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# EFFECTS OF HIGH ENERGY PARTICLE (HZE) RADIATION ON THE DISTAL LUNG

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## Abstract

Major sources of concern for manned space travel are the effects of high energy particle (HZE) radiation on various biological systems, and the consequences of major solar activity. To date, considerable attention has been directed toward HZE-induced alterations both on non-dividing systems, such as the retina, cornea and brain, and on dividing systems, such as the gut and testis. This paper is focused on the morphologically detectable late-occurring alterations in the distal lung, and toward a comparison of the changes with those induced by x-irradiation. Briefly, the salient alterations involve an increase in 1) the width of the septal walls and the capillary and alveolar basal laminae, and 2) the irregularity of the luminal surface of the capillaries, as exemplified by the presence of filipodial projections and blebbing. All alterations were focal in their localization, and no cells of any type (e.g., epithelial, endothelial or stromal) appeared to undergo damage, an observation quite unlike the cellular changes induced by x-irradiation.

KEY WORDS: High Energy Particle Radiation (HZE), Lung, Transmission Electron Microscopy, Scanning Electron Microscopy, Fibrosis, X-Irradiation, Mouse.

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## Introduction

Exploding solar sunspots and flares have been considered among the sources of high energy particle (HZE) radiation. For most terrestrial environs such accidental radiation is of little consequence, since much is trapped in the earth's radiation belts and atmosphere (Todd, 1983). However, those individuals who participate in space flights, particularly the polar orbital, geostationary and deep-space (high orbital) flights, will be exposed to various quantities of HZE radiation, depending on the altitude and inclination of the mission. Moreover, those individuals who may need to labor in an extra-vehicular environment will lose any protection from radiation which the space capsule/station might provide. HZE particle tracks have relatively narrow cores, but fairly large penumbrae, which increase with atomic number. For biological systems, this translates into a significant potential for biological (cellular) damage, which is usually expressed as a microlesion (Todd, 1983; Nelson and Tobias, 1983; Lett, 1986). For example, in one hypothetical situation involving the cornea, Todd (1983) proposed that 9000 microlesions per $cm^3$ of tissue per month would be induced in a geostationary orbit. Although total dose may be low, concern may exist if the relative biological effectiveness (RBE) is high. Present allowable astronaut exposures per lifetime are 400 rads, but may be lowered considerably in the near future. Moreover, more attention is now being directed toward the latent effects of low exposures occurring over long periods of time, particularly as they may involve compromises in the normal functioning of biological systems.

Prior studies have directed attention toward corneal damage and the induction of cataracts (Cox and Kraft, 1984; Lett et al., 1984; Philpott et al., 1985; Lett, 1986), retinal damage (Philpott et al., 1985) and central nervous system dysfunction (Philpott et al., 1985). Nelson and Tobias (1983) have shown that HZE radiation induced plasmalemmal damage rapidly after exposure. The rapidity with which cells are able to repair such damage influences significantly the degree and magnitude of injury that might ensue.

The purpose of this study is to concentrate on the late effects that HZE radiation might induce in the distal lung, and to compare those changes to alterations induced by ionizing radiation. To date no studies of HZE exposure to the lung have been reported, although numerous studies of the effects of

x-irradiation, both acute and chronic, to this organ system exist.

## Material and Methods

### Animals

Forty eight experimental and twenty four sham control male C57BL6 black mice were transported from Ames Research Center to the Lawrence Berkeley Radiation Laboratory. Body weights were recorded and tail markings were made at irradiation time. Throughout the experimental period, animals were maintained on standard laboratory chow and water ad libitum.

### Irradiation Procedures

For each irradiation the mice were anesthetized with Nembutal, diluted 1:2 in saline using 0.1 ml/10 grams body weight. Using the BEVALAC of the Lawrence Berkeley Laboratory (University of California), twenty four mice were exposed to 0.5 rad, and twenty four mice to 50 rads of iron (Fe) at 600 MeV/$\mu$m on the "Bragg" plateau, with an LET of approximately 180 keV/$\mu$m. An additional twenty four age-matched mice were sham irradiated, and served as controls. The two radiation doses were chosen to evoke an average of 1 hit/cell and 1 hit/100 cells at the higher and lower exposures, respectively.

