Biochemical Pharmacology, Vol. 34, No. 22, pp. 3999–4003, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECTS OF 6-AMINONICOTINAMIDE ON CELL GROWTH, POLY(ADP-RIBOSE) SYNTHESIS AND NUCLEOTIDE METABOLISM*

DAREL HUNTING, BONNIE GOWANS and J. FRANK HENDERSON†
Cancer Research Group (McEachern Laboratory) and Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

(*Received* 14 *November* 1984; *accepted* 16 *April* 1985)

Abstract—The purpose of this study was to determine if the cytotoxic effects of 6-aminonicotinamide are solely the result of an inhibition of poly(ADP-ribose) synthesis. The effects of 6-aminonicotinamide on cell growth, poly(ADP-ribose) synthesis and nucleotide concentrations were compared with the effect of 3-aminobenzamide, a more potent inhibitor of poly(ADP-ribose) synthesis. The growth of L1210 cells was not inhibited by 1 mM 3-aminobenzamide and was only slightly inhibited by 5 mM 3-aminobenzamide even though poly(ADP-ribose) synthesis, as measured by the *N*-methyl *N*-nitrosourea induced depletion of NAD^+, was inhibited substantially. In contrast, 6-aminonicotinamide was found to be a potent inhibitor of L1210 and CHO cell growth. A 5 mM concentration of 3-aminobenzamide had no effect on purine and pyrimidine ribonucleotide concentrations or on the ATP to ADP ratio, but it did cause a slight elevation of NAD^+. 6-Aminonicotinamide at 0.01 mM caused a depletion of purine and pyrimidine nucleotides and NAD^+ as well as a reduction in the ATP to ADP ratio. 6-Aminonicotinamide at 1 mM caused a substantial inhibition of purine nucleotide synthesis from [^{14}C] glycine but did not stimulate ATP breakdown. We conclude that inhibition of poly(ADP-ribose) synthesis caused little growth inhibition in itself and that the effects of 6-amininicotinamide on nucleotide metabolism were sufficient to produce an inhibition of both cell growth and DNA repair.

In 1977 Smulson *et al.* [1] suggested that, since poly(ADP-ribose) might play a role in the repair of alkylation damage to DNA, inhibitors of poly(ADP-ribose) synthesis might potentiate the antitumor effects of alkylating agents such as *N*-methyl *N*-nitrosourea. There is now good evidence for an association between poly(ADP-ribose) and DNA excision repair [2–5], and several studies have demonstrated that inhibitors of poly(ADP-ribose) synthesis potentiate the cytotoxic effects of alkylating agents in cultured cells [3, 6–9].

Recently, Berger *et al.* [10] evaluated the ability of several inhibitors of poly(ADP-ribose) synthesis to potentiate the cytotoxic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) against L1210 cells in culture and *in vivo*. Although 6-aminonicotinamide was the least effective inhibitor of poly(ADP-ribose) synthesis, it was the most effective potentiator of the cytotoxic effects of BCNU. Still, Berger *et al.* concluded that this potentiation was the result of inhibition of poly(ADP-ribose) synthesis. We have now evaluated this conclusion more directly by comparing the effects of 6-aminonicotinamide on cell growth, poly(ADP-ribose) synthesis and nucleotide concentrations in L1210 cells with those of 3-aminobenzamide, a more potent inhibitor of poly(ADP-ribose) synthesis. The effects of 6-aminonicotinamide on purine synthesis *de novo* and on ATP catabolism have also been determined. We conclude that substantial inhibition of poly(ADP-ribose) synthesis resulted in, at most, slight inhibition of cell growth, and that the cytotoxic effects of 6-aminonicotinamide were not solely the result of inhibition of poly(ADP-ribose) synthesis. Our data suggest an alternate explanation for the synergistic cytotoxic effects of the combination of BCNU and 6-aminonicotinamide.

* This work was supported by the National Cancer Institute of Canada.

† To whom requests for reprints should be addressed.

MATERIALS AND METHODS

Chinese hamster ovary-Kl cells were grown in suspension cultures in α-MEM medium containing 10% dialysed fetal calf serum (Grand Island Biological Co., Grand Island, NY), as described [11]. The population doubling time was *ca.* 12 hr. Murine leukemia L1210 cells were grown in static suspension cultures in Fischer's medium containing 10% horse serum (Grand Island Biological Co.). The population doubling time was *ca.* 13 hr. Cell density and the population volume distribution were determined using a model Z_f Coulter Counter equipped with a Coulter Channelyzer II.

