in the literature and have been attributed to the natural variation of background tropospheric aerosols (*2*); vertical transport and advection of natural and anthropogenic aerosols with greater instability and convection during summer months, particularly over continents (*15*); and annual migration of trajectories of stratospheric aerosol bands (*16*). We note that there is no change in the phase of the annual variation at Mauna Loa before and after a volcanic eruption.

A biennial periodicity in transmission is evident in the record for 1958 to 1962, when no explosive volcanic activity was reported (Fig. 2). Over this 5-year period, this oscillation is in phase with the stratospheric wind direction (*17*), and a westerly flow in the stratosphere over Mauna Loa is coincident with a larger decrease in transmission in summer. After Agung, the biennial variation in transmission is either nonexistent or masked by the larger episodic variations that followed.

Transmission recovery rates after the initial injections of volcanic effluent into the stratosphere are 0.61 percent per year after the Agung period, 0.33 percent per year after the Awu period, and 0.40 percent per year after the De Fuego period. The initial recovery rate after an episode seems to be more linear than exponential, whereas an exponential rate might be expected if only gravitational settling were acting on an originally Junge aerosol distribution (*18*). If additional aerosols are created sometime later from the gaseous effluent that accompanies the initial influx of aerosols into the stratosphere, they will undoubtedly affect the recovery times, as will the dynamics of natural cleansing processes between the troposphere and the stratosphere (*19, 20*). Complete recovery of atmospheric transmissions after volcanic eruptions, in times ranging from months to a few years, have been reported by others (*7, 21*).

Comparison of the transmission at the beginning (1958) and at the end (1977) of the Mauna Loa record shows no obvious long-term trend. Recovery from all volcanic eruptions in the 20-year period appears to be complete. However, a long-term linear decrease of 0.2 percent can be inferred from 1958 to 1976, excluding the transient decreases in the interim. Although such a small decrease in transmission is within the noise of the data, we attempted to account for it by computing the decrease in transmission that would be caused by a gradual increase in total atmospheric ozone from 270 to 280 m-atm-cm over Mauna Loa (*15, 22*). The calculated decrease amounted to only 0.03 percent and would not be enough to account for the total decrease, if any.

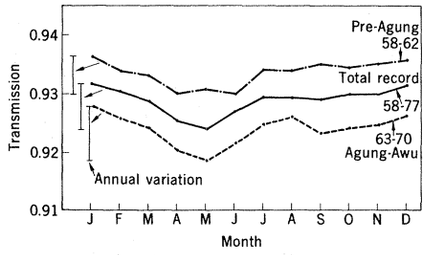

Fig. 3. Annual cycles in transmission for three periods at Mauna Loa. All the annual cycles are in phase. The magnitude of the annual cycle is largest for the period 1963 to 1970 and smallest for the pre-Agung period.

Annual, biennial, and transient variations in transmission have been observed in the continuous Mauna Loa record since 1958. The transient changes appear to be linked to episodic stratospheric aerosol increases due to injections of volcanic effluent into the stratosphere by sufficiently strong eruptions. The effect is greatest with eruptions originating near the equator, where the vertical motion and the subtropical jet and strong zonal stratospheric winds are conducive to rapid transport of aerosols over the equatorial belt. Volcanic injections that occurred north or south of 50° latitude can be detected at Mauna Loa, but are seen as gradual rather than sharp decreases in the transmission and as increases in the magnitude of the annual cycle.

A biennial cycle in the first 5 years of the record and an annual cycle in the entire record strongly suggest natural variations in atmospheric transmission, independent of the transient variations due to volcanic episodes. The cause or causes of these cycles observed at a remote site such as Mauna Loa Observatory are still not fully understood.