A translator capable of holding 5 anesthetized mice was used to hold and move the animals across the beam during exposure. A Lexan detector was placed behind each mouse for etching and verification of the mouse position at irradiation. A lead mask collimator was adjusted for whole body exposures. The radiation dose was recorded by computer at Berkeley. Upon the return of the mice to the laboratory at Ames Research Center, the body weights of the animals were recorded.

### Tissue Preparation

At 6 months following irradiation with 0.5 or 50 rads or following sham-irradiation, one animal from each group was anesthetized and perfused, first with oxygenated Tyrode's solution kept at body temperature, and then with Triple Fix (Philpott et al., 1980). Perfusion procedures employed a Harvard perfusion apparatus which maintains a positive pressure. At 12 months following irradiation or sham-irradiation, an additional five animals from each group were subjected to the same procedures. Following perfusion, the lungs were rapidly removed and placed in fresh Triple Fix, where they were further dissected and prepared for either scanning or transmission electron microscopy. For scanning electron microscopy, the tissues were processed as follows: a) 500 $\mu$m thick slices of lungs were sectioned using a DTK Microslicer, and allowed to remain in fixative from several hours to overnight, b) washed in 2-3 changes of 0.1 M phosphate buffer, pH 7.2, overnight, c) rinsed 2-3 more times in buffer, d) dehydrated in increasing concentrations of ethanol, and e) critically point dried using $CO_2$ as the transition fluid. Specimens were observed and photographed using a JEOL JSM-35CF scanning electron microscope at an accelerating voltage of 7 kV.

For transmission electron microscopy the tissues were cut into $mm^3$ blocks and processed as reported earlier (Penney and Rubin, 1977; Penney et al., 1981, 1982). Thick sections 0.5-1.0 $\mu$m thick were stained with methylene blue, azure II and basic fuchsin for light microscopy. Thin sections (40-90 nm) were stained with uranyl acetate and lead citrate and photographed with a Zeiss 10A transmission electron microscope using an accelerating voltage of 60 kV.

## Results

Throughout the course of the experiments, there was no significant difference in the body weight between the sham-treated control and irradiated animals.

The morphological changes of the distal lung of the mouse observed following HZE radiation exposures will be described according to the techniques employed: scanning and transmission electron microscopy.

### Scanning Electron Microscopy

0 Rad. The distal lungs of age-matched sham-treated control animals recovered 6 or 12 months following sham irradiation did not significantly differ from one another in morphologic organization, in that neither time period exhibited any damage. Figure 1 shows a representative low power view of control tissue in which the abundant vasculature, predominantly capillaries, is evident, as are the smooth surfaces of the alveoli. At higher magnification (not shown), types I and II pneumocytes can be differentiated, and the intimate juxtapositioning of capillaries to alveoli can be observed. The septal walls of the control lung are relatively thin, with an occasional alveolar macrophage present.

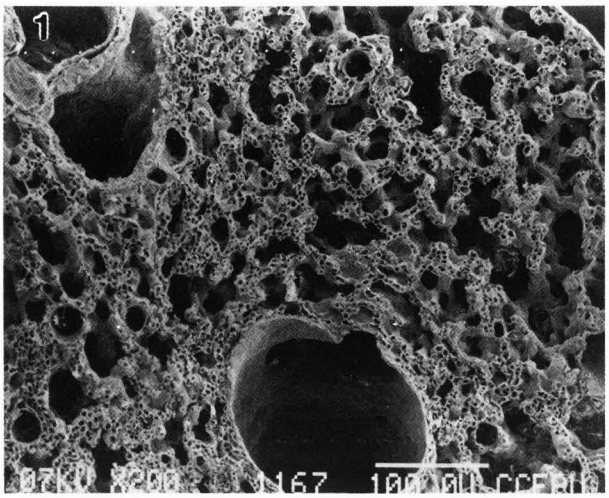
0.5 Rad. The structure of the distal lung is not significantly altered from control sham-treated tissues at either 6 or 12 months post-irradiation (PI) (Figure 2). Airways remain patent, capillaries abundant, alveolar walls smooth, and septal walls relatively thin.

