine oxidases, because they prefer free spermine to its acetyl derivatives as substrate. Transgenic mice and cells which lack spermine synthase revealed that spermine is not of vital importance for the mammalian organism, but its transformation into spermidine is a vitally important reaction, since in the absence of active polyamine oxidase, spermine accumulates in blood and causes lethal toxic effects.

Numerous metabolites of putrescine, spermidine and spermine, which are presumably the result of diamine oxidase-catalysed oxidative deaminations, are known as normal constituents of organs of vertebrates and of urine. Reasons for the apparent contradiction that spermine is *in vitro* a poor substrate of diamine oxidase, but is readily transformed into $N^8$-(2-carboxyethyl)spermidine *in vivo*, will need clarification.

Several attempts were made to establish diamine oxidase as a regulatory enzyme of polyamine metabolism. However, diamine oxidase has a slow turnover. This, together with the efficacy of the homeostatic regulation of the polyamines via the interconversion reactions and by transport pathways renders a role of diamine oxidase in the regulation of polyamine concentrations unlikely. 4-Aminobutyric acid, the product of putrescine catabolism has been reported to have antiproliferative properties. Since ornithine decarboxylase and diamine oxidase activities are frequently elevated in tumours, it may be hypothesised that diamine oxidase converts excessive putrescine into 4-aminobutyric acid and thus restricts tumour growth and prevents malignant transformation. This function of diamine oxidase is to be considered as part of a general defence function, of which the prevention of histamine and cadaverine accumulation from the gastrointestinal tract is a well-known aspect.

**Keywords:** Polyamines – Putrescine – Spermidine – Spermine – Polyamine oxidase – Diamine oxidase – AcetylCoA:spermidine $N^1$-acetyltransferase (SAT)

**Abbreviations:** ADH, aldehyde dehydrogenase; AdoMet, S-adenosylmethionine; AdoMetDC, S-adenosylmethionine decarboxylase; dAdoMet, decarboxylation product of AdoMet; BE 333, $N^1$,$N^{11}$-bis(ethyl)norspermine; BE 343, $N^1$,$N^{12}$-bis(ethyl)spermine; cAMP, cyclic adenosine monophosphate; CuAO, copper-amine oxidase; DAO, diamine oxidase; DFMO, (D,L)-2-(difluoromethyl)ornithine; GABA, 4-aminobutyric acid; MAO, monoamine oxidase; MDL 72527, $N^1$,$N^4$-bis(2,3-butadienyl)-1,4-butanediamine; NacPut, N-acetylputrescine; $N^1$acSpd, $N^1$-acetylspermidine; $N^8$acSpd, $N^8$-acetylspermidine; $N^1$acSpm, $N^1$-acetylspermine; $N^1$,$N^{12}$diacSpm, $N^1$,$N^{12}$-diacetylspermine; ODC, ornithine decarboxylase; Orn, ornithine; PA, polyamine; PAO, polyamine oxidase (FAD-dependent) Put putrescine (1,4-butanediamine); SAO, bovine (ruminant) serum amine oxidase; SAT, acetylCoA:spermidine $N^1$-acetyltransferase; SMO, spermine oxidase; Spd, spermidine (4-aza-1,8-octanediamine); Spm, spermine (4,9-diaza-1,12-dodecanediamine)

## 1 Introduction

The first enzymes that were recognised as polyamine (PA)-related, namely diamine oxidase (DAO) (Zeller, 1938a, b) and ruminant serum amine oxidase (SAO) (Hirsch, 1953), were catabolic enzymes. Since, however, PA biosynthesis attracted much more interest than PA degradation, physiological and pathological roles of these oxidases are still less well understood than functions of all other enzymes of PA metabolism. About 36 years ago it became obvious that the stepwise formation of spermine (Spm) from putrescine (Put) and spermidine (Spd) can be reversed (Siimes, 1967). Although the formation of Spd from Spm by an SAO-catalysed reaction had been demonstrated already in 1960 (Bachrach and Bar-Or, 1960), neither DAO nor SAO appear to play a significant role in the physiological back conversion of Spm to Put. As

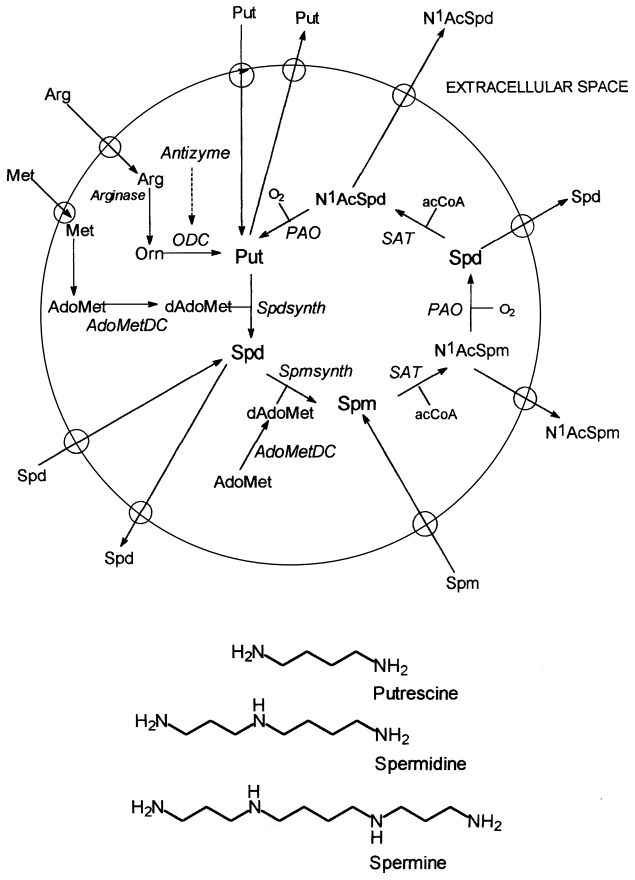


**Fig. 1.** Polyamine interconversion reactions and polyamine transport pathways of cells. (For non-standard abbreviations see page 217)

was first suggested in 1979 at a workshop (Seiler, 1981) acetylation and oxidative degradation of $N^1$-acetylspermidine ($N^1$acSpd) and $N^1$-acetylspermine ($N^1$acSpm) by a FAD-dependent polyamine oxidase (PAO) are the steps, which catalyse the conversion of Spm to Spd, and of Spd to Put. Together with the aminopropylation of Spd and Spm these reactions form the so-called interconversion cycle (Fig. 1). The regulated uptake and release of the PAs together with the interconversion pathway are the elements, which control PA homeostasis of most cells (Seiler, 1987).

It is a key aspect of PA interconversion, that Put and Spd, which were formed by degradation from Spd and Spm, can be re-utilised. The rate of the re-formation of Spd and Spm depends on the availability of dAdoMet. Therefore, the activity of S-adenosylmethionine decarboxylase (AdoMetDC) and the activity of acetylCoA: spermidine $N^1$-acetyltransferase (SAT) limit the flux rate through the cycle. Ornithine decarboxylase (ODC) has the function to provide Put at a rate, at which diaminobutane moieties are irreversibly lost by excretion or by oxidative deaminations (Seiler, 1987). The interconversion cycle appears to be the general system of PA regulation. Oxidative deaminations of the PAs are confined to those cell types, which are rich in DAO.

Basically the principles of PA regulation are the same in single cells and in the mammalian organism as an entity. Uptake of PAs from the gastrointestinal tract and their distribution into the individual organs by the bloodstream corresponds to the energy dependent, transporter mediated uptake from the cellular environment. The urinary excretion of PAs and of PA-derived amino acids corresponds to the controlled secretion through the cellular plasma membrane.

