HPLC and NMR Investigation of the Serum Amine Oxidase Catalyzed Oxidation of Polyamines

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In the presence of amine oxidases polyamines arrest cell proliferation owing to the generation of hydrogen peroxide and amino aldehydes. In this investigation the bovine serum amine oxidase catalyzed oxidation of polyamines has been studied by HPLC analysis of dansylated reaction products with or without $NaBH_4$ reduction and by NMR spectroscopy of the reaction products and 3-aminopropanal was found to be a major reaction product. These findings were further substantiated by analysis of the reaction products by ion-exchange chromatography and by analysis of the products formed by oxidation of polyamines by the cofactor of Cu amine oxidases, 6-hydroxydopa. 3-Aminopropanal is unstable and can give rise to acrolein by β-elimination.

In the presence of amine oxidases, polyamines are known to arrest cell proliferation[1-13] and to exert cytotoxic effects.[14-25] The cytotoxicity is generally accepted to result from the formation of hydrogen peroxide and amino aldehydes in the enzymatic oxidation of polyamines as shown in Scheme 1. However, the exact nature of the
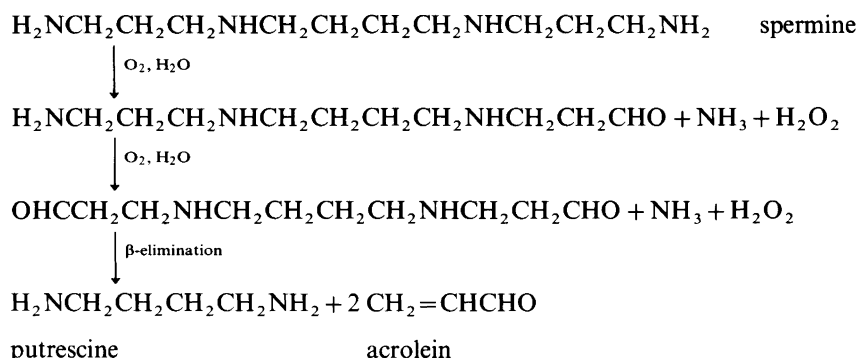
$$R^1CH_2NHR^2 + H_2O \rightarrow R^1CHO + H_2NR^2 + H_2O_2$$

Scheme 1.

cytotoxic aldehydes has been difficult to establish. In the case of the bovine serum amine oxidase (BSAO) catalyzed oxidation of spermine and spermidine the cytotoxic aldehydes have been found to be 4,9-diazadodecanedial

arising from spermidine or N-(4-aminobutyl)-3-amino-propanal arising from spermidine,[26,27] and acrolein, formed from the amino aldehydes by β-elimination[28-30] as shown in Scheme 2.[†]

In this investigation the bovine serum amine oxidase catalyzed oxidation of polyamines was studied by high performance liquid chromatography (HPLC) techniques and nuclear magnetic resonance (NMR) spectroscopy. Evidence for the formation of 3-aminopropanal as a major product during this reaction was obtained. This aldehyde is unstable and can give rise to acrolein and ammonia by β-elimination. Thus, the serum amine oxidase catalyzed oxidation of polyamines may give rise to different reaction products under different experimental conditions.

$$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2 \quad \text{spermine}$$

$$\downarrow O_2, H_2O$$

$$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CHO + NH_3 + H_2O_2$$

$$\downarrow O_2, H_2O$$

$$OHCCH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CHO + NH_3 + H_2O_2$$

$$\downarrow \text{β-elimination}$$

$$H_2NCH_2CH_2CH_2CH_2NH_2 + 2\ CH_2=CHCHO$$

putrescine   acrolein

Scheme 2.

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† 1,5-Diazabicyclo[4.3.0]nonane has also been suggested as the product of the enzymatic oxidation of spermine.[31]

Materials and methods

Chemicals. Spermine tetrahydrochloride, spermidine trihydrochloride, propanediamine and $NaBH_4$ were from Fluka (Buchs, Switzerland). Putrescine dihydrochloride, dansyl chloride, swine kidney diamine oxidase (SKDAO) and aldehyde dehydrogenase were from Sigma (St. Louis, USA). H_3PO_4 (80%), NaOH, NaCl, triethylamine, Na_2HPO_4, NaH_2PO_4, toluene, glycerol and acrolein were from Merck (Darmstadt, Germany). MeOH was from Rathburn (Walkerburn, Scotland). D_2O (99.9%) was from Stohler (Germany). N,N'-Bis-(3-hydroxypropyl)putrescine was synthesized as described.[26] Foetal calf serum was from Gibco (Paisley, Scotland). Purified bovine serum amine oxidase was a generous gift from Prof. B. Mondovi, or was purified by a slight modification of the method of Mondovi *et al.*[32]

NMR spectroscopy. NMR spectra were recorded on a 500 MHz AM-500 or on a 600 MHz AMX-600 Bruker instrument. Assignments were based on comparison with model compounds and $^{13}C-^1H$ correlation experiments. The enzyme kinetic experiments were performed using saturation of the HOD signal with the proton decoupler.

Enzyme reactions. For the enzyme reactions, 10 μl of 5 or 10 mM polyamine in 0.1 M phosphate buffer (PB), pH 7.2, were added to 1 ml foetal calf serum, 1 ml tissue extract or 1 ml 0.1 M phosphate buffer, pH 7.2, with 10 μg bovine serum amine oxidase and the mixtures were incubated at 37°C. Controls contained phosphate buffered saline, foetal calf serum or purified amine oxidase which had been boiled for 15 min, or an equal concentration of aldehyde dehydrogenase. Polyamine concentrations were then determined by HPLC analysis as described later. For NMR experiments enzyme, substrate and phosphate buffer were freeze-dried twice from D_2O before use. The reaction was carried out as described above except that 500 μg of enzyme were used and that samples were incubated at room temperature. Large-scale oxidation of spermidine was done by dissolving 10 mg substrate in 100 ml 0.1 M phosphate buffer, pH 7.2, and adding 1 mg bovine serum amine oxidase. When all of the substrate had been oxidized, the reaction mixture was lyophilized once, dissolved in D_2O (10 ml) and lyophilized again.

NaBH₄ reduction. $NaBH_4$ reduction was carried out essentially as described by Tabor *et al.*[26] by adding 10 μl of freshly prepared 0.2 M $NaBH_4$ in 1 M NaOH to 100 μl reaction mixture. After 20 min the reduction was stopped by addition of 10 μl 60% perchloric acid. The efficiency of the reduction was verified by reduction of a solution of benzaldehyde.

