Persistence of Methylated Bases in Ribonucleic Acid of Syrian Golden Hamster Liver after Administration of Dimethylnitrosamine

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1. Syrian golden hamster liver ribosomal RNA was isolated up to 96 h after administration of [^{14}C]dimethylnitrosamine at 25 mg/kg or 2.5 mg/kg body weight. 2. The chemical alkylation products, 7-methylguanine, 3-methylcytosine, O^6 -methylguanosine and 1-methyladenosine, were measured after acidic or enzymic hydrolysis of the RNA to bases or mononucleosides followed by ion-exchange chromatography. 3. Between 7 and 96 h, the relative amounts of alkylation products did not change with time even though the absolute amounts fell by approx. 80% and 51% after the high and low doses respectively. 4. The results suggest that base specific excision repair does not exist for RNA alkylation products in this experimental system.

In attempts to elucidate the molecular basis of the organ-specific toxic and carcinogenic effects of alkylating agents, the reaction of this class of compounds with cellular macromolecules has been extensively studied (see Magee *et al.* 1975, 1976; Montesano & Bartsch, 1976; Pegg, 1977a; Margison & O'Connor, 1979).

Dimethylnitrosamine (DMN), a potent carcinogen in many animal species (IARC, 1978), is not an alkylating agent *per se* but is able to methylate nucleic acids *in vivo* after α -carbon hydroxylation by the microsomal mixed-function oxidase system, a metabolic process which appears to be obligatory for the various biological effects of this agent (see Magee *et al.*, 1976; Montesano & Bartsch, 1976). The hypothesis that DNA may be a critical target in the initiation of malignant transformation has stimulated a closer examination of the products of alkylation of DNA by DMN and related agents. It has been found, by measuring the stability of alkylated bases in DNA, that excision processes exist for certain products and that the activity of these repair systems may be related to the tissue specificity of tumour production (Pegg, 1977a; Margison & O'Connor, 1979).

The chemical alkylation of RNA *in vivo*, which was first reported for DMN by Magee & Farber (1962), has also been examined in some detail by using this and related agents mainly in the rat (Craddock & Magee, 1963; Lawley *et al.*, 1968; Whittle, 1969; O'Connor *et al.*, 1972). Most of the alkylation prod-

ucts found in DNA have also been detected in RNA and the amounts present in rat liver ribosomal RNA after administration of DMN (O'Connor *et al.*, 1972), and in this and other RNA species after treatment with other alkylating agents, have been measured (see Margison & O'Connor, 1979).

Studies of the stability of alkylation products in RNA *in vivo* have been restricted to the major product 7-methylguanine, the concentrations of which in liver ribosomal RNA, of rats treated with DMN, decreased after the initial peak of alkylation. This apparent loss was attributed to the hepatotoxic effects of DMN after high doses and to normal RNA turnover after lower doses (Craddock & Magee, 1963; Craddock *et al.*, 1968; McElhone *et al.*, 1971; Craig & O'Connor, 1971; Capp's *et al.*, 1973). However, it has also been suggested that excision of 7-methylguanine does take place from liver ribosomal RNA in mice given DMN in the drinking water over a period of 4 weeks (Nemoto & Takayama, 1974) and from the metabolically stable species of liver chromatin RNA in rats given a single dose of DMN (Galbraith *et al.*, 1978).

From various studies (Ludlum *et al.*, 1964; Michelson & Pochon, 1966; Ludlum & Wilhelm, 1968; Loveless, 1969) it has been shown that, although 7-methylguanine does not constitute a miscoding or lethal lesion, alkylation occurring at position C-1 of adenine, C-3 of cytidine and O-6 of guanine appears to be responsible for such effects. If these products do have biological effects when present in ribosomal RNA it might be expected that they would be enzymically excised. On the other hand, although it would not exclude a possible role for RNA alkylation

Abbreviation used: DMN, dimethylnitrosamine.

