Summary.—Spermine interacted with serum polyamine oxidase (PAO) to arrest proliferation of cultured Briss lymphocytes. Arrest was independent of catalase activity and was not directly due to an H₂O₂ byproduct. Arrest was averted by 3-hydroxybenzylxoyamine, which inactivates the pyridoxal co-factor of PAO. The oxidation of spermine in the presence of different concentrations of PAO was non-linear, which implied complex intermediate events for conversion of spermine to labile di-oxidized spermine (N,N′-bis(3-propionaldehyde)-1,4-butanediamine) with, perhaps, overall generation of free radicals (O₂− and •OH) which are damaging to cells. Exogenous free radicals were apparently neither direct participants in cytostasis, nor in the chemiluminescence demonstrable for spermine oxidation. Thiourea, an •OH scavenger, protected against both proliferation arrest and luminescence. Many other powerful •OH scavengers, however, were ineffective. Though reaction mixtures reduced ferricytochrome c initially, reduction was not inhibited by superoxide dismutase (SOD) which indicated that the anion O₂− had not been generated. The powerful reducing capability of di-oxidized spermine itself could have competed against any O₂− for ferricytochrome c reduction. Nevertheless, O₂− was generated during further PAO conversion and/or auto-oxidation of di-oxidized spermine. Curiously, addition of SOD to destroy presumptive O₂− variably potentiated cytotoxicity. Blockage of any anion channels in the cell plasma membrane by stilbene derivatives did not influence cytotoxicity. Thus, findings support our previous evidence that cationic di-oxidized spermine is a potent G¹ inhibitor of cell proliferation. The possibility of intracellular free-radical and thiol involvement is discussed.

Polyamines are synthesized by eukaryotic cells during both G¹ and G² phases of the cell cycle, and are essential for proliferation (Bachrach, 1973; Fuller et al., 1977; Newton & Abdel-Monem, 1978). Polyamines are secreted by cells (Melvin & Keir, 1978; Newton & Abdel-Monem, 1978) and can be exogenously catabolized by polyamine oxidase (PAO) which is abundant in ruminant sera (Kapeller-Adler, 1970) human pregnancy sera (Gaugas & Curzen, 1978) human hepatitis sera (Morgan et al., 1980) and in liver (Hölttä, 1977). Enzymic deamination of the aliphatic polyamine, spermine⁴⁺(NH₂(CH₂)₃NH(CH₂)₄NH(CH₂)₃NH₂), which is the end-product of the biosynthesis (Tabor & Tabor, 1976) produces N,N′bis(3-propionaldehyde)-1,4-butanediamine (di-oxidized spermine⁴⁺) as the primary product (Tabor et al., 1964). Two electrons are possibly transferred in a two-stage reaction from the amine and O₂ is reduced to peroxide (Kapeller-Adler, 1970).

\[
(RHC=NH)₂ + 2O₂ \rightarrow (RHC=NH)₂ + 2H₂O₂
\]

spermine

\[
(RHC=NH)₂ + 2H₂O \rightarrow (RHC=O)₂ + 2NH₃
\]

di-oxidized spermine

The system in vitro somehow evoked potent G¹ arrest of cell proliferation by a transient (Gaugas & Dewey, 1979) and cytostatic mechanism (Byrd et al., 1977; Rijke & Ballieux, 1978). The di-oxidized...
spermine undergoes slow further oxidation, presumably of the remaining amine groups, to produce neutral aldehydes (Gaugas & Dewey, 1979) possibly via carbonyl compounds (RC=O)→→3+ which were capable of condensing to oligamines of 3+ at extreme pH values (Kimes & Morris, 1971). It is doubtful whether products of further oxidation by PAO, and/or auto-oxidation of chiefly the aldehyde moiety which could include production of some toxic acrolein (Alarcon, 1970; Kimes & Morris, 1971) are identical whether reaction occurs in the presence or absence of serum (Gaugas & Dewey, 1979). Enzyme-substrate kinetic and product-lability studies under precise culture conditions suggested that di-oxidized spermine, with a half-life of 2.3 h, arrested cell proliferation (Gaugas & Dewey, 1979). Conversion of the aldehyde moiety to its alcohol destroyed at least bacterial toxicity (Bachrach & Persky, 1964). Attempts to destroy cytotoxicity by addition of aldehyde dehydrogenase, however, failed (Gaugas, unpublished).