High performance liquid chromatography (HPLC). Samples for HPLC were dansylated and analyzed as described by Brossat *et al.*[33] using a linear gradient from 50% MeOH, 50% 50 mM triethylammonium phosphate pH 4,

to 100% MeOH over 15 min (A) or from 60% MeOH to 100% MeOH over 30 min (B). HPLC was done on a Waters system with two model 510 pumps, a model 420-AC fluorescence detector, a 710 B WISP, a Waters Data Module, a Z-module with an 8 mm × 10 cm RP8 column and a Waters System Controller.

Fast atom bombardment mass spectrometry. (FAB MS). Samples from the HPLC were dried, extracted into MeOH, dried again and dissolved in glycerol for FAB MS. FAB MS was done on a VG Masslab VG 20-250 Quadrupole mass spectrometer fitted with a VG FAB source and probe. The primary beam of xenon atoms was produced from an ion gun (Ion Tech. Ltd.), operating at 1.0 mA, 8 kV.

Malondialdehyde determination. Malondialdehyde was determined by reaction with thiobarbituric acid as described.[34]

Ion-exchange chromatography analyses. Samples for analysis by ion-exchange chromatography were mixed with an equal volume of start buffer (0.2 M sodium citrate, 0.2 M sodium nitrate, pH 3.1), filtered through Ultrafree MC filters (Millipore) and analyzed (100 μl) using an amino acid analysis system (Waters/Millipore) with post column OPA (*ortho*-phthalaldehyde) derivatization and fluorescence detection as described.[35]

Ion-exchange chromatography of polyamines. Samples were chromatographed on a 4 cm × 0.46 cm column packed with Hemabio 1000 SB (Tessek, Riskov, Denmark). Buffer A: 0.20 M sodium citrate, 4% isopropyl alcohol pH 3.1. Buffer B: 0.21 M sodium borate, 4% isopropyl alcohol, 1 M NaCl, pH 10.1. Gradient: 80% A, 20% B to 100% B over 20 min and then 100% B for 15 min. Post-column OPA detection and all other system parameters were as described.[35]

Results

The amine oxidase activity in foetal calf serum, BSAO, was found to oxidize spermine (SPM), N^1-Ac-SPM and spermidine (SPD) very efficiently (Table 1). BSAO was

Table 1. Oxidation of polyamines by bovine serum amine oxidase as determined by reversed-phase chromatography.[a]

Substrate	Retention time/min	% Oxidation	Product
N^1-Ac-PUT	8.64	45	N.i.[b]
PUT	16.93	11	N.i.[b]
N^1-Ac-SPD	17.33	0	—
SPD	21.85	100	PUT
N^1-Ac-SPM	22.09	100	N^1-Ac-SPD
SPM	24.92	100	PUT, SPD

[a] Substrate concentration was 0.1 mM. The reaction was allowed to proceed for 90 min before analysis as described in the Materials and methods section. [b] N.i. = not identified.
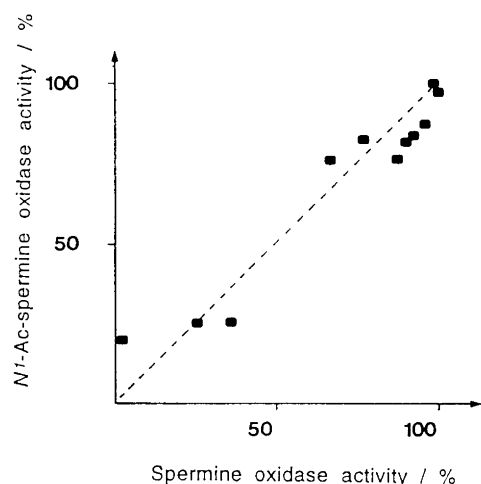
Fig. 1. Correlation between SPM oxidase and N^1-Ac-SPM oxidase activities in different bovine tissues. Samples from three different animals were homogenized in phosphate buffer, substrate was added and changes in substrate concentration were determined by dansylation and HPLC analysis.

observed to oxidize SPD, N^1-Ac-SPM and SPM to the same extent, a conclusion supported by the tissue distribution of SPM oxidase and N^1-Ac-SPM oxidase activity in the cow (Fig. 1), showing that there is a close correlation between these activities, and thus indicating that the same enzyme is responsible for these activities. Furthermore BSAO was found to catalyze the sequential
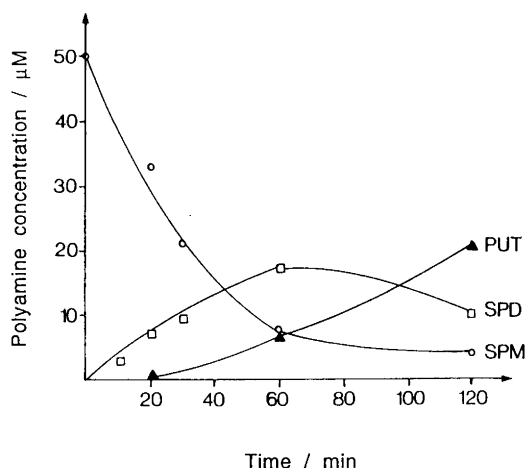

Fig. 2. Oxidation of spermine by foetal calf serum. At time zero 50 μl 1 mM SPM were added per milliliter foetal calf serum. At the times indicated duplicate 200 μl samples were removed and the reaction stopped by addition of 10 μl 60% PCA. To each sample were added 100 μl saturated Na_2CO_3 and 600 μl dansyl chloride (10 mg ml^{-1} in acetone). Dansylation proceeded for 1 h at 50°C or 2 h at 37°C. 100 μl proline (150 mg ml^{-1}) in H_2O were added and samples were incubated 4–12 h at 37°C. The dansylated polyamines were extracted into 2 ml toluene. The toluene was removed *in vacuo*, samples were redissolved in MeOH and analyzed by HPLC as described[33] using a gradient from 60% MeOH, 40% 50 mM triethylammonium phosphate, pH 3.5 to 100% MeOH over 20 min.
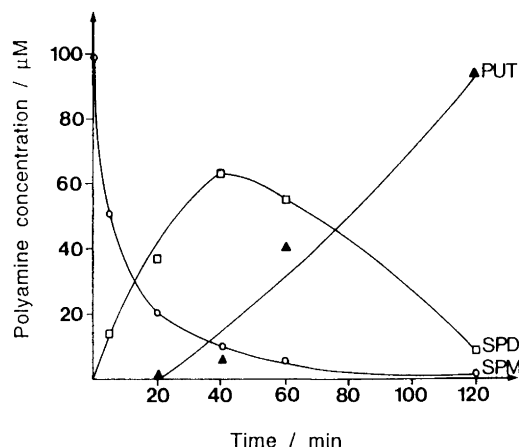

