polyamine synthesis are presently being analysed in further detail, and the information obtained may provide a basis for the rational development of novel and more effective anti-cancer and antiparasitic agents.

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Molecular mechanisms in polyamine biochemistry

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This paper is concerned with two aspects of polyamine biochemistry: (A) the mechanism of intracellular synthesis of spermidine [*N*-(3-aminopropyl)-1,4-diaminobutane, $H_2N(CH_2)_4NH(CH_2)_3NH_2$], a 'central' polyamine; (B) the nature of the polyamine uptake system found in rat lungs. We will show that spermidine is formed by an enzyme-catalysed S_N2 reaction between decarboxylated *S*-adenosylmethionine [$AdoS^+(CH_3)CH_2CH_2CH_2NH_2$; Ado, 5-adenosyl] and putrescine [1,4-diaminobutane, $H_2N(CH_2)_4NH_2$]. Results from studying the effects of a series of compounds on putrescine uptake by rat lung slices will be used to sketch some features of the uptake system.

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(A) Biosynthesis of spermidine

There are two obvious mechanisms for the reaction between decarboxylated *S*-adenosylmethionine and putrescine catalysed by putrescine aminopropyltransferase (spermidine synthase, EC 2.5.1.16).

(1) Enzyme-catalysed S_N2 reaction (single displacement). In this mechanism the function of the enzyme must be to align the substrates to enable the required colinearity of attacking nucleophile (one of the homotopic amino groups of putrescine), carbon centre undergoing attack (3-methylene group of the aminopropyl group of decarboxylated *S*-adenosylmethionine), and leaving group (sulphur). It is interesting that the *S*-methyl group should be intrinsically more reactive than either *S*-methylene group, yet one of the methylene groups is actually attacked. Although rotation about the adenosylmethylene to sulphur bond can place the *S*-methyl in the correct position for nucleophilic attack, this would require the 3-aminopropyl group (probably protonated on the amino) to occupy a site that previously con-

tained nothing but a lone pair (n.b. the sulphur is tetrahedral and presumably possesses *S* configuration, as in *S*-adenosylmethionine). During the reaction the attacking amino group becomes a protonated secondary amine, whilst the sulphonium centre becomes a dialkyl sulphide (5-methylthioadenosine). It may be supposed that the enzyme achieves further acceleration by placing a negatively charged group (probably carboxylate) near the developing ammonium group, and a hydrophobic environment near the 5-methylthioadenosine being formed. A model for the transition state that takes account of all the points discussed is shown in Fig. 1.

(2) Enzyme-catalysed double displacement (both steps S_N2) involving an intermediate in which the aminopropyl group becomes covalently bound to the enzyme. In such a mechanism a functional group (X) of the enzyme effects an S_N2 displacement on decarboxylated *S*-adenosylmethionine, with transfer of the aminopropyl group to the X group. Then an amino group of putrescine attacks the 3-methylene carbon of the X-bound aminopropyl with release of X and formation of spermidine. Possible candidates for X are the sulphur of methionine (leading to an intermediate sulphonium compound) and the oxygen of an amide function (leading to an intermediate imidate). For each S_N2 process in this pathway the same strict stereochemical controls apply as discussed in mechanism (1).

Determination of the stereochemical course of the spermidine synthase reaction provides in principle a clearcut method to distinguish between these possible mechanisms. The obvious approach is to label the 3-methylene of decarboxylated *S*-adenosylmethionine stereospecifically with deuterium and determine the chirality of the mono-deuteriospermidine [$H_2N(CH_2)_4NHCHDCH_2CH_2NH_2$] formed. We adopted a different approach in which dideuteriation at the 3,4-carbon atoms in methionine and knowledge of the relative stereochemistry of the deuterium atoms was used [1]. Thus, two samples of dideuteriomethionine were prepared by chemical synthesis from (*E*)- and (*Z*)-ethene, respectively. One [from (*E*)-ethene] contained equal parts of (2*R*,3*R*,4*R*)-, (2*S*,3*R*,4*R*)-, (2*R*,3*S*,4*S*)- and (2*S*,3*S*,4*S*)-[3,4-$^2H_2$]methionine (all 3,4-*erythro*-$^2H_2$), whilst the other contained equal parts of (2*R*,3*R*,4*S*)-, (2*S*,3*R*,4*S*)-, (2*R*,3*S*,4*R*)- and (2*S*,3*S*,4*R*)-[3,4-$^2H_2$]-methionine (all 3,4-*threo*-$^2H_2$). These labelled methionines were fed to cultures of *Escherichia coli* and the dideuteriospermidines produced were isolated. To determine the relative configurations of the deuteriums in these spermidines, authentic samples of *erythro*-1,2-dideuterio- [mixture of (1*S*,2*S*)- and (1*R*,2*R*)-dideuterio-] spermidine and the corresponding *threo* mixture were prepared by chemical synthesis from (*E*)- and (*Z*)-ethene, respectively. By comparison of the 400 MHz n.m.r. spectra of derivatives of the biosynthetic dideuteriospermidines with derivatives of the synthetic materials, it was concluded that the *erythro* dideuteriomethionines gave *threo* dideuteriospermidines, whilst the *threo* dideuteriomethionines gave *erythro* dideuteriospermidines. Hence the spermidine synthase reaction occurs by a single displacement and mechanism (1) above holds. The value of the *erythro* and *threo* dideuteriated methionines for studying other biosynthetic processes involving methionine has been demonstrated for the biosynthesis of 1-aminocyclopropanecarboxylic acid [2].

