The Effects of Inhaled Uranium Mine Air
Contaminants in Beagle Dogs*

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The growing number of cases of lung cancer among uranium miners of the Colorado plateau is a matter of national concern in a period of increasing demand for uranium ore. The miners are exposed to a variety of inhalation hazards, including radon daughters, uranium ore dust, and cigarette smoking that may cause or contribute to respiratory tract, ~~pathology~~ *pathologic changes in the*. Over 97% of the miners developing lung cancer have had histories of cigarette smoking.

The principal objective of the experiments to be described is to determine the biological effects of daily exposures to known levels of potentially carcinogenic uranium mine air contaminants and cigarette smoke in the beagle dog as a relatively long-lived experimental animal.

Slide # shows the experimental design of studies concerning the possible contributory or synergistic action of cigarette smoking and exposure to radon daughters with ore dust in the production of lung ~~changes~~ *changes*, pathology.

Each dog was trained to accept a mask for cigarette smoking that provides direct inhalation of smoke via the mouth only, with exhalation by either mouth or nose (*slide* Figure 2). The slight negative pressure in the

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TABLE 1. Experimental Design for Dog Studies.

Spile 1

Group	Number of Animals [§]	Exposure Regimen
1 ***	20	600 WL radon daughters with uranium ore dust (carnotite) 15 mg/m ³ , plus sham smoking
2*	20	Cigarette smoke ⁺ plus 600 WL radon daughters with uranium ore dust (carnotite) 15 mg/m ³
3*	20	Cigarette smoke ⁺
4*	9	Controls, with sham smoking

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+ Each dog receives ten cigarettes daily over two shifts, 7 days per week.

§ Two dogs in each group receive periodic lung washing for exfoliative cytology studies.

Figure 1. Modified cigarette smoke inhalation mask incorporating lighter weight materials and tapered barrel. Detached rubber dam for mask sealing is shown at lower left.

mask created at the beginning of each inspiration triggers a stepper-switch, which activates the rotory solenoid at a pre-set number of breaths. Thus, each dog smoked according to its own respiration patterns, drawing one breath (or puff) out of ten through the cigarette. Dogs receive smoking, or sham smoking, 7 days/week, over 16-hour periods.

Two groups of ~~20~~ dogs, ^{originally 20 per group,} each are continuing to receive lifespan, daily exposures for 4 hours daily to 600 WL of radon daughters with 15 mg/m³ of uranium ore dust, with and without concomitant cigarette smoking; these exposures have been in progress for ~~two~~ ^{4 1/2} years. The two ~~multiple~~ ^{multiple}-dog chambers constructed for these head-only exposures are continuing to provide constant and reproducible levels of radon (measured hourly in each chamber); radon daughters, and ore dust. Radon daughter and uranium ore dust concentrations are taken four times daily. Condensation nuclei counts are taken twice daily in each chamber and in room air; chamber counts range from 35,000 to 110,000 per cc. Samples for dust particle size distribution and percentage of unattached radon daughters are taken periodically.

The smoking patterns by individual dogs and by groups are determined periodically by careful

recording during repeated tests of the number of puffs

and the number of minutes required for each dog to smoke

several cigarettes (University of Kentucky Research

Cigarette 1R1) from 85 mm to the standardized 23 mm butt

length. Although the mean numbers of minutes required to

smoke a cigarette have changed very little during this

time, significantly increased numbers of puffs per cigarette

are being taken by dogs receiving cigarette smoke plus

radon daughters with ore (Group 2) compared to dogs

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receiving cigarette smoke alone (Group 3). The mean numbers of sham smoking puffs taken in successive twelve minute test periods by dogs that receive daily exposures to radon daughters attached to uranium ore dust (Group 1) have also increased, due to markedly increased respiration rates during the last few months of several dogs in this group.