Fig. 3. Effect of $NaBH_4$ reduction on the conversion pattern of the bovine serum amine oxidase oxidation of spermine as determined by HPLC of dansylated reaction products. The reaction was done at 37°C in 3 ml 0.2 M phosphate buffer, pH 7.2, with 0.1 mM spermine. At time zero 30 μl purified bovine serum amine oxidase ($A_{280} = 1.0$) were added. At the indicated times duplicate 100 μl samples were removed and treated with $NaBH_4$ before analysis as described in the Materials and methods section.

conversion of spermine into spermidine and of spermidine into putrescine (Fig. 2). The same conversion-pattern was seen in the oxidation of spermine by purified bovine serum oxidase even when the reaction products were treated with $NaBH_4$ before dansylation and analysis (Fig. 3). This result would not be expected if the 'terminally' oxidized products, shown in Scheme 2 were the main products under these reaction conditions. The dansyl derivatives of putrescine, synthetic N,N'-bis-(3-hydroxypropyl)putrescine and the product from the oxidation of spermine were found to be eluted with almost the same retention time under the standard conditions used for reversed-phase HPLC analysis (Table 2). Using another (less steep) gradient of acetonitrile, putrescine and N,N'-bis-(3-hydroxypropyl)putrescine were eluted with about 0.5 min difference in retention time, however, with this gradient, the product from both $NaBH_4$-treated and untreated samples were eluted at the position of putrescine (Table 3). The identity of the product was further confirmed by FAB MS analysis of the dansylated

Table 2. Effect of $NaBH_4$ reduction on product elution time in the bovine serum amine oxidase catalyzed oxidation of polyamines.

Compound	Retention time/min[a]	Amount – $NaBH_4$/mM	Amount + $NaBH_4$/mM
SPM	24.94	>0.01	0.02
SPD	21.87	>0.01	0.01
'Product'	17.04	0.33	0.39
PUT	17.02	—	—
N,N'-Bis-(3-hydroxypropyl)putrescine	17.15	—	—

[a] HPLC program a.

Table 3. Identification of the product from the bovine serum amine oxidase catalyzed oxidation of polyamines.

Compound	Retention time/min[a]	Mass (FAB MS)
Product	15.9(1)	554
PUT	15.73(1)	554
N,N'-Bis-(3-hydroxy-propyl)putrescine	16.21(3)	670

[a] HPLC program b.

Table 4. NMR chemical shifts (^{1}H and ^{13}C) for the polyamines.[a]

Compound	[1]H-chemical shifts/ppm	^{13}C-chemical shifts/ppm
Putrescine	1.78 (q), 3.06 (t)	24.7, 39.6
Spermidine	1.80 (m), 2.13 (q), 3.04–3.17 (m)	23.5, 24.6, 24.7, 37.4, 39.4, 39.7, 45.4, 47.9
Spermine	1.82 (q), 2.13 (q), 3.10–3.22 (m)	23.6, 24.6, 37.4, 45.4, 47.8

[a] q = quintet, t = triplet, m = multiplet.

compounds, collected from the HPLC (Table 3). Thus, there is unequivocal evidence for a major reaction pathway involving oxidation at the secondary amino groups of spermine since, otherwise, N,N'-bis-(3-hydroxypropyl)putrescine should have been observed instead of putrescine.

To investigate the reaction in more detail, the oxidation of spermidine by purified BSAO was followed by nuclear magnetic resonance (NMR) spectroscopy. Table 4 shows chemical shifts for putrescine, spermidine, spermine and acrolein. Fig. 4 shows the course of the reaction with time as followed by ^{1}H NMR spectroscopy. Compared with the ^{1}H spectrum of spermidine the most conspicuous

change in the beginning is the appearance of two singlets, one smaller than the other, at 3.18 ppm and two singlets, again one smaller than the other, at 5.24 ppm. As the reaction progresses, the smaller singlets disappear and the larger singlets grow in size. Concomitantly, the signals at 2.1 ppm almost disappear while the signals at 3.1 ppm change and become smaller. Also, the signals at 1.8 ppm move a little upfield and develop into a quintet at the same time as the signals at 3.06 develop into a triplet, a pattern characteristic for putrescine. These results show that when spermidine is oxidized by BSAO the asymmetrical spectrum disappears and the symmetrical spec-
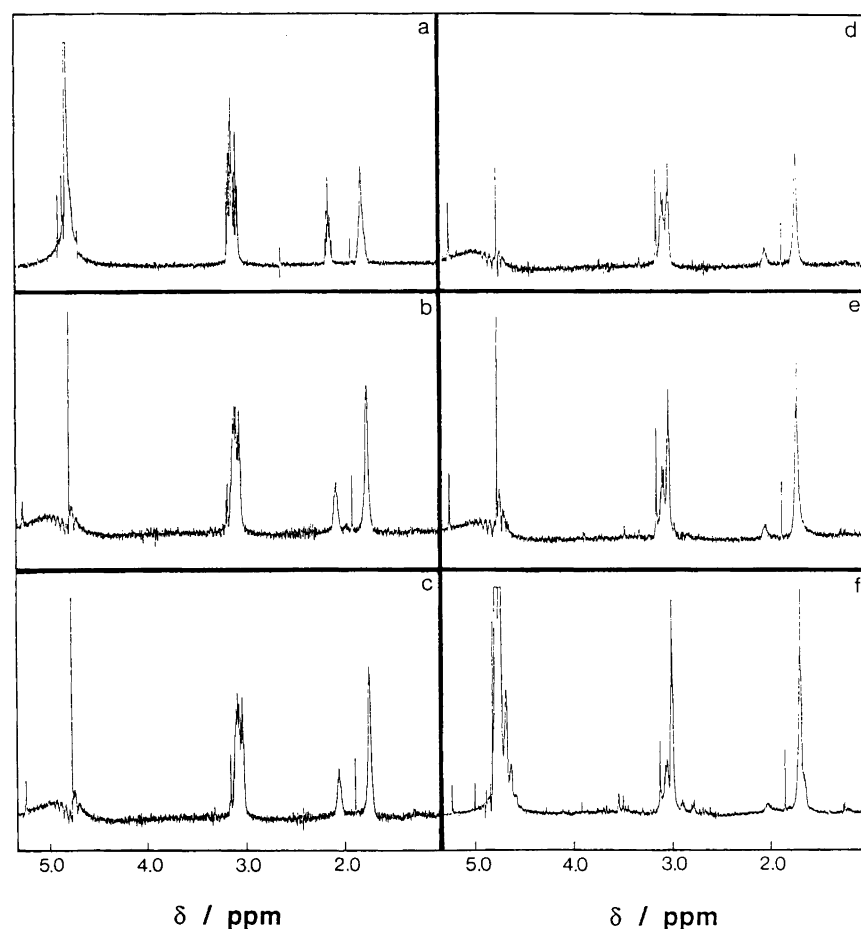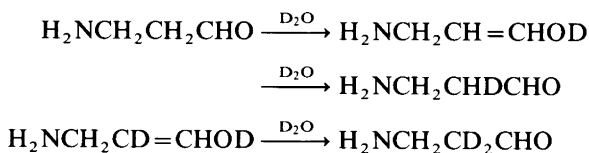

