DNA Replication and Unscheduled DNA Synthesis in Lungs of Mice Exposed to Cigarette Smoke

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ABSTRACT

Mice of the hybrid strain BC3F1/Cum (C57BL/Cum x C3H/AnfCum) were chronically exposed to measured amounts of machine-generated whole Kentucky reference 2A1 cigarette smoke. DNA replication and unscheduled DNA synthesis (UDS) were measured in lung tissue in vitro using a short-term organ culture method. Within one week of beginning smoke exposure, DNA replicative activity, as indicated by incorporation of [3H]-thymidine into total lung DNA, was increased more than two-fold over sham-exposed controls and remained elevated as long as smoke exposure was continued. Treatment of lung tissues in vitro with either the lung carcinogen 4-nitroquinoline-1-oxide or methylmethane sulfonate stimulated UDS, measured as incorporation of [3H]thymidine into lung DNA in the presence of hydroxyurea, presumably as the result of DNA repair activity. Until the 10th to 12th week of smoke exposure, at which time the accumulated deposition of total particulate material in the lung was approximately 40 mg, the level of UDS stimulated by the alkylating chemicals declined to approximately 50% of that seen in lung tissue from sham-exposed control mice. If the mice were removed from smoke exposure, DNA replicative activity returned to normal levels within one week, but the UDS response to DNA damage remained depressed up to five months after ending smoke exposure. The results show that both transient and apparently permanent changes are produced in mouse lung as the result of exposure to cigarette smoke. The role of these changes in lung neoplasia is under investigation.

INTRODUCTION

There is persuasive evidence that, in many cases, the initiating event for neoplasia may be a direct alteration of cellular DNA, i.e., somatic mutation (3, 4). There is also evidence that if the alteration can be repaired, the otherwise expected neoplasia will not occur (6). Thus, if cellular DNA repair capacity is somehow inhibited or reduced, the chance for the retention in cellular DNA of potentially oncogenic alterations is increased.

The results presented here are concerned with the effects of chronic cigarette smoke exposure on enzymatic activities in lung tissue associated with DNA replication and DNA repair. They form a part of a larger study directed toward characterization of the biological and biochemical changes in the lung which culminate in oncogenesis. The present studies have shown that some enzymatic activities were unchanged in lung tissues of chronically smoke-exposed mice, while marked differences were seen in vivo and in vitro in DNA replication and UDS3. While these studies have not established the role of these changes in oncogenesis, they do show that persistent changes in the replication and repair of genetic material are caused by chronic smoke exposure.

MATERIALS AND METHODS

Animals. Female BC3F1/Cum mice (C57BL x C3H/Anf; Cumberland View Farms, Clinton, Tenn.) were purchased at 4 to 6 weeks of age, housed 5 mice/cage in stainless steel cages equipped with plastic fronts and filter bonnets on Bed-O-Cob corn cob bedding (Chesapeake Feed Co., Beltsville, Md.), and allowed free access to Purina Lab Chow and water. Racks containing the animal cages were kept in a room at 21–23° with a light cycle of 12 hr of darkness and 12 hr of fluorescent lights. Upon arrival at Microbiological Associates, the mice were quarantined for 3 weeks and vaccinated against Sendai virus with a vaccine developed at Microbiological Associates. Throughout the experimental exposures, the mice were maintained in a barrier-type facility and were out of contact with other strains to avoid infection with adventitious agents.

Smoke Exposure. Mice were exposed to whole cigarette smoke using a large capacity Smoke Exposure Machine (SEM II and animal containment, Process & Instruments Corp., Brooklyn, N. Y.). More complete descriptions of this system may be found elsewhere (9, 10, 20); however, a brief description will be given here. The SEM II operates in a manner simulating human smoking and automatically and sequentially loads, lights, puffs, ejects, and extinguishes a series of 30 cigarettes over a programmed interval. The standard smoking conditions used were: puff frequency, 1/min; puff duration, 2 sec; average puff volume, 35 ml; and butt length, 23 mm. Cigarette smoke was delivered to the test animals within 2 sec after generation at the cigarette. The particle size has been determined by methylcyanoacrylate fixation and found to be log normally distributed with a geometric mean diameter of 0.34 μm. A smoke-monitoring system was used with the SEM II in order to provide instantaneous puff profiles of the smoke TPM in order to provide documentation of exposure levels and duration of exposure. A monitor provided continuous measurement of TPM through use of an optical-type scattering detector. The detector signals were processed to interface with appropriate safety apparatus to protect animals from overexposure. The signal from the detector was converted to mg TPM by using a corre-
The animal containment system used with the SEM II provides for “nose-only” exposure of the mice. Groups of 5 animals are held in a “stock-type” holder using a combination of a neck slot and restraining neck spring. A chin rest insures that the nose of the animal is aligned with a conical shaped opening on the exposure modules. The nose of each animal passes through a dental rubber dam diaphragm (Process & Instruments Corp.) forming a seal that prevents exposure of the body to smoke aerosol. When adequate air and/or smoke is provided to the exposure module, daily restraint for up to 3 hr (twice per day) resulted in no mortality and no obvious ill effects to the animals.

