

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.

WILLIAM A. HASELTINE
KWOK MING LO
ALAN D. D'ANDREA

Sidney Farber Cancer Institute,
Harvard Medical School,
Harvard School of Public Health,
Boston, Massachusetts 02115

References and Notes

1. National Academy of Sciences, "Particulate polycyclic 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. Grzesbowski, 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.).

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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.

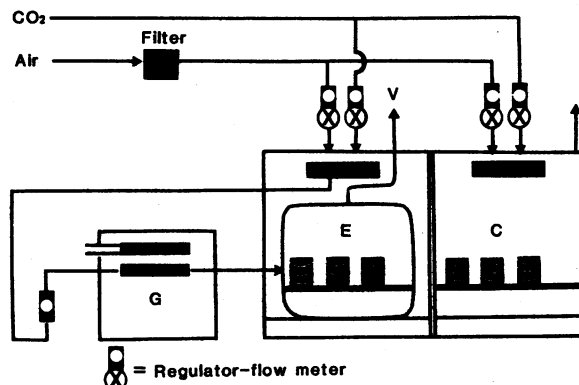
The effects of ozone on human health have been a focus of public concern and scientific investigation for more than two decades (1-4). Considerable attention has been devoted to assessing its cellular effects (5) because it is the major constituent of the ground-level oxidants in polluted air. Much has 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.



causing cellular damage and may decrease the growth rate of the aging fibroblast colony. However, in ozone at 0.5 ppm, all of the human cancer cells (which do not age) had growth rates several times lower than that of the aged, noncancerous cells (Fig. 2).

Evidently, cancer cells are less able to compensate for the oxidative burden of ozone than normal cells. The marked sensitivity of cancer cells to ozone raises questions about the possible mechanisms of oxidative inhibition of their growth. Virtually every major com-

ponent of normal cells has been found to be affected by elevated ozone levels (5). However, glutathione in its reduced form (GSH) has been credited with providing the first line of defense against the peroxides and free radicals generated in all cells by ozone and oxygen (1, 13-15). It deactivates peroxides and radicals by donating one hydrogen atom to the reactive species. Loss of a GSH hydrogen (oxidation) results in formation of oxidized glutathione (GS-SG). The cellular respiratory system is responsible for reducing GS-SG to GSH. The GSH-linked respiratory system in normal and cancer cells, before and after exposure to ozone, must be examined to learn whether a functional impairment of this system is associated with the marked sensitivity of cancer cells to the oxidant.

These findings lead us to believe that ozone—alone, in combination with radiation therapy (16), or in chemotherapy utilizing electrophilic compounds (17)—may have therapeutic value for patients with certain forms of lung cancer.

FREDERICK SWEET
MING-SHAN KAO
SONG-CHIAU D. LEE

Department of Obstetrics and
Gynecology, Washington University
School of Medicine,
St. Louis, Missouri 63110

WILL L. HAGAR
City of St. Louis Air Pollution
Control, St. Louis 63103

WILEEN E. SWEET
Air Quality Section, East-West
Gateway Coordinating Council,
St. Louis 63102

References and Notes

1. D. L. Dunsworth, C. E. Cross, J. R. Gillespie, C. G. Plopper, in *Ozone Chemistry and Technology*, J. S. Murphy and J. R. Orr, Eds. (Franklin Institute, Philadelphia, 1975), chap. 2.
2. H. E. Stokinger and D. Coffin, in *Air Pollution*, A. C. Stern, Ed. (Academic Press, New York, 1968), vol. 1, pp. 446-546.
3. H. D. Kerr et al., *Am. Rev. Respir. Dis.* 111, 763 (1975).
4. J. D. Hackney, W. S. Linn, C. D. Law, S. K. Karuza, Greenberg, R. D. Buckley, E. E. Pedersen, *Arch. Environ. Health* 30, 385 (1975).
5. B. D. Goldstein, *Rev. Environ. Health* 2, 177 (1977).
6. Normal human subjects tolerated breathing 0.5 ppm ozone in air 2 hours per day for 1 week or 0.25 ppm ozone 2 hours per day for 3 weeks (4). The two groups engaged in light exercise during exposure. Although both groups developed chest discomfort and moderately decreased respiratory function during exposure, their removal from the oxidative environment resulted in rapid disappearance of the symptoms. The mean dose-response curves from this study show a no-detectable-effect level at 0.25 to 0.30 ppm. A similar study (3) found that human subjects tolerated exposure to 0.5 ppm ozone for up to 6 hours. Pulmonary function was affected and chest discomfort developed at this level, with no significant differences observed between smokers and nonsmokers.
7. These cells (IMR-90) were obtained from the Human Aging Cell Repository and plated 48 hours after shipping. This cell type was characterized by W. W. Nichols, D. G. Murphy, V. J. Cristofalo, L. H. Toji, A. E. Greene, and S. A. Dwight [*Science* 196, 60 (1977)].

