at 5500 Å in killing bacteria. This lethal efficiency approximates that for absorption by DNA. Airborne viruses or those carried by small insects may be greatly altered by ultraviolet radiation. Host populations for these viruses could be greatly affected either by the production of numerous new mutants or by the return of such viruses after many generations of suppression by the intense ultraviolet radiation. Water, per se, is not a particularly good shield against that part of ultraviolet spectrum involved (in pure water the absorption coefficient at 2900 Å is only 7×10^{-3} cm^{-1}) (*4*). Since a high proportion of animal species is nocturnal, the effects of increased solar ultraviolet flux might be manifest in a differential depletion of diurnal species. There seems to be no compelling fossil evidence for past "biological cataclysms" (*18*), but the effects of ultraviolet deluges on past and future evolution may be more subtle.

M. A. RUDERMAN

Department of Physics,
Columbia University, New York 10027

References and Notes

1. H. Johnson, *Science* **173**, 517 (1971).
2. P. Crutzen, *Q. J. R. Meteorol. Soc.* **96**, 320 (1970).
3. K. D. Terry and W. H. Tucker, *Science* **159**, 421 (1968).
4. L. Koller, *Ultraviolet Radiation* (Wiley, New York, 1952).
5. R. C. Smith, in *Proceeding of Survey Conference of Climatic Impact Assessment Program* (15–16 Feb. 1972), A. Barrington, Ed. (Department of Transportation, Washington, D.C., 1972), p. 243.
6. S. Chapman, *Mem. R. Meteorol. Soc.* **3**, 103 (1930).
7. H. Foley and M. Ruderman, *J. Geophys. Res.* **78**, 4441 (1973).
8. H. Johnston, G. Whitten, J. Birks, Lawrence Berkeley Laboratory preprint No. 1421; *J. Geophys. Res.*, in press.
9. J. Angell and J. Korshover, *Mon. Weather Rev.*, in press.
10. M. Ruderman and J. Chamberlain, *Planet. Space Sci.*, in press.
11. G. Brasseur and M. Nicolet, *ibid.* **21**, 939 (1973).
12. P. Warneck, *J. Geophys. Res.* **77**, 6589 (1972).
13. I. Shklovsky, *Supernovae* (Wiley-Interscience, New York, 1969).
14. W. H. Tucker and K. D. Terry, *Science* **160**, 1138 (1968).
15. S. Colgate, *Can. J. Phys.* **46**, 5476 (1968).
16. R. Klebesadel, I. Strong, R. Olsen, *Astrophys. J. Lett.* **182**, L85 (1973).
17. H. Laster, *Science* **160**, 1138 (1968).
18. G. G. Simpson, *ibid.* **162**, 140 (1968).
19. I thank Dr. W. Bock and Prof. G. M. Spruch for informative discussions about biological "cataclysms." This research was supported in part by NSF grant GP 32336X.

28 February 1974

Nicotine Inhibition of the Metabolism of 3,4-Benzopyrene, a Carcinogen in Tobacco Smoke

Abstract. *A decreased rate of biliary excretion of radioactive metabolites of 3,4-benzopyrene was observed in rats given a single dose of nicotine. Prior treatment of rats with nicotine decreased benzopyrene hydroxylase activity in homogenates of liver, lung, and small intestine. The addition of nicotine to incubated tissues also decreased benzopyrene hydroxylase activity. These findings show that nicotine inhibits the metabolism of 3,4-benzopyrene in vivo and in vitro.*

In tobacco the alkaloid nicotine shares a physical and possibly a metabolic relation to the carcinogenic polycyclic hydrocarbon 3,4-benzopyrene (BP). Nicotine and BP are both constituents of inhaled tobacco smoke (*1*), and nicotine is reported to alter the activity of hepatic microsomal enzymes that metabolize many foreign chemicals including drugs and carcinogens (*2–4*). Thus, nicotine may also influence microsomal enzyme systems required for the metabolism of BP. Although BP is a potent carcinogen in tobacco smoke (*5*), information is limited concerning its metabolic interactions with nicotine. Only a single report was found showing that a large, single dose of nicotine (40 mg/kg) given intraperitoneally enhanced BP hydroxylase activity in rat liver microsomes measured in vitro 24 hours after treatment with nicotine (*3*).

