Inflammatory Sequences in Acute Pulmonary Radiation Injury

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The histopathologic events in the developing acute pulmonary inflammatory reaction to inhaled particles of Yttrium 90 are detailed. In animals that died or were sacrificed during the first year after inhalation exposure, microscopic findings of acute inflammation predominated and included vascular congestion; stasis; focal hemorrhage; edema; various inflammatory cell infiltrates; cytolysis and desquamation of bronchiolar and alveolar epithelium followed by regeneration; vascular injury and repair; and the eventual development of pulmonary fibrosis. Accumulation of alveolar fibrin deposits was an additional characteristic, though not a constant feature of the early stages of radiation pneumonitis. In addition to the direct effects of radiation on pulmonary cell populations, the histopathologic findings were suggestive of diverse activation of various cellular and humoral mediation systems in their pathogenesis. The potential interrelationships of systems responsible for increased vascular permeability, coagulation and fibrinolysis, chemotaxis, and direct cellular injury were discussed and related to the pathogenesis of the microscopic findings characteristic of early pulmonary radiation injury. (Am J Pathol 82:549–572, 1976)

Since the early recognition of radiation effects on the respiratory tract, a number of articles have described both the experimental and clinical development of pulmonary radiation injury. Some of this material has been reviewed and supports the fact that intensive radiation delivered to the thorax can produce a severe and sometimes fatal pulmonary inflammatory reaction. Little progress has been made, however, in our understanding of the pathogenesis of this condition, and the lung remains a dose-limiting structure in the radiotherapeutic treatment of thoracic and chest wall malignancies. In addition, the past several years have been marked by increasing concern for the potential toxicity of various radionuclides associated with nuclear power systems which may play an important role in the energy future of the United States. A large scale move to nuclear power as an energy source will result in a marked...

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increase in the quantities of these radionuclides present in nuclear fuel cycle inventories, thus increasing the potential for the exposure of man to these radionuclides at both the environmental and industrial levels. The most likely route of accidental internal deposition in man in such situations is through inhalation exposure. A better understanding of the pathogenesis of pulmonary radiation damage could lead to the development of improved preventive measures and better therapeutic means for the treatment of radiation pneumonitis or it could suggest certain modifications in the methodology of thoracic radiation therapy. Thus, an increased understanding of the sequence of events in pulmonary radiation injury will be of benefit to both the nuclear industry and to the medical profession.

The histopathology of the pulmonary radiation reaction in many respects resembles a classic inflammatory response. A certain combination of tissue changes presents a histologic picture which is considered characteristic for radiation pneumonitis, although many of these histologic changes may be found in other conditions. These changes include: edema; congestion; varied inflammatory cell infiltrates; desquamation of bronchiolar and alveolar epithelium followed by regeneration; hypertrophy and atypia of fixed pulmonary cells; vascular injury and repair; and the eventual development of fibrous connective tissue. If the initial insult and the ensuing reaction are mild, the changes may subside in a few weeks or months, leaving little or no residual evidence of inflammation. In other cases, the inflammatory changes can become chronic and may persist for months or years. During this period, fibrosis and extensive proliferation of connective tissue elements predominate. Little is known of the precise sequence of events which may determine the resolution or progression of the radiation-induced inflammatory response once it is initiated. The present report details the histopathologic findings in pulmonary radiation injury in dogs caused by the internal deposition of $^{90}\text{Y}$ in fused aluminosilicate particles following inhalation exposure and relates these tissue changes to present knowledge of the participation of various cells and humoral factors in the pathogenesis of the inflammatory response.