Fig. 2. Inhibition by ozone of growth of malignant and non-malignant cells in culture on day 8. Each of the cell types were grown in 10 ml of Dulbecco's modified Eagle's minimum essential medium containing 10 percent calf serum. In a typical experiment, 12 dishes per cell line (usually three or four cell lines were tested per experiment) were loaded into the environmental chamber with an equal number of control dishes in the adjoining compartment (Fig. 1). The initial population was 3×10^5 cells per dish. Every 48 hours three dishes for each cell type were removed from both compartments and the cells were tested for viability with 0.4 percent trypan blue and counted with a hemocytometer. Each data point represents the number of experimental cells divided by the number of corresponding control cells per dish multiplied by 100 (the percentage of control growth) and is plotted against the measured level of ozone in the environmental chamber. The percentage of growth inhibition is calculated by subtracting the percentage of growth from 100. The data are from cell counting on day 8 of incubation. There is a nearly linear relation between inhibition of the growth of the cancer cells and increasing ozone levels. The noncancerous cell line IMR-90 (Δ) began to display measurable growth inhibition only when ozone levels exceeded 0.5 ppm, a level that produced approximately 60 percent inhibition in all of the cancer cell lines tested [Δ , alveolar adenocarcinoma (A-549); \circ , breast adenocarcinoma (MCF-7); \square , uterine carcinosarcoma (10); \otimes , endometrial adenocarcinoma (10)]. There was some growth inhibition in noncancerous cells aged through 14 passages (\bullet). The mean populations of the cells serving as controls were as follows (per dish on day 8): IMR-90, 34.8×10^5 ; A-549, 36.5×10^5 ; MCF-7, 57.0×10^5 ; endometrial adenocarcinoma, 64.2×10^5 ; myometrial carcinosarcoma, 121.1×10^5 .

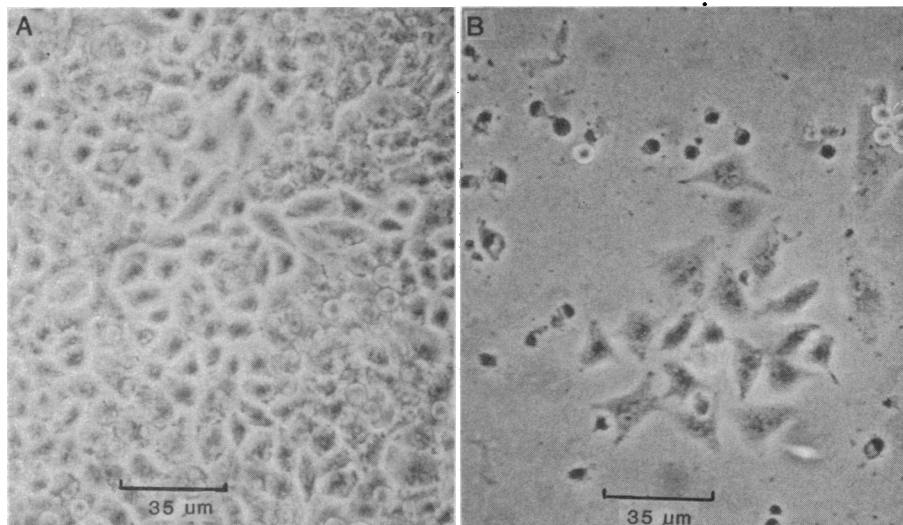
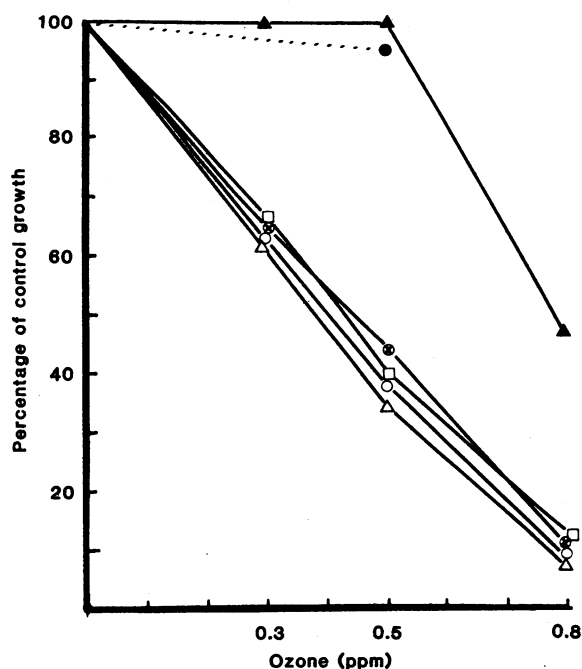


