2. L. B. Chen, A. Murray, R. A. Segal, A. Bushnell, M. L. Walsh, *Cell* **14**, 377 (1978); E. Crouch, G. Balian, K. Holbrook, D. Duksin, P. Bornstein, *J. Cell Biol.* **78**, 701 (1978); L. T. Furcht, D. F. Mosher, G. Wendelschafer-Crabb, *Cell* **13**, 263 (1978); K. Hedman, A. Vaheri, J. Wartiovaara, *J. Cell Biol.* **76**, 748 (1978).
3. P. Kuusela, *Nature (London)* **276**, 718 (1978).
4. R. A. Proctor, E. Prendergast, D. F. Mosher, *Clin. Res.* **27**, 650A (1979).
5. M. B. Furie and D. B. Rifkin, *J. Biol. Chem.* **255**, 3134 (1980).
6. D. F. Mosher, P. E. Schad, J. M. Vann, *ibid.*, p. 255.
7. F. Jilek and H. Hörmann, *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 1165 (1977).
8. D. D. Wagner and R. O. Hynes, *J. Biol. Chem.* **254**, 6746 (1979).
9. K. M. Yamada, K. Olden, L.-H. E. Hahn, in *The Cell Surface: Mediator of Developmental Processes*, S. Subtenly and N. K. Wessells, Eds. (Academic Press, New York, 1980), p. 43.
10. E. Ruoslahti and E. G. Hayman, *FEBS Lett.* **97**, 221 (1979); L.-H. E. Hahn and K. M. Yamada, *Cell* **18**, 1043 (1979).
11. L. Anderson and N. G. Anderson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5421 (1977).
12. E. Ruoslahti, E. G. Hayman, E. Engvall, in *Tumor Cell Surfaces and Malignancy*, R. O. Hynes and C. F. Fox, Eds. (Liss, New York, in press).
13. D. F. Mosher, *J. Biol. Chem.* **250**, 6614 (1975).
14. _____, P. E. Schad, H. K. Kleinman, *J. Clin. Invest.* **64**, 781 (1979).
15. T. M. Saba, F. A. Blumenstock, P. Weber, J. E. Kaplan, *Ann. N.Y. Acad. Sci.* **312**, 43 (1978); F. Jilek and H. Hörmann, *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1603 (1978); H. Hörmann and V. Jelinić, *ibid.*, in press.
16. M. W. Mosesson and R. A. Umfleet, *J. Biol. Chem.* **245**, 5728 (1970).
17. Supported by NIH grants HL 21644 and HL 24885, a grant from the University of Wisconsin Graduate School, and (to D.F.M.) an established investigatorship of the American Heart Association and its Wisconsin affiliate. We thank P. Olbranz, P. Schad, and R. Johnson for their assistance.

24 March 1980; revised 7 May 1980

Preferred Sites of Strand Scission in DNA Modified by *anti*-Diol Epoxide of Benzo[*a*]pyrene

Abstract. *The sites of DNA modification by the* anti-*diol epoxide of benzo*[a]*pyrene were investigated with the use of a DNA substrate of defined sequence. The modified DNA was labile to strand scission at alkaline and neutral* pH *at guanine, adenine, and cytosine positions.*

Benzo[*a*]pyrene, a potent mutagen and carcinogen is present in combustion products of gasoline and other fuels, and more than 1300 tons are liberated into the atmosphere of the United States per year (*1*, *2*). It requires metabolic conversion for activity in biological systems. One of the oxidative metabolites, the (±) *trans*-7β,8α-dihydroxy-9α,10α-epoxy-7, 8, 9, 10-tetrahydrobenzo[*a*]pyrene (*anti*-BP-diol epoxide) accounts for most of the benzo[*a*]pyrene DNA binding activity in animal feeding studies and in in vitro reactions (*3*, *4*). The *anti*-BP-diol epoxide is mutagenic and carcinogenic (*2*, *5*, *6*).

Incubation of DNA with *anti*-BP-diol epoxide results in breakage of the phosphodiester backbone of DNA (*7*). To determine whether DNA breakage occurred at a preferred nucleotide or nucleotide sequences, we incubated the *anti* isomer of *trans*-BP-diol epoxide with DNA fragments of defined sequence that were terminally labeled with ^{32}P. The reaction products were analyzed on high-resolution, denaturing polyacrylamide gels of the type used for DNA sequencing. As the DNA is labeled at only one end, and as the sequence of the DNA substrate is known, the lengths of the cleavage products should reveal the sites at which strand scission occurs.

