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FINAL REPORT

Study of the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs

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September 1, 1978

Prepared for
National Institute of
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INTRODUCTION

When mining of uranium-bearing ores began in the United States (1898)[1] it was known that radioactivity was associated with the ore, but no health hazards from this activity were suspected. In 1921, Uhlig[2] suggested that the large numbers of lung cancers found among European uranium miners might be due to ionizing radiation.

Lorenz[3] reported (in 1944) the growing conviction in the U.S. and abroad that the radiation emitted by radon that was inhaled for long periods of time, or that emitted from its daughters that were deposited in the lungs, might cause cancer of the lung in man. This conviction was based on the fact that primary carcinoma of the lung accounted for about 50% (reported rates ranged from 30 to 70%) of the deaths of the miners of Schneeberg (Germany) and Joachimsthal (Czechoslovakia).[4] As early as the Middle Ages, premature deaths among the miners in Joachimsthal were noted by a physician, who observed that miners' wives often married 5 or 6 times.[5] Since it was not known at that time what caused the miners' deaths, the cause was simply referred to as "miner's consumption." Much later (in 1879), Harting and Hesse[3] discovered, in the course of autopsies of 20 miners from Schneeberg, that the cause of death was uncontrolled lung tumor growth, which they thought was lymphosarcoma. In 1882 the tumors were identified as carcinomas.[5] Since then, many other investigators have found lung cancer among European uranium miners. In his report,[5] Pacina states that Sikl, et al., for example, found lung cancer to be the cause of death in 53% of the cases studied in the

years from 1929 to 1939. At that time, the average life expectancy of miners was 46 years, 14 years less than the average in the control population.

According to Pacina,[5] after World War II the European production of uranium increased tremendously, as it did in the U.S. Because of the tremendous influx of new employees, the number of deaths due to lung cancer was small, and drew little attention. However, in the late '50s, when the employment situation stabilized and the work force consisted of more long-term employees, the number of lung cancer cases observed began to increase. This gradually increasing incidence was attributed by Pacina to the induction-latency period in the onset of the disease.

Historically, the materials that have been mined are silver, uranium, copper, iron, cobalt, arsenic, bismuth and nickel.[6] Consequently, miners have been exposed to a considerable variety of materials, some of which are carcinogenic, although not necessarily to the lung. According to Albert,[7] most European miners lived far from the mines and had to walk for several hours across the mountains in the cold to get to work. The mines were deep, very damp and usually quite cold. And because the work was very hard, the miners were drenched with sweat when they left the mines for the long walk back to their homes. In addition, they suffered from malnutrition. It is not surprising, therefore, that the incidence of chronic lung disease was very high. In earlier times, pneumoconiosis from exposure to mine dusts associated with arsenic and cobalt was assumed to be the cause of the respiratory cancers, but there

is little question today that the radioactivity of the ore (in the form of airborne radon daughters) is the primary cause.

Even though mining of uranium-bearing ores began in the United States in 1898, this mining was on a very small scale until domestic uranium mining for nuclear weapons began in 1948.[4] The first reported death by respiratory cancer among U.S. uranium miners appeared in 1945,[8] but it was not until 1963 that sufficient additional deaths had been observed to confirm the excess risk from respiratory cancer noted during the intervening time.[9,10]

Following World War II, the Atomic Energy Commission encouraged the mining of uranium ores, resulting in a relatively rapid, large-scale expansion of uranium mining in the United States. In 1949, a formal request for a health study of uranium miners and millers was submitted to the Surgeon General of the U.S. Public Health Service by the Colorado State Health Department and by several companies engaged in the mining and processing of uranium ore.[11] This request received its stimulus from knowledge that 1) radiation was capable of inducing tumors in humans and animals (the European mining experience, in particular, lent credence to this notion--the field of radiobiology was well established by this time); and 2) that mines containing uranium ore had measurable amounts of airborne radioactivity. A pilot study, conducted in 1949, had also indicated a need for assessing the scope and nature of radiation hazards to uranium miners.[11] Because of these observations, the U.S. Public Health Service, in cooperation with Federal and State agencies,

initiated a program in 1950 to evaluate the health problems associated with the uranium mining industry.

The finding that uranium miners had proportionately more tumors of the small-cell, undifferentiated type was established as evidence for the causal role of airborne radioactivity.[12] The demonstration of an exposure-response relationship between airborne radioactivity and the incidence of respiratory cancer, as well as the exclusion of age, smoking, national origin, heredity, urbanization, self-selection and prior mining of hard rock or other ore constituents (including silica dust) as confounding factors, largely dispelled any remaining uncertainty as to the cause of the U.S. uranium miner problem.[13,14]

In spite of these studies, the role of cigarette smoking in respiratory cancer of uranium miners remained an enigma. A higher prevalence of lung cancer among cigarette smokers has been noted time and again in the literature, suggesting that tobacco smoking is the most important factor in the development of atypias of the respiratory epithelium and lung cancer among both miners and nonminers. Although this is supported by some mortality observations,[15] an early sputum cytology analysis indicated that radiation exposure in uranium mines was the most important factor.[16] A more recent sputum cytology analysis[17] supports the mortality data compiled by the National Institute for Occupational Safety and Health/National Institute of Environmental Health Sciences (NIOSH/NIEHS)[4], which show that age at which uranium mining is begun and cumulative radiation exposure are the paramount factors influencing incidence and age at appearance of bronchogenic carcinoma among miners.

A most comprehensive report on the epidemiology of U.S. uranium miners appeared in 1973.[18] All indications and implications of previous analyses were corroborated and strengthened, and many of the environmental factors and possible biases in the data were treated further. The conclusions were:

1. "A statistically significant excess of respiratory cancer was observed among uranium miners at each cumulative radiation exposure category down to and including 120-359 WLM.[a]
2. "A review of analyses devoted to exploring possible errors and factors, which might alter the exposure-response relationship, revealed that there were some which might distort the shape of the curve, but none that seriously affect the conclusion that there is a radiation-caused excess of lung cancer in the 120-359 and higher WLM categories. Most of the possible errors and distortions would indicate, rather, that the risk at low cumulative WLM may be underestimated.
3. "Cigarette smoking, diesel smoke and exposure to miscellaneous dusts may have contributed to the development of lung cancer in some miners by affecting the distribution of radon daughters within the lung or by acting as promoting agents. Cigarette smoking should be discouraged among uranium miners, as among other people, and other noxious agents in mine atmospheres

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[a] The Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3×10^5 MeV of potential alpha energy. The Working Level Month (WLM), as used in this report, is an exposure for 170 hours to a 1-WL concentration.

should be reduced to a minimum. Permissible levels for radon daughters must be based on data derived from the environment in which miners continue to work and live.

4. "Continued appearance of respiratory cancer cases in the Study Group indicate that the 'epidemic' among uranium miners is not subsiding at this time."

Shortly after the appearance of the mortality analyses, the Federal Radiation Council (FRC) began its review of the radiation hazards in uranium mining. This, as well as many previous and subsequent reviews, were attempts to reach a consensus as to the permissible radiation exposure for miners. FRC Report No. 8, revised,[1] states that animal experiments had demonstrated that doses of ionizing radiation delivered to the lungs may reduce pulmonary dust clearance, produce emphysema, cause loss of pulmonary function, and cause pulmonary neoplasia, fibrosis, or changes in bronchial epithelium. It was concluded that, despite several attempts, it had not been generally possible to systematically produce pulmonary carcinomas in animals from controlled exposure to radon daughters. In an interim review of pre-1971 literature on the biological effects of radon and radon daughters, Richmond and Boecker[19] conclude:

> "The literature on the biological effects of radon and its progeny has not, to this time, provided firm evidence on the dose-response relationship between the inhalation of radon and daughters and the subsequent development of lung tumors in experimental animals."

Had these reports been written later, some of the positive conclusions would have been somewhat softened: more recent studies have produced squamous carcinoma, as well as a wide variety of other severe lung lesions, in experimental animals that received daily inhalation exposures to radon daughters and other uranium mine air contaminants.[20,21] Such experiments have yielded valuable information on the effects of inhaling radon and other agents such as ore dust, and diesel and cigarette smoke. Once the relationships become established in animals, extrapolation of these results to man will require great care. Parker[22] has stated:

> "No matter how compelling the animal results, they would be suspect for application to cancer induction in humans. This arises from the growing belief that radiation carcinogenesis requires not only some radiation dose, but some as yet unidentified environmental factor. In man, such a factor could apply in the smaller bronchi but not in the bronchioles, trachea, or nasal sinuses. It could apply equally to any component of the human tracheo-bronchial apparatus vis-à-vis an animal. Nevertheless, components of the total problem can surely be attacked with confidence through animal systems. As an example, the main thrust of the attack on synergistic interactions between radiation, diesel smoke, tobacco smoke, and other irritants must be done through animals...because animals can be sacrificed, there can be a systematic search for progressive changes in irradiated lung tissue."

Therein lies the utility of animal experimentation in investigation of lung diseases.

The experiments described in this report were designed to elucidate the role of many of the factors implicated in radiation carcinogenesis and other associative lung diseases in man. The work has been cosponsored by DOE (formerly ERDA and AEC) under the title, "Inhalation Hazards to Uranium Miners"; and by NIEHS under the title, "Research on the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs." The two projects were integrated into one experimental effort, at the request of the two funding agencies, to insure maximum effort and efficiency.

These interdependent experiments involved life-span exposures of beagle dogs and Syrian golden hamsters to characterized aerosols of the potentially carcinogenic air contaminants, singly and in combination, that are believed to be the primary causative agents of pulmonary pathology seen in uranium ore miners. Part I of the report describes the experiments utilizing hamsters; Part II, those utilizing beagle dogs.

PART I - HAMSTER STUDIES

A. Methods

The experimental design for the hamster studies is shown in Table 1. In a preliminary study, sponsored by the AEC, hamsters were exposed to levels of radon and radon daughters (with and without the presence of uranium ore dust) that produced only limited pulmonary change, no life-span shortening, and no modification of body weights in the exposed animals when compared to controls. For the present studies, it was decided to raise the radon and radon daughter exposure levels in order to increase the severity of pulmonary pathology, hopefully to a point where neoplasia might develop.

1. Exposure Chambers

The six controlled-atmosphere test chambers used in this study are plexiglass spheres, 178 cm in diameter, with a volume of 3000 liters (Figure 1). Room air enters at the top of each sphere, is drawn through an opening at the bottom to a rotameter and flow control valve, and then exhausted through a filter. The inlet is a 10-cm lucite pipe fitted with a conical plastic end plate. Air enters the chamber through a series of holes around the circumference of this pipe above the end cap, and is dispersed by a small fan suspended below the inlet (in the case of the 2 chambers using diesel exhaust). Normal diffusion was adequate to disperse the air, dust and/or radon in the other four chambers. Radon was admitted

TABLE 1. Experimental Design for Hamster Studies

Group No.*	No. of Animals**	Exposure Chamber Contents
1	102	Room air
2	102	Radon and radon daughters
3	102	Radon, radon daughters, and uranium ore dust
4	102	Uranium ore dust
5	102	Diesel engine exhaust
6	102	Diesel engine exhaust, radon, radon daughters and uranium ore dust

* Groups 1-4 were supported by the AEC; groups 5 and 6 by NIEHS.
** Fifty-four hamsters were added to each group 16 months after the start of exposures.

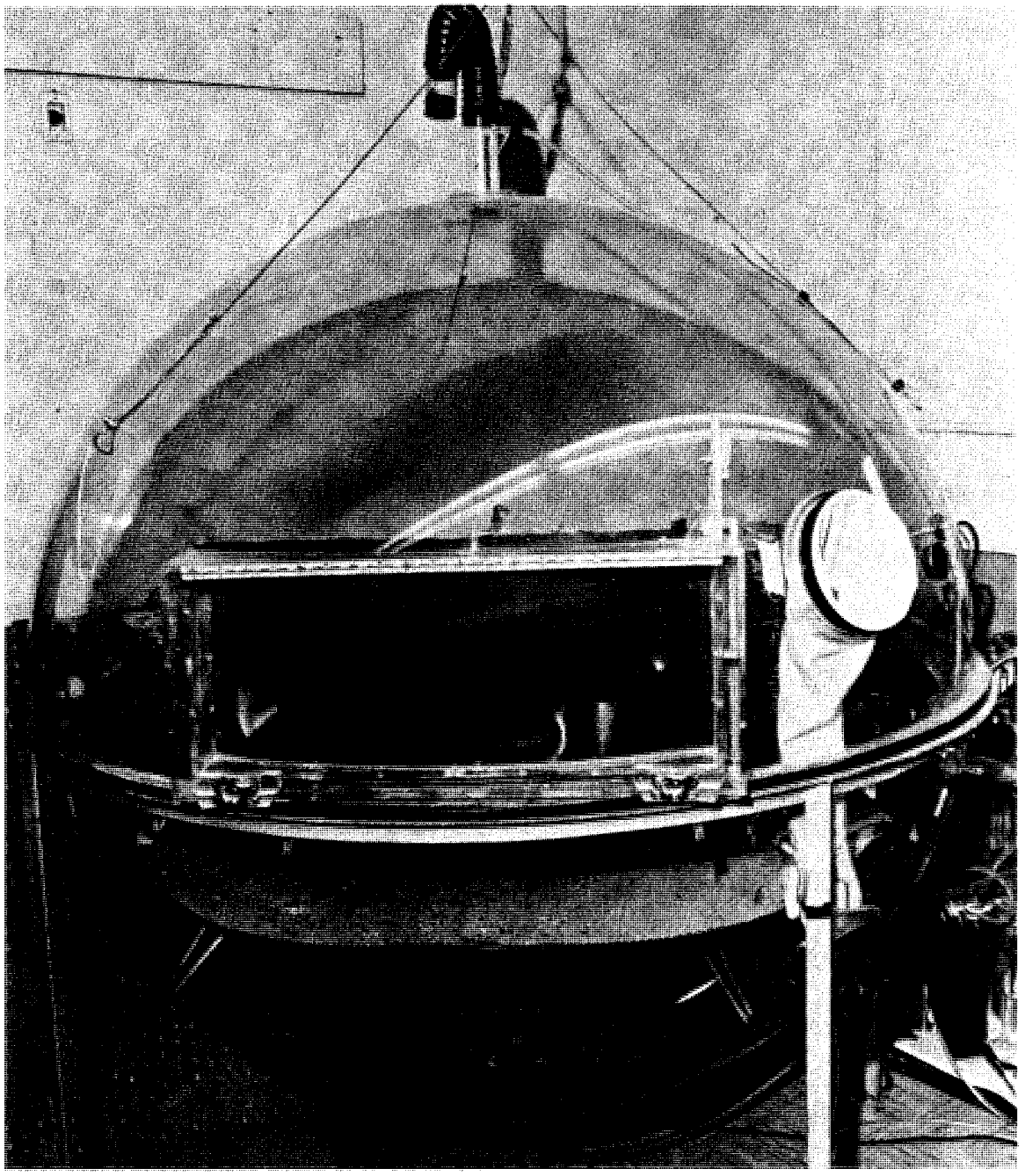

FIGURE 1. Spherical, Plexiglass Exposure Chamber

to chambers 2, 3 and 6 through fittings near the top of the inlet pipes. Radon was generated separately for each of the three chambers by flowing compressed air through a prehumidifying buffer solution, a radium solution trap, the radium solution itself, a second trap, then a final membrane filter mounted at the chamber inlet fitting. Each of the three radon generators is housed in a glove box for contamination control. Radium concentrations were adjusted to maintain radon concentrations in the chambers between 210 and 250 nanocuries/liter, at a flow rate through the chambers of 50 liters/minute. This was sufficient to maintain the radon daughter concentration at a level of about 800 WL in chambers 3 and 6. In chamber 2, which contained only room air aerosols and added radon, the unattached fraction of RaA was relatively high, causing a loss of radon daughters from diffusion to exposed surfaces. In an attempt to maintain the desired working levels of radon daughters, the flow rate through the chamber was reduced. Although this resulted in Rn levels between 265 and 310 nCi/ℓ, the radon daughter concentrations still averaged less than 700 WL.

Three chambers were equipped with Wright Dust Feed Mechanisms* (WDFM), which added carnotite ore dust to the inlet air stream in the 10-cm inlet pipe. The uranium ore had been broken up with a hammer mill and then ground for 60 hours in a rubber-lined ball mill with borundum rods. The resulting dust was compacted at 2,000 psi in the dust containers of the WDFM.

Chambers 5 and 6 were equipped to supply diluted diesel engine exhaust to the incoming air. The source of this exhaust was a 3-cylinder, 43-bhp diesel engine, driving a 15-kilowatt generator connected to a

* L. Adams, Ltd., Minerva Rd., London England

series of resistance coils. To simulate typical operating patterns of such engines in mines, a reversible motor was attached to the generator's voltage-controlling rheostat, and a solenoid was connected to the engine's governor, in order to vary both the load on the engine and its speed. Mode-regulating microswitches controlling the reversible motor and the solenoid were adjusted to provide the following speed-load cycle: the engine idled at 1400 rpm for 20 sec; accelerated to 1800 rpm with no load and held at this speed for 20 sec; decelerated to 1400 rpm as a load of approximately 9 kilowatts was applied, and held there for 80 sec; accelerated to 1800 rpm as the load was removed, then decelerated to 1400 rpm to start the cycle again. The engine was accelerating or decelerating during approximately half of each 4-minute cycle.

As shown in Figure 2, a portion of the engine's exhaust was diluted with compressed air, passed through a stainless steel surge tank and an orifice meter, then divided into two streams leading to the two exposure chambers. Just before entering the chambers, a controlled amount of compressed air was added to each exhaust stream through motorized needle valves. The level of "control air" for each exhaust stream was adjusted in response to the CO level in the chambers as measured by an infrared photometer that sampled each chamber during alternate 2-minute periods. Chamber pressures were regulated to approximately 1.3 cm of water below atmospheric pressure, and total flow through each of the 3,000-liter chambers was adjusted to 50 liters/min with by-pass valves.

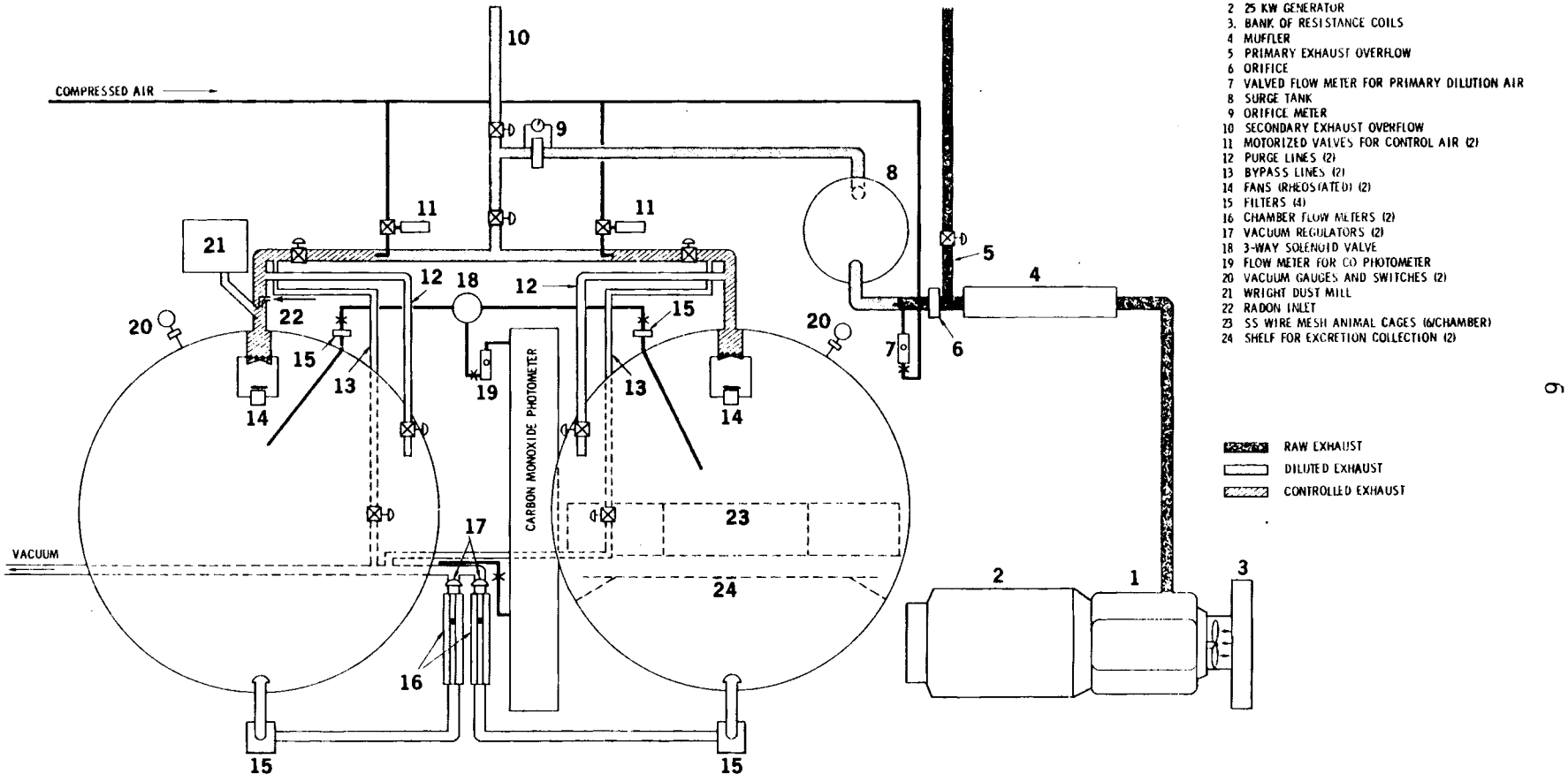

FIGURE 2. Diesel Exhaust Exposure System

Initial analyses for NO_2 levels in the chambers showed CO/NO_2 ratios of 2:1. These high levels of NO_2 may have been caused by oxidation in the surge tank, where the raw exhaust is initially diluted with air. In order to maintain the CO level at the desired 50 ppm and lower the NO_2 concentration to less toxic levels, the engine was modified to yield a higher fuel-to-air mixture to increase the CO level in the exhaust. This was accomplished by filing the fuel-injector tips to cause decreased combustion efficiency in the cylinders, thereby increasing the CO content of the engine exhaust with no increase in NO_2 content. This modification, which simulated a "worn" or out-of-time engine, resulted in an NO_2 level in the exposure chambers about one-tenth that of the CO.

Minor modifications were made in the diesel engine exhaust exposure system during the course of the study. An orifice was installed in the raw exhaust line upstream of the surge tank to reduce fluctuations in exhaust flow during various modes of engine operation, thus stabilizing CO levels in the chambers. A refrigerated aftercooler-dryer, placed in the compressed air line that supplied dilution air, served to reduce chamber relative humidity to <80% and temperature to <78°F. The diesel system operated satisfactorily for more than two years, providing automatically regulated exhaust for exposure of hamsters. Chamber air concentrations of carbon monoxide during each day's 6-hour exposure period were controlled to 50 ± 3 ppm. The NO_2 content of the chambers ranged from 4 to 6 ppm, while SO_2 and aliphatic aldehyde levels remained below our 1 ppm detection level.

The radon levels in chambers 2, 3, and 6 were monitored automatically from a sample port about 2/5 of the way from the top of the chamber. The monitor consisted of a flow-through scintillation flask fitted with a prefilter. Radon from one chamber was drawn through the flask continuously for 10 min and the level of alpha radioactivity was monitored with a strip chart recorder. After the 10-min sampling period, the inlet to the scintillation flask was automatically valved to flush room air through it for 10 min; another test chamber was then sampled for 10 min. The cycle was repeated to measure the radon content of the three test chambers containing radon. The scintillation flask was cross-calibrated against "grab" ionization chamber radon measurements.

Radon daughter concentrations were measured daily in chambers 2, 3 and 6, using small membrane filters that were decay counted with an alpha energy analyzer. Condensation nuclei measurements were routinely made with a Gardner, Type CN Small-Particle Detector.* The particle sizes of the ore dust and the soot from the diesel exhaust were occasionally measured using electron microscopy on grids from a Walkenhurst thermal precipitator or from a point impingement electrostatic precipitator. A low-pressure cascade impactor, designed by Parker and Buchholtz[23] at the Oak Ridge National Laboratory (ORNL), was used to study radon daughter particle size distributions. This is a 7-stage (6, plus a prestage), multihole impactor, operated at a reduced pressure by drawing about 8 liter/min through a critical orifice. An Andersen cascade impactor[24] was also used in a few studies of chambers 2, 3 and 6 aerosols. The

* Gardner Associates, Inc., Schenectady, NY.

fraction of unattached radon daughters in the atmospheres of chambers 2, 3 and 6 were measured with a Mercer-type diffusion chamber[25,26] and a screen sampler similar to those developed by Thomas and Hinchliffe[27] and James et al.[28]

2. Animals

The original 102 male, Syrian golden hamsters in each of the six exposure groups were 12 weeks old at the start of exposures. They were housed separately in six, 17-compartment cages per exposure group (Figure 3). Each day (five days per week), the cages of animals were transferred from the racks in the animal holding room to the exposure chambers. After sealing the chamber, room air, radon, uranium ore dust, and/or diesel engine exhaust were turned into the chambers for 6 hours. Following each 6-hour exposure, the chambers were flushed with room air for 3 hours, and the cages of animals were then returned to the animal holding room. The animals had food and water available at all times and were weighed biweekly. Bimonthly hematology samples were obtained from the venous orbital plexus of five animals randomly selected from each exposure group. When animals were found dead, or when moribund animals were sacrificed, the lungs, trachea, larynx, liver, kidney, spleen, heart and any abnormal tissue were removed and fixed in 10% neutral buffered formalin.

Sixteen months after the start of exposures, at least 60 hamsters from each of the six groups had died or had been sacrificed. At that

FIGURE 3. Stainless Steel, 17-Compartment Animal Cage

time 54 replacement animals were added to each group. Nine of these replacement animals from each group were sacrificed after 1, 2, 4, 6, 8 and 11 months of exposure, respectively, for studies of pulmonary pathology without possible complication due to morbidity, and to allow direct comparison of the pathogenesis of lesions in animals exposed to the various agents for the same period of time.

B. Results

Table 2 shows the averages of the daily measurements of several exposure chamber parameters taken in the 27 months during which hamsters were exposed to the various uranium mine air contaminants. Standard deviations (rather than standard errors) are shown to give a better indication of the variability among the measurements. Seasonal variations in temperature and humidity influenced radon daughter concentrations, as did the total number of animals in the chamber.

The radon daughter aerodynamic equivalent particle size distributions reported are based on the cascade impactor stage constants. These constants are defined in terms of the diameter of unit density spheres, for which the stage collection efficiency is 50%. Particles collected on a particular stage are referred to as having an aerodynamic diameter equivalent to a unit density sphere collected on that stage with 50% efficiency. It is important to emphasize the point that particles on a given stage will not necessarily be spheres nor will they necessarily have equal densities or diameters. Aerodynamic equivalent diameters (not actual diameter) are reported in this study.

TABLE 2. Exposure Chamber Parameters Measured Daily
(All values = mean ± S.D.)

Chamber No.	RaA (nCi/ℓ)	RaB (nCi/ℓ)	RaC (nCi/ℓ)	Working Level	Particulate Concentration (mg/m^3)	Condensation Nuclei ($10^3/cm^3$)
1	---	---	---	---	---	3.9 ± 2.8
2	150 ± 60	70 ± 50	40 ± 30	690 ± 380	---	5.1 ± 4.0
3	150 ± 60	90 ± 40	50 ± 20	790 ± 330	22 ± 7	130 ± 50
4	---	---	---	---	19 ± 8	36* ± 24
5	---	---	---	---	7.3 ± 3.0	160 ± 60
6	160 ± 60	90 ± 40	50 ± 20	810 ± 310	23 ± 10	210 ± 90

* The smaller condensation nuclei count is possibly due to charge effects not present in Chambers 3 and 6, which contain radioactivity.

The radon daughter distributions (shown in Figures 4, 5 and 6) are presented as log-probability plots of the aerodynamic equivalent diameter versus the cumulative percentage of total radioactivity. (These curves will be referred to as cumulative activity distribution curves--CAD curves.) This method has traditionally been used to represent particle size distributions because many stable aerosols are distributed normally with respect to the logarithm of particle size and exhibit linear plots on log-probability paper. When this is the case, the median diameter of the distribution (diameter at the 50th percentile) and the geometric standard deviation (ratio of the diameters at the 84th percentile and the 50th percentile) suffice to define the entire size distribution.

The CAD curves revealed no difference in the distribution of radium A, B or C in any one sample. The nonlinearity of the activity distribution in Chambers 2 (Figure 4) and 3 (Figure 5) cannot be ignored. In Figure 4, which shows a sample from the mixture of radon gas with room air and animal dust, the points at 2.8 and 6.4 μm aerodynamic size represent less than 1% of the total activity sampled and cannot be given the same weight on the graph as the activity on the other stages of the cascade impactor. The points from these other stages indicate that 84% of the activity collected in the impactor had an activity median aerodynamic diameter (AMAD) of 0.36 μm (no doubt reflecting the presence of animal dust), and a geometric standard deviation (GSD) of 2.0. Sixteen percent of the activity collected in the impactor was associated with particles less than 0.07 μm aerodynamic size.