Fig. 4. NMR investigation of the bovine serum amine oxidase catalyzed oxidation of spermidine: A, $t = 0$; B, 2 h; C, 3 h; D, 20 h; E, 50 h; F, 200 h. The experiment was performed as described in the Materials and methods section.

trum of putrescine appears together with a singlet at 5.24 (presumably from a hydrated–CHO), a singlet at 3.18 and additional signals at 3.10 to 3.18. These signals are characteristic of 3-aminopropanal, which in D_2O under the experimental conditions (pH 7.2), can easily undergo deuterium–proton exchange at the C-2 position owing to enolization (Scheme 3).

$$H_2NCH_2CH_2CHO \xrightarrow{\text{D}_2\text{O}} H_2NCH_2CH=CHOD$$

$$\xrightarrow{\text{D}_2\text{O}} H_2NCH_2CHDCHO$$

$$H_2NCH_2CD=CHOD \xrightarrow{\text{D}_2\text{O}} H_2NCH_2CD_2CHO$$

Scheme 3.

Furthermore, when a large portion of spermidine was oxidized by BSAO until complete conversion into putrescine (as determined by reversed-phase HPLC analysis of dansylated samples drawn from the reaction mixture) and finally lyophilized, only the spectrum of putrescine was seen in the ^1H and ^{13}C NMR spectra of the reaction mixture, owing to decomposition of 3-aminopropanal and evaporation of the products formed (e.g., acrolein and ammonia). Since lyophilization is the mildest post-treatment to which the reaction mixture can possibly be subjected, involving only freezing of the reaction mixture and subsequent freeze-drying, this result, in itself, represents strong evidence for oxidation at the secondary amino group.
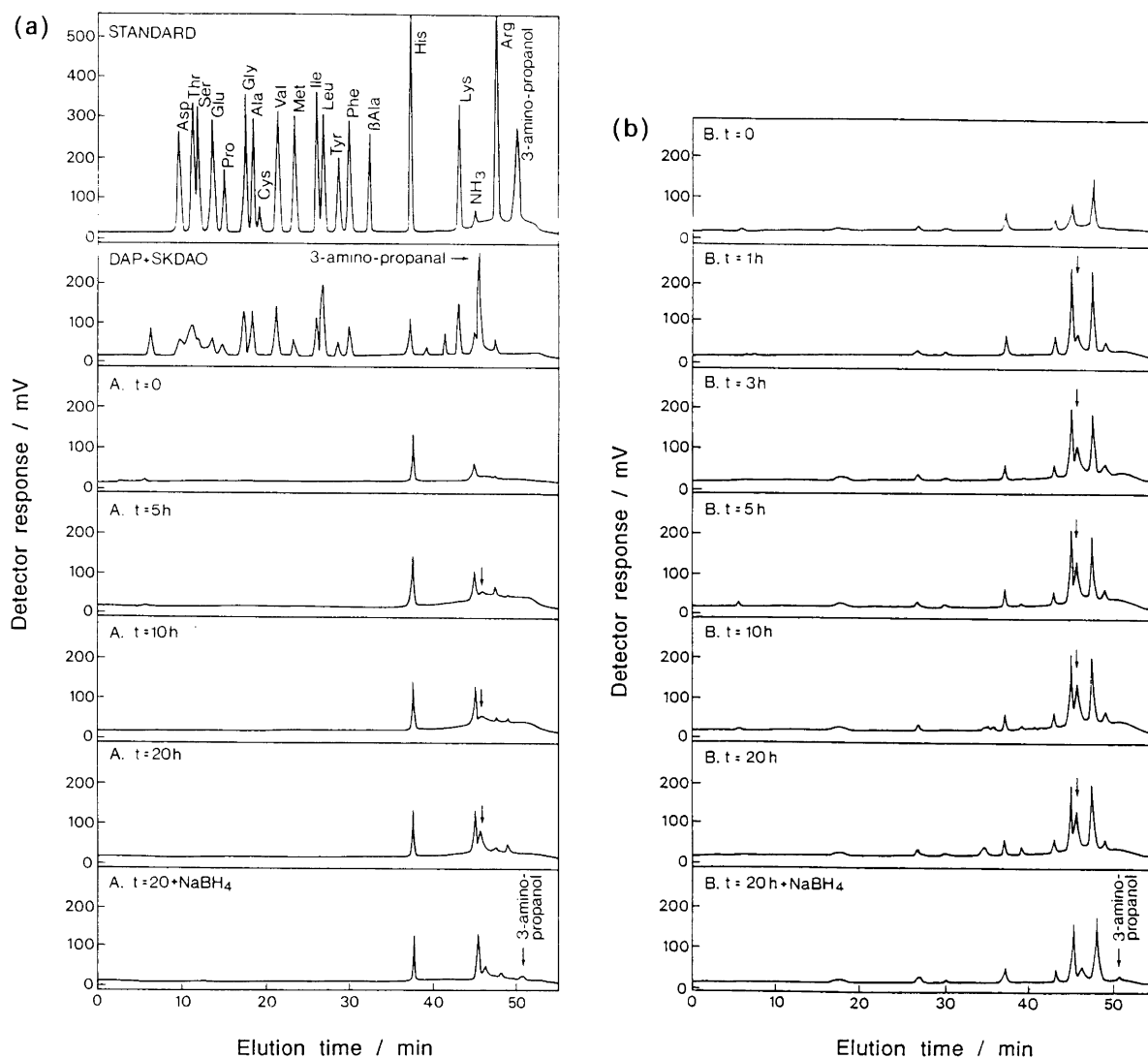

Fig. 5. Identification of 3-aminopropanal by ion-exchange chromatography. To 1 ml of 0.2 mM spermidine in 0.1 M phosphate buffer, pH 7.2, were added 50 units of purified bovine serum amine oxidase (a) or 500 units of partially purified bovine serum amine oxidase (b). The reaction mixtures were incubated at 37°C and, at the times indicated, 100 μl samples were drawn and analyzed on an amino acid analyzer as described in the Materials and methods section. Samples for $NaBH_4$ reduction were treated with 10 μl 0.2 M $NaBH_4$ for 1 h at room temperature before analysis. For identification of 3-aminopropanal 1 ml of 0.2 mM propanediamine (PDA) was incubated at 37°C with 2 mg of swine kidney diamine oxidase (SKDAO) for 2 h. The standard contained 1 nmol each of the indicated compounds.