Carboxyhemoglobin levels are evaluated periodically for each dog in all groups, with dogs of two groups smoking 10 cigarettes per day, and dogs of the two other groups receiving identical periods of sham smoking, ~~as shown in Table 3.~~ Each dog's smoking performance is evaluated by comparing its HbCO levels to its behavior during smoking. ~~Reactivity~~ ~~Reactivity~~; all dogs were sampled just before beginning the day's smoking or sham smoking periods and were again sampled after completion of the day's smoking. ^{Slide 3} As shown in Figure 2, group mean post-exposure carboxyhemoglobin levels for Groups 2 and 3 (smoking with radon daughters and uranium ore dust, and cigarette smoking alone, respectively) were significantly higher at the 5% probability level than those of Groups 1 and 4 (radon daughters with ore dust plus sham smoking and controls with sham smoking). Mean pre-exposure carboxyhemoglobin levels for dogs of Groups 1, 2, 3, and 4 were similar, with experimental groups not different from controls. Carboxyhemoglobin levels in both groups of dogs that receive daily cigarette exposures are markedly increased after smoking. Mean levels of % HbCO in Groups 2 and 3 are similar to those found in humans

Slide 3

Figure 2. Percent carboxyhemoglobin levels in peripheral blood of beagle dogs receiving daily smoking or sham smoking of cigarettes. Standard errors about mean values are shown.

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at these smoking levels, with apparently less variability than occurs between individual human cigarette smokers.

As a possible additional index of smoke constituent deposition, plasma thiocyanate levels were determined at the same sampling time as the ~~November 1976~~ HbCO determinations. ~~As shown in Table 4,~~ ^M mean values obtained from cigarette smoking dogs of Groups 2 and 3 are nearly double those from control dogs (Group 4) or dogs inhaling radon daughters with uranium ore and receiving sham smoking (Group 1). Significant differences did not appear between pre-smoking and post-smoking levels for each group.

Human levels of SCN- for non-smokers are 36 $\mu\text{M}/\ell$ (Pettigrew, AR and G. S. Fell, Clin. Chem. 18, 996, 1972), while those for control dogs average 26.7 $\mu\text{M}/\ell$. This difference may be due to variations in dietary patterns of the two species. Thiocyanate levels of the groups of dogs receiving smoke were about 40 $\mu\text{M}/\ell$ while human smokers levels were 65; thus cigarette smoking by these dogs and by humans appear to produce comparable fractional increases in plasma thiocyanate values.

The value of this test as an index of the cumulative exposure to cigarette smoking and determination of the absorbed dose of this smoke component is being investigated. It is planned to perform several analyses of build-up and

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clearance of SCN- in plasma during smoking and non-smoking period over several weeks.

Minute volumes, tidal volumes and respiration rates continue to be measured every month on each dog. Increasing numbers of dogs in each experimental group are beginning to show dyspnea. Mean minute volumes of each of the exposed groups have to date not differed significantly from controls. During the last ^{several} ~~few~~ months, increasing numbers of dogs that daily inhale cigarette smoke with radon daughters and ore, and also dogs that daily inhale radon daughters and ore, are showing continuously elevated respiratory rates, as shown in the group mean values in Table 5, and in Figure 3. The extent of the 95% confidence limits about mean values for Group 1 and Group 2 dogs demonstrate the fact that rates between individual dogs have begun to vary widely, with some animals reaching 100 to 120 respirations per minute. These changes are reflected in consistently reduced tidal volumes in Group 1 and Group 2 dogs during recent months. (Table 5).

Four dogs exposed to radon daughters with uranium ore dust and four dogs exposed to radon daughters with uranium ore dust plus cigarette smoke were euthanized when death was imminent due to respiratory insufficiency during the past ^{1 year,} ~~six~~ months. Lesions were detected in the thoracic radiograph

examination ~~was made~~ in five of the eight dogs. Radiographs were taken periodically to observe the progression of lesions in these dogs. The first lesions were uniform graying of the lung field, probably due to pulmonary fibrosis. Areas of uniformly increased density appeared in the apical lobes and pericardial regions followed by smaller, more dense 0.25 to 0.5 cm diameter spots, presenting a mottled appearance in the lungs. Some of the spots appeared to coalesce forming 1 to 2 cm diameter poorly defined densities. Two of the dogs showed areas of ^{bullous} ~~bullous~~ emphysema. As the lesions became more severe, the heart shadow was enlarged showing prominent right ventricle and pulmonary artery shadows with dorsal deviation and dilatation of the intrathoracic trachea. The radiographic changes were concomitant with severe respiratory insufficiency, including increased respiration rates, decreased arterial pO_2 , increased arterial pCO_2 , anorexia and decreased body weight in individual dogs.