A 30-minute incubation of the DNA fragment with *anti*-BP-diol epoxide modified the DNA so that the electrophoretic mobility was reduced (compare lanes 1 and 2 to lane 3 in Fig. 1). The higher the concentration of the *anti*-BP-diol epoxide (in the range from 10 to 100 μg/ml), the greater the observed reduction in the electrophoretic mobility of the DNA. Since the DNA was denatured prior to being layered on the gel, this experiment demonstrates that the phosphodiester bonds are not broken by this treatment.

In contrast, the DNA modified by exposure to *anti*-BP-diol epoxide (90 μg/ml) for 30 minutes was subject to strand scission on subsequent treatment at elevated *p*H. Treatment of the modified DNA with either 0.1*M* NaOH or 1*M* piperidine (Fig. 1, lanes 4 and 5, respectively) for 30 minutes at 90°C resulted in DNA products that migrated more rapidly than did unmodified DNA.

The length of the reaction products provides information regarding the sites of DNA cleavage. Comparison of the electrophoretic mobilities of the short DNA fragments that resulted from treatment of the DNA with *anti*-BP-diol epoxide to those produced in sequencing reactions shows that breakage occurred most frequently at guanine positions (Figs. 1 and 2). Cleavage products were also observed that would correspond to scission at cytosine and adenine and less frequently at thymine positions. The relative rates of strand scission at each nucleotide were determined by measurement of the amount of radioactivity in each band. The amount of radioactivity in bands corresponding to breakage at guanine positions was consistently three to four times higher than that observed for any other base.

Modification of DNA at lower concentrations (10 to 50 μg/ml) of *anti*-BP-diol epoxide for 30 minutes also resulted in lesions that caused strand breakage on alkaline hydrolysis and that were qualitatively similar to those observed at the higher concentration. In contrast, longer incubations (6 hours) with *anti*-BP-diol epoxide resulted in breakage of DNA that was not subject to subsequent alkaline hydrolysis (Fig. 2, lanes 7 and 8). Thus, the phosphodiester bonds were broken during the prolonged reaction. Again, the most frequent site of strand scission was at guanine positions. A similar pattern of DNA breakage was also observed in experiments in which DNA modified in 30-minute reactions with *anti*-BP-diol epoxide at 10.0 μg/ml was separated from the reactants by passage over a Sephadex G-100 column and then incubated for 6 hours more. These experiments demonstrated that modified DNA is labile to strand breakage at neutral *p*H in the absence of the aqueous hydrolysis products of the *anti*-BP-diol epoxide.

From these experiments we conclude that DNA modified by the *anti*-BP-diol epoxide is labile to strand scission both at alkaline and neutral *p*H. The question arises as to what are the modifications that result in the observed cleavage pattern. The principal DNA adduct of the *anti*-BP-diol epoxide is to the exocylic N^2-amino group of guanine (*3*, *4*, *8*, *9*). Formation of an adduct to the N-6 position of adenine has also been reported. Binding of benzo[*a*]pyrene to DNA at guanine and adenine probably accounts for the observed decrease in the electrophoretic mobility of the modified DNA, but it is not evident that such adducts would increase the lability of the phosphodiester bonds adjacent to the modified base. Osborne *et al.* (*10*) and King *et al.* (*11*) suggest that the *anti*-BP-diol epoxide forms an adduct at the N-7 position of guanine and that the lability of the corresponding glycosylic bond is greatly increased even at neutral *p*H. Such a lesion could result in the cleavage at the observed guanine positions. Adducts of the *anti*-BP-diol epoxide to adenine and cytosine residues of DNA have also been reported (*4*, *8*, *9*), but the site of modification is not known. We suggest that adducts at the N-3 position of cytosine and adenine could result in the cleavage pattern observed here. Gamper *et al.* (*7*) suggest that the *anti*-BP-diol epoxide may form phosphotriesters with