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in the process of carcinogenesis, the absence of excision processes for RNA alkylation products would provide indirect support for the hypothesis that the excision of DNA products is an important factor in the protection of tissues against the carcinogenic effects of alkylating agents.

In the present experiments we have investigated the possibility that active excision of alkylation products from liver ribosomal RNA takes place in Syrian golden hamsters, since the excision of alkylated DNA bases has been shown to occur in this species after a hepatocarcinogenic dose of DMN (Margison *et al.*, 1976).

Materials and Methods

[¹⁴C]Dimethylnitrosamine was synthesized from [¹⁴C]dimethylamine hydrochloride (57 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) by the method of Dutton & Heath (1956), or obtained from Dr J. A. Kepler, Research Triangle Institute, at 35 mCi/mmol. For injection, it was dissolved in 0.9% NaCl and adjusted to a specific radioactivity of 3.17 mCi/mmol (high dose) or 29.3 mCi/mmol (low dose) by using unlabelled DMN (Schuchardt, Munich, Federal Republic of Germany).

Dowex 50 (X4; -400 mesh) was obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A. *Escherichia coli* alkaline phosphatase, phosphodiesterase from *Crotalus atrox*, bovine pancreatic ribonuclease A and the marker compounds *N*⁶-methyladenosine, 1-methyladenosine, 3-methylcytosine, 7-methylguanine and 3-methyladenine were all obtained from Sigma (St. Louis, MO, U.S.A.).

Animal experiments

Male Syrian golden hamsters (104–136 g), obtained from Animalerie des Essertines, Rochetaillee, France, were allowed access to food and water at all times. [¹⁴C]Dimethylnitrosamine was injected intraperitoneally at a dose of 25 mg/kg or 2.5 mg/kg at 09.00 h. Groups of three or four animals were killed at various times later by bleeding from the abdominal aorta under ether anaesthesia. Organs were rapidly removed and frozen in liquid N₂.

Isolation of RNA

Livers were thawed and homogenized in 8 vol. of 6% (w/v) sodium 4-aminosalicylate/1% (w/v) NaCl, then shaken with an equal volume of phenol mixture (phenol/*m*-cresol/8-hydroxyquinoline/water, 50:7:0.5:55, w/v/w/v) for 30 min at room temperature. After centrifugation at 15000g at 5°C for 30 min and removal of the upper layer, the bottom (phenol) layer was re-extracted as described above with 0.5 vol. of the homogenizing solution. The upper layers were pooled and, after addition of 30 mg of NaCl/ml, re-

extracted with 0.5 vol. of the phenol mixture. The upper layer was mixed with 2 vol. of ethanol/*m*-cresol (9:1, v/v), and the precipitate of DNA and RNA spooled on a glass rod and washed three times with 20 ml of 70% (v/v) ethanol containing 2% (w/v) sodium acetate. The precipitate was dissolved in water, the solution was made 3.0M with respect to NaCl, and then stored at 4°C for at least 15 h. The precipitated RNA was collected by centrifugation and washed five times with 3M-sodium acetate, dehydrated by washing in 70% (v/v) ethanol containing 2% (w/v) sodium acetate (three times), in ethanol, ethanol/ether (1:1, v/v) and finally in ether, and then dried in a stream of N₂ and stored at -25°C.