Finally, it should be noted that acrolein was not observed during the reaction, and no indication of malondialdehyde production was found, either by NMR spectroscopy or by the thiobarbituric acid reaction for malondialdehyde. Since the investigation of the reaction by NMR spectroscopy provides a mild and completely non-invasive method of observation this constitutes unequivocal proof of oxidation at the secondary amino group of spermidine and agrees completely with the results from HPLC analysis of $NaBH_4$-reduced oxidation products. Fig. 5 shows the formation of 3-aminopropanal from spermidine as seen by direct identification and analysis by ion-exchange chromatography. The identity of 3-aminopropanal was verified by analysis of the reaction product from the oxidation of propane 1,3-diamine (PDA) by porcine kidney diamine oxidase. Recoveries of 3-aminopropanal on the amino acid analyser were low, owing to the instability of this compound which also afforded ammonia upon preparation of samples for analysis and during chromatography. On amino acid analysis, ammonia is eluted just before 3-aminopropanal. Treatment of the reaction mixture with $NaBH_4$ resulted in the disappearance of 3-aminopropanal and the formation of 3-aminopropanol, identified by its elution at a position corresponding to authentic 3-aminopropanol. Yields of 3-aminopropanol from 3-aminopropanal were also low, again reflecting the instability of this compound.

To substantiate further the above findings a com-parison by NMR spectroscopy of the aldehyde protons generated by BSAO oxidation of spermidine and by SKDAO oxidation of spermidine and propane diamine was made. The reactions were performed in 10% water in order to observe the aldehyde protons and it should be noted that the SKDAO oxidation of propanediamine can only result in formation of 3-aminopropanal. As seen in Fig. 6 only one 'new' prominent aldehyde peak is seen in the BSAO and SKDAO oxidation of SPD. This peak is also seen in the SKDAO oxidation of PDA. Furthermore, the same set of additional peaks resulting from further secondary reactions of the aldehydes are seen in the prolonged BSAO or SKDAO oxidation of SPD and in the SKDAO oxidation of PDA. Thus, several independent sets of NMR spectral and HPLC data point to the conclusion that BSAO can oxidize polyamines at the secondary amino group.

Since the reaction products have been shown by others to be stable under conditions of ion-exchange chromatography,[1,14] we also analyzed the reaction by ion-exchange chromatography using a column from which the polyamines can be eluted under 'mild' conditions, not involving extremes of pH. These experiments showed that 3-aminopropanal, and also small amounts of 3-aminopropanol, ammonia and β-alanine were formed in the SKDAO oxidation of PDA, as seen by amino acid analysis (not shown). Under these conditions a direct conversion of SPM into SPD and of SPD into PUT by
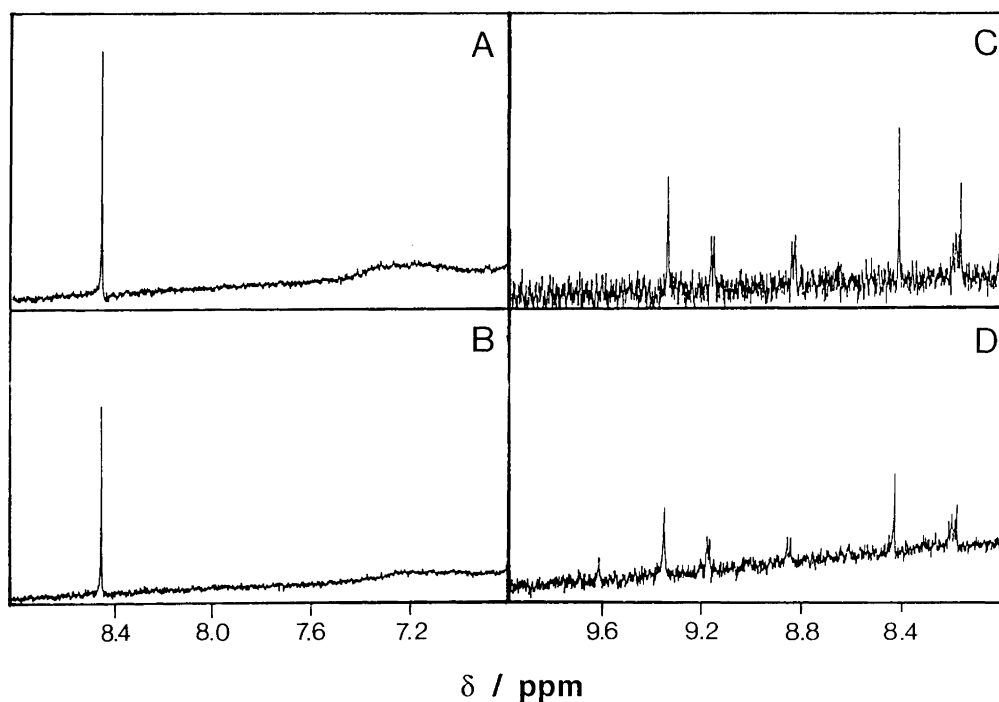