**Materials and Methods**

**Experimental Approach**

The animals used in this study were drawn from one of a large number of inhalation studies involving the beagle dog and the Syrian hamster being conducted at the Inhalation Toxicology Research Institute. Study of the toxicity of inhaled radionuclides is based upon evaluation of the influence of radiation dose rate and total radiation dose on dose-response...
relationships. The initial lung burdens used were predicted to result in: early deaths due to inflammatory pulmonary changes; deaths at later times due to moderate to marked pathologic changes, such as pulmonary fibrosis, at intermediate dose levels; and more subtle and late occurring changes, including neoplasia, at lower initial lung burden levels. For the preparation of this report, 37 dogs exposed to relatively high initial lung burden levels of $^{90}$Y inhaled in aluminosilicate particles which died or were sacrificed during the first year after exposure have been used. Detailed descriptions of the aerosol apparatus, exposure methods, isotope and aerosol preparation, and other experimental variables have been previously reported.10-18

Experimental Procedures and Design

All dogs used in these studies were derived from our own closed breeding colony of beagle dogs,14,18 and were exposed as young adults of 12 to 14 months of age. Inhalation exposures were conducted individually using an apparatus previously described.10 For preparation of the aerosol, $^{90}$Y was separated from its parent isotope, $^{89}$Sr, by the selective formation of colloidal hydrogen phosphate at a pH of 5.0.16 The separated $^{90}$Y was transferred into montmorillonite clay by ion-exchange. The clay was then filtered, resuspended into distilled water, and placed in a nebulizer type aerosol generator.15 The nebulized aerosol was passed through a column heated to 1100 C and then cooled by the addition of diluting air before delivery to the dog for inhalation. The resultant fused aluminosilicate particles containing $^{90}$Y were spherical in shape, with an activity median aerodynamic diameter ranging from 0.8 to 1.2 $\mu$m with geometric standard deviations of 1.6 to 1.9; the size distribution curve was a normal logarithmic curve. Further details on the experimental procedures have been reported.17

Yttrium 90 was selected for study because it represents a typical short-lived high-energy ($E_{\text{max}} = 2.28$ MeV) beta emitter with a physical half-life of about 64.2 hours, and because it represents one of the fission product radionuclides found in significant quantities in a nuclear reactor inventory after a sustained period of operation. The retention and tissue distribution pattern of $^{90}$Y inhaled in fused aluminosilicate particles matches what would be anticipated for an insoluble material of the particle size being studied.17 The lung is, therefore, the primary organ irradiated by the beta particles from $^{90}$Y. The retention half-life of $^{90}$Y particles in the lung approximates the physical half-life of $^{90}$Y; that is, 90% of the infinite dose is delivered in about 9 days and the irradiation is essentially complete by 28 days after deposition. Certain of the early biologic effects of inhaled $^{90}$Y in the dog is and its effects on pulmonary function and related parameters have been reported. The present report is based on histopathologic study of dogs with radiation pneumonitis that died during the first year after $^{90}$Y exposure and includes 37 animals which received initial lung burdens ranging from 590 to 5200 $\mu$Ci/kg and died at 7.5 to 277 days after exposure with total cumulative radiation doses to lung that ranged from 9300 to 70,000 rads.

Dose and Dose Rate

The methods of dose calculation have been previously reported.17 The total radiation dose to lung was basically determined by the nature of the radionuclide and its decay characteristics, its physical half-life, the retention of the particles in the lung (biologic or effective half-life), and the duration of radiation exposure or time to death. In essence, the relatively insoluble $^{90}$Y particles were inhaled and a portion was deposited deep in the lung and was not cleared or cleared slowly; hence they decayed and irradiated lung tissue at a relatively high but rapidly decreasing dose rate (see Text-figure 1).

Pathology

A complete necropsy was performed on each animal at the time of death or after sacrifice when death was imminent. Tissues for histopathologic study were routinely fixed in 10%
neutral buffered formalin. The lungs were fixed by bronchial perfusion and reexpanded to their approximate dimensions in an inflated state. After routine processing and paraffin embedding, sections of tissue were cut at 5 μ and routinely stained with hematoxylin and eosin. Selected tissues were also stained with elastic Masson’s trichrome and phosphotungstic acid stains.

The lungs of many of the dogs were cultured at necropsy. No significant pathogens were isolated from these lungs by routine microbiologic methods.