Uptake and *de novo* synthesis on one hand, and release and transformation into excretory products by oxidative deamination (“terminal catabolism”) on the other, fulfil the same purpose. The importance of uptake and release, vs. *de novo* synthesis and degradation differs from cell type to cell type, and changes with the physiological and pathological situation. As a rule, in rapidly proliferating cells the proportion of Put formed by degradation of $N^1$acSpd is small compared with its formation by decarboxylation of Orn. In contrast, slow or non-growing cells produce PAs at a slow rate, and ODC activity is correspondingly low.

Since this sketchy picture of PA catabolism is in essence generally accepted, this overview will concentrate on less well known observations, which support or complete our pertinent knowledge of PA catabolism. Uptake and release, although intimately entangled with PA regulation will not be a topic of this review.

## 2 The catabolic branch of the interconversion pathway

The first step of the catabolic branch of the interconversion pathway is the SAT – catalysed acetylation of the aminopropyl moieties of Spd and Spm (Casero and Pegg, 1993). The $N^1$-acetyl-PAs are substrates of a PAO, which removes the aminopropyl moiety as an aldehyde (3-acetamidopropanal) (Bolkenius and Seiler, 1981). Figure 2 shows the reactions of Spd acetylation and oxidative deaminations of the acetyl derivatives of Spd. The analogous reactions of Spm are shown in Fig. 3. 3-Acetamidopropanal is metabolised by an aldehyde dehydrogenase (ADH) – catalysed reaction to form N-acetyl-$\beta$-alanine. After its deacetylation by a selective hydrolase, $\beta$-alanine may be further metabolised by transamination. In situations of enhanced PA acetylation

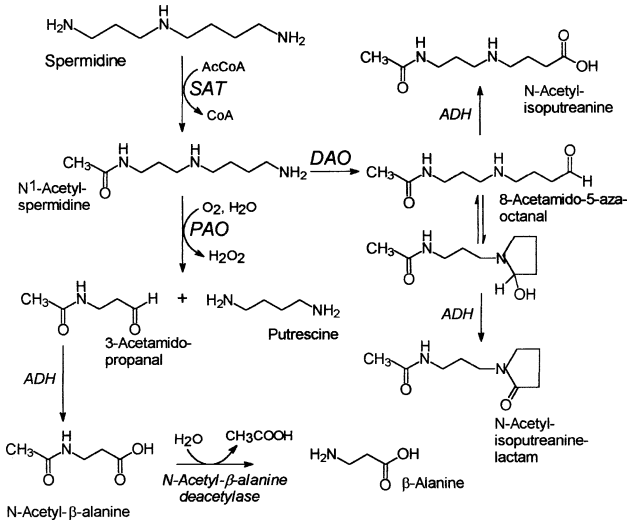


**Fig. 2.** Scheme of the oxidative degradation of spermidine via $N^1$-acetylspermidine. For non-standard abbreviations see page 217. For details of putrescine degradation see Fig. 6

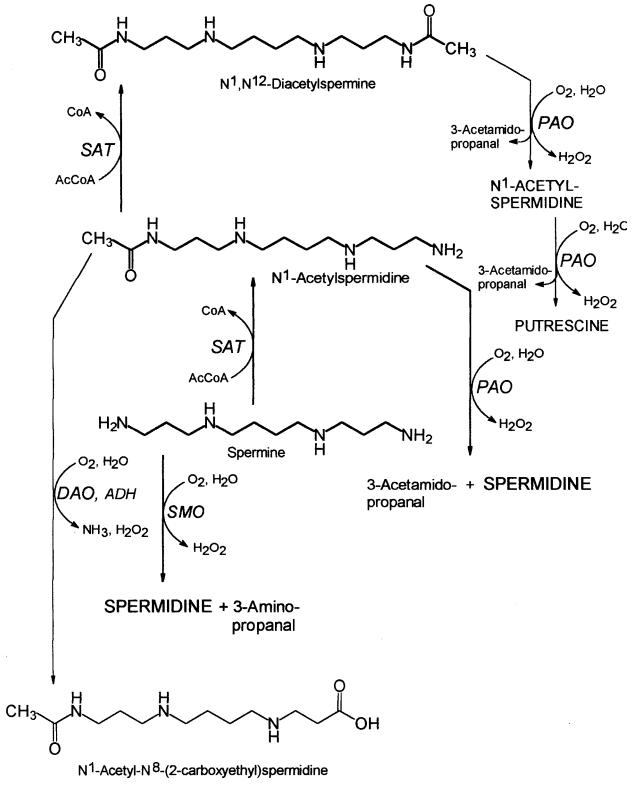


**Fig. 3.** Scheme of spermine acetylation, and oxidative deamination of its acetyl derivatives. Non-standard abbreviations are summarised on page 217. For details of $N^1$-acetylspermidine degradation see Fig. 2, for putrescine degradation see Fig. 6

the accumulation of $\beta$-alanine can be observed, for example in the pancreas of rats after treatment with a stable analogue of cAMP (Table 1).

### *2.1 Properties of acetylCoA:spermidine $N^1$-acetyltransferase (SAT)*

SAT is a highly regulated enzyme. Owing to its low basal activity, its short biological half-life (<30 min), which is comparable to that of ODC and AdoMetDC, it usually limits (together with AdoMetDC) the flux rate through the interconversion cycle.

SAT has been purified from several sources, and DNA sequences have been established. Its molecular weight differs in different species (chicken 36000; mammalian 65000–80000; subunit size 18000–20000). The active enzyme is a dimer or tetramer. Substrates have the general structure $R–NH–(CH_2)_3–NH_2$. Physiological substrates are 1,3-propanediamine, Spd, Spm and $N^1$acSpm (Casero and Pegg, 1993 and references therein). $Arg^{101}$, $Arg^{142}$ and $Arg^{143}$ are important for acetylCoA binding (Coleman et al., 1996; Lu et al., 1996). The GC-box located in the human SAT gene 42 to 51 base pairs upstream from the transcription start site appears to be important for Spd binding and transcription (Tomitori et al., 2002).

Constitutively expressed SAT activity is usually low, but in addition to physiological stimulation by Spd and Spm (Erwin and Pegg, 1986; Shappell et al., 1993), antiproliferative (catabolic) stimuli, hormones, growth factors, toxins and numerous drugs are capable of causing enormous increases of SAT activity (Seiler, 1987; Casero and Pegg, 1993; Fogel-Petrovic et al., 1997).

SAT is degraded through the ubiquitin-proteasomal pathway. Inducers with a PA like structure, such as $N^1$,$N^{12}$-bis(ethyl)spermine (BE 343) greatly stabilise the enzyme due to conformational changes, and prevent efficient polyubiquitination (Coleman and Pegg, 2001). The acetyltransferase of cells that were made resistant to a PA analogue was less efficient (decreased $V_{max}$ and decreased affinity for Spd and Spm) than SAT from the parental cells (McCloskey and Pegg, 2003).