Fig. 3. Photomicrographs ($\times 100$) showing growth inhibition and morphological changes in lung alveolar adenocarcinoma cells after 8 days of incubation in ozone at 0.5 ppm. (A) Control A-549 cells. (B) Ozone-treated A-549 cells showing vacuole formation, a typical morphological change associated with growth inhibition.

8. This cell line (A-549) was described by D. J. Giard, S. A. Aaronson, G. J. Todard, P. Arnstein, J. H. Kersey, H. Dorsik, and W. P. Parks [*J. Natl. Cancer Inst.* 51, 1417 (1973)]; M. Lieber, B. Smith, A. Szakal, W. Nelson-Rees, and G. A. Todaro [*Int. J. Cancer* 17, 62 (1976)]; and K. L. Jones, N. S. Anderson III, and J. Addison [*Cancer Res.* 38, 1688 (1978)].
9. This cell line (MCF-7, estrogen-sensitive) was described by H. D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennan [*J. Natl. Cancer Inst.* 51, 1409 (1973)] and by K. B. Horwitz, M. E. Kostlow, and W. I. McGuire [*Steroids* 26, 785 (1975)].
10. Human uterine carcinosarcoma cells and endometrial adenocarcinoma cells were obtained from pathologically confirmed gynecologic tumors and developed as new cell lines. The endometrial adenocarcinoma cell line is estrogen-sensitive. Both were described by M. S. Kao and S. C. D. Lee (27th Annual Meeting of the Society for Gynecologic Investigation, Denver, 20 to 23 March 1980), abstr. 7.
11. J. R. Smith and R. G. Whitney, *Science* 207, 82 (1980); S. C. D. Lee, P. M. Bemiller, J. N. Bemiller, A. J. Pappelis, *Mech. Ageing Dev.* 7, 417 (1978).
12. P. M. Bemiller and L. H. Lee, *ibid.* 8, 417 (1978).
13. C. K. Chow and A. L. Tappel, *Lipids* 1, 518 (1972).
14. C. K. Chow, *Nature (London)* 260, 721 (1976).
15. R. E. Kimball et al., *Am. J. Physiol.* 230, 1425 (1976).
16. R. E. Lee, *Semin. Oncol.* 1, 254 (1974).
17. O. S. Selawry, *ibid.*, p. 259.
18. Parts of this report were presented at the 27th Annual Meeting of the Society for Gynecologic Investigation, Denver, 20 to 23 March 1980 (abstracts 7 and 150), and at the 73rd Annual Meeting of the Air Pollution Control Association, Montreal, 24 June 1980 (poster session 27). We thank W. Nelson-Rees for his gift of A-549 cells; the MCF-7 cells were obtained from E. M. Jensen. We also thank C. M. Copley, Jr., H. M. Camel, and T. Morgan for their constructive criticism of the manuscript. Correspondence should be addressed to F.S.

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Two Coronaviruses Isolated from Central Nervous System Tissue of Two Multiple Sclerosis Patients

Abstract. Two coronaviruses were isolated from brain material obtained at autopsy from two multiple sclerosis patients. The viruses were neutralized by serum and spinal fluid from these patients. Although most of the population have antibody to these virus isolates, multiple sclerosis patients have slightly higher concentrations of serum antibody than controls. The results suggest that coronaviruses should be considered as one additional virus with a potential implication in the etiology of multiple sclerosis.