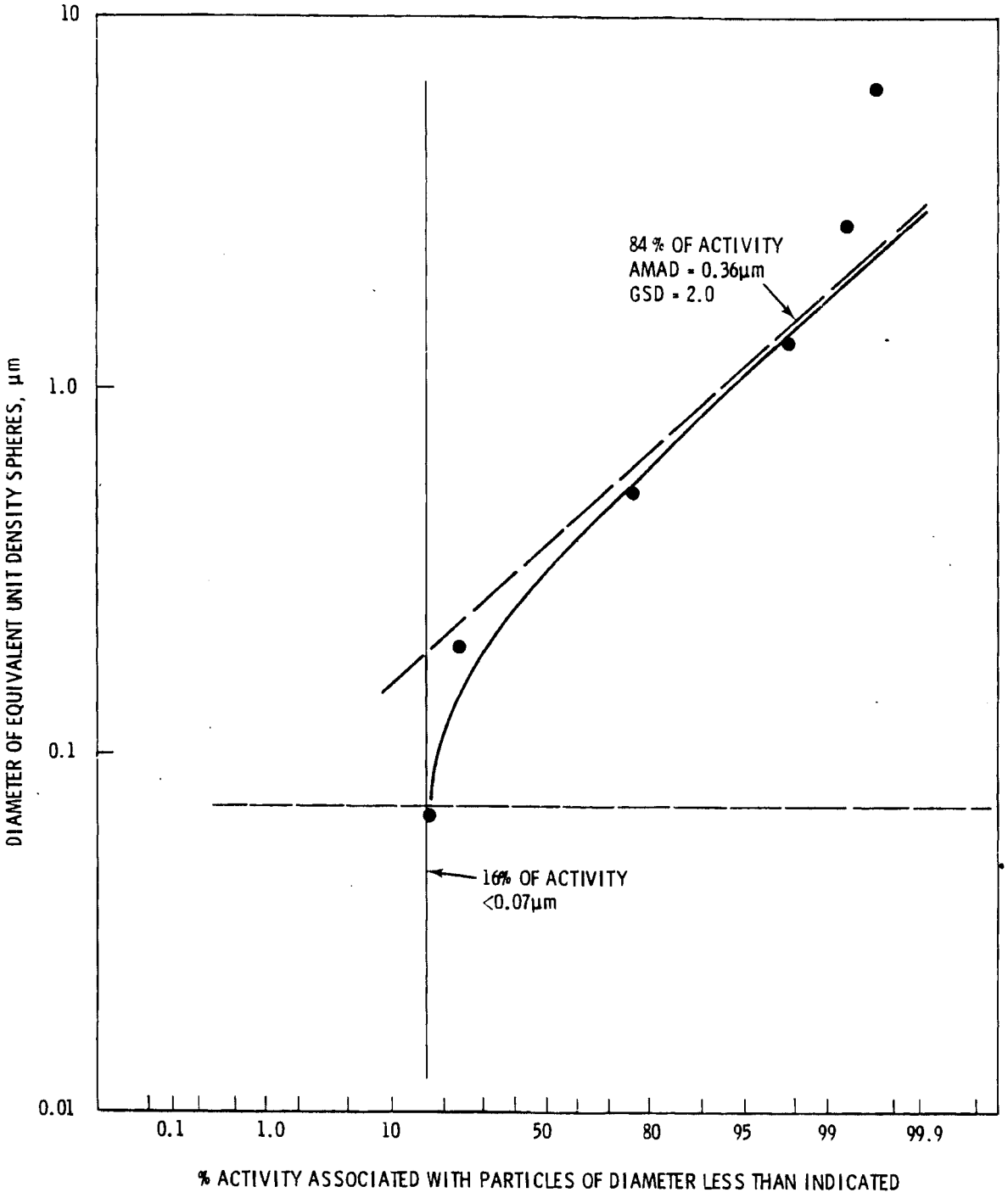

FIGURE 4. Cumulative Activity Distributions for Radon Daughters in Hamster Exposure Chamber 2 (Radon and Radon Daughters)

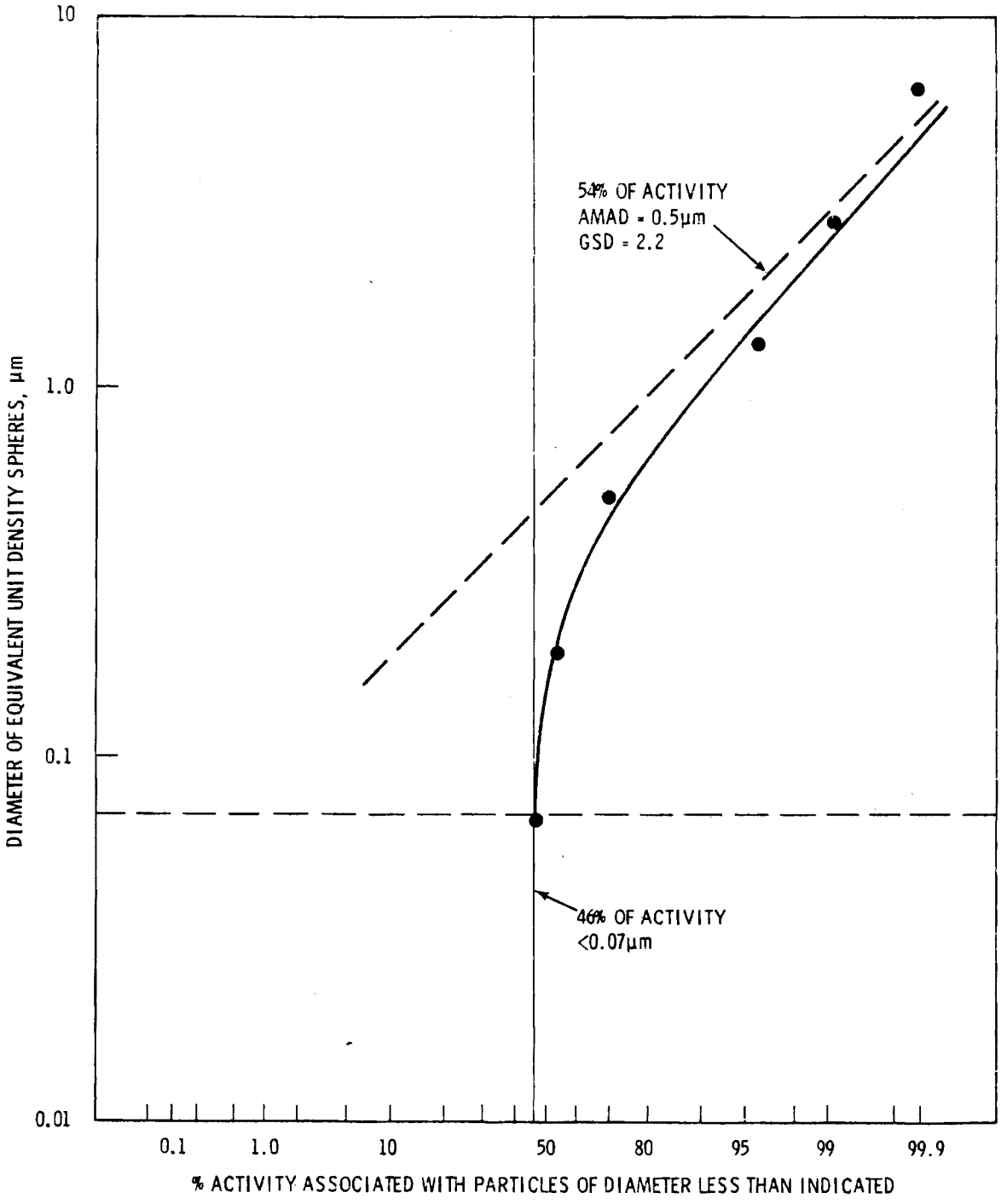

FIGURE 5. Cumulative Activity Distributions for Radon Daughters in Hamster Exposure Chamber 3 (Radon, Radon Daughters and Uranium Ore Dust)

In Figure 5, which shows a sample from the mixture of radon gas, room air, animal dust and uranium ore dust, 54% of the activity collected in the impactor had an AMAD of 0.5 μm (no doubt reflecting the presence of the ore dust), and a GSD of 2.2. Forty-six percent of the activity in this chamber (#3) was associated with particles less than 0.07 μm aerodynamic size. In Figure 6, which shows a sample from the mixture of radon gas, room air, animal dust, uranium ore dust and diesel exhaust fumes, all of the activity collected in the impactor could be described by a distribution having an AMAD of 0.31 μm and a GSD of 2.2.

The increased nuclei count between Chambers 2 and 3 (Table 2) would explain the increased fraction (46%, from 16%) of the sampled activity in Chamber 3 being associated with particles less than 0.07 μm in size, but does not explain the activity distribution seen for Chamber 6 (Figure 6). The Chamber 6 activity distribution demonstrates that all of the daughters are attached to diesel smoke particles of about 0.3 μm in diameter, exactly as found in mines extensively employing diesel equipment.

Nearly identical increases in body weights gradually occurred in all groups, reaching plateaus of 130-140 grams after four months of exposures. As shown in Figure 7, none of the exposure treatments (except for the radon daughter group) caused weight gains or losses markedly different from mean control values. The data of Table 3 indicate that life-span exposures to radon daughters, uranium ore dust, and diesel engine exhaust--singly or in combination--caused no significant (at the 0.05 level of significance) changes in mortality patterns compared to controls.

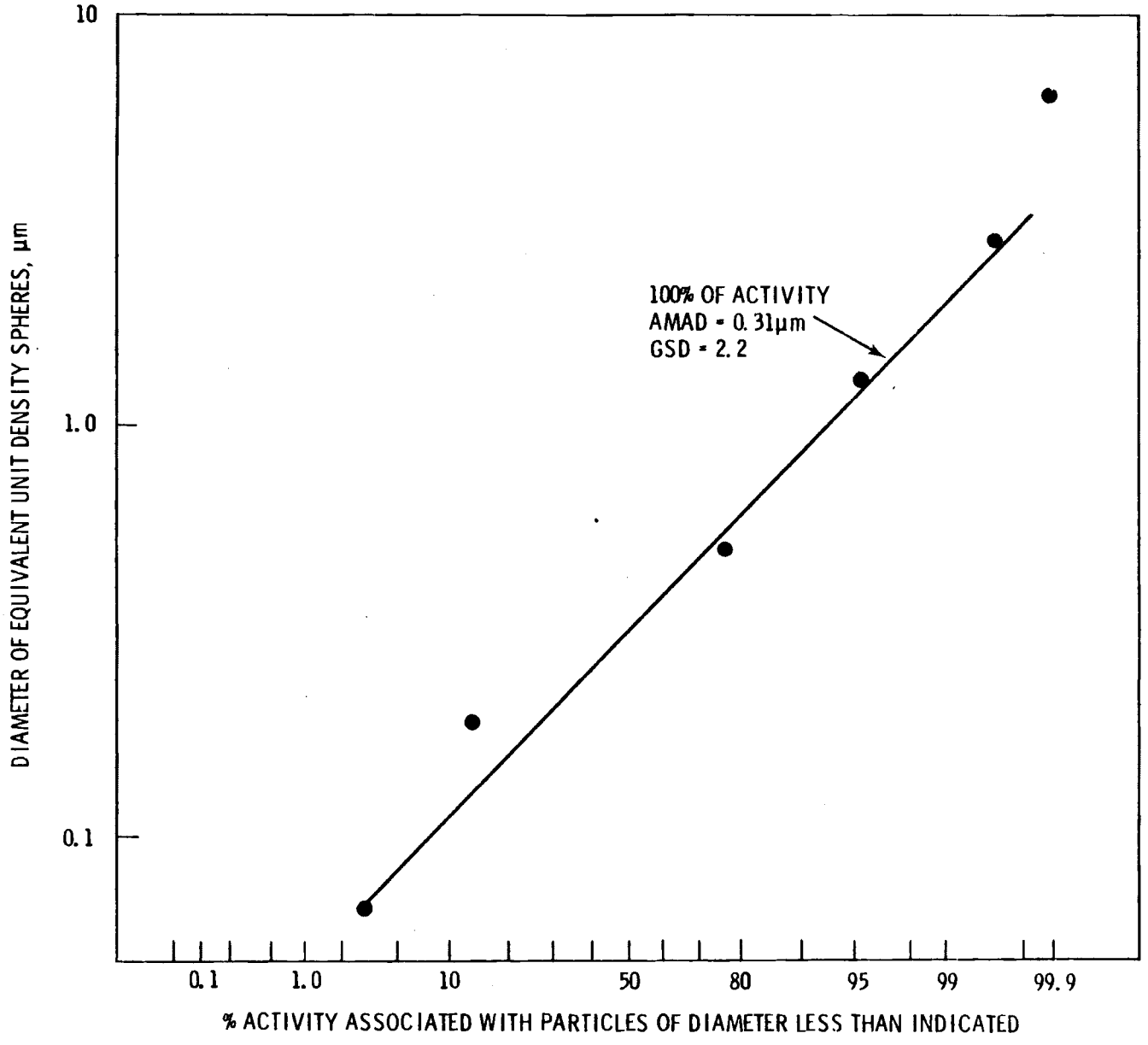

FIGURE 6. Cumulative Activity Distributions for Radon Daughters in Hamster Exposure Chamber 6 (Radon, Radon Daughters, Uranium Ore Dust and Diesel Engine Exhaust)

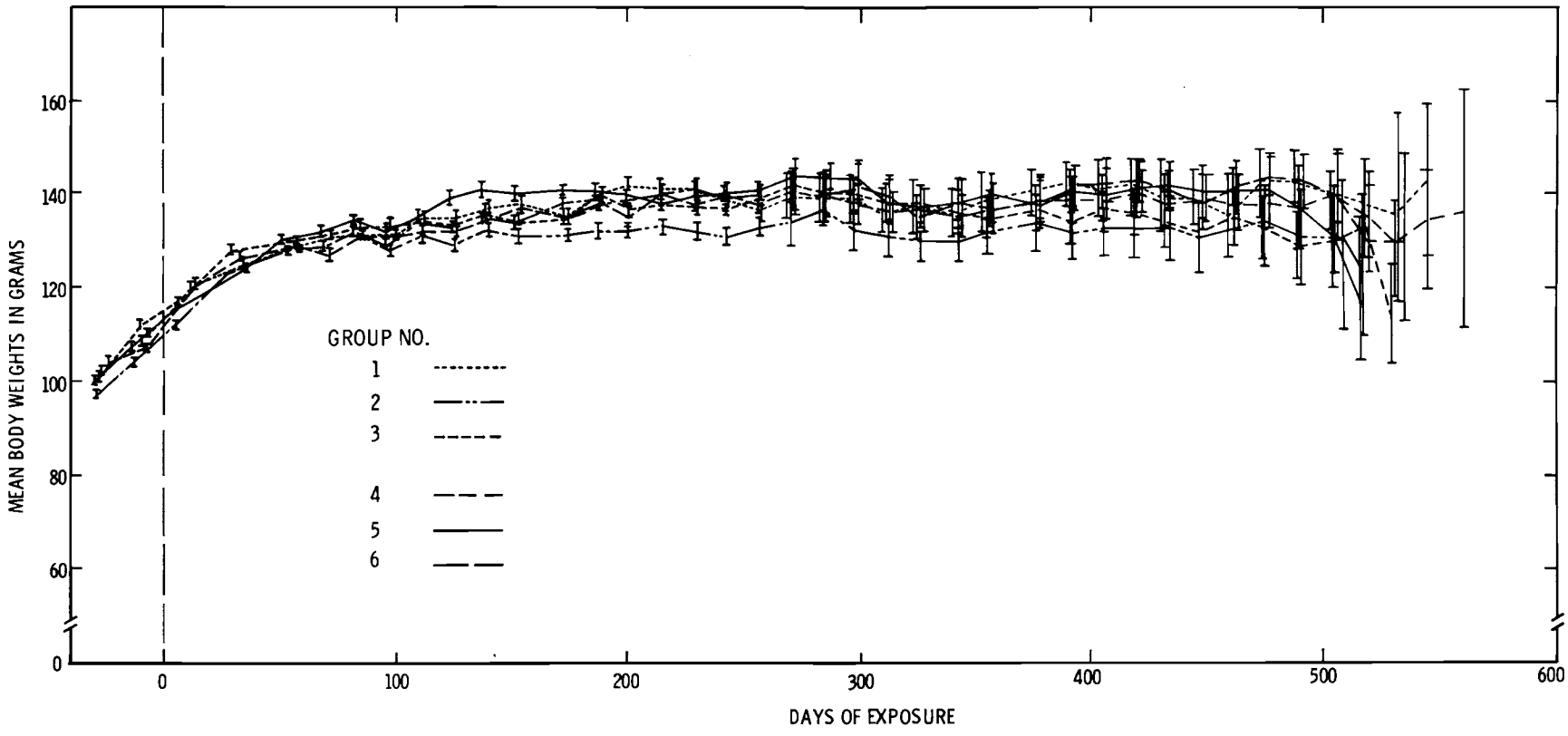

FIGURE 7. Mean Body Weight (Shown with 95% Confidence Limits) of Hamsters Exposed to Aerosols of Uranium Mine Air Contaminants

TABLE 3. Hamster Mortality Patterns (Percent Survival at Given Times After Start of Exposures)

Days of Exposure*	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
0	100	100	100	100	100	100
100	97	96	100	97	97	98
200	60	68	68	67	70	71
300	54	61	63	58	61	62
400	33	34	44	45	40	43
500	13	16	26	23	16	17
600	0	0	6	4	3	2

* Lifespan exposures, 6 hours per day, 5 days per week.

The carboxyhemoglobin levels measured in peripheral blood from hamsters sacrificed after 4, 6, 8 and 11 months of exposures to diesel engine exhaust (Groups 5 and 6) are shown in Table 4, along with those of the control animals. As expected, Groups 5 and 6 animals showed elevated carboxyhemoglobin levels compared with controls.

The bimonthly blood samples obtained from the five randomly selected hamsters from each of the exposure groups showed no significant changes in hematocrit, erythrocyte or differential leukocyte levels, when compared to levels measured in control animals.

C. Pathology

After 6 months of exposure, 15 to 20% of the hamsters in all groups, including control animals, developed enteritis of an unknown etiology. No bacterial pathogens were found and the condition was ascribed to stress-associated disturbances in the normal balance of intestinal microbes. The disease persisted for approximately 3 weeks, after which the incidence subsided to a very low level, where it remained for the first several months of exposure.

The nonpulmonary neoplasms observed in the hamsters are listed in Table 5. All are considered spontaneous, rather than exposure-related, with one exception, osteosarcoma (2, in Group 3), which is rare in hamsters. Although osteosarcoma has been induced in mice by exposure to such radionuclides as ^{226}Ra, ^{239}Pu, ^{232}U, ^{233}U, and ^{90}Sr, no radiation induction of this tumor in hamsters has previously been reported, to our knowledge.

TABLE 4. Carboxyhemoglobin Levels in Hamsters

Exposure Period (Months)	Percent Carboxyhemoglobin*		
	Group 1	Group 5	Group 6
4	4.5 ± 0.4	7.8 ± 0.3	8.2 ± 0.8
6	4.4 ± 0.5	7.2 ± 0.6	6.6 ± 0.3
8	4.5 ± 0.2	7.4 ± 0.1	7.8
11**	3.1 ± 0.4	6.1 ± 1.0	6.9 ± 1.0

* Mean ± standard deviation
**Optical filters changed and instrument recalibrated.

TABLE 5. Incidence of Nonpulmonary Neoplasms in Hamster Exposure Groups

Group 1 - CONTROL
- 3 Reticulum Cell Sarcomas
- 1 Myelogenous Leukemia
- 1 Nephroma (embryonal)
- 1 Hemangioendothelioma

Group 2 - RADON DAUGHTERS, alone
- 3 Reticulum Cell Sarcomas
- 1 Myelogenous Leukemia
- 1 Sarcoma (undifferentiated)

Group 3 - RADON DAUGHTERS AND URANIUM ORE DUST
- 4 Reticulum Cell Sarcomas
- 1 Adrenal Carcinoma
- 2 Osteosarcomas

Group 4 - URANIUM ORE DUST, alone
- 2 Reticulum Cell Sarcomas
- 1 Adrenal Carcinoma
- 1 Pheochromocytoma
- 1 Melanoma
- 1 Hemangioendothelioma

Group 5 - DIESEL ENGINE EXHAUST, only
- 4 Reticulum Cell Sarcomas
- 1 Carcinoma (undifferentiated)
- 1 Hepatic Sarcoma

Group 6 - RADON DAUGHTERS, URANIUM ORE DUST, AND DIESEL ENGINE EXHAUST
- 7 Reticulum Cell Sarcomas
- 1 Lymphosarcoma

Overall incidence of reticulum cell sarcomas was about 4% in all hamsters, including controls. No statistical significance of the 7% incidence of this tumor type in Group 6 (radon daughters, uranium ore dust and diesel engine exhaust) could be assigned, however, because of the high spontaneous incidence (2-4%) in each of the other 5 groups.

Pulmonary neoplastic lesions were limited to 3 hamsters of Group 2 and one hamster of Group 3. The apparent pathogenesis of the pulmonary squamous tumors is traced in a sequence of photomicrographs (Figures 8 through 19). Table 6 shows the cumulative exposures for hamsters with lung tumors, and for selected hamsters with pulmonary lesions considered preneoplastic. The hamsters with lung tumors all had cumulative exposures greater than 8000 WLM, while those with severe metaplastic lesions received less exposure. The tumor in the lung of Group 3 hamster #98 was very extensive, indicating that it had probably originated a considerable time before the death of the animal.

Within the first year of exposure, lesions of the type shown in Figure 8 were quite frequently observed in hamsters exposed to radon daughters alone or in combination with uranium ore or diesel engine exhaust. The alveolar lining cells were displaced or replaced by ciliated cuboidal epithelium such as that in terminal and respiratory bronchioles. This adenomatous proliferation of alveolar epithelium was significantly higher in all animals exposed to radon daughters (Groups 2, 3 and 6). In animals with longer exposure histories, those cells began to show vertical and horizontal crowding as they took on a columnar appearance (Figure 9). This stage was followed by squamous metaplasia

TABLE 6. Hamster Exposure Levels Resulting in Lung Tumors or Severe Pulmonary Epithelial Metaplasia (all values = mean ± S.D.)

Group	Hamster Identification No.	Death Date	Hours Exposed	Exposure Rate (WL)	Cumulative Exposure (WLM)	Pulmonary Change
2	38	4/24/72	2096	690 ± 380	8500 ± 4700	Squamous Carcinoma
2	11	4/23/72	2090	690 ± 380	8500 ± 4700	Carcinoma in situ
2	63	5/29/72	2239	690 ± 380	9100 ± 5000	Carcinoma in situ
2	22	10/1/71	1250	690 ± 380	5100 ± 2800	Severe Squamous Metaplasia
2	59	2/1/72	1750	690 ± 380	7100 ± 3900	Severe Squamous Metaplasia
3	98	9/1/72	2625	790 ± 330	12,000 ± 5100	Squamous Carcinoma
3	22	12/8/71	1550	790 ± 330	7200 ± 3000	Squamous Metaplasia
6	79	4/19/72	2066	810 ± 310	9800 ± 3800	Severe Squamous Metaplasia

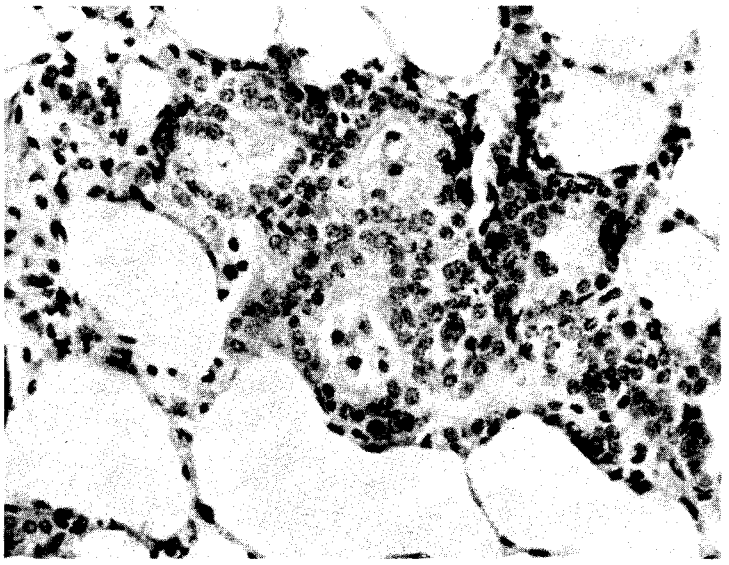

FIGURE 8. An Early Alveolar Epithelial Hyperplastic Lesion in a Group 2 Hamster After One Month of Exposure to Radon Daughters. H&E. 325X.

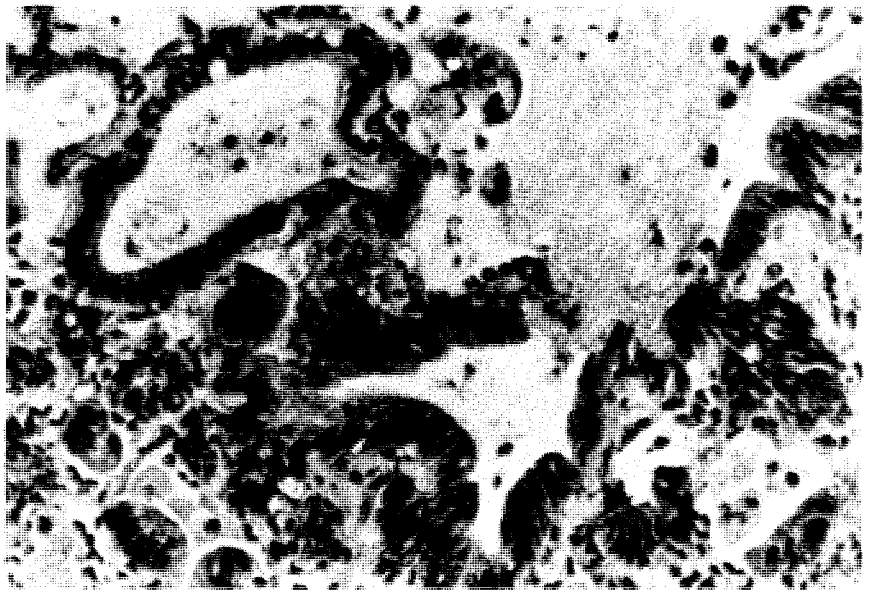

FIGURE 9. Bronchiolization of Alveolar Epithelium in the Lungs of a Hamster After 6500 WLM of Exposure to Radon Daughters. Note vertical and horizontal crowding of cells of the hyperplastic epithelium. H&E. 300X.

of the cells, accompanied by a loss of cilia (Figure 10). In the lungs of many animals, all three stages of the progression could be seen in the same section; two stages are illustrated in Figure 11. Additionally, cells of metaplastic lesions began to exhibit nuclear atypia, characterized by increase in nuclear size and irregularity of the nuclear outline, with clumping and margination of chromatin (Figures 12 and 13).

The stage at which the lesions became frank carcinomas could not be ascertained, but a precancerous status is suspected for at least the later stages in this progression. Two of the squamous tumors found in the lungs of hamsters from Group 2 were diagnosed as carcinoma in situ according to cytological criteria, but no evidence of invasion of pulmonary vessels or of supportive structure could be found (Figures 14, 15, and 16). An invasive squamous carcinoma from one hamster of Group 2 is shown in Figures 17, 18 and 19. An epidermoid carcinoma from a hamster of Group 3 is shown in Figures 20 and 21.

In Tables 7A, 7B and 7C, pulmonary lesions other than neoplasia, plus statistical analysis of this data, are listed by hamster exposure group. The animals that died in 1971 were exposed from 2-14 months, while those listed for 1972 had longer than 14 months exposure. Figure 22 is a photomicrograph of the lung section from one of the control hamsters. As indicated in Table 7A, the lungs of control hamsters remained essentially lesion-free, with one exception. The incidence of chronic interstitial pneumonitis was considered high, although no cause for it could be

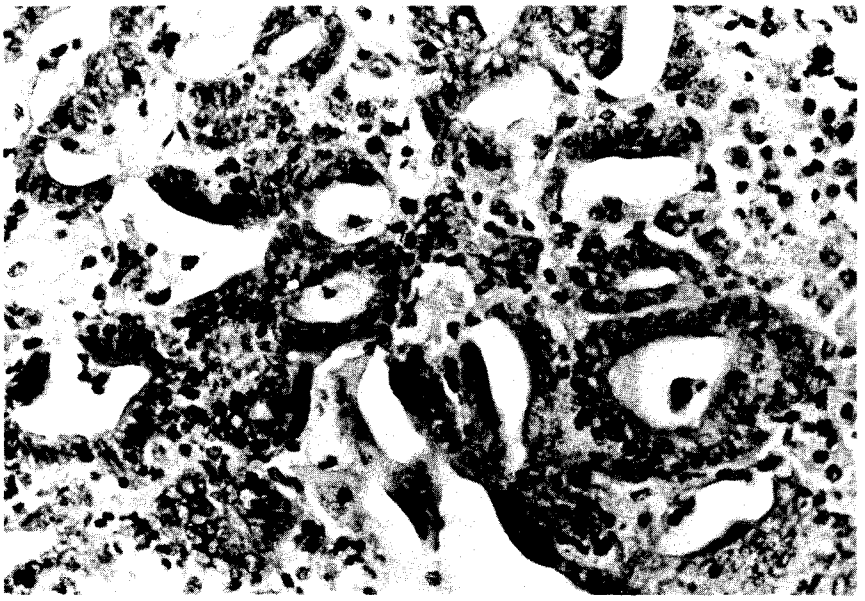

FIGURE 10. Squamous Metaplasia of Alveolar Epithelium in the Lungs of a Group 3 Hamster After 12.5 Months of Exposure to Uranium Ore Dust and 7200 WLM of Radon Daughters. H&E. 300X.

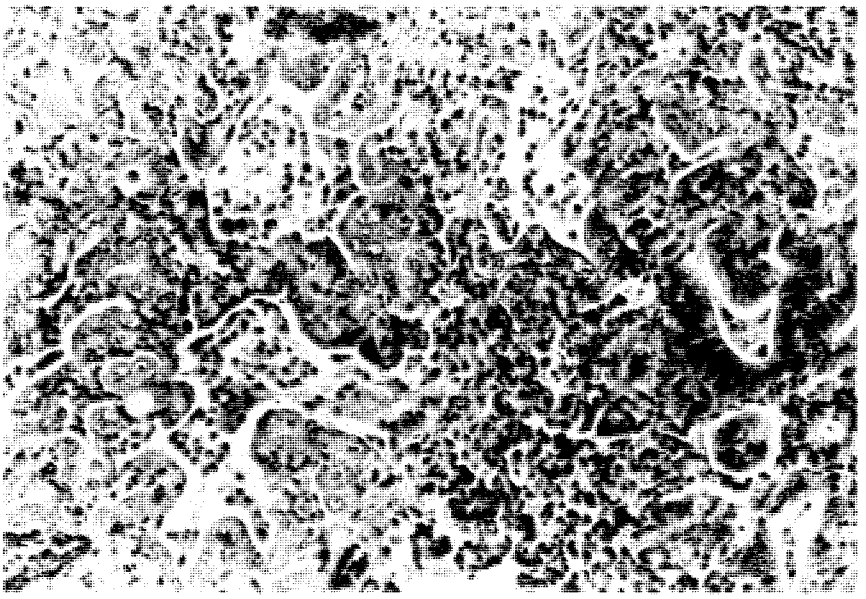

FIGURE 11. Adjacent Areas of Alveolar Epithelial Bronchiolization and Squamous Metaplasia in the Lung of a Group 2 Hamster After 5100 WLM of Exposure to Radon Daughters. H&E. 150X.

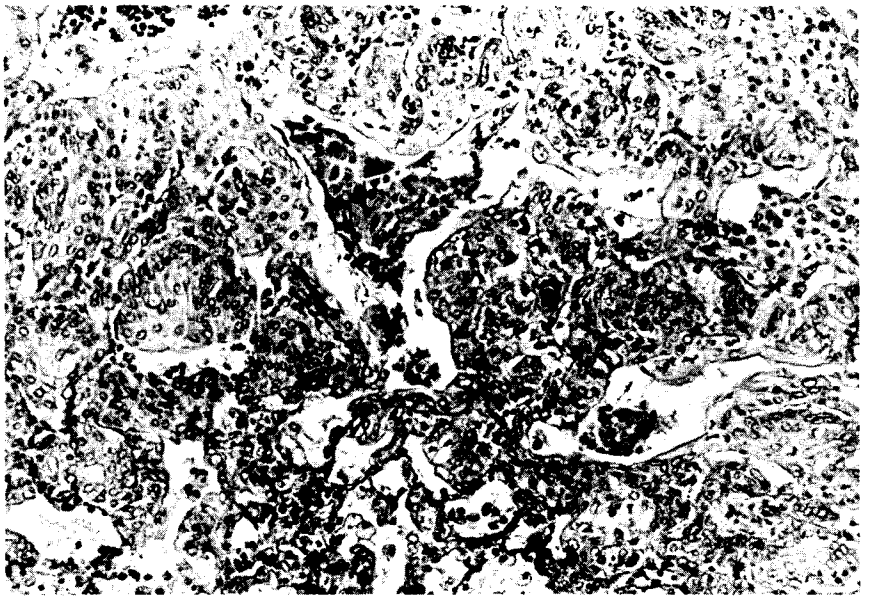

FIGURE 12. Squamous Metaplasia of Alveolar Epithelium in the Lung of a Group 2 Hamster after 7100 WLM of Exposure to Radon Daughters. H&E. 150X.

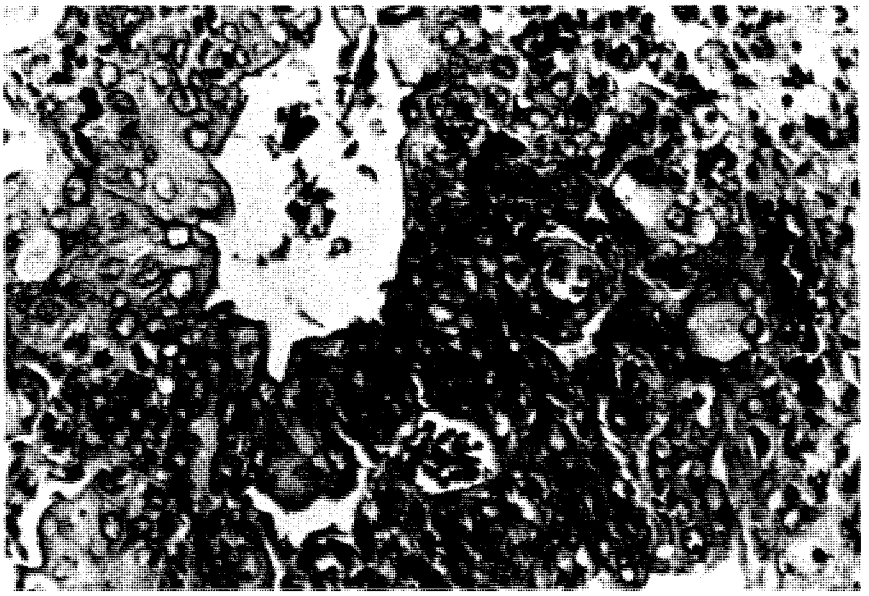

FIGURE 13. Squamous Metaplasia of Alveolar Epithelium With Nuclear Atypia Characterized by Clumping and Margination of Chromatin, Increase in Nuclear Size and Irregularity of Nuclear Outline. From a Group 2 hamster exposed to 5100 WLM of radon daughters. H&E. 300X.

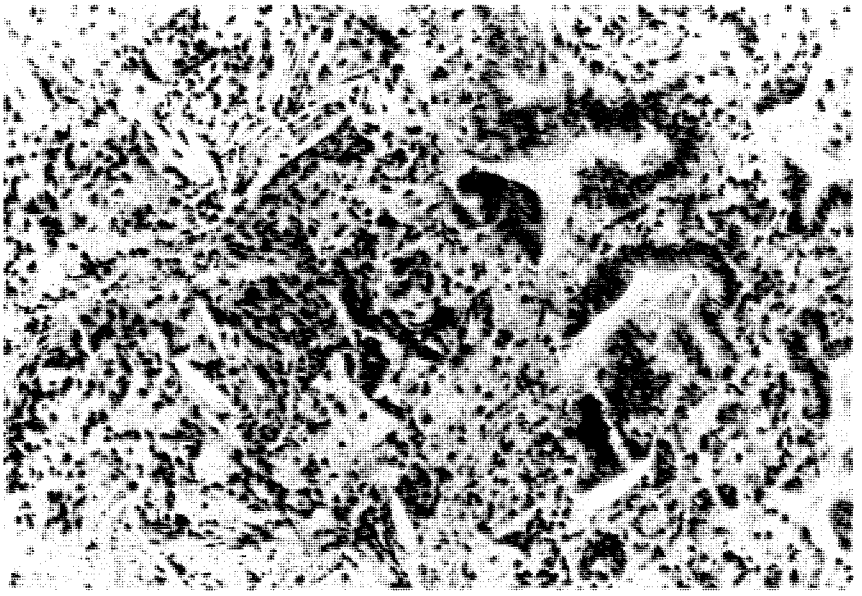

FIGURE 14. Carcinoma In Situ in the Lung of a Group 2 Hamster After 9100 WLM of Exposure to Radon Daughters. H&E. 150X.

