Polyamines are oxidized in plants, bacteria, protozoa, fungi and animals, by a variety of oxidases with differing modes of action and cofactor requirements [1-5]. The nomenclature in this area is confused and too often in the past it has been a question of 'When I use a word, it means just what I want it to mean', to quote Humpty Dumpty [6]. The confusion has arisen partly as a consequence of limited investigation of the substrate specificity of particular enzyme preparations and partly from a tendency to name an enzyme from whichever substrate was used in the assay. Polyamine oxidases are defined here as enzymes able to oxidize spermidine or spermine, whether or not they also act on di- or monoamines.

The polyamine oxidases can be divided into those that act at the primary (terminal) amino groups of di- and poly-amines and form ammonia as one of the products (Group I; Scheme 1a); and those (the majority) that act at the secondary amino group(s) of the aminopropyl moieties of spermine or spermidine (Group II; Scheme 1b). Oxidases acting at a secondary amino group can be further subdivided, depending on whether diaminopropane (Group IIa) or 3-acetamidopropanal (Group IIb) are among the products (Scheme 1).

Bovine plasma polyamine oxidase (EC 1.4.3.6.), an enzyme that has been considered the prototypic mammalian copper-dependent amine oxidase, has been the subject of extensive study (for reviews, see [4,7]). The enzyme has long been considered to act on spermidine and spermine to produce respectively an aminomonoaldehyde [N'-4-aminobutyl]-iminopropanal or a dialdehyde [N-N'-bis (3-propanal) 1,4-diaminobutane], ammonia and hydrogen peroxide (Scheme 1a; [8]), putting it in Group I of the classification proposed here. Reports that acrolein is a product of the oxidation of spermine or spermidine by bovine plasma polyamine oxidase persist [9-12] despite the careful work of Israel et al. [13,14] who were unable to detect acrolein formation...
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when synthetic spermine dialdehyde, prepared by unambiguous synthesis, was allowed to break down under physiological conditions. A more recent report, based on NMR and MS data, suggests that the products of spermine oxidation by the bovine plasma enzyme are 3-aminopropanal and spermidine [15], placing this enzyme in Group IIb. Again, no trace of acrolein was found under physiological conditions.

The polyamine oxidase of *Pichia pastoris* oxidizes spermine to a dialdehyde (Scheme 1a), and also forms a dialdehyde from spermidine [16], making this a Group I enzyme. Rat liver polyamine oxidase (E.C. 1.5.3.11; [4]) catalyses the oxidative cleavage of spermine or spermidine at a secondary amino group with the production of 3-aminopropanal (Scheme 1b), placing this enzyme in Group IIb. The barley (*Hordenum vulgare*) polyamine oxidase forms dianinopropane as one of the products spermine and spermidine oxidation (Scheme 1b; [17]), hence this enzyme is in Group IIa. Polyamine oxidases are widely distributed, a partial list of organisms in which activity has been detected is given in Table 1.

In excess, extracellular polyamines are toxic, both *in vivo* and *in vitro* [26,27]. Hence one function of the polyamine oxidases could be the regulation of both extracellular and intracellular polyamine levels, with N-acetylation as the initial, detoxifying, step [28–30]. However, some cells (and species) do not possess a polyamine acetylase, cells can excrete polyamines [31,32], and antizyme can protect against abnormal accumulation [33]. In most mammalian cells the intracellular flavin-containing polyamine oxidase is part of a recycling pathway that may not lead to a reduction in total intracellular polyamines. Inhi-

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**Scheme 1**

Alternative sites of action on spermine by polyamine oxidases from different sources

(a) Oxidation of terminal (primary) amino groups with the production of ammonia; the arrows indicate the bond that is broken. (b) Cleavage of the molecule at a secondary amino group to produce either dianinopropane (enzyme E₁) or aminopropanal (E₂).

(a)

(b)
bition of this enzyme did not affect growth rate in cultured cells [34] and rats treated for prolonged periods with a polyamine oxidase inhibitor showed no observable toxicity or change in body weight [5]. Is this enzyme important only in mammalian brain (almost a closed system for polyamines, which do not readily cross the blood-brain barrier; [5]) and redundant elsewhere?

The copper-containing polyamine oxidases, present in plasma and in some, but not all, mammalian tissues, initiate degradation of the polyamines to a variety of metabolites (Scheme 2), the polyamine origins of which have now all been demonstrated by isotopic labelling experiments ([35–37] and references therein). Very high levels of polyamine oxidase activity are present in the plasma of ruminants and in humans during pregnancy (for a review, see [4]) and it has been suggested that these are necessary to regulate extracellular polyamine levels. However, the aminoaldehydes produced are highly cytotoxic [4], in addition to killing bacteria, viruses and parasites [1,20]. Inactivation of these potentially lethal compounds would require immediate deactivation by plasma aldehydehydrogenases before the aminoaldehydes could bind to cell surfaces. In contrast N-acetylaminoaldehydes are much less cytotoxic [4,28,30]. It seems unlikely that a pathway committed solely to degradation should commence by producing potentially toxic compounds.

In some bacteria and lower eukaryotes polyamine oxidases can be induced when these organisms are grown on polyamines as the sole carbon or nitrogen source. Thus, under these circumstances, the induction of polyamine oxidases is an adaptation to the environment [16] and this may also be the case in some helminths [23] and other parasites [24,25].