### *2.2 Properties of tissue polyamine oxidases (PAOs)*

Until recently it was believed that the FAD-dependent PAO, which was first purified by Hölttä (1977) from rat liver, is a constitutive enzyme, of which $N^1$acSpd, $N^1$acSpm, $N^1$,$N^{12}$-diacetylspermine ($N^1$,$N^{12}$diacSpm) and Spm are physiological substrates, and $N^1$,$N^4$-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72527) is a selective inactivator (Seiler, 1995). Usually PAO activity is abundantly expressed in nearly all tissues of the vertebrate organism, and no evidence was found for its induction in tissues and organs. The

**Table 1.** Putrescine, $N^1$-acetylspermidine and $\beta$-alanine in the pancreas of rats after induction of spermidine degradation by IBMX. Effect of inhibition of polyamine oxidase by MDL 72527

| Treatment | Putrescine | $N^1$-acetyl spermidine | $\beta$-Alanine |
|---|---|---|---|
| | | nmol/g pancreas* | |
| Physiol. saline | $29 \pm 5$ | <2 | $36 \pm 2$ |
| IBMX | $370 \pm 40$ | $47 \pm 6$ | $150 \pm 13$ |
| MDL 72527 | $17 \pm 3$ | $25 \pm 8$ | $27 \pm 2$ |
| MDL 72527 + IBMX | $31 \pm 5$ | $385 \pm 120$ | $31 \pm 3$ |

* Mean value $\pm$ S.D. (n = 5). Fasted rats received 5 mg/kg MDL 72527, 16 and 2 h before 10 mg/kg IBMX. Four hours later polyamine analyses were performed. (Data from Seiler et al., 1985a)

existence of isoenzymes with differing substrate properties (one preferring Spm over $N^1$acSpd) has been reported (Libby and Porter, 1987). Recently several groups cloned PAOs of mammalian cell origin (Wang et al., 2001; Vujcic et al., 2002; Murray-Stewart et al., 2002; Vujcic et al., 2003; Cervelli et al., 2003; Wu et al., 2003). The PAO obtained by transient transfection of the cDNAs from human brain and a mouse mammary tumour into HEK 293 human kidney cells (Vujcic et al., 2003) and a murine peroxisomal flavoprotein expressed in an E. coli system (Wu et al., 2003) have substrate properties identical with those reported for the rat liver and macrophage enzymes (Bolkenius and Seiler, 1981; Seiler et al., 1995): $N^1$acSpd = $N^1$acSpm > $N^1$,$N^{12}$-diacetylspermine >> Spm >>>> Spd. Most probably this enzyme is identical with that responsible for the conversion of $N^1$acSpd to Put. The other recently cloned oxidases have a strong preference for Spm as substrate. They may be considered as spermine oxidase(s) (SMO), and catalyse the oxidative splitting of Spm to Spd and 3-aminopropanal, as is formulated in Fig. 3. In agreement with this suggestion, transfection of the cDNA into HEK 293 cells (Vujcic et al., 2002) caused a depletion of the Spm pool, whereas the pools of Put, Spd but also of $N^1$acSpd increased. Exposure of cells to inducers of SAT cause an increase of the activity of SMO (Vujcic et al., 2002; Wang et al., 2001; Casero et al., 2003). Since three splice variants of the enzyme of Wang et al. (2001) were detected, the existence of at least four isoenzymes of SMO was predicted (Murray-Stewart et al., 2002). It will be a task for the future to identify their functions.

## 3 AcetylCoA:spermidine $N^8$-acetyltransferase (Spd $N^8$ acetylase) and $N^8$-acetylspermidine deacetylase

A second acetyltransferase, which is located in the nuclear fraction of cells accepts Put as substrate (Seiler and Al Therib, 1974), and acetylates Spd exclusively at the aminobutyl moiety (Blankenship and Walle, 1978; Desiderio et al., 1992) (Fig. 4). Nuclear localisation and substrate specificity suggest that Spd $N^8$-acetyltransferase is at the same time a histone acetylase (Desiderio et al., 1992).

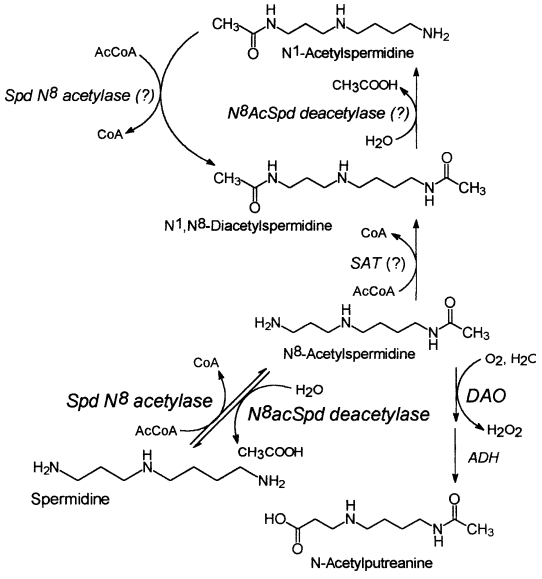


**Fig. 4.** Reaction scheme of spermidine $N^8$-acetylation, and metabolism of $N^8$-acetylspermidine. The question marks indicate that the reaction has not been verified. Non-standard abbreviations are shown on page 217

Since $N^1$,$N^8$-diacetylspermidine was identified as normal urinary constituent (Hiramatsu et al., 1995) it is likely that $N^1$acSpd is a substrate of Spd $N^8$-acetylase, but it is also possible that $N^8$acSpd is a substrate of SAT (Fig. 4).

N-Acetylputrescine (NacPut) and $N^8$acSpd are normal urinary constituents (Seiler et al., 1981a). $N^8$acSpd was also identified as an excretory product of cells (Wallace et al., 1988). The organ that is responsible for the formation of NacPut and $N^8$acSpd as excretory products has not been identified. The liver is likely to perform this function.

NacPut is a substrate of monoamine oxidase B (MAOB) and $N^8$-acSpd of DAO (Seiler, 1992). A cytosolic hydrolase, $N^8$-acetylspermidine deacetylase, regenerates Spd from $N^8$acSpd (Fig. 4). $N^1$acSpd is not a substrate of this enzyme (Libby, 1978; Marchant et al., 1986). A function of $N^8$-acetylation is presumably the removal of Spd from the cell nucleus (Seiler, 1987).

The selective inhibition of $N^8$-acetylspermidine deacetylase enhances the growth of L1210 cells (Wang et al., 1999). It will be of considerable interest to determine whether deacetylase inhibition affects intranuclear Spd pools. The transfer of Spd from intranuclear to extranuclear compartments could be involved in the regulation of cell growth by affecting histone acetylation. In this connection it is of interest to note that $N^8$-acSpd has an antiapoptotic effect (Berry, 1999).

## 4 Consequences of changing the activity of SAT

### *4.1 SAT-deficient cells*

Owing to its low constitutive expression, inhibitors of SAT had no great influence on our knowledge of SAT function. Niiranen et al. (2002) reported observations on PA interconversion in mouse embryonic stem cells with a disrupted SAT gene. Even though the targeted cells did not contain any SAT protein, they proliferated normally, and their Spd pool was elevated by about 30 percent. They were unable to convert [$^{14}$C]Spd into [$^{14}$C]Put, but converted [$^{14}$C]Spm into [$^{14}$C]Spd even more readily than the parental cells. Since this reaction was incompletely inhibited by MDL 72527, the embryonic stem cells contain most probably one or several of the above mentioned SMO isoenzymes.

### *4.2 How important is the formation of spermidine from spermine?*

Most microorganisms contain Put and Spd, but no Spm. The latter appeared in higher organisms during evolution together with the cell nucleus. The clarification of Spm functions vs. those of Spd is of considerable importance.

Depletion of Put and Spd pools by exposure of cells to a selective ODC inhibitor causes growth arrest, although Spm concentrations remain unaffected or may even be elevated (see e.g. Mamont et al., 1978). Selective inhibition of spermine synthase (Spmsynth) had little effect on cell growth, even if Spm depletion was profound, obviously because the compensatory increase of Put and Spd (together with an increase of dAdoMet) concentrations supports growth (see e.g. Pegg et al., 1995). These and related observations threw doubts on the importance of growth-related functions of Spm.

Recently transgenic (Gy) mice became available, which completely lack Spm due to the absence of a Spmsynth gene (together with the neighbouring phosphate regulating phex gene) (Lorenz et al., 1998). The existence of Gy mice demonstrates that Spm is not of vital importance for vertebrates.