Multiple sclerosis (MS) is a disease characterized by a variety of neurologic signs and symptoms resulting from damage to myelin in the central nervous system. The suspicion that MS has a viral etiology has intensified as a result of epidemiologic observations that MS occurs more frequently in specific geographic locations and that higher risk is related to exposure to an environmental agent before age 15 (1). Furthermore, the spinal fluids of MS patients contain antibody to viruses which is not present in the spinal fluids of control patients (2).

Virus isolations from MS tissue have been reported (3), but none has been confirmed and no single infectious agent has been consistently associated with the disease. When suckling mice or cell cultures particularly sensitive to coronaviruses were inoculated with fresh brain specimens obtained at autopsy from two MS patients, we were able to isolate two coronaviruses. Viruses of this family are known to cause upper respiratory infections in humans (4), but they had not previously been isolated from human brain material. Coronaviruses represent an attractive candidate for a role in the etiology of MS since viruses of this group cause a demyelinating-remyelinating disease in mice (5). Furthermore, using electron microscopy, Tanaka *et al.* have identified coronavirus-like particles in the brain of one MS patient (6).

Our first virus (S.D. virus) was isolated from a 55-year-old MS patient with brainstem dysfunction. An autopsy performed within 4 hours after death revealed typical MS plaques in the cerebrum, cerebellum, brainstem, and spinal cord. Histologic sections of medulla and pons showed areas of demyelination surrounded by reactive astrocytes indicating active disease. A 10 percent homogenate of fresh, unfrozen brainstem material in saline solution was inoculated intracerebrally into weanling BALB/c mice. Seven of the ten mice died between 2 and 6 months after inoculation. Seizures, myoclonic jerks, and limb pa-

ralysis were observed. When fresh brainstem material from a mouse that died 99 days after inoculation was inoculated intracerebrally into weanling mice, they developed neurological signs in approximately 50 days. Brainstem material obtained from these mice was inoculated intracerebrally into newborn BALB/c mice, and they died in 12 days. In subsequent serial passages the time between inoculation and death decreased to 3 to 5 days. We discovered during early passage attempts that the freezing of brain material from weanling and newborn mice resulted in the loss of transmissibility. Reinoculation of suckling mice with the original brainstem homogenate, which had been prepared after autopsy and frozen, failed to produce illness.

After failing to produce viral-induced changes in 16 different cell culture systems with homogenates prepared from brain material of infected suckling mice, we observed formation of syncytial tissue (giant cells) in a spontaneously transformed 3T3 Balb/c mouse cell line (17C1-1). Electron microscopy revealed coronavirus-like particles in the cell culture system and in the livers and brains of infected suckling mice. Negative-stain electron microscopy of virus released into supernatants in the infected cell culture revealed typical coronavirus particles (Fig. 1).

The second virus (S.K. virus) was isolated from an 89-year-old woman with weakness, spasticity, cerebellar dysfunction, and extraocular movement dysfunction. The disease was slowly progressive over a 30-year period excluding an initial period of 20 years of episodes of exacerbations and remissions. An autopsy performed within 4 hours after death revealed demyelinated areas in paraventricular white matter, brainstem, cerebellum, and spinal cord. A mild glial reaction was noted around

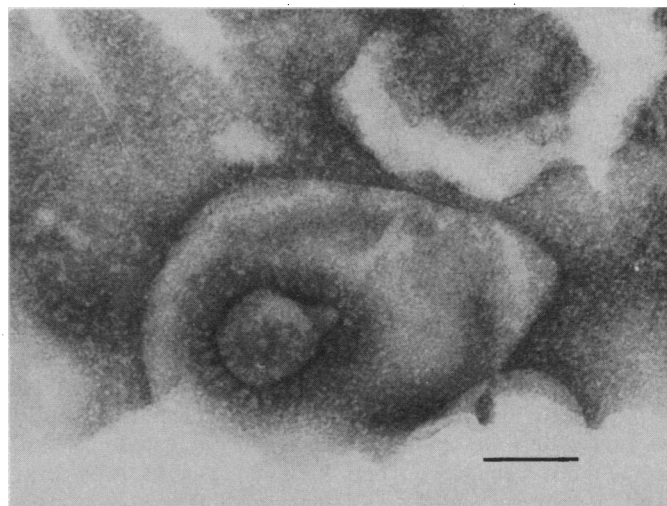


Fig. 1. Electron micrograph of virus isolate S.D. negatively stained with phosphotungstic acid. Average diameter of the virus particle is 100 nm with 20-nm corona-like peplomers. Scale bar, 100 nm.