Poly(ADP-ribose) Metabolism in Ultraviolet Irradiated Human Fibroblasts*

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Exposure of human fibroblasts to 5 J/m² of UV light resulted in a rapid increase of up to 1500% in the intracellular content of poly(ADP-ribose) and a rapid depletion of its metabolic precursor, NAD. When added just prior to UV treatment, the poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide, totally blocked both the increase of poly(ADP-ribose) and decrease in NAD for up to 2.5 h. Addition of 3-aminobenzamide at the time of maximal accumulation of poly(ADP-ribose) resulted in a decrease to basal levels with a half-life of 5-30 min. The rates of accumulation of poly(ADP-ribose) and depletion of NAD were increased in the presence of either 1-β-arabinofuranosylcytosine or hydroxyurea. Since these agents are known to cause an additional accumulation of DNA strand breaks following UV irradiation, these data provide evidence for a mechanism in which the rate of poly(ADP-ribose) synthesis following DNA damage is regulated in intact cells by the number of DNA strand breaks. Under conditions in which the synthesis of poly(ADP-ribose) was blocked, DNA repair replication induced by UV light was neither stimulated nor inhibited.

NAD is the substrate for the synthesis of the chromosomal polymer, poly(ADP-ribose) (1, 2). The synthesis of poly(ADP-ribose) is a DNA-dependent reaction (1, 2), and agents that damage DNA stimulate poly(ADP-ribose) polymerase activity in vitro (3-12) and stimulate the conversion of NAD to poly(ADP-ribose) in vivo (13). While the factors that regulate the synthesis and breakdown of poly(ADP-ribose) in vivo are not known, studies of poly(ADP-ribose) polymerase have shown that the enzyme is activated by DNA strand breaks (14-16).

We describe here the kinetics of accumulation of poly(ADP-ribose) and depletion of NAD in vivo in normal human fibroblasts following UV irradiation. These studies suggest a mechanism in which the rate of synthesis of poly(ADP-ribose) in response to DNA damage is regulated by the cellular content of DNA strand breaks and that once synthesized poly(ADP-ribose) is turned over extremely rapidly.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—Normal human diploid fibroblasts, CF-3, obtained from newborn foreskin tissue were a gift from Dr. R. Dell’Orco, The Samuel Roberts Noble Foundation, Ardmore, OK. All experiments were conducted on cell populations which had undergone 28 or less population doublings. The cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and 50 µg/ml of gentamycin. For experiments, cells were seeded in dishes and grown to confluence. Confluent cultures were mitotically arrested by lowering the serum concentration to 0.5% for 5-7 days (17). Medium was replenished twice weekly.

Procedures for DNA Damage—To treat with UV light, the medium was removed and the cells were washed with phosphate-buffered saline (0.01 M sodium phosphate, pH 7.2, 0.15 M NaCl). The cells were placed under a General Electric germicidal lamp (G15T8) with an incident flux of 0.5 J/m² for 10 s. Control cultures were treated in the same manner except that the G15T8 lamp was not on. For experiments, a modified Eagle’s medium was then added to the cells which were returned to the incubator. Hydroxyurea, when used, was added to a final concentration of 10 mM following UV treatment. Cells treated with araC or 3-aminobenzamide were preincubated for 1 h with 0.1 mM araC and/or 3 mM 3-aminobenzamide in serum-free nicotinamide-free medium.

Measurement of NAD Levels—The total NAD pool was extracted as described previously (18). NAD was measured by an enzymatic cycling assay described in detail elsewhere (19).