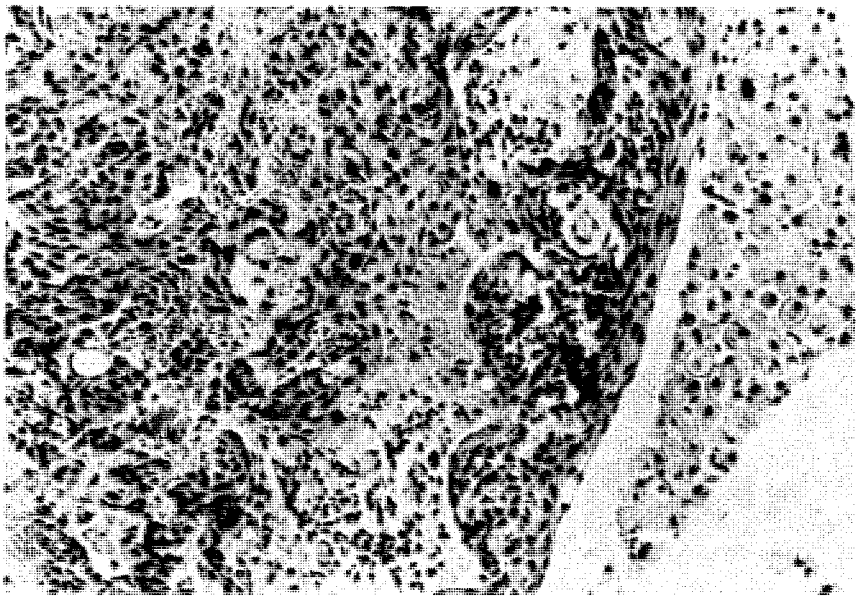

FIGURE 15. Carcinoma In Situ in the Lung of the Same Hamster as in Figure 14. H&E. 150X.

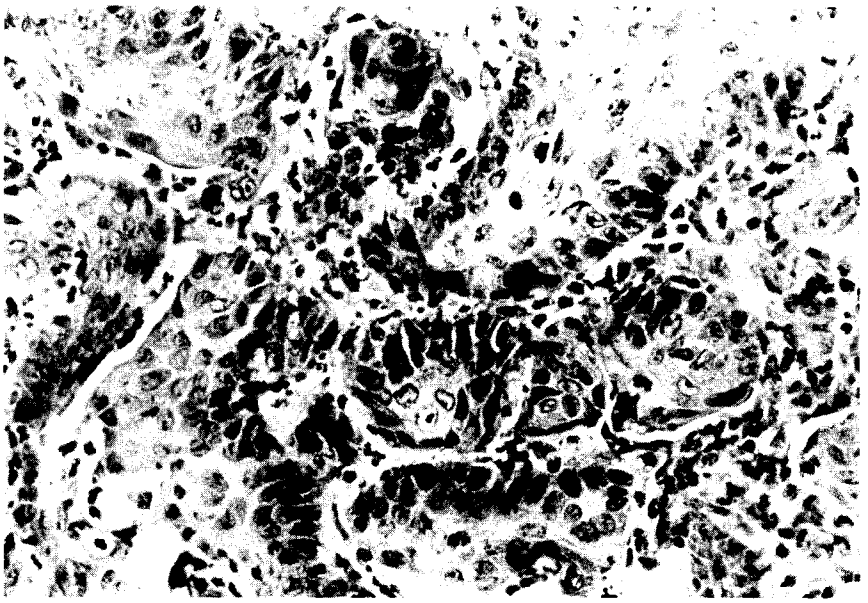

FIGURE 16. Carcinoma In Situ in the Lungs of a Group 2 Hamster After 8500 WLM of Exposure to Radon Daughters. H&E. 300X.

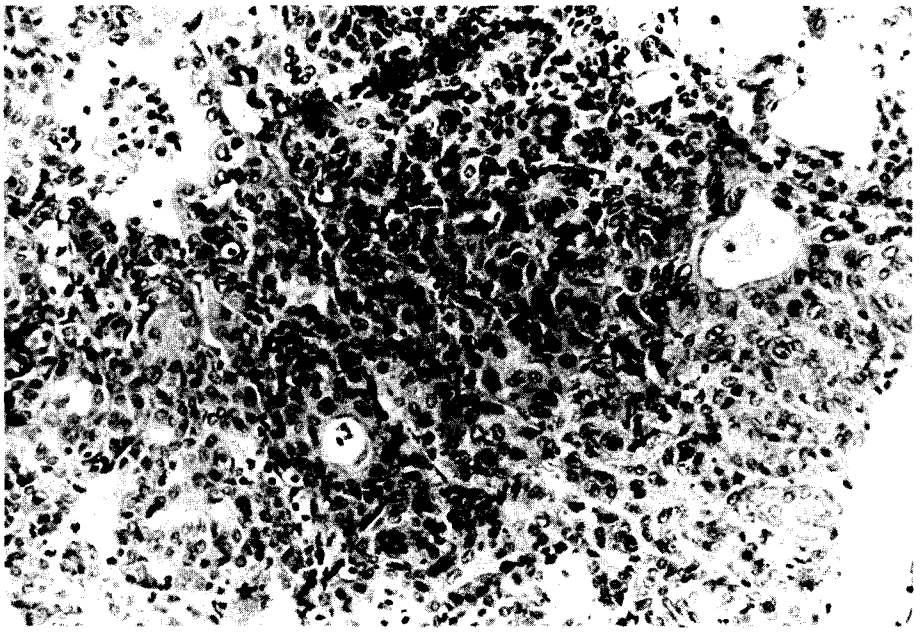

FIGURE 17. Pulmonary Squamous Carcinoma in a Group 2 Hamster After 8500 WLM of Exposure to Radon Daughters. H&E. 150X.

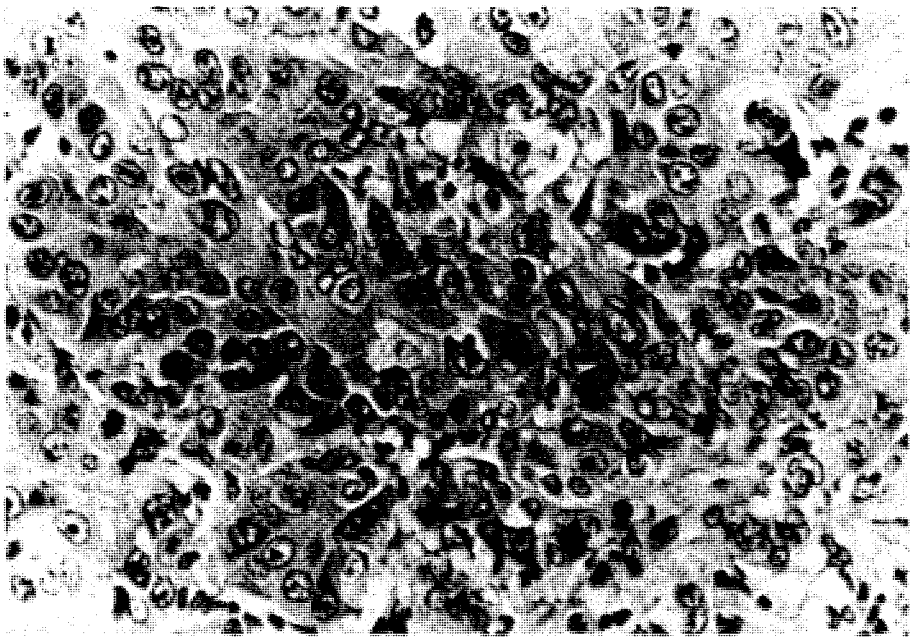

FIGURE 18. Higher Magnification of the Tumor Shown in Figure 17. H&E. 300X.

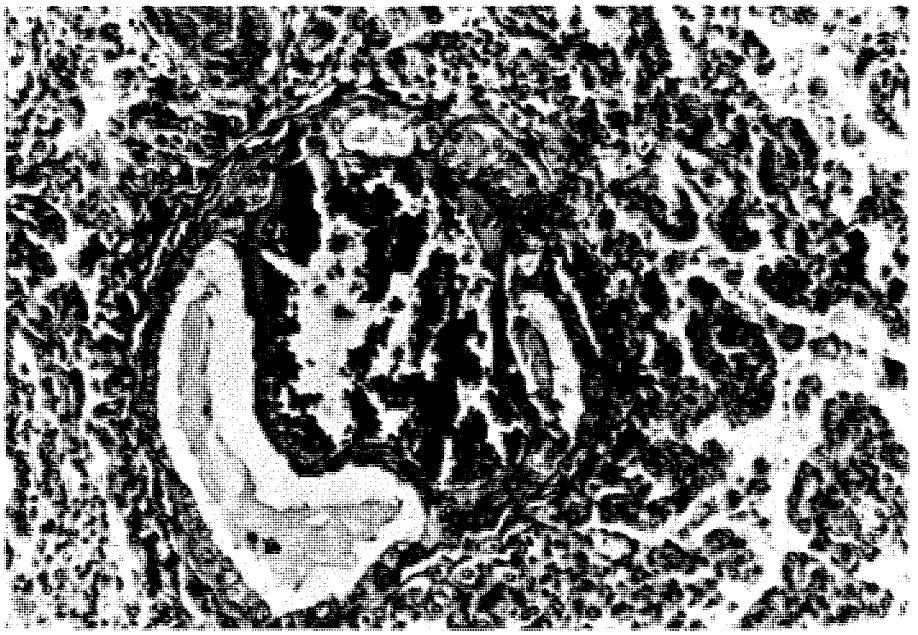

FIGURE 19. Tumor Cell Invasion of a Blood Vessel (same tumor as shown in Figures 17 and 18). H&E. 150X.

TABLE 7A. Incidence of Respiratory Tract Lesions in Hamsters

Exposure Group:	1	2		3		4		5		6	
	(Control)	(Radon daughters)		(Radon daughters + uranium ore)		(Uranium ore)		(Diesel exhaust)		(Radon daughters, uranium ore and diesel exhaust)	
Year of Death:		1971	1972	1971	1972	1971	1972	1971	1972	1971	1972
No. of Animals:	82	51	45	55	46	50	49	55	40	57	49
A. Macrophage Accumulations											
1-2*	0	[7	8]	[36	34]	[27	35]	[46	22]	[54	38]
3-4+	0	1	12	2	8	1	6	3	17	3	8
More°	0	0]	1]	0]	1]	1]	0]	0]	0]	0]	0]
B. Emphysema											
1-2	5	[16	10]	[17	19]	[9	18]	[17	5]	[11	12]
3-4	2	2	8	2	15	0	9	3	7	2	13
More	0	0]	0]	0]	0]	0]	0]	0]	1]	0]	0]
C. Alveolar Septal Cell Hyperplasia											
1-2	1	[9	10]	[9	26]	[11	21]	[23	12]	[20	26]
3-4		0	12	1	6	0	4	2	5	1	8
More		0]	1]	0]	1]	0]	0]	0]	0]	0]	0]
D. Adenomatous Proliferation of Alveolar Epithelium											
1-2	2	[8	11]	[12	20]	[2	11]	[6	11]	[13	23]
3-4	0	3	13	3	15	2	6	0	3	0	11
More	0	0]	1]	0]	0]	0]	0]	0]	0]	0]	0]
E. Alveolar Epithelial Squamous Metaplasia	0	[2	7]	[1	12]	0	0	0	0	0	4
F. Bronchial or Bronchiolar Epithelial Hyperplasia											
1-2	1	[19	14]	[22	30]	[11	18]	[11	15]	[26	25]
3-4		0	15	2	3	1	9	3	6	0	14
More		0]	1]	0]	0]	0]	0]	0]	0]	0]	0]
G. Bronchiolar Epithelial Hyperplasia with Atypia	0	0	6	[2	6]	2	1	0	1	[1	10]
H. Bronchiolar Epithelial Hyperplasia with Squamous Metaplasia	0	[8	1]	6	0	1	0	0	0	[9	1]
I. Tracheal or Laryngeal Epithelial Hyperplasia	0	0	4	2	4	1	3	1	2	0	3
J. Bronchitis or Bronchiolitis	0	0	0	2	2	2	1	0	0	0	1
K. Laryngitis	0	0	5	[0	11]	1	5	1	4	1	1
L. Interstitial Pneumonitis											
1-2	12	5	3	10	19	8	21	2	12	6	14
3-4	9	3	6	1	4	0	4	3	6	1	10
More	0	0	0	0	0	0	0	0	0	0	0
M. Alveolar Septal Fibrosis (Pneumoconiosis)	0	0	0	0	0	[1	15]	0	1	[2	19]

* Very slight to slight
\+ Moderate to marked
° Severe

[ ] All severities of lesions combined are significantly different from controls (combined) at the 0.05 level of significance.

TABLE 7B. Comparison of Exposure Groups by the Chi-Square Statistic

Respiratory Tract Lesion†	Comparison Groups†									
	2 & 3	2 & 4	2 & 5	2 & 6	3 & 4	3 & 5	3 & 6	4 & 5	4 & 6	5 & 6
A	**	**	**	**		*	**	**	**	
B	*				*	*	*			
C				**					*	
D		*	*		**	**			**	**
E		**	**		**	**	*			
F			*		*	**			**	**
G						*				*
H		*	**			*			*	**
I										
J										
K							*			
L	*	*								
M		**		**	**		**	**		**

† See Table 7A for description
* 0.05 level of significance (all severities combined)
** 0.01 level of significance (all severities combined)

TABLE 7C. Comparison of 1971 vs 1972 Hamster Deaths by the Chi-Square Statistic

Respiratory Tract Lesion†	Exposure Group†				
	2	3	4	5	6
A	**	**	**		
B		**	**		**
C	**	**	**		**
D	**	**	**	**	**
E		**			*
F	**	**	**	**	**
G	**				**
H††	*	*			*
I	*				
J					
K	*	**			
L		**	**	**	**
M			**		**

† See Table 7A for description
†† All lesions, except H, appeared with greater frequency in 1972.
* 0.05 level of significance (all severities combined).
** 0.01 level of significance (all severities combined).

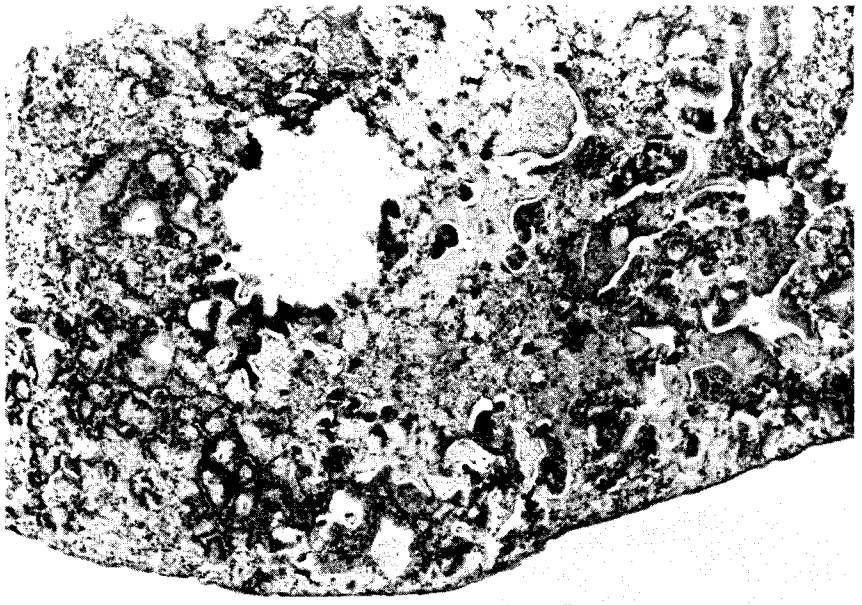

FIGURE 20. Keratinizing Squamous Carcinoma in the Lung of a Group 3 Hamster. The hamster was exposed for 21.5 months to uranium ore dust with 12,200 WLM of exposure to radon daughters. H&E. 60X.

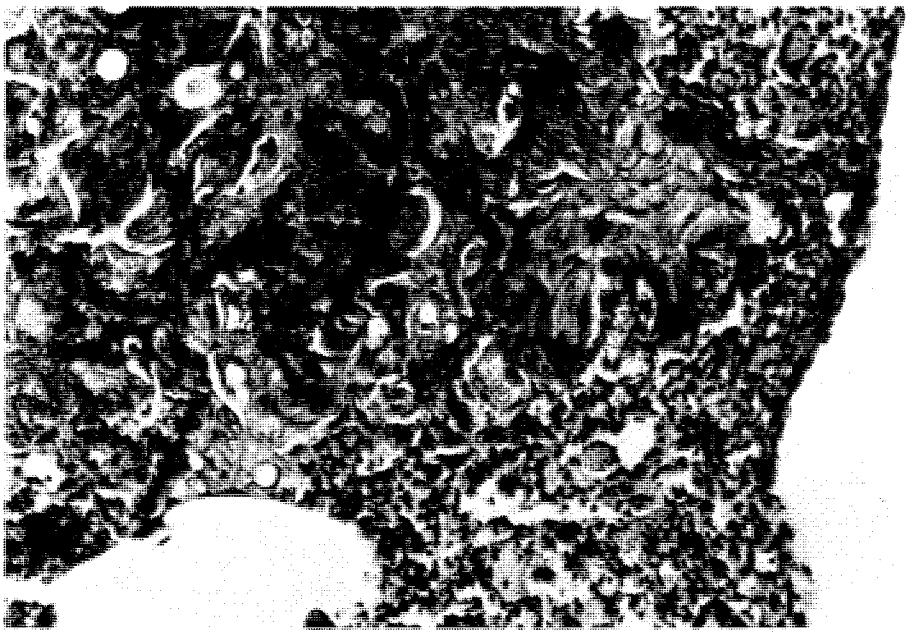

FIGURE 21. Higher Magnification of the Tumor Shown in Figure 20. H&E. 150X.

FIGURE 22. Lung Section From a Control Hamster. H&E. 60X.

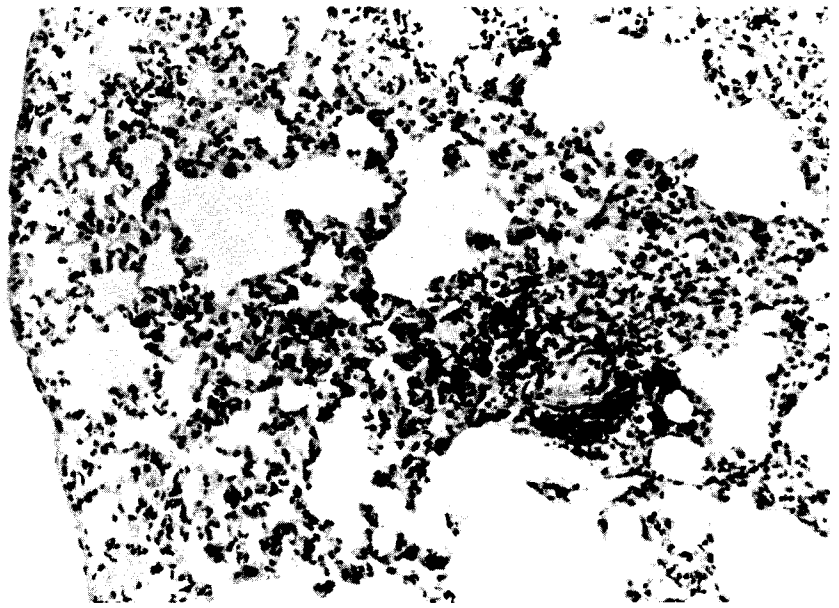

FIGURE 23. Black Pigment in Macrophages That Accumulated Adjacent to a Small Pulmonary Blood Vessel. The hamster was exposed to diesel engine exhaust for 1 month. H&E. 150X.

determined. Even with the high frequency of that diagnosis for control animals, it is evident that those animals exposed to ore alone or in combination with radon daughters and/or diesel engine exhaust (Groups 3, 4, and 6) had a slightly higher (in categories 1-2) than normal incidence of the disease.

Accumulation of particulate material in the lungs was most pronounced throughout the experiment in those hamsters exposed to diesel engine exhaust, either alone or in combination with radon daughters and uranium ore (Groups 5 and 6). As illustrated in Figures 23, 24, and 25, carbon may accumulate to the point where alveoli are filled. No carcinomas occurred, although metaplastic changes in the alveolar epithelium were frequent in these two groups (Figures 25 and 26). Pulmonary consolidation and/or emphysema were observed for the shorter exposure times, and accumulation of uranium ore dust was also seen. After longer exposure, a characteristic granulomatous response, referred to as uranium ore pneumoconiosis, was consistently diagnosed (Figures 27, 28 and 29).

Apparently in response to pulmonary irritation by inhaled particulates, a significantly high degree of macrophage accumulation was noted in lungs of hamsters from Groups 3 through 6 (see Table 7B). An increased number of macrophages, together with alveolar septal cell hyperplasia in the same groups, constitute the heavy pulmonary consolidation evident in Figures 27 through 29. The consolidative processes were most evident in Group 6, and occurred with similar frequency in hamsters of Groups 3 through 5. Even though there was notable septal cell hyperplasia in the

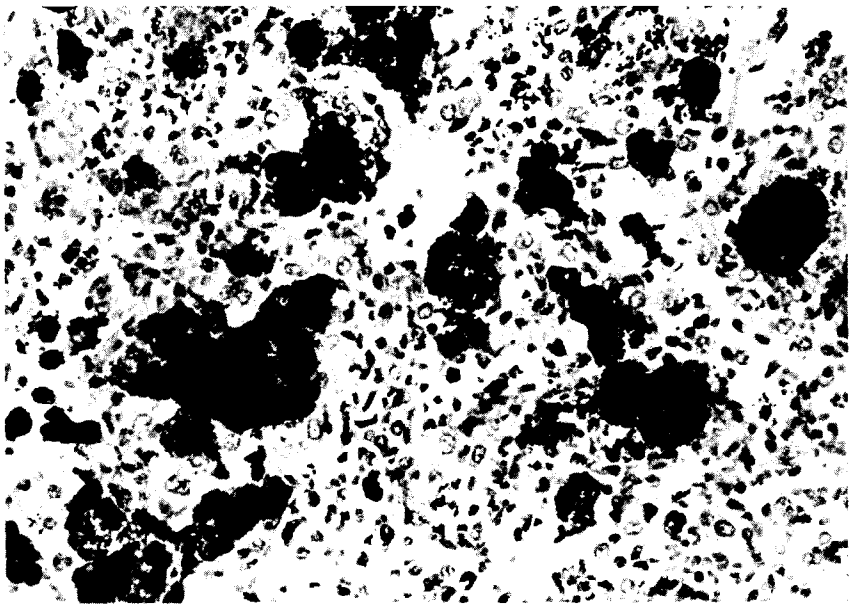

FIGURE 24. Diesel Engine Exhaust Pigment in the Lungs of a Hamster Exposed for 11 Months. H&E. 300X.

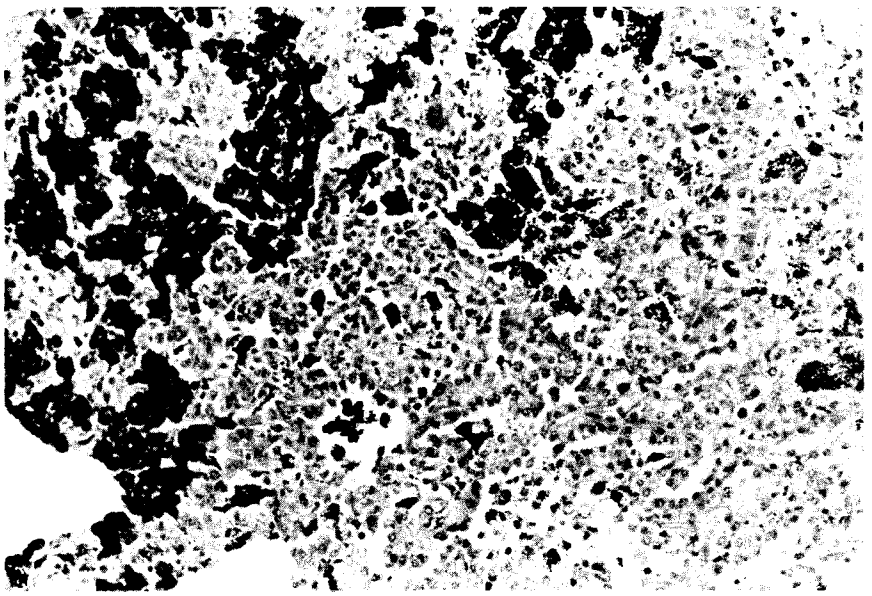

FIGURE 25. Squamous Metaplasia of Alveolar Epithelium, Adjacent to an Accumulation of Diesel Exhaust Pigment in the Lung. Group 6 hamster, exposed to uranium ore dust and diesel engine exhaust for 17 months with 9800 WLM of exposure to radon daughters. H&E. 150X.

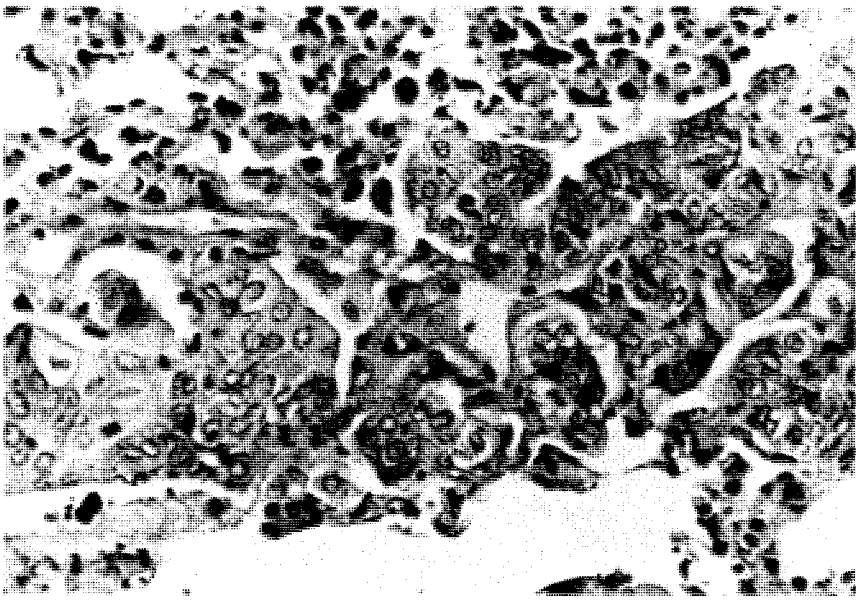

FIGURE 26. Higher Magnification of Alveolar Epithelial Squamous Metaplasia Shown in Figure 25. H&E. 300X.

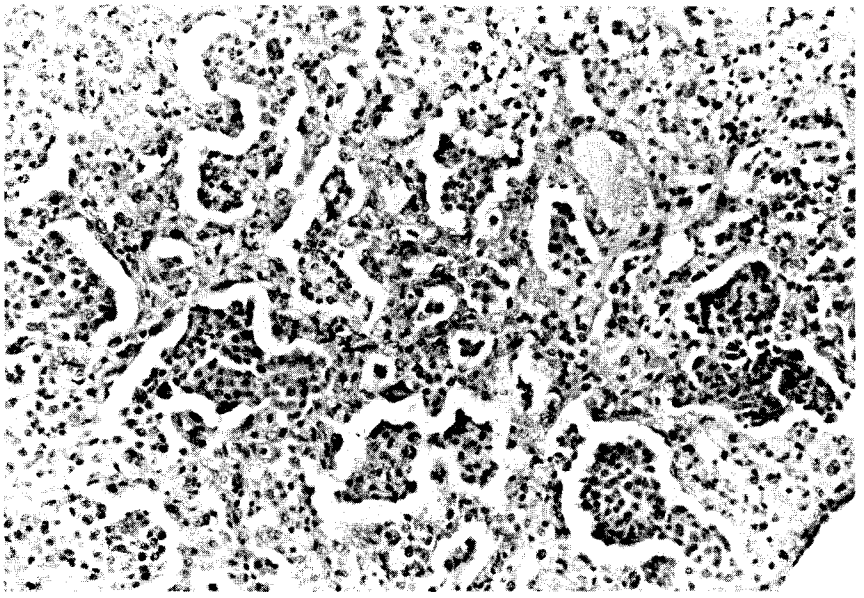

FIGURE 27. Uranium Ore Pneumoconiosis, Characterized by Large Accumulations of Macrophages, Alveolar Septal Cell Hyperplasia and Alveolar Septal Fibrosis. Group 3 hamster, exposed to uranium ore dust for 12.5 months with 7200 WLM of radon daughters. H&E. 150X.

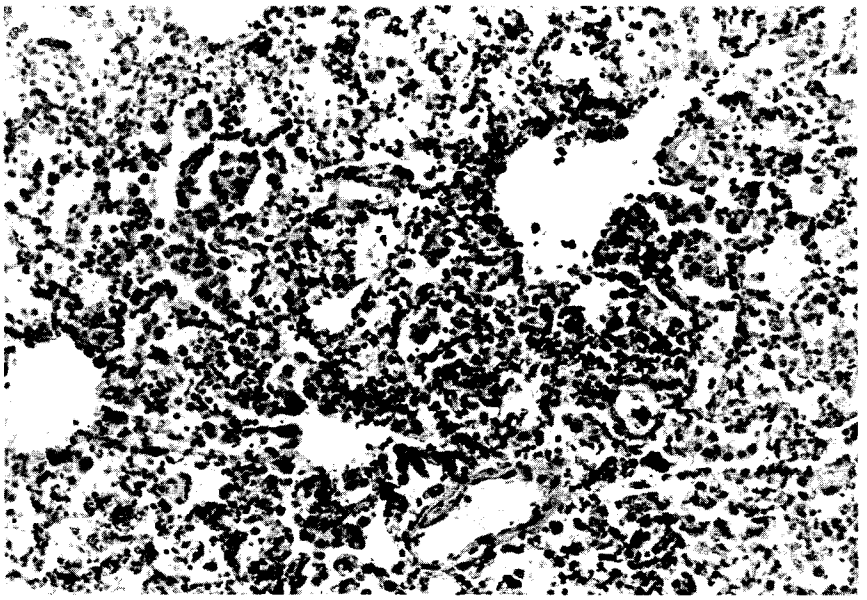

FIGURE 28. Uranium Ore Pneumoconiosis in a Group 4 Hamster After 14.5 Months of Exposure to Uranium Ore Dust. H&E. 150X.

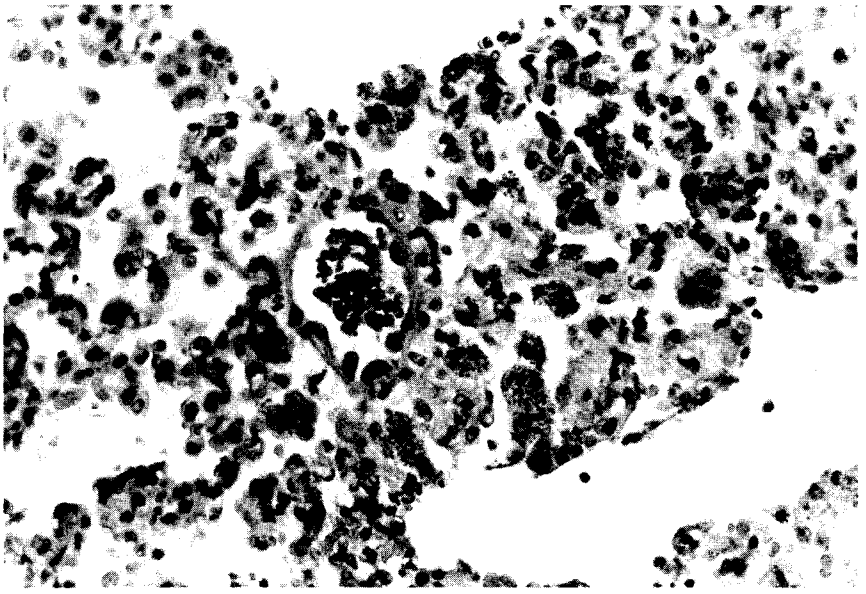

FIGURE 29. Higher Magnification of the Lung Section Shown in Figure 28. H&E. 300X.

lungs of hamsters from Group 2, macrophage accumulation was not as evident. The lungs of Group 2 animals, therefore, did not exhibit consolidation as severe as those of the other groups.

Pulmonary emphysema (Figure 30) appeared with similar frequency in all treatment groups, although the incidence was significantly higher (see Table 7B) in animals of Group 3 (radon daughters and uranium ore) than in the other groups. A higher incidence of moderately severe cases of this lesion was noted in Groups 3 and 6, also suggesting the involvement of the combination of radon daughters and uranium ore as a causative agent.

Exposure to radon daughters appears to be a primary factor in bronchial and/or bronchiolar epithelial hyperplasia (Figure 31), accompanied by squamous metaplasia with cellular atypia (Figure 32). The data presented in Tables 7A and 7B indicate significantly greater frequency of this lesion for hamsters exposed to radon daughters alone or in combination with uranium ore dust and diesel engine exhaust (Groups 2, 3 and 6). The incidence of squamous metaplastic lesions was significantly higher (see Table 7C) in animals of Groups 2, 3 and 6 that died during 1971 rather than in 1972. The reason for the lower frequency of this lesion after longer exposure is unknown.