Gy mice differ from their normal littermates by a considerably reduced size. Growth retardation was attributed in part to the absence of Spm. Tissue ODC and AdoMetDC activities of Gy mice are increased, and the pools of Put and Spd are elevated to an extent that total PAs are practically unchanged. Similarly transgenic Spm deficient cells exhibit PA metabolic changes, which resemble those, observed in Gy mice, and in Spmsynth inhibitor-treated cells. Growth rates of Spm-less cells and of parental cells were about the same, however, Spm deficient cells were more sensitive to BE 343, DFMO and MGBG (Korhonen et al., 2001), presumably because Spm is lacking as a source of Spd.

In spite of the non-vital role of Spm, its transformation into Spd is nevertheless a general physiological process in vertebrates. It was even observed in a microorganism (Yarlett et al., 2000).

In rapid growth, which is usually characterised by a high ODC activity, the conversion of Spm to Spd has no detectable function, but it exists, both physiologically, as has been demonstrated for the growing brain (Bolkenius and Seiler, 1986), as well as in tumour cells: In the presence of active PAO, Spm added to the culture medium reverses growth inhibition of several cell lines by DFMO. However, inhibition of PAO prevents Spm from reversing growth arrest because its degradation to Spd is blocked (Kramer, 1996).

A physiological example of the conversion of Spm to Spd is presumably early embryonic development. This is suggested from the PA changes in reproductive tissues. In the deciduomata of pregnant mice one observes during the first three days of gestation a gradual decrease of the Spm concentration. This decrease coincides with a peak of Spd concentration and of AdoMetDC activity on day 2, whereas ODC increases only a day later (Fozard, 1987), suggesting that Spd is initially formed from the available Spm and from Put, before Put is provided by decarboxylation of Orn. This interpretation of the PA concentration changes in reproductive tissues during early pregnancy is supported by the fact that the PAO inactivator MDL 72527 arrests pregnancy in mouse and hamster (Mehrotra et al., 1998), similar to DFMO (Fozard, 1987). However, in view of the cytotoxic properties of MDL 72527 (Dai et al., 1999; Duranton et al., 2002; Seiler et al., 2002), mechanisms other than blockade of Spm retroconversion are also possible.

All available information is in favour of Spd degradation to Put via an obligatory acetylation step. As far as Spm is concerned, little is known about the importance of the direct oxidation of Spm vs. its degradation via $N^1$acSpm as intermediate. *In situ* substrate specificity determinations of PAO in rat liver, kidney and duodenum support the view that Spm is mainly degraded via $N^1$acSpm, since hydrogen peroxide formation was only found with $N^1$acSpm and $N^1$acSpd, but not with Spm, Spd and Put as substrates (van den Munckhof et al., 1995). However, these experiments do not exclude direct oxidation of Spm, since the method may not have been sufficiently sensitive to allow the detection of a slow rate of Spm oxidation. It is known that homogenates of liver degrade Spm (Hölttä, 1977). The following observation is also in favour of Spm conversion to Spd without an intermediary acetylation step: If mice are treated chronically with the PAO inactivator MDL 72527, Spm accumulates gradually in red blood cells and plasma. If cell death rate is enhanced (i.e. if numerous cells release their PA content into the extracellular space), Spm may accumulate to lethally toxic concentrations. Figure 5A illustrates Spm toxicity and Fig. 5B the accumulation of Spm in blood due to prevention of its degradation. Discontinuation of MDL 72527-administration results in the transformation of Spm into Spd within the red blood cells (Sarhan et al., 1991). This observation demonstrates not only the vital importance of Spm degradation, but at the same time the inability of the vertebrate organism to get rid of free Spm by urinary excretion.

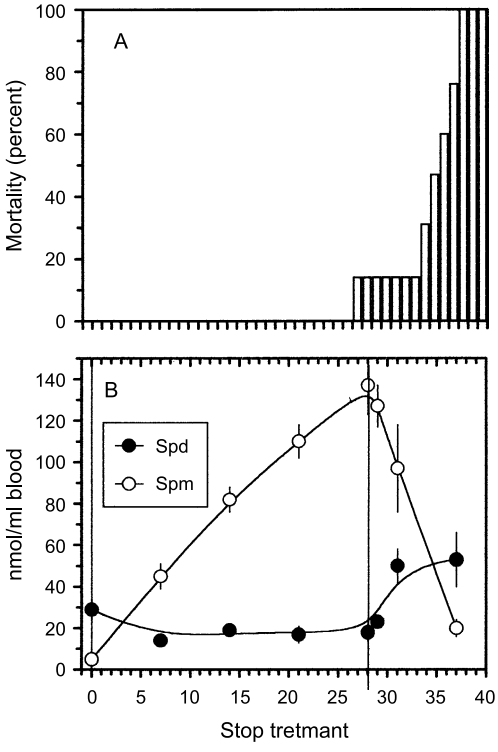


**Fig. 5.** Toxicity of 0.05% MDL 72527 plus 3% DFMO in the drinking water on mortality and blood polyamine content of C57 CL black mice **A**. Cumulative mortality. **B**. Spermidine and spermine concentrations in whole blood. (Treatment of this group was stopped on day 28). The error bars indicate S.D. (Data from Sarhan et al., 1991)

As soon as the exploration of SMO isoenzymes will have advanced, particularly if we know more about their activity in different tissues, their subcellular distribution, and their inducibility, our present ideas about Spm catabolism may considerably change.

A theoretical alternative to the PAO-catalysed formation of Spd from Spm (and of Put from Spd) is a DAO-catalysed reaction (see Section 5.2). The importance of this reaction under physiological or pathological conditions is at present unknown.

Even though Spm is not of vital importance, it has in addition to being a precursor of Spd, several important biological functions, which rely on its structural features. Their discussion is, however, beyond the scope of this review.

### *4.3 Increased SAT activity*

Following a physiological stimulus the immediate consequence of an increased SAT activity is an increase of the turnover rate of Spd. If the compensatory increase of *de novo* PA formation is inadequate to cope with the enhanced catabolic rate, SAT stimulation reduces preferentially Spd concentrations due $N^1$acSpd secretion and oxidative degradation. Since Spd is the growth-promoting member of the PA family, the cell growth rate decreases.

Elevation of SAT, decrease of PAO activity, and increase of $N^1$acSpd and $N^1$acSpm concentrations accompany the loss of ribosomal RNA and of proteins in the liver of fasting rodents (Seiler et al., 1981b). Similar changes of PA metabolism were observed in breast cancer tissue (Persson and Rosengren, 1989; Wallace et al., 2000). Enhanced formation of $N^1$acSpd was also found in hepatic preneoplastic nodules and hepatomas (Sessa and Perin, 1991). A sharp increase in retinal SAT mRNA in Müller glial cells was reported for the transition stage from proliferation to early differentiation (Witte and Godbout, 2002), and light modulates retinal $N^1$acSpd and $N^1$acSpm concentrations (Macaione et al., 1993). Increased rates of PA acetylation and Put formation from Spd regularly accompany brain lesions (Rao et al., 2000). These few examples indicate that elevation of SAT activity and enhancement of PA acetylation is a very frequent physiological and pathological event.