Poly(ADP-ribose) Content—The total intracellular content of poly(ADP-ribose) was determined by modifications of a previously published procedure (20). Acid-insoluble cell pellets from 1.5 x 10⁶ to 1.0 x 10⁷ cells were obtained as described previously (20) and dissolved by the aid of sonification in 10 ml of 50 mM 4-morpholinepropanesulfonic acid buffer, 6 mM guanidine-HCl adjusted to pH 8.8 with KOH. After dissolution the pH was checked and readjusted, if necessary, to pH 8.8 to 9.0. Dihydroxyboryl BioRex 70 resin prepared as described by Wielckens et al. (21), 0.2 ml packed volume, was added, and the mixture was shaken for 4 h to adsorb poly(ADP-ribose) to the resin. The resin was collected by centrifugation and washed successively with 5 ml of 50 mM MOPS, 6 mM guanidine-HCl, pH 8.8, and with 2.5 ml of 0.25 M ammonium acetate buffer, pH 8.8. Poly(ADP-ribose) was then eluted from the resin by washing with water. The polymer was converted to ribosyladenosine which was reacted with chloroacetaldehyde to form 1,N⁸-ethenobosyladenosine as described previously (20). Following chloroacetaldehyde treatment, the reaction mixtures (approximately 3 ml) were diluted to 10 ml and adjusted to 0.25 M in ammonium acetate, and the pH was adjusted to 9.0. The mixture was then diluted through a 0.5-ml column of dihydroxyboryl polycrlyamide resin (Affigel 601, Bio-Rad) to absorb ethenobosyladenosine. The column was washed with 5.0 ml of 0.25 M ammonium acetate buffer, pH 9.0, and the ethenobosyladenosine was eluted with 2.0 ml of 200 mM sodium citrate buffer, pH 4.5. High pressure liquid chromatography was performed with a Beckman 110A liquid chromatograph equipped with an Altex Ultrasphere-ODS reversed phase column (250 mm x 4.6 mm x 0.25 inch). Fluorescence was detected with a Varian Fluorochrom filter; fluorometer equipped with a deuterium light source and a Varian interference filter on the excitation side. A Varian 3-75 emission filter was used. All samples were injected in 200 µM sodium citrate buffer, pH 4.5, and elution was performed isocratically at room temperature with 7 mM ammonium formate buffer, pH 5.9/100% methanol, 83:7 (v/v). The values obtained were corrected for recovery as described previously (20).

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1 The abbreviations used are: araC, 1-β-D-arabinofuranosylcytosine; MNG, N,N-methyl-N' nitro-N-nitrosoguanidine; rAdo, 2'-ribosyladenosine.
DNA Repair Replication—DNA repair replication was measured by modifications of the method of Smith and Hanawalt (22). This method involves the incubation of cells with 10 μM bromodeoxyuridine as a density label and 10 μCi/ml of [methyl-3H]thymidine (65 Ci/m mole) radioisotopic label. All procedures were as described (22) except that fluorodeoxyuridine was omitted and the cells were pretreated for 1 h with bromodeoxyuridine. In selected experiments, hydroxyurea was also omitted. Cell lysates were mixed with CsCl and adjusted to a refractive index of 1.406 to 1.407 and the samples were subjected to centrifugation at 44,000 rpm at 20 °C for 17 h in a Beckman T-65 rotor. The gradients were fractionated onto Whatman No. 3MM paper circles which were washed twice with 10% trichloroacetic acid, twice with 5% trichloroacetic acid, and three times with 95% ethanol. The filters were air dried and counted in a toluene-based liquid scintillation cocktail. Radioactivity banding at the density of light DNA was taken as the measure of DNA repair replication.

RESULTS

Fig. 1 shows the total cellular content of poly(ADP-ribose) (A) and its precursor NAD (B) in normal human fibroblasts following treatment with 5 J/m² of UV light. The total amount of poly(ADP-ribose) in untreated cells was 12 pmol/10⁶ cells of ribosyladenosine derived from poly(ADP-ribose). Following UV treatment the level of poly(ADP-ribose) increased rapidly to approximately 200 pmol/10⁶ cells and remained elevated for 5 h. The increase over basal level was approximately 100% at 15 min, 1500% at 1 h, and 1100% at 5 h following UV treatment. The initial content of NAD was approximately 100 nmol/10⁶ cells, and no change in NAD content was detected at 15 min following UV treatment after which a progressive decrease in the cellular NAD pool was observed. Between 1 and 5 h the NAD pool size was decreasing at an approximate rate of 200 pmol/min/10⁶ cells. The effects of the poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide, when added to the culture medium are also shown. When added prior to UV treatment, no detectable increase in poly(ADP-ribose) content or decrease in NAD was observed for 2.5 h. By 5 h, however, both an increase of poly(ADP-ribose) and decrease in NAD were observed. When 3-aminobenzamide was added to the culture medium at 1 h following UV treatment, no further loss of NAD occurred and poly(ADP-ribose) disappeared with a half-life of approximately 6 min.