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References and Notes

1. This is part of research being conducted by the Geophysical Monitoring for Climatic Change program of the NOAA Air Resources Laboratories.
2. H. T. Ellis and R. F. Pueschel, *Science* **172**, 845 (1971).
3. A. J. Dyer and B. B. Hicks, *Nature (London)* **208**, 131 (1965).
4. H. J. Viebrock and E. C. Flowers, *Tellus* **20**, 400 (1968).
5. W. M. Irvine and F. W. Peterson, *J. Atmos. Sci.* **27**, 62 (1970).
6. F. E. Volz, *J. Geophys. Res.* **75**, 5185 (1970).
7. A. J. Dyer, *Q. J. R. Meteorol. Soc.* **100**, 563 (1974).
8. M. I. Budyko and Z. I. Pivovarova, *Meteorol. Hydrol.* **10**, 3 (1967).
9. R. Yamamoto, T. Iwashima, M. Hoshiai, *J. Meteorol. Soc. Jpn.* **53**, 482 (1975).
10. R. C. Oliver, *J. Appl. Meteorol.* **15**, 933 (1976).
11. R. E. Newell and B. C. Weare, *Science* **194**, 1413 (1976).
12. J. A. Dobrowolski, G. E. Marsh, D. G. Charbonneau, J. Eng, P. D. Josephy, *Appl. Opt.* **16**, 1491 (1977).
13. J. A. Watkins, Ed., *Geophysical Monitoring for Climatic Change* No. 4, *Summary Report 1975* (Environmental Research Laboratories, National Oceanic and Atmospheric Administration, Boulder, Colo., 1976), pp. 58–62.
14. *Report of the Study of Man's Impact on Climate* (MIT Press, Cambridge, Mass., 1971), pp. 196–201.
15. E. C. Flowers, R. A. McCormick, K. R. Kurfis, *J. Appl. Meteorol.* **8**, 955 (1969).
16. H. W. Ellsaesser, *Science* **176**, 814 (1972).
17. K. R. Ramanathan, *Q. J. R. Meteorol. Soc.* **89**, 540 (1963).
18. C. E. Junge, *Air Chemistry and Radioactivity* (Academic Press, New York, 1963).
19. R. D. Cadle, *Trans. Am. Geophys. Union* **53**, 812 (1972).
20. ——, P. Crutzen, D. Ehholt, *J. Geophys. Res.* **80**, 3381 (1975).
21. H. H. Kimball, *Mon. Weather Rev.* **46**, 355 (1918).
22. J. Angell and J. Korshover, *ibid.* **104**, 63 (1977).

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Atmospheric Reactions of Polycyclic Aromatic Hydrocarbons: Facile Formation of Mutagenic Nitro Derivatives

Abstract. *Directly active mutagens are formed on exposure of the promutagen benzo*[a]*pyrene to gaseous pollutants in smog. In simulated atmospheres containing 1 part per million nitrogen dioxide and traces of nitric acid, directly mutagenic nitro derivatives are readily formed from both benzo*[a]*pyrene and perylene, a nonmutagen in the Ames reversion assay. Possible formation of direct mutagens by such reactions on sample collection filters, in exhaust effluents, and in the atmosphere should be recognized.*

The carcinogenicity of atmospheric organic particulate matter in experimental animals has been known for more than 30 years (*1, 2*). It has generally been attributed to the presence in polluted air of benzo[*a*]pyrene (BP) and other polycyclic aromatic hydrocarbons (PAH) as well as their azaheterocyclic analogs (*3, 4*). Several investigators, however, have noted that the carcinogenicity of both urban air and exhaust particulates from spark-ignition engines could not be ac-

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counted for solely on the basis of their measured content of BP and other carcinogenic PAH (*2, 5, 6*). In fact, there was a significant "excess carcinogenicity," presumably due to unidentified compounds present.

The mutagenic activity of organic particulates that we collected at a number of sites throughout the Los Angeles air basin (*7–9*), as studied by the Ames microbiological reversion assay (*10*), may be related to these observations. All urban and suburban samples tested to date induce frameshift mutations and are directly active—that is, are mutagenic without requiring metabolic activation. This clearly indicates the presence in the total samples of frameshift mutagens other than BP and other promutagens that require metabolic activation in the Ames test (*10*).

Furthermore, after chemical separation of two recently collected particulate samples from this air basin, we found that the acidic, neutral, and basic fractions each showed significant specific mutagenicity (that is, revertants per gram of sample). However, since the basic fraction of the organic extract usually accounts for only ~1 to 2 percent of the total weight of organics, the mutagenicity of the total sample is mainly due to acidic and neutral compounds. In addition, more than half of the mutagenicity of the acidic and neutral fractions was of the direct type.

We recently proposed (*9, 11–13*) that the presence of these direct mutagens in ambient particulates may be due in part to the reactions of BP and other PAH with O_3, NO_2, peroxyacetyl nitrate (PAN), and free radicals present in polluted atmospheres (*14–18*). If some of the products formed in these reactions are analogous to the metabolites of BP and PAH in mammalian cells (*19*), reactions of PAH in exhaust effluents and in the atmosphere could account for the presence of directly active mutagens in the urban air.

There are several reports that BP and other PAH undergo photochemical transformations when adsorbed on a variety of support materials such as filters, silica gel, and carbon (soot) particles (*2, 3, 20*). However, statements to the effect that PAH are chemically inert also appear in the literature (*21*), including a recent comprehensive review on atmospheric mutagens (*22*).

In order to test our hypothesis that oxidative reactions of PAH in the atmosphere may lead to the formation of directly active mutagens, we conducted one set of experiments in which the carcinogen, BP, and perylene, reportedly a noncarcinogen (*3, 4*), were each exposed to pollutant gases under simulated atmospheric conditions and one set in which BP was exposed to the gases in ambient photochemical smog.