Fig. 6. Oxidation of spermidine by BSAO (and SKDAO) and oxidation of PDA by SKDAO: A and B, aldehyde peak seen in the oxidation of SPD by BSAO (and SKDAO) and in the oxidation of PDA by SKDAO (B), after a reaction time of 1 h; C and D, secondary aldehyde peaks seen after prolonged incubation (24 h) of SPD with BSAO and PDA with SKDAO.
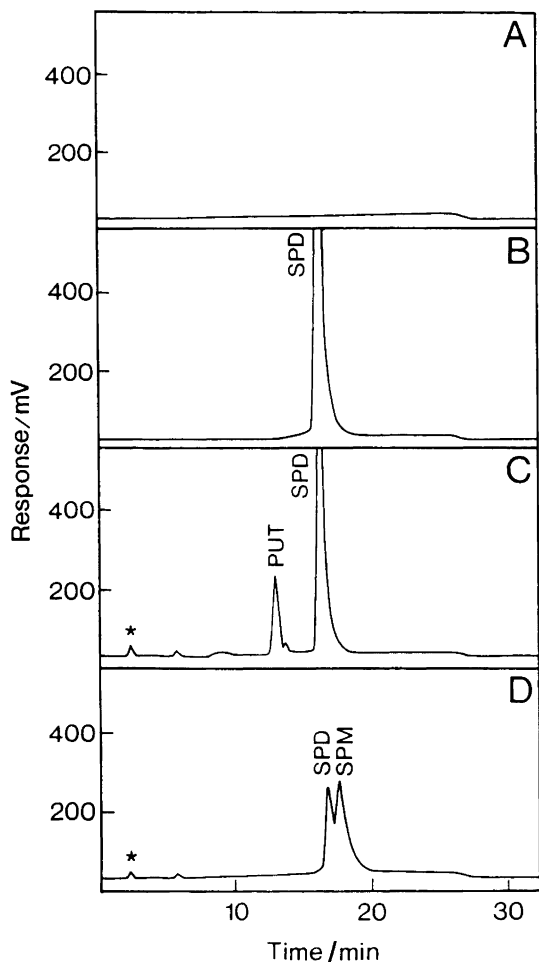
Fig. 7. Oxidation of spermidine and spermine by BSAO: A, control with BSAO and without substrate; B, control with substrate and without enzyme; C, oxidation of spermidine with BSAO after 1 h; D, oxidation of spermine with BSAO after 1 h. * indicates the position of 3-aminopropanal.

BSAO was observed (Fig. 7) and small amounts of 3-aminopropanal were observed.

The cofactor of Cu–amine oxidases has recently been identified as 6-hydroxydopa.[36] The cofactor was previously thought to be pyrroloquinoline quinone (PQQ)[37-39] and we have shown that the product of PQQ oxidation of SPM or SPD is PDA, thus implying oxidation at the secondary, more nucleophilic amino group by PQQ.[40] For this reason we investigated the oxidation of SPD by 6-hydroxydopa itself. As seen in Fig. 8, 3-aminopropanal was again seen to be a product (not PDA), and no ammonia or putrescine was seen in the chromatograms. The reason for the missing putrescine was found in a heavy precipitate composed of 6-hydroxydopa and putrescine. This precipitate was only observed when 6-hydroxydopa was allowed to react with SPD and not with PUT. This again implies reaction of 6-hydroxydopa with the more nucleophilic secondary amino group, and is reminiscent of the substrate specificity of BSAO for which PUT is a poor substrate.
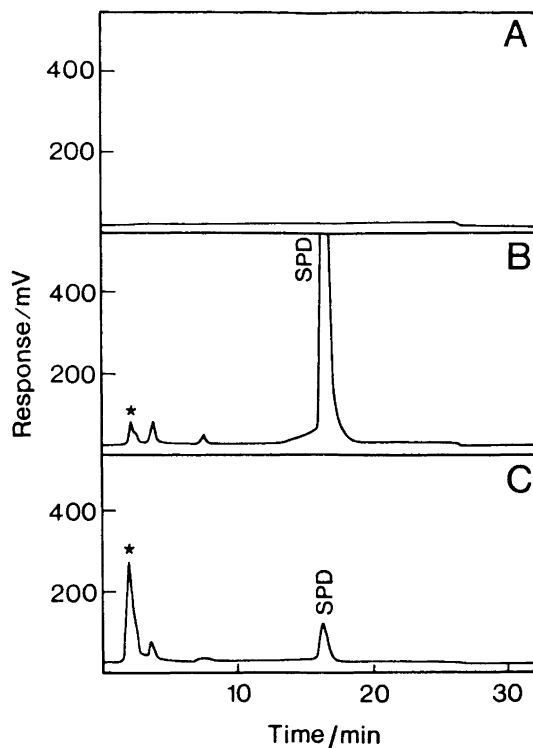

Fig. 8. Oxidation of spermidine by 6-hydroxydopa as determined by ion-exchange chromatography: A, control with 10 mM 6-hydroxydopa; B, 1 mM spermidine + 1 mM 6-hydroxydopa, 37°C, 20 h; C, 1 mM spermidine + 10 mM 6-hydroxydopa, 37°C, 20 h. * indicates the position of 3-aminopropanal.

Finally the results presented here imply that BSAO can oxidize secondary amines such as for example N-ethylbenzylamine. This was actually found to be the case as seen in Fig. 9 which shows the production of benzaldehyde from N-ethylbenzylamine.
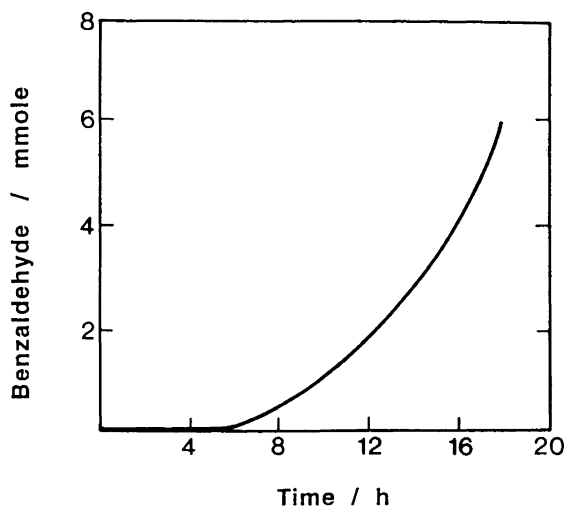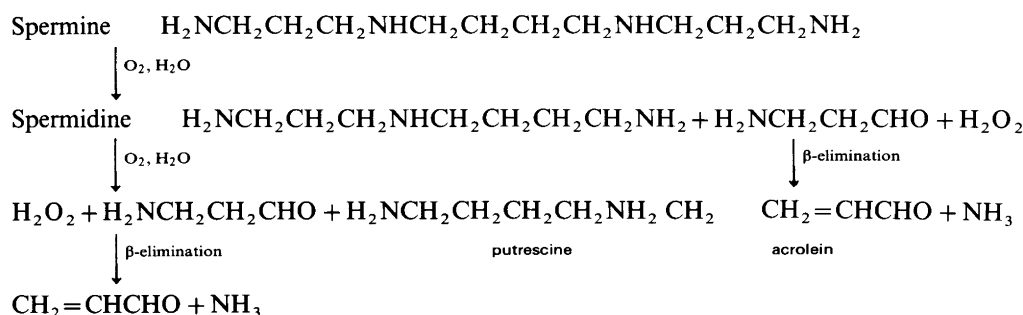

Fig. 9. Oxidation of N-ethylbenzylamine by BSAO as seen by production of benzaldehyde. Substrate concentration was 1 mM and 0.1 μg of enzyme was added to 1 ml at time zero. Controls with enzyme or substrate alone did not show an increase in absorbance at 250 nm.