When BP is metabolized by hepatic enzymes the end products are hydroxylated metabolites (*6*). An active epoxide intermediate, however, is also formed (*7*); it binds readily to DNA (*8*) and is implicated in the formation of tumors at target organs. Thus, an important aspect of carcinogenesis is the formation of a reactive epoxide of BP, the concentration of which might be influenced by other chemical agents: for example, nicotine, which may affect the microsomal enzyme activity required for the metabolism of BP. The accumulation of evidence that heavy, prolonged cigarette smoking in humans plays a role in the etiology of cancer of the lung emphasizes the need for more extensive studies of the metabolic interactions between a known carcinogen and other chemical agents in tobacco smoke.

We investigated the effects of a single dose of nicotine on hepatic and nonhepatic metabolism of the carcinogen BP in vivo and in vitro. Since BP is metabolized by hepatic microsomes and appears in the bile almost entirely as metabolic products (*9*), we first studied the effects of nicotine on the biliary excretion of BP metabolites. Sprague-Dawley male rats (220 ± 10 g) were used and treated with a single dose of nicotine bitartrate (25 mg/kg; nicotine base, 8.13 mg/kg) administered intraperitoneally in a volume of 0.5 ml per 100 g of body weight. The corresponding controls were given an equivalent amount of saline. The animals were anesthetized with ether 24 hours after the preliminary nicotine treatment, and the common bile duct was cannulated (*10*). Bile was collected in tared scintillation vials during a 15-minute control period. In the standard procedure, BP (2.5 mg/kg) containing tracer quantities of ^{14}C-labeled BP (0.8 μc/kg) was injected into the right femoral vein after the control period, after which bile samples were collected for six additional 15-minute periods. The ^{14}C in the bile (20 μl) samples was counted in a Packard Tri-Carb liquid scintillation counter with a Triton fluor (*11*).

For the metabolism of BP in vitro in a separate experiment rats were killed 24 hours after treatment with nicotine or saline, and homogenates of liver, lung, and small intestine were made (3 percent in ice-cold 0.25*M* sucrose). Tissue BP hydroxylase activity was determined by measuring the fluorescence of hydroxylated metabolites of BP (*12*). The homogenates (0.5 ml) of liver,

Table 1. Effect of treatment with nicotine on tissue BP hydroxylase activity. Rats were given nicotine bitartrate (25 mg/kg) intraperitoneally, and the enzyme activity of each tissue was determined 24 hours later. The final incubation mixture had a volume of 3.1 ml which was composed of: 0.5 ml of glucose-6-phosphate dehydrogenase (5 Kornberg units), 0.2 ml of 0.03*M* glucose 6-phosphate, 0.1 ml of nicotinamide adenine dinucleotide phosphate solution (4 mg/ml), 0.1 ml of nicotinamide adenine dinucleotide solution (4 mg/ml), 0.2 ml of 0.01*M* adenosine triphosphate, 0.2 ml of 0.6*M* nicotinamide, 0.1 ml of 2.0*M* KCl, 0.1 ml of 0.1*M* $MgCl_2$, 0.1*M* KH_2PO_4–K_2HPO_4 buffer at *p*H 7.4, 0.5 ml of tissue homogenate, and 0.1 ml of BP (50 μg). The results are expressed as nanograms of 3-hydroxybenzopyrene per milligram of tissue per 20 minutes. Each value represents the mean ± S.E. of data from five or six rats.

Treatment	BP hydroxylase activity*		
	Lung	Liver	Small intestine
None	14.55 ± 1.3	2.64 ± .42	0.92 ± .25
Nicotine*	8.65 ± 0.7	1.46 ± .15	0.25 ± .03

* Nicotine produced a significant reduction in all three tissues ($P < .05$).

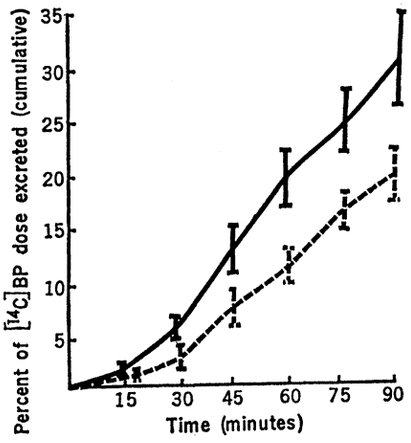

Fig. 1. Cumulative biliary excretion of ^{14}C-labeled BP metabolites in control rats (solid line) and nicotine-treated (dashed line) rats over a 90-minute period. Each point represents the mean ± S.E. of data from six rats.

lung, or small intestine were incubated with BP (50 μg in 0.1 ml of acetone) in the presence of cofactors (2.5 ml) required for an NADPH (reduced nicotinamide adenine dinucleotide phosphate) generating system. Values for tissue BP hydroxylase activity represent the amount (in nanograms), the reference standard, of 3-hydroxybenzopyrene causing fluorescence equivalent to the total hydroxylated metabolites produced per milligram of tissue from BP during 20 minutes of incubation.