Kentucky reference 2A1 cigarettes (University of Kentucky, Lexington, Ky.) were used throughout this study. This cigarette type delivered an average of 40 mg TPM, 0.5 mg nicotine, and 19 cc cm carbon monoxide under the conditions of this study. Mice were initially exposed once in the morning and once in the afternoon to two 120-sec exposures of 10% 2A1 cigarette smoke with a 10-min rest between the 120-sec exposures. Exposure was increased daily until, at 10 weeks after the initial exposures, a total of 1500 sec of smoke was being given over 90 min in the morning and again in the afternoon. Mice were then maintained at this level of 3000 sec of smoke exposure per day for the remainder of the study. Dosimetry studies (9, 10) using radiolabeled cigarette smoke particulates indicated that: (a) TPM deposition was quite reproducible, averaging 145 µg/mouse lung/cigarette for the smoke exposure conditions utilized in these studies, and (b) 80 to 90% of the deposited TPM was distributed in the respiratory tract of the mice. The total daily dose of smoke received by the mice in the studies presented here was about 1.4 mg TPM per lung per day.

Control animals were subjected to the same manipulations as the smoke-exposed mice, including sham exposure on an identical smoking machine in the presence of unlit cigarettes. Additional control animals were held without manipulation, to permit estimation of possible stress effects of the exposure procedures.

Chemicals. Fetal bovine serum was from Grand Island Biological Co., Grand Island, N. Y. Radioactive materials [3H]dThd, [3H]BrdUrd, and [14C]MMS were from Schwarz/Mann, Orangeburg, N. Y. Fluoroxyuridine and BrdUrd were from Sigma Chemical Co., St. Louis, Mo. HU and MMS were from ICN Corp., Irvine, Calif. Other chemicals used for buffers, scintillation solutions, and DNA preparations were from a variety of sources and were of at least reagent grade. RNase was from Worthington Biochemical Corp. (Freehold, N. J.).

Assay of DNA Replication and UDS. DNA replication and UDS were measured in vitro by using a short-term organ culture method. Freshly excised lungs were trimmed free of trachea, heart, etc. and washed in ice-cold Dulbecco’s phosphate-buffered saline, and the separated and trimmed lobes from individual lungs (wet weight, 0.6 to 1 g) were minced using sharp scissors or crossed scalpels to pieces of approximately 1 cu mm. Portions (20 mg wet weight) of the minced tissue were incubated in 2 ml Dulbecco’s phosphate-buffered saline, containing 1% fetal bovine serum, and which included [3H]-dThd (5 µCi/ml; 60 Ci/mmol), HU (10⁻² M), fluorodeoxyuridine (10⁻³ M), and either MMS or 4NQO as indicated in the text and table legends. In most experiments, MMS was used as the DNA-damaging agent because of its lack of a requirement for metabolic activation. In some experiments, 4NQO was used to: (a) confirm that the smoke-induced UDS reduction was not limited to MMS damage, and (b) to extend the observations to include a known lung carcinogen (11).

The lungs from different mice were not pooled. Triplicate samples of the minced lungs were prepared for each of the incubation groups; i.e., DNA replication and UDS were determined in individual mice in triplicate. UDS was calculated for each mouse as the difference between the mean value of [3H]dThd incorporation into lung DNA in the presence of HU (2) and MMS or 4NQO minus the mean value of [3H]dThd incorporation into lung DNA in the presence of only HU. The data shown in the tables are the pooled mean values obtained from 3 mice ± S.D. For measurement of DNA replication, HU and the DNA-damaging agent were omitted. After incubation for 2 hr at 37°C in air, the tissue was collected by centrifugation. DNA content and radioactivity were measured by modification of methods described previously (21, 27). In brief, the tissues were digested with 1 N NaOH for 1 hr at 37°C; DNA and some proteins were precipitated by the addition of one-fifth volume 6 N HCl, and after washing twice with 1 M NaCl, pH 2.0, the precipitate was digested with 10% (w/v) HClO₄ for 10 min at 60°C. The absorbance of the supernatant solution at 265 nm gave a measure of the solubilized DNA (21), and an aliquot was taken for radioactivity determination in a scintillation spectrometer. The specific radioactivity was calculated as 3H dpm/µg of DNA.