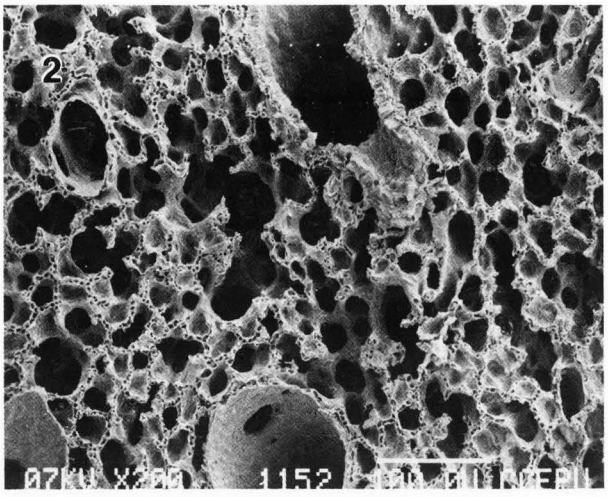
50 Rad. By 6 months PI, the alveolar airspaces were focally reduced. Although this observation was not quantitated as has been done for $^{60}Co$ exposures of 300-1300 rad (Penney et al., 1982, 1986; Siemann et al., 1982), the overall appearance of the lung was similar (Figure 3). By 12 months PI, the thickening of the septal walls was more pronounced, and there were areas in which fibrous and cellular components of the connective tissue were extensively developed (Figure 4). Macrophages were also prominent.

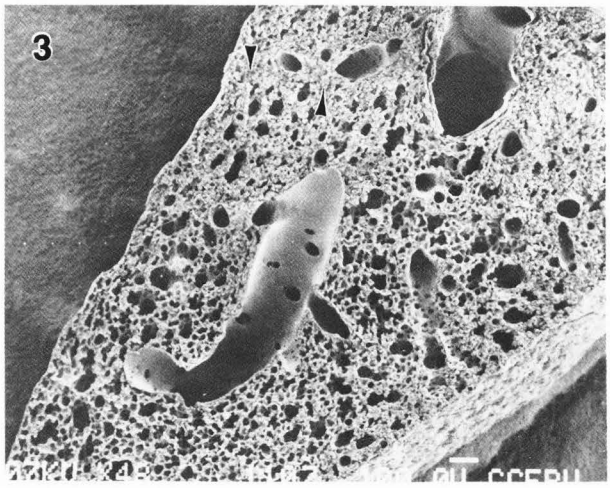
### Transmission Electron Microscopy

0 Rad. Fine structural studies of the distal lungs of sham-irradiated mice exhibited normal morphologic organization at both 6 and 12 months following sham treatment. Type II pneumocytes possessed numerous surfactant-containing lamellar bodies, and occasional alveolar macrophages were encountered. Occasionally tubular myelin, proteinaceous precipitate and evidence of type II cell secretion were observed within the alveoli (Figure 5). The capillary endothelium and the alveolar type I cells were normal in appearance, and consistent with numerous other descriptions of these cell types. An occasional blood cell was noted within the capillary lumina.

0.5 Rad. Morphologic alterations following 0.5 rad exposure were more evident 12 months PI. Increased amounts of collagen and elastin were observed in the septal walls (Figure 6), lipofibroblasts invaded the interstitium, and the basal laminae of both alveoli and capillaries were focally



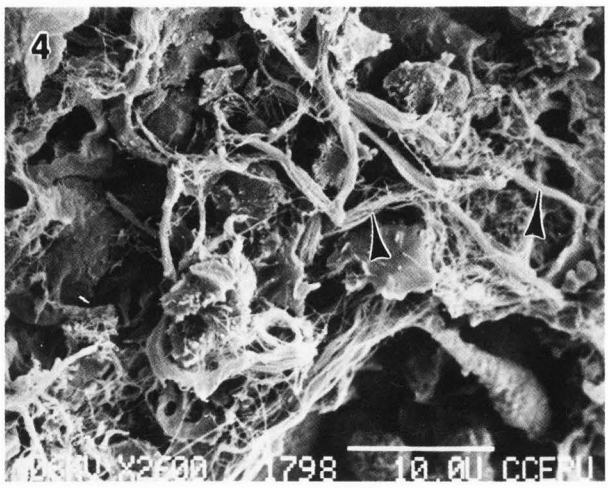




**Figure 1**: SEM, 0 rad, 12 months. The normal architecture of the lung can be observed, including larger blood vessels (lower center), airways (upper left) and the alveolar organization. An extensive capillary network surrounds each alveolus. The pleural surface is in the upper right. Bar = 100 μm.