The effect of nicotine on BP hydroxylase activity in tissue (liver, lung, and small intestine) from normal untreated animals was determined by adding nicotine directly to the incubation medium in vitro. The assay was similar to that described above for treated animals with the following modifications. A stock solution of nicotine bitartrate (149.4 μg/0.1 ml) was serially diluted (tenfold) prior to the assay. A solution of cofactors was made up in a reduced volume of 2.4 ml, and when 0.1 ml of nicotine bitartrate was added to the incubation medium, the final concentration of nicotine bitartrate ranged from 10^{-4} to $10^{-6}M$ in 3.1 ml of reaction mixture. All data in these studies were statistically analyzed by Student's *t*-test.

When [^{14}C]BP was injected intravenously into rats 24 hours after a single dose of nicotine, the cumulative excretion of radioactive metabolites of BP was decreased in the bile (Fig. 1). The decrease in radioactivity in bile caused by the nicotine treatment was statistically significant throughout the interval from 15 to 90 minutes. Measuring [^{14}C]BP metabolites in the bile is an appropriate way to follow the metabolism of BP since Levine (*10*) has shown that the rate of biliary excretion of BP metabolites reflects directly the rate of hepatic metabolism of BP. To eliminate the possibility that the reduced excretion of biliary metabolites was merely a function of bile flow, the bile of the control rats and the nicotine-treated rats was monitored. No significant difference was found between the bile flow of the control group (2.91 ± 0.09 ml/hour per kilogram of body weight) and that of the nicotine-treated group (2.49 ± 0.19 ml/hour per kilogram of body weight) during the 90-minute collection period.

Rats treated with nicotine and killed 24 hours later showed a decrease of BP hydroxylase activity in homogenates of liver, lung, and small intestine when compared to control (no treatment) groups (Table 1). The enzyme activity in liver and small intestine decreased approximately 40 and 45 percent, respectively, while the lung showed a reduction of almost 73 percent.

Nicotine caused inhibition of BP hydroxylase in liver and lung, and with increasing concentration of the inhibitor (10^{-6} to $10^{-4}M$) there was a progressive increase in the percent inhibition of the enzyme (Table 2). A similar inhibitory effect on enzyme activity by nicotine was observed in small intestine. In this tissue, however, nicotine concentrations of $10^{-5}M$ and higher did not exhibit increasing inhibitory effects on enzyme activity.

Our studies show that a single dose of nicotine inhibits the metabolism of BP as evidenced by a decreased biliary excretion of BP metabolites. In addition, nicotine inhibits the metabolism of BP by liver, lung, and small intestine both in vitro and in vivo. At variance with our results Yamamoto *et al.* (*3*) showed that a high dose of nicotine (40 mg/kg) in corn oil given intraperitoneally stimulated the activity of hepatic BP hydroxylase. This difference might possibly be related to both the vehicle and dose of administered nicotine, since in our studies we administered in saline a relatively low dose of nicotine (8.13 mg/kg), calculated as the free base. On the other hand, Stälhandske and Slanina (*2*) report that nicotine given intraperitoneally at low levels, 15 mg/kg per day for 3 days, significantly reduced its metabolism. Though it has been reported that rats exposed to cigarette smoke show a stimulation of the activity of enzymes necessary for the metabolism of BP (*13*), our studies show that at least one component of cigarette smoke, namely nicotine, can inhibit BP metabolism.

Table 2. Effect of nicotine on the metabolism of BP in vitro. Rat tissue homogenates were incubated with increasing molar concentrations of nicotine bitartrate. Nicotine bitartrate was added in 0.1 μl of distilled water and the incubation mixture was incubated for 3 minutes before the addition of 0.1 ml of BP (50 ng). The assay was carried out as described with the cofactors listed in Table 1. Each value represents the mean ± S.E. from the data of four rats. Enzyme activity of liver, lung, and small intestine respectively (no inhibitor added) was 16.20, 3.84, and 1.15 ng of 3-hydroxybenzopyrene per milligram of tissue per 20 minutes.