We first performed experiments in which BP, deposited on washed and fired glass fiber filters of the type used in ambient sampling, was exposed in the dark to particle-free, pure air containing (i) O_3 [11 parts per million (ppm); exposure time, 24 hours; flow rate, 3 cubic feet (0.084 m^3) per minute (ft^3/min)], (ii) NO_2 (1.3 ppm, 24 hours, 1 ft^3/min), and (iii) PAN (1.1 ppm, 16 hours, 3 ft^3/min). Control runs with BP exposed to pure air (24 hours at 3 ft^3/min) and with blank fil-

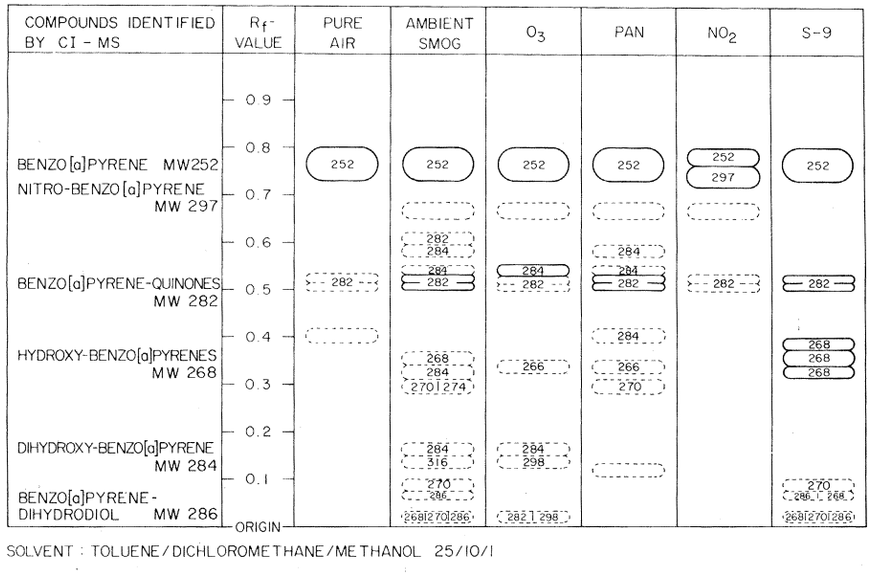

Fig. 1. Thin-layer chromatograms of products formed on exposure of BP (6 to 10 mg, deposited on two 8 by 10 inch Gelman AE glass fiber filters) to pure air, ambient smog, O_3, PAN, and NO_2 and on treatment of BP with liver S-9 mix. Each TLC band (separation on Merck silica gel plates, No. 5763) was recovered in methanol and analyzed by methane chemical ionization–mass spectrometry (*CI-MS*, direct introduction probe). Each band (solid line, major; dashed line, minor) shows the molecular weight (*MW*) of each product found in it. Also shown are the products identified by CI-MS in each band and the TLC R_F values. See text for exposure levels, experimental conditions, and results of mutagenicity assay.

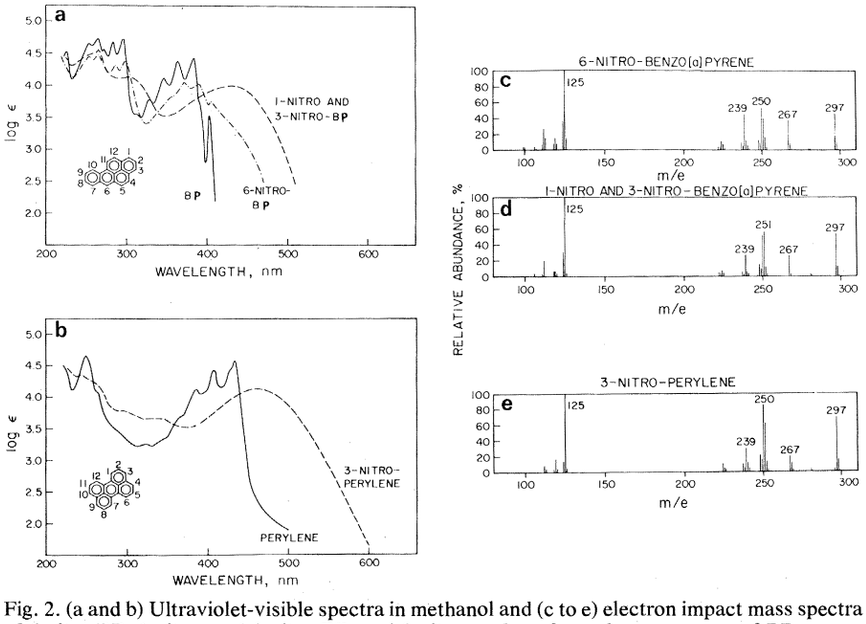

Fig. 2. (a and b) Ultraviolet-visible spectra in methanol and (c to e) electron impact mass spectra of 6-nitro-BP, 1-nitro- and 3-nitro-BP, and 3-nitroperylene formed on exposure of BP or perylene to NO_2. Also shown (a and b) are the formulas and ultraviolet-visible spectra of the parent PAH.

ters exposed to NO_2, O_3, or PAN were also included (*23*). Experiments were also run at 0.25 ppm of NO_2 and 0.1 ppm of O_3 in air with similar results.