Extraction of DNA for CsCl Gradient Analysis. In some experiments, [3H]BrdUrd (5 µCi/ml; adjusted to 6 Ci/mmol with unlabeled BrdUrd) was substituted for [3H]dThd. Following incubation for 3 hr, DNA was extracted from the tissues using a modified technique of Marmor (16). The tissues were homogenized in 3 to 4 volumes of ice-cold SSC which contained 1% sodium dodecyl sulfate using 6 to 8 strokes of a Potter-Elvehjem Teflon-glass homogenizer. In order to inactivate nucleases but not denature the DNA, the homogenate was rapidly warmed in a water bath to 55°C and held there for 1 min. After rechilling, the homogenate was shaken repeatedly with equal volumes of CHCl₃:isoamyl alcohol (24:1), until only a very small amount of protein was seen at the interface upon centrifugation (10 min; 200 x g) of the mixture. The aqueous layer was removed and dialyzed for 24 hr in the cold against 100 volumes of SSC, with at least 2 changes of the bath. At this point, the DNA solution was incubated for 30 min at 37°C, with 50µg RNase per ml followed by a repetition of the CHCl₃ treatment and dialysis against SSC as above. Finally, the DNA content was estimated by absorbance readings at 260 nm. Exactly 4.50 ml of each of the DNA solutions which contained no more than 1 mg of DNA was mixed with 5.800 g CsCl so as to give a final specific gravity of approximately 1.700 in a total volume of 6.3 ml. The resulting solutions were centrifuged at 37.5 krpm (Beckman 50Ti rotor) for 60 to 65 hr, and the resulting CsCl gradients were collected into about 20 fractions as described elsewhere (24). The positions of fractions containing replicated (hybrid) DNA and unreplicated DNA were determined by refractive index and absorbance measurements. The fraction containing the bulk of the unreplicated DNA as indicated by absorbance measurements was pooled together with the 2 fractions on either side, and the mixture was dialyzed against...
1000 volumes of SSC for 24 hr. The DNA content was estimated from absorbance readings at 260 nm or by the diphenylamine reaction (26). Radioactivity was measured by scintillation spectrosopy of an aliquot of the solution. Protein contamination was estimated by the method of Lowry et al. (15).

**Assay of Apurinic Endonuclease Activity.** A substrate for this enzyme activity was prepared by incubating a thymine-requiring strain of *Escherichia coli* K-12 (provided by Dr. D. Kingsbury, University of California, Irvine) with [3H]dThd (1 μCi/ml; 2 μg/ml) and isolating the DNA by a modification of the method of Marmur (16). In order to produce apurinic sites, the DNA was alkylated in vitro with MMS (0.3 M; 1 hr; pH 7.0) followed by mild hydrolysis (pH 7.0; 50°; 6 hr) (31). After dialysis, the product could be completely precipitated by 0.5 M HCIO4. Apurinic endonuclease activity was measured in homogenates of lung tissue. The lungs were homogenized in 3 volumes of buffer (0.05 M Tris:0.1 mM EDTA:0.1 mM 2-mercaptoethanol, pH 8.0), and the homogenate centrifuged at 15,000 × g for 20 min. The supernatant solution was removed and dialyzed against 100 volumes of SSC for 1 hr, and the protein concentration was measured (15) and adjusted to 2 mg/ml with SSC. The reaction mixture contained 0.2 mg protein, normal or depurinated *E. coli* DNA having approximately 105 3H dpm, and 0.01 M MgCl2, in a total volume of 0.20 ml SSC. At intervals during incubation at 37°, aliquots of 0.025 ml were taken and mixed with 0.2 mg of salmon sperm DNA which served as carrier. An equal volume of ice-cold 1.0 M HClO4 was added, and the mixture was centrifuged. An aliquot of the clear supernatant solution was taken for radioactivity determination.