While the enhancement of PA acetylation by physiologic catabolic stimuli, such as fasting and differentiation, has an obvious function, namely the diminution of Spd pools, the accumulation of $N^1$acSpd in pre-cancerous nodules and tumours has no obvious purpose. The fact that PAO activity is lower in tumours than in the surrounding normal tissue (see e.g. Romano and Bonelli, 1986; Nikolov et al., 1991; Linsalata et al., 1997; Wallace et al., 2000), contributes to the elevation of $N^1$acSpd concentration. In large, poorly vascularised tumours the inadequate $O_2$-supply is a factor that contributes to the elevation of $N^1$acSpd, because the rate of $O_2$-dependent reactions is diminished. In agreement with this suggestion it was found that the urinary excretion of $N^1$acSpd by tumour bearing rats increases with increasing tumour mass (Seiler et al., 1981c). Lacking $O_2$ will not only cause a diminution of the oxidation rate of $N^1$acSpd, but also a reduction of the rate of Spm degradation by SMO. Since Spm in contrast with the acetylated PAs is not secreted, a relative accumulation of Spm over Spd is observed. In addition, tumour cells may in general have higher levels of SAT than normal cells, as could be suspected from the fact that transformed chick embryo fibroblast acetylate Spd at a higher rate, and convert Spd more rapidly into Put than their non-transformed counterparts (Bachrach and Seiler, 1981).

One may interpret the reduction in oxidative metabolism of the PAs as a protective mechanism: The diminished formation of cytotoxic products (hydrogen peroxide, 3-aminopropanal) reduces the probability of metabolic stress generation. In agreement with this notion is the fact that the PAO inhibitor MDL 72527 reduces the infarct volume and other consequences of brain lesions (see references in Seiler, 2000). In addition sparing of Spm in situations of Spd losses is most probably important for cell survival. By stabilising against degradation the chromatin structure, and the structure of other vitally important macromolecules with anionic binding sites, Spm preserves cells from irreversible damage (see e.g. Basu et al., 1992). The higher binding energy of Spm in comparison with that of Spd is important in this function.

The identification of those isoforms of PAO and SMO, which change their activity under the above-discussed conditions will improve our understanding of the regulatory role of PA oxidation in situations of activated catabolism.

In the course of the last two decades the synthesis of numerous polyamine analogues was reported which strongly induce SAT, and impair cell growth (see e.g. Kramer, 1996; Seiler, 2003). The detailed study of these compounds has considerably contributed to our knowledge of consequences of high acetylation capacities of cells and tissues. However, it is not possible to distinguish between effects of high SAT activities and effects, which are due to the interaction of the Spm-like tetramines with functionally important PA binding sites. The generation of transgenic animals, and of cells over-expressing SAT was, therefore, instrumental for studying long-term consequences of activated PA catabolism.

Transgenic mice over-expressing ODC or AdoMetDC do not exhibit important changes of their tissue PA patterns (Heljasvaara et al., 1997). This may be taken as evidence for the efficient regulation of PA homeostasis, and as an indication for its importance. In contrast, overexpression of the SAT gene causes in rodent organs the excessive accumulation of Put due to degradation of $N^1$acSpd, and a concomitant depletion of Spd and Spm concentrations (Pietila et al., 1997; Alhonen et al., 1999).

The PA patterns of cells overexpressing SAT resemble those observed in cells exposed to inducers of SAT. In Table 2 an example is shown. In addition to the depletion of Spd and Spm pools, and the elevation of Put, the accumulation of acetyl-PAs, including $N^1$,$N^{12}$-diacSpm, has been shown. In cells with stably transfected SAT an increase of ODC and AdoMet DC activities was observed (Alhonen et al., 1998; McCloskey et al., 1999). These changes are accompanied by a reduction, though not a profound reduction of the cell growth rate, probably because high Put concentrations (Seiler et al., 1991), and even some structural analogues of Put (Sarhan et al., 1987) are able to substitute Spd to some extent in growth related processes.

Cells and transgenic animals which overexpress SAT are significantly more sensitive to PA analogues, such as $N^1$,$N^{11}$-bis(ethyl)norspermine (BE 333), than the respective parental cells, and normal littermates, respectively (McCloskey et al., 1999; Alhonen et al., 1998; Vujcic et al., 2000; Alhonen et al., 1999), because the Spm analogues activate acetylation dramatically. Since in this situation mRNA is not increasing proportionately to the increase of enzyme protein, translational regulation of SAT must occur (Fogel-Petrovic et al., 1996; McCloskey et al., 1999). An interesting detail: At high SAT activity exogenous PAs are not able to affect much the endogenous PA pools, because they are rapidly eliminated via acetylation and secretion (Vujcic et al., 2000).

In view of these observations it is not surprising that the previously mentioned cells with a disrupted SAT gene are more resistant to BE 333 than their parental cells (Niiranen et al., 2002).

**Table 2.** Effect of overexpression of acetylcoenzymeA:spermidine $N^1$-acetyltransferase (SAT) and induction of SAT by $N^1$,$N^{11}$-bis(ethyl)norspermine (BE 333) on the polyamine pattern of cells

| | Parental cells* MCF-7 | Cell clone* H-10 | SK-MEL-28 cells** | |
|---|---|---|---|---|
| | | | | + BE 333 |
| SAT | 20 ± 5 | 271 ± 46 | 15 | 24035 |
| Put | 0.84 ± 0.08 | 0.45 ± 0.05 | 0.05 | 0.26 |
| Spd | 4.1 ± 0.2 | 1.9 ± 0.1 | 1.6 | 0.07 |
| Spm | 2.8 ± 0.2 | 2.6 ± 10.1 | 7.3 | 0.31 |
| $N^1$acSpd | <0.005 | 1.8 ± 0.1 | <0.01 | 0.39 |
| $N^1$acSpm | <0.005 | 0.14 ± 0.01 | <0.01 | 0.06 |
| $N^1$,$N^{12}$diacSpm | <0.005 | 0.11 ± 0.01 | <0.01 | 0.22 |

SAT activity is expressed in pmol/min per mg protein; polyamine concentrations are expressed in nmol per $10^6$ cells. The polyamine determinations in the H-10 clone were performed 48 h after Doxocyline removal. Polyamines in SK-MEL-28 cells were determined 24 h after exposure to 10 $\mu$M BE 333. At the time of polyamine determinations BE 333 was present in cells at 25.3 nmol. Data are from * Vujcic et al., 2000; ** Chen et al., 2001

Since excessive SAT expression cannot be outbalanced by a compensatory increase of biosynthesis and transport rates, it may not surprise that pathologic alterations were observed in transgenic rodents as a consequence of SAT overexpression. For example, expression of SAT in mouse epidermis caused a 10-fold increase in the number of 7,12-dimethylbenz[a]anthracene-induced skin neoplasias (Coleman et al., 2002). In a different strain hair loss due to dermal follicular cysts was observed (Pietila et al., 1997). The pathology of these mice resembles the skin and hair abnormalities of an X chromosome-linked human disease (Siemens-1 syndrome). Polyamine changes in cells of patients with this disease were consistent with the overexpression of the SAT gene (Gimelli et al., 2002). Expression of SAT in pancreas caused the development of pancreatitis (Alhonen et al., 2000). In view of these observations, one may suspect that chronic administration of SAT-inducing PA analogues may produce similar pathologic effects. However, no pertinent observations have been reported.

## 5 Diamine oxidase (DAO) in mammalian polyamine catabolism

DAO belongs to the family of copper containing amine oxidases (CuAOs), of which 2,4,5-trihydroxyphenylalanine quinone (topa quinone) is the cofactor. The copper atom is essential for catalytic activity. For reviews of mammalian CuAOs, and the mechanism of topa quinone-assisted deaminations see e.g. Buffoni and Ignesti, 2000; Houen, 1999; Klinman and Mu, 1994. Three mammalian CuAOs are able to use diamines and the natural PAs, including most acetyl derivatives of the PAs, as substrates: DAO, (human) pregnancy-associated amine oxidase (hpaAO), and SAO. Among these only DAO appears to play a role in the management of PA concentrations in tissues of a variety of vertebrate species, whereas the two other enzymes are confined to functions in pregnancy, respectively in ruminants, with their particular digestive system. This review will focus on DAO.