### Table 1

<table>
<thead>
<tr>
<th>Organism Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>Ila</td>
</tr>
<tr>
<td><em>Neisseria perflavia</em></td>
<td>Ila</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Ila</td>
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<tr>
<td><em>Serratia marcescens</em></td>
<td>Ila</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
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<tr>
<td><em>Penicillium PO-I</em></td>
<td>Iib</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em></td>
<td>Iib</td>
</tr>
<tr>
<td>Yeasts</td>
<td></td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>I</td>
</tr>
<tr>
<td><em>Candida boidinii</em></td>
<td>Iib</td>
</tr>
<tr>
<td><em>Pichia pastoris</em></td>
<td>I</td>
</tr>
<tr>
<td><em>Hansenula polymorpha</em></td>
<td>Iib</td>
</tr>
<tr>
<td>Plants</td>
<td></td>
</tr>
<tr>
<td><em>Avena sativa</em> (oats)</td>
<td>Ila</td>
</tr>
<tr>
<td><em>Zea mays</em> (maize)</td>
<td>Ila</td>
</tr>
<tr>
<td><em>Lens culinaris</em> (lentil)</td>
<td>I</td>
</tr>
<tr>
<td><em>Eichhornia crusipes</em> (water hyacinth)</td>
<td>Ila(?)</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
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<tr>
<td><em>Parasilurus asotus</em> (catfish)</td>
<td>Iib (?)</td>
</tr>
<tr>
<td>Helminths</td>
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<tr>
<td><em>Seta cervi</em></td>
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<tr>
<td><em>Ankylostoma ceylanicum</em></td>
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<tr>
<td><em>Hymenolepis diminuta</em></td>
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<tr>
<td>Parasites</td>
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<tr>
<td><em>Ascaris suum</em></td>
<td>Iib</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em></td>
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</tr>
</tbody>
</table>
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Scheme 2

Metabolites of spermine (a), spermidine (b) and putrescine (c)

(a) Spermine monoaldehyde, N-(3-aminopropyl)-N'-(3-propanal)-1,4-diaminobutane; spermine dialdehyde, NV'-bis(3-propanal)-1,4-diaminobutane; spermine acid 1, N-(3-aminopropyl)-NN'-bis(2-carboxyethyl)-1,4-diaminobutane; spermine acid 2. (b) Spermidine monoaldehyde 1, N-(4-aminobutyl)-aminopropanal; spermidine monoaldehyde 2, N-(3-aminopropyl)-aminobutanal; spermidine dialdehyde, N-(3-propanal)-4-aminobutanal; spermidic acid, N-(2-carboxyethyl)-4-aminobutyric acid; putreanine, (4-aminobutyl)-3-aminopropionic acid; isoputreanine, N-(3-aminopropyl)-4-aminobutyric acid. (c) Putrescine metabolites are given the names by which they are commonly known in the literature.
An amine oxidase has been implicated in cross-linking of proteins in pea seedlings [38], and also in the incorporation of spermine into protein [39,40]. Polyamine-dependent mucosal healing is known to involve transglutaminase catalysed cross-linking of proteins [41] and aminoaldehydes resulting from polyamine oxidation are known to play a part in wound healing in plants [42]. Immunoregulatory roles have been proposed for polyamine oxidases in pregnancy [43], and in some autoimmune diseases [44]. Oxidized polyamines have antimicrobial [20], antiviral [20], fungicidal [45], and antiparasitic properties [46–48], and appear to be part of the macrophage defence armamentarium [49,50]. Polyamine oxidases have been implicated in apoptotic processes in both mammalian [51] and plant [52] cells. The presence of elevated levels of acetylated polyamines in a variety of tumours is associated with reduced polyamine oxidase activity, suggesting that a product of polyamine oxidation may regulate cell replication [3,20].

The polyamine oxidases are well conserved in evolutionary terms and this indicates that these enzymes play a fundamental role in cell function. The complex and varied pathways of polyamine catabolism and the number of compounds produced suggest that some of these products may have biological functions of which we are, at present, unaware. To paraphrase Cofino [53]: 'Polyamine oxidases are doing some interesting things but we do not know why.'

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Abbreviations used: ODC, ornithine decarboxylase; WR1065, N-(2-mercaptoethyl)-1,3-diaminopropane.


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Antizyme modifications affecting polyamine homoeostasis
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Introduction
Putrescine and the polyamines spermidine and spermine are ubiquitous organic cations that affect the structure and activity of multiple cell components. Because of this central role in cellular physiology, a complex network of feedback reactions is required to co-ordinate polyamine synthesis, catabolism, and transport, and thereby maintain polyamine homoeostasis. Recent investigations in mammalian tissues have identified a small regulatory protein, antizyme, as a critical component in the maintenance of cellular polyamine levels (see reviews by Hayashi, [1,2]). Increases in cell polyamines stimulate the synthesis of antizyme, which, in turn, down-regulates both of the mechanisms for increasing cell polyamines: (i) antizyme blocks polyamines synthesis by binding, inactivating and stimulating the rapid degradation of ornithine decarboxylase (ODC), the first enzyme in polyamine biosynthesis [1]. Antizyme also appears to diminish translation of ODC from its mRNA [3]; (ii) antizyme reversibly inhibits the cytoplasmic membrane polyamine transport system, thereby preventing utilization of external sources of polyamines [4–6]. Antizyme is clearly a key factor in mammalian cell polyamine homoeostasis, yet at this point very little is known about the synthesis, degradation, and structural modifications affecting the activity of this most strategic regulatory protein. In this report we summarize recent studies of factors affecting cellular antizyme levels and activity.

Antizyme synthesis
One of the most interesting aspects of antizyme is the unique mechanism whereby polyamines enhance the synthesis of this protein. As initially reported by Matsufuji et al. [7], translation of the generally abundant antizyme mRNA is