Enzymatic activity was measured in terms of the conversion of apurinic DNA to 0.5 M HClO4-soluble fragments with time of incubation at 37°. Control mixtures contained 3H-labeled *E. coli* DNA that had not been treated with MMS but otherwise treated as the alkylated DNA.

**In Vivo Alkylation of Lung DNA with [14C]MMS.** BC3F1/Cum sham- or smoke-exposed mice were given a single intratracheal instillation of 5 μCi [14C]MMS (0.52 mCi/mmol) in 0.02 ml of 0.9% NaCl solution. At 2 and 24 hr posttreatment, sample animals were killed with an overdose of sodium pentobarbital, and lungs were removed and immediately frozen in glass vials immersed in a solid CO2:isopropanol bath. Frozen tissues were stored on solid CO2 or liquid N2 until DNA extraction. DNA was isolated and purified by the methods described above and finally by centrifugation in CsCl. After dialysis of the CsCl gradient fractions containing the DNA against SSC, absorbance (260 nm) and radioactivity measurements were used as described above to determine the specific radioactivity which served as an estimate of the extent of DNA alkylation.

**RESULTS**

**DNA Replication and DNA Repair in Tissues of Smoke-exposed Mice.** UDS and replicative DNA synthesis data are presented in Table 1 for mice exposed to whole cigarette smoke for periods of 1 to 17 weeks. The averages of dpm values obtained from 3 animals/time point indicate that both UDS and replicative DNA synthesis appeared to be increased after the first week of exposure to cigarette smoke. At Week 4, however, UDS was depressed and appeared to remain depressed through 17 weeks of smoke exposure. Regression analysis showed that the UDS values from smoke-exposed mice decreased significantly with time (p = 0.03), while the UDS values from sham-exposed mice did not change significantly with time (p = 0.18). DNA replicative synthesis in lung tissue of smoke-exposed mice was stimulated after the first week and remained at a constant elevated level over the sham controls for the entire 17-week period.

Data in Table 1 are also presented as the ratio of UDS and replicative DNA synthesis in the smoke- and sham-exposed animals. The smoke:sham-exposed UDS ratio declined after the first week and continued to decline significantly with time (p = 0.03) reaching 0.47 by 17 weeks. The smoke:sham-exposed DNA replicative synthesis ratio did not change significantly (p = 0.13) with time during the observation period, suggesting that the elevated replicative DNA synthesis resulting from smoke exposure remained unchanged.

**Persistence of Depressed UDS.** DNA replication and UDS were measured in lung tissue of mice exposed in a separate study for up to 35 weeks. At the termination of smoke exposure, the mice were housed at Microbiological Associates under usual conditions with no further treatment. At 5 months postexposure, sample groups were assayed for UDS and DNA replication in lung. Table 2 shows that, by 5 months postexposure, replicative DNA synthesis was not different between smoke- and sham-exposed mice, but UDS in the smoke-exposed mice was about one-half that seen in the controls. A repeat assay using mice from the same experiment 2 weeks later gave the same result. The results of this experiment and results found with mice exposed for even longer periods have indicated that

### Table 1

**UDS and DNA replication in lung tissue in vitro from BC3F1/Cum mice exposed to 2A1 cigarette smoke**

<table>
<thead>
<tr>
<th>Wk</th>
<th>mg TPM/lung</th>
<th>Smoke-exposed UDS 3H dpm/μg DNA</th>
<th>Sham-exposed UDS 3H dpm/μg DNA</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>25 ± 63</td>
<td>189 ± 17</td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
<td>66 ± 12</td>
<td>101 ± 20</td>
</tr>
<tr>
<td>6</td>
<td>4.3</td>
<td>123 ± 28</td>
<td>138 ± 15</td>
</tr>
<tr>
<td>8</td>
<td>26.4</td>
<td>94 ± 45</td>
<td>125 ± 30</td>
</tr>
<tr>
<td>11</td>
<td>45.2</td>
<td>101 ± 22</td>
<td>176 ± 32</td>
</tr>
<tr>
<td>15</td>
<td>70.2</td>
<td>89 ± 15</td>
<td>131 ± 32</td>
</tr>
<tr>
<td>17</td>
<td>82.7</td>
<td>119 ± 23</td>
<td>254 ± 19</td>
</tr>
</tbody>
</table>