Procedures for the extraction and quantitation of nucleotides by high performance liquid chromatography (HPLC) have been described [11]. NAD^+ was quantitated separately from the other ribonucleotides using a Waters Resolve C-18 reversed phase HPLC column (Waters Scientific Ltd., Mississauga, Ontario) which was eluted using the following gradient system, at a flow rate of 1 ml/min: solvent A, 100 mM $(NH_4)_3PO_4$, pH 5.1; solvent B, 25% methanol in water; 0–7 min, 100% A; 13 min, 70% A; 16 min, 70% A; 18 min, 100% A. Detection was at 254 nm, and the peaks were integrated auto-

matically by a Spectra-Physics 4100 integrator. The reproducibility of the NAD^+ determinations (standard deviation, %) was 5.1% (independent determinations on five cultures).

[^{14}C]Glycine incorporation into purine nucleotides in L1210 cells was determined in Fischer's medium containing 0.1 mM [^{14}C]glycine (100 mCi/mmole; New England Nuclear Corp., Boston, MA).

ATP catabolism was determined essentially as described previously [12]. L1210 cells were incubated for 30 min in Fischer's medium (Grand Island Biological Co.) containing 0.1 mM [8-^{14}C]adenine (59 mCi/mmole; Moravek Biochemicals Inc., Brea, CA) to label ATP. The cells were centrifuged and resuspended in fresh medium with or without 6-aminonicotinamide and incubated at 37°. Samples were removed at several times, the cells were centrifuged, and the cells and medium were extracted separately with 0.4 M perchloric acid. The radioactivity in cellular ATP and in hypoxanthine in the medium was determined. Procedures for the separation and measurement of radioactivity in purine ribonucleotides have been described [13].

6-Aminonicotinamide and 3-aminobenzamide were obtained from the Sigma Chemical Co., St. Louis, MO.

RESULTS

Cell growth. 6-Aminonicotinamide at 0.01 mM reduced the growth of L1210 cells over 6- and 24-hr periods to 68 and 20% of control respectively. 6-Aminonicotinamide at 0.1 and 1 mM not only completely inhibited cell growth but also produced a slight reduction in cell number and caused the accumulation of cellular debris, as detected on the Coulter Channelyzer. In contrast, 3-aminobenzamide at 1 mM had no effect on L1210 cell growth, and at 5 mM reduced cell growth to 80% of control over a 24-hr period. 6-Aminonicotinamide was less toxic in CHO-Kl cells than in L1210 cells in that at a concentration of 0.01 mM cell growth was only slightly inhibited (93% of control over a 23-hr period), while concentrations of 0.1 and 1 mM had little effect on cell growth over a 6-hr period but completely inhibited cell growth after 23 hr of treatment. 3-Aminobenzamide (5 mM) had no effect on the growth of CHO cells over a 30-hr period.

Inhibition of poly(ADP-ribose) synthesis. Poly(ADP-ribose) synthesis is difficult to measure in intact cells both because of the lack of a specific precursor and because of rapid turnover of poly(ADP-ribose) in cells treated with DNA-damaging agents [4, 14]. The rapid turnover of poly(ADP-ribose) argues against using poly(ADP-ribose) accumulation as a measure of its synthesis. Since previous studies have provided evidence that the NAD^+ depletion caused by alkylating agents is the result of poly(ADP-ribose) synthesis and turnover [4, 14–17], we have used inhibition of *N*-methyl *N*-nitrosourea-induced depletion of NAD^+ as a measure of inhibition of poly(ADP-ribose) synthesis.

As shown in Table 1, treatment of L1210 cells with 1 mM *N*-methyl *N*-nitrosourea for 1 hr caused the NAD^+ concentration to decline to 32% of control. 3-Aminobenzamide at 5 mM prevented most of the NAD^+ depletion, whereas 6-aminonicotinamide at 0.01 mM was only moderately effective and, interestingly, was less effective at 0.1 and 1 mM. At concentrations of 0.1 and 1 mM a 1.5-hr treatment with 6-aminonicotinamide caused a slight depletion of NAD^+ in the absence of *N*-methyl *N*-nitrosourea treatment, suggesting a possible effect on NAD^+ synthesis. This effect was apparently not related to inhibition of poly(ADP-ribose) synthesis since it was not observed with 3-aminobenzamide. Even when the results were corrected for the reduction in NAD^+ caused by 6-aminonicotinamide, 5 mM 3-aminobenzamide caused more inhibition of poly(ADP-ribose) synthesis than 1 mM 6-aminonicotinamide, consistent with previous results in permeable cells [10]. This conclusion, taken together with the growth data reported above, suggests that inhibition of poly(ADP-ribose) synthesis causes little growth inhibition in itself and, therefore, that the potent growth