It is feasible that superoxide (O₂−) and/or hydroxyl (·OH) free radicals, which are deleterious to cells (Myers, 1973; McCord, 1974; Oberley & Buettner, 1979) could somehow be generated during biological oxidations (Cohen, 1978; Borg et al., 1978) and in polyamine oxidation at least contribute to cytostasis. Though ·OH has never been found in such oxidations, its presence has been inferred (Cohen, 1978) and postulated, for example, in the much-disputed Haber–Weiss reaction shown below (Haber & Weiss, 1934; Fee & Valentine, 1977).

\[ \text{H}_2\text{O}_2 + \text{O}_2^{-} \rightarrow \cdot \text{OH} + \text{OH}^{-} + \text{O}_2 \]

Superoxide dismutase (SOD) and possibly catalase activities should obviate the reaction. Because of the current interest in free radicals in biological oxidations (e.g. Fee & Valentine, 1977; Borg et al., 1978; Sagone et al., 1978) investigations were carried out to determine any role for O₂− dependent free radicals in the cytostasis of spermine oxidation.

**Materials and Methods**

**Enzymes**

Purified bovine-serum amine oxidase or PAO (EC 1.4.3.4) Batch 7028 (Miles Labs. Ltd) 29.2 u/g, where the unit is defined as the amount required to produce 1·0 μmol benzaldehyde/min at 25°C by oxidation of benzylamine.

Catalase.—From bovine liver (EC 1.11.1.6) Batch 26C-7650, 2,000 u/g where one unit decomposes 1·0 μmol H₂O₂/min at pH 7·0 and 25°C.

Superoxide dismutase.—Cu-Zn (SOD) (EC 1.15.1.1) Batch 38C-8190, 2,900 u/mg (Sigma Ltd) and Batch 7017, 5,500 u/mg (Miles Ltd) assayed per McCord & Fridovich (1969).

**Reagents**

Ferricytochrome c Type III, from horse heart (Sigma Ltd), 3-hydroxybenzylxoyla-amine (Sandev Ltd). Pargyline, N-methyl-N-propargylbenzylamine HCl (Aldrich Ltd); nialamide (N-isonicotinoyl-N’ [β-N-benzyl-carbamido]ethyl) hydrazine (Pfizer Ltd). Iproniazid (isonicotinic acid 2-isopropyl-hydrazine PO₂); semicarbazide HCL; aminoguanidine HCO₃; luminol (5-amino-2,3-di-hydro-1,4-phthalazinedione); spermine (N, N’-bis [3-aminopropyl]-1,4-butenediamine)-tetra-HCl (Sigma Ltd), 1-phenyl-3-(2-thiazylol)-2-thiourea (PTTU) (Aldrich Ltd). Thiourea, dimethyl sulphoxide (DMSO), Na benzoate, butanol, ethanol, standardized H₂O₂ and NH₃, Na dithionite; 1-anilino-naphthalene-8-sulphonic acid Mg salt (ANS); 4-acetamido-4’-iso-thiocyanato-stilbene-2,2’-disulphonic acid di-Na salt (SITS) (BDH Ltd). RPMI 1640 20-mm L-glutamine medium; 0·1M phosphate-buffered saline with Earle’s salts, pH 7·2 (PBS) (Flow Ltd). Foetal calf serum (Flow Ltd, Sera Labs. Ltd).

**Methods**

Cell proliferation assay.—Briefly, triplicate 1·0 ml cultures of Bri8 human lymphocytes (Searle Ltd) in RPMI 1640 medium plus 10% foetal calf serum, which contains PAO, were inoculated with 10⁴ cells at Day 0 and after 4–5 days’ incubation (37°C) just before reaching the plateau phase of the growth curve cells were counted. Full details have been presented elsewhere (Gaugas & Dewey, 1979).

Radiochemical assay of ³H-spermine conversion.—Spermine substrate and products of reaction-mixture were isolated by ion-ex-
change chromatography and measured as previously described (Gaugas & Dewey, 1979).

Detection of $O_2^-$ by ferricytochrome $c$ reduction: by $O_2^-$-dependent (i.e. SOD-inhibitable) reduction of ferricytochrome $c$ measured at 550 nm (Fee & Valentine, 1977).

Chemiluminescence of PAO-substrate interaction.—Reagents, luminol, PAO and substrate were mixed at varying concentrations in 0.1M phosphate-buffered saline (pH 7.2) and in a total volume of 10 ml in a low-K glass scintillation vial which was immediately placed into a Scintillation Counter (Beckman Ltd). The counter was set at repeat 1-0-min counts for 0.2% accuracy in the $^3$H window. The temperature of the dark vial chamber was 28°C. Luminosity was recorded and expressed as ct/min x 10^3.