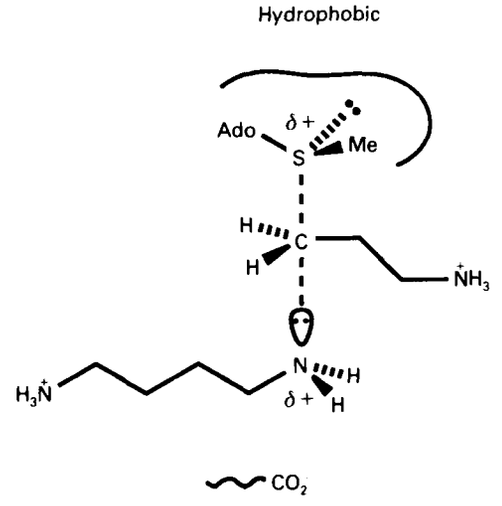


Fig. 1. *Transition state for the reaction between decarboxylated S-adenosylmethionine and 1,4-diaminobutane catalysed by spermidine synthase*

Our mechanistic conclusion was confirmed by a study using the 'obvious approach' [3], in which decarboxylated *S*-adenosylmethionine, chirally deuteriated at the 3-methylene, was processed by spermidine synthase isolated from *E. coli*. The potent inhibition of spermidine synthase by *S*-adenosyl-1,8-diamino-3-thiooctane, which can resemble the transition state of mechanism (1), has been cited [4] as further evidence for this mechanism. The conversion of methionine into nocardicin A, in which sulphur is displaced by an oxygen function, also proceeds with inversion of configuration [5]. Methyl transfers from *S*-adenosylmethionine likewise proceed by a direct S_N2 mechanism [6].

(B) Polyamine receptor studies

Polyamine uptake systems have been identified for many cell types. That characterized for type I and type II epithelial cells of the rat and human lung has been described by L. L. Smith and co-workers [7]. The uptake system found in tumour cells (e.g. L1210 leukemia cells) has potential for the delivery of cytotoxic agents into the cells [8]. Ingestion of the herbicide paraquat causes anoxia in humans resulting in the accumulation of this herbicide into lung cells through the polyamine uptake system [9]. Studies of the ability of a variety of compounds (e.g. 1,n-diaminoalkanes, whose amino groups are protonated at physiological pH) to inhibit paraquat uptake by rat lung slices, led to the conclusion that the transport system recognizes paraquat as a di-cation in which the charges are separated by the near optimum distance of 7.02 Å [10]. This may be due to the presence in the putative receptor of two negatively charged groups (probably protein carboxylates) that interact by ionic binding with the positively charged nitrogens. It can also be concluded that hydrophobic interactions may be important between the methylene chain of 1,n-diaminoalkanes or aromatic rings of paraquat and the receptor. A model incorporating these features for the receptor is shown in Fig. 2.

In order to define the receptor (cf. Fig. 2) better we have synthesized a series of analogues of purescine (see Table 1) and have determined the ability of these compounds to inhibit the uptake of putrescine by rat lung slices. We have explored the effect of alkyl substitution and we have also attempted to achieve covalent labelling of the putative carboxylates. The synthetic methodology for preparing the *N*-alkylputrescines (cf. Table 1) has been described [11]. We have found the reported [12] one-pot conversion of alcohols into amines to be exceptionally useful for preparing putrescine analogues. *N*-(4-Aminobutyl)aziridine was presumed to be highly toxic and we have therefore devised a method for the generation of this compound *in situ*. The stable, crystalline *N*-(2-bromoethyl)-1,4-diaminobutane dihydrobromide was prepared essentially as described in [13]. Monitoring a solution of the salt in pH 7.4 phosphate buffer by 300 mHz n.m.r. showed $t_{1/2}$ for generation of the aziridine to be