The incidence of nonpulmonary lesions in hamsters is presented in Table 8. Few lesions are consistent for any exposure group, with the exception of necrotic hepatic lesions, which are significantly (0.05 level of significance) more frequent in Groups 4, 5 and 6 than in other

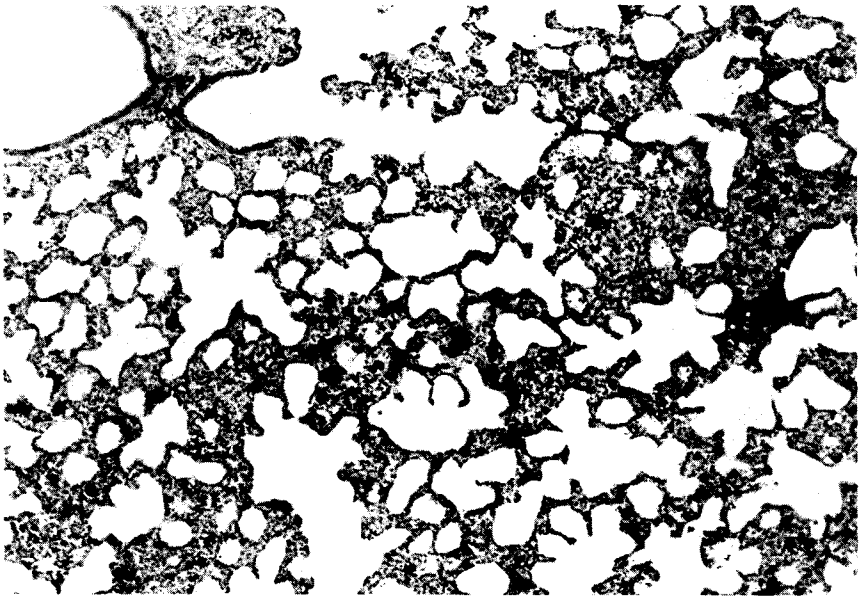

FIGURE 30. A Moderate to Severe Degree of Pulmonary Emphysema in a Hamster Exposed for 11 Months to Uranium Ore Dust and Diesel Engine Exhaust with 7900 WLM of Radon Daughters. H&E. 60X.

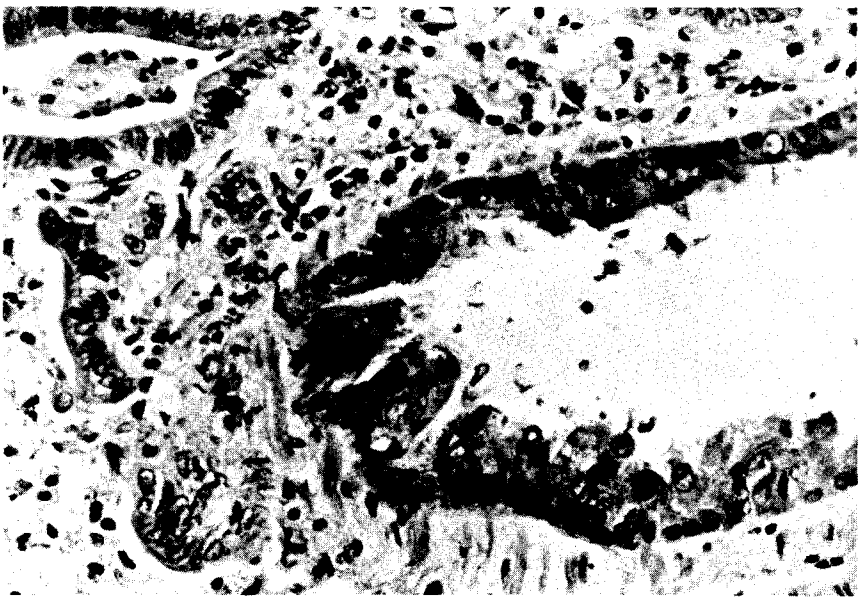

FIGURE 31. Bronchiolar Epithelial Hyperplasia in the Lung of a Group 3 Hamster After 17 Months of Exposure to Uranium Ore Dust with 9600 WLM of Radon Daughters. Atypical and degenerating cells are present. H&E. 300X.

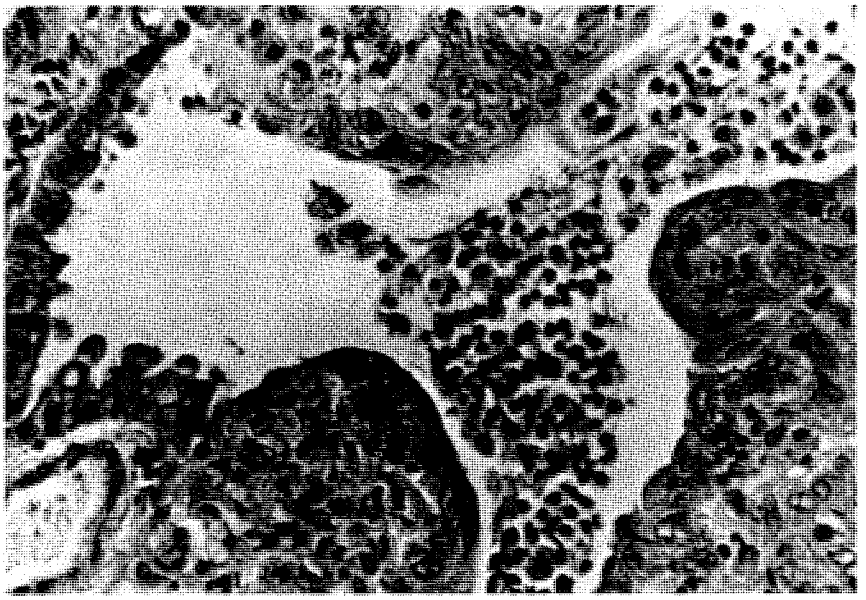

FIGURE 32. Squamous Metaplasia of Bronchiolar Epithelium in the Lung of a Group 3 Hamster. The hamster received 12.5 months of exposure to uranium ore dust with 7200 WLM of radon daughters. H&E. 300X.

TABLE 8. Incidence of Nonpulmonary Lesions in Hamsters

Exposure Group:	1	2		3		4		5		6	
	(Controls)	(Radon daughters)		(Radon daughters + uranium ore)		(Uranium ore)		(Diesel exhaust)		(Radon daughters, uranium ore and diesel exhaust)	
Year of Death:		1971	1972	1971	1972	1971	1972	1971	1972	1971	1972
No. of Animals:	82	51	45	55	46	50	49	55	40	57	49
Liver											
1. Bile stasis	3	0	4	2	3	2	1	0	7	2	3
2. Bile duct proliferation	2	5	2	0	6	0	5	2	7	2	3
3. Cirrhosis	3	2	2	2	5	0	3	0	2	3	2
4. Necrosis	0	0	4	1	4	2	6	5	6	3	7
5. Hepatitis	1	0	0	0	0	0	0	0	0	0	0
6. Amyloidosis	9	6	14	6	34	6	30	3	22	4	26
Kidney											
7. Glomerular nephritis	6	1	6	2	3	4	3	3	0	5	3
8. Tubular nephrosis	3	0	1	1	2	0	0	1	0	1	2
9. Amyloidosis	13	7	19	7	37	8	32	4	32	6	27
Spleen											
10. Amyloidosis	9	4	14	4	29	3	25	1	21	4	10
Adrenal											
11. Amyloidosis	10	6	16	3	30	7	27	3	23	6	22

groups. The frequency of amyloidosis of the liver, kidney, adrenals, and spleen is significantly (generally at the 0.01 level of significance) higher for all animals exposed to particulates (Groups 3 through 6) than for controls. In several older animals, very severe amyloidosis was noted, which probably contributed heavily to their deaths. However, since amyloidosis is common in all hamsters, it is probable that stress from exposure merely increased the incidence.

The 54 replacement hamsters added to each group, which were periodically sacrificed in groups of nine for studies of pulmonary changes, allowed direct comparison of the pathogenesis of lesions in animals exposed to various agents for the same periods of time. The cumulative exposure for each radon daughter-exposed group is listed in Table 9. Generally, the most significant lesions in these hamsters were in the lungs, apparently representing early stages of the types of lesions reported in the hamsters of the original group. The incidences of various pulmonary lesions are listed in Table 10 for each exposure group at the time of sacrifice. Only those changes that were present with more than a slight degree of severity were enumerated; the table does not otherwise reflect the severity of pulmonary changes.

Most nonpulmonary changes observed in these hamsters were considered age-related and occurred in animals of Group 1 (control) with the same frequency and degree of severity as in the exposure groups. Nonpulmonary lesions included amyloidosis of the kidneys, spleen, liver and adrenals. There was no indication that the amyloid conditions were initiated by

TABLE 9. Cumulative Exposures for Each Group Exposed to Radon Daughters

Group	Months of Exposure	Cumulative Exposure (WLM)
2	4	1750 ± 680
	6	
	6	3090 ± 1270
	8	4570 ± 1850
	11	7020 ± 2890
3	4	2840 ± 1080
	6	4130 ± 1460
	8	5820 ± 1970
	11	8150 ± 2440
6D	4	2930 ± 400
	6	4460 ± 750
	8	6020 ± 950
	11	7900 ± 1440

TABLE 10. Incidence of Pulmonary Lesions in Hamsters Added to the Exposure Groups and Sacrificed Periodically

Exposure Group:	1 (Control)						2 (Radon daughters)						3 (Radon daughters + uranium ore)						4 (Uranium ore)						5 (Diesel exhaust)						6 (Radon daughters, uranium ore and diesel exhaust)					
No. of Months Exposure	1	2	4	6	8	11	1	2	4	6	8	11	1	2	4	6	8	11	1	2	4	6	8	11	1	2	4	6	8	11	1	2	4	6	8	11
No. of Animals	5	8	6	6	6	5	7	6	6	6	3	5	6	6	7	6	6	4	6	6	9	5	6	4	6	8	6	6	5	5	6	6	6	6	6	4
Focal Accumulation of Macrophages	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	2	6	4	0	2	8	1	4	3	0	5	4	4	4	5	0	5	6	6	6	4
Emphysema	0	0	0	1	1	0	0	0	0	0	0	0	0	1	3	3	2	2	0	1	7	0	2	0	0	2	3	2	4	5	0	2	4	4	6	4
Interstitial Pneumonitis	1	0	0	0	1	2	0	0	1	1	1	2	0	2	1	1	3	3	0	0	9	0	3	3	0	0	4	0	1	3	0	2	4	2	5	4
Septal Cell Hyperplasia	1	0	0	0	1	0	0	0	0	0	0	0	0	1	4	0	0	1	1	1	5	0	0	0	0	4	1	2	2	2	2	3	5	4	3	2
Adenomatous Proliferation of Alveolar Epithelium	0	1	0	2	2	0	2	3	0	0	0	2	0	2	1	1	3	4	1	3	5	1	1	3	1	5	4	4	3	3	3	3	3	4	5	3
With Squamous Metaplasia												2						3																		1
Bronchiolar Epithelial Hyperplasia	1	3	1	0	0	0	2	0	1	1	0	0	0	0	1	0	0	0	4	2	0	1	1	2	0	2	1	0	0	2	1	1	0	0	2	2

any of the exposures in this experiment. Centrilobular hepatocyte degenerative changes and renal tubular nephrosis were the other conditions common to animals of all groups.

One osteosarcoma was present in a Group 5 animal that died after 4 months of exposure to diesel exhaust. The tumor was considered to be spontaneous, since it occurred only once.

The lungs of control animals remained essentially lesion-free throughout the 11 months of the experiment. Only two cases of vesicular emphysema and septal cell hyperplasia, and four cases of moderate acute interstitial pneumonitis, were noted during the 11-month period. Lungs of five of the hamsters contained small foci of adenomatous hyperplasia, and focal bronchiolar epithelial hyperplasia was present in the lungs of five others. These lesions were minimal and were regarded as spontaneous changes (not exposure-induced).

Group 2 animals were relatively lesion-free for the first 8 months of the experiment. Pulmonary emphysema was slight and infrequent, and a low incidence of slight septal cell hyperplasia was observed during the 11 months of the experiment. Five cases of interstitial pneumonitis appeared during the last 7 months of the experiment.

Probably the most significant lesions discovered in Group 2 animals were the areas of alveolar epithelial bronchiolization in the lungs of two hamsters killed after 11 months of exposure. In both animals there was progression to squamous metaplasia.

The lungs of Group 3 animals were characterized by early, rapid increases in the frequency and severity of both emphysema and acute

interstitial pneumonitis, which stabilized to gradual increases after 4 months of exposure. A granulomatous response to the uranium ore dust was observed in all ore dust exposure groups (3, 4, and 6). This was characterized early in the exposure period by large foci of macrophages containing ore dust; as exposure time increased, pneumoconiotic lesions became apparent.

Adenomatous proliferation of alveolar epithelium appeared at 4 months of exposure and continued to be a finding throughout the experimental period. Squamous metaplasia of hyperplastic epithelium occurred in the lungs of 3 of 4 hamsters at 11 months in Group 3. In that respect, this group of animals was the most severely affected during the 11-month experimental period.

The lungs of Group 4 animals were affected by lesions at much the same rate as that described for Group 3, particularly with regard to emphysema, septal cell hyperplasia, acute interstitial pneumonitis, and accumulation of pulmonary macrophages. Adenomatosis of alveolar epithelium occurred slightly more frequently but with slightly less severity than in Group 3 animals. During the 11 months of the experiment, no squamous metaplastic changes of the bronchiolized alveolar epithelium were found in this group, in contrast to the findings for hamsters of Groups 2 and 3.

Probably the most striking feature of the lungs of animals exposed to diesel engine exhaust (Group 5) was the heavy accumulation of soot over the longer exposure periods, both in alveolar macrophages and in alveolar air spaces. Pulmonary emphysema was a significant lesion in

these animals, with a relatively high frequency after only 2-4 months of exposure and, by 8 months, a moderate degree of severity. The diesel engine exhaust exposure also caused pulmonary consolidation, due to septal cell hyperplasia and acute interstitial pneumonitis, appearing as early as 2 to 3 months after the beginning of exposures. Moderately severe bronchiolization of alveolar epithelium also occurred in this group, with a relatively high frequency early in the exposure. Only a gradual increase in severity was seen between 4 and 11 months, although the incidence of the lesion remained high.

The lungs of Group 6 animals exhibited very similar lesions to those of Group 5, with slightly higher frequency of occurrence and slightly greater severity throughout the experiment. Severe lesions of adenomatous proliferation of the alveolar epithelium were observed, many of which contained cholesterol clefts. There were, however, fewer indications of squamous metaplasia than were noted in Groups 2 and 3. The possibility exists that proliferative changes of this nature were partly obscured by the very heavy soot accumulations by the 11th month of exposure.

DISCUSSION AND SUMMARY

From examination of the data presented in Tables 7A, 7B and 8 it can be appreciated that exposure to particulates, i.e., uranium ore dust and diesel exhaust soot, provoked inflammatory and proliferative responses in lungs consisting of macrophage accumulation, alveolar cell hyperplasia,

and adenomatous alteration of alveolar epithelium. Additionally, exposure to radon + radon daughters was associated with increased occurrence of bronchiolar epithelial hyperplasia and with metaplastic changes of alveolar epithelium.

Squamous carcinoma developed in four hamsters, three of which received exposure to radon + radon daughters, and one to radon + radon daughters + uranium ore dust. Squamous carcinoma occurred only in association with squamous metaplasia of alveolar epithelium which, as stated above, occurred only in hamsters receiving exposure to radon + radon daughters alone or in combination with uranium ore. Several animals in Groups 2 and 3 developed squamous metaplasia of alveolar epithelium, but carcinoma was not diagnosed. Thus, it appears that exposure to radon + radon daughters, development of squamous metaplasia and development of carcinoma were related.

The sequence of events leading to development of carcinoma appears to be dependent upon the change to the squamous form of epithelium, which in turn appears to have developed in areas that underwent adenomatous change (adenomatoid proliferation: bronchiolization of alveolar epithelium). The adenomatous change occurred in hamsters that received exposure to particulates alone without exposure to radon daughters, i.e., Groups 4 and 5 hamsters receiving uranium ore dust and diesel exhaust exposure. In these groups, however, the adenomatous lesions did not undergo further morphologic change. Thus, it appears that not only was squamous metaplasia induced by radon + radon daughter exposure, it was a prerequisite stage in development of carcinoma. The question of whether the altered

epithelium was committed to malignancy once it became squamous is not answerable by the results of the experiments. This change is generally regarded as a necessary stage in the cellular events whereby normal epithelial cells of the air passageways of humans are transformed to malignant squamous cell carcinoma.

The data from this experiment at least suggest that this cellular change is also a precursor or premalignant change in hamsters; that this is true of other species (i.e., rats and dogs) is supported by data from experiments discussed later in this report.

An additional conclusion may be drawn from the data obtained in this study: an animal model other than the hamster should be selected for study of the pulmonary carcinogenic potential of uranium ore alone or diesel exhaust alone. This conclusion is based on two observations: (1) that the Syrian golden hamster has, by this time, definitely been shown to be highly refractory to carcinoma induction, especially by agents with low carcinogenicity; (2) that when it is exposed to "realistic" levels of these agents in life-span exposure regimens, the hamster does not develop lesions that could be classified as precancerous, much less as malignant.

PART II - DOG STUDIES

A. Methods

The experimental design for the dog studies is shown in Table 11. The procedures used for exposing beagle dogs to cigarette smoke in a manner similar to that of a man smoking were developed in previous studies in our laboratory.[29,30] Beagle dogs raised in our laboratory (both male and female) were trained to accept daily smoking of 10 cigarettes* (Groups 2 and 3) or to sham smoke unlighted cigarettes (Groups 1 and 4) for identical periods. Individual smoking masks fitted to each dog permitted direct oral smoke inhalation and nose-plus-mouth exhalation (Figure 33). The slight negative pressure in the mask at the start of each breath was detected by a pressure transducer that sent a signal to an electromechanical stepper switch. This switch advanced with each breath, and was adjusted to activate the rotary solenoid at each tenth breath. Activation of the solenoid operated a valve that cause the inspired air to be drawn through the cigarette rather than through the room-air port, as was the case for the other nine breaths.

One of two identical 10-dog exposure chambers used in these studies is shown in Figure 34. These chambers provided space for simultaneous head-only exposures of 20 dogs to radon daughters and carnotite ore dust. An aerosol diffusion system was incorporated into each chamber in order to channel fresh aerosol past each dog's head. As in the hamster chambers, radon was generated separately for each of the

* University of Kentucky, 1R1 research cigarettes.

TABLE 11. Experimental Design for Dog Studies

Group No.*	No. of Animals	Exposure Chamber Contents
1	20	Radon, radon daughters, and uranium ore dust
2	20	Radon, radon daughters, uranium ore dust and cigarette smoke
3	20	Cigarette smoke
4	9	Room air

* Group 1 was supported by the AEC; Groups 2, 3 and 4 by the NIEHS.

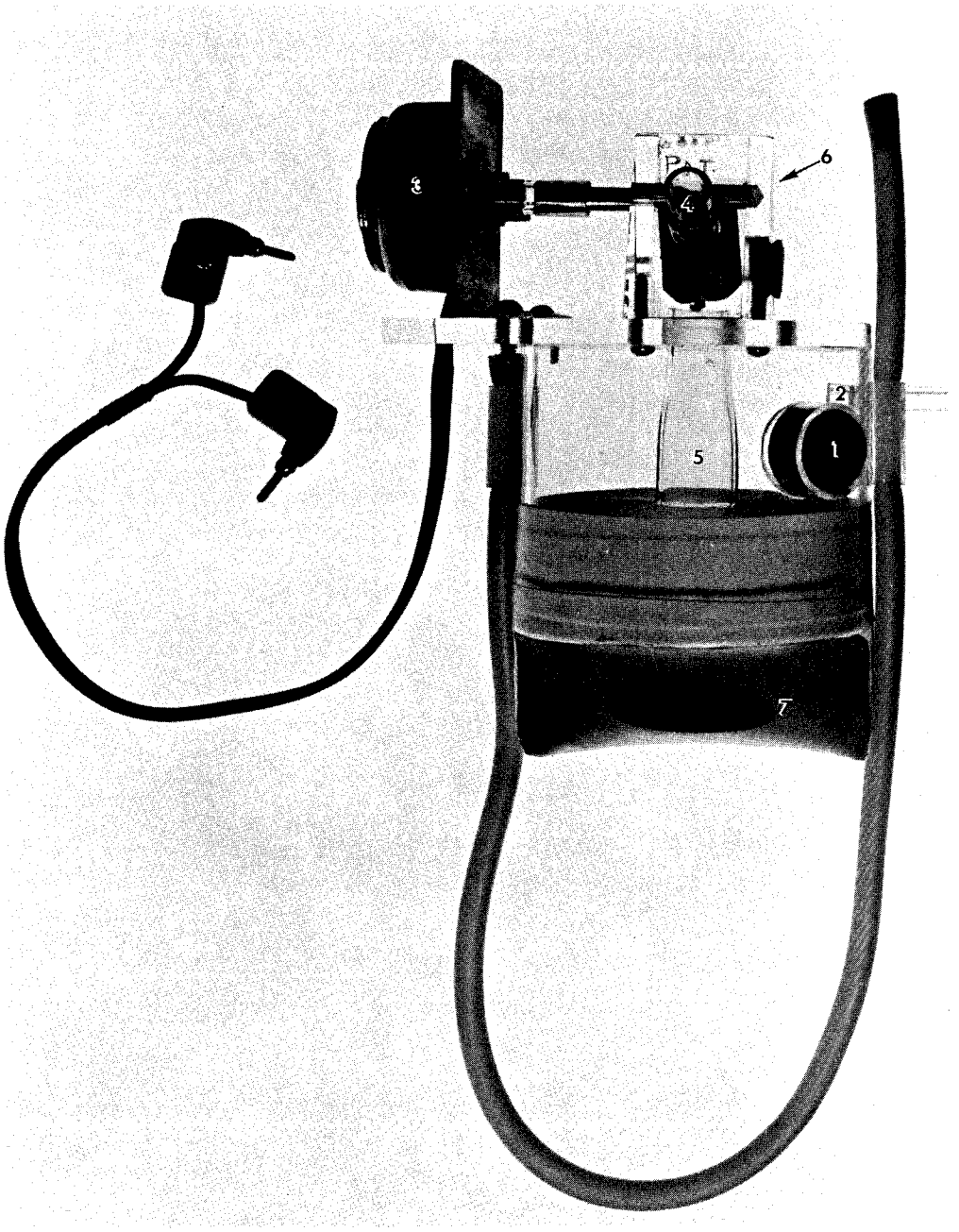

FIGURE 33. Mask Used for Smoking Cigarettes. Note the flutter valve for nasal exhalation from the mask (1) and the tube connected to a negative pressure sensor (2). A solenoid valve (3) controls the frequency with which smoke is inhaled through the cigarette-holder (4) into the plastic mouthpiece (5). Room air enters at (6), beneath the cigarette holder, at a frequency controlled by the pressure and electro-mechanical stepper switches. The dog's muzzle is surrounded by a latex sheet (7) that prevents escape of air and smoke from the mask.

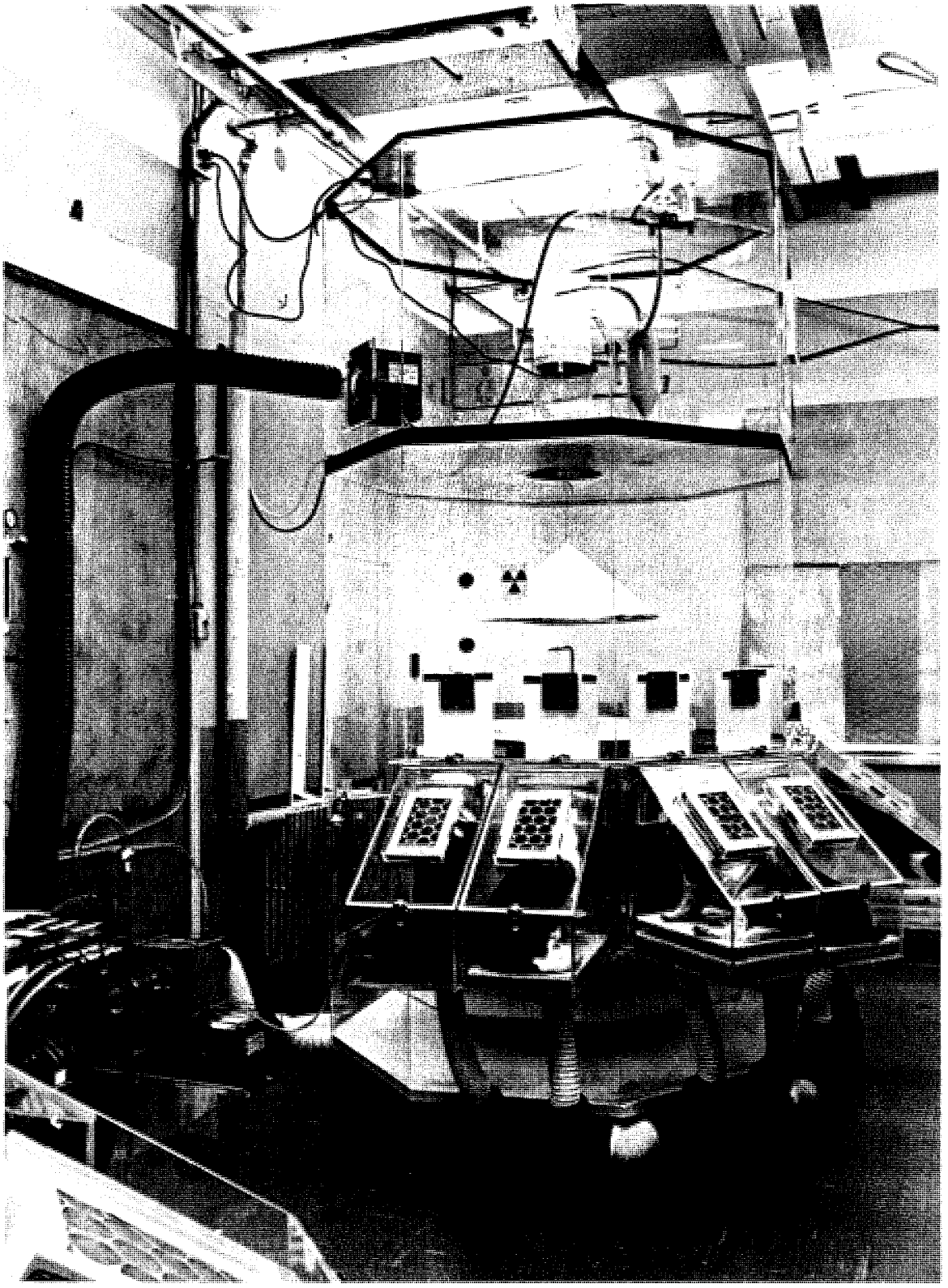

FIGURE 34. Exposure Chamber Designed for Head-Only Exposures of 10 Dogs to Radon Daughters with Uranium Ore Dust

two chambers. The uranium ore dust was added to the inlet room air and radon air streams with WDFMs. Magnetically closed doors were designed to ensure tight seals between chamber and room during aerosol concentration-buildup periods. The chambers were equipped with instrumentation for hourly radon measurements. Radon daughter, uranium ore dust and condensation nuclei concentrations were measured daily. Chamber aerosols were occasionally monitored for particle size distributions and for the fraction of unattached RaA, as described earlier for the hamster chambers.

A typical 16-hour daily exposure regimen proceeded in the following manner: Group 2 dogs smoked 3 cigarettes in a 60-minute period and were returned to their cages for 30 to 60 minutes prior to exposure to radon daughters with uranium ore dust. After four hours exposure, the dogs' heads were cleaned and they were returned to their cages for a 90- to 120-minute period, permitting decay of the short-lived radon daughters so as to eliminate radioactive contamination of personnel handling the dogs during subsequent smoking procedures. The boxes that held the dogs during exposure to radon daughters with uranium ore dust were cleaned after decay of the short-lived radon daughters, after which Group 1 dogs were exposed to radon daughters and uranium ore dust for 4 hours. Following the 90- to 120-minute decay period, Group 2 dogs began a second period of smoking 4 cigarettes over a 90-minute period. In the third smoking period, begun 4 to 5 hours after the second smoking period, the dogs were exposed to 3 cigarettes over a 60-minute period. Group 3 animals smoked 10 cigarettes over the same intervals but at different

times in the 16-hour period. Groups 1 and 4 dogs sham-smoked 10 cigarettes over the same time intervals but during different times in the 16-hour period; the cigarettes were not lighted. Exposures were conducted 5 days per week; over weekends dogs were exposed to smoking and sham smoking, to simulate the exposure received by a uranium miner who smokes.

The dogs' health was monitored on a day-to-day basis by exposure technicians and animal care personnel; periodically each dog received a complete physical examination, including thoracic X-ray. All animals were weighed biweekly; respiration rates, minute and tidal volumes were measured monthly; blood was sampled quarterly to permit hematology and clinical chemistry measurements, and periodically for measurement of carboxyhemoglobin and plasma thiocyanate.

In the majority of cases, Groups 1 and 2 animals were sacrificed when death appeared imminent because of pulmonary insufficiency, which was characterized by rapid, shallow breathing. Groups 3 and 4 animals were sacrificed at periods corresponding to high mortality peaks in Groups 1 and 2 so that tissues might be compared.

Animals were sacrificed by exsanguination under deep pentothal anesthesia. At necropsy a sufficient number of samples was taken to permit detailed histopathological evaluation of the respiratory tract: turbinates, larynx, trachea, and all lung lobes. Other organ systems were sampled less extensively; however, all organs have been preserved for possible future examination. Tissues were fixed with 10% neutral buffered formalin.

In an ancillary study, three dogs smoked 20 cigarettes per day, seven days per week for nine months to compare pulmonary histopathology in a very heavy smoker to that seen in Group 3 dogs, which received only half of that smoke exposure. Immediately before sacrifice, each of the three dogs smoked three cigarettes, identically labeled with ^{14}C-dotriacontane, to obtain a measure of smoke deposition in each dog. Dotriacontane is a 32-carbon hydrocarbon with a boiling point of 310°C. It is volatilized during smoking and is adsorbed on the particulate phase of the smoke. Each of these dogs smoked the first of its three cigarettes through a filter that removed the particulate phase from the mainstream smoke prior to the dog's inhalation. The second and third cigarettes were smoked in the usual manner without a filter. The measured amount of ^{14}C-dotriacontane on the filter from the first cigarette was taken to be one-half of the amount that the dog inhaled while smoking the second and third cigarettes. (Previous tests had shown that greater than 98% of the ^{14}C-dotriacontane in the mainstream smoke was retained by the filter.) During these tests, the particulate fraction of the air exhaled by the dog was collected and was subsequently analyzed for its ^{14}C-dotriacontane content.

At necropsy, multiple weighed aliquots from each lobe of the dogs' lungs were collected, and were subsequently analyzed for their ^{14}C-dotriacontane content to obtain a measure of its average concentration in the lung. Multiplication of the concentration by the total lung weight gave a measure of the amount of the particulate phase that was deposited in the dog's lungs from the second and third labeled cigarettes.

B. Results

During the 4-1/2 years that Group 1 and 2 dogs were exposed to radon daughters and uranium ore dust, radon daughter concentrations averaged 605 Working Levels (WL), with a standard deviation of 169 WL. During the same period of time the average radon concentration was 105 $\pm$ 20 nCi/ℓ; the uranium ore dust, 12.9 $\pm$ 6.7 mg/m^3; and the condensation nuclei, 73,000 $\pm$ 20,000 per cm^3. Mass median aerodynamic diameters of the uranium ore dust ranged from 0.6 to 1.2 μm, with geometric standard deviations of 1.8 to 2.6. Activity median aerodynamic diameters, measured subsequent to exposure, averaged 0.60 μm, with a geometric standard deviation of 1.7. The unattachment of RaA averaged <3%. Unattached percentages of RaB and RaC were only fractions of RaA levels.

Tables 12 and 13 show the exposure history of Groups 1 and 2 dogs up to the time of their death. Cumulative exposures (WLM) were calculated by multiplying the actual number of hours each dog was exposed by the average radon daughter concentration (605 WL), and dividing by 170. Some animals show equivalent or higher exposure months but lower cumulative exposure than others because they were removed from experiment for a period of time due to illness or injury. After they had recovered, their exposures were continued. No significant (at the 0.05 level of significance) differences between survival curves (Figure 35) were noted for these animals.

Table 14 shows the average number of puffs and length of time required for Groups 2 and 3 dogs to smoke 85-mm, 1R1 cigarettes to the standard 23-mm butt length. These values, measured about one year after

TABLE 12. Cumulative Exposure and Interval Until Death of Group 1 Dogs (mean ± S.D.)

Dog No.	Months of Exposure	Interval From End of Exposure to Death (Months)*	Cumulative Exposure (WLM)	Neoplasms
610	34	0	9410 ± 2630	
505	37	0	10,400 ± 2910	
509	39	0	11,400 ± 3180	
520	42	0	10,800 ± 3030	
577	43	0	11,000 ± 3070	
642	44	1	12,800 ± 3590	
615	46	2	13,300 ± 3730	Nasal carcinoma
539	48	0	11,900 ± 3320	
608	48	9	14,000 ± 3920	Epidermoid carcinoma and nasal carcinoma
524	51	0	13,100 ± 3660	
512	51	0	14,200 ± 3970	Bronchioloalveolar carcinoma
567	52	0	14,900 ± 4150	
514	54	2	15,700 ± 4380	
522	54	2	15,600 ± 4370	
523	54	0	15,300 ± 4260	Epidermoid carcinoma
531	54	0	15,700 ± 4390	Bronchioloalveolar carcinoma
541	54	3	15,700 ± 4390	Epidermoid carcinoma
540	54	8	15,700 ± 4400	Fibrosarcoma
525	54	11	15,700 ± 4390	Bronchioloalveolar carcinoma

* Exposure to radon daughters and uranium ore stopped after 4-1/2 years.

