Oxidized polyamines and the growth of human vascular endothelial cells

Prevention of cytotoxic effects by selective acetylation

D. M. L. MORGAN

Section of Vascular Biology, M.R.C. Clinical Research Centre, Harrow, Middx. HA1 3UJ, U.K.

The responses of human umbilical-vein vascular endothelial cells in culture to the naturally occurring polyamines spermine, spermidine and putrescine, their acetyl derivatives and oxidation products were examined. In the absence of human polyamine oxidase, exposure of cells to polyamines (up to 160 μ M) had no adverse effects. In the presence of polyamine oxidase, spermine and spermidine were cytotoxic, but putrescine was not. Acetylation of the aminopropyl group of spermidine or both aminopropyl groups of spermine prevented this cytotoxicity. The amino acids corresponding to the polyamines, representing a further stage of oxidation, were also without effect. The cytotoxic effects were irreversible. Use of bovine serum amine oxidase in place of the human enzyme gave qualitatively similar results.

INTRODUCTION

The polyamines spermine [$\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$], spermidine [$\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$] and putrescine [$\text{NH}_2(\text{CH}_2)_4\text{NH}_2$] are essential for the normal growth and differentiation of eukaryotic cells (Heby, 1981; Pegg, 1986), although the mechanisms involved are not clear. The intracellular concentrations of these compounds are high (around millimolar) and fluctuate according to the stage of the cell cycle (Scalabrino & Ferioli, 1981, 1982). The major catabolic pathway for polyamines is oxidation by polyamine oxidase (PAO), which is present in all mammalian cells (Seiler *et al.*, 1980a) and in human maternal serum (Illei & Morgan, 1979b). Oxidation of spermine or spermidine by tissue PAO leads to the formation of spermidine or putrescine respectively, plus H_2O_2 and 3-aminopropionaldehyde (Holttä, 1977; Morgan, 1985a,b; Scheme 1). Products of this reaction have been shown to inhibit cell proliferation (Morgan & Illei, 1980a).

Bovine serum contains an amine oxidase (EC 1.4.3.6), which acts on spermidine or spermine to produce respectively an aminomonoaldehyde [N' -(4-aminobutyl)-aminopropionaldehyde] or a dialdehyde [NN' -bis-(3-propionaldehyde)-1,4-diaminobutane], plus NH_3 and H_2O_2 in each case (Tabor *et al.*, 1964; Scheme 2). The products of this reaction are also cytotoxic (for reviews see Bachrach, 1970, 1981; Morgan, 1980). In both instances the aminoaldehydes are the active agents.

The growth-regulatory effects of the polyamines are well recognized in several cell types, but nothing is known of the effects of polyamines or their oxidation products on endothelial cells, although the endothelium would be the first tissue with which the products of serum PAO would interact. Furthermore, the effects of the oxidation products of acetyl-polyamines on any cell type have not been investigated, although the first, and rate-limiting, step in the intracellular degradation of polyamines in mammalian cells is acetylation of an aminopropyl moiety of spermine or spermidine by the

readily inducible spermidine/spermine acetyltransferase (Pegg, 1986; Pegg & Erwin, 1985; Matsui-Yuasa *et al.*, 1985; and references therein). Therefore these effects have been examined by using an experimental system designed to monitor the incorporation of radiolabelled precursors into protein, RNA and DNA by human umbilical-vein endothelial cells in culture.

EXPERIMENTAL

Chemicals

Spermine, spermidine, putrescine, N^1 -acetylspermidine, N^8 -acetylspermidine, N^1 -acetylspermine, N^1 -acetylputrescine (as the hydrochlorides), Dowex-1 ion-exchange resin (Cl^- form; 2% cross-linked, 200–400 mesh), and collagenase (Type II) were from Sigma Chemical Co., Poole, Dorset, U.K.; calf serum and tissue-culture media (in powder form) were from Flow Laboratories, Irvine, Scotland, U.K.; L-[4,5- ^3H]leucine (1 Ci/mmol), [methyl- ^3H]thymidine (5 Ci/mmol) and [5,6- ^3H]uridine (51 Ci/mmol) were from Amersham International, Amersham, Bucks., U.K.; scintillation fluid NE 265 was from Nuclear Enterprises, Edinburgh, Scotland, U.K.

