1,N²-Propanodeoxyguanosine Adduct Formation in Aortic DNA Following Inhalation of Acrolein

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Recent reports indicate that many of the cytotoxic and health-threatening components of environmental tobacco smoke (ETS) reside in the vapor phase of the smoke. We have reported previously that inhalation of 1,3-butadiene, a prominent vapor phase component of ETS, accelerates arteriosclerotic plaque development in cockerels. In this study we asked whether inhaled acrolein, a reactive aldehyde that is also a prominent vapor-phase component of ETS, damages artery-wall DNA and accelerates plaque development. Cockerels inhaled 0, 1, or 10 ppm acrolein mixed with HEPA-filtered air for 6 hr. Half were killed immediately (day 1 group) for detection of the stable, premutagenic 1,N²-propanodeoxyguanosine acrolein adduct (AdG3) in aortic DNA via a ³²P-postlabeling/HPLC method, and half were killed after 10 days (day 10 group) for indirect assessment of adduct repair. In the day 1 group, acrolein–DNA adducts were 5 times higher in the 1 and 10 ppm groups than in HEPA-filtered air controls. However, in the day 10 group, adduct levels in the 1 and 10 ppm acrolein groups were reduced to the control adduct level. For the plaque studies, cockerels inhaled 1 ppm acrolein (6 hr/day, 8 weeks), mixed with the same HEPA-filtered air inhaled by controls. Plaque development was measured blind by computerized morphometry. Unlike butadiene inhalation, acrolein inhalation did not accelerate plaque development. Thus, even though repeated exposure to acrolein alone has no effect on plaque size under the exposure conditions described here, a single, brief inhalation exposure to acrolein elicits repairable DNA damage to the artery wall. These results suggest that frequent exposure to ETS may lead to persistent artery-wall DNA damage and thus provide sites on which other ETS plaque accelerants can act. ***Key words:*** **acrolein–DNA adducts, adduct repair, arteriosclerotic plaque, environmental tobacco smoke.** ***Environ Health Perspect*** **109:219–224 (2001). [Online 14 February 2001]**

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Cardiovascular disease (CVD) remains the greatest killer in the United States (~ 700,000 deaths/year) and in most of the Western world (*1*). Ischemic heart disease is responsible for about two-thirds of the CVD deaths in the United States. Arteriosclerotic plaque is the arterial lesion most often associated with ischemic heart disease.

Cigarette smoking is a major risk factor for CVD (*2*) and is associated with > 25% of the annual CVD deaths in the United States. Environmental tobacco smoke (second-hand smoke; ETS) also is recognized now as a major contributing factor to CVD. The American Heart Association has listed it as a major pollutant and cardiovascular toxin (*3*). Additionally, 40,000–60,000 excess heart disease deaths are estimated to occur yearly in the United States as a result of long-term exposure to ETS (*4,5*). In cockerels, inhalation of environmentally relevant levels of sidestream smoke, a surrogate for ETS, produces statistically significant acceleration of plaque development, even in the absence of diets augmented with cholesterol and/or saturated fats (*6*). Cockerels (*6,7*), rabbits (*8*), and humans (*3–5*) all display accelerated plaque development as a result of chronic exposure to ETS. Similar findings were reported recently for apo E-/-transgenic female mice fed a high-fat diet while being exposed to ETS (*9*).

Other *in vivo* studies demonstrated that ETS tar, with its high concentration of polycyclic aromatic hydrocarbons, has no effect on plaque development and strongly suggested that the plaque-promoting activity of ETS, and likely of whole smoke as well, resides in the vapor-phase (*10*). Inhalation of 1,3-butadiene, a "top 20" U.S. industrial chemical and a prominent vapor phase component of ETS (~400 µg/cigarette), produces a profound, statistically significant increase in plaque size (*11*). Other investigators have focused recently on the vapor phase as the major source of ETS toxicants contributing to respiratory disease and lung cancer (*12*).

Acrolein (CH_2 = CH-CHO), a highly reactive bifunctional aldehyde, is a ubiquitous toxic air pollutant. Acrolein is used widely in the chemical industry, especially in the production of acrylic acid. About 0.9 billion pounds of acrolein are produced in the United States each year (*13*). Acrolein also is produced during incomplete combustion of organic material and, like 1,3-butadiene, is a prominent vapor-phase component of ETS. About 1,200 µg of acrolein are present in the ETS from just one moderate tar-filtered cigarette, versus about 70 µg in the mainstream smoke from the same cigarette (*14*). These values are for the standardized, filtered 1R4F cigarettes [Tobacco and Health Research Institute (THRI), University of Kentucky, Lexington, KY], which we have used in our previous studies (*6,7*).

Acrolein is the principal cytotoxic metabolite of the anticancer and arthritis drug cyclophosphamide and is the principal and most physiologically active metabolite of allylamine (*15*), which is metabolized intracellularly to acrolein via semicarbazide sensitive amine oxidase. The activity of this enzyme is high in artery wall cells (*16*). Allylamine has long been recognized as an agent that elicits cardiotoxic and vasculotoxic responses. Avian artery smooth-muscle cells are more sensitive to treatment with allylamine and acrolein than are those of rat artery. Among the reported changes are reductions in cellular glutathione levels (*17*). Pathologic alterations to the artery wall *in vivo* and to artery wall cells *in vitro* after treatment with allylamine or acrolein have been reported (*18*) that are similar to changes known to occur during development of arteriosclerosis.