After exposure, the products and unreacted BP were separated by thin-layer chromatography (TLC). The major TLC bands were then analyzed by mass spectrometry and were tested for mutagenic activity with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without addition of a mammalian metabolic activation system [0.5 ml of rat liver S9 mix (*24*)]. For comparison, a sample of 9 mg of BP incubated for 30 minutes at 37°C with liver S9 mix was analyzed and tested in the same way.

Our tester strains are routinely monitored for the presence of the plasmid (TA98 and TA100) by ampicillin resistance, for the deep rough mutation by the crystal violet test (all strains), and for the *uvr*B deletion by sensitivity to ultraviolet radiation (all strains). In each experiment, all strains are plated for the spontaneous mutation frequency (this value is subtracted from the test frequencies), and each strain is plated with various known mutagens (ICR-191, hycanthone, sodium azide, and so on) as quantitative controls and to ensure that the strains are responding properly. For example, TA98 gives 110 to 120 revertants per nanomole of BP added. As a check on phenocopies, which might yield false positive results, five colonies are picked from selected test plates that have been scored as positive and are checked on histidine-free medium to ensure that they are true revertants.

To prepare S9 mix we follow the Ames procedure of giving rats a single injection of Aroclor 1254 (500 mg/kg), allow them free access to water and feed for 5 days, starve them for 12 hours, and kill them. Our S9 preparations activate BP, so that our reversion frequencies per nanomole are in good agreement with those published by Ames *et al.* (*10*).

As shown from the TLC bands in Fig. 1, BP reacted with O_3 and PAN to form a variety of oxygenated products and with NO_2 to form nitro derivatives. As expected, the TLC bands containing the unreacted BP (R_F = 0.77) were not directly active and required metabolic activation. The bands containing the BP-quinones (R_F = 0.54) were complex and contained, in addition to the inactive quinones (*25*), a directly active compound of molecular weight 284, as yet unidentified.

Treatment of BP with liver S9 mix resulted in the appearance of a complex TLC band such as that seen with ambient smog but not with the single gases (Fig. 1). This contained directly active mutagens. Its R_F (0.35) and the molecular weight of its components (268) are consistent with those of isomers of hydroxybenzo[*a*]pyrene.

Exposure of BP to NO_2 (1 ppm) containing traces of nitric acid [~10 parts per billion (ppb)] resulted in the appearance of only one major TLC band. This contained a directly active mutagen whose R_F (0.74) and molecular weight (297) were consistent with the nitrobenzo[*a*]pyrene (nitro-BP) structure.

By changing the TLC solvent system described in the caption of Fig. 1 to toluene alone, the band containing nitro-BP was further resolved into two bands, one yellow and one orange, the latter having a lower R_F value. From the mass spectra and the ultraviolet-visible spectra shown in Fig. 2, and by comparison with those of authentic samples synthesized as described by Dewar *et al.* (*26*), we assign the structure 6-nitro-BP to the component present in the yellow TLC band; the orange TLC band consists of a mixture of the 1-nitro and 3-nitro isomers (*27*).

As shown in Fig. 3, both 6-nitro-BP and the mixture of the 1-nitro and 3-nitro isomers are direct mutagens in the Ames test. They are directly active with strains TA1537, TA1538, TA98, and TA100, which are reverted by frameshift mutagens. Addition of liver S9 significantly enhanced their activity in strains TA1537, TA1538, and TA98, and reduced the activity of the 1-nitro and 3-nitro mixture with strain TA100 (*28*).

Dose-response curves for the authentic samples, also shown in Fig. 3, are in good agreement with those of the nitro-BP isomers formed on exposure of BP to NO_2. No activity was found with strain TA1535, which is reverted by base-pair substitution mutations.

Samples purified by high-pressure liquid chromatography gave substantially the same results as the earlier tests. The purified 6-nitro-BP still showed a substantial increase in reversion frequency with metabolic activation. In these latter experiments we also tested amounts up to 150 nmole. With TA98, the effect of both BP and 6-nitro-BP was saturated at 10 nmole, but the effect of the mixture of 1- and 3-nitro isomers continued to rise out to 50 nmole. At this saturating amount, the total number of revertants was approximately 2.5 times higher than that of the 6-nitro isomer and approximately 4 times higher than that of the parent BP. The complex dose-response curves from the 1-nitro and 3-nitro isomers for TA98 are thought to be the result of the interaction of the two isomers.