Poly(ADP-ribose) polymerase activity is stimulated in vitro by DNA strand breaks (14-16). It has also been shown that there is a transient increase in the number of DNA strand breaks in human fibroblasts following UV irradiation (23-28) and that the presence of araC or hydroxyurea following UV irradiation results in an increased number over UV treatment alone (27, 28). Fig. 2 shows the effect of 0.1 mM araC on cellular NAD and poly(ADP-ribose) content following UV treatment. The rate of depletion of the NAD pool was stimulated by the presence of araC (Fig. 2A). In the period from 15 min to 1 h following UV treatment, the amount of NAD depletion was increased by 150% by the presence of araC. Fig. 2B shows that the content of poly(ADP-ribose) at 15 min following UV irradiation was similar in the presence or absence of araC but that in the presence of araC the content was increased 120% at 30 min and 80% at 1 h relative to cells treated with UV alone. By 2.5 h, the intracellular content of poly(ADP-ribose) was lower in the presence of araC, presumably due to turnover of poly(ADP-ribose) and substrate depletion in the ara-C-treated cells which had only 5% of their initial NAD content remaining at that time. We have observed identical results with those shown in Fig. 2 if 10 mM hydroxyurea was present instead of araC.

Since hydroxyurea and araC cause a significant stimulation of poly(ADP-ribose) accumulation and NAD depletion, we have examined the effectiveness of 3-aminobenzamide on the inhibition of poly(ADP-ribose) metabolism in the presence of araC or hydroxyurea. Fig. 3 shows the results obtained with araC. While the presence of 3-aminobenzamide alone blocked any detectable increase in poly(ADP-ribose) levels for 2.5 h, in the presence of araC poly(ADP-ribose) levels are elevated over 200% at 1 h and over 400% at 2.5 h. Likewise, significant decreases in the NAD pool are observed at 1 and 2.5 h in the presence of araC and 3-aminobenzamide. Again, very similar results were observed in the presence of 5 mM hydroxyurea.

The effect of inhibitors of poly(ADP-ribose) polymerase on unscheduled DNA synthesis following DNA damage have been examined in a number of previous studies (29-33). In each of these studies, hydroxyurea was utilized to suppress replicative DNA synthesis. Since we have observed that 3-aminobenzamide does not effectively block poly(ADP-ribose) synthesis in the presence of hydroxyurea under our conditions, we have conducted experiments to determine whether DNA repair replication in these cells can be quantified in the
absence of hydroxyurea. Fig. 4 shows profiles of alkaline cesium chloride gradients of control cells and cells treated with UV in the absence or presence of hydroxyurea. The cells were incubated for 2.5 h in the presence of [3H]thyminide and 5-bromodeoxyuridine and analyzed by a modification of the method of Smith and Hanawalt (22). The profile of the untreated control cells (Fig. 4A) shows a small peak of high density which represents semiconservative DNA replication. Following UV treatment (Fig. 4B) a peak of repair replicative DNA synthesis was observed at a lower density. This material is well separated from the DNA which banded at the position of semiconservative replication. These data show that repair replication can be quantified in the absence of hydroxyurea. We then assessed the effect of 3-aminobenzamide on repair replication in the absence of hydroxyurea. Under these conditions, we have shown poly(ADP-ribose) synthesis to be completely blocked for at least 2.5 h (Fig. 1). Fig. 5 shows the time course of DNA repair replication following UV treatment in the absence or presence of 3-aminobenzamide. Very little difference in amount or time course of repair replication was observed due to the presence of 3-aminobenzamide.

**FIG. 3. Effect of 0.1 mM araC on NAD (A) and poly(ADP-ribose) (B) content in the presence of 3 mM 3-aminobenzamide.**

The circles represent NAD (A) or poly(ADP-ribose) (B) content of unirradiated (C) or UV irradiated (●) cells in the absence of araC. The triangles represent NAD (A) or poly(ADP-ribose) (B) in the presence of araC of unirradiated (△) or UV irradiated (▲) cells. The data shown are mean values from two separate experiments.

**FIG. 4. Alkaline cesium chloride equilibrium sedimentation analyses of cell extracts from human fibroblasts incubated for 2.5 h in the presence of [3H]thyminide (10 μCi/ml, 65 Ci/mmol), 10 μM 5-bromodeoxyuridine, and 10 mM hydroxyurea (C and D only).** Circles represent [3H]radioactivity and the triangles represent radioactivity from cells grown in the presence of [14C]thyminide alone to serve as a density marker. A and C are from unirradiated cells and B and D are from UV irradiated cells. Each gradient contained DNA from 1.7 x 10^6 cells. Increasing density is from right to left.