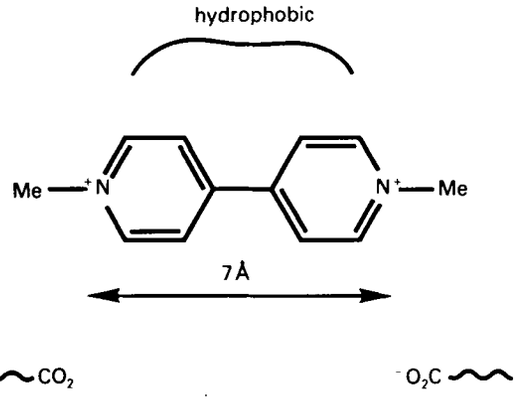
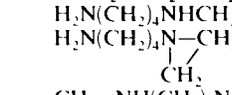
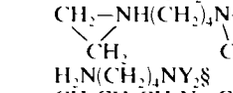


Fig. 2. *Binding sites for paraquat within the putative receptor of the transport system of lung*

Table 1. *Inhibitory effects of putrescine analogues on the uptake of putrescine or paraquat into rat lung slices*

The values given are derived from the mean of three determinations at three different concentrations of inhibitor (typically 10, 25 and 100 μM). The K_i for each inhibitor was calculated from Lineweaver–Burk plots of 1/concentration of putrescine or *paraquat (abscissa) compared with 1/uptake of radioactive putrescine or paraquat by lung slices (ordinate). †The buffer conditions used in the uptake studies resulted in the formation of *N*-(4-aminobutyl)-aziridine from this substrate, see text, ‡X = 1,1-dichlorocyclopropan-2,3-diyl, §Y = norbornan-2,3-diyl.

Compound	K_i against putrescine uptake (μM)
$H_2N(CH_2)_4NH_2$	10*
$H_2N(CH_2)_4NHCH_3$	8
$H_2N(CH_2)_4N(CH_3)_2$	11.5
$CH_3NH(CH_2)_4NHCH_3$	25.5
$CH_3NH(CH_2)_4N(CH_3)_2$	41.5
$(CH_3)_2N(CH_2)_4N(CH_3)_2$	100
$H_2N(CH_2)_4NHCH_2CH_2CH_3$	23
$H_2N(CH_2)_4NHCH_2CH(CH_3)_2$	51
(*E*)-$H_2NCH_2CH{=}CHCH_2NH_2$	31
(*Z*)-$H_2NCH_2CH{=}CHCH_2NH_2$	40
meso-$H_2NCH_2XCH_2NH_2$‡	no inhibition
rac-$H_2NCH_2XCH_2NH_2$‡	no inhibition
$H_2N(CH_2)_4NHCH_2CH_2Br$	9†
	7.5, 5*
	31.5
$H_2N(CH_2)_4NY_2$§	197
$CH_3CH_2CH_2N{=}C{=}N(CH_2)_4N(CH_3)_2$	no inhibition

approx. 80 min at 37°C. This approach obviated the difficult safe isolation of the free liquid aziridine. For the synthesis of [^{3}H] *N*-(4-aminobutyl)aziridine, *N*-(2-hydroxyethyl)-1,4-diaminobut-(2*Z*)-ene was reacted with tritium gas over palladium/charcoal (prepared by Amersham International p.l.c.) to give [2,3-^{3}H]-*N*-(2-hydroxyethyl)-1,4-diaminobutane. This was brominated and then converted into the aziridine by the *in situ* method described for the unlabelled compound. The syntheses of the other compounds in Table 1 will be reported in a full paper on these studies.

The K_i values in Table 1 were determined by measuring the ability of a given compound to inhibit the uptake of [^{14}C]putrescine or [^{14}C]paraquat into rat lung slices. With the exception of three non-inhibitory compounds, all of the compounds tested displayed competitive inhibition of putrescine uptake. The results show that limited *N*-alkyl substitution at an amino group of putrescine is tolerated, but substitution within the putrescine chain is not (note: this is based on only two examples). The most interestng result is that *N*-(4-aminobutyl)aziridine behaves very similarly to *N*,*N*-dimethylputrescine and is a better inhibitor of paraquat uptake than putrescine. No evidence was obtained for covalent labelling of the receptor by the aziridine. Pre-incubation of lung slices with 100 μM-*N*-(4-aminobutyl)-aziridine for 15 min did not influence their subsequent ability to take up putrescine. Autoradiography of lung slices that had been incubated with the tritiated aziridine showed that this compound was selectively taken up into Clara cells and type I and II epithelial cells (I. Wyatt & A. Soames, unpublished work). This confirms that *N*-(4-aminobutyl)-aziridine is a substrate for the polyamine transporter because these cell types have been shown to accumulate putrescine and paraquat [9]. It is concluded that the aziridine (protonated at both its amino and aziridino functions) binds in a similar manner to *N*,*N*-dimethylputrescine, but if this binding involves nucleophilic groups (e.g. carboxylates) then they are not correctly disposed (see discussion on S_N2 reactions in section A) to attack a methylene group of the aziridine. Our results are in good agreement with those of Heston *et al.* [14], who determined the ability of *N*-(4-aminobutyl)-aziridine to inhibit uptake of putrescine into PC-3 human prostatic carcinoma cells.

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