**DNA replicative synthesis**

<table>
<thead>
<tr>
<th>Wk</th>
<th>mg TPM/lung</th>
<th>Smoke-exposed</th>
<th>Sham-exposed</th>
<th>Smoke: sham</th>
<th>Sham: sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>1838 ± 615</td>
<td>622 ± 208</td>
<td>2.95</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
<td>927 ± 160</td>
<td>369 ± 102</td>
<td>2.51</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.3</td>
<td>963 ± 127</td>
<td>556 ± 97</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>26.4</td>
<td>1240 ± 13</td>
<td>581 ± 116</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>45.2</td>
<td>1300 ± 172</td>
<td>737 ± 202</td>
<td>1.76</td>
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<tr>
<td>15</td>
<td>70.2</td>
<td>1237 ± 92</td>
<td>1082 ± 226</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>82.7</td>
<td>1532 ± 46</td>
<td>696 ± 365</td>
<td>2.19</td>
<td></td>
</tr>
</tbody>
</table>

* TPM dose estimated from quantitative dosimetry results (9, 10).
Confirmation of Alkylation-induced UDS as DNA Repair Replication. Cell culture studies have shown that excision-type DNA repair replication consists of insertion of single-stranded runs of nucleotides in previously existing double-stranded DNA (24, 25). One method of demonstration of repair replication involves substitution of the dThd analog, BrdUrd, during the period when repair is underway. When [3H]BrdUrd is used, it is incorporated into both newly replicated double-stranded DNA and the relatively short runs of nucleotides inserted during repair replication. The presence of BrdUrd increases the buoyant density of the DNA molecules which undergo normal semiconservative replication but does not significantly change the buoyant density of those molecules which incorporated [3H]-BrdUrd as the result of repair replication. This is because the runs of nucleotides incorporated during repair replication are very short in relation to the size of DNA molecules isolated from the cells or tissue for analysis (25).

Lungs were removed from 3 mice exposed to smoke for 17 weeks and from their corresponding sham-exposed controls. After incubation of the pooled minced lungs with 10⁻⁴ M 4NQQ, 10⁻² M HU, and [3H]BrdUrd for 3 hr, the DNA was isolated and analyzed using CsCl gradient methods as described above. Since HU was present in the incubation mixture, normal DNA replication was inhibited. The extent of DNA repair replication was quantitated by determining the specific radioactivity of the DNA banding in the region of the CsCl gradient corresponding to normal density (i.e., unreplicated DNA). The results are given in Table 3. They confirm that DNA repair replication can be observed using these methods and also show the same relative difference in UDS between tissues from smoke-exposed and control mice which was seen using the HClO₄ extraction procedure.

Repair of DNA Alkylation In Vivo. A major question was whether the UDS-reduction shown in vitro was also present in the intact animal. This question was approached by measuring the change in specific radioactivity of lung DNA with time after intratracheal instillation of [¹⁴C]MMS. Groups of 3 animals from smoke-exposed (36 weeks; 201.4 mg TPM/lung) and control groups were killed at 2 and 24 hr following [¹⁴C]MMS instillation. The lung DNA was isolated, and radioactivity was measured as described in “Materials and Methods.” The radioactivity associated with the DNA was assumed to be the result of base alkylation, probably largely at the 7 position of guanine and the 5 position of cytosine (29). However, this has not as yet been confirmed by hydrolysis of the DNA and chromatography of the nucleosides. Table 4 provides evidence that the reduced UDS (presumed to be repair) seen in short-term organ cultures of lung tissue can also be demonstrated in vivo. At 2 hr, the specific radioactivity of DNA was the same in lungs from both the smoke-exposed and control animals. However, 24 hr later, the specific radioactivity of the DNA from control mouse lung had declined by more than 60% but that of the smoke-exposed lung had changed little. This result has been confirmed in a repeat experiment. It is also of interest that essentially the same result was observed when the [¹⁴C]MMS was given i.p. (data not shown).

Assay of Apurinic Endonuclease Activity. Homogenates of lung tissue were incubated with [³H]-labeled apurinic E. coli DNA prepared as described. The results of a typical experiment showing the conversion with time of the labeled DNA to acid-soluble fractions are presented in Chart 1. Homogenates from smoke-exposed or control lung tissues were equally active in showing the conversion with time of the labeled DNA to soluble fractions during the incubation period. This assay was repeated 5 times using lung tissue from mice exposed to smoke for various periods with always the same results.