**FIG. 5. DNA repair replication following UV irradiation in the absence of hydroxyurea.** Amount of [3H]thyminide incorporation in light DNA is shown following UV irradiation in the absence (●) or presence (▲) of 3 mM 3-aminobenzamide. Each gradient contained DNA from 3.2 x 10^6 cells.

**TABLE I**

| Additions       | Repair replication | Per cent |
|-----------------|--------------------|----------|
| None            | 4695               | 100      |
| Hydroxyurea (10 mM) | 2987               | 64       |
| 3-Aminobenzamide (3 mM) | 4976               | 106      |
| Hydroxyurea (10 mM) and 3-aminobenzamide (3 mM) | 4876 | 104 |

It is of interest that studies (29-32) which have examined the effect of poly(ADP-ribose) polymerase inhibitors on unscheduled DNA synthesis in the presence of hydroxyurea have observed that these inhibitors stimulate unscheduled DNA synthesis. Table I shows a representative experiment in which DNA repair replication was measured in the presence of hydroxyurea or 3-aminobenzamide alone and in combination. We observed that hydroxyurea inhibited repair replication by 36% in this experiment and we routinely observed 30 to 40% suppression in a number of other experiments. However, in the presence of 3-aminobenzamide the amount of repair replication was not inhibited by the presence of hydroxyurea. Therefore, in the presence of hydroxyurea, 3-aminobenzamide caused an apparent stimulation of repair replication.

**DISCUSSION**

These data provide the first direct demonstration that the UV treatment of cells results in a rapid increase in the intracellular content of poly(ADP-ribose). This also represents the first study that has examined in detail the effectiveness of the poly(ADP-ribose) polymerase inhibitor 3-aminobenzamide on poly(ADP-ribose) synthesis in intact cells. We have observed conditions where 3-aminobenzamide effectively blocks both the accumulation of poly(ADP-ribose) and the decrease of NAD for up to 2.5 h (Fig. 1). Further, we have observed several conditions in which decreases in NAD levels are closely correlated with increases in poly(ADP-ribose) content (Figs. 1-3). The data presented here demonstrate that poly(ADP-ribose) synthesized following UV treatment is turned over very rapidly in vivo. In an experiment in which 3-aminobenzamide was added to the culture medium at the
time of maximal accumulation of poly(ADP-ribose), we have measured a half-life of approximately 6 min. This represents a maximum estimate of half-life since it cannot be determined how rapidly 3-aminobenzamide blocks synthesis. Between 1 and 6 h following UV irradiation, the intracellular content of poly(ADP-ribose) remains at 150 to 200 pmol/10^6 cells during which time the NAD pool is decreasing at a rate of approximately 200 pmol/min/10^6 cells. Thus, assuming that all the NAD is being converted to poly(ADP-ribose), the half-life of poly(ADP-ribose) would actually be less than 1 min. A rapid turnover of poly(ADP-ribose) in vivo has also been observed in 3T3 cells following MNNG treatment (13). In general, these observations in vivo are in contrast to the nature of poly(ADP-ribose) synthesis in isolated nuclei or permeable cells in which the polymer is quite stable (34, 35), although Benjamin and Gill (14, 15) have observed rapid turnover of poly(ADP-ribose) in vitro following treatment with x-rays.

Studies of the factors that affect the activity of poly(ADP-ribose) polymerase in vitro have indicated that DNA strand breaks affect the activity of the enzyme. Activity is stimulated by nuclease (3, 4, 14, 15) and Benjamin and Gill (14, 15) have quantitatively related polymerase activity to the number of nuclease-induced strand breaks. Cohen and Berger (16) have shown that the presence of UV damage in the SV40 minichromosome is not sufficient to support polymerase activity but that the enzyme is active following treatment of the minichromosome with Micrococcus luteus UV endonuclease. The results of the present study provide evidence that the number of DNA strand breaks also regulates the rate of poly(ADP-ribose) synthesis in vivo. Many studies have shown that following UV irradiation of human fibroblasts there is a transient increase in the number of DNA single strand breaks which are associated with DNA excision repair (23–28). The formation of DNA strand breaks following UV damage is dependent on endonuclease activity that appears to be the rate-limiting step in repair of pyrimidine dimers (27). It has also been shown that the presence of either hydroxyurea or araC results in an increase in the cellular content of DNA strand breaks by decreasing the rate of the polymerization step at sites under repair without altering the rate of incision (27). Our observations that UV causes a rapid increase in the cellular content of poly(ADP-ribose) and that the presence of either araC or hydroxyurea stimulates both the accumulation of poly(ADP-ribose) and rate of NAD depletion are consistent with a role of increased DNA damage on poly(ADP-ribose) metabolism following UV irradiation. In 3T3 cells following MNNG treatment (13). In general, these observations in vivo are in contrast to the nature of poly(ADP-ribose) synthesis in isolated nuclei or permeable cells in which the polymer is quite stable (34, 35), although Benjamin and Gill (14, 15) have observed rapid turnover of poly(ADP-ribose) in vitro following treatment with x-rays.