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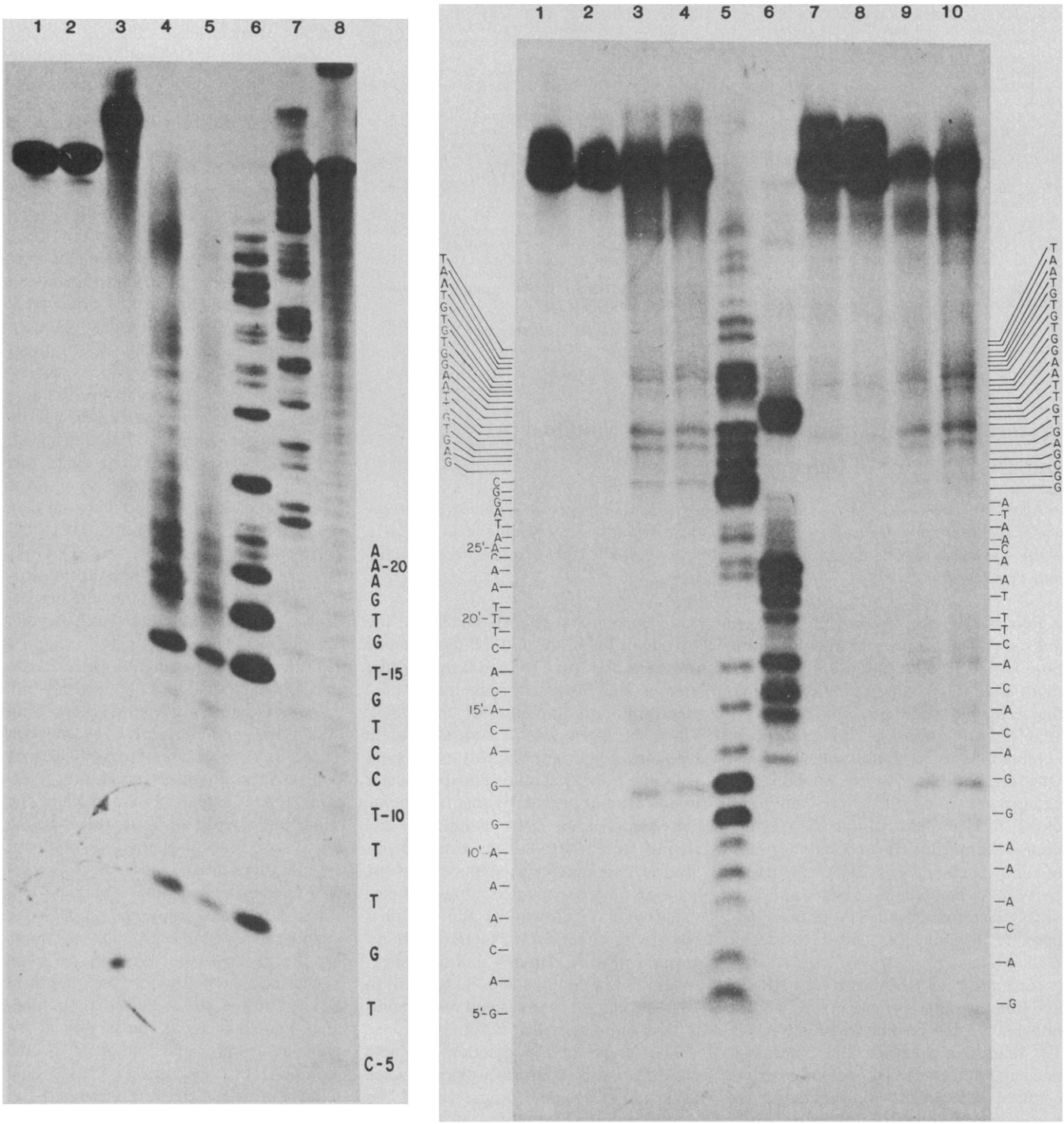