DISCUSSION

Evidence for the preventive role of DNA repair in oncogenesis has been obtained primarily from studies of hereditary human disease in which exist both a predisposition to cancers of various types and demonstrable abnormalities in DNA repair activities (13, 22). A major difficulty in the experimental demonstration of the role of DNA repair in carcinogenesis is that there are no known animal models which resemble the DNA repair-deficient diseases in humans. Thus, it has not been possible to show unequivocally that inhibition of the DNA repair contributes to oncogenesis.

Cigarette smoke is a complex biological material that con...
Inhibition of UDS during smoke exposure was progressive and became very obvious after the 11th week of exposure (Table 1). Cumulative deposition of at least 40 mg TPM had occurred before this inhibition of UDS capacity was observed. That this effect was not reversible was indicated by studies of mice taken off smoke after long-term exposure (Table 2).

There are many possible explanations for the observation of decreased UDS capacity, only some of which have been tested in the present study. It does not seem to result from decreased accessibility of lung DNA to damage by external agents. This was shown by the experiments in which \[^{14}C\]MMS was instilled intratracheally or injected i.p. In both cases, the extent of alkylation of lung DNA was similar in smoke-exposed and control mice. The loss of labeled \[^{14}C\]MMS from control lung DNA with time has been interpreted in this study as the result of a repair process and the retention of label in lung DNA from smoke-exposed mice as a deficiency in repair, induced by the chronic smoke exposure. A large fraction of \[^{14}C\]MMS-induced labeling of DNA is thought to be in the form of N-7-[\[^{14}C\]\]-methylguanine, which is subject to chemical hydrolysis, and it is difficult to explain how smoke exposure could affect this hydrolysis. Since there is evidence for the enzymatic removal of some improper methylated bases in DNA (14), it may be that N-7-methylguanine is subject to such enzymatic attack as well as chemical hydrolysis at the glycosidic bond. In either case, the resulting apurinic site would stimulate DNA repair leading to detectable UDS. In the present study, apurinic endonuclease activity was not different in homogenates of lung from smoke-exposed and control animals. However, this did not indicate whether the damaged DNA was equally accessible to repair in both smoke-exposed and control lung but only that this soluble enzyme activity was not affected by the smoke exposure.

A decrease in \[^{3}H\]dThd incorporation might be due to lowered dThd kinase activity or other toxic effect that indirectly affects DNA synthesis. However, the apparent increase in DNA replication in smoke-exposed lung makes this an unlikely explanation. Preliminary tests indicated that specific activities of NADH-dependent cytochrome c reductase and dThd kinase were the same in smoke-exposed and control lung, as were DNA:protein ratios (data not shown).

Two hypotheses are suggested to explain the observed reduced pulmonary UDS seen in smoke-exposed mice. Either an overall reduction in UDS capacity of those cells normally capable of repair has occurred, or there has been an accumulation of a stable population of cells that has a substantially reduced repair capacity. If the decreased UDS capacity was the result of an intracellular accumulation of toxins in a cell population capable of further proliferation, then it might be expected that recovery of UDS capacity would occur some time after smoke exposure was stopped as the toxins became diluted out. This was not observed. Therefore, it would seem that some kind of stable change has occurred in the lung.

Histological observations by others (18) of lungs from smoke-exposed hamsters and by us5 of lungs from long-term smoke-exposed mice have revealed aggregations of what appear to be alveolar macrophages swollen with particulate materials,

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often having very small almost pyknotic nuclei. These clumps of macrophages persist when smoke exposure has stopped. We have not yet quantified these clumps in mouse lung to determine their relative proportion to other lung cells and whether they may contribute to a significant fraction of the total lung DNA. It is not known whether these macrophages are capable of UDS. In cross-sections of lung from mice exposed to 2A1 cigarette smoke for 20 to 30 weeks, several of these aggregations are seen in every section. The choice between the above alternatives must await the results of autoradiographic studies which are presently underway to quantify UDS capacity in individual cell types after in vivo and in vitro treatment with DNA-alkylating agents. These studies may provide a cellular explanation for the observed effects of cigarette smoke on DNA replication and repair in intact lung.

Whether the accumulation of unrepaired or unreparable DNA in lung (or other tissue) has any relevance to oncogenesis cannot be determined from these experiments. Although the strain of mice used in these studies is susceptible to lung carcinogenesis by polynuclear hydrocarbons administered intratracheally (8), no lung tumors have been observed to date which could be attributed to exposure to cigarette smoke alone (7). Experiments in which carciogenic hydrocarbons are administered to mice showing reduced UDS in the lung as a result of smoke exposure have yet to be completed.

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