Hydrolysis and chromatography

For the determination of 3-methylcytosine, 7-methylguanine and the incorporation of radioactivity into guanine, purine and pyrimidine bases were released from samples of RNA (3–8 mg) by hydrolysis in 0.3 ml of 72% (w/v) perchloric acid at 100°C for 60 min. The hydrolysate was diluted 10 times with water, clarified by centrifugation and, after addition of marker 7-methylguanine, applied to a column (25 cm × 1 cm) of Dowex 50 (H⁺ form). Elution was with a linear gradient of 0.75–3.0M-HCl (250 ml of each concentration) at a flow rate of 15 ml/h. The identity of 3-methylcytosine, which elutes just after cytosine (see Fig. 1), was confirmed by rechromatography on a column (85 cm × 1 cm) of Dowex 50 (NH₄⁺ form, -400 mesh), eluted with 0.3M-ammonium formate, pH 8.9, after addition of marker 3-methylcytosine. The relative amounts of 7-methylguanine and 3-methyladenine [which co-chromatograph in the Dowex 50 (H⁺ form) system; see Fig. 1] were determined by rechromatography on a column (40 cm × 1 cm) of Sephadex G-10 [eluted with 0.05M-ammonium formate, pH 6.75, containing 0.02% (w/v) sodium azide] after addition of marker 3-methyladenine. The 3-methyladenine/7-methylguanine ratio was found to be 0.005, and no correction was applied to the 7-methylguanine values.

For the determination of *O*⁶-methylguanosine, 1-methyladenosine and the incorporation of radioactivity into adenine, RNA was degraded to ribonucleosides: 3–15 mg of RNA was dissolved in 1 ml of water, then incubated with ribonuclease A (200 mg/ml) for 1 h at 37°C. Phosphodiesterase type IV (approx. 0.02 unit), alkaline phosphatase type III (approx. 3.0 units) and Tris/HCl buffer (pH 8.9, 0.2M; 1.1 ml) were added and the mixture was adjusted to pH 8.9 with 1.0M-Tris base. The pH was re-adjusted to pH 8.9 after 2–3 h, again after the mixture had stood overnight and after 20 h incubation at 37°C. The markers *O*⁶-methylguanosine (a gift from Dr. P. D. Lawley), *N*⁶-methyladenosine and 1-methyladenosine were added and the sample was

applied to a column (85cm $\times$ 1cm) of Dowex 50 (NH_4^+ form, -400 mesh). Elution at 8ml/h was with 0.3M-ammonium formate, pH 8.9 (80 fractions), and then 1.0M-ammonium formate, pH 8.9 (50 fractions).

In each type of chromatography, the A_{260} of each fraction (4.5–5.5ml) was determined. The fractions were then evaporated in a stream of air and the residues redissolved in Hyamine (0.3–0.1ml of a 1M solution in methanol) or water (1.0ml) for measurement of radioactivity after addition of scintillation mixture [toluene or Triton X-100/toluene (1:1, v/v) respectively] containing 0.5% (w/v) diphenyloxazole. Quench corrections were by automatic external standardization or by addition of internal standard.

Results

Two types of hydrolysis and chromatography were used. An example of the Dowex 50 (H^+ form) fractionation of the purine and pyrimidine bases released by perchloric acid hydrolysis of RNA is shown in

Fig. 1. The various products were eluted as reported elsewhere (Lawley & Shah, 1972; O'Connor *et al.*, 1972) and their identity was confirmed by rechromatography on alternative systems (see the Materials and Methods section). The material eluting just before cytosine on the Dowex 50 (H^+ form) eluted in a similar position on Dowex 50 (NH_4^+ form) and has not been further identified.

The other type of chromatography was the Dowex 50 (NH_4^+ form), which separated the ribonucleosides produced by phosphodiesterase and alkaline phosphatase degradation of RNA, as shown in Fig. 2. This method was routinely used for the determination of O^6 -methylguanosine and 1-methyladenosine. The former is destroyed by strong acid hydrolysis and the product, methanol, is eluted in the first few fractions on Dowex 50 (H^+ form) chromatography, but is evaporated during the preparation of the fractions for radioactivity measurements. During hydrolysis at pH 8.9, 1-methyladenosine undergoes partial rearrangement to N^6 -methyladenosine (see O'Connor

Fig. 1. Dowex 50 (H^+ form) chromatography of a perchloric acid hydrolysate of RNA isolated from the livers of hamsters killed 12h after administration of [^{14}C]dimethylnitrosamine

The dose of [^{14}C]dimethylnitrosamine was 25mg/kg (3.17mCi/mmol). ●, $A_{260}^{1\text{cm}}$; ○, radioactivity. Ura, Uracil; Cyt, cytosine; 3-meCyt, 3-methylcytosine; Gua, guanine; 7-meGua + 3-meAde, 7-methylguanine plus 3-methyladenine. For details see the Materials and Methods section.