RESULTS

The ability of spermine to interact with PAO in foetal calf serum in medium supporting Br18 lymphocytes in a way to arrest cell proliferation (Gaugas & Dewey, 1979) was confirmed (included in Fig. 4). The spermine concentration required to evoke 50% arrest of proliferation (ID_{50}) was about 6.0 $\mu$M.

Byproduct cytotoxicity

$H_2O_2$ was a byproduct of the enzyme-substrate reaction. When commercial $H_2O_2$ was added to cultured cells at the onset of incubation, its extreme toxicity was confirmed (Table I). When bovine spleen catalase (250 u/ml) was added to cultures containing spermine and PAO the arrest of cell proliferation was not prevented. The amount of catalase inactivated the toxicity of $> 300 \mu$M $H_2O_2$ which had been mixed with completed medium before addition of cells at the onset of incubation. Thus the $H_2O_2$ generated during spermine oxidation could not have been responsible for cytostasis. Catalase, normally present in the serum supplement for cultures, therefore had a potential for $H_2O_2$ destruction at a greater rate than its production by the system. If $H_2O_2$ were involved in $OH$ production, catalase might have ablated the reaction (e.g. Haber–Weiss reaction). No evidence was obtained for such ablation by added catalase.

Enzyme inhibitors

Arrest of lymphocyte proliferation by spermine oxidation was prevented by addition of 3-hydroxybenzylxoyamine which inactivates PAO pyridoxal co-factor (Table II) but not by culture-tolerated

| Table II.—List of reagents which reverse cell proliferation arrest by PAO-spermine interaction (enzyme inhibitors) |
|---------------------------------------------------------------|
| Pyridoxal inactivator:                                        |
| 3-hydroxybenzylxoyamine                                       | 0.1 |
| PAO/diamine oxidase inhibitors:                               |
| Na semicarbazide                                               | 50.0 |
| aminoguanidine                                                | 150.0 |
| Flavin inactivators:                                          |
| nialamide                                                     | non-inhibitory† |
| pargyline                                                     | non-inhibitory† |
| iproniazid                                                    | non-inhibitory† |

* Dose causing 50% reversal of arrest of lymphocyte proliferation.
† At maximal tolerated dosage (~250 $\mu$M) to cell culture.

levels of drugs which inactivate flavin co-factor and thereby inhibit human monoamine oxidase (Knoll, 1976). Hence PAO was indeed the enzyme causing the oxidation of spermine.

Since PAO is more effective against polyamine than monoamine substrates (Gaugas & Dewey, 1979) it should be reclassified, as it is currently described as either “monoamine oxidase” or, more acceptably, “amine oxidase” (EC 1.4.3.4). For the foregoing reasons we always refer to the bovine-serum enzyme as PAO.
Free radicals

Studies have shown that the velocity of \(^{3}\text{H}\)-spermine conversion by PAO was inexplicably nonlinear with respect to PAO concentration, using either purified PAO (Fig. 1) or foetal calf serum containing the enzyme. This implied uneven side-atom \(^{3}\text{H}\)-labelling of the substrate, or complex intermediate events culminating in the formation of di-oxidized spermine. Hence the possibility arose that \(\text{O}_2^{--}\) and/or \(\text{OH}\) could have been generated. Supraphysiological concentrations of PAO-spermine mixture, essentially in the presence of catalase to destroy the \(\text{H}_2\text{O}_2\) byproduct, reduced ferricytochrome \(c\) (Fig. 2). This was not inhibitable by SOD, so was attributed to the powerful reducing capability of the aldehyde moiety of di-oxidized spermine. During further PAO oxidation of di-oxidized spermine and in the absence of catalase, SOD-inhibitable reduction of the cytochrome was observed, which indeed showed that \(\text{O}_2^{--}\) had probably been produced. Reduction was nevertheless limited to 20–25\% of the cytochrome (Fig. 3).