TABLE 13. Cumulative Exposure and Interval Until Death of Group 2 Dogs (mean ± S.D.)

Dog No.	Months of Exposure	Interval From End of Exposure to Death (Months)*	Cumulative Exposure (WLM)	Neoplasms
855	33	15	9780 ± 2730	
591	37	0	10,200 ± 2860	
629	38	0	9240 ± 2580	
585	42	0	11,200 ± 3130	
595	42	0	11,500 ± 3210	
552	44	0	12,700 ± 3540	
587	46	0	11,800 ± 3300	
627	46	0	13,000 ± 3630	
516	46	0	12,200 ± 3420	
562	46	0	12,900 ± 3610	
637	46	1	13,200 ± 3650	
504	47	0	13,200 ± 3690	
593	50	0	14,300 ± 4000	
544	51	0	13,800 ± 3840	Nasal carcinoma
573	52	0	14,900 ± 4160	
545	52	1	15,100 ± 4210	
551	52	3	15,000 ± 4200	
530	52	8	15,100 ± 4210	
518	52	28	12,000 ± 3340	Bronchioloalveolar carcinoma

* Exposures to radon daughters and uranium ore stopped after 4-1/3 years, but smoke exposures continued until death.

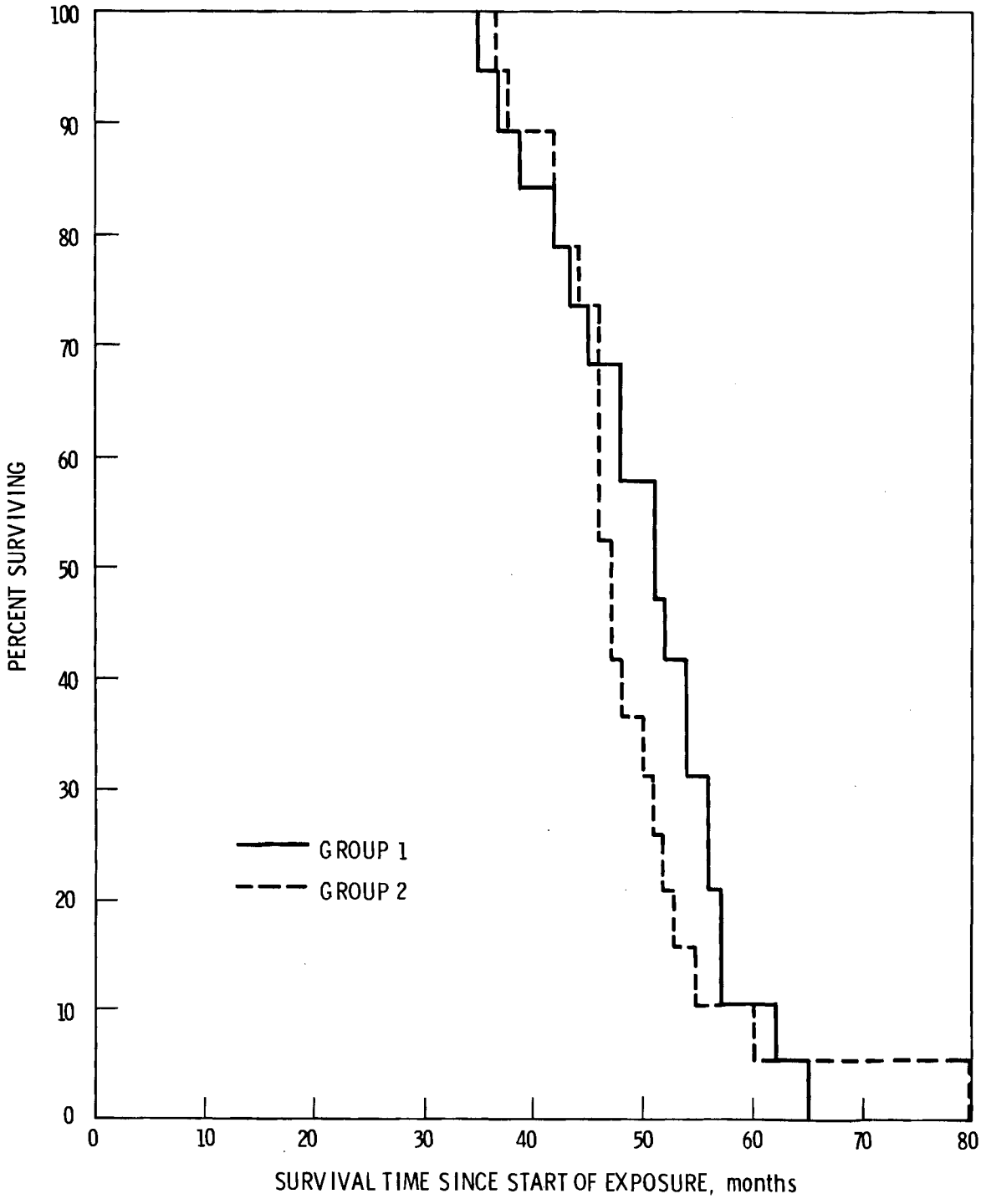

FIGURE 35. Survival Curves for Animals of Groups 1 and 2

TABLE 14. Cigarette Smoking Patterns in Beagle Dogs

Group	Date	Number of Puffs per Cigarette*	Number of Minutes per Cigarette*	Number of Puffs per Minute
1	10-2-71	19 ± 6	12**	1.6 ± 0.5
	11-6-73	36 ± 8	12	3.0 ± 0.7
2	10-2-71	20 ± 6	12 ± 1	1.7 ± 0.5
	11-6-73	28 ± 7	11 ± 1	2.6 ± 0.6
3	10-2-71	18 ± 5	12 ± 1	1.5 ± 0.4
	11-6-73	21 ± 3	11 ± 1	1.9 ± 0.3
4	10-2-71	16 ± 5	12**	1.3 ± 0.4
	11-6-73	19 ± 3	12	1.6 ± 0.3

* Mean values ± standard deviation.
** Period of sham smoking using unlighted cigarettes.

the start of smoke exposures, and again approximately 2 years later, reflect an increase in respiratory rate with exposure to radon daughters and ore dust.

Table 15 lists percentage levels of carboxyhemoglobin, measured periodically during the course of exposures, in the blood of dogs exposed to cigarette smoking or sham smoking. As expected, carboxyhemoglobin levels were increased in the blood of smoking dogs. A one-time measurement of plasma thiocyanate levels showed levels in Groups 2 and 3 dogs nearly double those in the sham smokers (40 $\pm$ 2 micromoles per liter, vs 25 $\pm$ 2).

Table 16 shows the results of measurements of particulate deposition from cigarettes in the lungs of the three dogs that had smoked 20 cigarettes per day for 9 months. The finding of 30% deposition in two of the three dogs correlates well with deposition patterns in man for particle sizes of 0.2 to 0.3 microns. The close agreement between the values for the amount of smoke deposited, obtained by measuring the amount in the lung and by subtracting the amount exhaled from that inhaled, shows that very little lung clearance occurred during the few minutes between smoke exposure and sacrifice.

TABLE 15. Carboxyhemoglobin Levels (%)* in Dogs

Date	Group 1	Group 2	Group 3	Group 4
5-13-71	1.2 ± 0.2	2.0 ± 0.6	2.0 ± 0.3	1.4 ± 0.4
8-12-71	1.5 ± 0.3	2.3 ± 0.2	3.2 ± 0.4	1.1 ± 0.5
4-18-72**	2.2 ± 0.1	2.6 ± 0.1	3.2 ± 0.2	2.2 ± 0.1
5-8-73	2.6 ± 0.1	4.2 ± 0.3	4.6 ± 0.3	2.3 ± 0.2
11-27-73	2.0 ± 0.8	5.3 ± 1.7	4.7 ± 0.3	1.8 ± 0.2

* Mean ± Standard Deviation
** New circuit board and filters installed in analyzer.

TABLE 16. Deposition of Cigarette Smoke Particulates in the Lungs of Smoking Dogs

	Dog #1117	Dog #1119	Dog #1123
Cigarette #1, through filter			
^{14}C on filter	4.88 μCi	4.60 μCi	5.83 μCi
Cigarettes #2 and #3, no filter			
Total ^{14}C Inhaled (two times above)	9.76 μCi	9.20 μCi	11.66 μCi
^{14}C in Exhaled Air	*	6.44 μCi	7.96 μCi
Total ^{14}C Deposited (Inhaled minus Exhaled)	*	2.76 μCi	3.70 μCi
^{14}C Measured in Lungs	1.53 μCi	2.73 μCi	3.53 μCi
% Inhaled that Deposited in Lung	15.7%	29.7%	30.3%

* No value obtained due to malfunction of the filter collecting the exhaled air from Dog #1117.

C. Pathology

Histopathologic changes present in the tissues of the dogs of Group 4 (sham-exposed controls) will be discussed first, to be followed by a discussion of changes induced by cigarette smoke only (Group 3). Because the changes present in the respiratory tracts in dogs from Group 1 (radon daughters and uranium ore dust) and Group 2 (radon daughters, uranium ore dust, and cigarette smoke) were similar, they will be discussed together. The probable progressions of many exposure-induced respiratory tract changes, including neoplasia, are traced and reported below.

1. Group 4 (Sham-Exposed Controls)

Minimal changes occurred in the respiratory tracts of the sham-exposed control dogs examined. One dog (#526) from Group 4 was sacrificed after 52 months of sham exposure for comparison of histopathologic data with those from the large number of exposed dogs requiring sacrifice due to respiratory distress. The respiratory tract of this dog was generally normal in appearance. There were a few small foci of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and metaplasia (Figure 36) in the lungs. Those lesions are considered spontaneous in nature, and are commonly found in older beagle dogs.[31]

Three other control dogs (#517, #570, and #574) were killed after 65 months of sham exposure. Pulmonary changes included a slight degree of subpleural vesicular emphysema in two of the three dogs, and small amounts of focal mononuclear cellular infiltration in all three dogs.

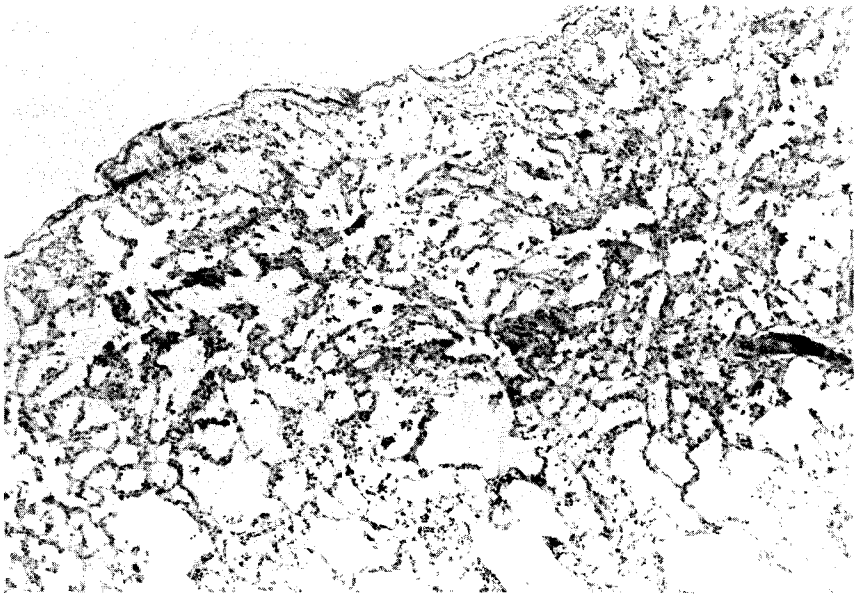

FIGURE 36. Subpleural Interstitial Fibrosis with Associated Alveolar Epithelial Hyperplasia in the Lungs of Dog 526 (Control) after 52 Months of Sham Exposure. H&E. 70X.

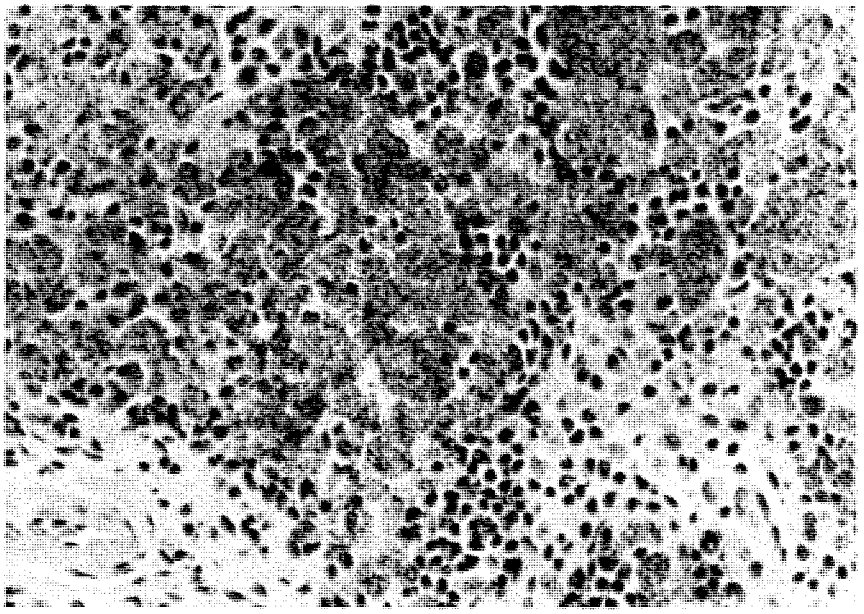

FIGURE 37. Tracheobronchial Lymph Node from Dog 536 (Cigarette Smoke - 49 Months). Histiocytosis-containing pigment considered to be related to cigarette smoking is visible in a large area (H). H&E. 350X.

The lungs of the three dogs also contained slight to moderate degrees of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and metaplasia. All three dogs had a very slight degree of basal cell hyperplasia in both the tracheal and laryngeal mucosa, as well as slight glandular hyperplasia in these organs. In addition, the tracheal epithelium had a slightly flattened appearance in each of the three dogs.

2. Group 3 (Cigarette Smoke Only)

Dog #536 from Group 3 was killed after 49 months of exposure for histological comparison with dogs from Groups 1 and 2 that had died or were killed.

Relatively small amounts of an exogenous yellow pigment that is considered to be associated with cigarette smoking were found in alveolar macrophages in the lungs of this animal. The tracheobronchial lymph nodes, however, contained large amounts of phagocytized exogenous yellow pigment in histiocytes (Figure 37). Pulmonary lesions in this dog included slight vesicular emphysema (Figure 38) and occasional foci of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and metaplasia (Figure 39). The latter lesion was also seen in control dogs of this experiment, although the alveolar epithelial metaplasia in this dog was slightly more advanced, with very small foci of squamous metaplasia (Figure 40).

One dog from Group 3 (#550) died of foreign-body pneumonia subsequent to vomition during smoke exposure. In lung sections, numerous alveolar

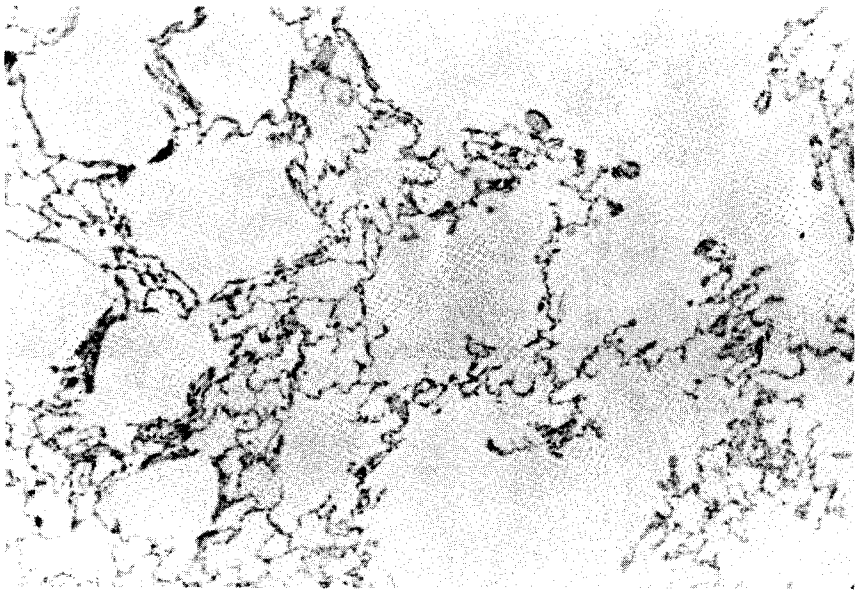

FIGURE 38. Vesicular Emphysema in the Lung of Dog 536 After 49 Months of Exposure to Cigarette Smoke Only. H&E. 70X.

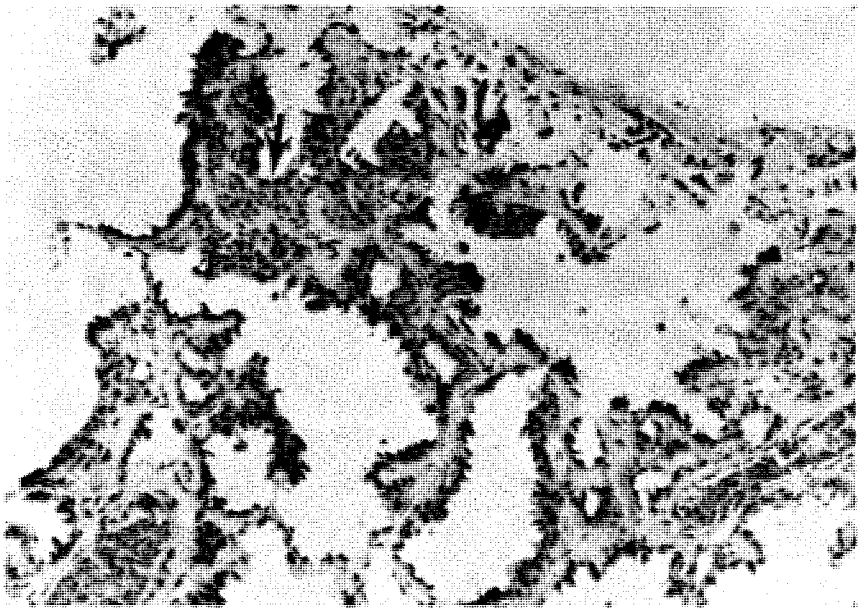

FIGURE 39. Subpleural Interstitial Fibrosis with Alveolar Epithelial Metaplasia from the Lung of Dog 536, Exposed to Cigarette Smoke Only for 49 Months. The metaplastic epithelium has assumed a squamous nature in a portion of the area shown (arrow). H&E. 175X.

macrophages adjacent to small blood vessels and bronchioles contained a black pigment, probably particulate material from the cigarette smoke (Figure 41). Slight dilation of bronchial mucus glands and a slight degree of subpleural vesicular emphysema were also present. Extensive inflammatory changes, including numerous microgranulomata, were seen. Microgranulomata were present in the pulmonary parenchyma of three other dogs exposed to smoke from 20 cigarettes per day and are described subsequently. The lesions are associated with cigarette smoking but were not observed in the lungs of dogs from Groups 1 or 2, probably because they were obscured by more severe inflammatory and fibrotic changes. None were present in a Group 3 dog after 49 months of exposure.

Three other dogs from Group 3 (#557, #561, and #589) were killed after 65 months of exposure to cigarette smoke. Tissues from these dogs were directly compared with tissues from the three sham-exposed dogs killed at the same time (previously discussed).

There were mild chronic inflammatory changes in the lungs of dogs from the smoke-exposed group. A moderate bronchiolitis in one dog (#561) was characterized by a mononuclear inflammatory infiltrate surrounding respiratory and terminal bronchioles, and extended into the surrounding alveolar septa, forming an associated chronic alveolitis. The smoke-exposed dogs had an increased amount of phagocytized yellow pigment found primarily in peribronchiolar and perivascular areas. There was a tendency toward more subpleural vesicular emphysema in the smoke-exposed dogs than in the sham-exposed dogs. Focal interstitial pneumonitis, focal

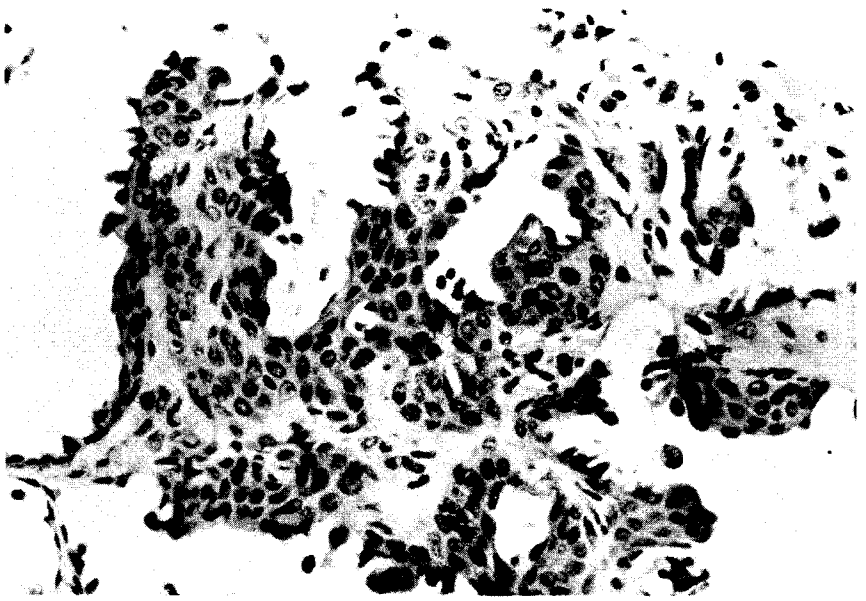

FIGURE 40. Higher Magnification of Alveolar Epithelial Squamous Metaplasia Shown in Figure 39. H&E. 350X.

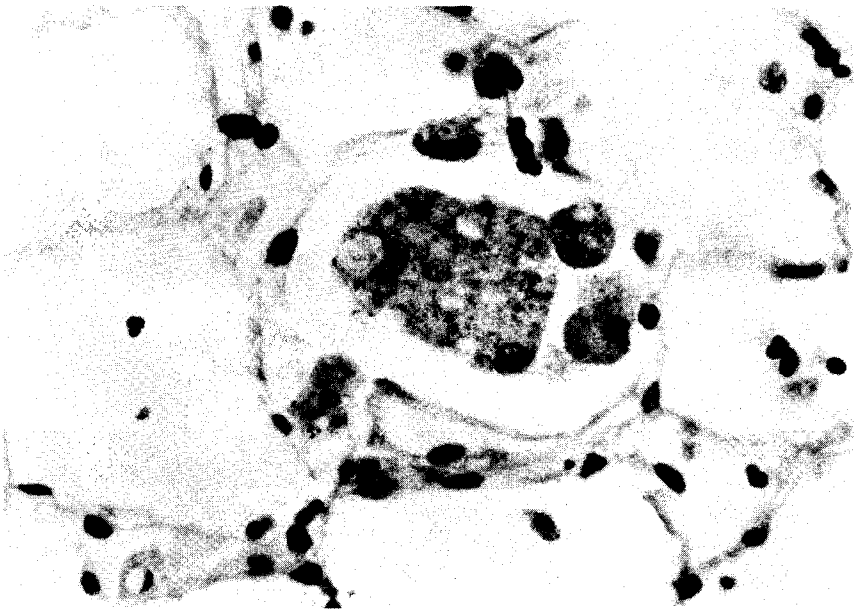

FIGURE 41. Alveolar Macrophages Containing Cigarette Smoke Pigment, from the Lung of Dog 550, Exposed to the Smoke of 10 Cigarettes Per Day for 60 Months. H&E. 700X.

mononuclear cellular infiltration, subpleural interstitial fibrosis with associated alveolar epithelial cell hyperplasia, and focal calcification occurred in both smoke-exposed and sham-treated dogs.

The tracheas of two smoke-exposed dogs and the larynx of one smoke-exposed dog had thickened basement membranes, which were suggestive of chronic irritation, possibly related to smoke inhalation. Other changes in the trachea and larynx were present in both sham-exposed and smoke-exposed animals and were, therefore, probably not related to treatment; these include basal cell hyperplasia, glandular hyperplasia, cellular infiltration and flattened epithelium. The larynx of one control dog had a small ulcer.

The tracheobronchial lymph nodes of all three smoke-exposed dogs had lesions clearly related to treatment. Two dogs (#557 and #589) had moderate amounts of yellow pigment, which appeared to be the same as the smoke-related pigment found in the lungs. The moderate reactive lymphoid hyperplasia in the tracheobronchial lymph nodes and the moderate histiocytosis in the mediastinal lymph nodes of dog #561 were probably related to the chronic inflammatory changes in the lung of this smoking dog.

In the three dogs (#1117, #1119, and #1123) that were exposed to smoke from 20 cigarettes per day, respiratory tract changes included focal areas of pleural thickening, alveolar septal fibrosis, and subpleural chronic inflammation (Figure 42). Pulmonary vesicular emphysema (Figure 38) ranged in severity from very slight in one of the dogs to slight-to-moderate in another, and two of the dogs had large areas of acute interstitial pneumonitis (Figure 43). Additional changes observed in the

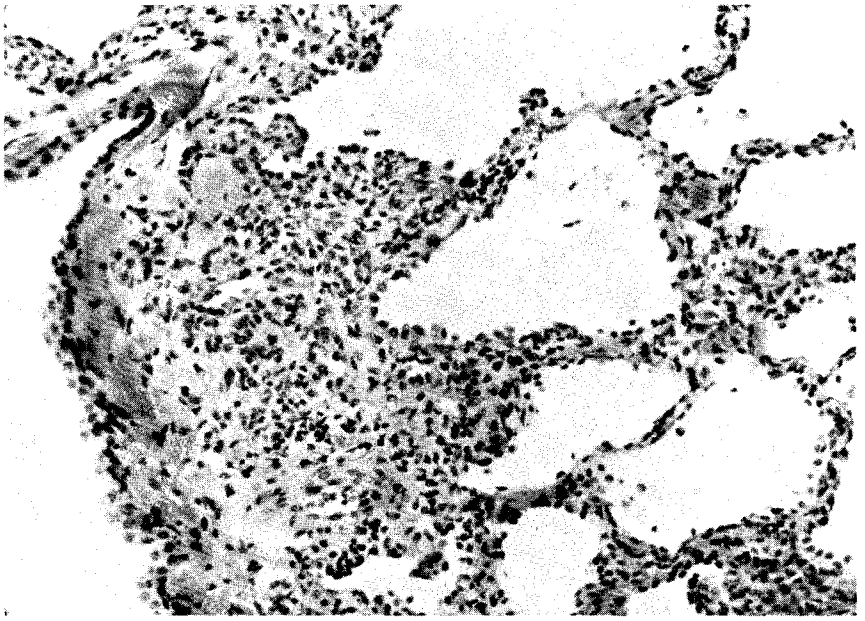

FIGURE 42. A Focus of Pleural Thickening, Subpleural Inflammation, Alveolar Septal Fibrosis, and Vesicular Emphysema. This lung section is from a dog that smoked 20 cigarettes per day for 9 months. H&E. 175X.

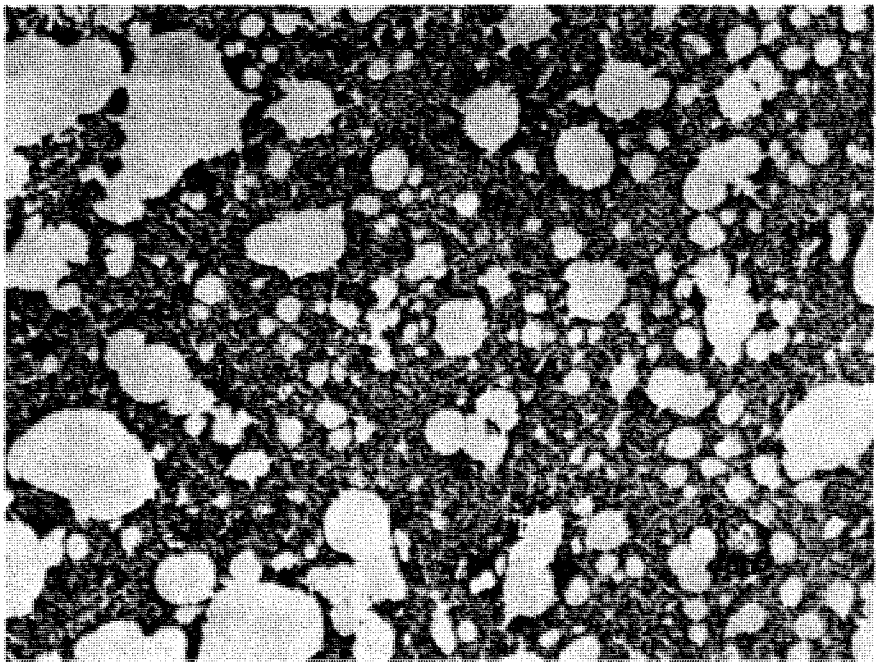

FIGURE 43. Acute Interstitial Pneumonitis in the Lung of a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 80X.

pulmonary parenchyma of these dogs included numerous focal granulomata (Figures 44 and 45), which appeared to contain fat cells. The micro-granulomata were not present in the lungs of a dog examined after 49 months of smoking 10 cigarettes per day (Group 3), but have been observed in the lungs of dogs in other studies involving administration of cigarette smoke.[32] Slight to moderate chronic bronchitis and bronchiolitis were present in the lungs of each of the three dogs (Figure 46). Those lesions have been reported in other investigations and, to a lesser degree, in the lungs of dogs from Group 3 in this study.

Lesions of the upper respiratory tract of these three dogs included an ulceration of the tracheal mucosa in two cases (Figure 47), and squamous metaplasia of the tracheal epithelium in all three dogs (Figures 48 and 49). A subepithelial inflammatory focus (Figure 50) in the larynx was found in one, although the surrounding epithelium was essentially normal in appearance.

The histopathologic data obtained from the three dogs of the ancillary study show that the higher dose of cigarette smoke had a more destructive effect. The quantity of smoke from that number of cigarettes is, however, very high (when compared on an organ or body weight basis), and may be unparalleled in all but the most avid of human cigarette smokers.

3. Groups 1 (Radon Daughters and Uranium Ore Dust) and 2 (Radon Daughters, Uranium Ore Dust, and Cigarette Smoke)

Pulmonary macrophages containing a brown pigment, considered to be ore dust, were the earliest histological manifestations of exposure of

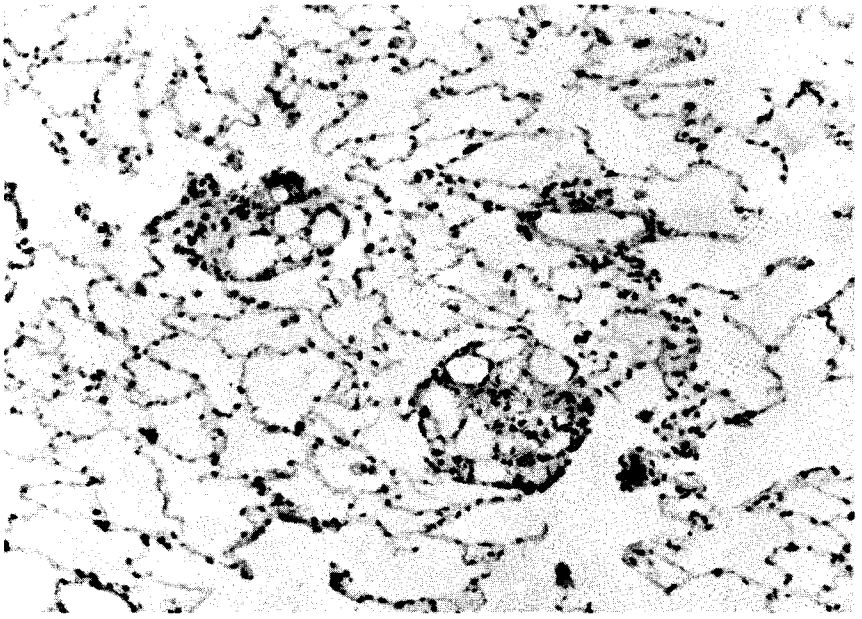

FIGURE 44. Focal Granulomata in a Lung Section From a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 175X.