Nicotine bitartrate (*M*)	Inhibition (percent) of BP hydroxylase activity*		
	Liver	Lung	Small intestine
10^{-6}	21.8 ± 3.2	15.5 ± 7.5	34.1 ± 1.5
10^{-5}	29.0 ± 2.4	17.8 ± 5.5	41.6 ± 8.6
10^{-4}	38.8 ± 1.0	34.8 ± 7.9	39.0 ± 8.3

Nicotine, by inhibiting the metabolism of BP, possibly reduces the amount of epoxide formed and thus decreases the carcinogenic action of BP. In support of this concept, studies by Gelboin *et al.* (*14*) show that 7,8-benzoflavone added to homogenates of skin epidermis in vitro inhibits BP hydroxylase activity, and that topically applied 7,8-benzoflavone inhibits the formation of mouse skin tumors after repeated treatment with 9,10-dimethylbenzanthracene. Whether a reduction of the nicotine content of tobacco will offset a potential chemoprophylactic mechanism for protecting against the carcinogenic action of BP and whether prolonged exposure to low concentrations of nicotine may have any effect on the formation of epoxides and the consequent carcinogenicity of BP remain to be determined.

ROCKY P. WEBER
JULIUS M. COON
ANTHONY J. TRIOLO
Department of Pharmacology,
Jefferson Medical College,
Thomas Jefferson University,
Philadelphia, Pennsylvania 19107

References and Notes

1. D. Hoffmann and E. Wynder, *Science* **178**, 1197 (1972).
2. T. Stälhandske and P. Slanina, *Acta Pharmacol. Toxicol.* **28**, 75 (1970).
3. I. Yamamoto, K. Nagai, H. Kimura, K. Iwatsubo, *Jap. J. Pharmacol.* **16**, 183 (1966).
4. R. W. Ruddon and A. M. Cohen, *Toxicol. Appl. Pharmacol.* **16**, 613 (1970); D. G. Wenzel and L. L. Broadie, *ibid.* **8**, 455 (1966).

5. Department of Health, Education, and Welfare, *Smoking and Health* (Government Printing Office, Washington, D.C., 1964), p. 57.
6. P. Sims, *Biochem. Pharmacol.* **19**, 795 (1970).
7. P. L. Grover, A. Hewer, P. Sims, *ibid.* **21**, 2713 (1972).
8. H. V. Gelboin, *Cancer Res.* **29**, 1272 (1969).
9. P. Kotin, H. L. Falk, R. Busser, *J. Natl. Cancer Inst.* **23**, 541 (1959); H. L. Falk, P. Kotin, S. S. Lee, A. Nathan, *ibid.* **28**, 669 (1962).
10. W. G. Levine, *J. Pharmacol. Exp. Ther.* **175**, 301 (1970).
11. R. H. Benson, *Anal. Chem.* **38**, 1353 (1966).
12. R. Kuntzman, L. C. Mark, L. Brand, M. Jacobson, W. Levin, A. H. Conney, *J. Pharmacol. Exp. Ther.* **152**, 151 (1966).
13. R. W. Welch, Y. E. Harrison, B. W. Gommi, P. J. Poppers, M. Finster, A. H. Conney, *Clin. Pharmacol. Ther.* **10**, 100 (1969).
14. H. V. Gelboin, F. Wiebel, L. Diamond, *Science* **170**, 169 (1970).
15. We thank H. V. Gelboin for supplying us with the reference standard 3-hydroxybenzopyrene. Supported by PHS support grant RR-5415 and PHS grant ES00507.

10 December 1973; revised 1 February 1974

Bacterial Stromatolites: Origin of Laminations

Abstract. *Laminated mats composed of motile filamentous photosynthetic bacteria and nonmotile unicellular blue-green algae occur in a large number of Yellowstone hot springs at temperatures between 55° and 70°C. Field studies indicate that the bacteria are the predominant mat-forming component. Under low light intensities, mats composed exclusively of bacteria can be formed. The bacteria undergo a diurnal migration, moving on top of the algae during the night and becoming mixed again with the algae during the day as a result of algal growth. Thus, the laminations probably arise as a result of differential migration of the bacteria in daily response to reduced light intensities. This response to light is exactly opposite to that previously reported for filamentous stromatolite-forming, blue-green algae, but the net result is the same—formation of a laminated mat.*