**Figure 2**: SEM, 0.5 rad, 12 months. No significant changes can be observed when compared to Figure 1. A branching airway is located at the top center, and a blood vessel at the lower center. Bar = 100 μm.

**Figure 3**: SEM, 50 rads, 6 months. At this low power, areas of focal loss of alveolar surface area and fibrotic development can be noted (arrowheads). Bar = 100 μm.

**Figure 4**: SEM, 50 rads, 12 months. In areas where the septal wall is thickened, there is a prominence of extracellular fibrous components (arrowheads), which are not observed in unexposed tissues. Bar = 10 μm.

thickened. The lamellar body morphology was not atypical, and evidence of secretory activity was prominent. Endothelial cells exhibited an apparent increased pinocytic activity, although this observation was not quantitated. Noteworthy is the microvillous or pseudopodial projections of the endothelial cells following radiation exposure, an observation noted previously for x-irradiation (Penney et al., 1982, 1986). Alveolar macrophages were occasionally found, and often possessed long, needle-like, electron-opaque particles, which, at higher magnification, possessed a highly ordered internal structure (see Fig. 9 for similar observation).

50 Rad. By 6 months following the greater exposure, the amount of fibrous material in septal walls had increased (Figure 7). To some extent collagenous and elastin deposition was focally more prominent in subpleural regions than in the deeper zones of the lung. The type II pneumocytes contained abundant lamellar bodies, with no evidence of atypical lamellar body formation. As with other times, there was evidence of type II cell secretion, suggestive of the viability of the cells. No evidence of type II cells sloughing was evident, as has been

described following x-irradiation (Penney et al., 1981, 1982, 1984, 1986). Prominent lipofibroblasts were present in the interstitium. Endothelial cells possessed more irregular surfaces, and occasional blebbing. No widespread degeneration of any cell type was observed.

By 12 months PI the extent of fibrous invasion of septal walls was significantly increased (Figure 8), although as in prior descriptions, the collagen was not uniformly distributed. Endothelial cells possessed numerous pinocytic vesicles, microvillous projections, and blebbing (Figures 7 and 8). The alveoli contained tubular myelin and surfactant, indicating type II cell viability. Lamellar body organization was focally aberrant in that the normal lamellated pattern was not present. Often the septal walls were invaded by lipid-containing cells, similar in appearance to lipofibroblasts as described by Kaplan et al. (1985). No evidence of contractile cells was noted within septal walls. Alveolar macrophages were prominent, often containing rod-like inclusions (Figure 9), which at higher magnification (Inset) proved to be composed of phospholipid-like lamellae arranged in parallel in different planes (i.e., at right angles, parallel and diagonal to the long axis of the structure).

## Discussion

Of the primary responses of the distal lung to x-irradiation, pneumonitis and fibrosis are most intimately involved in the morbidity and mortality of the host; and these responses make the lung the dose-limiting organ for thoracic radiation. Numerous other studies have described radiation-induced pneumonitic, fibrotic, survival, breathing rate, collagen and enzyme alterations (Penney and Rubin, 1977; Penney et al., 1981, 1982, 1986; Travis et al., 1977, 1980, 1985; Rubin et al., 1980, 1983; Ts'ao et al. , 1983; Ward et al., 1985; Maisin et al., 1982). The purpose of this study was to compare the effects on lung of radiation from high energy particle irradiation and x-irradiation, albeit the total exposures are unequal.

A significant effect of x-irradiation on lung is sloughing and disintegration of type II pneumocytes. Their putative role in the development of pneumonitis has been described in detail (Penney and Rubin, 1977; Penney et al., 1981, 1982; Travis et al., 1977, 1980, 1985; Bellet-Barthos et al., 1980; Maisin et al., 1982; Rubin et al., 1983). The doses utilized in the aforementioned studies (500-3000 rad) were substantially greater than the HZE exposures used in the experiments described herein. HZE radiation did not evoke any identifiable alterations in type II pneumocytes or other parenchymal cells of the distal lung, and active secretion of surfactant appeared to be unaffected. Thus, even at 50 rad exposures, cell killing by HZE radiation was not identified. In other unpublished studies (Penney and Rosenkrans, in preparation), x-irradiation at exposures of 100 and 300 rad, more closely approximating exposures used in these HZE studies, also did not evoke significant cell killing.