### *5.1 Some characteristics of DAO*

DAO is unevenly distributed in the vertebrate organism. Highest activities are found in placenta, small intestine, kidney and liver (Houen, 1999). In contrast with Shaff and Beaven (1976), Burkard et al. (1963) found very low DAO activities in brains of different species.

DAO has a slow turnover rate. From experiments with heparin and cyloheximide a biological half-life of 15–18 h was determined in rat small intestine (Shaff and Beaven, 1976; see also Sourkes and Missala, 1981). The enzyme is under the control of different hormones (see e.g. Sourkes and Missala, 1981; Andersson et al., 1980a; Hougaard et al., 1992; Jotova et al., 2000).

It was generally accepted that species and tissue specific DAOs exist, which can be distinguished, among others, by their biochemical properties, such as K(m) values (see e.g. Romijn et al., 1986). DAOs have repeatedly been purified from different sources (see references in Houen, 1999). Presently large quantities of DAO can be purified (Schwelberger and Bodner, 1997; Elmore et al., 2002), so that the detailed characterisation of the enzyme and the generation of specific antibodies is now possible. The puzzling uncertainties concerning species and tissue specific DAOs will soon be a matter of the past.

Native DAO is a homodimeric glycoprotein (with an apparent Mr 186 kDa for the porcine kidney enzyme). The protein of porcine kidney and intestinal DAO is identical (Schwelberger and Bodner, 1998). The extent of glycosylation changes with organ and species. In the recombinant DAO from human kidney cDNA a tightly bound $Ca^{2+}$ was identified in addition to 1 mol Cu per subunit, suggesting a second metal-binding site. One may speculate that this site is capable of binding PAs.

Substrate specificity of kidney and intestinal DAO was found identical. K(m) values were as follows: 0.02 mM (histamine), 0.35 mM (Put), 3.3 mM (Spd). Spm is considered a poor substrate (Zeller, 1938b; Schwelberger and Bodner, 1997), but $N^1$acSpd, $N^1$acSpm and $N^8$acSpd are substrates (Suzuki et al., 1981; Seiler, 1985).

### *5.2 Reactions that are likely to be catalysed by DAO*

The metabolic interrelationships between Put and 4-aminobutyric acid (GABA) have been clarified more than 25 years ago. In reviews (Seiler, 1980, 1992) the observations are described, which demonstrated the role of DAO and MAO in the formation of GABA in vertebrates, and possible regulatory interrelationships between PA metabolism and GABA. In Fig. 6 the GABA-forming reactions are shown. An aldehyde dehydrogenase with high affinity for 4-aminobutanal has been purified (Testore et al., 1999).

Putreanine (N-(4-aminobutyl)-3-aminopropionic acid) is a normal constituent of vertebrate brains (Kakimoto et al., 1969). Together with isoputreanine (N-(3-aminopropyl) 4-aminobutyric acid) and the Spm derivatives $N^8$-(2-carboxyethyl)spermidine and spermic acid ($N^1$,$N^4$-bis(2-carboxyethyl)-1,4-butanediamine) were detected in rat urine after [$^{14}$C]Spd and [$^{14}$C]Spm injections (Noto et al., 1978). Using hexadeuterospermidine as precursor resulted in the urinary excretion of putreanine, isoputreanine, Put, 1,3-propanediamine, $\beta$-alanine, GABA and 2-hydroxyputrescine (van Berg et al., 1984). $N^8$-(2-carboxyethyl)spermidine accumulated in rodent tissue after injection of Spm in a time- and dose-dependent manner (Seiler et al., 1981d). These and related observations allow the construction of the reaction schemes

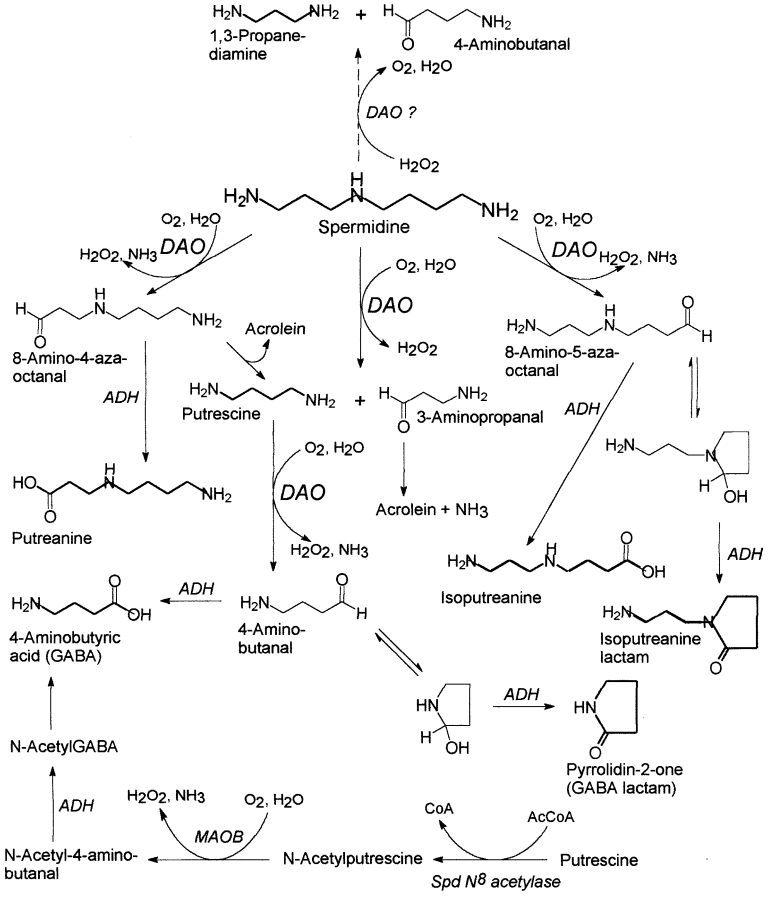


**Fig. 6.** Scheme of possible diamine oxidase-catalysed oxidative deaminations of spermidine and putrescine, and consecutive transformations of the primary reaction products. Non-standard abbreviations are summarised on page 217

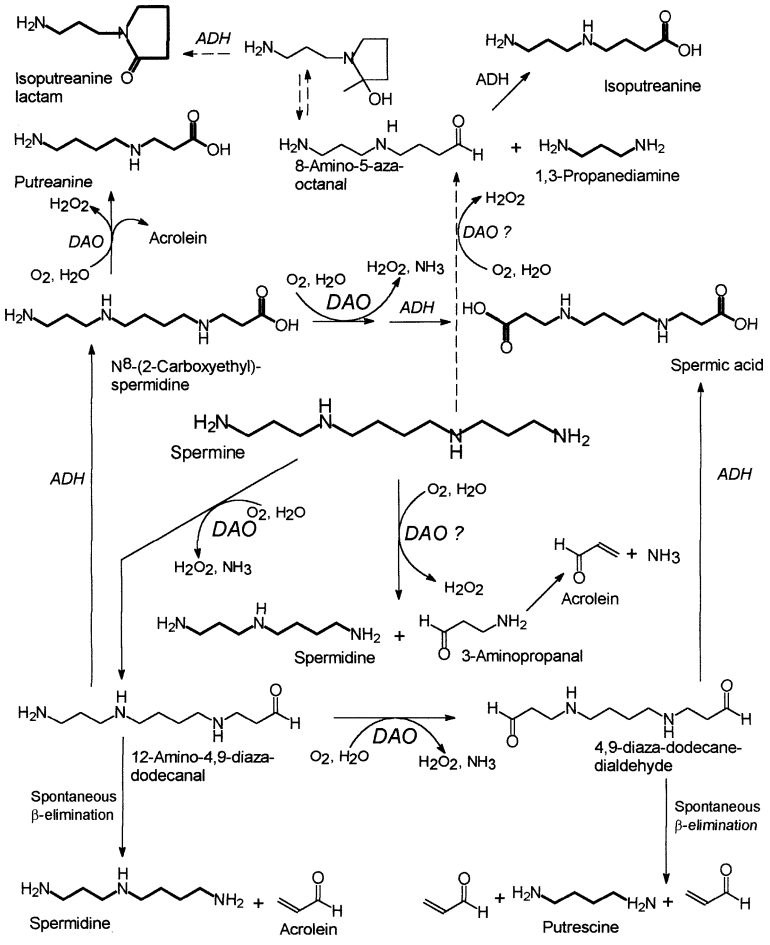


**Fig. 7.** Scheme of possible diamine oxidase-catalysed oxidative deaminations of spermine, and consecutive transformations of the primary reaction products. (For non-standard abbreviations see page 217)

