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LONG TERM EFFECTS OF RADIATION AND COMBINED MODALITIES ON MOUSE LUNG

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Abstract

The lung appears to be the major dose-limiting organ in radiation of the thorax. Early responses (<1 week) involve the type II pneumocyte and increased surfactant biosynthesis and secretion. Later changes, which appear to be related to the surfactant response, lead to classical radiation pneumonitis, which is often fatal. Animals which survive radiation pneumonitis develop progressive fibrosis, a late-appearing response, which reduces compliance and available air space, and is usually fatal. This study centers on the fine structural changes in the lungs of LAF_1 mice, 63 weeks following various radiation exposures (5-13 Gy). Doses which are subthreshold in evoking surfactant and pneumonitic responses precipitate fibrosis and atelectasis by 63 weeks, and involve type II pneumocyte sloughing and degeneration. Of the two major deterrents to lung irradiation (pneumonitis and fibrosis), these results suggest that fibrosis always follows pneumonitis, but pneumonitis is not a necessary preliminary step to fibrosis. Bleomycin elicits several morphological alterations characteristic of radiation, and, when combined with the latter, appears to exacerbate radiation effects.

Key words: Radiation, Bleomycin, Combined Modalities, Lung, Pneumonitis, Fibrosis, Mouse

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Introduction

It is well understood that irradiation can evoke deleterious effects on normal tissues, which become dose-limiting in the treatment of neoplasia by radiation. A site of considerable concern to the radiation therapist is the lung, especially the more distal respiratory regions. Previous studies on the effects of radiation on lung tissue have indicated early (pneumonitic), intermediate and late (fibrotic) responses (Rubin and Casarett, 1968; Travis, 1980a). To date many investigations in animal models have concentrated on the radiation pneumonitis phase. For mouse studies, a commonly used parameter, the $LD_{50/180}$ (dose lethal to 50% of the animals by 180 days) assay, reflects lung damage during the acute radiation pneumonitis phase (Phillips and Margolis, 1972; Field and Hornsey, 1977; Travis, 1980a; Siemann et al., 1982). Studies evaluating lung lavage surfactant levels soon after irradiation suggest an apparent relationship between the acute radiation pneumonitis syndrome and type II cell surfactant release (Rubin et al., 1980; Rubin et al., 1983). These and other studies (Travis et al., 1977; Travis, 1980b; Gross, 1978; Penney and Rubin, 1977; Penney et al., 1980, 1981, and 1982a and b) suggest that type II pneumocytes play an important role in the early phase of the radiation response by the lung. This acute phase of radiation-induced pulmonary toxicity may progress to irreversible fibrosis (Rubin and Casarett, 1968; Law et al., 1976; Travis, 1980a). Alternatively under certain conditions the radiation pneumonitis phase may be bypassed; yet, even under these circumstances, lung damage can occur (Travis, 1980a; Travis et al., 1980; Penney et al., 1982b; Siemann et al., 1982). It is the latent fibrosis, apparently irreversible, which may ultimately be radiation dose-limiting for this organ. Consequently, the present investigation, an extension of previous short-term studies (Penney et al., 1981, 1982 a and b; Siemann et al., 1982), was directed in particular toward evaluating in detail the ultrastructural and morphometric changes associated with the fibrotic lung.

Discovered in 1966, bleomycin (Blenoxane; BLM) has been effective in the treatment of various malignant diseases, such as lymphoma and testicular and squamous cell carcinomas. Its advantage in circumventing neurotoxicity, a particularly useful characteristic in multi-drug regimens, is offset by its pulmonary toxicity, which becomes the dose-limiting factor (Comis, 1978). BLM-induced pulmonary fibrosis, type I pneumocyte destruction and squamous metaplasia have been studied extensively in animals and man (Adamson and Bowden, 1974, 1977, 1979; Jones and Reeve, 1978; Luna et al., 1972; Bedrossian et al., 1973; Sikic et al., 1978a and b; Aso et al., 1976; Burger et al., 1981; Samuels

et al., 1976; Raisfeld, 1979; Moseley et al., 1984; Thrall et al., 1979; Daskal and Gyorkey 1978; Comis, 1978; Catane et al., 1979). In more recent studies (van Houtte, personal communication), bleomycin has been shown to evoke a significant dose response increase in surfactant secretion by day 6 post-administration as measured by phophatidylcholine (PC) concentrations in bronchoalveolar lavages. Correspondingly, unlike radiation, there was a progressive increase in lung tissue PC, indicating that surfactant synthesis was probably stimulated. Van Houtte also noted a slight, but progressive dose:response increase in hydroxyproline content of lung tissue. The studies of van Houtte et al. concentrated on early events, and the purpose of this report is to focus on more latent changes.