Bovine serum amine oxidase (Tabor *et al.*, 1954; Bachrach, 1974) (specific activity 25 units/mg) was a gift from Professor U. Bachrach, Hadassah Medical School, Jerusalem, Israel. Samples of authentic N^1N^{12} -diacetylspermine, N^1 -acetylspermidine and N^8 -acetylspermidine were given by Dr. N. Seiler, Centre de Recherche Merrell, Strasbourg, France. Retroplacental serum (RPS) was used as a source of human PAO (Illei & Morgan, 1979a) and assayed for activity as described by Morgan & Illei (1980b); only samples with activity $> 2 \mu\text{mol/min per litre}$ were used.

N^1N^{12} -Diacetylspermine

Acetic anhydride (3 ml) was added during 30 min to a stirred mixture of spermine tetrahydrochloride

Scheme 1. Oxidation of spermine and spermidine by bovine serum amine oxidase

Scheme 2. Cleavage of spermine and spermidine by PAO

(10 mmol) in water (75 μ l), methanol (7.5 ml) and Dowex-1 (CO_3^{2-}) resin (25 g; 2% cross-linked, 200–400 mesh) at 0 °C. After 90 min the mixture was poured into a column (3 cm $\times$ 10 cm) and the resin washed with water (3 $\times$ 10 ml). The filtrate and washings were combined, acidified with HCl and evaporated to dryness in a rotary evaporator. The dry residue was digested with propan-1-ol (1 l) at the boiling point, insoluble material removed by filtration and the filtrate left at 4 °C for 16 h. The product was collected, washed with cold propan-1-ol and dried in air at room temperature for 3 days, when it had m.p. 229.5–230.5 °C. A solution of this material (1 mg/ml, in water) had an absorption maximum at 204 nm. Treatment of the product with dinitrofluorobenzene (Dubin, 1960) resulted in a derivative with A_{350}/A_{390} of 0.72 (predicted 0.63). Dansylation of the product gave a derivative that co-migrated on t.l.c. with the dansyl derivative of authentic N^1N^{12} -diacetylspermine dihydrochloride (Seiler & Knödgen, 1979). Found: C, 46.18; H, 8.83; N, 15.84. $\text{C}_{14}\text{H}_{34}\text{N}_4\text{O}_2 \cdot 2\text{HCl}$ requires C, 46.79; H, 8.97; N, 15.59.

Cell cultures

Endothelial cells were isolated from human umbilical cords and cultured essentially as described by Jaffe *et al.* (1973). Briefly, endothelial cells were detached from the interior of the umbilical vein of a 20 cm segment of cord by treatment for 10 min at 37 °C with collagenase (0.05% in Medium 199 containing 100 units each of penicillin and streptomycin/ml). After brief centrifugation, cells were resuspended in 5 ml of Medium 199 supplemented with 10% (v/v) fetal-calf serum, 10% (v/v) newborn-calf serum, 2 mM-glutamine and antibiotics as above, plated in a 25 cm² tissue-culture flask and incubated in an atmosphere of air/ CO_2 (19:1). When confluent, cells were subcultured at a split ratio of 1:2 by brief treatment with trypsin (0.1%) plus EDTA (0.02%) in PBS. Confluent first-passage cells were subcultured similarly and the cells resuspended at a concentration of 5×10^4 cells/ml in Ham's F-12 medium containing 10% (v/v) pooled human serum, or 10% fetal-calf serum, or 10% (v/v) RPS (prepared as described elsewhere; Kloppner & Hughes, 1978; Illei & Morgan, 1979a), glutamine and antibiotics. Portions of the cell suspension (200 μ l) were dispensed into 60 wells of a 96-well flat-bottomed micro-test plate, the outer 36 wells of which each contained 200 μ l of PBS. The plates were then incubated in air/ CO_2 (19:1) at 100% humidity. Experiments were conducted at an initial cell concentration of 1×10^4

cells/well, thus ensuring that the cells were in continuous growth for the duration of the experiment and had not reached confluence when harvested. After 24 h the test material was added in 10 μ l of serum-free medium (controls received 10 μ l of medium only). After a further 24 h, 1 μ Ci (1 μ l) of radiolabelled marker was added to each well. The experiment was terminated 24 h later as follows: after removal of the medium, the cell monolayer in each well was washed twice with PBS (200 μ l), exposed to 5% (v/v) trichloroacetic acid (200 μ l) for

Fig. 1. Effects of spermidine (a), spermine (b) or putrescine (c), with (○) or without (●) human PAO, on the incorporation of [^3H]leucine by human umbilical-vein endothelial cells in culture

For putrescine, similar results were obtained in the presence or absence of the enzyme, hence only the results with enzyme are shown. For experimental details see the text. Results are given as means $\pm$ s.d. ($n = 10$ –12).