A control dog was sacrificed after approximately 35 months for histological comparison with the ^{sacrificed} ~~latter two~~ dogs (all experimental and control dogs in the project began exposures or sham exposures at 22-24 months of age). Only minimal ^{focal} lesions were present in the lungs of this animal and they consisted of a few small foci of subpleural interstitial fibrosis with slight pleural thickening (Slide 4).
 Generally, The lungs of This dog appeared quite normal (Slide 5).
 (Figure 12).

Slide 4~~Figure 12.~~

Minimal lesions in the lungs of a control dog with sham smoking for 35 months, consisting of a few small foci of subpleural interstitial fibrosis with associated alveolar epithelial metaplasia and very slight pleural thickening. H&E 185

Slide 5~~Figure 6.~~

Lung section from dog used as a sham smoking control for 35 months. The pleura is shown at the bottom of the photomicrograph. H&E 185X

Because of the similarity of lesions within each group and because these deaths occurred within the last ¹⁵ ~~nine~~ months, discussion of the pulmonary lesions of these dogs is structured according to specific exposure groups.

A. Group 1. Radon Daughters with Uranium Ore Dust.

All lungs of dogs from this group displayed large numbers of alveolar macrophages containing uranium ore dust, and in all cases there was considerable accumulation of these macrophages around the bronchi, bronchioles, and smaller blood vessels. In three of the five cases, this accumulation had produced a chronic inflammatory response characterized by infiltration of mononuclear cells. In alveoli adjacent to these areas, pronounced septal fibrosis and extensive epithelial hyperplasia had occurred.

The most striking feature of the lungs of this group was the pleural thickening and septal fibrosis of subpleural alveoli ^{Slide 6} (~~Figures 13 and 14~~). The septal fibrosis was always accompanied by alveolar epithelial hyperplasia similar to that observed at perivascular and peribronchiolar areas but with a higher degree of severity. Numerous small blood vessels at the periphery of the lung lobes were found to be occluded by fibrosis of the vascular walls, endothelial proliferation, or both (~~Figure 15~~). ^{Slide 7}

Slide 6
Figure 14. Pleural thickening and peripheral alveolar septal fibrosis from the lung of a dog after 47 months exposure to radon daughters with uranium ore dust. Also shown are emphysematous vesicles (E). H&E 95X

Slide 7
Figure 15. An arteriole nearly completely occluded by endothelial cell hyperplasia and fibrosis of the vessel wall. The section is from the lung of a dog exposed to radon daughters with uranium ore dust for 47 months. H&E

Established neoplastic lesions have not been found to date, but four of the five animals had developed numerous foci of squamous metaplasia of alveolar epithelium as shown in ^{Slide 8 + 9} Figures ~~16 and 17~~, ranging from slight to moderate severity; in two of these dogs there were foci of very advanced squamous metaplasia. Atypical nuclei were noted in these lesions although no evidence of invasion of adjacent tissues was present. These lesions are very similar to the pathology shown in plutonium-exposed dogs prior to tumor development. These proliferative lesions of the ^{alveolar} ~~bronchiolar~~ epithelium are similar to the squamous metaplasia appearing in hamsters as a stage of progression immediately preceding observed squamous carcinoma, (~~see Progress Report of May 31, 1973~~).

B. Group 2. Daily Cigarette Smoking plus Inhalation of Uranium Ore Dust.

Detailed histopathological examination of the lungs of the three dogs from this group showed that all had developed the peribronchiolar and perivascular accumulations of macrophages with focal infiltration of mononuclear cells described for the Group 1 dogs. Large numbers of alveolar macrophages were also observed, especially in one dog having 48 months of daily exposures, as shown in ^{Slide 10} ~~Figure 7 and Figure 18~~.

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Figure-16: Squamous metaplasia of alveolar epithelium from the lung of a dog after 46 months exposure to radon daughters with uranium ore dust. H&E 375X

Slide 9

Figure-17: Higher magnification of the lesion shown in Figure 16. Atypical nuclei are present in cells lining the alveolar lumen (L). H&E 750X

Slide 10

~~Figure 18.~~ Numerous alveolar macrophages from the lung of a dog exposed to radon daughters with uranium ore dust and cigarette smoke for 48 months. H&E 375X

Slide 11

~~Figure 19.~~ Cuboidal metaplasia of alveolar epithelium and associated alveolar septal fibrosis from the lung of a dog after exposure to radon daughters with uranium ore dust and cigarette smoke for 48 months. The lesion has progressed to squamous metaplasia as shown in the upper left portion of the photomicrograph. H&E 185X

Subpleural interstitial fibrosis with associated alveolar epithelial metaplasia was observed in two of the three dogs of this group, involving extensive areas in the lungs of one of the dogs exposed for 48 months ^{Slide 11} (~~Figure 19~~). In a few foci, a progression from cuboidal metaplasia to squamous metaplasia of the alveolar epithelium was present, (~~Figure 20~~).