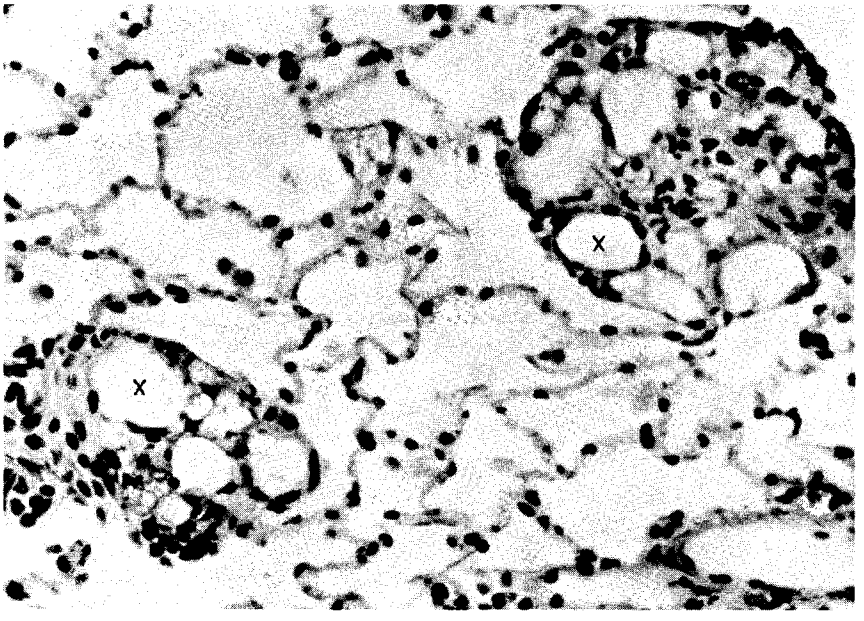

FIGURE 45. Higher Magnification of the Same Lesions Shown in Figure 44. Note the open spaces (X), probably occupied by lipids before histotechnical processing. H&E. 350X.

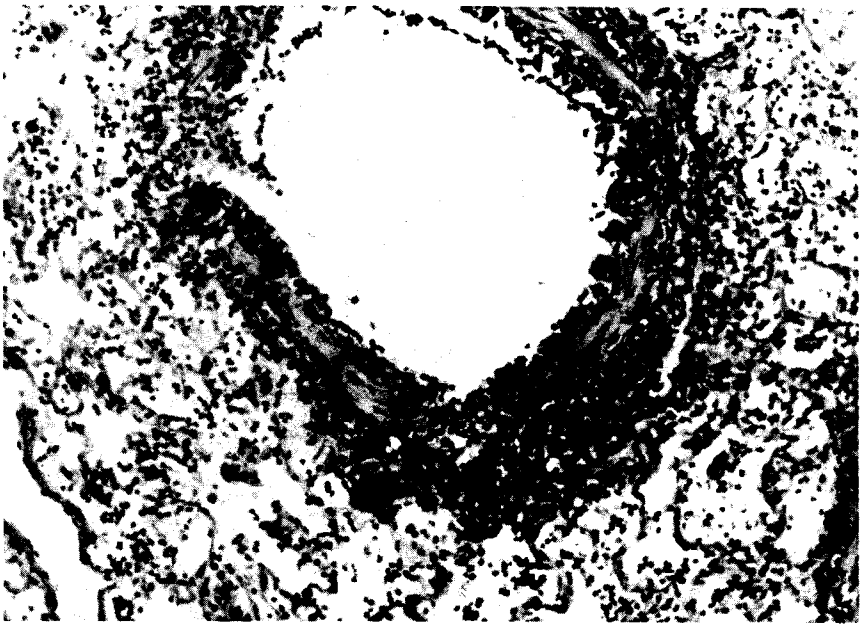

FIGURE 46. Chronic Bronchiolitis in a Lung Section From a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 175X.

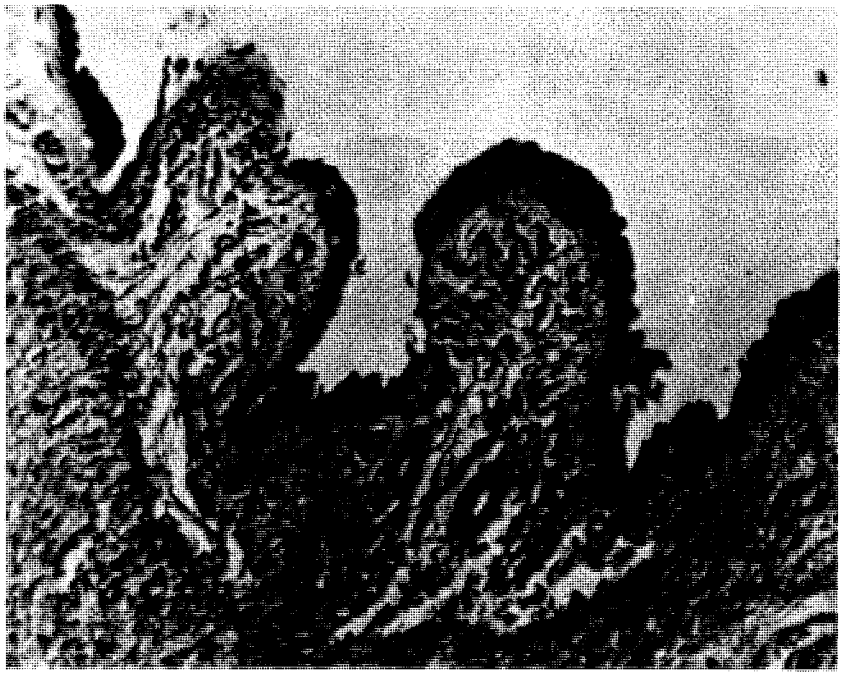

FIGURE 47. Ulcerative Lesion of the Tracheal Mucosa of a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 200X.

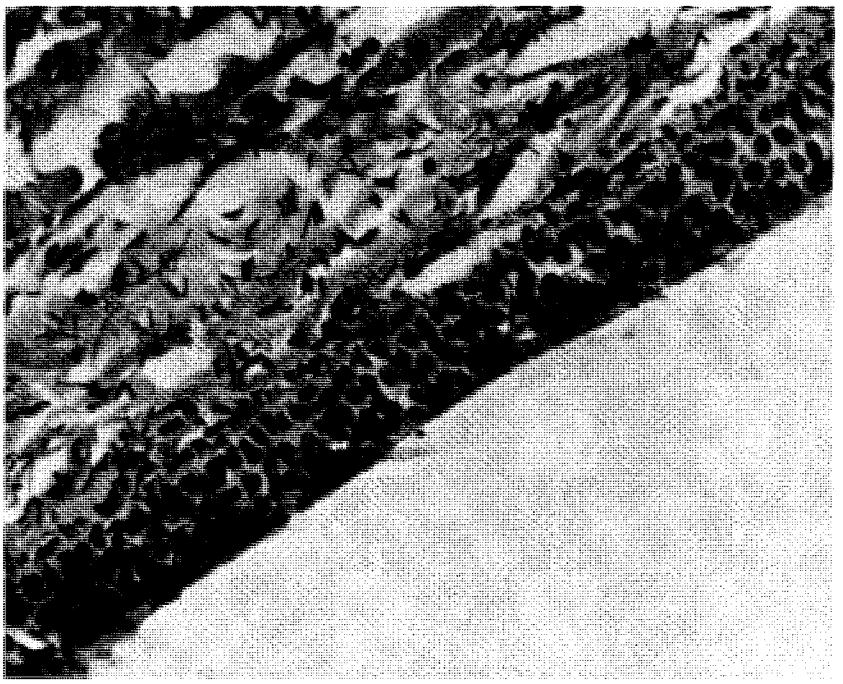

FIGURE 48. Hyperplasia and Slight Squamous Metaplasia of the Tracheal Epithelium in a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 400X.

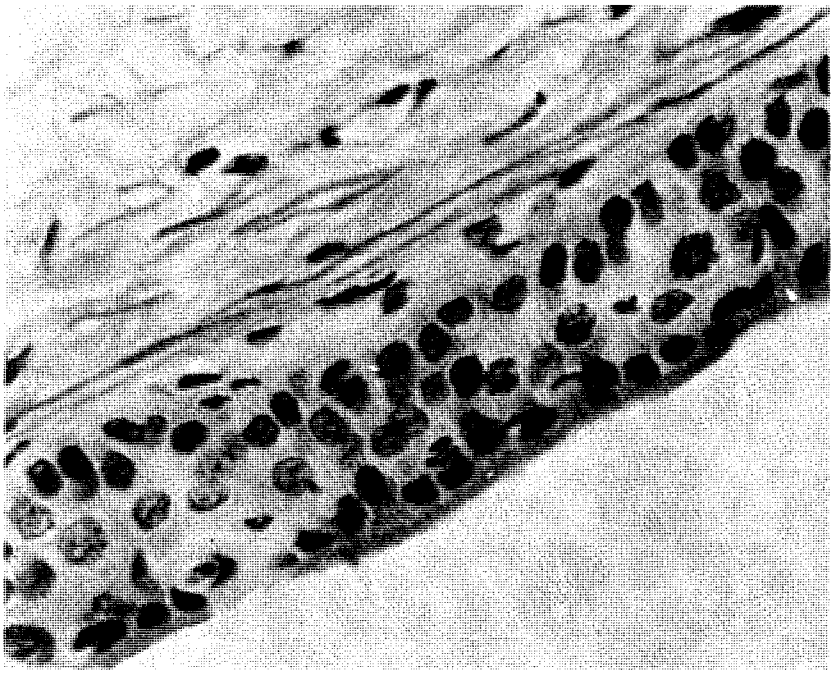

FIGURE 49. Higher Magnification of the Tracheal Epithelium Shown in Figure 48. H&E. 800X.

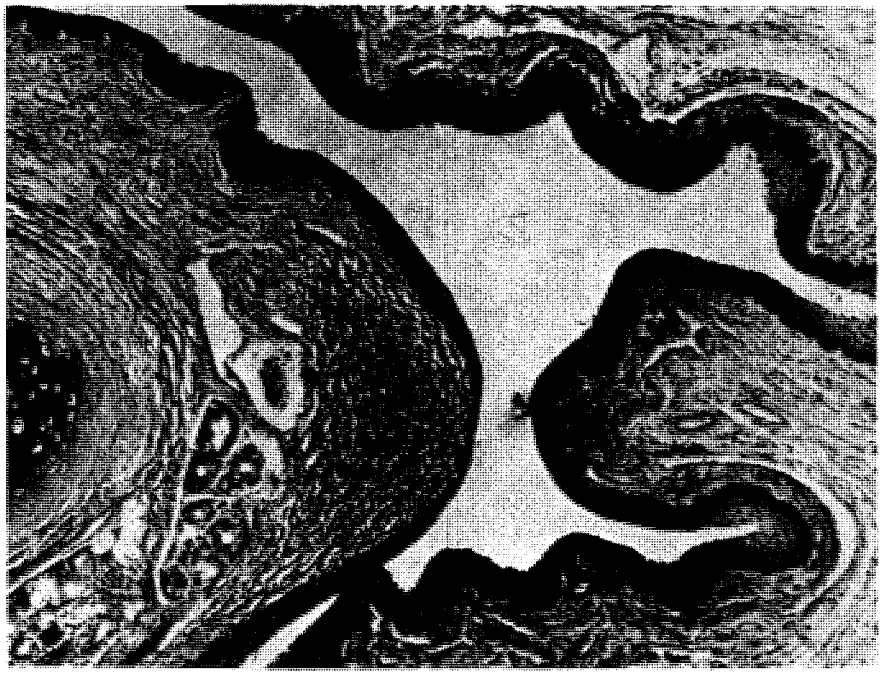

FIGURE 50. Focal Laryngitis in a Dog that had Smoked 20 Cigarettes Per Day for 9 Months. H&E. 80X.

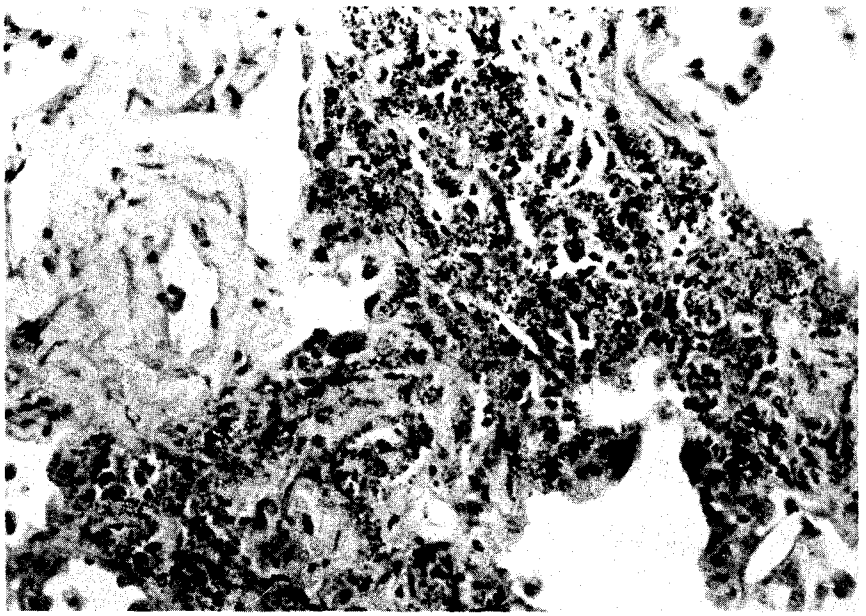

FIGURE 51. Alveolar Macrophage Containing Particles of Uranium Ore Dust. This section is from Group 2 Dog 552. H&E. 350X.

the dogs to radon daughters and uranium ore dust. The macrophages were accumulated in perivascular, peribronchiolar, and peribronchial areas (Figure 51), and those areas were frequently infiltrated with mononuclear inflammatory cells. Tracheobronchial lymph nodes of those dogs contained large amounts of uranium ore dust (Figure 52). After prolonged exposure to ore dust, the lymph nodes became markedly enlarged and dark in color. Microscopically, they contained large numbers of macrophages at the medullary cords (Figure 53) and slight to moderate lymphoid hyperplasia. Accumulations of macrophages bearing ore dust were evident in the lungs and lymph nodes of two dogs killed after only 6 months of exposure to radon daughters and uranium ore dust, and in all dogs that died or were killed after longer exposures.

Pulmonary hyalinosis was a common microscopic change in dogs from Groups 1 and 2 that died or were killed after exposure for 27 months or longer. The lesion was present in the lungs of 17 of 20 dogs from Group 1, and in 19 of 19 dogs from Group 2. The least severe form of the lesion consisted of individual alveolar macrophages containing the hyaline material (Figure 54). In the lungs of dogs with long exposure histories, large areas of some lobes were so affected (Figure 55). Those areas were frequently associated with a granulomatous reaction (Figure 56) and were palpated at gross examination. Frequently, masses of the hyaline material were encapsulated by fibrous connective tissue (Figure 57).

The hyaline material was characterized on the basis of histochemical reactions as a calcium-lipid-immunopolysaccharide complex by Billups,

FIGURE 52. Tracheobronchial Lymph Node From Group 2 Dog 551, 3 Months After Cessation of Exposures to Radon Daughters

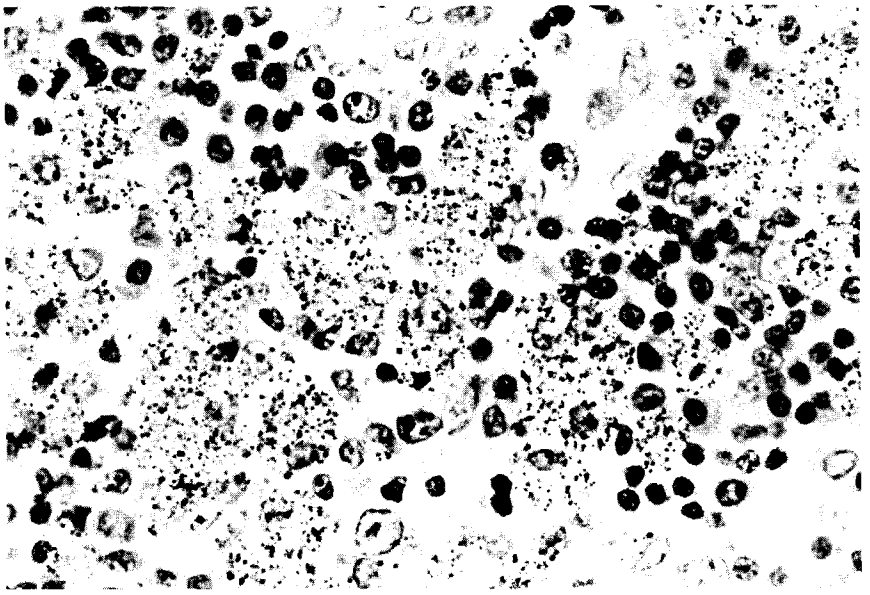

FIGURE 53. Higher Magnification Photomicrograph of the Same Lymph Node as in Figure 52. H&E. 700X.

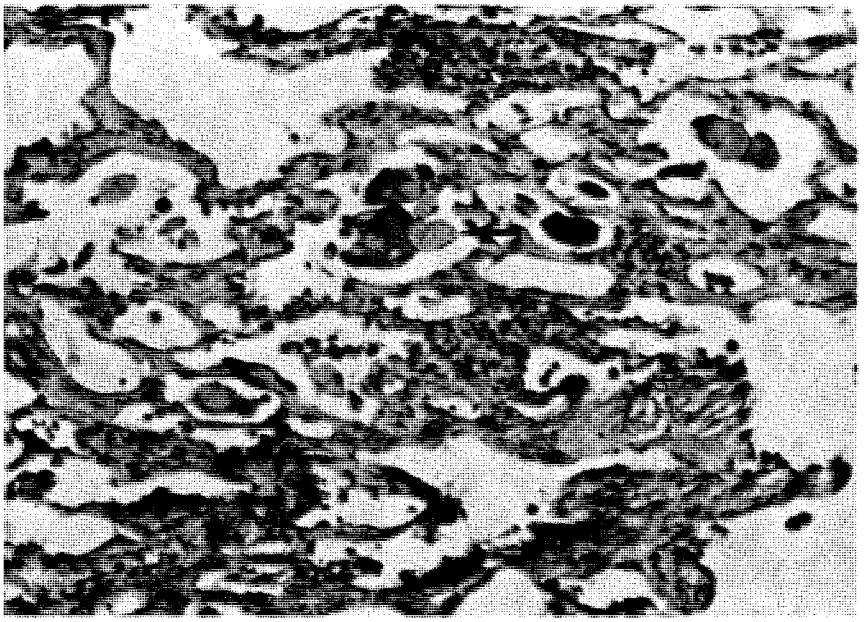

FIGURE 54. Hyaline Structure (arrow) Contained Within the Pulmonary Alveoli of Dog 505 Exposed to Radon Daughters with Uranium Ore Dust for 37 Months. H&E. 175X.

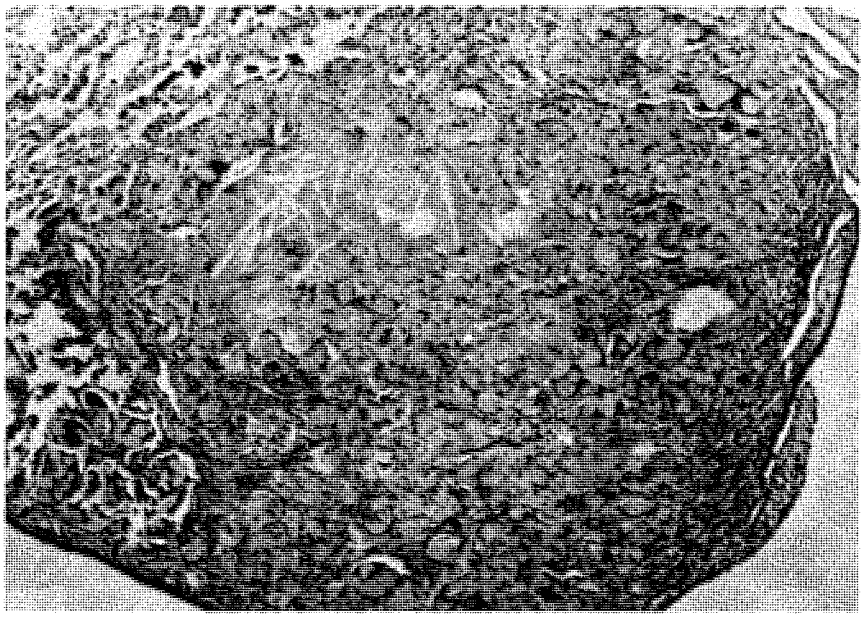

FIGURE 55. A Dense Accumulation of the Hyaline Structures with Associated Granulomatous Response from the Lung of Dog 577, Exposed to Radon Daughters With Uranium Ore Dust for 43 Months. H&E. 90X.

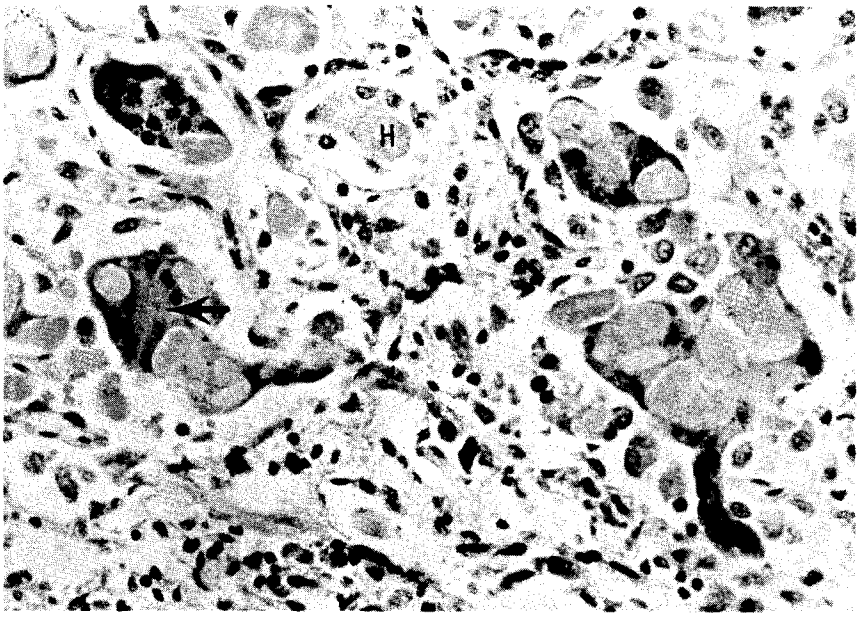

FIGURE 56. Hyaline Structures (H) Within the Pulmonary Alveoli of Dog 509, Exposed to Radon Daughters with Uranium Ore Dust for 39 Months. Giant foreign-body cells are shown (arrow). H&E. 350X.

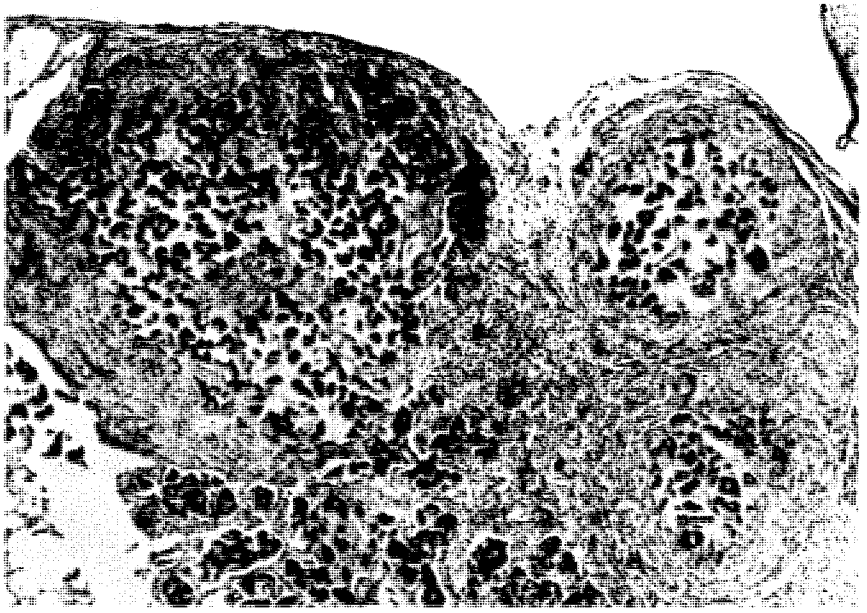

FIGURE 57. Encysted PAS-Positive Bodies From the Lung of Dog 567, Exposed to Radon Daughters and Uranium Ore Dust for 52 Months. H&E. 70X.

et al.[33] They suggested that the lesions were caused by aspirated material. Dagle et al.[34] described the pulmonary hyalinosis that occurred in the dogs of Groups 1 and 2; the ultrastructural appearance (Figure 58) suggested to them that the lesion was more likely a result of cellular degradation in the lungs.

Vesicular emphysema was present in the lungs of all dogs from Groups 1 and 2. In dogs that died or were killed early in the experiment, the lesion was of a less severe form: alveolar septa were interrupted, especially in the subpleural parenchyma (Figure 59). The lesions were more severe in dogs that had been exposed to radon daughters and uranium ore dust for 30 to 45 months (Figure 60); and, in dogs with exposure histories longer than 45 months, bullous emphysema was common. Subpleural cavities were visible at gross examination, with dimensions occasionally exceeding one centimeter (Figure 61). Bullous emphysema was present in the lungs of 11 of 20 dogs from Group 1 and in 13 of 19 dogs from Group 2.

Pulmonary fibrosis was prevalent in all dogs from Groups 1 and 2. Alveolar septal fibrosis was apparent to a slight degree in the two dogs killed after only 6 months exposure, and was progressively worse after longer exposure. The condition was characterized by large fibrotic areas in the parenchyma (Figure 62), which occasionally involved the major portion of some lung lobes.

Pleural thickening due to fibrosis (Figure 63) was consistently severe in dogs exposed to radon daughters and uranium ore dust. It was visible at gross examination as gray-white "scars," many of which were

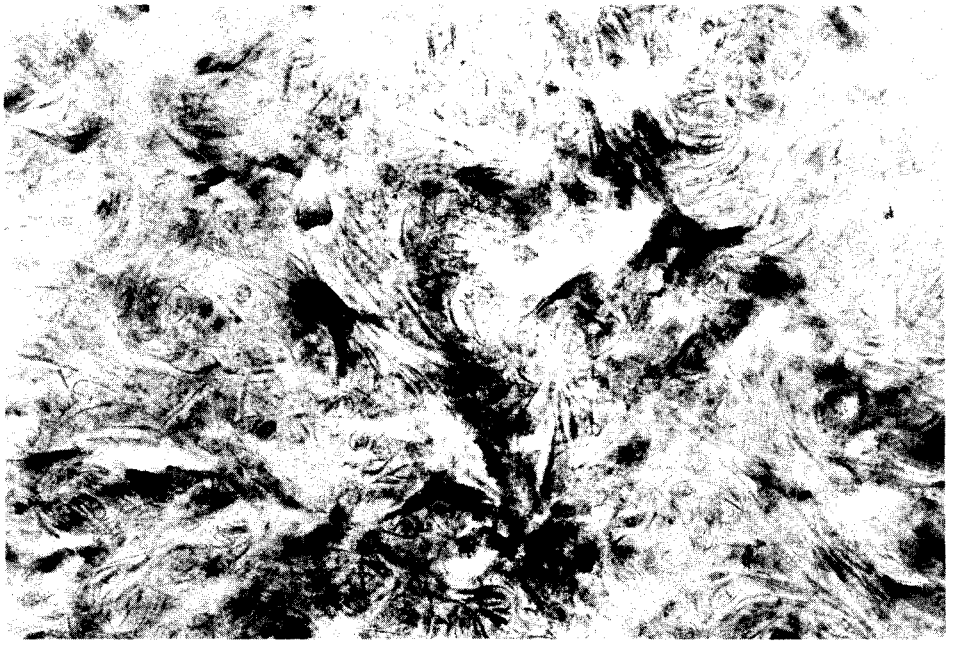

FIGURE 58. Electron Photomicrograph of the Hyaline Structures in the Lung of Dog 573 after 52 Months of Exposure to Radon Daughters and Uranium Ore Dust, 58 Months of Exposure to Cigarette Smoke. H&E. 41,000X.

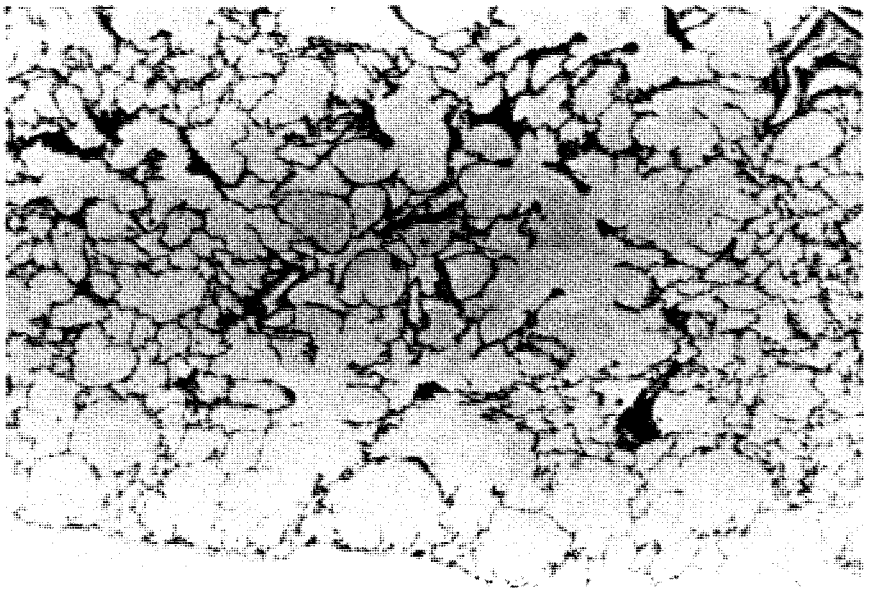

FIGURE 59. Subpleural Vesicular Emphysema in the Lungs of Dog 556 After 3 Months Exposure to Radon Daughters and Uranium Ore Dust, and 8 Months of Exposure to Cigarette Smoke. H&E. 70X.

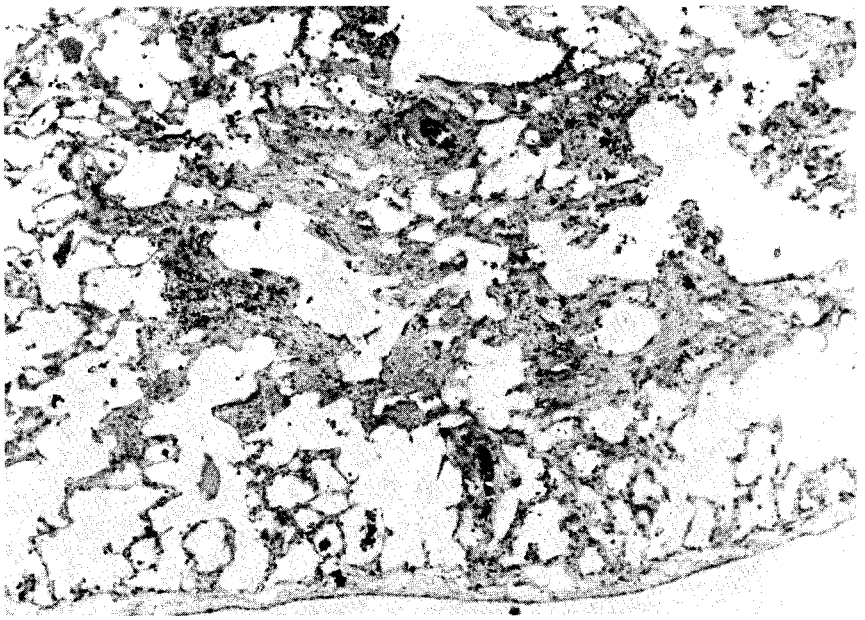

FIGURE 60. Peripheral Alveolar Septal Fibrosis and Emphysema from the Lung of Dog 505 after 37 Months of Exposure to Radon Daughters with Uranium Ore Dust. Slightly thickened pleura is shown along the bottom of the photomicrograph. H&E. 70X.

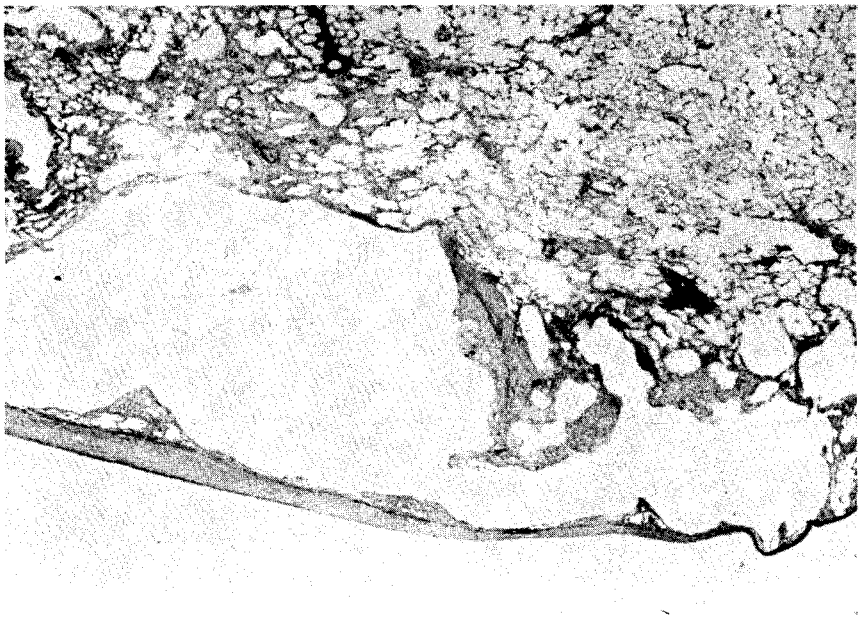

FIGURE 61. Bullous Emphysema in a Lung Section from Dog 539 after 48 Months of Exposure to Radon Daughters with Uranium Ore Dust and Sham Smoking. H&E. 21X.