Discussion

The substrate specificity of bovine serum amine oxidase observed here is in agreement with previous studies showing that BSAO oxidizes spermine, spermidine, and N^8-Ac-spermidine efficiently but putrescine, Ac-putrescine and N^1-Ac-spermidine less efficiently.[32,41] This substrate specificity has, in one case, been interpreted as representing the presence of tissue type (FAD-dependent) polyamine oxidase activity in bovine serum.[42] Previous studies with natural and synthetic polyamines have established that the structural requirement for maximum cytotoxicity in the presence of BSAO is $RNH(CH_2)_xNHCH_2CH_2CH_2NH_2$, where $x \geqslant 3$[13,43] and that spermine, spermidine, N^8-Ac-spermidine and N^1-Ac-spermine are almost equally cytotoxic in the presence of serum amine oxidase, while putrescine, N^1-Ac-spermidine and N^1,N^{12}-diacetylspermine are much less cytotoxic.[14] Also, 3-aminopropanal has been shown to be as cytotoxic as the reaction products from oxidation of SPD and SPM.[44] These results are most readily interpreted as reflecting the substrate specificity of bovine serum amine oxidase as described in the present work and not the differential cytotoxicity of the oxidation products of the various polyamines. The structure of the cytotoxic aldehydes produced by the oxidation of the polyamines is controversial. Some authors have found evidence for the production of acrolein and others for the existence of 'terminally' oxidized polyamines.[26-30] There can be little doubt that the various aldehydes in question are almost equally cytotoxic (acrolein, 3-aminopropanal, and the oxidation products from the polyamines),[1-25] reflecting the generally high cytotoxicity of aldehydes. The conclusion that the 'terminally' oxidized polyamines are the major reaction products[26] was based on comparison of R_f values on TLC plates, melting points, elemental analyses and IR spectra of authentic and $NaBH_4$-reduction products. However, such analyses, although being then the only methods available, are inadequate by today's standards in an unambiguous characterization of the reaction products. Furthermore, these results are in conflict with other reported results[45-47] and the results presented here. Most investigations of the serum amine oxidase catalyzed oxidation of spermine seem to indicate a sequential conversion of spermine into spermidine and

of spermidine into putrescine.[45-50] This was also found in this investigation but neither acrolein nor the 'terminally' oxidized polyamines could be identified under physiological conditions by the various techniques employed, including NMR, HPLC and FAB MS. On the other hand, the formation of putrescine and 3-aminopropanal could be demonstrated (Scheme 4). These data, particularly the formation of putrescine without formation of acrolein in the NMR reaction, where the reaction mixture is undisturbed, are only compatible with oxidation occurring at the secondary amino group and agree well with other reported results, demonstrating the presence of 3-aminopropanal in human serum and its formation from spermidine,[51] and that among many other metabolites β-alanine is quantitatively the most important metabolite from spermidine and spermine in rat plasma.[52] The formation of 3-aminopropanal from spermidine has also previously been demonstrated in normal and virus-transformed rat kidney cells[53] and in Pseudomonas sp.[54] and as the product of tissue type (FAD-dependent) polyamine oxidase catalyzed oxidation of spermidine and spermine.[55]

Owing to its structure, 3-aminopropanal is very reactive and unstable under non-physiological conditions, explaining the conflicting results reported in the literature and explaining the difficulties in its identification and isolation (e.g., it disappears just as a result of freeze–drying). Under physiological conditions it seems to be quite stable as no acrolein is formed (Ref. 42, this work), but under non-physiological conditions β-elimination can be expected to occur readily, resulting in the formation of acrolein and ammonia during most procedures for isolation and characterization of the reaction products. In vivo, 3-aminopropanal is probably further oxidized to β-alanine under most conditions as would also be expected for the precursor aldehydes of putreanine and spermic acid, which would be the end products if the polyamines are oxidized at the primary amino groups. The distribution of putreanine in mammals, with highest concentrations in brain and in liver,[56] would be compatible with their formation by a monoamine oxidase involving pathway, but not with their formation by a Cu–amine oxidase catalyzed pathway. Under non-physiological conditions, such as rotary evaporation, acrolein will be formed by β-elimination. Reaction of

Spermine $H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$

$\Big\downarrow O_2, H_2O$

Spermidine $H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2 + H_2NCH_2CH_2CHO + H_2O_2$

$\Big\downarrow O_2, H_2O$ $\Big\downarrow$ β-elimination

$H_2O_2 + H_2NCH_2CH_2CHO + H_2NCH_2CH_2CH_2CH_2NH_2 \quad CH_2$ $CH_2=CHCHO + NH_3$

$\Big\downarrow$ β-elimination   putrescine   acrolein

$CH_2=CHCHO + NH_3$

Scheme 4.

acrolein with putrescine by addition of the amino group to the double bond can lead to the formation of both 3-(4-aminobutylamino)propanal and 3,3'-(butane-1,4-diamino)dipropanal and hence their detection in the reaction mixture following rotary evaporation and work-up of reaction mixtures.

In all, the results presented here, together with other available evidence, point to the conclusion that serum amine oxidase can oxidize polyamines at the secondary amino group.