Fig. 2. Incorporation of [^3H]leucine by human endothelial cells after exposure to spermine for various periods in the presence of bovine serum amine oxidase

Culture medium contained 10% pooled normal human serum plus 120 μg of purified bovine serum amine oxidase/ml. Spermine concentration was 100 μM . Cells were exposed to enzyme plus substrate for the times shown, after which the cell sheet was washed twice with serum-free medium, and then fresh medium was applied. After 24 h, [^3H]leucine was added to each culture and the amount of label incorporated was determined 24 h later. Controls contained enzyme but no substrate and were otherwise treated the same as test cultures. Results are given as percentages of control values, means $\pm$ S.D., $n = 10$.

5 min, then washed with methanol (200 μl). Finally, the cells were digested by the addition of 200 μl of 25 M-formic acid. Each formic acid digest was transferred, with one rinse of PBS, to a vial containing 3.5 ml of NE 265 scintillation fluid. Radioactivity was determined in a Wallac/Rackbeta liquid-scintillation counter.

RESULTS

The polyamines spermine, spermidine and putrescine had little effect on the incorporation of [^3H]leucine into trichloroacetic acid-insoluble material by cells cultured in the absence of PAO (Fig. 1). When human PAO, in the form of RPS, was added to the cultures in place of pooled normal human serum, a profound and dose-dependent inhibition of leucine incorporation occurred in those cultures exposed for 24 h to spermine or spermidine (Fig. 1). Incorporation of thymidine or uridine was similarly affected (results not shown). In appearance the cells, although still adherent, were contracted and extensively crenellated. The changes induced in cell growth by the aminoaldehyde products of polyamine oxidation were progressive and irreversible (Fig. 2), virtually all cells being affected after 4 h exposure. Putrescine, a substrate for both human PAO (Morgan, 1985b) and bovine amine oxidase (Gahl & Pitot, 1982), had little effect on the incorporation of leucine (Fig. 1, Table 1), thymidine or uridine (results not shown), in the presence of either enzyme.

Acetylation of both aminopropyl moieties of spermine, or the single aminopropyl group of spermidine, although resulting in an increase in their rate of metabolism by both the human (Morgan, 1985b) or the bovine (Gahl & Pitot, 1982; D. M. L. Morgan, unpublished work) enzyme, rendered the products of their oxidation non-cytotoxic (Table 1). Monoacetylation of spermine, however, did not abolish the cytotoxic effect. When human serum was replaced by fetal-calf serum (Table 1), the results were qualitatively similar to those obtained with human PAO. Addition of purified bovine serum amine oxidase to cultures containing polyamines in the presence of normal human serum (Table 2) also gave similar results.

The amino acids corresponding to spermine, spermidine and putrescine, representing a further stage of oxidation of the amino aldehydes formed by the bovine enzyme, were without effect (Table 3), as were the oxidation products of 3-aminopropionaldehyde.

Acetamide, a putative product of the action of bovine serum amine oxidase on N^1 -acetylspermidine or N^1N^{12} -diacetylspermine (Scheme 3), was non-cytotoxic (Table 4),

Table 1. Effects of polyamines and acetylpolyamines on the incorporation of [^3H]leucine into macromolecules by human endothelial cells cultured in the presence of 10% normal human serum, RPS or fetal-calf serum

Concentration of test materials was 100 μM in each case. Quantitative differences in effect between RPS and fetal-calf serum reflect differences in enzyme activity of the sera. Results are expressed as percentages of control values, as means $\pm$ S.D., $n = 10$ –12; n.d., not done.