Acrolein is one of the most biologically reactive of all the aldehydes. It has been reported as a carcinogen [(*19*); see "Discussion" below] and forms stable 1,N²-propanodeoxyguanosine (acrolein-dG)–DNA adducts (*20,21*), at least one of which is mutagenic (*22–25*).

Acrolein induces lipid peroxidation, possibly via metabolism to glutathionylpropionaldehyde, which stimulates oxygen radical formation (*26*). Peroxidized low-density lipoprotein (LDL) is present in rabbit and

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human atherosclerotic plaques (*27*). Oxidation of polyunsaturated fatty acids associated with LDL also generates reactive aldehydes (*28*). These, in turn, can modify the protein (apo B) moiety of LDL, facilitating its uptake by macrophages and setting the stage for the development of foam cell lesions in the artery wall (*29*). Acrolein reactivity with thiols, such as reduced glutathione (*26*) and cysteine sulfhydryl groups, is largely responsible for the potent protein cross-linking potential of acrolein (*30*). Acrolein forms protein adducts at the ε amino group of lysine (e.g., in apo B of LDL) (*29*) and at the imidazole nitrogen of histidine. Studies on human plasma with vapor-phase cigarette smoke aldehydes reveal that acrolein inhibits the activity of lecithin-cholesterol acyltransferase, a key enzyme in reverse cholesterol transport. Acrolein adduction of cysteine sulfhydryls near the active site of this enzyme has been implicated in the inhibitory effect of acrolein. Acrolein may promote plaque development by inhibiting reverse cholesterol transport (*31,32*).

Autopsy studies of children and young adults clearly show that plaque development begins early in life (*33–35*). Thus, sensitive individuals (e.g., children) exposed chronically to ETS may be at increased risk for damage to arterial DNA, protein, and lipids due to 1,3-butadiene and acrolein in ETS. Several reports over the past 30 years have implicated chemical carcinogens or viruses as etiologic or major contributing factors to plaque development in both humans and animals, including avian models (*36–41*).

The hypothesis we tested is that inhalation of acrolein from ETS and other environmental sources should increase the likelihood that plaque will develop through formation of stable premutagenic adducts in artery wall cells. The experiments described here focused on the connection between inhalation exposure to environmentally relevant levels of acrolein, acrolein–DNA adduct formation in the aorta, and aortic plaque development. We posed four questions: Are acrolein-specific DNA adducts formed in arterial cell DNA after *in vivo* exposure to environmentally relevant levels of acrolein? Is there a dose dependence for acrolein associated with adduct formation? Is there evidence for repair of the acrolein-specific DNA adducts? And does extended inhalation exposure to acrolein, at levels comparable to those found in ETS, accelerate arteriosclerotic plaque development?

Materials and Methods

Animals. Four-week-old white leghorn cockerels (Avian Services, Frenchtown, NJ) were quarantined for 2 weeks at New York University's (NYU) Nelson Institute of Environmental Medicine Animal Facility in Tuxedo, New York, before exposures began. During this 2-week period they were acclimated to a 12-hr light/12-hr dark cycle and observed for anomalous behavior and disease. They were housed in large stainless-steel cages, and Association for Assessment and Accreditation of Laboratory Animal Care guidelines were followed for animal housing and care. Food (Chick Starter Grower; Purina, St. Louis, MO) and water were available *ad libitum* except when the cockerels were in the exposure chambers. All experimental protocols were approved by the Institutional Animal Care and Use Committee of NYU Medical Center.

Acute* in vivo *exposures. The general exposure protocols described previously for ETS and its components (*6,7,11*) were followed here with acrolein. Exposures were carried out in 1.3-m^3 dynamic exposure chambers at the Inhalation Facility of NYU's Nelson Institute of Environmental Medicine. Twelve test cockerels were exposed to 1 ppm acrolein (> 97% pure; Sigma-Aldrich, St. Louis, MO) mixed with HEPA-filtered air, while another 12 were exposed to 10 ppm acrolein mixed with filtered air. Exposures were for 6 hr on 1 day. Twelve control cockerels in adjoining chambers were exposed to HEPA-filtered air alone. There were 14 air changes/hr in each chamber. Air was conditioned to maintain the temperature in each chamber at 21°C. Relative humidity was ambient.

Subchronic* in vivo *exposures. Eight cockerels were exposed to acrolein (> 97% pure; Sigma-Aldrich) mixed with HEPA-filtered air. Eight control cockerels in an adjacent chamber were exposed simultaneously to HEPA-filtered air. The acrolein exposure level selected for these studies was based on the acrolein level in ETS produced during the steady-state combustion of five filtered 1R4F reference cigarettes (THRI). A striking, statistically significant acceleration of plaque development results from exposure to this level of ETS (*6*). The chamber acrolein concentration during this ETS study was 0.52 ppm. This was the targeted steady-state acrolein level for each chamber in the present acrolein study. These subchronic acrolein exposures lasted 6 hr/day, 5 days/week, for 8 weeks.