The synthesis of poly(ADP-ribose) appears to be a general response to DNA damage (3–16, 40) and Nduka et al. have shown that inhibitors of poly(ADP-ribose) polymerase potentiate the cytotoxicity of DNA-damaging chemicals (41). Since the repair of DNA lesions can reduce the cytotoxic effects of DNA-damaging agents (42), it seems reasonable to propose that one possible function of poly(ADP-ribose) synthesis may be related to DNA repair. A number of recent studies have examined the effect of inhibitors of poly(ADP-ribose) polymerase on unscheduled DNA synthesis (29–33). Each of these studies have utilized hydroxyurea to suppress replicative DNA synthesis. Since we have observed that under our conditions 3-aminobenzamide does not completely inhibit poly(ADP-ribose) synthesis in the presence of hydroxyurea, we have examined the effect of 3-aminobenzamide on DNA repair replication following UV irradiation in the absence of hydroxyurea. We do not observe any significant effect of poly(ADP-ribose) synthesis on the amount or time course of DNA repair replication following UV irradiation. Further, our observations suggest that a stimulatory effect of poly(ADP-ribose) polymerase inhibitors on unscheduled DNA synthesis may be due to the presence of hydroxyurea.

Previously, we have observed that mouse cells depleted of NAD are deficient in unscheduled DNA synthesis caused by MNNG (43, 44), a result consistent with a requirement for poly(ADP-ribose) synthesis for polynucleotide synthesis during DNA excision repair. Our present studies do not support such a requirement for repair of UV damage to DNA in human fibroblasts; however, they do not rule out a role for poly(ADP-ribose) synthesis in a later step in excision repair. Durkacz et al. (45) and Creissen and Shall (46) have provided evidence consistent with a requirement for poly(ADP-ribose) synthesis for the rejoining of DNA strand breaks. The kinetics of poly(ADP-ribose) synthesis in vivo following UV is also entirely consistent with an involvement of poly(ADP-ribose) in the regulation of some aspect of DNA repair or as a regulatory signal associated with some process that must be coordinated with DNA repair.