	Incorporation (%)		
	Human serum	RPS	Fetal-calf serum
Control	100 $\pm$ 3 (9656 $\pm$ 292 c.p.m.)	100 $\pm$ 5 (7535 $\pm$ 379 c.p.m.)	100 $\pm$ 6 (7115 $\pm$ 434 c.p.m.)
Spermine	103 $\pm$ 19	36 $\pm$ 7	1 $\pm$ 2
Spermidine	140 $\pm$ 20	43 $\pm$ 9	2 $\pm$ 0.4
Putrescine	n.d.	100 $\pm$ 11	99 $\pm$ 11
N^1 -Acetylspermidine	100 $\pm$ 5	93 $\pm$ 9	95 $\pm$ 7
N^8 -Acetylspermidine	102 $\pm$ 9	46 $\pm$ 7	6 $\pm$ 2
N^1 -Acetylspermine	100 $\pm$ 6	53 $\pm$ 8	2 $\pm$ 0.4
N^1N^{12} -Diacetylspermine	100 $\pm$ 6	90 $\pm$ 7	81 $\pm$ 6
N^1 -Acetylputrescine	n.d.	97 $\pm$ 8	90 $\pm$ 6

Table 2. Effects of acetyl derivatives of spermine and spermidine on the incorporation of [³H]-uridine, -thymidine or -leucine into macromolecules by human endothelial cells in the presence of bovine serum amine oxidase

The culture medium contained 10% pooled normal human serum plus 120 μg of purified bovine serum amine oxidase/ml; polyamine concentration was 100 μM. Controls contained enzyme without polyamines. Results are expressed as percentages of control values, as means ± S.D., *n* = 12.

	Incorporation (%)		
	Uridine	Thymidine	Leucine
Control	100 ± 29 (364 ± 104 c.p.m.)	100 ± 15 (4402 ± 659 c.p.m.)	100 ± 11 (8467 ± 955 c.p.m.)
<i>N</i> ¹ -Acetylspermidine	95 ± 22	95 ± 5	109 ± 5
<i>N</i> ⁸ -Acetylspermidine	17 ± 4	1.3 ± 0.5	0.7 ± 0.2
<i>N</i> ¹ -Acetylspermine	16 ± 4	0.7 ± 0.5	0.5 ± 0.1
<i>N</i> ¹ <i>N</i> ¹² -Diacetylspermine	71 ± 11	84 ± 8	101 ± 7

Table 3. Effects of the amino acids corresponding to spermine, spermidine and putrescine, and of 3-aminopropan-1-ol and β-alanine, on the incorporation of [³H]leucine by human endothelial cells in culture

The culture medium contained 10% pooled normal human serum. Concentration of test compounds was 100 μM. Results are expressed as percentages of control values, as means ± S.D., *n* = 12.

	Incorporation (%)
Control	100 ± 3 (7182 ± 640 c.p.m.)
Spermic acid	97 ± 7
Putrescine	97 ± 7
γ-Aminobutyric acid	97 ± 10
β-Alanine	98 ± 10
3-Aminopropan-1-ol	112 ± 9

nor did it exert any protective effect against the cytotoxicity of oxidized spermine (Table 4).

DISCUSSION

Human umbilical-vein endothelial cells were, as expected, susceptible to damage by the products formed from the oxidation of spermine or spermidine by both types of amine oxidase (Fig. 1; Table 1). Aminoaldehydes or further metabolic products arising from them, H₂O₂, and, in the case of the bovine enzyme, NH₃, are all possible candidates for the cytotoxic agent. However, NH₄Cl, at concentrations up to 1 mM, has been shown to have little or no effect on the growth of *Plasmodium falciparum* (Rzepczyk *et al.*, 1984). Vascular endothelial cells are known to be sensitive to H₂O₂ (Ager & Gordon, 1984; Whorton *et al.*, 1985; Shingu *et al.*, 1985), but these

effects can be greatly decreased or abolished in the presence of serum (Bishop *et al.*, 1985). *N*¹-Acetylspermidine or *N*¹*N*¹²-diacetylspermine are both metabolized by human PAO (Morgan, 1985b) and by bovine serum amine oxidase (Gahl & Pitot, 1982; D. M. L. Morgan, unpublished work), at a greater rate than the parent compounds, with the production of H₂O₂ in each case. The lack of effect of the oxidation products of these acetylated polyamines (Tables 1 and 2) demonstrates that the cytotoxicity observed with spermine or spermidine is not due to concomitantly formed H₂O₂. Furthermore, addition of the amino acids *NN'*-bis(2-carboxyethyl)-1,4-diaminobutane (spermic acid), *N*-(2-carboxyethyl)-1,4-diaminobutane (putrescine) or 4-amino-*n*-butyric acid (γ-aminobutyric acid), corresponding to spermine, spermidine or putrescine:

and representing a further stage in the oxidation of the polyamines, or of 3-aminopropan-1-ol or 3-amino-*n*-propionic acid (β-alanine), oxidation products of 3-aminopropionaldehyde, had no effect on the incorporation of leucine by endothelial cells cultured in medium containing pooled normal human serum (Table 3). In contrast, there is a large and growing body of evidence to support the concept that the cytotoxic effects resulting from polyamine oxidation are due to aminoaldehyde formation (Bachrach, 1970, 1981; Allen *et al.*, 1979; Hussain *et al.*, 1983; Ferrante *et al.*, 1984; Rzepczyk *et al.*, 1984; Morgan *et al.*, 1986).

There are conflicting reports of the effects of putrescine oxidation products on cell proliferation (Gaugas & Curzen, 1978; Byrd *et al.*, 1978; Allen *et al.*, 1979). Addition of putrescine at concentrations up to

Scheme 3. Oxidation of *N*¹*N*¹²-diacetylspermine by bovine serum amine oxidase

Table 4. Effect of acetamide, alone or in the presence of spermine, on the incorporation of [³H]leucine by human endothelial cells in cultures containing 10% fetal-calf serum

Results are given as percentages of control values, as means $\pm$ S.D., $n = 6$.

	Incorporation (%)
Control	100 $\pm$ 14 (11131 $\pm$ 1490 c.p.m.)
Acetamide (10 μ M)	107 $\pm$ 23
Acetamide (100 μ M)	104 $\pm$ 31
Acetamide (200 μ M)	101 $\pm$ 16
Acetamide (1000 μ M)	99 $\pm$ 22
Spermine (100 μ M)	1 $\pm$ 0.2
Spermine (100 μ M) + acetamide (200 μ M)	1 $\pm$ 1
N ¹ N ¹² -Diacetylspermine (100 μ M)	92 $\pm$ 11

160 μ M to cultures of human umbilical-vein endothelial cells in the presence of either bovine serum or human RPS failed to inhibit leucine incorporation to any great extent in conditions where both spermine and spermidine were effective (Table 1). Putrescine was also without effect on incorporation of uridine and thymidine (results not shown).

Oxidation of N¹-acetylspermidine or N¹N¹²-diacetylspermine by bovine plasma amine oxidase or human pregnancy-associated PAO did not result in the formation of cytotoxic products (Tables 1 and 2), although both substrates are oxidized at a greater rate than the non-acetylated polyamines. Oxidation of N⁸-acetylspermidine or N¹-acetylspermine by either enzyme, however, resulted in products that were cytotoxic. For the human enzyme these findings are in accord with the reaction sequence described above (Scheme 1); acetylation of the aminopropyl nitrogen results in the formation of N-acetylaminopropionaldehyde on oxidation. Loss of the cytotoxic effect may be due to the decreased charge on the nitrogen resulting from acylation. Acetylation of the aminobutyl nitrogen in the parent compound does not modify the resulting aldehyde, and hence would not be expected to alter the biological effect.

The results obtained with the bovine serum enzyme are more difficult to interpret, the more so as there are conflicting reports in the literature (Mamont *et al.*, 1981; Gahl & Pitot, 1982) as to whether or not N¹-acetylspermidine is a substrate for this enzyme. Acetylation of the primary amino groups of spermine or spermidine should have no effect on the structure of the aldehyde formed (Scheme 3), if the reaction proceeds according to Scheme 2. The consequence of modifying the substrate should be that acetamide becomes the leaving group instead of ammonia. Investigation of the effects of adding acetamide to cultures, alone or in the presence of spermine, led to the results shown in Table 4. Acetamide at concentrations up to 1 mM had no effect on leucine incorporation by endothelial cells cultured in medium containing fetal-calf serum, nor did it exert any protective effect if spermine were added to the cultures.