One of the most striking pathological changes found in the lungs of this group of dogs was that of pulmonary emphysema, as shown in ^{Slide 12} ~~Figures 21 and 22~~. In the dog exposed for 43 months and one of the dogs exposed for 48 months, slight to moderate vesicular emphysema was observed; however, in the lungs of the other dog with 48 months exposure, large emphysematous bullae ^{ge} were found (^{Slide 13} ~~Figure 23~~). These were evident grossly as large, soft elevations on the pleural surface. The yellow pigment, tentatively identified as hematoidin, was found to be associated with the emphysematous bullae in large quantities. A few foci of cuboidal to squamous metaplasia of alveolar epithelium were also found in one lobe to the lungs of this dog. Another dog from this group has been very recently sacrificed. Large subpleural bullae ^{ge} were visible on its lungs; all respiratory and systemic tissues are being prepared for detailed histopathologic examination.

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Figure 22.

Photograph of a lung section from a dog after 48 months exposure to radon daughters with uranium ore dust and cigarette smoke. The emphysematous bulli (B) are a prominent feature of the lungs from this dog. H&E 4.5X

Slide 13

Figure 23.

Photomicrograph of a portion of the same lung section shown in Figure 22. Alveolar septal fibrosis is present in the area of the emphysematous bulli (B). H&E 75X

C. Group 3. Daily Cigarette Smoking.

After 49 months of exposure, a dog from this group was sacrificed for comparison of pathological lesions with those of the dogs from Group 1 and Group 2 which had died or were sacrificed. Pulmonary lesions of this cigarette smoking only dog included vesicular emphysema, and also occasional foci of subpleural interstitial fibrosis with associated alveolar epithelial metaplasia as shown in ^{slide 14} Figure 24. This lesion was a frequent observation in the lungs of dogs from Group 2, also exposed to cigarette smoke, and has been considered neoplastic by others studying respiratory carcinogenesis and its relationship to smoking. It is very interesting that a similar lesion was observed in one of the Group 1 dogs after eight months of exposure to radon daughters with uranium ore dust, precluding it as a lesion specific to cigarette smoke inhalation. These experimental findings indicate, however, that cigarette smoke may effect the frequency of occurrence and severity of the lesion. It is also possible that the heavy peripheral fibrosis in the lungs of Group 1 dogs has obliterated the lesion with exposures longer than 30 months.

Relatively small amounts of the exogenous yellow pigment considered to be associated with cigarette smoking was found in alveolar macrophages in the lungs of this animal. The tracheobronchial lymph nodes,

Slide 14

Figure 24. Subpleural interstitial fibrosis with alveolar epithelial metaplasia from the lung of a dog exposed to cigarette smoke only for 49 months. The metaplasia epithelium has assumed a squamous nature in a portion of the area shown (arrow). H&E 185X

Slide 15

Figure 25. Tracheobronchial lymph node from the same dog for which the lung in Figure 16 is shown. Histiocytes containing pigment considered to be related to cigarette smoking are visible in a large area (H). H&E 360X

however, contained large amounts of phagocytized exogenous yellow pigment in histiocytes as shown in ^{Slide 15} Figure 25. Pulmonary clearance of the pigment via the lymphatic system is thus apparently quite efficient in beagle dogs.

This dog that received cigarette smoking only also showed foci of tracheal epithelial squamous metaplasia, as shown in ^{Slide 16} Figure 26. ~~Tracheas of all dogs receiving cigarette smoke alone or with radon daughters and uranium ore dust are currently being examined for further evidence of development of this lesion.~~

^{Slide 16}

Figure 26. Squamous metaplasia of tracheal epithelium from a dog exposed to cigarette smoke, alone, for 49 months. The transition from normal ciliated columnar to metaplastic squamous cells is shown.

Exposures of the surviving dogs of this experiment are continuing. The post-mortem examination of a dog sacrificed very recently revealed palpable masses in the anterior one-third of each diaphragmatic lobes^{of the lung.} Samples of the lung are presently being prepared for microscopic examination.~~and~~