Steady-state chamber levels of acrolein were monitored via an HPLC method (*42*). Briefly, we used cartridges of silica gel coated with 2,4-dinitrophenylhydrazine for collection of acrolein and HPLC analysis of the hydrazone derivatives. This method has been used to measure levels of aldehydes, including acrolein, in exposure chamber studies of second-hand smoke (*43*).

DNA purification. We extracted DNA from individual abdominal aortas of half the cockerels immediately following the 1-day exposures to 1 ppm and 10 ppm acrolein. We extracted aortic DNA from the other half of the 1 ppm and 10 ppm acrolein-exposed cockerels after sacrifice 10 days later to provide a measure of adduct repair. We isolated aortic DNA from half the air control cockerels at each of these two time points as well. Following humane sacrifice (Nembutal overdose), aortas were excised, cleaned, and frozen in liquid nitrogen. They were stored at –80°C until all samples were collected. Frozen tissue in ice-cold Tris-EDTA (TE) buffer, pH 7.4, was homogenized with a Tissuemizer (Tekmar, Cincinnati, OH) and centrifuged to collect the nuclear pellet. This was resuspended in TE buffer, digested (37°C, 30 min) with RNase A and dialyzed RNase T1 (Sigma-Aldrich), followed by addition of 10% sodium dodecyl sulfate and proteinase K for 30 min more. The sample was extracted sequentially with freshly prepared TE-saturated phenol, phenol/chloroform-isoamyl alcohol (24:1), and chloroform-isoamyl alcohol. DNA was precipitated with 5M NaCl and ice-cold ethanol, washed to remove traces of salt, resuspended in 1.5 mM NaCl, 0.15 mM sodium citrate, and 0.1 mM disodium EDTA and stored at –20°C. DNA purity was assessed at 260/280 µm. Typical DNA yields from the elastin- and collagen-rich aortas were 250–300 µg/g tissue.

Plaque size measurements. We measured plaque size as described previously (*6,7,10,11*). Briefly, after Nembutal overdose of the cockerels, aortas were excised, fixed in buffered formalin, cut into 5-mm segments, embedded, sectioned, and stained with hematoxylin and eosin. Plaque size measurements on coded samples were made blind, via computerized morphometry. The PC-based BioQuant IV (R&M Biometrics, Nashville, TN) software package was used to quantitate plaque sizes. We measured sections from each of the six most distal (beginning at the aortic bifurcation) segments from each aorta because this is where the most pronounced effects of environmental agents on cockerel plaque size have been detected (*10*). Plaque prevalence in the two groups was compared by the chi-square test. For each section, plaque cross-sectional area (if plaque was visible) and the luminal circumference were measured directly in triplicate. We calculated plaque indexes (mean plaque cross-sectional area [mm^2]/mean luminal circumference [mm] × 100) from the means of each triplicate determination for each of the six distal aortic segments from each cockerel. Prior studies have shown that plaque sizes are log-normally distributed (*6,7,10,11*). The skewed plaque sizes were log-transformed for statistical evaluation. To analyze plaque size by group, we plotted values on

log-probability coordinates. Linear regression lines were calculated via least squares analysis and drawn for each data set. We used analysis of covariance to test for differences between the two regression lines.

Detection and measurement of acrolein–DNA adducts. The acrolein-DNA adduct analysis (Figure 1) was carried out via a ^{32}P-postlabeling/HPLC method (*21,44*). Briefly, the first step after enzymatic hydrolysis of the DNA to 3´-monophosphates was an HPLC prepurification on a reversed-phase C18 column (Burdick and Jackson, Baxter Healthcare, McGaw Park, IL) with 1 mM Tris-HCl and methanol:H_2O (50:50) as buffer systems. Fractions with retention times corresponding to acrolein-specific adducts of deoxyguanosine were collected. Before ^{32}P-postlabeling, unmodified nucleotides were removed by treatment with nuclease P1. Unlabeled monophosphates were converted to labeled 3´,5´-bisphosphates by treatment with ^{32}P-ATP and T4 kinase. Adducted bisphosphates were purified on thin-layer chromatography (TLC) plates, excised, extracted, and reconstituted with distilled water. The sample was spiked with authentic acrolein-specific standards as UV markers, and purified first on a reversed-phase system with 50 mM sodium phosphate (pH 5.2) and methanol:H_2O (50:50) as buffer systems, and then on an ion-pair HPLC system using 25 mM triethylamine phosphate and methanol:H_2O (1:1) as buffers. Finally, the purified adducts were analyzed on a reversed-phase HPLC system with 50 mM sodium phosphate (pH 5.8) and methanol:H_2O (1:1) as buffers with detection by a radioflow detector. Co-migration of purified radiolabeled adduct peaks obtained from aortic DNA with the authentic UV standards was considered confirmation of the identity of the *in vivo* adducts (*21,44,45*). The method is sufficiently sensitive to detect 1 nmol adduct/mol guanine in DNA (*44*).

We determined statistically significant differences ($p < 0.05$) in acrolein–DNA adduct levels between acrolein-exposed and air control cockerels in each data set by a one-way analysis of variance with Dunnett's *post-hoc* test (*46*).