Byrd *et al.* (1977, 1978) and Hussain *et al.* (1983) concluded that the inhibitory effects of oxidized

polyamines on mitogen-stimulated lymphocyte proliferation were reversible, but in each case the experimental design was such that interpretation of the data is difficult. Exposure of human vascular endothelial cells to spermine in the presence of bovine serum amine oxidase resulted in irreversible damage after 1–2 h (Fig. 2), demonstrated by a marked decrease in [³H]leucine incorporation after incubation for 48 h in fresh medium. Hence it would appear that the polyamine/PAO system, as suggested by Byrd *et al.* (1977, 1978), affects some event in actively proliferating cells rather than having a more general effect on both quiescent and growing cells.

The discovery that acetylation of the aminopropyl groups of spermine or spermidine prevents the products of oxidation being cytotoxic may go some way to providing the answer to what has been a puzzling question: why should polyamine degradation apparently proceed by a pathway that results in the production of such potentially hazardous compounds as the aminoaldehydes? The reported values of the kinetic constants of rat liver PAO for N¹-acetylspermine (K_m 0.6 μ M, V_{max} 2.5 nmol of H₂O₂/min per mg of protein), N¹N¹²-diacetylspermine (5 μ M, 4.0 nmol/min per mg) and spermine (40 μ M, 0.1 nmol/min per mg; Bolkenius & Seiler, 1981) suggest that the acetyl derivatives will be metabolized more rapidly than the parent compound. The spermidine formed by oxidation of N¹-acetylspermine would be acetylated to form N¹-acetylspermidine (K_m 14 μ M, V_{max} 2.5 nmol/min per mg), oxidation of which would also result in products that were not cytotoxic.

Acetylation of the primary amino groups prevented the uptake of polyamines by cultured L1210 leukaemia cells (Porter *et al.*, 1982, 1985) and this, together with the occurrence of acetylated polyamines in normal urine (e.g. Aigner-Held & Daves, 1980, and references therein; Seiler, 1981), suggests that acetylation may be an important step in polyamine elimination (Wallace *et al.*, 1985) as well as in degradation via the PAO pathway (Seiler *et al.*, 1980b).

The results reported here are the first demonstration that the products formed from the oxidation of N¹-acetylspermidine or N¹N¹²-diacetylspermine by either human PAO or bovine serum amine oxidase are not cytotoxic. This finding, together with evidence that acetylation is the first step in the intracellular metabolism of spermine and spermidine and that acetylpolyamines are rapidly metabolized by PAO, suggests a solution to an enigma in polyamine metabolism and indicates that polyamine degradation probably proceeds via a pathway that does not involve the formation of toxic aminoaldehydes.

I thank Professor U. Bachrach for the gift of purified bovine amine oxidase, Dr. N. Seiler for samples of acetylated polyamines, and the staff of the Delivery Suite, Northwick Park Hospital, for their co-operation.

REFERENCES

- Ager, A. & Gordon, J. L. (1984) *J. Exp. Med.* **159**, 592–603
- Aigner-Held, R. & Daves, G. D. (1980) *Physiol. Chem. Phys.* **12**, 389–400
- Allen, J. C., Smith, C. J., Hussain, J. L., Thomas, J. M. & Gaugas, J. M. (1979) *Eur. J. Biochem.* **102**, 153–158
- Bachrach, U. (1970) *Ann. N.Y. Acad. Sci.* **171**, 939–956