However, exposure to HZE radiation at the exposures selected for these experiments did evoke significant increases in the thickness of the septal wall, with increased collagen and elastin being the principal components. Additionally, there was focal thickening of the basal laminae of capillaries and alveoli following x-irradiation, as reported previously (Penney et al,. 1982, 1986) - basal laminar alterations are expressed as both acute ( 24 h) and late ( 63 weeks) post-irradiation effects (Penney and Rosenkrans, 1984; Rosenkrans and Penney, 1985, 1986), and may play a significant role in both pneumonitic and fibrotic responses.

Although the times and HZE doses used in this study would not be expected to induce either a pneumonitic response or type II cell sloughing, the long-range effects of HZE radiation on the distal lung are similar in some respects to the effects of x-irradiation. In particular, fibrotic induction, blebbing and filipodial extensions of endothelial cells, invasion of lipofibroblasts into the septal wall, and thickening of the septal walls are similar to late effects induced by low dose x-irradiation (500 rad or less) (Penney et al., 1986). The potential role of endothelial responses in evoking sluggish blood flow through the capillaries has been described earlier (Penney et al., 1986). Lipofibroblasts, or lipid-containing interstitial cells (Kaplan et al., 1985), have been described in developing lung. Their role in either repair of lung injury or the development of the fibrotic response remains under investigation. Lipofibroblasts have been shown to increase in irradiated lungs (Penney et al. 1986).

It has been suggested by several investigators that radiation may be an environmental factor which can enhance the rate of aging of cells and tissues.

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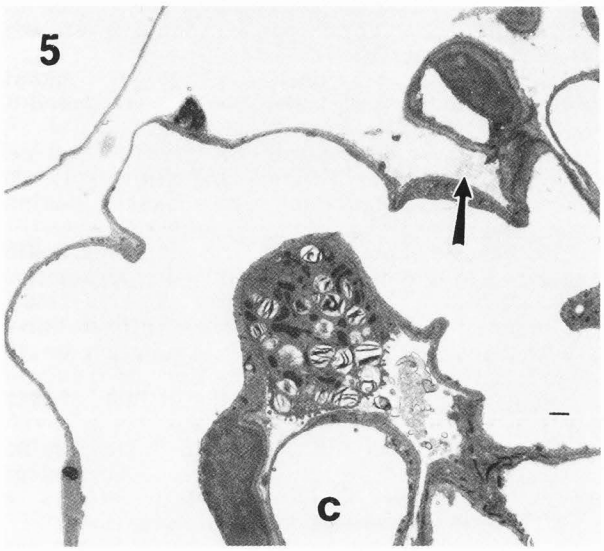
**Figure 5**: TEM, 0 rad, 12 months. A type II pneumocyte, showing evidence of surfactant secretion into the alveolus, is noted in the center. Some alveoli also contain a proteinaceous flocculent precipitate (arrow). The capillary (c) luminal surfaces are smooth. Bar = 1.0 μm.

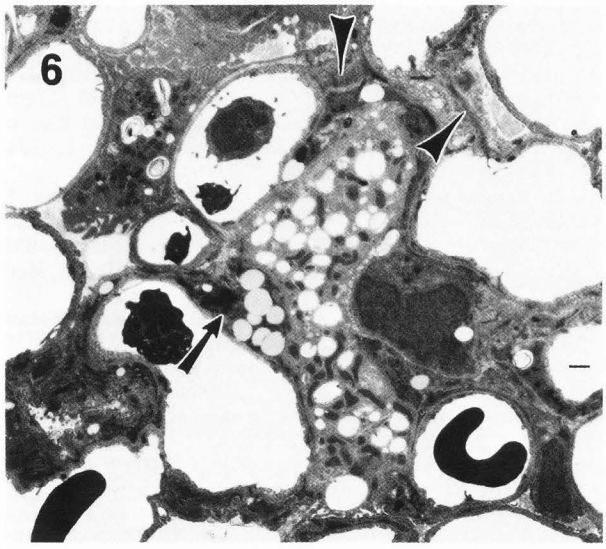
**Figure 6**: TEM, 0.5 rad, 12 months. Increased fibrous deposition (arrowheads) in septal walls, the septal invasion of lipofibroblasts (arrow) and minor irregularities of capillary lumina can be observed. There does not appear to be any destruction of any cell type. Bar = 1.0 μm.