Fig. 2. Dowex 50 (NH_4^+ form) chromatography of an enzymic hydrolysate of RNA isolated from the livers of hamsters killed 7 h after administration of [^{14}C]dimethylnitrosamine

The dose of [^{14}C]dimethylnitrosamine was 25 mg/kg (3.17 mCi/mmol). ●, $A_{260}^{1\text{cm}}$; ○, radioactivity. Urd, Uracil; Cyd, cytosine; Guo, guanosine; Ado, adenosine; N^6 -meAdo, N^6 -methyladenosine; O^6 -meGuo, O^6 -methylguanosine; 3-meCyd, 3-methylcytosine; 1-meAdo, 1-methyladenosine. For details see the Material and Methods section.

et al., 1972, and Fig. 2) and values for 1-methyladenosine were corrected accordingly. During incubation at pH 8.9, 7-methylguanosine residues undergo imidazole ring fission (Lawley & Brookes, 1963) and the products elute between fractions 4 and 35 during Dowex 50 (NH_4^+ form) chromatography.

The amounts of 7-methylguanine, 3-methylcytosine, O^6 -methylguanosine and 1-methyladenosine in hamster liver RNA between 7 h and 96 h after administration of dimethylnitrosamine are shown in Fig. 3. After the high dose, alkylation appears to be at a maximum at about 12 h, followed by a decrease, which was most rapid between 24 and 48 h. This may have been a reflection of the hepatotoxicity of this dose of dimethylnitrosamine (see the Discussion section). After the low dose, the amounts of alkylation products were approximately seven times lower and no changes in the rates of loss of the various alkylation products were observed between 7 and 96 h. Furthermore, after both the high and low dose, the relative amounts of the four alkylation products appear not to change be-

tween 7 and 96 h after treatment. This is confirmed by the alkylated base ratios (see Table 1), which, though showing slight variations (probably due to experimental error in determining the three or four parameters contributing to the ratios), are relatively constant with time and dose. These results contrast markedly with those of hamster liver DNA after 25 mg of dimethylnitrosamine/kg (Margison *et al.*, 1976; see Fig. 4 and the Discussion section).

Incorporation of radioactivity into the normal purines in RNA takes place when labelled, one-carbon-atom breakdown products of [^{14}C]dimethylnitrosamine are used during synthesis *de novo*. The extents of this labelling in guanine and adenine in RNA (Table 2) were initially low after the high dose of dimethylnitrosamine, but generally increased up to 96 h. After the low dose, incorporation increased between 7 and 48 h, then decreased at a rate which was similar to that observed for the alkylation products, indicating that both may have been the result of RNA turnover (see the Discussion section).

Table 1. Alkylation product ratios in hamster liver RNA at various times after administration of dimethylnitrosamine
Groups of three or four hamsters received [¹⁴C]dimethylnitrosamine (3.17 mCi/mmol at 25 mg/kg; 29.3 mCi/mmol at 2.5 mg/kg) and were killed after the times indicated. rRNA was extracted from the pooled livers and analysed as described in the Materials and Methods section.

Dose (mg/kg)	Time after administration (h)	Product ratios		
		3-Methylcytosine: 7-methylguanine	O ⁶ -Methylguanosine: 7-methylguanine	1-Methyladenosine: 7-methylguanine
25	7	0.034	0.043	0.039
	12	0.036	0.048	0.037
	24	0.035	0.048	0.040
	36	0.036	0.041	0.034
	48	0.038	0.046	0.037
	96	0.033	0.052	0.038
2.5	7	0.037	0.047	0.039
	48	0.032	0.046	0.033
	96	0.032	0.042	0.033

Fig. 3. Amounts of alkylated bases in hamster liver RNA at various times after administration of [¹⁴C]dimethylnitrosamine

[¹⁴C]Dimethylnitrosamine was given at 25 mg/kg (3.17 mCi/mmol) (continuous line) or 2.5 mg/kg (29.3 mCi/mmol) (broken line). ●, 7-Methylguanine; ○, O⁶-methylguanosine; △, 1-methyladenosine; ▲, 3-methylcytosine. For details see the Materials and Methods section.