References

1. Kimes, B. W. and Morris, D. R. *Biochim. Biophys. Acta 228* (1971) 235.
2. Morgan, D. M. L. In: Calderera, C. M. *et al.*, Eds., *Adv. Polyamine Res.*, Raven Press, New York 1981, Vol. 3, p. 65.
3. Higgins, M. L., Tillman, M. C., Rupp, J. P. and Leach, F. R. *J. Cell. Physiol. 74* (1969) 149.
4. Frolik, C. A., Roller, P. P., Cone, J. L., Dart, L. L., Smith, D. M. and Sporn, M. *Arch. Biochem. Biophys. 230* (1984) 93.
5. Byrd, W. J., Jacobs, D. M. and Amoss, M. S. *Nature (London) 267* (1967) 621.
6. Allen, J. C., Smith, C. J., Hussain, J. I., Thomas, J. M. and Gaugas, J. M. *Eur. J. Biochem. 102* (1979) 153.
7. Gaugas, J. M. and Curzen, P. *Lancet 8054* (1978) 18.
8. Gaugas, J. M. and Dewey, D. L. *Br. J. Cancer 39* (1978) 548.
9. Gaugas, J. M. and Dewey, D. L. *Br. J. Cancer 41* (1980) 946.
10. Smith, C. J., Maschler, R., Maurer, H. R. and Allen, J. C. *Cell Tissue Kinet. 16* (1983) 269.
11. Hussain, J. I., Smith, C. J. and Allen, J. C. *Cell Tissue Kinet. 16* (1983) 583.
12. Kuramochi, H., Hiratsuka, M., Nagamine, S., Takahashi, K., Nakamura, T., Takeuchi, T. and Umezawa, H. *J. Antibiot. XLI* (1988) 234.
13. Israel, M. and Modest, E. J. *J. Med. Chem. 14* (1971) 1042.
14. Bachrach, U., Abzug, S. and Bekierkunst, A. *Biochim. Biophys. Acta 134* (1967) 174.
15. Morgan, D. M. L. *Biochem. J. 242* (1987) 347.
16. Morgan, D. M. L., Bachrach, U., Assaraf, Y. G., Harari, E. and Golenser, J. *Biochem. J. 236* (1986) 97.
17. Morgan, D. M. L. and Christensen, J. In: Bachrach, U., Kaye, A. and Chayen, R., Eds., *Adv. Polyamine Res.*, Raven Press, New York 1983, Vol. 4, p. 169.
18. Swanson, T. L. and Gibbs, G. *In Vitro 16* (1980) 761.
19. Nishida, Y. and Miyamoto, T. *Toxicol. Lett. 31* (1986) 203.
20. Gilad, G. M. and Gilad, V. H. *Int. J. Developm. Neurosci. 4* (1986) 195.
21. Hegre, O. D., Marshall, S. and Hickey, G. E. *In Vitro 20* (1984) 198.
22. Ferrante, A., Maxwell, G. M., Rencis, V. O., Allison, A. C. and Morgan, D. M. L. *Int. J. Immunopharmacol. 8* (1986) 411.
23. Ferrante, A., Allison, A. C. and Hirumi, H. *Parasite Immunol. 4* (1982) 340.
24. Ferrante, A. *Immunology 54* (1985) 785.
25. Ferrante, A., Rzepczyk, C. M. and Saul, A. J. *J. Immunol. 133* (1984) 2157.
26. Tabor, C. W., Tabor, H. and Bachrach, U. *J. Biol. Chem. 239* (1964) 2194.
27. Kimes, B. W. and Morris, D. R. *Biochim. Biophys. Acta 228* (1971) 223.
28. Alarcon, R. A. *Arch. Biochem. Biophys. 106* (1964) 240.
29. Alarcon, R. A. *Arch. Biochem. Biophys. 137* (1980) 365.
30. Dewey, D. L. In: Gaugas, J. M., Eds., *Polyamines in Biomedical Research*, Wiley, Chichester 1980, p. 309.
31. Croker, S. J., Loeffler, R. S. T. and Smith, T. A. *Tetrahedron Lett. 24* (1983) 1559.
32. Mondovi, B., Turini, P., Befani, O. and Sabatini, S. *Methods Enzymol. 94* (1983) 314.
33. Brossat, B., Straczek, J., Belleville, F. and Nabet, P. *J. Chromatogr. 277* (1983) 87.
34. Sinnkuber, R. O., Yu, T. C. and Yu, T. C. E. *Food Res. 23* (1958) 626.
35. Barkholt, V. and Jensen, A. L. *Anal. Biochem. 177* (1989) 318.
36. Janes, S. M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Maltby, D., Burlingame, A. L. and Klinman, J. *Science 248* (1990) 981.
37. Lobenstein-Verbeek, C. L., Longejan, J. A. and Duine, J. A. *FEBS Lett. 170* (1984) 305.
38. Knowles, P. F., Pandega, K. B., Rius, F. X., Spencer, C. M., Moog, R. S., McGuirl, M. A. and Dooley, D. M. *Biochem. J. 241* (1987) 603.
39. Hartman, C. and Klinman, J. *J. Biol. Chem. 262* (1987) 962.
40. Houen, G., Larsen, C. and Larsson, L.-I. *Tetrahedron 45* (1989) 4235.
41. Gahl, W. A. and Pitot, H. C. *Biochem. J. 202* (1982) 603.
42. MacGowan, S. H., Keir, H. M. and Wallace, H. M. *Med. Sci. Res. 15* (1987) 687.
43. Israel, M., Zoll, E. C., Muhammad, N. and Modest, E. J. *J. Med. Chem. 16* (1973) 1.
44. Morgan, D. M. L., Bachrach, U., Assaraf, Y. G., Harari, E. and Golenser, J. *Biochem. J. 236* (1986) 97.
45. Unemoto, T. *Chem. Pharm. Bull. 11* (1963) 1255.
46. Tabor, C. W., Tabor, H. and Rosenthal, S. M. *J. Biol. Chem. 208* (1954) 645.
47. Tabor, C. W. and Rosenthal, S. M. *J. Pharmacol. Exp. Ther. 116* (1956) 139.
48. Bachrach, U. and Bar-Or, R. *Biochim. Biophys. Acta 40* (1960) 545.
49. Seiler, N., Bolkenius, F. N. and Rennert, O. M. *Med. Biol. 59* (1981) 334.
50. Labib, R. S. and Romasi Jr., T. B. *Eur. J. Immunol. 11* (1981) 266.
51. Quash, G. and Taylor, D. R. *Clin. Chim. Acta 30* (1970) 17.
52. Van den Berg, G. A., Elzinga, H., Nagel, G. T., Kingma, A. W. and Miskiet, F. A. J. *Biochim. Biophys. Acta 802* (1984) 175.
53. Quash, G., Ripoll, H., Gazzalo, L., Doutheau, A., Saba, A. and Gore, J. *Biochimie 69* (1987) 101.
54. Padmanabhan, R. and Kiltan, K. *Biochem. Biophys. Res. Commun. 19* (1965) 1.
55. Höltta, E. *Biochemistry 16* (1977) 91.
56. Nakajima, T. *J. Neurochem. 20* (1973) 735.