If the present is the key to the past, a study of modern growing stromatolites may help in interpreting the origin and significance of Precambrian stromatolites and the environmental conditions under which they were formed. It has been conventionally assumed that Precambrian stromatolites are formed by filamentous blue-green algae (*1*), yet laminated mats are also being formed today by filamentous bacteria in the effluent channels of hot springs. In earlier work (*2*) it was shown that the main structural component of these mats was bacterial, but at that time it was not known that the bacteria were photosynthetic. Since then it has been shown (*3, 4*) that these filamentous bacteria contain bacteriochlorophylls and are capable of light-stimulated fixation of CO_2. These bacteria, now classified in the genus *Chloroflexus* (*5*), are widespread in hot springs of neutral to alkaline *p*H throughout the world. They are capable of both anaerobic and aerobic growth, and can grow photosynthetically as well as heterotrophically. Under conditions of high sulfide, mats are formed composed exclusively of these bacteria (*6*), but in most cases they are found in nature in association with unicellular, nonmotile blue-green algae of the genus *Synechococcus*. It is our purpose here to provide data showing that it is the bacterial component which controls mat structure, and that the laminae arise as a result of differential response of the bacteria to changing light conditions over the diurnal cycle.

The distinction between the *Chloroflexus* mats under study in this report and the Conophyton-like stromatolitic structures described earlier from hot springs by Walter *et al.* (*7*) should be emphasized.The Conophyton-like structures are formed by an association of a filamentous blue-green alga *Phormidium tenue* and *Chloroflexus*, but in most cases the alga is dominant and is capable of forming the structures in the complete absence of the bacteria (*8*). The mats under study in this report are generally flat rather than conical, although, as noted below, nodular structures are sometimes formed (*9*). Detrital silica derived from the siliceous sinter of the geyser basins is frequently incorporated into the *Chloroflexus* mats, being carried onto the mats with runoff from occasional thunderstorms. Between storms, the mats are relatively undisturbed, and *Chloroflexus* migrates rapidly around and on top of the detrital material which falls on a mat, as can be readily observed by sprinkling carborundum powder onto the mat. *Chloroflexus* is thus a sediment-trapping organism, as are the stromatolite-forming blue-green algae.

Details of methods will be published elsewhere (*10*). Growth rates of the mats were followed by marking with carborundum powder. *Chloroflexus* contains both bacteriochlorophylls a and c. Bacteriochlorophyll a was assayed by extraction into methanol and measurement of absorption with a Beckman DB-G spectrophotometer at 770 nm, the absorption peak of bacteriochlorophyll a in this solvent. Bacteriochlorophyll c (which absorbs in solvents at virtually the same wavelength as algal chlorophyll a) was measured in vivo by homogenizing mat material in a solution of sucrose (1.4 g/ml) to reduce scatter and reading the in vivo absorption spectrum of bacteriochlorophyll c at 740 nm. Algal chlorophyll a was determined similarly, by using the absorption peak at 665 nm in methanol or the in vivo peak in sucrose suspension at 680 nm. Photosynthesis was measured by the ^{14}C method as described by Brock and Brock (*11*), and the effect of light intensity on photosynthesis was determined by using calibrated nylon mesh as described by Brock and Brock (*12*). To distinguish between algal and bacterial photosynthesis, the inhibitor of algal photosynthesis 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) was used, at a final concentration of about $5 \times 10^{-5} M$. In preliminary experiments it was shown that at this concentration light-stimulated uptake of CO_2 by the alga is completely inhibited, whereas bacterial CO_2 fixation is unaffected. In some photosynthesis experiments, an infrared filter (Tiffen 87 C) was used which eliminated all wavelengths of light to which the alga responds, but passed infrared radiation used by the bacterium. These two methods of measuring bacterial photosynthesis gave similar results. Mats were sampled with cork borers of various sizes. In some experiments, layers of the mat were cut in the field by using a dissecting microscope. To preserve delicate structures for later microscopy, cores were allowed to fall gently into vials containing melted 2 percent water agar. After the agar had hardened, it was removed from the vial and the core was cut longitudinally. The study areas in Yellowstone Park—Pool A, Toadstool Geyser, and Twin Butte Vista—have been described by Bauld and Brock (*4*). These areas may be considered representative of hundreds of springs in Yellowstone Park where *Chloroflexus* is present. In the study areas, the laminated mats develop in shallow, gently flowing water, the flow characteristics being essentially