Fig. 4. O⁶-Methylguanine:7-methylguanine ratios in hamster liver nucleic acids at various times after administration of [¹⁴C]dimethylnitrosamine

The dose of [¹⁴C]dimethylnitrosamine was 25 mg/kg (3.17 mCi/mmol). ●, DNA (taken from Margison *et al.*, 1976); ○, RNA (present results).

Discussion

In the present experiments, the concentrations of 7-methylguanine, 1-methyladenosine, 3-methylcytosine and O⁶-methylguanosine have been measured in the liver ribosomal RNA of Syrian golden hamsters at various times after administration of DMN in order to determine whether there is any evidence that specific damaged bases are actively removed, a process which has been observed for chemical alkylation products in the DNA of this animal species (Margison *et al.*, 1976). In the case of RNA, the interpretation of such results is less com-

Table 2. *Incorporation of radioactivity into normal purines in hamster liver RNA at various times after administration of dimethylnitrosamine*

Groups of three or four hamsters received [^{14}C]-dimethylnitrosamine (3.17 mCi/mmol at 25 mg/kg; 29.3 mCi/mmol at 2.5 mg/kg) and were killed after the times indicated. RNA was extracted from the pooled livers and analysed as described in the Materials and Methods section.

Dose (mg/kg)	Time after administration (h)	Radioactivity (d.p.m./ μmol)	
		Guanine	Adenosine
25	7	128	301
	12	197	437
	24	252	1149
	36	861	1322
	48	808	1061
	96	1979	2421
2.5	7	1077	1240
	48	3384	5070
	96	2332	2279

plicated than for DNA, since the *N*-glycosidic bonds of the RNA products are chemically stable at 37°C (Kriek & Emmelot, 1964; Shooter *et al.*, 1971). The concentrations of the minor products can thus be expressed in relation to the major and therefore most accurately determined product, 7-methylguanine, without the need to use methylated RNA incubated *in vitro* as a control, since changes in the base ratios can only be attributed to biochemical processes. The calculation of product ratios compensates for differences between the concentrations of individual products that might arise from biological variation between animals in, for example, DMN-activating enzymes, which would affect the initial amounts of products or RNA turnover, which would affect their apparent rate of loss.

In some animal species, 1-methyladenosine and 7-methylguanosine have been found as the products of enzymic methylation of normal bases in ribosomal RNA. However, only very small amounts are present (approx. 0.1 % or less of the parent base; Hall, 1971; Pegg, 1977a) and, unless hamster liver ribosomal RNA contained vastly greater quantities of these products, biomethylation of metabolically labelled nucleosides in newly synthesized RNA would not contribute significantly to the measurement of chemical methylation. Although 3-methylcytidine is present in transfer RNA it has not been reported to occur in ribosomal RNA and *O*⁶-methylguanine is not known as a biomethylation product.

The amounts relative to 7-methylguanine of 1-methyladenine, 3-methylcytidine and *O*⁶-methylguanine in hamster liver ribosomal RNA were found to be very similar to those reported for rat liver ribosomal RNA after administration of DMN (O'Connor

et al., 1972), thus supporting the general concept that the product ratio is determined principally by the alkylating agent (Lawley, 1974; Margison & O'Connor, 1979). Between 7 and 96 h after DMN administration, the absolute concentrations of the products fell by 80 and 51 % after the high and low dose respectively. However, since this applied to each alkylation product, the relative amounts did not change over this period (see Table 1) so that there was no evidence for base-specific excision.