Results

Identification of acrolein–dG DNA adducts in cockerel aortas. A typical HPLC chromatogram showing co-migration of the exocyclic acrolein–dG adduct peak (UV and radioactivity) from the synthetic standards and from cockerel aorta DNA is shown in Figure 2. The UV absorbance of the major standard-AdG3, the acrolein-specific 1,N^2-propanodeoxyguanosine adduct, is shown in (*A*). The UV absorbances of two crotonaldehyde-specific adducts, CdG1 and CdG2, for which we did not assay here, also are shown in (*A*). The three rapidly migrating peaks that appear before the AdG3 peak in the standards were not identified. The radioactive peak representing AdG3, the acrolein-specific adduct that was isolated from cockerel aorta, is shown in (*B*). Thus, the acrolein–DNA adduct, AdG3, is formed in cockerel artery wall DNA after *in vivo* exposure to acrolein.

Measurement of acrolein–dG adduct levels in cockerel aortas. The target values for the 1-day (6 hr) acute exposure studies were 1 ppm and 10 ppm, respectively. Steady-state chamber concentrations of acrolein were measured 5 times, at regular intervals, during the 1 ppm exposures and 6 times during the 10 ppm exposures. The actual exposure concentrations (mean + SE) were measured at 0.84 ± 0.27 ppm and 8.7 ± 0.79 ppm, respectively—in both cases about 15% lower than predicted.

All cockerels in the two exposure groups survived the single 6-hr exposures without any apparent deleterious health effects, according to observation of the animals and gross inspection of internal organs at necropsy. The exposure results (Figure 3) reveal that a single 6-hr inhalation exposure to a steady-state of 1 ppm acrolein in HEPA-filtered air was sufficient to increase acrolein–dG adduct levels in aortic DNA by about 5 times over background levels. Adduct levels in the 10 ppm group were slightly but not significantly increased ($p > 0.05$) over

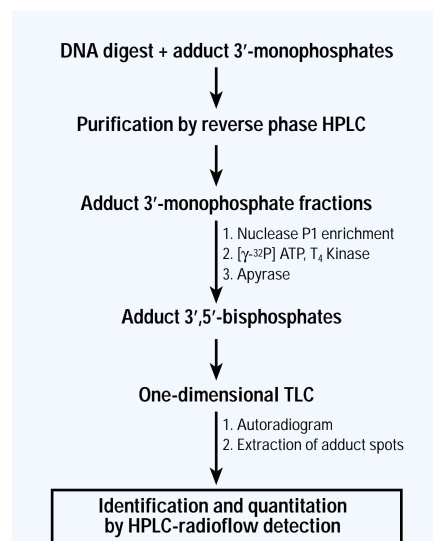

Figure 1. Flow chart for acrolein–DNA adduct analysis.

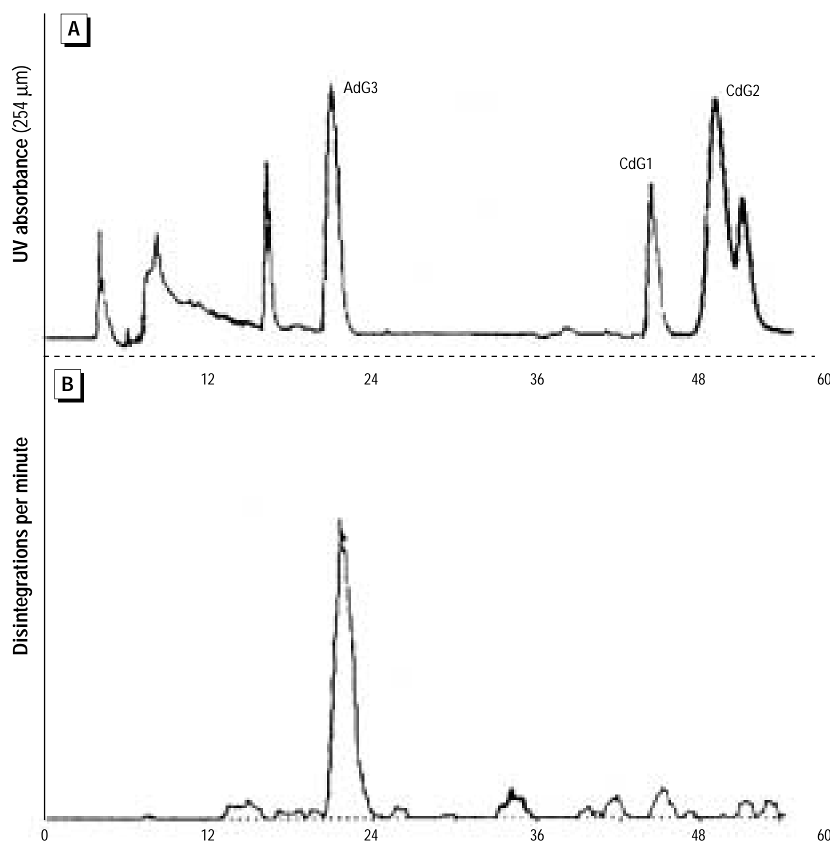

Figure 2. Typical HPLC chromatograms showing co-migration of the single purified radioactive peak, corresponding to AdG3, the cyclic acrolein–dG adduct, from aortic DNA of an acrolein-exposed cockerel (*B*) with AdG3 in the authentic UV standard (*A*). The peaks labeled CdG1 and CdG2 in (*A*) represent standards for crotonaldehyde-specific DNA adducts, which were not investigated in this study.

those in the 1 ppm group. Thus, there likely is a dose dependence for acrolein associated with adduct formation. However, acrolein exposures in the parts per billion range would have to be performed to confirm this.