Results

Gross Pathologic Findings

Typical gross findings in dogs dying of acute radiation pneumonitis were generally confined to the lungs and contiguous tissues. The lungs were commonly rubbery, dark in color or mottled light and dark, edematous, moderately firm in consistency, and often failed to collapse normally when the thorax was opened (Figure 1). The lung weight in general was two to three times that observed in necropsy of normal animals in this colony. The lungs of animals dying of radiation pneumonitis of somewhat longer duration after exposure frequently exhibited well-delineated, cream-colored, irregularly shaped areas of variable size on the pleural surface. Animals surviving long enough to develop pulmonary fibrosis commonly exhibited irregular pleural contractions with underlying firm pale zones of scar tissue development. Irregular blotchy discoloration of the cut surface was a common finding in the lung injured by radiation.
Histopathologic Findings

In animals dying relatively soon (up to 75 days), an acute inflammatory reaction predominated with vascular congestion, stasis, variable accumulations of edema fluid and fibrin in alveoli and terminal bronchioles, and occasional necrotizing inflammatory changes in pulmonary vessels. A variable but occasionally marked focal infiltration of granulocytic leukocytes in the interstitial tissues and alveoli was sometimes observed. Bronchiolar and alveolar duct epithelium was usually markedly altered and assumed a disorganized appearance with irregular hypertrophic features within cells and in some cases complete denudation of the terminal bronchiolar and alveolar duct epithelial lining cells.

Congestion of alveolar septal capillaries, commonly seen in the acute stages, was often marked and uniform in the irradiated portions of lung tissue, and often was associated with patchy leukocytic infiltrates (Figure 2). Focal alveolar hemorrhages and red cell diapedesis were also noted and confirmed the presence of microvascular damage. Exudation of protein-rich fluid into alveolar spaces as well as edema of alveolar walls was commonly seen in the acute phases of the radiation pneumonitis but often was irregular in distribution. Swelling of endothelial cells and increased thickness and cellularity of alveolar septae also highlighted the acute lesions. Leukocytic exudation was often noted in acute lesions, but accumulations of neutrophils were generally a focal change. More often a few macrophages or lymphocytes were seen in alveolar septae, and accumulations of alveolar macrophages were often seen filling alveolar spaces and injured terminal bronchioles and alveolar ducts (Figure 3). Pavementing of neutrophils along swollen vascular endothelium was also an irregularly distributed but commonly encountered acute event. Partial but irregular atelectasis and small areas of focal emphysema were occasionally noted, particularly in regions in which marked accumulations of cellular debris filled alveoli and dependent small airways.

Another characteristic, though not constant, feature of the acute to subacute stages of radiation pneumonitis was the accumulation of intraalveolar fibrin either free in the alveoli along with a few macrophages or lying against the alveolar wall in the form of a hyaline membrane. In these areas, necrotic alveolar cells were often noted to be adjacent to the fibrin deposits. Such fibrin deposits were seen to extend up into denuded alveolar ducts and terminal bronchioles where the fibrin deposits sometimes became attached to the uncovered basement membrane (Figure 4). Occasionally swollen septal cells appeared to extend into or overlay the fibrin deposits, suggesting organization into the
alveolar wall. In nearly all cases in which early fibrin deposits were seen, exudation of serum proteins and leukocytes could generally be found in nearby areas, suggesting that an active process was operating. In contrast to this situation, however, rather extensive fibrin accumulations were sometimes encountered in areas of lung exhibiting features of chronicity including septal fibrosis. Such a histologic picture raised questions of locally aberrant fibrinolysis. Proliferative epithelial changes and bizarre hypertropy of alveolar cells commonly were encountered adjacent to areas of fibrin exudation and suggested that injury to alveolar walls and lining epithelial cells played a major role in setting the stage for fibrin deposition.