The nitration of BP by part per million levels of NO_2 in air is catalyzed by part

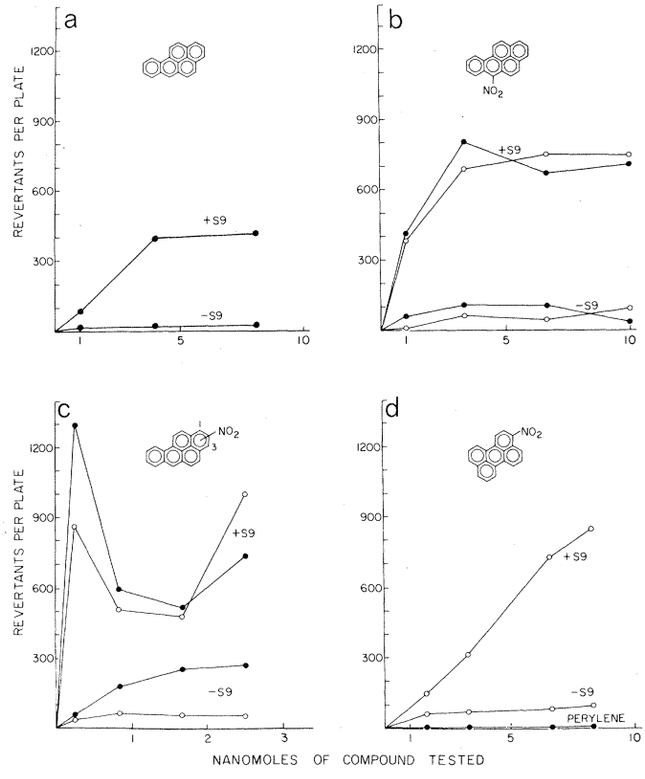

Fig. 3. Mutagenic activity of (a) BP, (b) 6-nitro-BP, (c) 1-nitro- and 3-nitro-BP, and (d) 3-nitroperylene in the Ames assay system. Dose-response curves are shown only for the most sensitive strains, TA100 (a and b), TA98 (c), and TA1538 (d), with (+*S9*) and without (−*S9*) metabolic activation. Each point represents the mean of at least triplicate plates. Subtracted spontaneous revertant backgrounds were: TA100, 118 to 130 (−S9) and 130 to 152 (+S9); TA98, 24 (−S9) and 44 (+S9); and TA1538, 12 (−S9) and 41 (+S9). Also shown (•) are the correreresponding curves for perylene (d) and for the nitro-BP isomers (b and c) synthesized according to Dewar *et al.* (*26*).

per billion levels of HNO_3. This species was shown by long-path (100 m) Fourier transform infrared spectroscopy to be present in our tanks containing known amounts of reportedly pure NO_2 in nitrogen. In 8-hour exposures, typical yields for the conversion of BP to the nitro derivatives ranged from ~18 percent at 0.25 ppm of NO_2 (~3 ppb of HNO_3) in air to ~40 percent at 1.0 ppm of NO_2 (~11 ppb of HNO_3). The lower value of 0.25 ppm is the air quality standard for NO_2 (1-hour average) in California and is commonly exceeded there.

Since exposure of BP, a known carcinogen and activable mutagen, to part per million levels of NO_2 resulted in the formation of mutagenic nitro derivatives, it seemed interesting to see whether similar products could also be formed from a noncarcinogenic PAH under the same conditions. To address this point, NO_2 exposure studies were repeated with perylene, an isomer of BP present in ambient air and emitted in many combustion processes (*3, 4*), including vehicle exhaust. Unlike BP, we found perylene to be nonmutagenic with or without S9 in the Ames reversion assay (Fig. 3d).

Perylene deposited on glass fiber filters was exposed to 1 ppm of NO_2 (containing trace levels of HNO_3) for 24 hours at a flow rate of 1 ft^3/min. The major resulting TLC band (brick-red color on silica gel) consisted of 3-nitroperylene, identified on the basis of its mass spectrum and by comparison of its ultraviolet spectrum with published data (*29*). Both spectra are shown in Fig. 2. When tested with strains TA98 and TA1538, 3-nitroperylene was found to be a directly active mutagen whose activity was significantly enhanced by the addition of liver S9 (Fig. 3d).

These studies conducted in simulated atmospheres clearly demonstrate that directly active mutagens, including nitro derivatives, can form on exposure of PAH to gaseous pollutants. Additional evidence that such transformations may occur in real atmospheres was obtained by drawing ambient photochemical smog (flow rate, 40 ft^3/min) through two glass fiber filters mounted in series, using BP as our model PAH. The upstream filter collected ambient particulates, thus allowing BP deposited on the downstream filter to be exposed only to gaseous pollutants (*30*).

Five such experiments were carried out. The first four were conducted simultaneously on 17 to 20 July 1977 at two locations in southern California, Los Angeles and Riverside (*31*), with BP exposed to daytime (6 a.m. to 9 p.m.) and nighttime (9 p.m. to 6 a.m.) ambient air for periods of 40 hours. The filter samples were then extracted and the concentrated organic extracts were tested with strains TA98 and TA100. Typically, assay of 10 μl of these extracts yielded 150 to 250 revertants per plate with strain TA98 in the absence of metabolic activation. Thus, exposure of BP to polluted ambient air results in the formation of directly active mutagens.