Table 1. Effects of 3-aminobenzamide (3-ABA) and 6-aminonicotinamide (6-ANA) on *N*-methyl *N*-nitrosourea (MNU)-induced NAD^+ depletion in L1210 cells*

		NAD^+	
MNU treatment	Drug	% of Untreated control	% of Inhibitor-treated control
−	5 mM 3-ABA	135	100
−	0.01 mM 6-ANA	112	100
−	0.1 mM 6-ANA	81	100
−	1 mM 6-ANA	76	100
+	None	32	
+	5 mM 3-ABA	95	70
+	0.01 mM 6-ANA	60	54
+	0.1 mM 6-ANA	42	52
+	1 mM 6-ANA	45	59

* Cells were incubated for 30 min with or without drugs and then incubated for 60 min with or without 1 mM MNU. The cells were then centrifuged and extracted on ice with 0.4 M perchloric acid (PCA). The control value for NAD^+ in pmoles/10^6 cells was 355.

Table 2. Effects of 3-aminobenzamide (3-ABA) and 6-aminonicotinamide (6-ANA) on ribonucleotide concentrations in L1210 cells*

Treatment time (hr)	Drug	NAD^+	UTP	CTP	GTP	ATP	ATP/ADP†
		(% of control)					
6	5 mM 3-ABA	114	101	98	105	115	132
6	0.01 mM 6-ANA	76	32	38	64	45	65
6	0.1 mM 6-ANA	37	22	24	80	42	40
6	1 mM 6-ANA	24	18	20	55	31	47
24	5 mM 6-ABA	122	90	109	101	96	115
24	0.01 mM 6-ANA	110	43	28	50	39	34
24	0.1 mM 6-ANA	36	14	24	51	31	56
24	1 mM 6-ANA	18	10	20	34	14	63

* Cells were incubated with or without drugs for the times indicated and then centrifuged and extracted on ice with 0.4 M PCA. Control values for nucleotide concentrations in nmoles/10^6 cells at 6 and 24 hr, respectively, were: NAD^+, 0.56, 0.46; UTP, 0.80, 1; CTP, 2, 2.5; GTP, 0.54, 0.85; and ATP, 2.4, 3.2. The control values for the ATP/ADP ratio at 6 and 24 hr were 15.4 and 15.5 respectively.

† The ATP/ADP ratio is a useful measure of energy metabolism; however, because this value is large in control cells (e.g. 15:1), small conversions of ATP to ADP will have relatively large effects on the ratio. In general, we interpret ATP/ADP ratios of less than 75% of control as indicative of impaired energy metabolism.

inhibitory effects of 6-aminonicotinamide must result from other metabolic effects.

Nucleotide concentrations. The effect of 6-aminonicotinamide on NAD^+ concentrations in the absence of *N*-methyl *N*-nitrosourea treatment, together with the fact that 6-aminonicotinamide is a potent inhibitor of the oxidative pentose phosphate pathway [18], led us to consider the possibility that 6-aminonicotinamide might alter the concentrations of other nucleotides.

The effects of 6-aminonicotinamide and 3-aminobenzamide on purine and pyrimidine ribonucleoside triphosphate concentrations in L1210 cells are shown in Table 2. A 6-hr treatment with 0.01 mM 6-aminonicotinamide caused a substantial reduction in the concentrations of all four nucleoside triphosphates and of NAD^+. As well, the ATP:ADP ratio, a measure of energy metabolism, also declined. At concentrations of 0.1 and 1 mM, 6-aminonicotinamide was slightly more effective at depleting nucleotides than at 0.01 mM. As well, the effect of 0.01 mM 6-aminonicotinamide on NAD^+ concentration was substantially less than on the concentrations of the other nucleotides. Comparison of these values with of cell growth observed with 1 mM 6-aminonicotinamide was not solely the result of an inhibition nucleotide concentrations are not related to inhibition of poly(ADP-ribose) synthesis. Continued treatment of the cells with 6-aminonicotinamide to 24 hr had little further effect on nucleotide concentrations, except that the NAD^+ concentration increased to control levels.