An acute-to-subacute change in alveolar epithelial cells was swelling of the cytoplasm and overall hypertrophy. The relationship to the underlying basement membrane appeared intact at early stages. This alveolar cell change was variable in that sometimes it involved virtually all of the cells in a given area whereas other areas might exhibit only an occasional hypertrophied cell. Increased vacuolation was sometimes noted. From this initial cell swelling, there was a progression of cytopathologic changes in which a variety of bizarre epithelial elements were visible including some extremely large atypical cells (Figure 5). These epithelial cell changes were particularly characteristic of radiation injury. Necrosis and separation of epithelial elements from the underlying basement membrane and sloughing of degenerate cells into the alveolar lumen was also noted. Hyperplastic changes were encountered in the subacute phases of lung injury and included proliferations of vacuolated and swollen cells resembling Type 2 pneumocytes within the injured alveoli (Figure 6). In regions of such Type 2 cell proliferations, similar appearing cells were often noted in the alveolar space, suggesting that the proliferating cells occasionally slough off. Other patterns of hyperplasia were occasionally encountered at later stages in the development of radiation pneumonitis, and the cells became quite bizarre in appearance. The sloughing of epithelial elements and the death of other pulmonary cells led to an accumulation of debris in the alveoli. Occasional areas contained masses of lipid-filled macrophages and cholesterol clefts resembling endogenous lipid granulomas (Figure 7).

Acute changes in larger bronchi were rarely encountered, and larger bronchioles were often less injured than smaller airways in the deep lung parenchyma. These bronchiolar changes depended in large part on the overall severity of the pulmonary radiation injury. In lower airways, partial desquamation was commonly encountered with focal denudation
and exposure of underlying basement membrane. Smaller bronchioles and alveolar ducts often showed pathologic features of comparable severity to those seen in alveolar lining cells. Bizarre bronchiolar epithelial cells were commonly encountered and were markedly swollen and denuded of cilia (Figure 8). Subepithelial leukocytic infiltrates were rarely seen in airways. Pathologic changes were generally not detected in the muscular layers or in the cartilage or supporting connective tissues of larger airways.

Pulmonary lesions observed in animals that died with progressive radiation pneumonitis of somewhat longer duration (75 to 150 days) were variable; however, several major categories of histopathologic change were encountered. A progressive and apparently active inflammatory reaction was noted and was characterized by persisting congestion, edema, and variable leukocytic infiltration with continuing evidence of capillary injury including swelling of endothelial lining cells, and finally, massive intraalveolar accumulation of dense fibrin deposits, macrophages, and the proliferation and bizarre hypertrophy of alveolar lining cells. The maximum inflammatory reaction was generally related to the total dose and the time to death after exposure, but the active process was detectable in virtually all animals. The inflammatory reaction in animals receiving higher doses was more diffuse and more severe when compared to a more focal or irregular distribution of inflammatory change in animals which had somewhat lower radiation dose levels delivered to the lungs. Injury to alveolar duct lining epithelium and bronchiolar cells was prominent. Bronchiolar cells were lost in patches, exposing underlying basement membrane, and more distal terminal bronchiolar epithelial cells commonly exhibited hyperchromasia with focal areas of regenerative hyperplasia. The bronchial epithelium lining the larger airways rarely showed pathologic changes approaching the severity of those seen in the bronchiolar and alveolar duct lining epithelium. Within these larger airways, only mild surface changes were detected.

Lesions in vessels were often marked. The earliest detectable change was edema of vessel walls often accompanied by dilation of perivascular lymphatic channels and occasional periarterial lymphangiectasia. Endothelial swelling and vacuolation was sometimes noted. At higher dose levels, splitting and reduplication of the elastica of muscular vessels was occasionally visible. The most striking vascular change seen was frank vasculitis which resembled immunologically mediated arteritis. These lesions were often segmental and were characterized by necrotizing changes in the vessel wall accompanied by variable amounts of leukocytic infiltration in and around the actively involved lesions (Figure 9). Such
Changes were most commonly seen in small muscular arterioles but occasionally veins and venules demonstrated similar necrotizing and infiltrative lesions.

