- **Inhalation Hazard to Uranium Miners**

This project will determine the specific biological effects of daily exposures to known levels of pathogenic uranium mine air contaminants, using both large and small experimental animal models of human respiratory disease. Lung cancer and deaths by degenerative lung diseases have reached epidemic proportions among uranium miners, but the cause-effect relationships for these diseases are based on inadequate epidemiological data. This project will identify the agents or combinations of agents and their levels which are responsible for severe respiratory tract pathology, including respiratory epithelial carcinoma, pneumonconiosis, and emphysema. Determination of actual absorbed radiological and chemical doses of inhaled materials is essential to establish cause and effect relationships.

BIOLOGICAL EFFECTS OF INHALED CIGARETTE SMOKE IN BEAGLE DOGS

Investigators:

B. O. Stuart, R. F. Palmer, R. E. Filipy, and G. E. Dagle

Technical Assistance:

W. Skinner, C. Petty, K. C. Upton, and D. Teats

A group of twenty dogs has received up to 7 yr of daily cigarette smoking (10 cigarettes per day, 5 days per week), using realistic methods of oral inhalation and nose-plus-mouth exhalation. Three dogs that received 20 cigarettes per day over 9 mo developed respiratory tract lesions, including pleural thickening, alveolar septal fibrosis, vesicular emphysema, and chronic bronchitis, more rapidly than dogs receiving 10 cigarettes per day.

These experiments (Table 3.26) were initiated to examine cause and effect relationships in the development of respiratory tract pathology as a result of lifespan daily inhalation exposures of a large experimental animal to radon daughters with uranium ore dust and cigarette smoking, both combined and separately, under conditions that closely simulate conditions of human exposure in uranium mines. This report will concentrate on effects of chronic cigarette smoking alone.

Beagle dogs were trained to accept daily smoking of 10 cigarettes (Groups 2 and 3) or to receive identical daily periods of sham smoking of unlighted cigarettes (Controls, Groups 1 and 4). Fresh smoke was inhaled using masks specifically designed to simulate human patterns of cigarette smoking; i.e., oral smoke inhalation and nose plus mouth exhalation. These dogs were not anesthetized or tranquilized during smoke exposures, and received their repeated daily

TABLE 3.26. Experimental Design

Group	Number of Animals	
1	20	600 WL Radon Daughters with Uranium Ore Dust (Carnotite) 15 mg/m³ with Sham Smoking
2	20	600 WL Radon Daughters with Uranium Ore Dust (Carnotite) with Cigarette Smoking
3	20	Cigarette Smoking
4	9	Controls, with Sham Smoking

inhalation of smoke in response to their individual respiration rates.

Carboxyhemoglobin levels in both groups of dogs that received daily cigarette exposures were markedly increased after smoking, with mean levels of 5 to 6% COHb, similar to those found in humans at these smoking levels, and apparently with less variability. As a possible additional index of smoke constituent deposition, plasma thiocyanate levels were determined. Mean values obtained from cigarette smoking dogs were nearly double those from control dogs or sham-smoking dogs inhaling radon daughters with uranium ore. Differences were not significant between presmoking and postsmoking levels for each group.

Minimal changes had occurred in the respiratory tracts of the sham-exposed control dogs that were examined. One control dog was sacrificed after 52 mo of sham exposure for comparison of histopathologic data with that from the large number of dogs from Groups 1 and 2 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 in the lungs. Those lesions are considered spontaneous, and are commonly found in older beagle dogs.

Pulmonary changes in three control dogs killed after 65 mo of sham exposure included slight subpleural vesicular emphysema in two, and slight focal mononuclear cellular infiltration in all three dogs. Lungs of all three dogs also contained slight to moderate degrees of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and metaplasia. All three dogs had very slight degrees of basal cell hyperplasia, as well as slight glandular hyperplasia, in both tracheal and laryngeal mucosa.

In one dog from Group 3, killed after 49 mo of exposure, there was seen in alveolar macrophages a relatively small amount of exogenous brownish-yellow pigment, considered to be associated with cigarette smoking. Tracheobronchial lymph nodes contained large amounts of phagocytized exogenous yellow pigment in histiocytes. Pulmonary lesions in this dog included slight vesicular emphysema, and occasional foci of subpleural interstitial fibrosis with associated alveolar epithelial hyperplasia and metaplasia.

One dog from Group 3 died, after 60 mo of daily cigarette exposure, from foreign body pneumonia subsequent to vomition during smoke exposure. In lung sections, numerous alveolar macrophages adjacent to small blood vessels and bronchioles contained black pigment, probably particulate material from cigarette smoke. Slight dilation of bronchial mucous glands and a slight degree of subpleural vesicular emphysema were also present. Extensive inflammatory changes were seen, including numerous microgranulomata. These lesions are associated with cigarette smoking and have not been observed in the lungs from Group 1 dogs.