Presumptive \(\text{O}_2^{--}\) in cultures with PAO-substrate mixture was destroyed by prior addition of much SOD. Paradoxically, rather than any reversal of arrest, a significant enhancement of cytotoxicity was demonstrated (Fig. 4). Unfortunately, any \(\text{O}_2^{--}\) generated in cultures was unmeasurably low, so qualitatively similar results to those obtained using much reaction mixture in the assay for ferricytochrome \(c\) reduction (see Fig. 2) had to be assumed.
Thus, rather than contribute to cell damage as suspected, destruction of any $O_2^-$ generated during spermine catabolism apparently enhanced cytotoxicity, perhaps by delaying the further oxidation of di-oxidized spermine. It is therefore unlikely that $O_2^-$, if generated in cultures, reached a level sufficient to elicit cytotoxicity directly. It is noteworthy that extremely high levels of PAO-substrate mixture were needed to show cytochrome c reduction, but the number of sites on the relatively large cytochrome molecule that are reducible is not taken into account.

The SOD preparations were not contaminated by PAO as shown by the radiochemical assay. Nonetheless it is doubtful whether the different batches were comparable in activity or purity (Wardman, 1979).

Thiourea, which is a well-known $\cdot$OH scavenger, reproducibly afforded significant protection of cells against the cytostasis due to spermine oxidation (50% protection at 1-0 mM). In marked contrast, other at least equally powerful $\cdot$OH scavengers afforded no discernible protection at maximal concentrations tolerated for cell cultures (PTTU, 0-2 mM; DMSO, 2-0% v/v; benzoate, 2-0 mM; butanol, 0-1% v/v; ethanol, 0-75% v/v). Thiourea apparently produced this reversal by competing in great excess with natural substrate for PAO, since it also suppressed the chemiluminescence of the PAO-substrate interaction (see below).

**Inhibitors of anion permeability**

SITS and ANS suppress exchange of anions, including $O_2^-$, across plasma membranes of those cells which possess anion
Chemiluminescence of reaction mixture in absence of luminol (B). Autoluminescence of reaction mixture in absence of PAO (C).

1. Chemiluminescence following interaction of 300 μM spermine with 1.0 mg/10 ml purified PAO in the presence of 100 μM luminol in PBS (pH 7.2 at 28°C) (A). Luminescence was equal when the Luminal was added 13.0 min after mixing reagents (arrowed) showing that light emission was not attributable to short-lived O2-dependent free radicals. Autoluminescence of reaction mixture in absence of luminol (B). Autoluminescence of reaction mixture in the absence of PAO (C).

Channels (vide Gennaro & Romeo, 1979). When added to cultured lymphocytes at maximal tolerated concentrations of 500 μM SITS and 5.0 μM ANS, they did not modulate cytotoxicity elicited by spermine oxidation. Thus anions either did not participate directly in the cytotoxicity or (less likely), anion channels do not exist in B18 lymphocytes.

Chemiluminescence

PAO-substrate interaction produced much chemiluminescence in the presence of luminol (Fig. 5). Oxidation of luminol produces light emission (Weber et al., 1943). Two agents could be formed which should oxidize luminol: \( \text{OH}^- \) (e.g. \( \text{NH}_3 + \text{H}_2\text{O} \Rightarrow \text{NH}_4^+ + \text{OH}^- \)) and \( \text{H}_2\text{O}_2 \). The luminescence was ablated by added catalase (Fig. 7) and so was attributed to \( \text{H}_2\text{O}_2 \) byproduct and not free radicals. The luminescence was bi-phasic. Whereas the initial phase of luminescence was in accord with the process of spermine oxidation as judged by a radiochemical assay, despite extensive investigation the cause of the
final phase was not ascertained. Enzyme activity was indicated by the height of the primary luminescence peak (inverse relationship). The chemiluminescence was inhibited by thiourea (Fig. 6) but not by PTTU, DMSO, benzoate or alcohols, at concentrations tolerated for lymphocyte culture. Chemiluminescence was also inhibited by 3-hydroxybenzoyloxyamine (ID$_{50}$ < 1.0 µM), semicarbazide (ID$_{50}$ = 50-0 µM) and aminoguanidine (ID$_{50}$ < 250 µM). Relatively low but nonetheless measurable chemiluminescence was produced by PAO-substrate interaction in the absence of luminol (Fig. 5).

Though requiring some clarification, the technique is remarkably accurate and simple and could be adapted for assay of both PAO and substrate in tissues.