Conspicuous vascular and interstitial inflammatory reactions developed with time into an extensive dense fibrous accumulation around blood vessels in which a leukocytic infiltrate was commonly detected at the periphery. Such changes were noted around blood vessels, alveolar ducts, and smaller bronchioles. Progressive vascular lesions included endothelial swelling, intimal and medial edema, and intramural infiltration of leukocytes which were both mononuclear and polymorphonuclear in character. Segmental fibrinoid necrosis similar to that seen in the pulmonary arterioles was occasionally detected in the bronchial arteries of some animals, particularly in animals dying soon after exposure to high dose levels. In addition to the fibrin deposits detailed above, thrombi were sometimes seen within small vessels where they partially or completely occluded the lumen. The vascular injury process appeared progressive, and at later stages both medium- and small-sized blood vessels often developed prominent fibrous obliterator intimal thickening. Major pulmonary arterial branches were relatively spared in this process and often failed to exhibit an inflammatory reaction, although in late-occurring deaths, a prominent but irregular intimal sclerosis was sometimes seen.

Most animals surviving 6 months after exposure developed irregular or diffuse pulmonary fibrosis. Fibrous parenchymal changes, in addition to the chronic obliterator vasculitis, were characteristic features in the lungs of animals dying at protracted times after exposure. The pattern of pulmonary fibrosis was not consistent. Generally, two histologic patterns were observed: a) a focal or diffuse interstitial fibrosis with thickening of alveolar septal walls arranged in a patchy manner such as to produce a web-like pattern in the distorted alveolar tissue or b) large and small stellate-shaped areas of fibrous scar tissue with obliteration of the normal alveolar pattern. The latter were usually found in terminal bronchioles and alveolar ducts, where they sometimes enveloped and obliterated the structure (Figure 10). Such obliterator changes were often widely distributed in the deeper portions of the lung parenchyma. The presence of pulmonary fibrosis appeared to increase in quantity, amount, and density with increased survival time after exposure.

Animals that survived for longer periods after exposure often had adenomatous proliferation of alveolar epithelium. The amount of atypical alveolar epithelial proliferation varied considerably from animal to animal. Such proliferative regions were sometimes seen at the margins of areas of pulmonary fibrosis or in alveolar remnants which had become
embedded in fibrous connective tissue scars. Such lesions were also observed as small clusters of proliferative alveolar cells adjacent to a terminal bronchiole or alveolar duct. Squamous metaplastic change filling the alveoli with nodules of cells was an uncommon finding in most animals, and these epithelial proliferations were often not reflected in nor related to similar changes in the alveolar duct. That is, the squamous metaplastic change often appeared to be an isolated reaction peculiar to alveoli.

Discussion
Most of the pathologic changes observed in these dogs are consistent with those previously reported as being induced in the lung by ionizing radiation.\textsuperscript{1,3} The pulmonary vascular lesions—particularly the more chronic, occlusive lesions—appear to be more prominent in these animals than in animals in other studies of experimentally induced radiation pneumonitis. However, similar vascular lesions have been reported in dogs that inhaled \textsuperscript{144}Ce in aluminosilicate particles\textsuperscript{8} as well as in animals and man that received external radiation.\textsuperscript{6,7}

The pulmonary inflammatory response is a complex reaction in which a variety of cells and humoral factors participate. Even in models of well-defined injury,\textsuperscript{20} the morphologic pattern of reaction can change quickly. Direct injury to pulmonary cell populations is marked in radiation pneumonitis and no doubt constitutes the major basis for the developing reactions. The inflammatory sequences in acute pulmonary radiation injury caused by inhaled \textsuperscript{90}Y particles that are detailed here, however, provide morphologic evidence for the participation of various mediation systems in the pathogenesis of the lesions. This is particularly evident when one considers that the inflammatory changes were present in variable degrees in the lungs of dogs dying much later after exposure than the 28 days required to deliver the total infinite dose to lung from inhaled \textsuperscript{90}Y particles. Thus, while direct cellular injury initiates the inflammatory process, the reaction persists well after the dose has been delivered, suggesting that processes other than those associated with continuing radiation-induced cell injury are involved in the maintenance and amplification of the inflammatory process.