shown in Figs. 6 and 7. N-acetylisoputreanine lactam (N-(3acetamidopropyl)pyrrolidin-2-one) is the product of the oxidative deamination of $N^1$-acetylspermidine (Seiler et al., 1982) (Fig. 2). It is also a normal excretory product (van den Berg et al., 1986).

GABA, although found in urine, is to a considerable proportion metabolised by transamination to succinic semialdehyde, which is further metabolised to succinic acid. The latter enters the tricarboxylic acid cycle. According to a postulate of Bardocz et al. (1998) this reaction sequence is a source of instant energy in the small intestine. Putreanine and isoputreanine lactam may also be further metabolised to the respective amino acids N-(2-carboxyethyl)-4-aminobutyric acid and 2-oxo-1-pyrrolidinepropionic acid (not shown), which were detected in urine (van den Berg et al., 1984; Kawase et al., 1994).

Figures 6 and 7 show undoubted products of Spd, Spm and Put metabolism. However, the importance of the individual reactions within the mammalian organism is with few exceptions not known. It is not even possible to prove the exclusive responsibility of DAO for all reactions shown in these figures. Samples of pure DAO will help to clarify, which of the compounds are substrates. The following observations are arguments in favour of DAO: (a) In contrast with high activities of DAO in some tissues, particularly in intestine, SAO (which is capable of forming most of the products of DAO) is low in human and rodent plasma (see references quoted by Houen, 1999) (b) The dose of aminoguanidine ($ED_{50} = 0.16\ \mu mol/kg$) necessary for reducing putreanine formation from injected Spd in mice was similar to the dose required for 50 percent reduction of intestinal DAO activity (Seiler et al., 1983). (Aminoguanidine is the most frequently used inhibitor of DAO. For details of its properties and biological effects see Nilsson, 1999). (c) SAO deaminates the aminopropyl moiety of Spd, forming 8-amino-4-azaoctanal, hydrogen peroxide and ammonia. The aldehyde may be transformed into putreanine, or, depending on pH and other conditions, Put may be formed due to spontaneous elimination of acrolein (Tabor et al., 1964; Lee and Sayre, 1998). In contrast, injection of Spd to mice is followed by

a time-dependent accumulation of putreanine and of isoputreanine lactam in several organs (Seiler et al., 1982), indicating that *in vivo* each primary amino group of Spd may be oxidatively deaminated. $\beta$-Elimination of acrolein from the aminoaldehydes that are generated by DAO-catalysed oxidative deaminations is in principle suitable to convert Spd and Spm into Put (see Figs. 6 and 7). In theory this reaction is an alternative to the acetylation-dependent PA degradation by PAO. However, evidence for its significance in PA interconversion does not exist.

The identification of deuterium labelled 1,3-propanediamine and of Put as products of *in vivo* Spd degradation (Van den Berg et al., 1984) suggests the possibility that the secondary amino group was attacked. More recently the formation of 3-aminopropanal in ischaemic brain was reported (Ivanova et al., 1998), and Houen (1999) presented evidence for the analogous splitting of Spm by purified SAO. A careful study of this reaction, using highly purified DAO and timely methodology appears important, particularly with regard to the mechanism of oxidative deaminations by CuAOs, which are believed to require imine formation with topa quinone (Klinman and Mu, 1994; Bellelli et al., 2000).

There is an apparent contradiction between the poor substrate property of Spm *in vitro* (Zeller, 1938b; Schwelberger and Bodner, 1997) and the rapid formation of $N^8$-(2-carboxyethyl)spermidine from injected Spm *in vivo* (Seiler et al., 1981d). One may speculate that *in vitro* the rate of Spm oxidation is reduced by product inhibition. 12-Amino-4,9-diaza-dodecanal, the aldehyde, which is generated from Spm by DAO-catalysis (Fig. 6) may bind to the enzyme, whereas in cells, which contain (with few exceptions, e.g. hepatomas (Sessa et al., 1981)) high activities of aldehyde metabolising enzymes, the rapid transformation of aldehydes into amino acids is favoured.

In contrast with intracellular oxidative deaminations, extracellular deaminations of the PAs by SAO, DAO and PAO give rise to cytotoxic effects. A controversial literature on functions and mechanisms of "polyamine cytotoxicity" was generated during the last decades (see e.g. Parchment, 1996). Which of the cytotoxic products, hydrogen peroxide, aminoaldehydes, or acrolein (generated non-enzymatically by $\beta$-elimination from 12-amino-4,9-diaza-dodecanal, 8-amino-4-azaoctanal, and from 3-aminopropanal (see Figs. 6 and 7)) contributes most to the cytotoxicity of PAs was, and still is, a debated question. A discussion of this topic is beyond the scope of this review. However, it should be mentioned that in recent papers well founded answers were given to those questions, which concern the cytotoxic role of acrolein (Sharmin et al., 2001), the potential of SAO in generating cytotoxic products for therapeutic purposes (Calcabrini et al., 2002), and the mechanism of cell death induced by products of Spm oxidation (Bonneau and Poulin, 2000). Morgan (1987) has pointed out earlier that the products of the extracellular oxidative catabolism of acetyl PAs are considerably less cytotoxic than the oxidation products of non-derivatised PAs. A likely explanation for the observation is that 3-acetamidopropanal, in contrast with 3-aminopropanal, is not a precursor of acrolein. This difference suggests an advantage of interconversion via acetylation over the direct oxidation of free PAs.

### *5.3 Diamine oxidase, polyamine oxidase, and polyamine excretion*

The products of CuAO-catalysed reactions are normal urinary excretory products, indicating that a certain proportion of the PAs destined for eliminated undergoes terminal catabolism.