The apparent loss of alkylated bases from liver ribosomal RNA can be attributed to two factors, namely (a) cell death and a consequently increased RNA catabolism due to the hepatotoxicity of DMN and (b) normal ribosomal RNA turnover. The former appears to take place after the higher dose of DMN and the effects are most marked between 36 h and 48 h, when there was a considerable reduction in the concentrations of alkylation products together with a fall in the metabolic labelling of normal purines. Between 48 and 96 h, metabolic labelling increased whereas the amounts of alkylation products continued to decrease (see Table 2 and Fig. 3).

This may have been due either to an increased rate of RNA synthesis as a result of the release of an initial DMN-mediated inhibition of RNA synthesis (similar effects, i.e. an initial inhibition followed by a stimulation of RNA synthesis, have been reported for hamster liver after a high dose of diethylnitrosamine; Witschi, 1973) or to an increase in the labelling of the RNA precursor pool as a result of the extensive RNA catabolism. Possibly both of these effects combine to produce the results obtained. After the low dose of DMN, there was a constant loss of products with a half-life of 3–4 days, which was probably a consequence of the normal turnover of RNA. Similar findings and conclusions regarding the rate of loss of 7-methylguanine were described for liver ribosomal RNA of rats treated with low doses of DMN (Cradock *et al.*, 1968; McElhone *et al.*, 1971; Craig & O'Connor, 1971).

Excision of 7-methylguanine from ribosomal RNA has been suggested to occur in mouse liver during chronic administration of DMN, since the amounts of the product in RNA decreased although RNA turnover, as measured by orotic acid incorporation, was unchanged. However, the authors also demonstrated that chronic DMN administration decreased the capacity of the liver to produce alkylating species from DMN and this may have contributed to an apparent loss of 7-methylguanine. On the other hand, the amounts of 7-methylguanine in rat liver chromatin RNA were found to decrease after administration of a single dose of DMN and since this species of RNA did not show significant incorporation of labelled precursors, i.e. it was metabolically stable, it was concluded that excision of 7-methylguanine was taking place (Galbraith *et al.*, 1978).

If base-specific repair reactions did affect ribosomal RNA bases, they might by analogy with rat liver DNA (Pegg, 1977b; Rogers & Pegg, 1977) or hamster liver DNA (Margison *et al.*, 1976) be expected to be more efficient after the low dose, whereas here the highest rate of loss was after the highest dose. In addition, as shown in Fig. 4, whereas no changes were observed in the *O*⁶-methylguanine/7-methylguanine ratio in the ribosomal RNA over a period of 96h, in the DNA this ratio increases considerably as a consequence of enzymic excision processes involving these alkylated DNA bases (see Margison *et al.*, 1976).

In conclusion, these results suggest that, in contrast to the observation in liver DNA of the same animal species, there is a lack of specific excision of alkylated bases from ribosomal RNA. Preliminary results obtained for rat liver ribosomal RNA also indicate a lack of excision, since the ratios *O*⁶-methylguanine:7-methylguanine and 1-methyladenine:7-methylguanine were the same at 5h and 69h after administration of 2mg of DMN/kg (O'Connor *et al.*, 1972; G. P. Margison, M. J. Capps, P. J. O'Connor & A. W. Craig, unpublished work). The present result does not exclude the possibility that excision of individual products takes place, but it must either occur at a level which does not significantly change the alkylated base ratios, or all the products are excised at the same rate. This latter possibility would appear to be unlikely, especially in view of the very different rates of excision of individual alkylated DNA bases under a wide variety of circumstances (see Pegg, 1977a; Margison & O'Connor, 1979). Such processes, if they occur to any extent in ribosomal RNA, may constitute an initial stage in a mechanism leading to the rapid and complete degradation of alkylated RNA containing potentially lethal damage. It would be of interest, however, to examine the persistence of various alkylation products in other forms of RNA, such as messenger or nuclear RNA.