Disappearance of acrolein–DNA adducts. Ten days after the single 6-hr exposure to either level of acrolein, adduct levels in acrolein-exposed cockerels were reduced to levels comparable to those in control cockerels (Figure 3). There were no differences ($p > 0.05$) in adduct levels between the three groups. Thus, there is evidence for repair of the acrolein-specific DNA adducts.

Plaque development after semichronic inhalation exposure to acrolein. On the basis of the acrolein levels in an earlier ETS study (*6*), we targeted the daily steady-state acrolein concentration in the chambers for the 8-week exposures here at 1 ppm. At this level, all the cockerels survived the 8-week exposures without any adverse health effects. Weight gain over the 8-week exposure period was similar in acrolein-exposed and air control groups. At necropsy, internal organs were grossly normal, with no apparent lesions.

Plaque prevalence was similar for both the 1 ppm acrolein-exposed and air control groups. There were slightly more aortic segments with plaque in the air controls than in the acrolein-exposed cockerels. However, these differences were not statistically significant (chi-square test, $\alpha = 0.05$; data not presented). The log-normally distributed plaque sizes were plotted versus cumulative percentage of plaque-containing segments on log-probability coordinates, as described previously (*6,7,10,11*) (Figure 4). The linear regression lines drawn for each set of values had nearly identical slopes, and there were no significant differences in plaque size between the two groups. Thus, in the absence of other toxicants, 8 weeks of daily (6 hr/day) exposure to 1 ppm acrolein, a moderately high (*47*) but still environmentally relevant dose, are insufficient to accelerate plaque development in a sensitive animal model.

Discussion

Five new findings emerge from the data presented in Figures 2–4. First, the acrolein-specific, 1,N^2-propanodeoxyguanosine adduct, AdG3, has been identified for the first time in vascular tissue. Although the experiments here were not designed to identify the site of acrolein–dG adduct formation, there is clear evidence that acrolein is metabolized by both avian and rodent arterial cells *in vivo* (*18*). Thus, it is reasonable to conclude that long-term exposure to acrolein or to mixtures containing high concentrations of acrolein, such as ETS, will damage vascular wall DNA. Previous studies with mammalian samples have identified three acrolein–DNA adducts. Of the three, AdG3 was the major adduct found in human tissues (*44*) and was the only one detected in this study. This may reflect a difference between avian and mammalian metabolism or might represent a tissue-specific response.

Second, the results suggest a dose response for adduct formation resulting from inhaled acrolein, and, if anything, they overestimate the acrolein levels necessary to elicit damage to vascular wall DNA. Because the mean adduct levels are both elevated and so similar in the 1 ppm and 10 ppm groups (Figure 3), the results strongly suggest that high adduct levels would be registered at acrolein levels even below 1 ppm. Although a single 6-hr exposure to 10 ppm acrolein produced no obvious adverse health effects on the cockerels, 6-hr exposures to 5 and 10 ppm acrolein for 3 consecutive days caused significant morbidity and mortality, respectively (data not presented). Thus, at least in cockerels, 1 ppm acrolein is below the threshold for systemic toxicity as well as sufficient for DNA adduct formation.

In an earlier ETS study (*6*), the steady-state ETS from five cigarettes was distributed to four chambers, each with seven or eight cockerels. The acrolein concentration per chamber in that study was 0.52 ppm. The target acrolein chamber concentrations in the studies reported here were only 2–20 times higher than that, and the measured concentrations were 1.65–16.5 times higher. Thus, these results strongly suggest that significantly elevated acrolein–dG levels will be produced and retained in artery-wall DNA within 1 day of exposure to environmentally relevant levels of ETS.

Third, the strikingly lower levels of AdG3 at 10 days compared to those at 1 day (Figure 3) are consistent with repair of the acrolein-induced DNA damage. Repair was not measured directly. However, cockerels in the 1-day and 10-day groups were exposed simultaneously, in the same set of chambers,

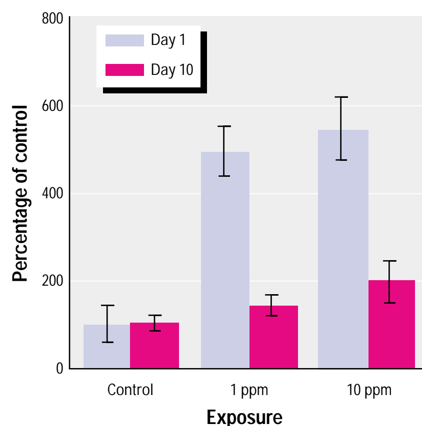

Figure 3. Levels of AdG3, the acrolein–dG adduct, in aortic DNA from acrolein-exposed cockerels on day 1 and day 10 after single 6-hr inhalation exposures to 1 ppm and 10 ppm acrolein. Values are expressed as a percentage of the respective control values at day 1 and day 10.