An idea of the extent of oxidative deaminations can be obtained by administration of aminoguanidine to humans and rats. In humans repeated doses of 50 mg aminoguanidine produced about a 2-fold increase in Put and a 4–8-fold increase in Spd excretion (Chayen et al., 1985). Determination of the urinary PAs in rats receiving 25 mg/kg aminoguanidine per day during 5 days exhibited a 2.5-fold increase in total PA excretion (Fig. 8),

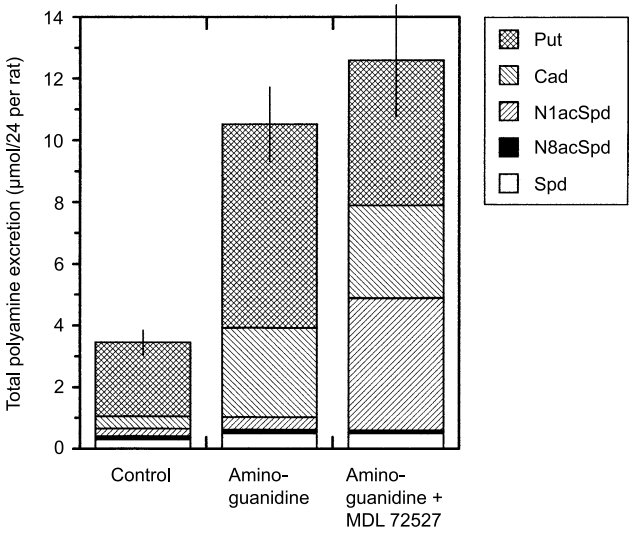


**Fig. 8.** Urinary polyamine excretion by rats. Effect of treatment with aminoguanidine and the polyamine oxidase inactivator MDL 72521. Rats were treated for 5 days by daily i.p. injections of 25 mg/kg aminoguanidine sulfate or a combination of 25 mg/kg aminoguanidine sulfate and 20 mg/kg MDL 72521. Polyamines were determined in 24 h urine samples. (For details see Seiler et al., 1985b). The error bars indicate S.D. (n = 3)

suggesting that about 60 percent of the PAs destined for elimination are oxidatively deaminated, and form the products, that were discussed in the preceding section. The highest increase was observed for Put and cadaverine, indicating that the diamines are quantitatively the most important substrates of DAO. However, urinary Spd and $N^1$acSpd were also significantly increased due to treatment with aminoguanidine. Additional inhibition of PAO decreased Put excretion by about 30 percent, but caused a more than proportionate increase of $N^1$acSpd excretion, demonstrating that about 30 percent of the Put destined to be eliminated were formed by degradation from $N^1$acSpd, and 70 percent by decarboxylation of Orn. The difference in total urinary polyamine excretion due to additional inhibition of PAO represents the portion of $N^1$acSpd-derived Put, which is normally re-used for Spd synthesis (Seiler et al., 1985b).

## 6 Diamine oxidase, putrescine and GABA

In rats the administration of a large dose of Put caused a measurable increase of DAO activity within 3–6 h in liver, heart and kidneys. Actinomycin D and cycloheximid prevented the increase of DAO activity, suggesting that the increase was due to *de novo* synthesis of the enzyme (Perin et al., 1986). This and related observations were interpreted as indications for the regulation of DAO by PAs.

A physiological relationship between ODC and DAO activities is suggested, among others, by the increase of ODC and DAO activities, and an enhanced formation of GABA upon stimulation of confluent glioma cells with serum containing medium (Bachrach, 1980), and by the fact that inhibition of rat bowel enterocyte ODC by DFMO is accompanied by a decrease of DAO activity. The latter observation was taken as an indication for a role of ODC in DAO expression (D'Agostino et al., 1990).

The simultaneous elevation of ODC and DAO activities is typical for numerous neoplasias. Baylin and Luk (1981) compiled the older literature, and examples of elevated DAO activities in established tumours (see e.g. Chanda and Ganguly, 1995; Keskinege et al., 2001; Rogers et al., 2002) and during carcinogenesis (see e.g. Sessa et al., 1990, 1993; Schwelberger et al., 1995; Fernandez et al., 1995) have been published up to the present. Organ hypertrophy (kidney (Desiderio et al., 1982), heart (Perin et al., 1983), pancreas (Rabellotti et al., 1998), as well as various injuries (see e.g. Erdmann et al., 1989; Schwelberger et al., 1995; Ivanova et al., 1998) belong also into the category of pathologic growth processes, which are characterised by increased DAO and ODC activities. Along with an increased DAO activity the excessive formation of GABA has been reported for several tumours (Andersson et al., 1980b; Nicholson-Guthrie et al., 2001). The oxidative deamination of Put is the most general GABA-forming reaction. A function for the putrescine-derived GABA is, however, unknown (Seiler and Lajtha, 1987).

In contrast with most other organs of the vertebrate organism intestinal mucosa has high constitutively expressed DAO and ODC activities. Interestingly DAO activity in the tumour tissue of patients with large bowel tumours was lower than in the surrounding mucosa (see e.g. Mennigen et al., 1988). Likewise intestinal DAO activity was reduced in gut mucosa after azoxymethane-induced carcinogenesis (see e.g. Kusche, 1986). These and related observation suggested DAO as an antiproliferative principle. The fact that inhibition of DAO improves the adaptive response after small bowel resection (Erdmann et al., 1989) supports this idea.

Cells overproducing ODC may undergo malignant transformation. Since ODC overproducers have usually high Put concentrations with only minor changes of Spd and Spm concentrations, it is likely that high Put concentrations are responsible for the acquisition of the malignant phenotype. Support for this suggestion comes from the observation that the inactivation of ODC by DFMO prevents the formation of transformed cells, respectively reverses the transformed phenotype into the non-transformed type (Hölttä et al., 1993; Shantz and Pegg, 1994; Tabib and Bachrach, 1998; Peralta-Soler et al., 1998). The idea that Put is responsible for malignant transformation is also supported by the observation that long-term inhibition of DAO by administration of aminoguanidine promotes colon tumour formation (Kusche et al., 1989).

In contrast with DAO, PAO activity was found at lower activity in tumours than in the surrounding normal tissue, and $N^1$acSpd concentrations were found elevated in some tumours (see Section 4.3). The diminished Put formation by PAO-catalysed degradation of $N^1$acSpd compensates for its excessive formation by ornithine decarboxylation in situations of high ODC and DAO activities.

Based on the enhanced formation of GABA from Put in intestines and tumours, one may suspect that GABA may not only be a passive metabolite. A report of Boggust and Al-Nakib (1986) supports this view. They observed inhibition of mouse carcinoma and HeLa cell proliferation by GABA at micromolar concentrations, as well as the inhibition of the growth of syngeneic

squamous carcinoma in mice. Growth inhibition by GABA was also reported for regenerating liver (Minuk and Gauthier, 1993), and HepG2 human hepatocellular carcinoma cells (Zhang et al., 2000). It is believed that GABA A-receptors mediate this effect. Since some cells of peripheral tissues produce significant amounts of GABA by decarboxylation of glutamate (Erdö and Wolff, 1990), putrescine is not the only GABA source to be considered in the future in connection with a growth regulatory function.

## 7 Conclusions

The interconversion reactions, together with the active transport systems excellently control PA concentrations in almost all tissues, irrespective of their DAO activity. An additional regulation of Put and PA concentrations by DAO appears, therefore, redundant. In addition its slow turnover rate argues against a regulatory role. DAO function seems better described as a key part of a general protective mechanism against pathologic accumulation of diamines and PAs in vertebrate tissues. Detoxification of diamines of gastrointestinal origin, particularly the catabolism of cadaverine and histamine within the intestinal mucosa, and the protection of the embryo against these amines by high placental DAO activities are accepted examples of the protective function of DAO.

Several groups own cells, which acquire the transformed phenotype due to over-expression of ODC. If one of these cell lines expresses DAO, it should not be difficult to confirm or refute a potential role of GABA in the transformation process. The demonstration of a function of GABA in malignant transformation will most probably have far-reaching therapeutic and chemopreventive consequences.

As far as polyamine interconversion is concerned, most of the basic questions have been addressed in the past. A gap in our knowledge remains the clarification of the importance of Spm oxidation by SMOs. Their role in tumour biology, but particularly the changes of the SMO isoenzymes in stroke and other brain lesion will most probably open new therapeutic possibilities. While the past work with transgenic animals and cell lines mainly confirmed results that had been obtained by using selective enzyme inhibitors of polyamine metabolism, it is likely that due to the presently available technology, cells with defective or lacking SMO genes will be sooner available than selective inhibitors of these amine oxidases.

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