In the fifth experiment, BP was exposed under the same conditions to ambient Riverside smog for 72 hours and the products were analyzed by TLC and mass spectrometry. The results are shown in Fig. 1 along with those of the BP exposure studies conducted in simulated atmospheres. Many of the resulting products were analogous to those formed in the laboratory exposures of BP to the individual gaseous pollutants or in solution to the liver S9 mix. On the basis of their molecular weights, determined by chemical ionization–mass spectrometry, and their R_F values, these oxidation products appear to include hydroxy, dihydroxy, and dihydrodiol derivatives. These are known metabolites of BP in mammalian cells (*19*).

No nitrobenzo[*a*]pyrenes could be detected in the 72-hour exposure. This is not unexpected in view of the relatively low NO_2 levels prevailing in Riverside and the fact that the catalyst HNO_3, which is present in photochemical smog, was removed by the upstream filter. Furthermore, it is possible that the prolonged exposure to oxidizing species would degrade any nitro-PAH compounds that might have been formed.

The fact that 9-nitroanthracene, on photolysis, forms 9,10-anthraquinone, both in solution (*32*) and in our experiments on silica gel (*33*), suggests that similar reactions may represent the fate of nitro-PAH in ambient atmospheres, where several quinones, including the three quinones derived from BP, have been identified (*34*). Thus, photooxidation of nitro derivatives should be considered as an alternative pathway, besides direct oxidation of PAH by ozone (*34*) or by sunlight in air (*20*), for the formation of polycyclic quinones in polluted air.

However, caution should be expressed in the interpretation of the formation and yields of quinones in ambient air or from BP-O_3 experiments. Thus, in the latter case we observed that when high-pressure liquid chromatographic techniques were used for separation instead of TLC, no quinones were observed as products.

In conclusion, directly active mutagens are formed on exposure of PAH to part per million levels of the major gaseous components of photochemical smog, NO_2, O_3, and PAN. Although we are aware that urban particulates contain several hundred organic compounds that have not yet been tested individually for carcinogenic or mutagenic activity, we feel that reactions of PAH such as those investigated here may account, in part, for our observations of directly active mutagens in airborne particulate matter.

The facile formation of mutagenic nitro derivatives by exposure of noncarcinogenic as well as carcinogenic PAH to part per million levels of NO_2 warrants further investigations of the modes of formation and atmospheric fate of these compounds, especially in situations where relatively high levels of PAH and oxides of nitrogen may coexist. These could include automobile and diesel exhausts as well as plumes from coal-fired power plants. Indeed, the presence of direct mutagens in auto exhaust and the direct mutagenicity of 6-nitrobenzo[*a*]pyrene have recently been reported (*35*).

Finally, we reemphasize that our studies were conducted with PAH deposited on the surface of glass fiber filters. Whether PAH adsorbed on the surface of airborne particles (soot, fly ash, and so on) will react in a similar fashion in the atmosphere is not yet known. This is a complex problem because atmospheric reactions of PAH may be influenced by many factors typical of surface chemistry as well as by pollutant levels, particle size, sunlight intensity, atmospheric mixing, and transport time. Similarly, little is known about the extent of possible reactions of PAH on glass fiber filters, which have been widely employed for decades to collect ambient particulates; our results suggest that they may indeed be significant. Therefore, the determination of possible filter "artifacts" is of major importance since most evaluations of the carcinogenic and mutagenic activity of organic particulates have been based on filter samples.