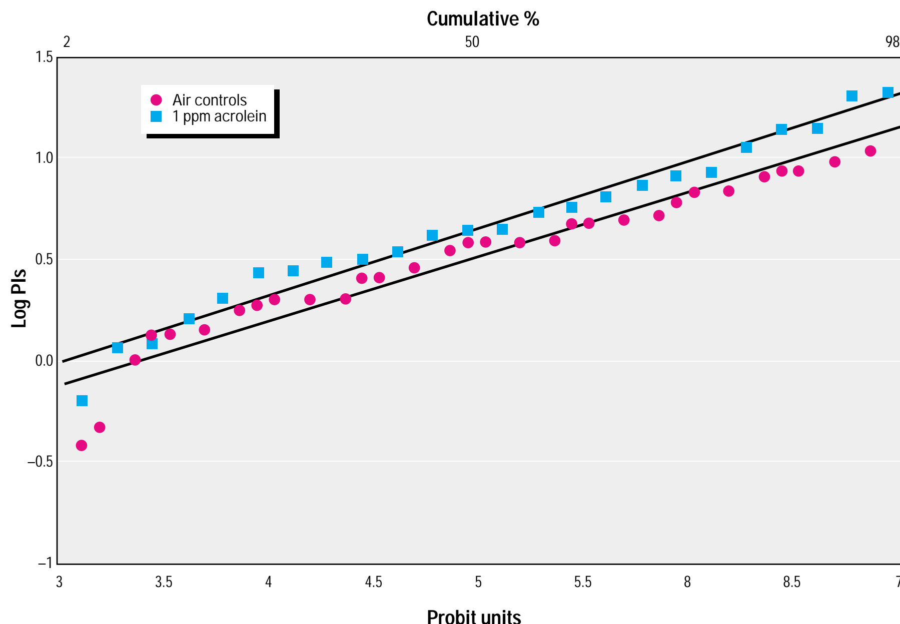

Figure 4. Log-normal distribution of plaque sizes. Plaque indexes (PIs) are plotted versus probit units (lower abscissa) and cumulative percentage of plaque-containing segments (upper abscissa) on log probability coordinates. Linear regression lines calculated via least squares analysis were drawn for each data set. Thirty-two plaque index values are represented on the lower line, and 24 plaque index values are represented on the upper line. The two lines have nearly identical slopes, and there were no significant differences between plaque size distributions in the two groups.

to the acrolein. The striking, statistically significant increases in aortic DNA adduct levels in the 1 ppm and 10 ppm groups compared to controls, which was evident in tissues harvested 6 hr after acrolein exposures ended, contrast strongly with the similarity in adduct levels among the three groups after 10 days. These results imply that the damage to aortic DNA represented by the AdG3 adduct was repaired *in situ* during the 9 days between exposure and sacrifice. Another interpretation of these 10-day results also should be considered: that they are caused, at least in part, by cell death. Large-scale necrosis is likely not involved, because the results from the 8-week exposures revealed no apparent histologic differences between acrolein-exposed and air control cockerels. However, apoptosis in response to the initial acrolein exposure followed by a compensatory cell proliferation also might explain the apparent repair at 10 days and the lack of obvious histologic differences after 8 weeks. Regardless of whether repair of DNA damage or cell death and subsequent proliferation best explains our results, the data indicate that transient exposure to even relatively high levels of acrolein, in the absence of concomitant exposure to other genotoxic agents, will not have long-term genotoxic effects, at least in this animal model.

Fourth, the increased acrolein–dG adduct levels in cockerel aorta DNA *in vivo* confirm and extend earlier *in vitro* findings that avian artery wall cells are more sensitive to treatment with acrolein, or its precursor allylamine, than are rat artery cells (*17*). This is consistent with what is known about susceptibility of animal models to environmentally associated cardiovascular disease. Rat arteries are generally refractory to plaque development, but cockerel arteries are not. Cockerels also are the only animals besides humans in whom exposure-related acrolein–dG adducts have been identified thus far. The one published study of humans reported elevated acrolein–dG adduct levels in the oral tissue of long-term cigarette smokers compared to controls (*45*). In earlier studies, the low but measurable background level of acrolein–dG adducts in both rodent and human tissues were attributed to endogenous lipid peroxidation (*21,44*). This likely explains the background levels of acrolein–dG adducts in cockerels as well.

The fifth finding (Figure 4) was that 8 weeks of daily (6 hr/day) exposure to 1 ppm acrolein had no effect on plaque development in a model system that is very sensitive to the plaque-modulating effects of a variety of chemicals and complex mixtures (*6,11,38*). The acrolein–dG adduct levels in the cockerel abdominal aortas were not measured after the 8 weeks of acrolein exposure because all the tissues were taken for morphometric analysis. However, given the results following the 1-day acrolein exposures, it is reasonable to assume that after 8 weeks of daily acrolein exposure, artery-wall DNA-adduct levels would, if anything, be higher than those found after 1 day. Thus, the presence of the DNA damage represented by the acrolein–dG adducts is by itself insufficient to augment plaque development. Although these results were disappointing, they were not totally unexpected.