REFERENCES

1. Hayashi, O., and Ueda, K. (1977) Annu. Rev. Biochem. 46, 95–116
2. Hiš, H., and Stone, P. (1976) Rev. Physiol. Biochem. Pharmacol. 76, 1–58
3. Miller, E. G. (1975) Biochem. Biophys. Res. Commun. 66, 280–286
4. Miller, E. G. (1975) Biochim. Biophys. Acta 395, 191–200
5. Smulson, M., Stärk, P., Gazzoli, M., and Roberts, J. (1975) Exp. Cell Res. 90, 175–182
6. Smulson, M. E., Schein, P., Mullins, D. W., and Sudhaker, S. (1977) Cancer Res. 37, 3006–3012
7. Benjamin, R. C., and Gill, D. M. (1978) J. Supramol. Struct. Suppl. 2, 74
8. Goodwin, P. M., Lewis, P. J., Davies, M. I., Skidmore, C. J., and Shall, S. (1978) Biochem. Biophys. Acta 543, 576–582
9. Benjamin, R. C., and Gill, D. M. (1979) Fed. Proc. 38, 619
10. Berger, N. A., Sikorski, G. W., Petzold, S. J., and Kurohara, K. (1979) Fed. Proc. 38, 619
11. Berger, N. A., Sikorski, G. W., Petzold, S. J., and Kurohara, K. (1979) J. Clin. Invest. 64, 1164–1171
12. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
13. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
14. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
15. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
16. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
17. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
18. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
19. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
20. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
21. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
22. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
23. Jacobson, M. K., Levi, V., and Jacobson, E. G. (1980) Biochim. Biophys. Acta 543, 576–582
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282, 740-741
14. Benjamin, R. C., and Gill, D. M. (1980) J. Biol. Chem. 255, 10493-10501
15. Benjamin, R. C., and Gill, D. M. (1980) J. Biol. Chem. 255, 10502-10508
16. Cohen, J. J., and Berger, N. A. (1981) Biochem. Biophys. Res. Commun. 98, 268-274
17. Dell’Orco, R. T., and Whittle, W. L. (1978) Mech. Ageing Dev. 8, 269-279
18. Jacobson, E. L., Lange, R. A., and Jacobson, M. K. (1979) J. Cell. Physiol. 99, 417-426
19. Jacobson, E. L., and Jacobson, M. K. (1976) Arch. Biochem. Biophys. 175, 627-634
20. Sims, J. L., Juarez-Salinas, H., and Jacobson, M. K. (1980) Anal. Biochem. 98, 268-274
21. Wielckens, K., Bredehorst, R., Adamietz, P., and Hilz, H. (1981) Biochemical Biophys. Acta 675, 282, 740-741
22. Smith, C. A., and Hanawalt, P. C. (1976) Biochim. Biophys. Acta 432, 336-347
23. Collins, A. R. S., Schor, S. L., and Johnson, R. T. (1977) Mutat. Res. 42, 413-432
24. Hiss, E. A., and Preston, R. J. (1977) Biochim. Biophys. Acta 478, 1-8
25. Johnson, R. T., and Collins, A. R. S. (1978) Biochim. Biophys. Res. Commun. 80, 361-369
26. Collins, A. R. S., and Johnson, R. T. (1969) J. Cell. Physiol. 99, 125-138
27. Erixon, K., and Ahnstrom, G. (1979) Mutat. Res. 50, 257-271
28. Collins, A. R. S., Downes, C. S., and Johnson, R. T. (1980) J. Cell. Physiol. 103, 179-191
29. Berger, N. A., and Sikorski, G. W. (1980) Biochim. Biophys. Res. Commun. 95, 67-72
30. Althaus, F. R., Lawrence, S. D., Sattler, G. L., and Pitot, H. C. (1980) Biochim. Biophys. Res. Commun. 95, 1063-1070
31. Miwa, M., Kanai, M., Kondo, T., Hoshine, H., Ishihara, K., and Sugimura, T. (1981) Biochem. Biophys. Res. Commun. 100, 463-470
32. Durkacz, B. W., Irwin, J., and Shall, S. (1981) Biochem. Biophys. Res. Commun. 101, 1433-1440
33. McCurry, L. S., and Jacobson, M. K. (1981) J. Supramol. Struct. Cell. Biochem. 17, 87-90
34. Berger, N. A., Petzold, S. J., and Berger, S. J. (1979) Biochim. Biophys. Acta 654, 90-104
35. Lei, V., Juarez-Salinas, H., and Jacobson, M. K. (1981) Fed. Proc. 40, 171
36. Hashem, N., Bootma, D., Keijzer, W., Greene, A., Coriell, L., Thomas, G., and Cleaver, J. E. (1980) Cancer Res. 40, 13-18
37. McCurry, L. S., and Jacobson, M. K. (1981) J. Biol. Chem. 256, 551-555
38. Oikawa, A., Tobda, H., Kanai, M., Miwa, M., and Sugimura, T. (1980) Biochim. Biophys. Res. Commun. 97, 1311-1316
39. Hori, T. (1981) Biochem. Biophys. Res. Commun. 102, 38-45
40. Rankin, P. W., Jacobson, M. K., Mitchell, V. R., and Busbee, D. L. (1980) Cancer Res. 40, 1803-1807
41. Nduka, N., Skidmore, C. J., and Shall, S. (1980) Eur. J. Biochem. 105, 525-530
42. Yang, L. L., Maher, V. M., and McCormick, J. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5933-5937
43. Jacobson, E. L., Narashimhan, G., and Jacobson, M. K. (1979) Tex. J. Sci. 31, 85-91
44. Jacobson, E. L., and Narashimhan, G. (1979) Fed. Proc. 38, 619
45. Durkacz, B., Omidiji, O., Gray, D., and Shall, S. (1980) Nature 283, 593-596
46. Creissen, D., and Shall, S. (1982) Nature 296, 271-272