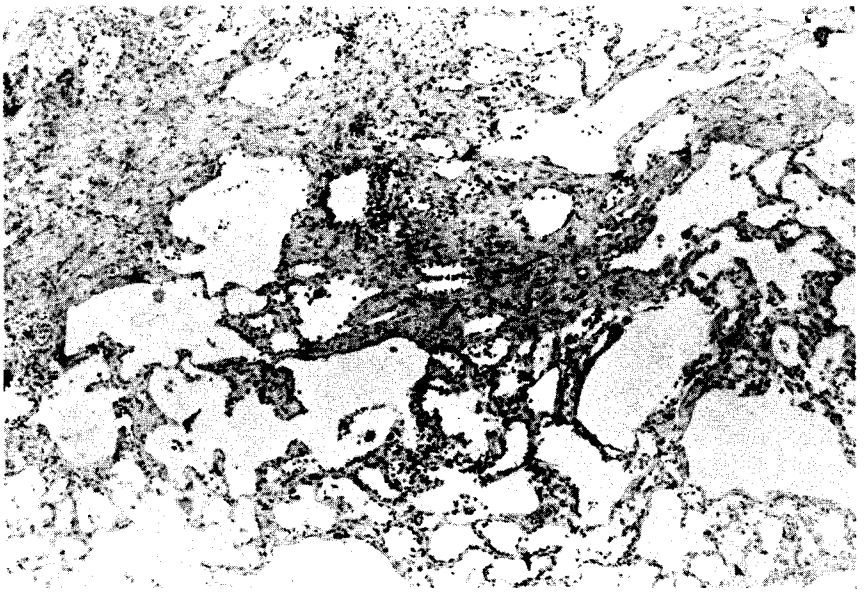

FIGURE 62. Alveolar Septal Fibrosis in the Lung of Dog 573 after 52 Months of Exposure to Radon Daughters and Uranium Ore Dust, and 58 Months of Exposure to Cigarette Smoke. H&E. 70X.

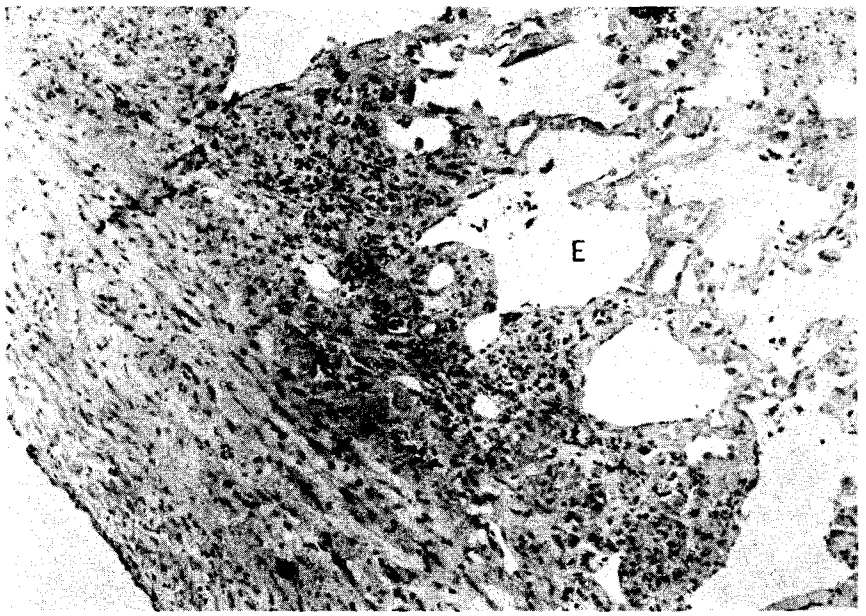

FIGURE 63. Pleural Thickening and Peripheral Alveolar Septal Fibrosis from the Lung of Dog 520 after 42 Months of Exposure to Radon Daughters with Uranium Ore Dust. Also shown are emphysematous vesicles (E). H&E. 90X.

depressed and appeared to be umbilicated (Figure 64). Small subpleural pulmonary arteries, especially in the areas of marked pleural and septal fibrosis, were often partially or completely occluded (Figure 65). The occlusion was considered a result of fibrosis of arterial walls and intimal hyperplasia. The arterial lesion was present, to a greater or lesser degree, in nearly all dogs of the two groups that were exposed to radon daughters and uranium ore dust for periods longer than 40 months.

Alveolar epithelial changes were prominent in the lungs of dogs from Groups 1 and 2 that were exposed for longer than 30 months. The least severe form of these changes consisted of small foci of cuboidal metaplasia (adenomatosis) adjacent to bronchioles and alveolar ducts (Figure 66). In dogs with longer exposure histories (40 months or more), large areas of adenomatosis were present (Figure 67), occasionally involving the major portion of a section through a lung lobe. Frequently, the lesion had progressed to squamous metaplasia of the alveolar epithelium (Figures 68 and 69), in which atypical cells were present. These cells were characterized by marginated nuclear chromatin, irregularly shaped nuclei, and decreased nuclear-to-cytoplasmic ratio.

After approximately 50 months of exposure to radon daughters and uranium ore dust, lungs from 11 of 21 Group 1 and 2 dogs that had died or were killed contained large cavities within the parenchyma. Each cavity was surrounded by a band of hyerplastic adenomatous epithelial cells (Figure 70 and 71).

In two of the Group 1 dogs, #523 and #608, there were epidermoid carcinomas associated with the cavities described above. The tumors

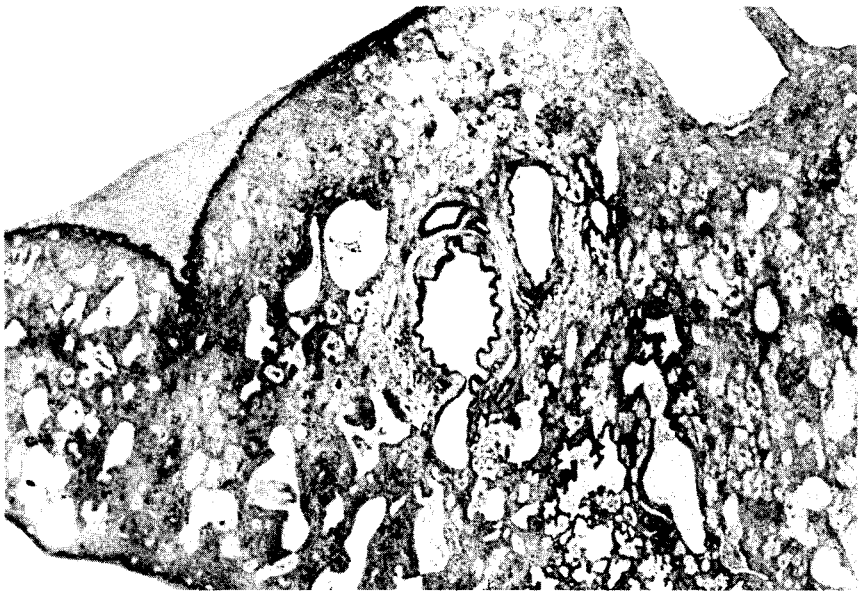

FIGURE 64. Pleural Fibrosis and Interstitial Fibrosis in the Lung of Dog 587 after 46 Months of Exposure to Radon Daughters and Uranium Ore Dust and 53 Months of Exposure to Cigarette Smoke. H&E. 21X.

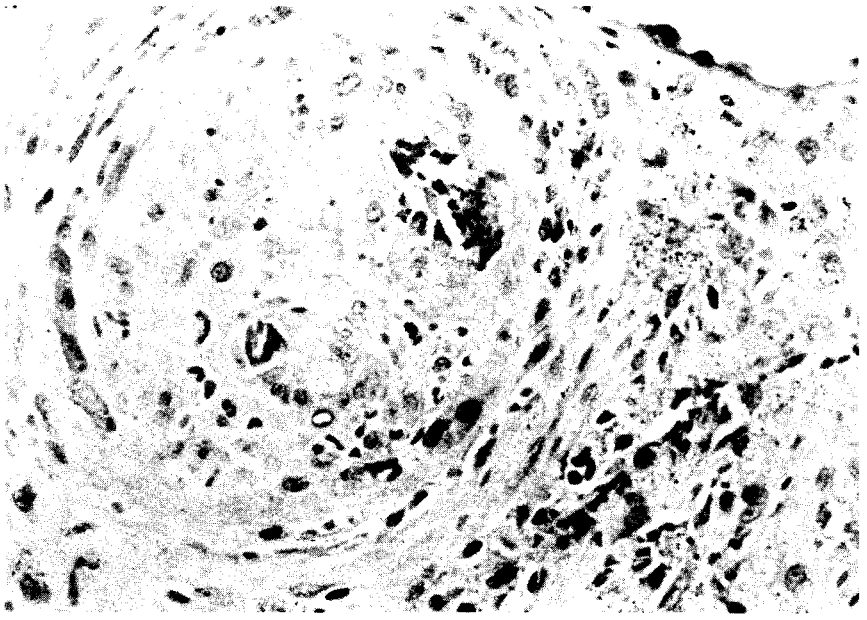

FIGURE 65. An Arteriole Almost Completely Occluded by Endothelial Cell Hyperplasia and Fibrosis of the Vessel Wall. The section is from the lung of Dog 520, exposed to radon daughters with uranium ore dust for 42 months. H&E. 375X.

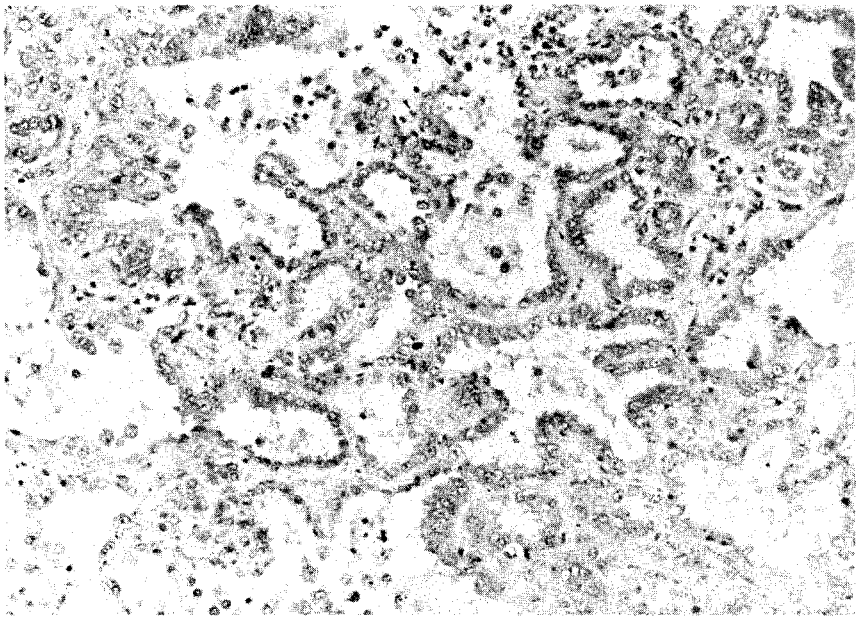

FIGURE 66. Cuboidal Metaplasia of Alveolar Epithelium and Associated Alveolar Septal Fibrosis from the Lung of Dog 585 after Exposure to Radon Daughters with Uranium Ore Dust for 42 Months, and to Cigarette Smoke for 48 Months. The lesion has progressed to squamous metaplasia (upper left portion of the photomicrograph). H&E. 175X.

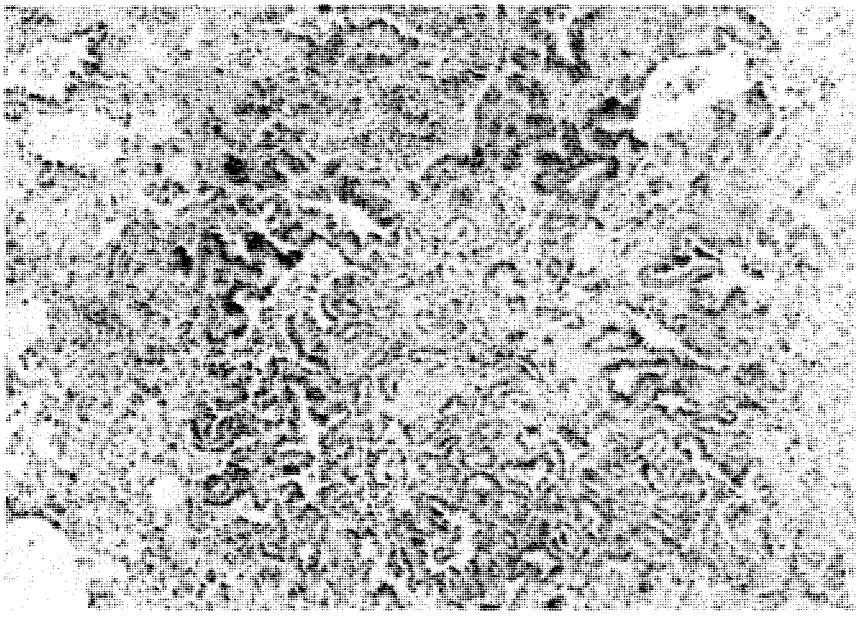

FIGURE 67. Adenomatous Proliferation of Alveolar Epithelium in a Section from the Lung of Dog 629, Exposed to Radon Daughters and Uranium Ore Dust, and Cigarette Smoke for 38 Months. H&E. 90X.

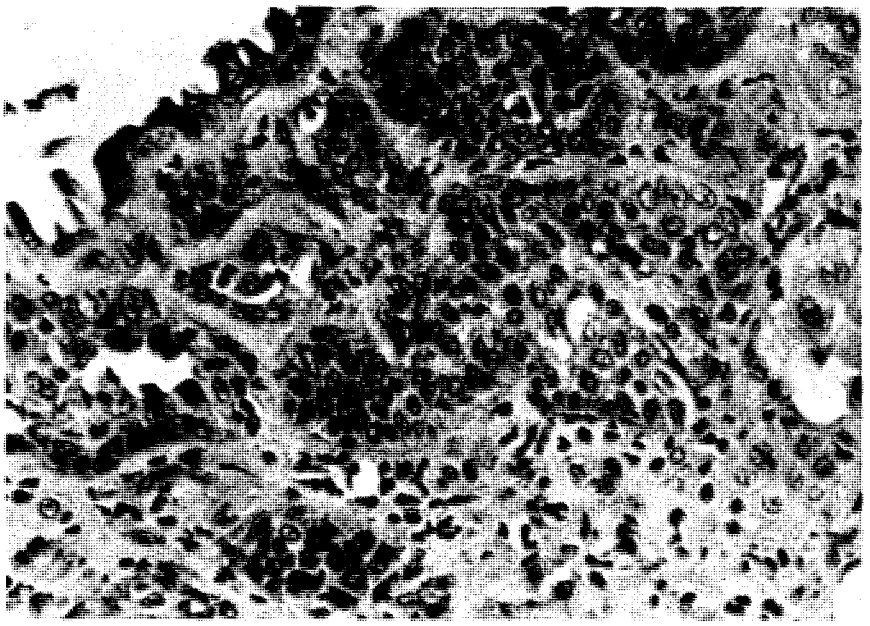

FIGURE 68. Squamous Metaplasia of Alveolar Epithelium from the Lung of Dog 520 After 42 Months of Exposure to Radon Daughters with Uranium Ore Dust. H&E. 350X.

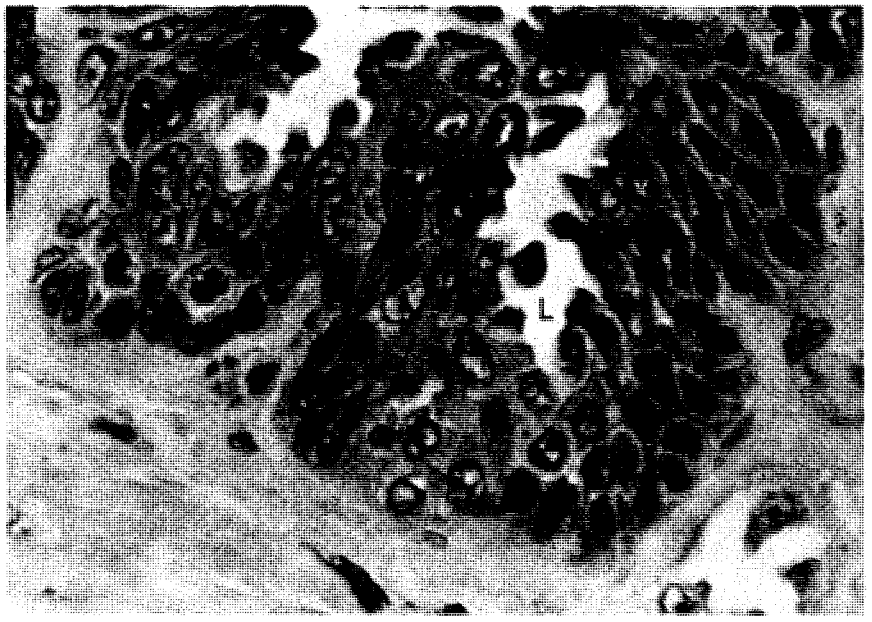

FIGURE 69. Higher Magnification of the Lesion Shown in Figure 68. Atypical nuclei are present in cells lining the alveolar lumen (L). H&E. 700X.

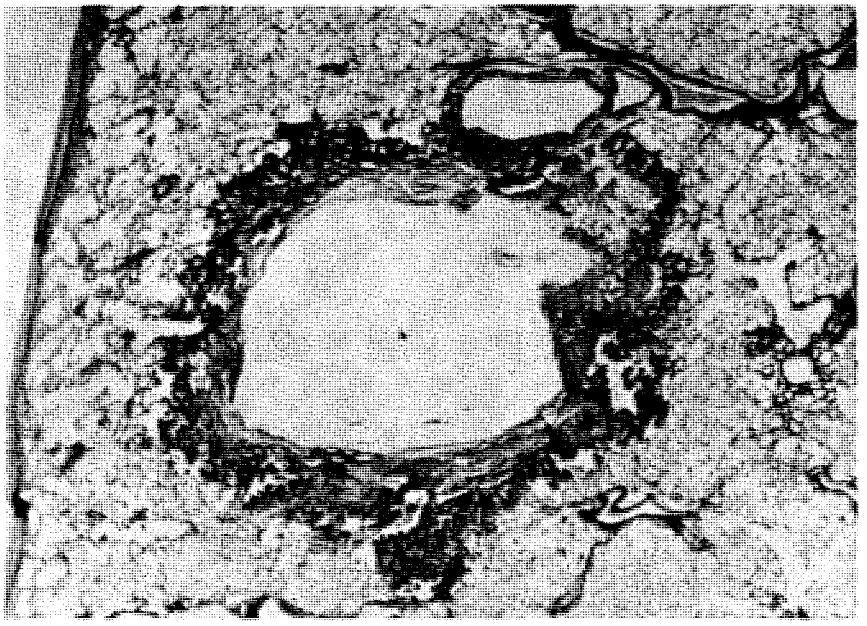

FIGURE 70. Alveolar Epithelial Cuboidal Metaplasia Surrounding an Emphysemic Bulla. This lesion was present in the lung of Dog 523. H&E. 21X.

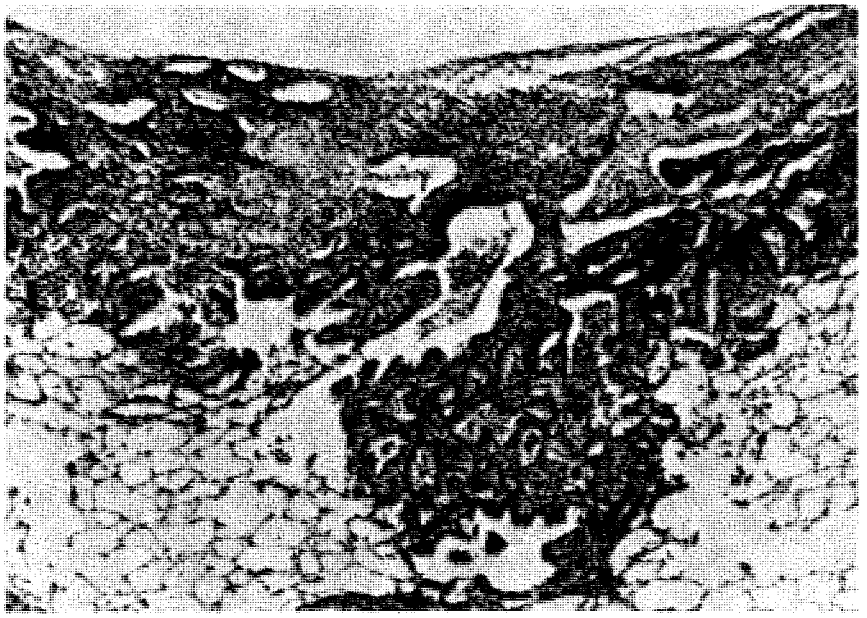

FIGURE 71. More Highly Magnified Photomicrograph of the Same Lesion Shown in Figure 70. H&E. 70X.

were solitary masses in peripheral areas of single lobes and, microscopically, consisted of irregularly shaped lobules of nonkeratinizing stratified squamous epithelium with a scant amount of connective tissue stroma. They were locally invasive into adjacent alveoli (Figure 72 and 73). Another epidermoid carcinoma was a well-circumscribed mass composed of anaplastic cells and numerous mitotic figures (Figure 74).

Three other primary lung tumors, bronchioloalveolar carcinomas, were present in Group 1 dogs #512, #525, and #531. The tumors were solitary masses associated with distal bronchioles in single lobes, which were locally invasive into adjacent alveoli, and had not metastasized (Figures 75 and 76). A bronchioloalveolar adenoma, composed of a papillary proliferation of epithelium, was present in Group 1 dog #541 (Figure 77); that dog also had an epidermoid carcinoma.

A fibrosarcoma was present in the anterior ventral portion of the right apical lobe of the lung from Group 1 dog #540. It consisted of a 3-cm-diameter, roughly spherical, well-circumscribed mass (Figures 78 and 79).

Pathologic changes in respiratory air passages of the dogs from Groups 1 and 2 were primarily in the nasal mucosa (discussed below). Changes in the bronchiolar and bronchial epithelium included slight to moderate basal cell hyperplasia, which occasionally led to formation of Helles-Zeller cells (Figure 80). In lungs of dogs with long exposure histories, slight to moderate degrees of bronchiolar epithelial squamous metaplasia occurred (Figure 81).

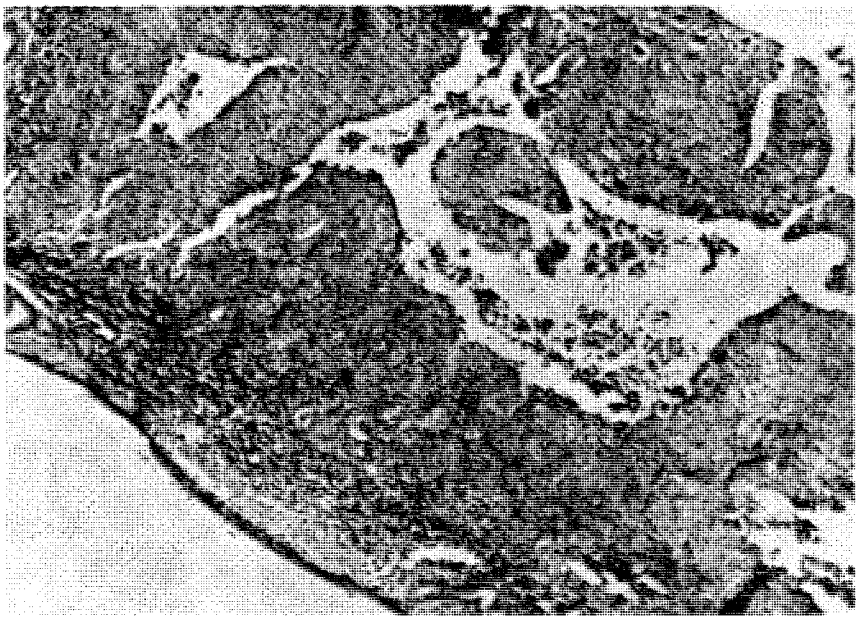

FIGURE 72. Pulmonary Squamous Cell Carcinoma from Dog 523 after 54 Months of Exposure to Radon Daughters and Uranium Ore Dust. H&E. 70X.

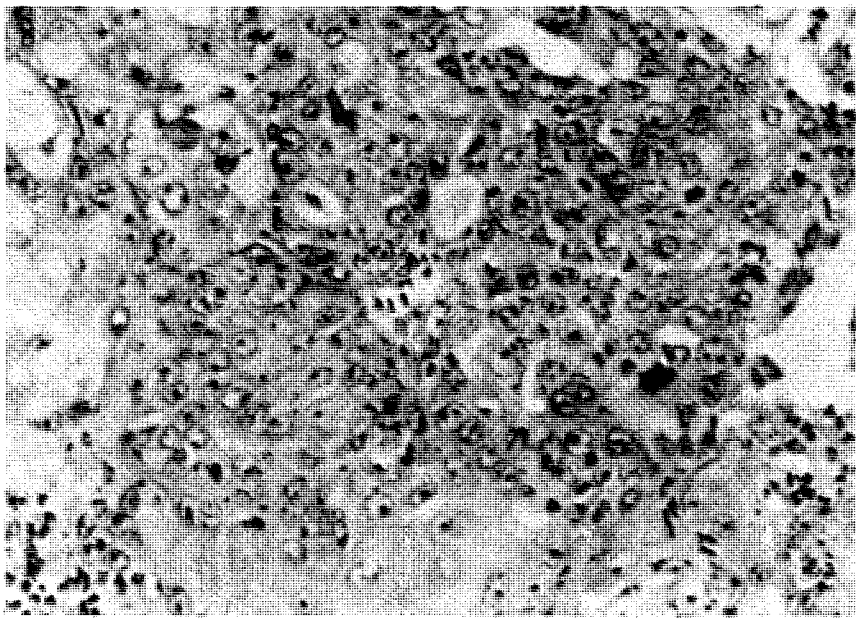

FIGURE 73. More Highly Magnified Photomicrograph of the Same Lesion Shown in Figure 72. H&E. 350X.

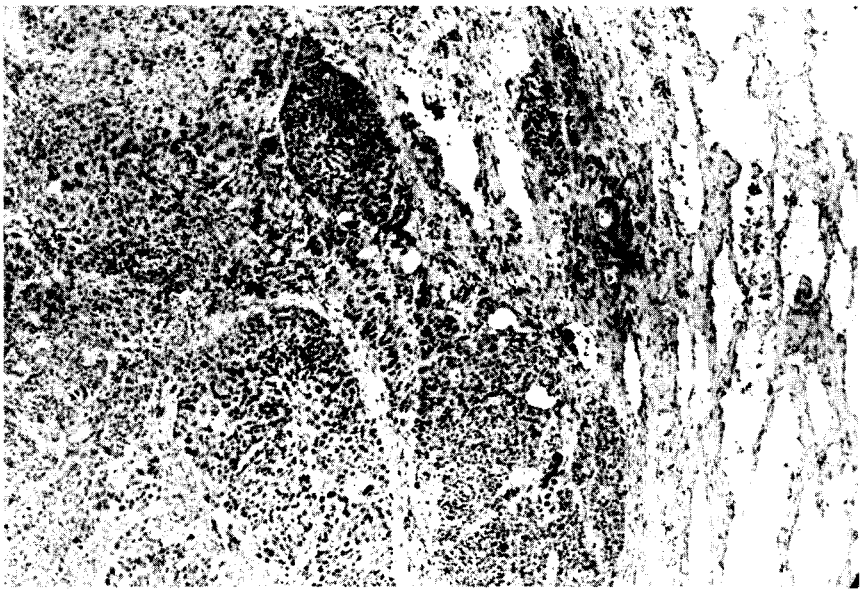

FIGURE 74. Epidermoid Carcinoma in the Lung of Dog 541 (3 Months after a 54-Month Exposure to Radon Daughter and Uranium Ore Dust). H&E. 70X.

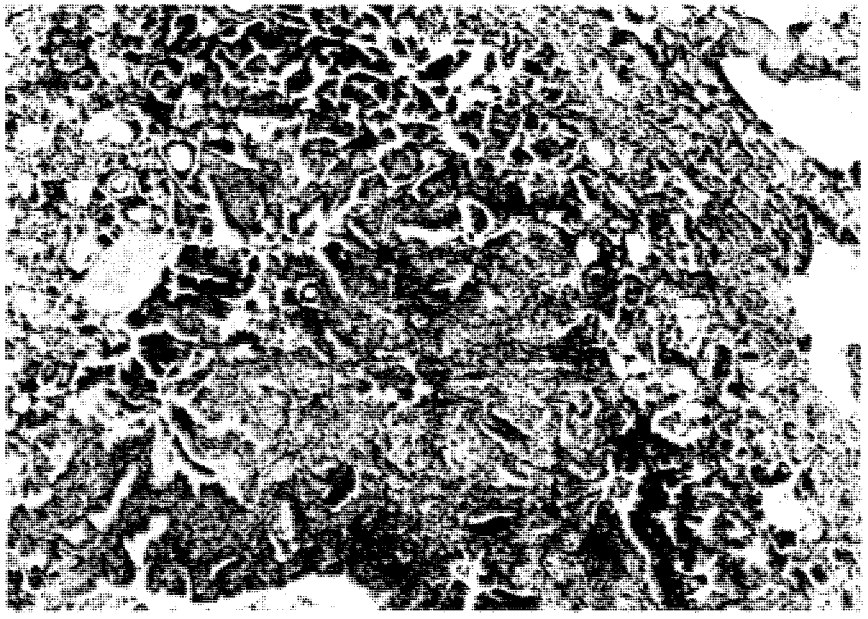

FIGURE 75. Bronchioloalveolar Carcinoma from the Lung of Dog 531 after 54 Months of Exposure to Radon Daughters with Uranium Ore Dust. H&E. 70X.

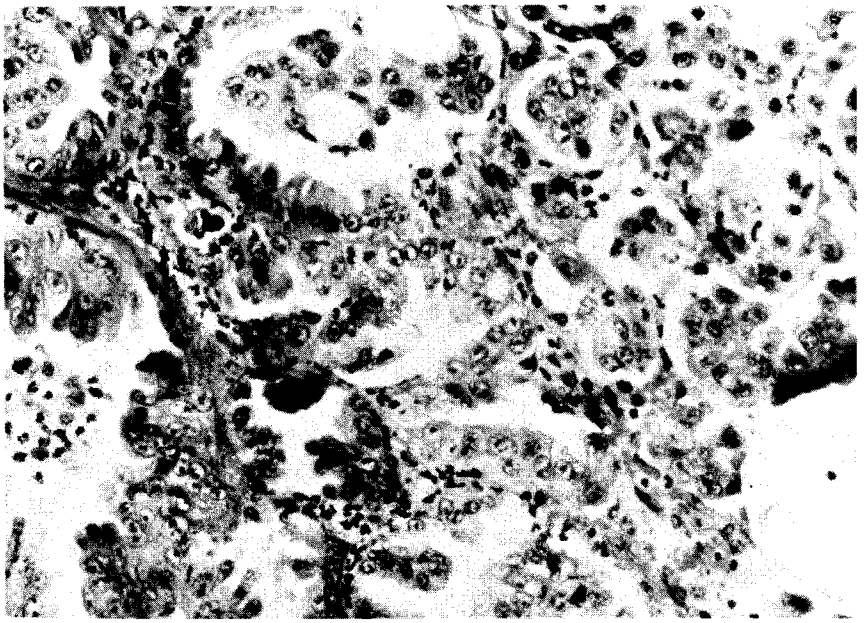

FIGURE 76. More Highly Magnified Photomicrograph of the Same Lesion Shown in Figure 75. H&E. 350X.

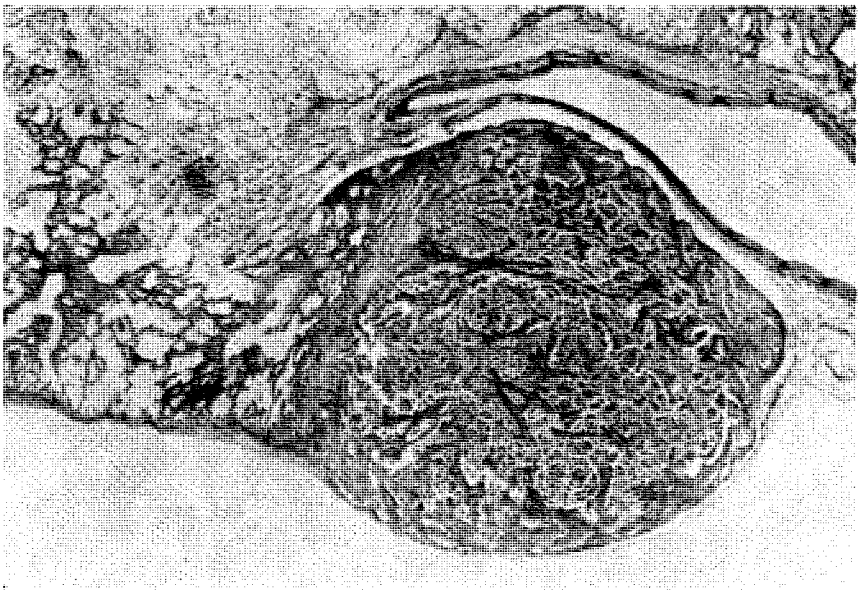

FIGURE 77. Bronchioloalveolar Adenoma in the Lung of Dog 541. The epidermoid carcinoma shown in Figure 74 was from the lungs of the same dog. H&E. 21X.