In a report describing acrolein carcinogenicity noted above (*19*), acrolein treatment (2 times/week, 6 weeks, followed by uracil feeding) yielded greater numbers of papillomas than were seen in controls. No carcinomas were observed. In the absence of uracil feeding, no increases in papilloma numbers were observed. Thus, by itself acrolein is a weak carcinogen. Acrolein-associated DNA damage was not investigated in that study. Although the mutagenicity of acrolein is well established, our results indicate that, at least *in vivo*, acrolein-associated DNA damage alone is insufficient to elicit long-term adverse health effects in the artery wall. These results suggest that frequent exposure to ETS may lead to persistent artery-wall DNA damage from the acrolein component of ETS and thus provide sites on which other ETS plaque accelerants, such as 1,3-butadiene, can act. In support, a recent report showed that acrolein alone was not nearly as effective as cigarette smoke, which is known to contain a number of potential DNA damaging agents, at inducing DNA damage in human lymphoid cells (*48*). The need for additional DNA damage to augment that provided by acrolein–dG formation was shown in an earlier study where acrolein was mutagenic to fibroblasts from xeroderma pigmentosum patients but not to fibroblasts from healthy controls (*49*). The mutagenic responses and possible health effects arising from combined *in vivo* administration of acrolein and other selected ETS components, such as 1,3-butadiene, should be investigated.

References and Notes

1. National Center for Health Statistics. National Vital Statistics Report, 1999, Vol 47, No 19 (PHS) 99-1120, Deaths: Final Data for 1997. Hyattsville, MD:National Center for Health Statistics, 1999.
2. Shopland DR. The Health Consequences of Smoking. Cardiovascular Disease: A Report of the Surgeon General. Rockville, MD:U.S. Public Health Service, Office on Smoking and Health, 1983.
3. Taylor A, Johnson D, Kazemi H. Environmental tobacco smoke and cardiovascular disease. Circulation 86:699–702 (1992).
4. Wells AJ. Passive smoking as a cause of heart disease. J Am Coll Cardiol 24:546–554 (1994).
5. Steenland K. Passive smoking and the risk of heart disease. JAMA 267:94–99 (1992).
6. Penn A, Snyder C. Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. Circulation 88:1820–1825 (1993).
7. Penn A, Chen LC, Snyder C. Inhalation of steady-state sidestream smoke from one cigarette promotes arteriosclerotic plaque development. Circulation 90:1363–1367 (1994).
8. Zhu B, Sun Y-P, Sievers R, Isenberg W, Glantz S, Parmley W. Passive smoking increases atherosclerosis in cholesterol-fed rabbits. J Am Coll Cardiol 21:225–232 (1993).
9. Gairola CG, Daugherty A. Acceleration of atherosclerotic plaque formation in apoE-/- mice by exposure to tobacco smoke. The Toxicologist 48(1-S):A1401 (1999).
10. Penn A, Keller K, Snyder C, Nadas A, Chen LC. The tar fraction of cigarette smoke does not promote arteriosclerotic plaque development. Environ Health Perspect 104:1108–1113 (1996).
11. Penn A, Snyder C. 1,3-Butadiene, a vapor phase constituent of sidestream cigarette smoke, accelerates arteriosclerotic plaque development. Circulation 93:552–557 (1996).
12. Witschi H, Espiritu I, Marenpot R, Pinkerton K, Jones A. The carcinogenic potential of the gas phase of environmental tobacco smoke. Carcinogenesis 18:2035–2042 (1997).
13. ATSDR. Toxicological Profile for Acrolein. TP-90-01. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1990.
14. R.J. Reynolds Tobacco Company. Chemical and Biological Studies on New Cigarette Prototypes that Heat Instead of Burn Tobacco. Winston-Salem, NC:R.J. Reynolds Tobacco Co.,1988.
15. Nelson T, Boor P. Allylamine cardiotoxicity IV. Metabolism to acrolein by cardiovascular tissues. Biochem Pharmacol 31:509–514 (1982).
16. Awasthi S, Boor P. Semicarbazide protection from in vivo oxidant injury of vascular tissue by allylamine. Toxicol Lett 66:157–163 (1993).
17. Ramos KS, Thurlow C. Comparative cytotoxic responses of cultured avian and rodent aortic smooth muscle cells to allylamine. J Toxicol Environ Health 40:61–76 (1993).
18. Ramos KS. Comparative angiotoxic responses of avian and rodent species *in vivo*: implications in atherogenesis. J Toxicol Environ Health 29:357–376 (1990).
19. Cohen S, Garland E, St. John M, Okamura T, Smith R. Acrolein initiates rat urinary bladder carcinogenesis. Cancer Res 52:3577–3581 (1992).
20. Chung F-L, Young R, Hecht S. Formation of cyclic 1, N^2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Cancer Res 44:990–995 (1984).
21. Nath RG, Chung F-L. Detection of exocyclic 1,N^2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc Natl Acad Sci USA 91:7491–7495 (1994).
22. Smith R, Cohen S, Lawson T. Acrolein mutagenicity in the V79 assay. Carcinogenesis 11:497–498 (1990).
23. Foiles P, Akerkar S, Chung F-L. Application of an immunoassay for cyclic acrolein deoxyguanosine adducts to assess their formation in DNA of *Salmonella typhimurium* under conditions of mutation by acrolein. Carcinogenesis 10:87–90 (1989).
24. Burcham P, Marnett L. Site-specific mutagenesis by a propanodeoxyguanosine adduct carried on an M13 genome. J Biol Chem 269:28844–28850 (1994).
25. Moriya M, Zhang W, Johnson F, Grollman A. Mutagenic potency of exocyclic DNA adducts: marked differences between *Escherichia coli* and simian kidney cells. Proc Natl Acad Sci USA 91:11899–11903 (1994).
26. Adams JD Jr, Klaidman L. Acrolein-induced oxygen radical formation. Free Radic Biol Med 15:187–193 (1993).
27. Yla-Herttuala S, Palinski W, Rosenfeld M, Parthasarathy S, Carew T, Butler S, Witztum J, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 84:1086–1095 (1989).
28. Esterbauer H, Jurgens G, Quehenberger O, Koller E. Autooxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J Lipid Res 28:495–509 (1987).
29. Steinbrecher U. Oxidation of human low density lipoprotein results in derivitization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. J Biol Chem 262:3603–3608 (1987).
30. Kuykendall J, Bogdanffy M. Efficiency of DNA-histone