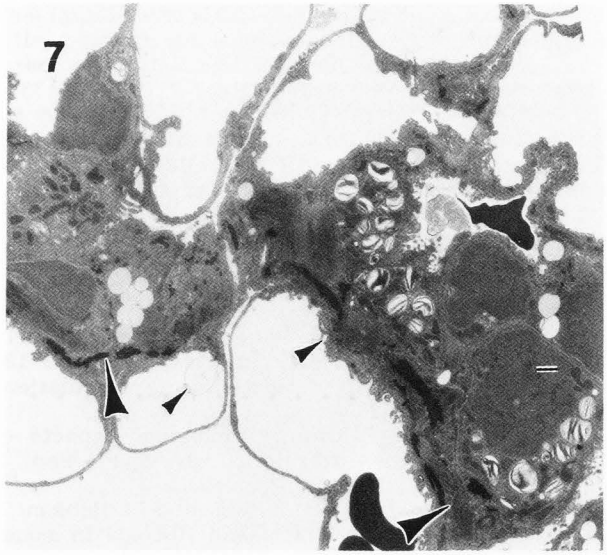
**Figure 7**: TEM, 50 rads, 6 months. Evidence of increased fibrous deposition in septal walls (large arrowheads) and endothelial blebbing (small arrowheads) can be observed, as well as viable type II pneumocytes and secretory activity, can be observed. No cellular destruction was observed. Bar = 1.0 μm.

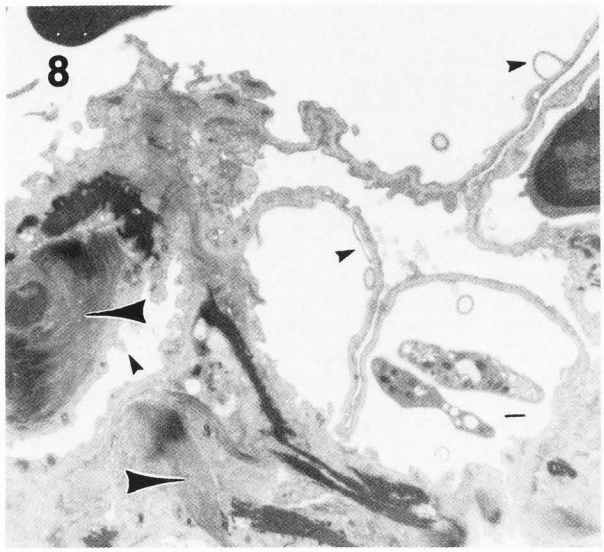
**Figure 8**: TEM, 50 rads, 12 months. Fibrotic development (large arrowheads) in septal walls is more clearly illustrated in this higher magnification micrograph, as are the blebbing and filipodial projections of endothelial cells (small arrowheads). Bar = 1.0 μm.

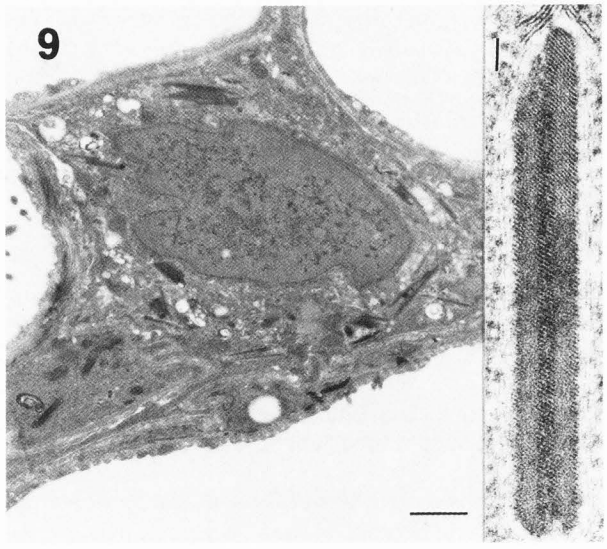
**Figure 9**: TEM, 50 rads, 12 months. Higher magnification of an alveolar macrophage containing rod-shaped crystalline-like inclusions. Bar = 1.0 m. Inset: Higher magnification of an inclusion illustrating the lamellations which, in this figure, are almost perpendicular to the long axis of the rod. Bar = 0.1 μm.