**DISCUSSION**

In a recent publication (Gaugas & Dewey, 1979) we have discussed the phenomenon of cytostasis of mammalian cells resulting from the *in vitro* enzymic oxidation of spermine. Evidence has now been presented that O$_2$-dependent free radicals were not apparently direct participants in the system. The findings generally support circumstantial evidence that labile di-oxidized spermine was the agent responsible for G$_1$ arrest of cell proliferation (Gaugas & Dewey, 1979). Nonetheless, it is feasible that the free radicals could be generated during the catabolism of di-oxidized spermine and provide an additional antimitotic agent. As we shall argue later, free-radical participation might accord with modifying intracellular events. Moreover, participation of free radicals on largely theoretical grounds cannot be ignored, and is therefore discussed. Enzymically produced radicals need not cause cell damage, because they may fail to detach from the enzyme and diffuse to a "wrong" substrate in order to react cytotoxically (Yamazaki, 1977); they are scavenged by thiols, or enzymes such as SOD, catalase and endogenous glutathione peroxidase (Fryor, 1978).

As a precautionary note, when thiourea was added to cultured lymphocytes because it is a well known scavenger of ‘OH, the results conflicted with those obtained for other known scavengers. Thiourea was alone in affording protection against the cytostasis system, yet undoubtedly reacted in some capacity other than as an ‘OH scavenger.

If O$_2$– was generated in a foetal calf serum (PAO) and spermine mixture in lymphocyte cultures, it did not arise until a further stage of oxidation of di-oxidized spermine. Such O$_2$– was not deleterious to cell viability when the concentration of the reaction mixture was optimal for cytostasis. Obviously, such cells had already been exposed to di-oxidized spermine, so evaluation of an O$_2$– effect was made by showing that the addition of SOD to destroy O$_2$– failed to reduce the cytotoxicity measured throughout a spermine dose–response curve. Thus antiproliferative ability due even in part to O$_2$– activity was not forthcoming. It seems likely that insufficient O$_2$– was generated for itself to contribute to the cytotoxicity. Serum ceruloplasmin should have influenced the results, since it scavenges O$_2$– (Goldstein *et al.*, 1979). Serum could also possibly contain traces of SOD able to destroy the relatively small amount of O$_2$–, as it was slowly generated by reaction mixtures.

Curiously, it was found that SOD potentiated the cytostasis of spermine oxidation. If O$_2$– is catalytic for oxidation of di-oxidized spermine by PAO, its destruction should help stabilize this otherwise labile primary product.

It is feasible that O$_2$– reacted with a likely non-cytotoxic and relatively stable carboxyl derivative of di-oxidized spermine (RHC=O + ½O$_2$→RCOOH) to generate a free radical (*i.e.* RCOOH + O$_2$–→RCO– + HO– + O$_2$) which could be the cytotoxic agent (see Peters & Foote, 1976). In other words, O$_2$– might regenerate a cytotoxin. Di-oxidized sper-
mine could also generate a free radical (RCO•) by auto-oxidation (Nonhebel & Walton, 1974).

The reaction pathway leading to cytotoxicity from O2•– is unknown. Of the relatively few biological pathways recognized for O2•– deployment (Oberley & Buettner, 1979) in respect of polyamine oxidation, the sulphhydryls are of particular interest,

\[
RSH + H^+ + O_2•– \rightarrow RS^- + H_2O_2 \rightarrow RSSR
\]

since they have been shown both to enhance and protect against polyamine-oxidation-elicited cytostasis, according to concentration (Dewey, unpublished).

Cytotoxicity of O2-dependent free radicals, due to irreversible cross-linkage of essential cell proteins at nucleophilic sulphhydryl residues, has been suggested (Tse et al., 1976). On the other hand, the aldehyde moiety of di-oxidized spermine could adduct with cysteine (Schauenstein et al., 1977).

Diminished endogenous levels of SOD, and O2•– production, occur almost invariably in tumours. Diminished SOD has been associated with rapid proliferation in non-malignant cells, thus implicating its O2•– substrate in the regulation of cell proliferation (reviewed by Oberley & Buettner, 1979). As well as exogenous PAO in cultures, amine oxidases also occur in cells (Kapeller-Adler, 1970; Quash et al., 1979; Morgan et al., 1980) so a source of O2-dependent free radicals could be from intracellular overall oxidation of polyamines, or indeed oxidized diamines (unpublished) or oxidized monoamines (Cohen, 1978; Borg et al., 1978). This hypothesis is subject to the unlikely event of O2•– being not merely a product of the reaction mixture in vitro, but an in vivo physiological or pathological product. The O2•– is very diffusible into cells and tissues (Lynch & Fridovich, 1978) and thereby could have evaded dismutation by extracellular SOD. The addition of SITS and ANS, which suppress anion but not cation permeability of cells (vide Gennaro & Romeo, 1979) failed to alter the susceptibility of lymphocytes to polyamine-oxidation cytostasis. Though anion channels are present in granulocytes, their existence in lymphocytes has not been ascertained (Gennaro & Romeo, 1979).