crosslinking induced by saturated and unsaturated aldehydes *in vitro*. Mutat Res 283:131–136 (1992).
31. McCall M, Tang J, Bielicki J, Forte T. Inhibition of lecithin-cholesterol acyl transferase and modification of HDL apolipoproteins by aldehydes. Arterioscler Thromb Vasc Biol 15:1599–1606 (1995).
32. Chen C, Loo G. Inhibition of lecithin:cholesterol acyltransferase activity in human blood plasma by cigarette smoke extract and reactive aldehydes. J Biochem Toxicol 10:121–128 (1995).
33. Enos W, Holmes R, Beyer J. Coronary disease among United States soldiers killed in action in Korea: preliminary report. JAMA 152:1090–1093 (1953).
34. Freedman D, Newman W III, Tracy R, Voors A, Srinivasan S, Webber L, Restrepo C, Strong J, Berenson G. Black-white differences in aortic fatty streaks in adolescence and early childhood: the Bogalusa Heart Study. Circulation 77:856–864 (1988).
35. PDAY Research Group. Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking. JAMA 264:3018–3024 (1990).
36. Benditt E, Benditt J. Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 70:1753–1756 (1973).
37. Majesky M, Yang H-Y, Benditt E, Juchau M. Carcinogenesis and atherogenesis: differences in monooxygenase inducibility and bioactivation of benzo(a)pyrene in aortic and hepatic tissues of atherosclerosis susceptible versus resistant pigeons. Carcinogenesis 4:647–652 (1983).
38. Penn A, Snyder C. Arteriosclerotic plaque development is "promoted" by polynuclear aromatic hydrocarbons. Carcinogenesis 9:2185–2189 (1988).
39. Minick CR, Fabricant C, Fabricant J, Litrenta M. Atherosclerosis induced by infection with a herpes virus. Am J Pathol 96:673–703 (1979).
40. Hajjar D, Pomerantz K, Falcone D, Weksler B, Grant A. Herpes simplex virus infection in human arterial cells: Implications in atherosclerosis. J Clin Investig 80:1317–1321 (1987).
41. Roivainen M, Viik-Kajander M, Palosuo T, Toivanen P, Leinonen M, Saikku P, Tenkanen L, Manninen V, Hovi T, Manttari M. Infections, inflammation and the risk of coronary heart disease. Circulation 101:252–257 (2000).
42. Tejada S. Evaluation of silica gel cartridges coated in situ with acidified 2,4-dinitrophenylhydrazine for sampling aldehydes and ketones in air. Int J Environ Anal Chem 26:167–185 (1986).
43. Lofroth G, Burton R, Forehand L, Hammond S, Seila R, Zweldinger R, Lewtas J. Characterization of environmental tobacco smoke. Environ Sci Tech 23:610–614 (1989).
44. Nath R, Ocando J, Chung F-L. Detection of 1, N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. Cancer Res 56:452-456 (1996).
45. Nath R, Ocando J, Guttenplan J, Chung F-L. 1,N^2-propanodeoxyguanosine adducts: potential new biomarkers of smoking-induced DNA damage in human oral tissue. Cancer Res 58:581–584 (1998).
46. Zar JH. Biostatistical Analysis. 4th ed. Upper Saddle River, NJ:Prentice Hall,1999;196–200.
47. U.S. EPA Unified Air Toxics Website (Acrolein). Available: http://www.epa.gov/ttn/uatw/hlthef/ acrolein.html [cited 8 August 2000].
48. Yang Q, Hergenhan MA, Weninger A, Bartsch H. Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. Carcinogenesis 20:1769–1775 (1999).
49. Curren R, Yang L, Conklin P, Grafstrom R, Harris C. Mutagenesis *ofxeroderma pigmentosum* fibroblasts by acrolein. Mutat Res 209:17–22 (1988).

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