Since a) BLM evokes several short term (<4 weeks) biochemical alterations in the lung which appear to replicate the radiation response (van Houtte, personal communication) and b) multimodality therapy is often the preferred treatment regimen, the potential latent additive or synergistic effects of BLM and radiation have become increasingly important. Catane et al. (1979), Jorgensen (1972) and Collis et al. (1983) have described the synergistic or additive effects of BLM and radiation, especially when both were administered together or within a short time interval. The present study addresses the responses when the time interval is relatively long (in the life of a mouse) to determine possible additive late effects. Prior to this report, no long-term animal studies of combined effects have extended beyond 30 weeks. Toward that end, the latent effects of combined modalities on the lung are described.

Materials and Methods

Animals: In concert with other ongoing and previous studies, the male LAF_1 mouse strain was used for all experiments. Two to ten animals were included in each group, the lower numbers being the BLM long term studies for 109 weeks.

Radiation: Radiation treatment conditions employed have been described in detail elsewhere (Siemann et al., 1982). Briefly, the thoraces of unanesthetized mice were locally irradiated using a ^{137}Cs irradiator (Siemann and Kochanski, 1981). The dosage rate was 4.13 Gy/min, and single total exposures of 5, 9 or 13 Gy were administered. Sham-irradiated animals served as the time-matched control group. Animals were sacrificed 63 weeks post-irradiation (PI).

Bleomycin Administration: Bleomycin was administered intravenously into the tail veins of mice at concentrations of 10, 30, 60, 100 or 150 mg/kg body weight in 0.9% sterile saline. Concentrations of the chemotherapeutic agent were prepared so that the volume (0.25 ml) of the final injection into the mice was constant. An equal volume of the vehicle was administered for the control groups. Animals were sacrificed 30 or 109 weeks following injection.

Bleomycin and Radiation: To determine whether or not pretreatment with BLM would significantly alter radiation-induced changes, other groups of animals were administered BLM (150 mg/kg) via tail vein and 34 weeks later were irradiated at doses of either 10 or 20 Gy. These two radiation doses were selected since earlier studies have shown that the doses were insufficient and adequate, respectively, to evoke surfactant secretion and pneumonitis. The interval of 34 weeks between BLM administration and radiation exposure was chosen because some investigators (Catane et al., 1979; Jones and Reeve, 1978; Iacovino et al., 1976) have postulated that BLM-induced pulmonary toxicity may be somewhat subthreshold in evoking clinical symptoms, but remains latent, becoming expressed when lungs are subsequently irradiated. At 19 weeks PI (53 weeks following BLM administration) the animals were sacrificed. The period of 19 weeks PI was selected to correlate with earlier studies in which fine structural changes associated with radiation pneumonitis were present (Penney et al., 1981 and 1982b).

Morphology: Mice were killed by cervical dislocation. Tissue specimens were immediately taken from right lungs and immersed in 2% glutaraldehyde fixative where mm^3 blocks were dissected and processed as previously reported (Penney and Rubin, 1977; Penney et al., 1981 and 1982b). Thick sections (0.5–1.0 μm) 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 electron microscope. For routine histologic observations, larger specimens were processed for embedment using the standard JB-4 plastic procedure. For scanning electron microscoy, specimens were fixed in 2% glutaraldehyde overnight, rinsed in 2–3 changes of 0.1 M phosphate buffer (pH 7.2), dehydrated in increasing concentrations of ethanol, and critically point dried using CO_2 as the transition fluid. Specimens were then sputter-coated with gold and observed and photographed in a JEOL JSM-35CF scanning electron microscope.

Stereology: Measurements of the relative percentages of area available for gaseous exchange per area of lung were made on a Zeiss Videoplan Image Analyzer, using thick (1 μm) plastic-embedded sections. In measuring the air spaces available for gaseous exchange, major blood vessels and air passages above the level of terminal bronchioles were deleted, and therefore account for the remaining percentages of the total lung area.