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References and Notes

1. J. Leiter, M. B. Shimkin, M. J. Shear, *J. Natl. Cancer Inst.* **3**, 155 (1942).
2. P. Kotin, H. L. Falk, P. Mader, M. Thomas, *Arch. Ind. Hyg. Occup. Med.* **9**, 153 (1954).
3. Committee on Biologic Effects of Atmospheric Pollutants, *Particulate Polycyclic Organic Matter* (National Academy of Sciences, Washington, D.C., 1972).
4. D. Hoffman and E. L. Wynder, in *Air Pollution*, A. C. Stern, Ed. (Academic Press, New York, ed. 3, 1977), vol. 2, pp. 361-455; R. C. Lao, R. S. Thomas, H. Oja, L. Dubois, *Anal. Chem.* **45**, 908 (1973); R. C. Pierce and M. Katz, *Environ. Sci. Technol.* **9**, 347 (1975); D. Grosjean, in *Ozone and Other Photochemical Oxidants* (National Academy of Sciences, Washington, D.C., 1977), pp. 45-125; W. Cautreels and K. Van Cauwenberghe, *Atmos. Environ.* **10**, 447 (1976); M. L. Lee, M. Novotny, K. D. Bartle, *Anal. Chem.* **48**, 1566 (1976).
5. W. C. Hueper, P. Kotin, E. C. Tabor, W. W. Payne, H. Falk, E. Sawicki, *Arch. Pathol.* **74**, 89 (1962).
6. S. S. Epstein, S. Josh, J. Andrea, N. Mantel, E. Sawicki, T. Stanley, E. C. Tabor, *Nature (London)* **212**, 1305 (1966); R. H. Rigdon and J. Neal, *Tex. Rep. Biol. Med.* **29**, 110 (1971); A. E. Freeman, P. J. Price, R. J. Bryan, R. J. Gordon, R. V. Gilden, G. J. Kelloff, R. J. Huebner, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 445 (1971); U. Mohr, H. Reznik-Schuller, G. Reznik, G. Grimmer, J. Misfeld, *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B* **163**, 425 (1976); G. Grimmer, *IARC (Int. Agency Res. Cancer) Sci. Publ. 16* (1977), pp. 29-39.
7. J. N. Pitts, Jr., Second Annual Progress Report, NSF-RANN grant AEN73-02904 A02, September 1975, p. V-8.
8. _____, D. Grosjean, T. M. Mischke, V. F. Simmon, D. Poole, *Toxicol. Lett.* **1**, 65 (1977).
9. _____, paper presented at the 174th national meeting of the American Chemical Society, Chicago, 28-31 August 1977; in *Biological Effects of Environmental Pollutants*, S. D. Lee, Ed. (Ann Arbor Science, Ann Arbor, Mich., in press).
10. B. N. Ames, J. McCann, E. Yamasaki, *Mutat. Res.* **31**, 347 (1975).
11. J. N. Pitts, Jr., paper presented at the Royal Society Meeting on Pathways of Pollutants in the Atmosphere, London, 3-4 November 1977; *Philos. Trans. R. Soc. London*, in press; paper presented at the Conference on Chemical Carcinogens in the Environment: Emissions and Control, Pasadena, Calif., 6-7 March 1978.
12. _____, W. L. Belser, K. Van Cauwenberghe, D. Grosjean, J. Schmid, D. R. Fitz, G. B. Knudson, P. Hynds, paper presented at the Environmental Protection Agency symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Va., 21-23 February 1978.
13. J. N. Pitts, Jr., D. Grosjean, K. Van Cauwenberghe, W. L. Belser, paper presented at the 175th national meeting of the American Chemical Society, Anaheim, Calif., 12-17 March 1978.
14. Also relevant to the postulated importance of oxidative processes during atmospheric transport of PAH are several reports of the carcinogenic activity of polar fractions of organic particulates (*5, 15*), products of ozonized gasolines (*16*), and products of oxidation of aliphatic hydrocarbons (*17*), and of the toxicity of the photooxidation products of a commercial fuel oil (*18*).
15. S. S. Epstein, N. Mantel, T. W. Stanley, *Environ. Sci. Technol.* **2**, 132 (1968); E. L. Wynder and D. Hoffman, *J. Air Pollut. Control Assoc.* **15**, 155 (1965); R. J. Gordon, R. J. Bryan, J. S. Rhim, C. Demoise, R. G. Wolford, A. E. Freeman, R. J. Huebner, *Int. J. Cancer* **12**, 223 (1973); S. Asashina, J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi, D. Coffin, S. S. Epstein, *Cancer Res.* **32**, 2263 (1972).
16. P. Kotin, H. L. Falk, C. J. McCammon, *Cancer* **11**, 473 (1958).
17. P. Kotin, H. L. Falk, M. Thomas, *ibid.* **9**, 905 (1956).
18. R. A. Larson, L. L. Hunt, D. W. Blankenship, *Environ. Sci. Technol.* **11**, 492 (1977).
19. D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, A. H. Conney, in *In Vitro Metabolic Activation in Mutagenesis Testing*, F. J. de Serres, J. R. Fouts, J. R. Bend, R. M. Philpot, Eds. (Elsevier, Amsterdam, 1976), pp. 159-177; O. G. Fahmy and M. J. Fahmy, *Cancer Res.* **33**, 302 (1977).
20. H. L. Falk, I. Markul, P. Kotin, *AMA Arch. Ind. Health* **13**, 13 (1956); B. D. Tebbens, J. F. Thomas, M. Mukai, *Am. Ind. Hyg. Assoc. J.* **27**, 415 (1966); B. D. Tebbens, M. Mukai, J. F. Thomas, *ibid.* **32**, 365 (1971); K. W. Boyer and H. A. Laitinen, *Environ. Sci. Technol.* **9**, 457 (1975).
21. R. S. Berry and P. A. Lehman, *Annu. Rev. Phys. Chem.* **22**, 47 (1971).
22. "PAH are chemically inert and hence are removed from the air only by rain or the slow sedimentation of the particulates," according to L. Fishbein, in *Chemical Mutagens*, A. Hollaender, Ed. (Plenum, New York, 1976), vol. 4, pp. 219-319; see p. 232.
23. No mutagenic activity was found in any of the control runs performed with blank filters.
24. A 0.5-ml portion of S9 mix contains 0.02 ml of liver S9 from Aroclor-induced rats. We recently stressed (*12*) the need for standardization of the widely used Ames test (number of cells per plate, plate volume, concentration of rat liver S9, and so on).
25. Authentic samples of the three purified quinones (BP-6,12-quinone, BP-1,3-quinone, and BP-3,6-quinone) were tested in our laboratory and were found to be nonmutagenic in the Ames assay system. Note that exposure of BP to pure air (control run, see Fig. 1) also yielded small amounts of BP-quinones.
26. M. J. S. Dewar, T. Mole, D. S. Urch, E. W. T. Wasford, *J. Chem. Soc. (London)* (1956), p. 3572.
27. Cochromatography of the two TLC bands with authentic samples further confirmed these assignments. The two isomers present in the orange TLC band were resolved by gas chromatography, using a glass capillary column. Relative concentrations of the two isomers were 1:1 in the mixture synthesized according to Dewar *et al.* (*26*) and 10:1 in the orange TLC band formed on exposure of BP to NO_2 (plus a trace of HNO_3).
28. The reduction in reversion frequency of TA100 may be explained by microsomal detoxification of the sample. The enzyme systems activated by Aroclor in rat liver have the general function of detoxifying the insulting compound or compounds. These reactions sometimes form mutagens from nonmutagenic chemicals, which is the basis for the metabolic activation in the Ames test. The same or related enzymes may also catalyze reactions that reduce the activity of direct chemical mutagens.
29. M. J. S. Dewar and T. Mole, *J. Chem. Soc. (London)* (1956), p. 1441; H. Hopff and H. R. Schweizer, *Helv. Chim. Acta* **42**, 2315 (1957).
30. Control runs were also performed at each location, replacing the downstream filters by blank filters. No activity was detected in these control runs, indicating that no mutagens were transferred from the upstream to the downstream filter and that no mutagens were formed by interaction of ambient gaseous pollutants with the blank filters during sampling.
31. Riverside is located about 100 km east of Los Angeles.
32. O. L. Chapman, D. C. Heckert, J. W. Reasoner, S. P. Thackaberry, *J. Am. Chem. Soc.* **88**, 5550 (1966).
33. We irradiated in air 9-nitroanthracene adsorbed on silica gel and found significant yields of 9,10-anthraquinone (unpublished work). This is consistent with the results obtained in solution by Chapman *et al.* (*32*) and supports our suggestion that nitro-PAH adsorbed on airborne particles may be further photooxidized to quinones in sunlight.
34. R. C. Pierce and M. Katz, *Environ. Sci. Technol.* **10**, 45 (1976).
35. Y. Wang, R. Talcott, R. Sawyer, S. Rappaport, E. Wei, paper presented at the Environmental Protection Agency symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Va., 21-23 February 1978.