Direct endothelial damage is an early effect and evidences itself by the presence of protein-rich edema fluid in alveoli and the subsequent appearance of fibrin deposits. Phillips\textsuperscript{21} and others\textsuperscript{22,23} have previously noted endothelial damage as an initial site of radiation damage. With vascular injury, platelets quickly stick to the damaged endothelium due to the interruption of the endothelial lining and the exposure of vascular basement membrane. In addition to their role in the extrinsic clotting
system, platelets may contain histamine and/or serotonin, which are liberated during clotting and enhance smooth muscle contraction and vascular permeability. However, we have no direct evidence that serotonin or histamine in the concentrations apt to be present in these injured lungs can cause pulmonary arterial changes.

The histopathology of acute pulmonary radiation injury includes many features which suggest participation of the kinin system in the pathogenesis of the lesions. Increased blood kinin and histamine levels occur following irradiation in animals. The literature, however, reveals sparse and conflicting information about the canine kinin system. Exposure of canine plasma to glass neither produced kinins nor resulted in a decrease of prekallikrein or kininogen. Subsequent data, however, suggested that in addition to containing Hageman factor, canine plasma contained a prekallikrein activable by purified bovine Hageman factor and did have kinin-forming ability following contact activation. The kinetics of the canine kallikrein–kinin system appear to differ quantitatively, however, from the human system. The kinins have important biologic properties including the ability to cause increased vascular permeability, dilation of small blood vessels, enhanced sticking of leukocytes to small blood vessels, and the migration of these cells into extravascular spaces. They have a variable effect on pulmonary arterial pressure. Many of these features are readily evident morphologically in acute radiation pneumonitis.

Increased vascular permeability, abundantly evident histologically in acute pulmonary radiation injury, leads to interstitial and alveolar edema. The consequences of this increased vascular permeability depend in part on the nature of the proteins leaking into the alveolus. Some may be reabsorbed and carried back into the general circulation via either the vascular or lymphatic systems. Studies of pulmonary lymphatic flow in dogs after 4000 to 5000 R external thoracic irradiation, however, showed no appreciable alteration in lymph flow either immediately after the completion of a course of radiation or after a delay of several months. Some proteins, like fibrinogen, may have a long residence time, with potential participation in the injury process through conversion to fibrin and binding to surface active lipoproteins. Fibrin deposits and hyaline membrane formation have long been noted to be a hallmark of pulmonary radiation injury. The presence of fibrin deposits calls into play the fibrinolytic system which has diverse biologic outcomes. Its role in radiation pneumonitis has not been clearly delineated, but delivery of 1500 to 2000 rads gamma radiation from a Co source to the right
hemithorax of dogs results in a marked decrease in lung plasminogen activator (PA) as assayed on fibrin plates, suggesting that paralysis of the pulmonary fibrinolytic system may be involved in the persistence of fibrin deposits. Since PA activity (and hence fibrinolysis) is intimately associated with the pulmonary endothelial cells, injury of such cells may relate to the pathogenesis of radiation pneumonitis in at least two ways: a) the injury itself can lead to increased vascular permeability enhancing edema formation and subsequent fibrin deposition, and b) defective fibrinolysis may result from diminished synthesis or release of PA from the injured endothelial cells. Direct evidence for decreased PA content in irradiated vessels has been reported in rats given 1000 rads external irradiation. It is of interest in this regard that stimulated macrophages can secrete PA. Since the macrophage is an ubiquitous infiltrating cell in radiation pneumonitis, PA secretion by such cells may in part counteract the decreased PA content of injured endothelial cells and thus assist in providing or restoring fibrinolytic capacity. In addition, fibrin deposition itself appears to lead to increased output of PA and potentially increased fibrinolysis.

The importance of the fibrinolytic system in pulmonary injury stems from the diverse biologic activities of the fibrin degradation products (FDP) produced through the action of plasmin on fibrinogen-fibrin substrates. The most pronounced biologic effect of FDP is their anticoagulant activity effected through antithrombin activity, but they can also affect platelet functions, cause smooth muscle contraction, enhance the activities of bradykinin, and cause increased capillary permeability directly. Their role in the chemotaxis of granulocytes may similarly be important in the pulmonary inflammatory response. Thus, in addition to their effects on hemostasis, the FDP may play an amplifying role in the inflammatory lesions of radiation pneumonitis.