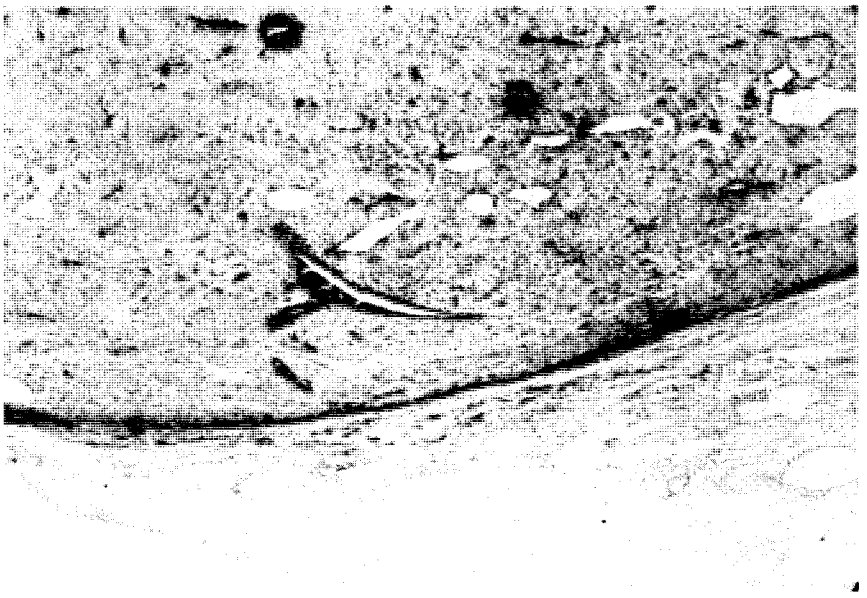

FIGURE 78. Pulmonary Fibrosarcoma from Dog 540, 8 Months after 54-Month Exposure to Radon Daughters and Uranium Ore Dust. H&E. 21X.

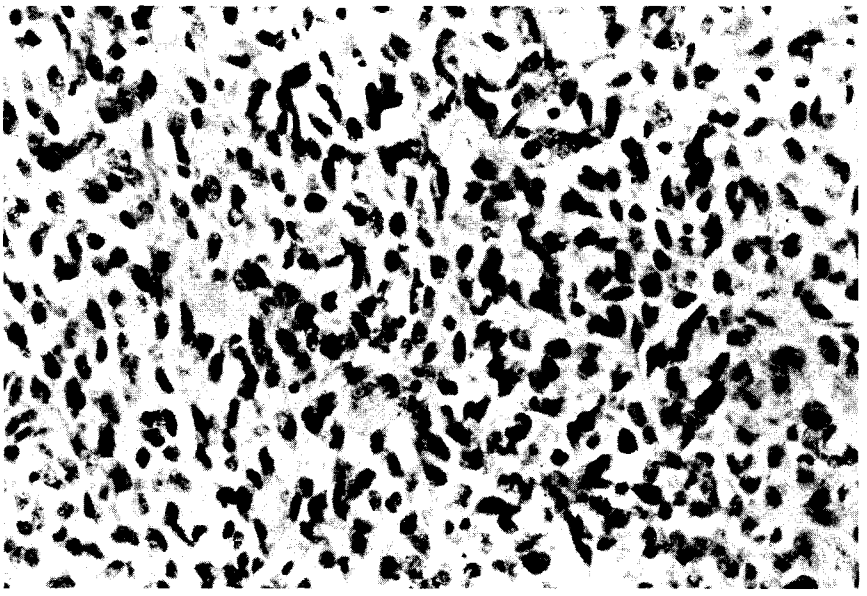

FIGURE 79. Higher Magnification of the Same Tumor Shown in Figure 78. H&E. 350X.

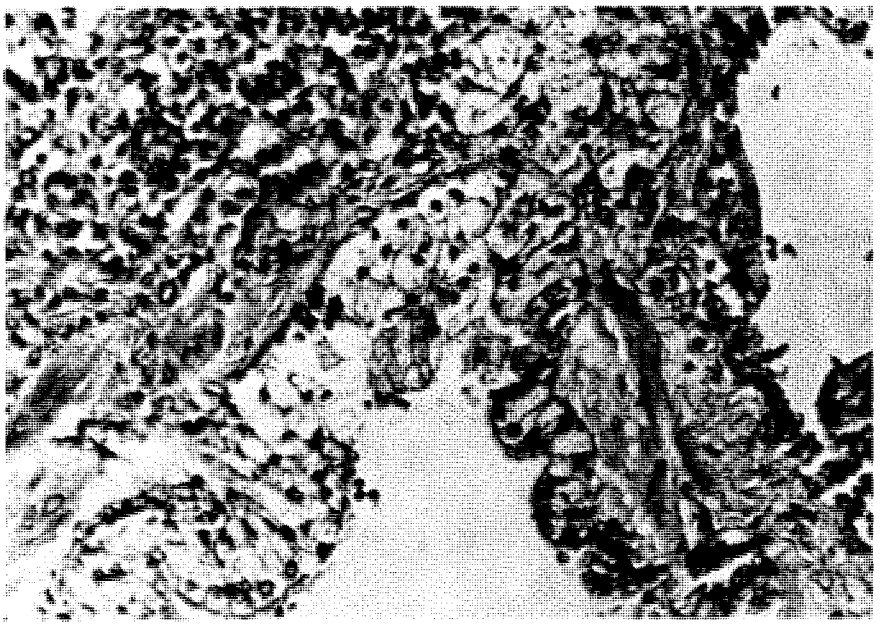

FIGURE 80. Atypical Bronchial Epithelial Cells from the Lungs of a Group 1 Dog after 27 Months of Exposure to Radon Daughters and Uranium Ore Dust. Note the irregular arrangement of the cells and the vacuolated cytoplasm. H&E. 500X.

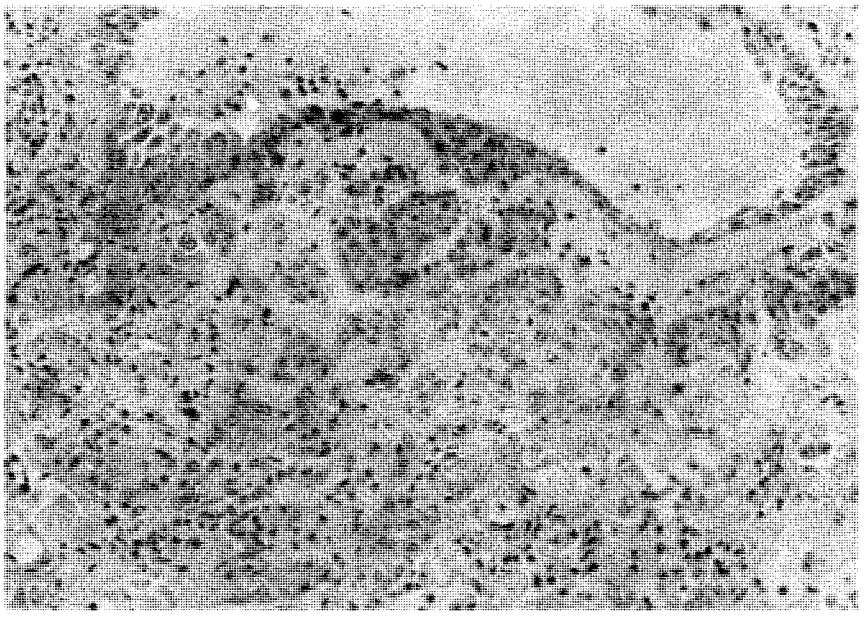

FIGURE 81. Squamous Metaplasia of Bronchiolar Epithelium and Epithelium of Adjacent Alveoli. This section is from the lung of Dog 629, exposed to radon daughters with uranium ore dust and cigarette smoke for 38 months. H&E. 175X.

Other changes in the airways were goblet cell hyperplasia in larger bronchi and bronchial mucus gland dilatation (Figure 82). Tracheal changes in dogs from Groups 1 and 2 included epithelial hyperplasia and focal squamous metaplasia, goblet cell hyperplasia, and dilatation of tracheal mucus glands. The changes were mild and generally more pronounced at the tracheal bifurcation. The laryngeal epithelium had slight basal cell hyperplasia and the thickened epithelial layer was apparent on gross examination. Occasionally, foci of laryngitis were present, but laryngeal changes were not considered significant.

The most pronounced upper respiratory changes were in the nasal mucosa. There was an apparent progression of epithelial changes from a normal to neoplastic state corresponding to length of exposure times in dogs from Groups 1 and 2 (Figures 83-89). Initial changes in the normally ciliated columnar epithelium (Figure 83) included flattening of cells and loss of cilia (Figure 84), followed by hyperplasia and squamous metaplasia of cells of the epithelial layer (Figure 85). Proliferation of the basal cell layer (Figure 86) resulted in lesions referred to as pseudoepitheliomatous hyperplasia (Figure 87). Marked progression of proliferation of the squamous epithelium may have resulted in squamous cell carcinomas such as those shown in Figures 88 and 89.

Squamous carcinomas arising from the epithelium of the nasal cavity occurred in three dogs. In the two Group 1 dogs, #608 and #615, the tumors were visible as swellings on the outside of the nose. The largest tumor, in dog #615, was composed of a soft, tan, oval, 4.5-cm-diameter mass arising from the area of the right maxilla and eroding into the

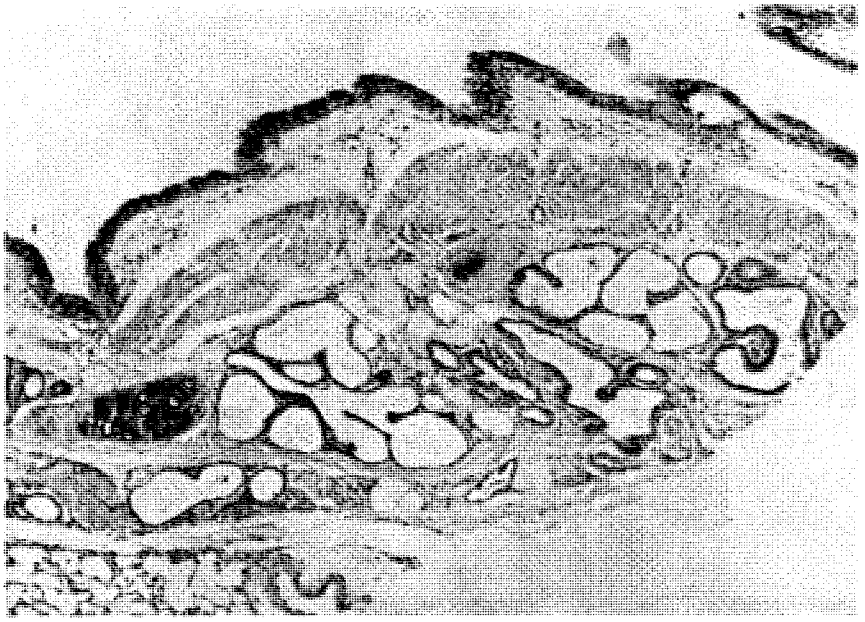

FIGURE 82. Dilated Bronchial Mucous Glands in a Section from the Lungs of Dog 552 after 44 Months of Exposure to Radon Daughters with Uranium Ore Dust and 51 Months of Exposure to Cigarette Smoke. H&E. 70X.

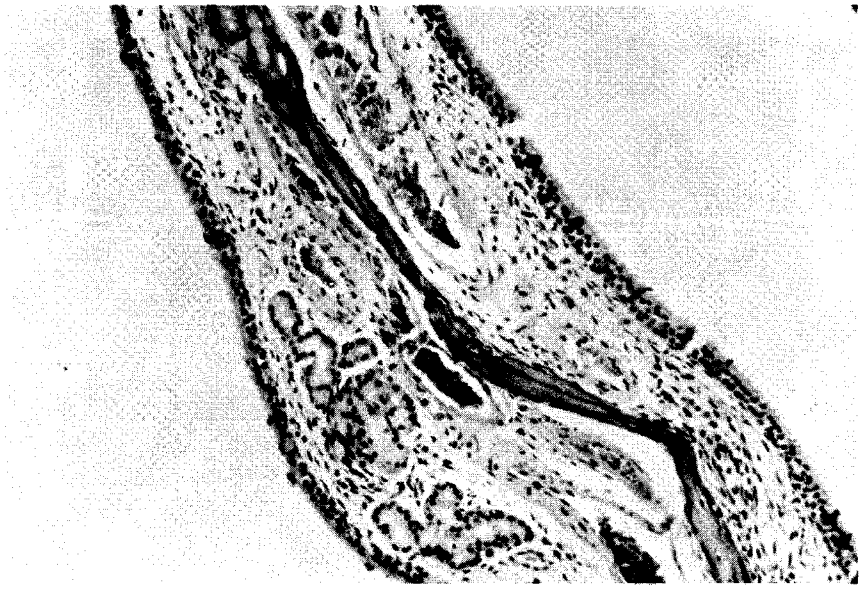

FIGURE 83. Normal Turbinate Epithelium from a Control Dog. H&E. 260X.

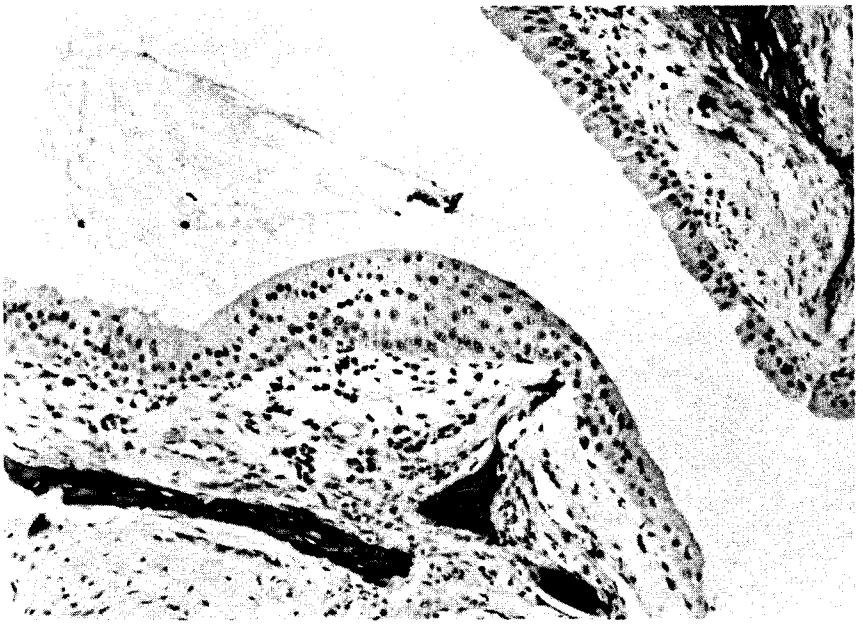

FIGURE 84. Aciliation and Squamous Metaplasia of Turbinate Epithelium from Dog 524, Exposed to Radon Daughters with Uranium Ore Dust for 51 Months. H&E. 175X.

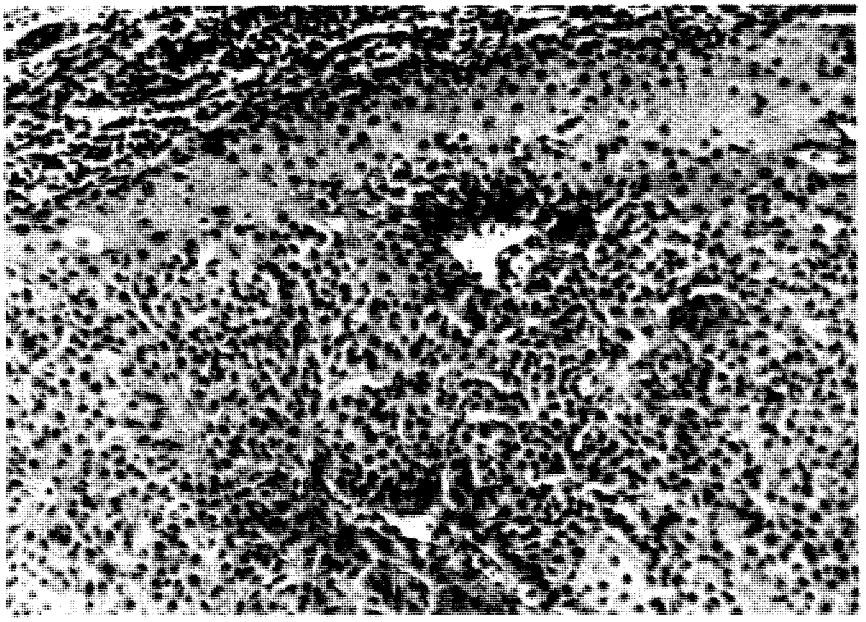

FIGURE 85. Severe Squamous Metaplasia of Turbinate Epithelium from Dog 522 after 56 Months of Exposure to Radon Daughters with Uranium Ore Dust. H&E. 175X.

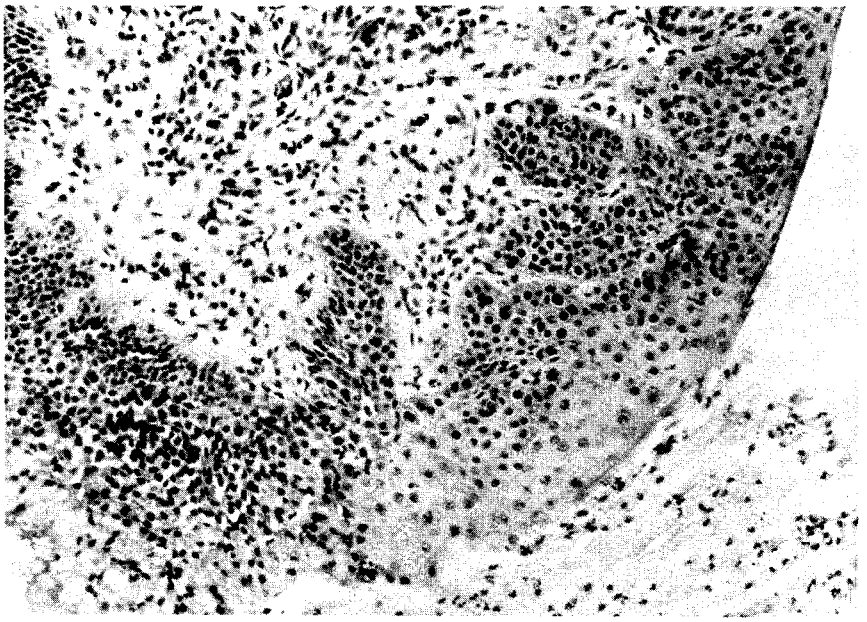

FIGURE 86. Photomicrograph Showing the Transition from Severe Squamous Metaplasia to Pseudoepitheliomatous Hyperplasia of Turbinate Epithelium. The tissue is from Dog 627, which had been exposed to radon daughters and uranium ore dust for 46 months, and to cigarette smoke for 52 months. H&E. 175X.

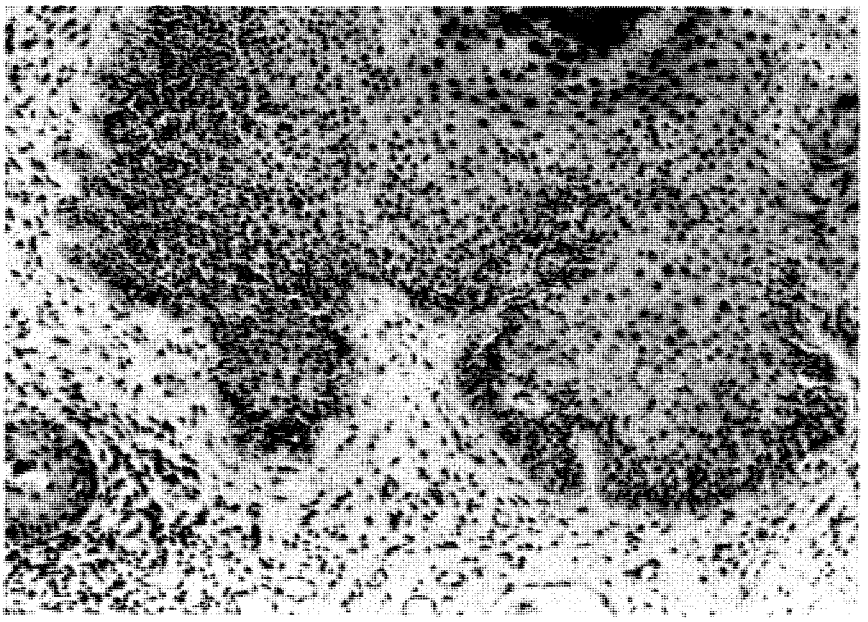

FIGURE 87. Pseudoepitheliomatous Hyperplasia of Turbinate Epithelium from Dog 504 after 47 Months of Exposure to Radon Daughters and Uranium Ore Dust, and to Cigarette Smoke for 53 Months. H&E. 175X.

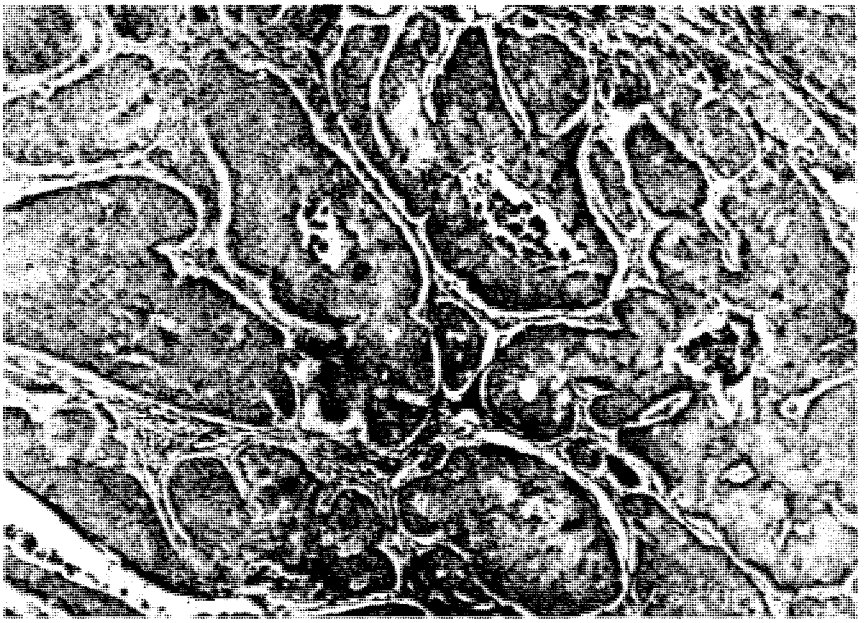

FIGURE 88. Squamous Carcinoma of the Nasal Epithelium of Dog 615, Which had been Exposed to Radon Daughters with Uranium Ore Dust for 46 Months. H&E. 70X.

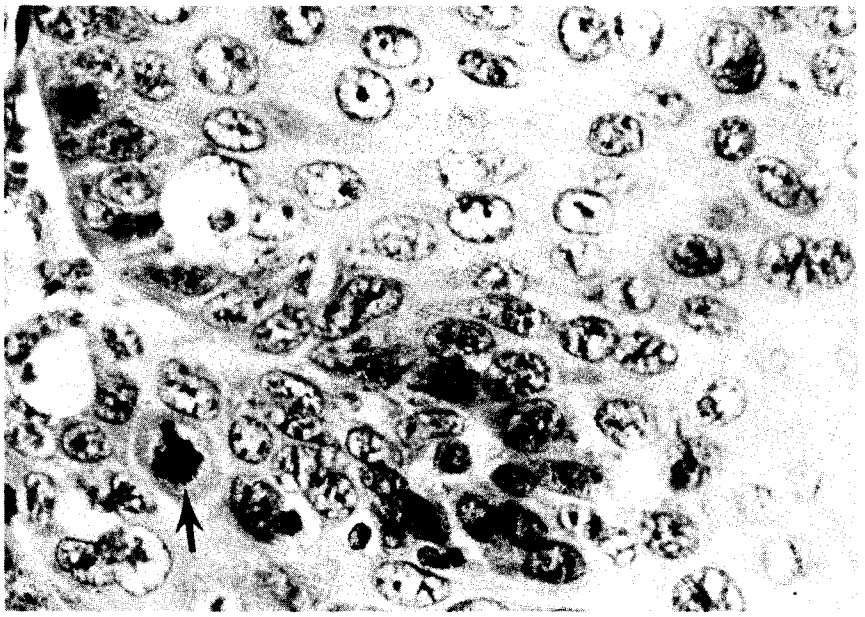

FIGURE 89. Higher Magnification of the Same Lesion Shown in Figure 88. Note the aberrant mitotic figure (arrow). H&E. 700X.

oral cavity (Figure 90). The smallest nasal tumor was in Group 2 dog #544, and was manifested clinically only as a profuse bloody discharge from the external nares. Histologically, the nasal carcinomas were composed of nonkeratinizing stratified squamous epithelium forming irregular cords infiltrating the submucosa and extending between spicules of bone. A large metastasis was present in the mandibular lymph node of dog #608 (Figure 91). Dog #608 also had an epidermoid carcinoma in the lungs, not necessarily metastasized from the nasal carcinoma.

DISCUSSION AND SUMMARY

From examination of the data in Tables 12 and 13 it is evident that all animals with lung tumors had cumulative exposures greater than 13,000 WLM, except dog #518. That dog was also unique in that it survived for 28 months after completion of exposures to radon daughters and uranium ore dust.

Results of this experiment indicate that cigarette smoke exposure had a mitigating effect on radon daughter-induced respiratory tract cancer in dogs. There were eight dogs with respiratory tract tumors in Group 1 (no smoke) and two dogs with respiratory tract tumors in Group 2 (smoke-exposed). Two possible reasons for that may be suggested, although no empirical evidence was collected. First, smoking could cause an increase in mucus production that would result in a smaller radiation dose to bronchial and bronchiolar epithelial proliferating cells. Second, the amount of cigarette smoke that the dogs inhaled could have had a net stimulatory effect on the mucociliary clearance in the smoke-exposed

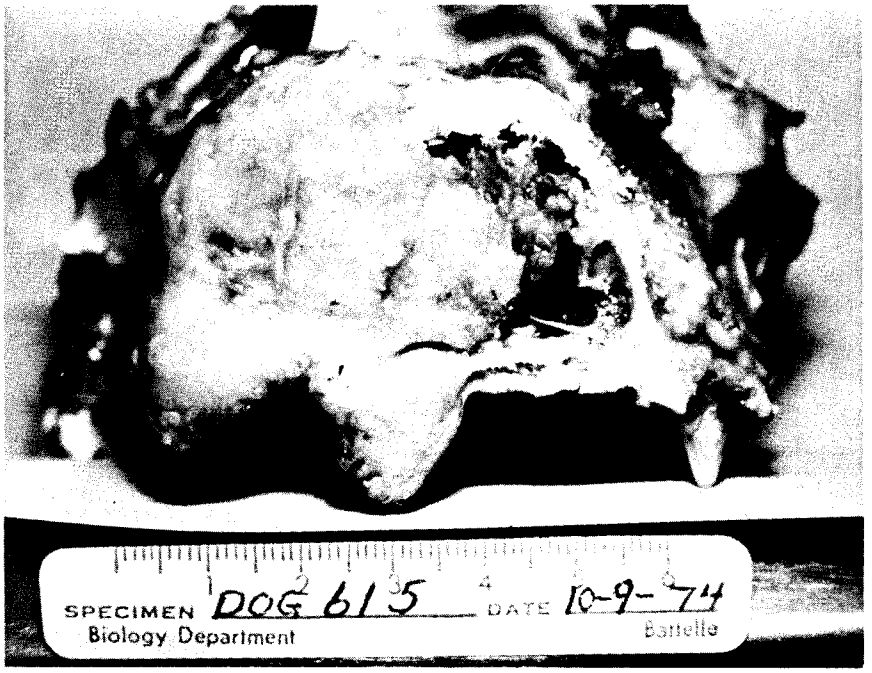

FIGURE 90. Gross Section Through a Nasal Tumor from Dog 615 after 48 Months of Exposure to Radon Daughters and Uranium Ore Dust.

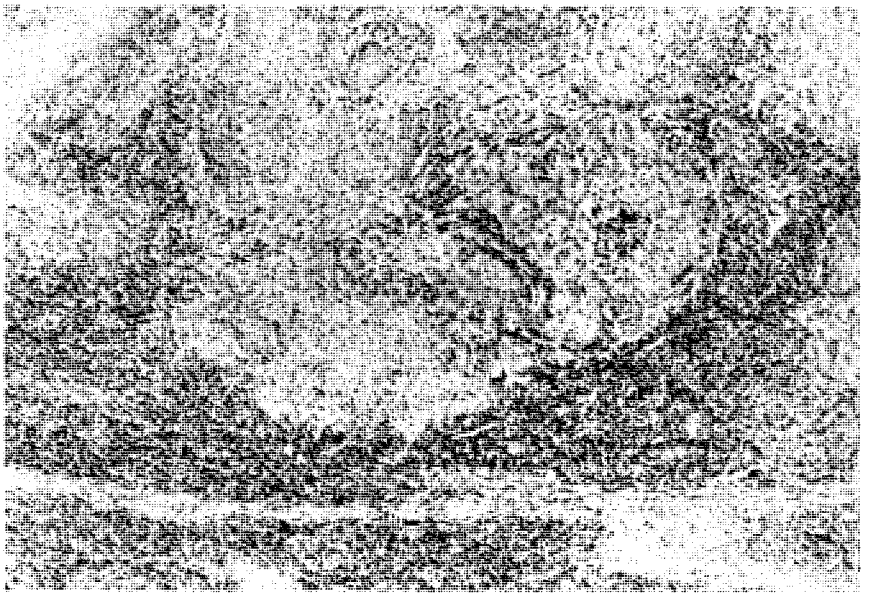

FIGURE 91. Metastatic Tumor Cells in the Mandibular Lymph Node of Dog 608. The primary tumor was a nasal carcinoma. H&E. 60X.

dogs. Published results from experiments investigating the effect of cigarette smoke on mucociliary clearance in animals are contradictory. Cigarette smoke has been frequently demonstrated to be cytotoxic and to impair ciliary action. There are published accounts, however, which indicate that the ciliary impairment is transitory and the net effect of smoke exposure is an increase in clearance rate.[35]

The amount of smoke actually inhaled by the dogs is questionable for two reasons: the lack of significant respiratory tract lesions in Group 3 dogs and the relatively small blood carboxyhemoglobin elevation in those dogs. Dogs of Group 3 had only very slight amounts of pulmonary emphysema and fibrosis after 4 or more years of exposure. Mild chronic inflammatory changes and slight alveolar epithelial hyperplasia were present in the lungs of three dogs exposed to smoke for 65 months but were only slightly more severe than in three control dogs sacrificed at the same time.

The most notable smoke-induced respiratory tract changes were present in the lungs of three dogs exposed to smoke from 20 cigarettes per day (twice the exposure of Group 3 dogs). These changes included pulmonary emphysema, fibrosis and chronic bronchitis and bronchiolitis, all of which have been attributed to smoke exposure in hamsters and in animals of other studies.[32]

Smoke deposition studies with ^{14}C-dotriacontane (Table 16) were performed on these three dogs and indicated pulmonary deposition of 15-30 percent of available cigarette smoke. Carboxyhemoglobin measurements in smoking dogs (Table 15) resulted in mean values up to 5.3% versus

mean values to 2.6% in nonexposed dogs. The values in exposed dogs were quite low when compared to the data of Zwicker, et al., who reported blood carboxyhemoglobin levels over 30% in dogs exposed to cigarette smoke by tracheostomy.[32] Measurements of ^{14}C-dotriacontane in those same tracheostomized dogs ranged between 17 to 48% (unpublished data), values which were not dissimilar from those obtained with the above three dogs.

Slight differences occurred between Groups 1 and 2 dogs that could be attributed to smoke exposure. Generally, the incidence and degree of severity of bullous emphysema were greater in the smoke-exposed dogs (Group 2). The onset of that condition was also noted earlier in the dogs of Group 2 than in those of Group 1.

We have no explanation for the very high cumulative exposures necessary to induce tumors in either hamsters or dogs. The exposures were nearly two orders of magnitude greater than those reported to cause lung cancer in man.[18] A possible explanation is associated with dose rate: while 120 to 359 WLM may be sufficient to cause lung cancer in man,[18] the longer human life span affords more time for carcinogenesis to proceed. Higher dose rates appear to be necessary in experimental animals in order to increase the probability of lung cancer over their shorter life span. As a result, much of the radiation may be wasted, i.e., not utilized in the formation of subsequent tumors. Nevertheless, this radiation is included in the animals' cumulative exposure. Absent from this explanation, however, is the suspicion that other environmental factors (p. ix) have not been

adequately taken into account in the comparison of animals with man. Respiratory tract tumors among uranium miners are bronchogenic (specifically, in the smaller bronchi), while in the animal experiments they are bronchiolo-alveolar in origin and also occur in the nasal epithelium. A strict comparison of human and animal exposures required to produce tumors is not possible at this time.

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