Three other dogs from Group 3 were killed after 65 mo of exposure to cigarette smoke. Moderate bronchiolitis in one dog was characterized by a mononuclear inflammatory infiltrate surrounding respiratory and terminal bronchioles, and extending into surrounding alveolar septa, forming an associated chronic alveolitis. The smoke-exposed dogs had an increased amount of phagocytized yellow pigment, 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 mononuclear cellular infiltration, subpleural interstitial fibrosis with associated alveolar epithelial cell hyperplasia, and focal calcification occurred in both smoke-exposed and sham-exposed dogs. The tracheobronchial lymph nodes of all three smoke-exposed dogs had lesions clearly related to treatment. Two dogs had moderate amounts of yellow pigment, apparently the same as the smoke-related pigment found in the lungs. Moderate reactive lymphoid hyperplasia in the tracheobronchial lymph nodes and moderate histiocytosis in the mediastinal lymph nodes of one smoke-exposed dog were probably related to chronic inflammatory changes in the lungs.

In an ancillary study, three dogs were exposed to smoke from 20 cigarettes per day for only 9 mo--double the dose rate of the dogs described above, and equivalent to four

packs per day smoked by man, as evidenced from comparative lung volumes, and measured COHb and nicotine levels. Pathology developed more rapidly in the respiratory tracts of these dogs. Focal areas of pleural thickening, alveolar septal fibrosis, and subpleural chronic inflammation were present in all three dogs (Figure 3.36). Vesicular emphysema (Figure 3.37), ranging in severity from very slight in one of the dogs to slight-to-moderate in another, was a feature of the lungs of this group of dogs; it was grossly visible in one dog. Two dogs had large areas of acute interstitial pneumonitis. Additional changes observed in the pulmonary parenchyma included numerous focal granulomata (Figure 3.38), which appeared to contain fat cells. Slight-to-moderate chronic bronchitis and bronchiolitis were present in the lungs of each of the three dogs (Figure 3.39), changes seen to a lesser degree in lungs of Group 3 dogs.

Lesions of the upper respiratory tract of the three 20 cigarette/day dogs included an ulceration of the tracheal mucosa in two cases, and focal, slight squamous metaplasia of the tracheal epithelium in all three dogs. A subepithelial inflammatory focus in the larynx was found in one of the dogs, although the surrounding epithelium was essentially normal in appearance.

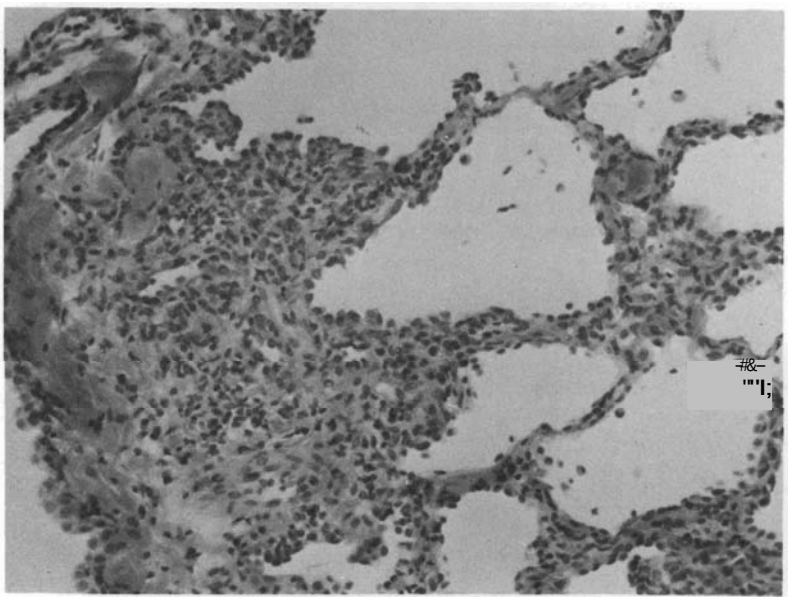

FIGURE 3.36. A Focus on Pleural Thickening, Subpleural Inflammation, Alveolar Septal Fibrosis, and Vesicular Emphysema, from a Dog that had Smoked 20 Cigarettes per Day for 9 Months. (H&E 200X)