Purine nucleotide synthesis de novo. The large reduction in nucleotide concentrations caused by 6-aminonicotinamide could have resulted from inhibition of nucleotide synthesis, stimulation of nucleotide catabolism, or both. [^{14}C]Glycine incorporation into ATP and GTP was used as a measure of purine synthesis *de novo* (see Materials and Methods). Cells were incubated with 1 mM 6-aminonicotinamide for 1.5 hr at 37° followed by a further 0.5-hr incubation with [^{14}C]glycine. Under these conditions, 6-aminonicotinamide (1 mM) reduced the synthesis of ATP and GTP to 8.1 and 12% of control respectively.

Catabolism of ATP. To determine the effect of 6-aminonicotinamide on ATP catabolism, L1210 cells were incubated with [8-^{14}C]adenine for 30 min to label the ATP pool (see Materials and Methods). The cells were resuspended in fresh medium with or without 1 mM 6-aminonicotinamide and incubated at 37°. Samples were removed at several times for determination of radioactivity in cellular ATP and in hypoxanthine in the medium. The results in Table 3 show that in control cells the rate of ATP turnover was such that 90% of the radioactivity in the ATP pool was lost during a 6-hr incubation, although no accumulation of radioactive hypoxanthine occurred.* While 6-aminonicotinamide (1 mM) did not stimulate the loss of radioactivity from the ATP pool, it did influence the fate of the radioactivity. No hypoxanthine accumulation was detected during the first 2 hr of treatment with 6-aminonicotinamide (1 mM); however, during the final 4 hr of a 6-hr treatment approximately 90% of the radioactivity in ATP was catabolized to hypoxanthine.

* We have calculated the turnover time for the ATP pool using the control data for the loss of radioactivity from the ATP pool and the following formula: turnover time = $(t_1 - t_1)/(\ln C_1 - \ln C_2)$, where t_1 and t_2 are the sampling times and C_1 and C_2 are the respective amounts of radioactivity in ATP per 10^6 cells. This calculation is based on the assumptions that the ATP pool behaved as a single compartment and that no recycling occurred. The calculated turnover time was 168 min.

Table 3. Effects of 6-aminonicotinamide (6-ANA) on ATP catabolism in L1210 cells*

Drug	Treatment time (hr)	Radioactivity in ATP (cpm/10^6 cells)	Radioactivity in ATP (% of 0 hr treatment)	Radioactivity in extracellular hypoxanthine (cpm/10^6 cells)
None	0	99,900	100	0
	2	65,100	65	0
	6	10,300	10	0
1 mM 6-ANA	0	83,100	100	0
	2	62,200	75	0
	6	13,400	16	43,000

* L1210 cells were incubated for 30 min in Fischer's medium containing 0.1 mM [8-^{14}C] adenine to label ATP and were then centrifuged and resuspended in fresh medium with or without 6-aminonicotinamide. The cells were incubated at 37°, and samples were removed at the times indicated. The samples were centrifuged, and the cells and medium were extracted separately with 0.4 M PCA.

DISCUSSION

Berger *et al.* [10] measured poly(ADP-ribose) synthesis on permeablized cells and found that 2 mM 3-aminobenzamide and 6-aminonicotinamide inhibit poly(ADP-ribose) synthesis by 96 and 70% respectively. This, together with our finding that 5 mM 3-aminobenzamide was only slightly growth inhibitory, suggests that the growth inhibitory effect of 0.01 mM 6-aminonicotinamide was not the result of inhibition of poly(ADP-ribose) synthesis. We determined the relative potency of 3-aminobenzamide and 6-aminonicotinamide as inhibitors of poly(ADP-ribose) synthesis in intact cells by using inhibition of the *N*-methyl *N*-nitrosourea-induced depletion of NAD^+ as an assay for the inhibition of poly(ADP-ribose) synthesis; however, 6-aminonicotinamide by itself also reduced NAD^+ concentrations. Even when the results were corrected for the depletion of NAD^+ caused by 6-aminonicotinamide, it was clear that 5 mM 3-aminobenzamide caused a greater inhibition of poly(ADP-ribose) synthesis than 1 mM 6-aminonicotinamide, suggesting that the complete inhibition of cell growth observed with 1 mM 6-aminonicotinamide was not solely the result of an inhibition of poly(ADP-ribose) synthesis. If poly(ADP-ribose) synthesis were involved only in DNA repair in cultured cells, then one might not expect inhibition of poly(ADP-ribose) synthesis to cause substantial growth inhibition, in the absence of a DNA damaging agent. Berger *et al.* have suggested that the lack of cytotoxicity of some inhibitors of poly(ADP-ribose) synthesis may be the result of metabolic inactivation [10]; however, we have found that 24-hr incubation with L1210 cells did not reduce the ability of 3-aminobenzamide to inhibit the *N*-methyl *N*-nitrosourea-induced depletion of NAD^+.*