Injury of cells on both the endothelial and epithelial sides of the alveolar wall is prominent in radiation pneumonitis and creates a situation in which serum proteins obtain access to denuded or exposed vascular and subepithelial basement membranes. The opportunity for activation of Hageman factor on these insoluble collagenous substrates thus exists. The biologic consequences of Hageman factor activation are diverse. They include initiation of the intrinsic clotting system through activation of plasma thromboplastin antecedent, kinin release from plasma precursors, activation of the complement system through C'1 esterase and initiation of the fibrinolytic pathways via plasminogen activator which converts plasminogen to plasmin. Plasmin itself activates both the kinin system and
the early-reacting components of the complement system and can inactivate C1 esterase inhibitor. These reactions can collectively enhance the pulmonary inflammatory response caused by radiation injury.

While activation of the complement system is most dramatic in immunologically mediated reactions, its role in the mediation of other nonspecific inflammatory reactions is clear. Decreased serum complement levels and complement depletion have occurred in radiation pneumonitis and complement has been immunohistologically demonstrated in the lesions. Complement is operative in normal blood coagulation since an intact complement system is essential for normal clotting. In addition, complement activation occurs when the clotting system is triggered and platelets are involved in the interaction between the coagulation and complement systems. By releasing histamine from mast cells, complement causes increased capillary permeability, edema, and contraction of smooth muscle. In histamine-independent reactions it causes directed migration of leukocytes and the opsonization of particles or membranes, and it produces a change in platelet properties, resulting in the appearance of platelet coagulation activity. The activated platelets then set in motion the coagulation system. All of these phenomena can collectively amplify the radiation-induced inflammatory reaction and lead to cellular injury and destruction as well as the accumulation of cellular debris, edema fluid, and leukocytes in injured tissues. Morphologic evidence for the participation of a complement-related system in radiation pneumonitis derives from the presence of histopathologic markers such as increased vascular permeability, blood coagulation, and leukocyte migration into the lesions. It may be further involved in the actual cytolysis of certain altered pulmonary cell elements and probably participates in the development of the striking vasculitis sometimes encountered in lungs injured by radiation.

The pulmonary surfactant, vital to the maintenance of normal alveolar fluid–gas dynamics, may also participate in the radiation-induced pulmonary injury process through its interaction with key serum proteins. Surface active lipoprotein (SAL) greatly inhibits activation of the fibrinolytic system, even when the proportion of SAL to fibrinogen is equimolar. In addition, fibrinogen binds to SAL, reducing the latter's surface active properties. This can increase the amount of alveolar proteinaceous debris and may contribute to the local atelectasis sometimes seen in acute pulmonary radiation injury. Of further interest relative to surfactant is the finding of an α-1-globulin trypsin inhibitor in canine surfactant protein which strongly resembles canine serum α-1-
antitrypsin (A1AT). Since A1AT is a plasmin inhibitor, further interaction between the surfactant system and fibrinolysis may be indicated.

In addition to injury on the vascular side of the alveolar membrane, with its many pathogenetic overtones, direct injury to the pulmonary epithelial elements is a prominent histopathologic finding in radiation pneumonitis. Direct cell killing not only exposes subepithelial basement membranes but contributes to the alveolar debris. Desquamation of injured epithelial cells leads to repopulation of the lining cells by atypical cellular elements as well as focal hyperplastic changes. The continuing injury created by inhaled internal emitters tends to make this a prolonged process, and the buildup of cellular debris, proteins, and lipids attracts scavenger cells such as neutrophils and macrophages which further contribute to the alveolar debris. The chemotactic properties of such membrane components and necrotic debris are well documented. Repair mechanisms occurring as part of the inflammatory response and attempts to organize the alveolar debris and fibrin deposits can enhance the development of interstitial fibrosis and other chronic sequelae.