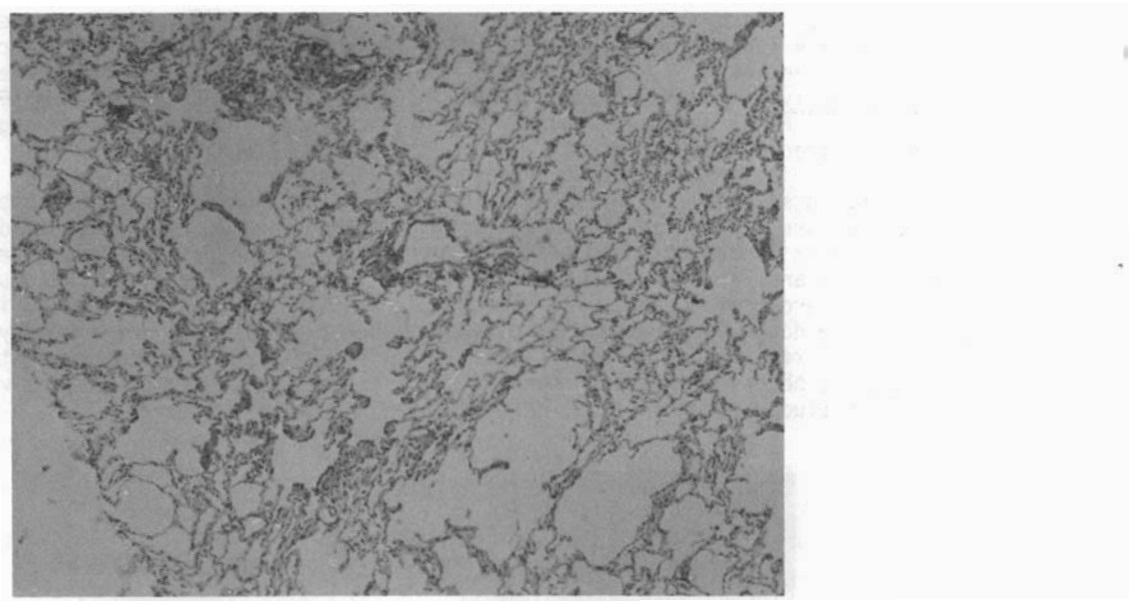

FIGURE 3.37. Vesicular Emphysema in a Section of the Same Lung Shown in Figure 3.35. (H&E 80X)

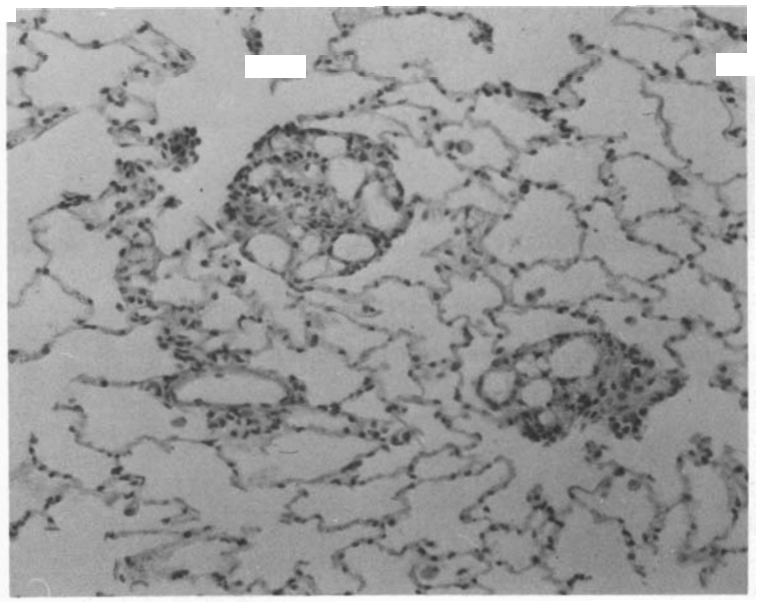

FIGURE 3.38. Focal Granulomata in a Lung Section from a Dog that had Smoked 20 Cigarettes per Day for 9 Months. (H&E 200X)

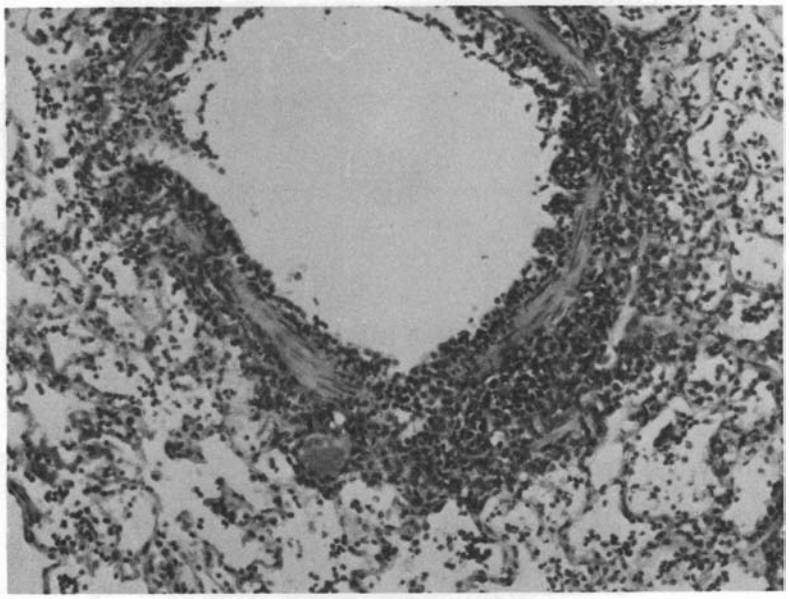

FIGURE 3.39. Chronic Bronchiolitis in a Lung Section from a Dog that had Smoked 20 Cigarettes per Day for 9 Months. (H&E 200X)