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Growth Lines in a Bivalve Mollusk: Subdaily Patterns and Dissolution of the Shell

Abstract. *Scanning electron micrographs of sections of the prismatic shell of the bivalve* Mercenaria mercenaria *reveal narrow subdaily growth striations. The width of these narrow lines, formed by concentrations of organic material, corresponds to the quantity of shell material that would be expected to dissolve during periods of anaerobic metabolism. The* pH *in the extrapallial fluid of the bivalve decreases when the valves are closed, and the amount of dissolution of shell is related to the duration of valve closure.*

The shells of numerous mollusks contain repeating microscopic structures that have been interpreted as records of periodic growth (*1*). In radial sections of bivalve shells these features appear as lines or striations parallel with growth surfaces (*2, 3*). The temporal frequency of striations has been analyzed in various species and correlated with seasonal growth rates, geochronometry, and behavior and, by inference, with physiological changes in the animals studied (*1-4*). Growth lines are visible in polished and etched shell sections as narrow bands in which calcium carbonate is absent or much lower in concentration than in adjacent areas (*5*).

Until quite recently, hypotheses concerning the origin of these lines were based on an assumption of periodic episodes of calcium carbonate deposition (*6*), although a few references were made to the possibility that dissolution of recently formed shell might play a part (*3, 6*). Last year, Lutz and Rhoads (*7*) published a theory of growth-line formation which held that organic striations are simply residues left behind as a result of dissolution of shell material during periods of anaerobiosis. To date, quantitative evidence confirming this mechanism has not appeared. In this report we present independent data in partial support of the hypothesis of Lutz and Rhoads, based on measurements of extrapallial fluid *p*H (*8*), published rates of shell dissolution during valve closure (*9*), thickness of subdaily growth lines, and duration of closure.

We installed glass microelectrodes, sealed by O rings from the surrounding seawater, through a drilled hole in the

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