In addition to abundant evidence of direct cellular injury, the inflammatory sequences visible in acute pulmonary radiation injury would

**Text-figure 2**—Possible pathogenetic pathways in experimental acute pulmonary radiation injury. Direct cellular injury on both sides of the alveolar membrane can lead to diverse activation of closely integrated mediation systems which collectively amplify the inflammatory reaction.
seem to suggest diverse secondary activation of many cellular and humoral mediation systems in their pathogenesis (Text-figure 2). The direct effects of radiation on many of these cells and systems are poorly understood. The neutrophil appears to be highly radioresistant while the alveolar macrophage exhibits some functional decrement following inhalation of certain alpha-emitters. The role of the mast cell in the development of radiation-induced septal fibrosis warrants further close attention. In addition, we have observed a transient lymphopenia in dogs after 90Y inhalation although this does not result in depressed peripheral lymphocyte responses to mitogens. Additional studies are required to delineate the relative contributions of these various cells and mediation systems as well as direct cellular injury in the pathogenesis of radiation pneumonitis. The relationship of these relatively early events in the pathogenesis of pulmonary radiation injury to late-occurring sequelae such as pulmonary fibrosis and lung cancer remains an area of considerable importance, particularly when the prolonged injury produced by inhaled internal emitters is taken into consideration.

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Figure 1—Radiation pneumonitis in a dog dying 205 days after inhalation of $^{90}$Y in alumino-silicate particles with a cumulative pulmonary radiation dose of 9300 rads. Histologic examination confirmed the presence of active inflammation as well as patchy pulmonary fibrosis.

Figure 2—Acute alveolar injury in a dog with radiation pneumonitis 23 days after exposure showing septal congestion and patchy leukocytic infiltrates into alveolar spaces surrounding an alveolar duct. Cumulative radiation dose to lung was 23,000 rads. (H&E, × 240)
Figure 3—Prominent infiltrations of alveolar macrophages into an injured alveolar area which has been largely denuded of epithelium. There is wispy thickening of the interalveolar septae. The animal died 75 days after exposure with a cumulative radiation dose to lung of 20,000 rads. (H&E, × 350)

Figure 4—Denudation of bronchiolar epithelium and lining of the injured bronchiole with fibrin deposits (arrow) in a dog dying of radiation pneumonitis 92 days after exposure with a cumulative radiation dose to lung of 17,000 rads. Fibrin deposits and thickening of interalveolar septae are apparent in adjacent alveoli. (H&E, × 150)
Figure 5—Marked alveolar cytopathology with swelling and atypia of alveolar cells in a dog with radiation pneumonitis that died 117 days after exposure with a cumulative radiation dose to lung of 12,000 rads. Note the swollen and disorganized interalveolar septae. (H&E, × 400) Figure 6—Diffuse alveolar injury in a dog with radiation pneumonitis at 121 days after exposure showing thickened interalveolar septae and proliferation of swollen alveolar lining cells resembling Type 2 pneumocytes (a) and sloughing of similar appearing cells into alveolar spaces (b). Cumulative radiation dose to lung was 11,000 rads. (H&E, × 420)
Figure 7—Cholesterol cleft formation and cellular accumulation in a region of scarring in a dog dying of radiation pneumonitis 214 days after exposure. The cumulative radiation dose to lung was 11,000 rads. (H&E, × 150)

Figure 8—Bronchiolar injury in a dog dying of radiation pneumonitis at 108 days after exposure with a cumulative radiation dose to lung of 15,000 rads. There is loss of cilia, swelling, and cytologic atypia. (H&E, × 320)
Figure 9—Segmental vasculitis in a dog dying of radiation pneumonitis at 38 days after exposure. There is marked leukocytic infiltration into the vessel wall as well as vascular edema and periarteritis. Cumulative radiation dose to lung 27,000 rads. (H&E, × 250)

Figure 10—Pulmonary fibrosis occurring around a denuded terminal airway. There is active inflammation in the surrounding parenchyma as well as persisting alveolar fibrin deposits. Dog died 143 days after exposure with a cumulative radiation dose to lung of 12,000 rads. (H&E, × 150)