It is well established that 6-aminonicotinamide is a potent inhibitor of the oxidative pentose phosphate pathway at the level of 6-phosphogluconate dehydrogenase [18]. In some cell types, inhibition of 6-phosphogluconate dehydrogenase increases the concentration of 6-phosphogluconate, which is a competitive inhibitor of glucose phosphate isomerase. For example, 0.07 mM 6-aminonicotinamide has been found to inhibit glucose consumption and lactate production by more than 50% in cultured C-6 glial cells [19]. Therefore, 6-aminonicotinamide might affect nucleotide metabolism either by inhibiting ATP generations or by inhibiting the synthesis of phosphoribosyl pyrophosphate. In some biological systems 6-aminonicotinamide has been found to reduce ATP concentrations [19–21], while in others it had no effect [19], or even produced an elevation in ATP [22].

In L1210 cells we found that 6-aminonicotinamide, even at 0.01 mM, caused a substantial reduction in purine and pyrimidine ribonucleotide and NAD^+ concentrations and in the ATP:ADP ratio. Since 5 mM 3-aminobenzamide had no effect on nucleotide concentrations, with the exception of a small but reproducible elevation in NAD^+, we conclude that the effect of 6-aminonicotinamide on nucleotide concentrations was not related to an inhibition of poly(ADP-ribose) synthesis.

The reduction in nucleotide concentrations and ATP to ADP ratio by 6-aminonicotinamide could have resulted from either a stimulation of nucleotide catabolism or from an inhibition of nucleotide synthesis. We tested both these possibilities and found that 6-aminonicotinamide inhibited [^{14}C]glycine incorporation into purine nucleotides, suggesting an inhibition of purine synthesis *de novo*. Although 6-aminonicotinamide did not increase the rate of loss of radioactivity from the ATP pool in cells prelabeled with [^{14}C]adenine, it did cause a substantial accumulation of radioactive hypoxanthine. Since the inhibition of purine synthesis *de novo* preceded the accumulation of hypoxanthine, we conclude that the reduction in nucleotide concentrations caused by 6-aminonicotinamide is mainly the result of inhibition of nucleotide synthesis, rather than a stimulation of nucleotide catabolism. An indirect inhibition of phosphoribosyl pyrophosphate synthesis by 6-aminonicotinamide, resulting from the inhibition of the pentose phosphate pathway, would be consistent with the depletion of both purine and pyrimidine nucleotide pools.

* D. Hunting and J. F. Henderson, in preparation.

Previous studies using inhibitors of nucleotide synthesis have shown that conditions which result in substantial reductions in nucleotide concentrations also produce growth inhibition, consistent with the possibility that the growth inhibitory effects of 6-aminonicotinamide are the result of effects on nucleotide metabolism rather than on poly(ADP-ribose) synthesis [11]. It has also been found that the drug-induced reduction in individual ribonucleoside triphosphate pools generally correlates with a reduction in the corresponding deoxyribonucleoside triphosphate pool [23]. Furthermore, several studies have shown that hydroxyurea, an inhibitor of deoxyribonucleotide synthesis, inhibits DNA excision repair [24–27], and a recent study has shown that ATP is required for the incision step during DNA repair [28]. Therefore, the potentiation of the cytotoxic effects of BCNU by 6-aminonicotinamide in L1210 cells may indeed have resulted from inhibition of DNA repair by 6-aminonicotinamide as proposed by Berger *et al.* [10], but the mechanism of this inhibition may have been the effect of 6-aminonicotinamide on nucleotide metabolism rather than directly on poly(ADP-ribose) synthesis.

We note that three recent reports from Cleaver's laboratory have argued that 3-aminobenzamide inhibits "the *de novo* synthesis of DNA purines", which presumably encompasses an inhibition of any of the following steps: purine synthesis *de novo*, nucleotide interconversion and phosphorylation, the synthesis of deoxyribonucleotides by ribonucleotide reductase and DNA synthesis [29–31]. In none of the three studies was the effect of 3-aminobenzamide specifically on purine synthesis *de novo* or on ribonucleotide concentrations actually determined. Our finding that 3-aminobenzamide (5 mM) had no effect on purine and pyrimidine ribonucleotide concentrations in L1210 cells suggests that 3-aminobenzamide is not a potent inhibitor of ribonucleotide synthesis.