Fig. 1 (left). Site-specific cleavage of *anti*-BP-diol epoxide modified DNA. The DNA substrate was a double-stranded DNA fragment 168 nucleotides long of the *lac* p-o gene labeled at the 5′ terminus with ^{32}P (*12*, *13*). Reactions contained 25 ng of the labeled DNA and 20 μg of unlabeled salmon sperm DNA. The reaction buffer contained 20 m*M* tris-HCl (*p*H 8.0), 0.01 m*M* disodium EDTA, and 5 percent dimethyl sulfoxide. DNA products were analyzed on 20 percent polyacrylamide gels (*14*). (Lane 1) Unmodified DNA before or (lane 2) after treatment with 0.1 NaOH at 90°C, for 30 minutes; (lane 3) DNA treated with *anti*-BP-diol epoxide (90 μg/ml) for 30 minutes at 37°C and layered on the gel without prolonged treatment at alkaline *p*H; (lane 4) after treatment at 90°C for 15 minutes in 10 m*M* sodium phosphate (*p*H 7.0), 0.1 m*M* disodium EDTA followed by treatment with 0.1 NaOH at 90°C for 30 minutes; (lane 5) after treatment with 1*M* piperidine for 30 minutes at 90°C. (Lane 6) Unmodified DNA was treated with dimethyl sulfate in the G>A reaction of Maxam and Gilbert (*14*), neocarzinostatin [breaks at adenine and thymine (*12*)], and 1 m*M* $FeSO_4$ [breaks at all bases (*12*)]. The numbers to the right indicate the position of the base from the 5′ terminus of the DNA strand. Fig. 2 (right). (Lane 1) A sample of 3′-end-labeled DNA (*15*) was treated with *anti*-BP-diol epoxide [10 μg/ml or (lane 2) 50 μg/ml] for 30 minutes at 37°C and layered on the gel without further treatment. DNA treated with (lane 3) 10 μg/ml or (lane 4) 50 μg/ml was subsequently heated at 90°C for 15 minutes at neutral *p*H followed by alkaline hydrolysis as described for DNA of Fig. 1, lane 5. Unmodified DNA was treated with dimethyl sulfate in the G>A reaction of Maxam and Gilbert (*14*) (lane 5) or neocarzinostatin (*12*) (lane 6). DNA was treated with 10 μg/ml (lane 7) and 50 μg/ml (lane 8) *anti*-BP-diol epoxide for 6 hours and layered on the gel. DNA was treated with 10 μg/ml (lane 9) and 50 μg/ml (lane 10) *anti*-BP-diol epoxide for 6 hours followed by alkaline hydrolysis before being layered on the gel.

DNA and propose that strand scission of modified DNA occurs by an attack of the C-9 hydroxyl group of the adduct on the triester group. The sequence preference of the observed cleavage can imply that phosphotriesters do not account for the majority of the scission events, as the rate of phosphotriester formation should not depend on nucleotide sequence.

Although a number of alterations created by benzo[*a*]pyrene in DNA have been characterized, it is not evident which if any are responsible for the cytotoxic, mutagenic, and carcinogenic effects of the compound. These effects could be due to strand scission events that occur at site of DNA modification. Although the base modifications that lead to strand scission may be a small fraction of the total number of alterations, they may have a major biological effect. The site specificity of strand breakage suggests that the susceptibility of different genes may differ with respect to their sensitivity to the effects of benzo[*a*]pyrene. The chemical nature of the alterations that lead to strand breakage and the differential effect of benzo[*a*]-pyrene on different genetic loci remain to be determined.

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References and Notes

1. National Academy of Sciences, "Particulate polycylic organic matter" (National Academy of Sciences, Washington, D.C., 1979).
2. C. Heidelberger, *Annu. Rev. Biochem.* **44**, 79 (1975).
3. I. B. Weinstein *et al.*, *Science* **193**, 592 (1976).
4. K. M. Straub, T. Meehan, A. L. Burlingame, M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5285 (1977).
5. E. Huberman, L. Sachs, S. K. Tang, H. V. Gelboin, *ibid.* **73**, 2594 (1976).
6. S. K. Yang, D. W. McCourt, P. P. Rollec, H. V. Gelboin, *ibid.*, p. 2594.
7. H. B. Gamper, A. S.-C. Tung, K. Straub, J. C. Bartholomew, M. Calvin, *Science* **197**, 671 (1977).
8. T. Meehan, K. Straub, M. Calvin, *Nature (London)* **269**, 725 (1977).
9. A. M. Jeffrey, K. Grzesbowiak, I. B. Weinstein, K. Nakanishi, P. Roller, R. G. Harvey, *Science* **206**, 1309 (1979).
10. M. R. Osborne, R. G. Harvey, P. Brooks, *Chem. Biol. Interact.* **20**, 123 (1978).
11. H. W. S. King, M. R. Osborne, P. Brooks, *ibid.* **24**, 345 (1979).
12. A. D'Andrea and W. A. Haseltine, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3608 (1978).
13. W. A. Haseltine, C. P. Lindan, A. D. D'Andrea, *Methods Enzymol.* **65**, 235 (1980).
14. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
15. W. A. Haseltine, L. K. Gordon, C. P. Lindan, *Nature (London)* **285**, 364 (1980).
16. We thank Eric Eisenstadt for helpful discussions. This work was supported by a grant from the National Cancer Institute (CA 26716) and an American Cancer Society faculty research award (W.A.H.).