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References

- Capps, M. J., O'Connor, P. J. & Craig, A. W. (1973) *Biochim. Biophys. Acta* **186**, 229–231
- Craddock, V. M. & Magee, P. N. (1963) *Biochem. J.* **89**, 32–37
- Craddock, V. M., Mattocks, A. R. & Magee, P. N. (1968) *Biochem. J.* **109**, 75–78
- Craig, A. W. & O'Connor, P. J. (1971) *Biochem. J.* **123**, 34–40
- Dutton, A. H. & Heath, D. F. (1956) *J. Chem. Soc.* 1892–1893
- Galbraith, A., Barker, M. & Itzhaki, R. F. (1978) *Biochim. Biophys. Acta* in the press
- Hall, R. H. (1971) *The Modified Nucleosides in Nucleic Acids*, Columbia University Press, New York and London
- IARC (1978) *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, vol. 17, pp. 125–175, International Agency for Research on Cancer, Lyon
- Kriek, E. & Emmelot, P. (1964) *Biochim. Biophys. Acta* **91**, 59–66
- Lawley, P. D. (1974) *Mutat. Res.* **23**, 283–295
- Lawley, P. D. & Brookes, P. (1963) *Biochem. J.* **89**, 127–138
- Lawley, P. D. & Shah, S. A. (1972) *Biochem. J.* **128**, 117–132
- Lawley, P. D., Brookes, P., Magee, P. N., Craddock, V. M. & Swann, P. F. (1968) *Biochim. Biophys. Acta* **157**, 646–648
- Loveless, A. (1969) *Nature (London)* **223**, 206–207
- Ludlum, D. B. & Wilhelm, R. C. (1968) *J. Biol. Chem.* **243**, 2750–2753
- Ludlum, D. B., Warner, R. C. & Wahba, A. T. (1964) *Science* **145**, 397–399
- Magee, P. N. & Farber, E. (1962) *Biochem. J.* **83**, 114–124
- Magee, P. N., Pegg, A. E. & Swann, P. F. (1975) in *Handbuch der Allgemeinen Pathologie II* (Altmann, H. W., Büchner, F., Cottier, H., Grundmann, E., Holle, G., Letterer, E., Masshoff, W., Meesen, H., Roulet, F., Seifert, G. & Siebert, G., eds.), pp. 329–419, Springer-Verlag, Heidelberg
- Magee, P. N., Montesano, R. & Preussmann, R. (1976) *ACS Monogr.* **173**, 491–625
- Margison, G. P. & O'Connor, P. J. (1979) in *Chemical Carcinogens and DNA* (Grover, P. L., ed.), CRC Press, Baltimore, in the press
- Margison, G. P., Margison, J. M. & Montesano, R. (1976) *Biochem. J.* **157**, 627–634
- McElhone, M. J., O'Connor, P. J. & Craig, A. W. (1971) *Biochem. J.* **125**, 821–827
- Michelson, A. M. & Pochon, F. (1966) *Biochim. Biophys. Acta* **114**, 469–480
- Montesano, R. & Bartsch, H. (1976) *Mutat. Res.* **32**, 179–228
- Nemoto, N. & Takayama, S. (1974) *Biochem. Biophys. Res. Commun.* **58**, 242–249
- O'Connor, P. J., Capps, M. J., Craig, A. W., Lawley, P. D. & Shah, S. A. (1972) *Biochem. J.* **129**, 519–528
- Pegg, A. E. (1977a) *Adv. Cancer Res.* **25**, 195–269
- Pegg, A. E. (1977b) *J. Natl. Cancer Inst.* **58**, 681–687
- Rogers, K. J. & Pegg, A. E. (1977) *Cancer Res.* **37**, 4082–4087
- Shooter, K. V., Edwards, P. A. & Lawley, P. D. (1971) *Biochem. J.* **125**, 829–840
- Whittle, E. (1969) *Biochim. Biophys. Acta* **195**, 381–388
- Witschi, H. (1973) *Biochem. J.* **136**, 781–788