As well as being a byproduct of enzymic spermine oxidation, additional H2O2 could be formed from both PAO oxidation of di-oxidized spermine and from O2•– (McCord & Fridovich, 1969).

\[
SOD
O_2•– + O_2•– + 2H^+ \rightarrow H_2O_2 + O_2
\]

Incidentally, this raises the important consideration of whether cells are either more or less vulnerable to exogenous rather than endogenous H2O2, or indeed free radicals. In the system, the participation of H2O2 from oxidation of spermine was readily excluded because of its destruction by added catalase. In contrast, the intracellularly generated H2O2, if any, could not be similarly excluded. However, the cell should be protected against any H2O2 toxicity by cytosol peroxidases (Salin & McCord, 1974).

Unlike aldehyde compounds in general, which are indiscriminately toxic throughout the cell cycle, N,N’-bis(3-propion-aldehyde)–1,4 butanediamine (di-oxidized spermine) apparently caused G1 arrest (Gaugas & Dewey, 1979). Intracellular conversion of di-oxidized spermine itself to a free-radical state (e.g. RCO•) or generation of O2•–, HO2•– or ‘OH and H2O2, could be mandatory for cytotoxic activity. If O2•– were generated, cells with diminished SOD (i.e. tumour cells) should then be more susceptible to cytostasis. Our results suggest, however, that such cells would be less susceptible to the cytotoxic activity of di-oxidized spermine. The products of biochemical events accompanying extracellular spermine catabolism would then be modified intracellularly, especially since PAO, SOD and catalase (but not the products) would be excluded by molecular size from cell entry. The
restricted location of endogenous macro-
molecular PAO, SOD and peroxidase, in
plasma membrane, organelles or cytosol
could determine the fate and any inter-
relationship function of di-oxidized sper-
mine and byproducts once incorporated
into a cell. Such states should alter in
different phases of the cell cycle, possibly
in accordance with the G₁ arrest elicited
by exogenous spermine oxidation. The
system could integrate into permutations
of biochemical pathways to arrest cell
proliferation, or to protect against arrest.
For an example so far not mentioned,
\( \text{RHC} = \text{O} \) could be converted to innocuous
carboxyl (RCOOH) by endogenous alde-
hyde reductase or xanthine oxidase but
with \( \text{O}_2^- \) generation.

Though many polyamine-unrelated alde-
hyde compounds are involved as inter-
mediates in normal cell metabolism, they
are not toxic (Schauenstein et al., 1977).
Rapid detoxification of di-oxidized sper-
mine in the cells is necessary if catabolism
of polyamines is a function of mitochon-
drial PAO. Thus it seems unlikely that
the relatively slow further oxidation of
di-oxidized spermine and therefore \( \text{O}_2^- \)
 generation would occur in vitro. Moreover,
because the carbonyl products of further
oxidation (Kimes & Morris, 1971) should
themselves be cytotoxic (Alarcon, 1970)
the total process would give no advantage
to the cell. Dioxidized spermine itself
might reduce a vital cell component
essential for G₁ metabolism.

In conclusion, \( \text{O}_2^- \)-dependent free rad-
cals or \( \text{H}_2\text{O}_2 \) generated by polyamine
catabolism appear not to be involved in
in vitro exogenous cytostasis. Their pro-
duction intracellularly from oxidized poly-
amine is, however, feasible. It was impor-
tant to determine any role for direct
participation of free radicals, since the
purported antiproliferative potency of
dioxidized spermine is of interest in de-
velopmental chemotherapeutics for mali-
gnancy. The synthesized and chemically
stable ethyl-acetal derivatives of oxidized
polyamines are thought to owe their latent
potent in vivo antileukaemic effects to
hydrolysis which generates an aldehyde
moiety (Allen et al., 1979). On the other
hand, a slow secondary oxidation by PAO
may occur, to generate toxic levels of
\( \text{O}_2^- \) and/or carbonyls. If so, our findings
suggest that on an equimolar basis the
ethyl-acetal derivatives would be less
antiproliferative than di-oxidized sper-
mine itself.

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