Indeed, such a view finds support in this study, since many of the HZE-induced morphologic changes described earlier resemble those changes which occur normally, but are temporally accelerated. Of interest, however, is the normal appearance of all lung cell types, particularly the type II pneumocytes, which undergo dose-dependent degeneration following exposure to x-irradiation, albeit at exposures considerably greater than those used here, if measured on a dose rather than a microlesion basis as proposed by Nelson and Tobias (1983). The ability of cells to repair microlesions rapidly, particularly those which do not involve nuclear chromatin, may be an important factor in preserving cellular viability with low dose HZE radiation. Nevertheless, based on the morphologic development of fibrosis and endothelial aberrations, the relative biological efficiency (RBE) of HZE radiation may be as high as 10 or greater.

Unresolved at present is the extent to which

fibrosis is a factor in the induction of host morbidity and mortality. In recent studies, Travis et al. (1985) have demonstrated that WR-2721 + radiation induced extensive fibrosis, as measured biochemically by hydroxyproline content, yet yielded little response insofar as breathing rates and morbidity were concerned. In contrast, radiation alone induced less fibrotic development, but more significant increases in breathing rates, morbidity and eventual mortality of the host, thus suggesting that localization and distribution may be more significant than the extent in determining the effect of fibrosis. Fibrosis resultant from HZE exposure was focal in its localization, being more prominent in septal walls, in the subpleura and in the walls of terminal bronchioles. Studies are continuing here and elsewhere to elucidate the relationships between the sites of focal fibrosis and the morbidity and mortality of the host.

Finally, the rod-like inclusions present in alveolar macrophages is somewhat suggestive of crocidolite asbestos (Crawford, 1980). However, the shape of the structures as rods, rather than irregular polygons, the lack of faults, and the orientation of the lamellae at various angles to the long axis of the rod are all incompatible with crocidolite asbestos, and their presence in both control and experimental animals indicates they are not a radiation-induced phenomenon, although macrophages possessing the rod-like structures were more numerous in the radiated lungs. The possible composition of these structures is under current study, employing non-morphologic techniques.

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## Discussion With Reviewers

J. Szekely: Do you think the focal loss of alveolar surface area and the focal nature of fibrosis are due to the discrete nature of the HZE particle tracks?
Authors: Not necessarily, since x-irradiation and other deleterious influences also evoke focal changes. It has been suggested by us and others that factors such as the metabolic state of the cells and matrices, oxygenation, etc., may influence the degree of response to exogenous insult.

J. Szekely: Since you did not identify any cell killing from the HZE-radiation exposure, do you think that the irradiated cells may become transformed? Is there any evidence in the literature for increased cancer production after low doses of HZE?
Authors: We have no first-hand data to answer the question directly. However, insofar as we could determine by our approach, there was no evidence of cellular transformation. In answer to the second part of your question, there is no evidence of which we are aware reporting increased cancer production after low doses of HZE radiation. However, a recent report (Coggle et al., Int. J. Radiat. Biol., 48, 95-106, 1985) has described tumor induction in x-irradiated mice, citing the peak of incidence curve at 5 Gy (500 rad) exposures. Our unreported data (Penney et al., unpublished data) confirm the work of Coggle et al. (1985) but we also observe a high tumor incidence following 9 Gy exposures, whereas Coggle et al. report a significant decrease at 7.5 Gy, the highest dose those investigators employed.

T. M. Seed: What was the long-term fate of the remaining 42 HZE-irradiated mice in terms of their overall mortality / morbidity rates, relative to sham-irradiated controls?
Authors: The experiments were set up to sacrifice the mice prior to the expression of any significant alteration in mortality or morbidity. Therefore, during the periods following exposure (6 or 12 months), no significant increase in mortality was observed in the exposed mice. Moreover, in some of our other studies, mortality rates are less than 10% by 88 weeks following doses of 9 Gy, a dose considerably greater than those HZE exposures used in these studies.