28 March 1979; revised 23 May 1980

Ozone Selectively Inhibits Growth of Human Cancer Cells

Abstract. *The growth of human cancer cells from lung, breast, and uterine tumors was selectively inhibited in a dose-dependent manner by ozone at 0.3 to 0.8 part per million of ozone in ambient air during 8 days of culture. Human lung diploid fibroblasts served as noncancerous control cells. The presence of ozone at 0.3 to 0.5 part per million inhibited cancer cell growth 40 and 60 percent, respectively. The noncancerous lung cells were unaffected at these levels. Exposure to ozone at 0.8 part per million inhibited cancer cell growth more than 90 percent and control cell growth less than 50 percent. Evidently, the mechanisms for defense against ozone damage are impaired in human cancer cells.*

The effects of ozone on human health have been a focus of public concern and scientific investigation for more than two decades (*1-4*). Considerable attention has been devoted to assessing its cellular effects (*5*) because it is the major constituent of the ground-level oxidants in polluted air. Much has been learned about the effects of ozone on normal tissues, but little is known about its action on cancer cells. We have conducted experiments in which continuous exposure to ozone at 0.3 ppm (*6*) selectively inhibited the growth of human cancer cells 40 percent in 8 days.

Controlled levels of ozone (0.3 to 0.8 ppm) were continuously generated by ultraviolet irradiation of filtered ambient air. The ozonated air, containing 5 percent carbon dioxide, was introduced at a constant flow rate of 4.0 liter/min into an environmental chamber in an incubator maintained at 37°C (Fig. 1). The ozone levels were assayed daily with a spectrophotometric ozone analyzer. For comparison, noncancerous human lung diploid fibroblasts (*7*) were cultured in the chamber along with the cancer cells. The cancer cells were from alveolar (lung) adenocarcinomas (*8*), breast adenocarcinomas (*9*), uterine carcinosarcomas, and endometrial carcinomas (*10*). All the cells were grown in 60-mm petri dishes in 10 ml of medium and were placed in the chamber at the same time. Control cells were incubated in an adjoining compartment receiving filtered ambient air containing 5 percent carbon dioxide (4.0 liter/min). Three petri dishes for each cell type were removed from each of the two compartments every 48 hours for 8 days, and the number of cells per plate were counted. All of the cancer cells showed marked dose-dependent growth inhibition in ozone at 0.3 and 0.5 ppm (Fig. 2). There was no growth inhibition of the noncancerous lung cells at these ozone levels, and they were morphologically identical to the corresponding control cells. At 0.8 ppm, the growth of the noncancerous cells was inhibited 50 percent, but all four types of cancer cells were inhibited more than 90 percent.

After being cultured through 14 passages, the noncancerous cells exhibited measurable growth inhibition and morphological changes (vacuolation) in ozone at 0.5 ppm, suggesting that aging increases the sensitivity of normal lung cells to ozone (Fig. 3). In cultured human diploid fibroblasts, morphological changes and a gradual decrease in rate of growth have been attributed to a buildup of cellular damage with each successive division (*11, 12*). Ozone may accelerate processes similar to those naturally

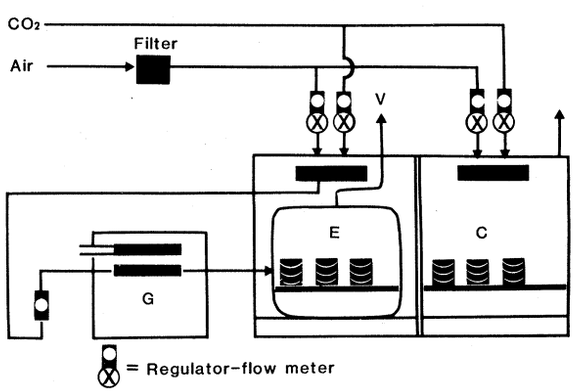

Fig. 1. Schematic diagram (not to scale) of the system used for culturing human cells in ozonated ambient air. Filtered ambient air was mixed with carbon dioxide (5 percent) and introduced into a dual chamber incubator (National 3331). Half was conducted through a calibrated ozone generator (*G*) consisting of a quartz glass tube irradiated with ultraviolet light and then into a hermetically sealed (20 by 20 by 20 cm) glass and stainless steel environmental chamber (*E*) containing a gasketed access door. Output of ozone from the generator varied less than 1 percent per day. The ozone content of the vented air (*V*) from the chamber was measured daily with a spectrophotometric ozone analyzer (Dasibi 1003-AH). Malignant and normal human cells were incubated in chamber *E* saturated with water vapor. Corresponding cells serving as controls were incubated in the adjoining compartment (*C*), also saturated with water vapor.

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