REFERENCES

1. M. E. Smulson, P. Schein, D. W. Mullins and S. Sudhaker, *Cancer Res.* **37**, 3006 (1977).
2. N. A. Berger and G. W. Sikorski, *Biochemistry* **20**, 3610 (1981).
3. B. W. Durkacz, O. Omidiji, D. A. Gray and S. Shall, *Nature, Lond.* **283**, 593 (1980).
4. E. L. Jacobson, K. M. Antol, H. Juarez-Saliras and M. K. Jacobson, *J. biol. Chem.* **258**, 103 (1983).
5. M. R. James and A. R. Lehmann, *Biochemistry* **21**, 4007 (1982).
6. N. Nduka, C. J. Skidmore and S. Shall, *Eur. J. Biochem.* **105**, 525 (1980).
7. B. W. Durkacz, N. Nduka, O. Omidiji, S. Shall and A. A. Zia'ee, in *Novel ADP-Ribosylations of Regulatory Enzymes and Proteins* (Eds. M. Smulson and T. Sugimura), p. 207. Elsevier/North-Holland, New York (1980).
8. L. G. Durrant and J. M. Boyle, *Chem. Biol. Interact.* **38**, 325 (1982).
9. H. Yamamoto and H. Okamoto, *Fedn. Eur. Biochem. Soc.* **145**, 298 (1982).
10. N. A. Berger, D. M. Catino and T. J. Vietti, *Cancer Res.* **42**, 4382 (1982).
11. D. Hunting and J. F. Henderson, *Biochem. Pharmac.* **31**, 1109 (1982).
12. C. A. Lomax and J. F. Henderson, *Cancer Res.* **33**, 2825 (1973).
13. G. W. Crabtree and J. F. Henderson, *Cancer Res.* **31**, 985 (1971).
14. K. Wielckens, E. George, T. Pless and H. Hilz, *J. biol. Chem.* **258**, 4098 (1983).
15. M. K. Jacobson, V. Levi, H. Juarez-Salinas, R. A. Barton and E. L. Jacobson, *Cancer Res.* **40**, 1797 (1980).
16. H. Juarez-Salinas, J. L. Sims and M. K. Jacobson, *Nature, Lond.* **282**, 740 (1979).
17. C. J. Skidmore, M. I. Davies, P. M. Goodwin, H. Halldorsson, P. J. Lewis, S. Shall and A. A. Zia'ee, *Eur. J. Biochem.* **101**, 135 (1979).
18. K. Lange, H. Kolbe, K. Keller and H. Herken, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1389 (1972).
19. H. Kolbe, K. Keller, K. Lange and H. Herken, *Biochem. biophys. Res. Commun.* **36**, 93 (1969).
20. V. C. Sheffield and R. E. Seegmiller, *J. Embryol. exp. Morph.* **59**, 217 (1980).
21. E. J. Ritter, W. J. Scott and J. G. Wilson, *Teratology* **12**, 233 (1975).
22. D. W. McCandless and W. J. Scott, *Teratology* **23**, 391 (1981).
23. D. Hunting, J. Hordern and J. F. Henderson, *Can. J. Biochem.* **59**, 821 (1981).
24. E. Ben-Hur and R. Ben-Ishai, *Photochem. Photobiol.* **13**, 337 (1971).
25. A. R. S. Collins, S. Squires and R. T. Johnson, *Nucleic Acids Res.* **10**, 1203 (1982).
26. A. A. Francis, R. D. Blevino, W. L. Carrier, D. P. Smith and J. D. Regan, *Biochim. biophys. Acta* **563**, 385 (1979).
27. R. D. Snyder, *Mutation Res.* **131**, 163 (1984).
28. S. L. Dresler and M. W. Lieberman, *J. biol. Chem.* **258**, 12269 (1983).
29. J. E. Cleaver, W. J. Bodell, W. F. Morgan and B. Zelle, *J. biol. Chem.* **258**, 9059 (1983).
30. K. M. Milan and J. E. Cleaver, *Science* **223**, 589 (1984).
31. J. E. Cleaver, *Mutation Res.* **131**, 123 (1984).