- Bachrach, U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., vol. 4, pp. 1744–1748, Verlag-Chemie, Weinheim
- Bachrach, U. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Martin, L. J., eds.), pp. 151–168, Marcel Dekker, New York and Basel
- Bishop, C. T., Mirza, Z., Crapo, J. D. & Freeman, B. A. (1985) *In Vitro Cell. Dev. Biol.* **21**, 229–236
- Bolkenius, F. N. & Seiler, N. (1981) *Int. J. Biochem.* **13**, 287–292
- Byrd, W. J., Jacobs, D. M. & Amos, M. S. (1977) *Nature (London)* **267**, 621–623
- Byrd, W. J., Jacobs, D. M. & Amos, M. S. (1978) *Adv. Polyamine Res.* **2**, 71–83
- Dubin, D. T. (1960) *J. Biol. Chem.* **235**, 783–786
- Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182
- Ferrante, A., Rzepczyk, C. M. & Saul, A. J. (1984) *J. Immunol.* **133**, 2157–2162
- Gahl, W. A. & Pitot, H. C. (1982) *Biochem. J.* **202**, 603–611
- Gaugas, J. M. & Curzen, P. (1978) *Lancet* **i**, 18–20
- Heby, O. (1981) *Differentiation* **19**, 1–20
- Holttä, E. (1977) *Biochemistry* **16**, 91–100
- Hussain, J. I., Smith, C. J. & Allen, J. C. (1983) *Cell Tissue Kinet.* **16**, 583–591
- Illei, G. & Morgan, D. M. L. (1979a) *Br. J. Obstet. Gynaecol.* **86**, 873–877
- Illei, G. & Morgan, D. M. L. (1979b) *Br. J. Obstet. Gynaecol.* **86**, 878–881
- Jaffe, E. A., Nachman, R. L., Becker, C. G. & Minick, C. R. (1973) *J. Clin. Invest.* **52**, 2745–2756
- Klopper, A. & Hughes, G. (1978) *Arch. Gynaekol.* **225**, 171–177
- Mamont, P. S., Seiler, N., Sient, M., Joden-Ohlenbusch, A.-M. & Knodgen, B. (1981) *Med. Biol.* **59**, 347–353
- Matsui-Yuasa, I., Otani, S., Morisawa, S., Takigawa, M., Enomoto, M. & Suzuki, F. (1985) *J. Biochem. (Tokyo)* **97**, 387–390
- Morgan, D. M. L. (1980) in *Polyamines in Biomedical Research* (Gaugas, J. M., ed.), pp. 285–302, Wiley, Chichester
- Morgan, D. M. L. (1985a) *Biochem. Soc. Trans.* **13**, 322–326
- Morgan, D. M. L. (1985b) *Biochem. Soc. Trans.* **13**, 351–352
- Morgan, D. M. L. & Illei, G. (1980a) *Br. Med. J.* **280**, 1295–1297
- Morgan, D. M. L. & Illei, G. (1980b) *Med. Lab. Sci.* **38**, 49–56
- Morgan, D. M. L., Bachrach, U., Assaraf, Y., Harari, E. & Golenser, J. (1986) *Biochem. J.* **236**, 97–101
- Pegg, A. E. (1986) *Biochem. J.* **234**, 249–262
- Pegg, A. E. & Erwin, B. G. (1985) *Biochem. J.* **231**, 285–289
- Porter, C. W., Bergeron, R. J. & Stolowich, N. J. (1982) *Cancer Res.* **42**, 4072–4078
- Porter, C. W., Cavanaugh, P. F., Stolowich, N., Ganis, B., Kelly, E. & Bergeron, R. J. (1985) *Cancer Res.* **45**, 2050–2057
- Rzepczyk, C. M., Saul, A. J. & Gerrante, A. (1984) *Infect. Immun.* **43**, 238–244
- Scalabrino, G. & Ferioli, M. E. (1981) *Adv. Cancer Res.* **35**, 151–268
- Scalabrino, G. & Ferioli, M. E. (1982) *Adv. Cancer Res.* **36**, 1–102
- Seiler, N. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 127–149, Marcel Dekker, Basel and New York
- Seiler, N. & Knodgen, B. (1979) *J. Chromatogr.* **164**, 155–168
- Seiler, N., Bolkenius, F. N., Knodgen, B. & Mamont, P. (1980a) *Biochim. Biophys. Acta* **615**, 480–488
- Seiler, N., Bolkenius, F. N. & Knodgen, B. (1980b) *Biochim. Biophys. Acta* **633**, 181–190
- Shingu, M., Yoshioka, E., Nobunaga, M. & Yoshida, K. (1985) *Inflammation* **9**, 309–320
- Tabor, C. W., Tabor, H. & Rosenthal, S. M. (1954) *J. Biol. Chem.* **208**, 654–661
- Tabor, C. W., Tabor, H. & Bachrach, U. (1964) *J. Biol. Chem.* **239**, 2194–2203
- Wallace, H. M., MacGowan, S. H. & Keir, H. M. (1985) *Biochem. Soc. Trans.* **13**, 329–330
- Whorton, A. R., Montgomery, M. E. & Kent, K. S. (1985) *J. Clin. Invest.* **76**, 295–302