Results

Radiation

Survival Rates: One of the criteria by which radiation damage is measured is animal survival. In these studies percentages of animals which survived various radiation exposures were determined (Fig. 1). A minimum of 70 animals was included in each group. By 63 weeks PI, animal survival was essentially unaltered following exposures of 9 Gy or less. However, doses >9 Gy elicit a significant dose-related decrement in survival by this time. At exposures of 15 Gy or greater the survival rate is 0% (Siemann et al., 1982; Penney et al., 1982b).

Areas Available for Gaseous Exchange: Figure 2 clearly shows decrements in available airspace by 63 weeks PI at all exposures. Stereologic data for 9 through 15 Gy have been previously reported (Penney et al., 1982b), but are included here for the purpose of comparing latent effects of 5 Gy exposures, which were initially thought to be sufficiently low to circumvent a latent fibrotic response. Although the severity of airspace loss appears to be dose-related, exposures as low as 5 Gy can affect available airspace and therefore lung function, albeit insufficient to evoke morbidity (as assessed by breathing rates, not reported here) or mortality by 63 weeks PI. These results confirm the hypothesis that all radiation inflicts lung injury (Gross, 1978).

Histology: Compared to non-irradiated animals, the histologic appearance of lungs recovered from mice 63 weeks following exposure to 5, 9 or 13 Gy was one of a dose-related thickening

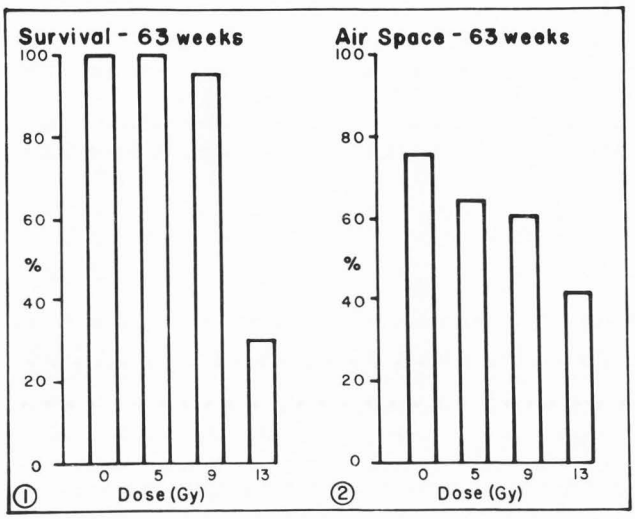


Fig. 1. Animal survival percentages 63 weeks post-irradiation (PI).

Fig. 2. Stereologic measurement of airspace surface available for gaseous exchange expressed in percentage of total lung area.

of septal walls, reduction in alveolar areas for gaseous exchange, and fusion of alveoli.

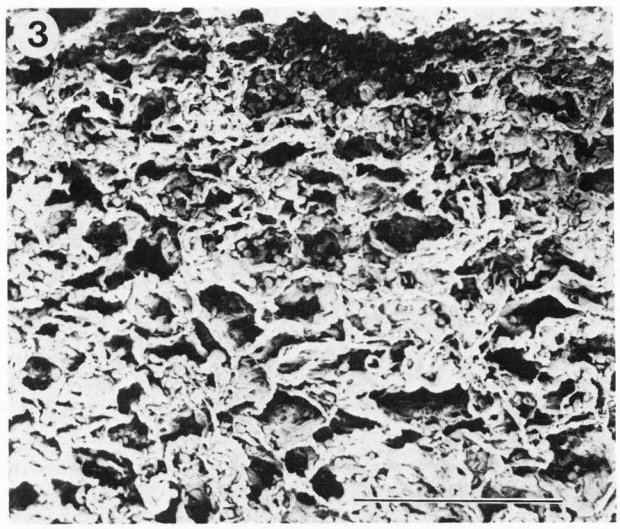
Scanning Electron Microscopy: Radiation-induced alterations in the architecture of the distal lung are rather dramatic when observed with scanning electron microscopy. Sham-irradiated mice at 63 weeks do not exhibit significant morphologic change when compared to younger animals. The alveoli are prominent with abundant microvascularization (Fig. 3).When compared to lungs of age-matched mice following 5 Gy (Fig. 4) or 13 Gy (Fig. 5) exposures, there is a) a progressive increase in the thickness of septal walls, b) an increased atelectasis, particularly in subpleural regions, and c) an increased size of alveoli. The latter loss in septation probably arises from the coalescence of numerous distal smaller alveoli, which would reduce, particularly following larger radiation exposures, the available surfaces for air exchange. These results further confirm our morphometric, histologic and fine structural data.

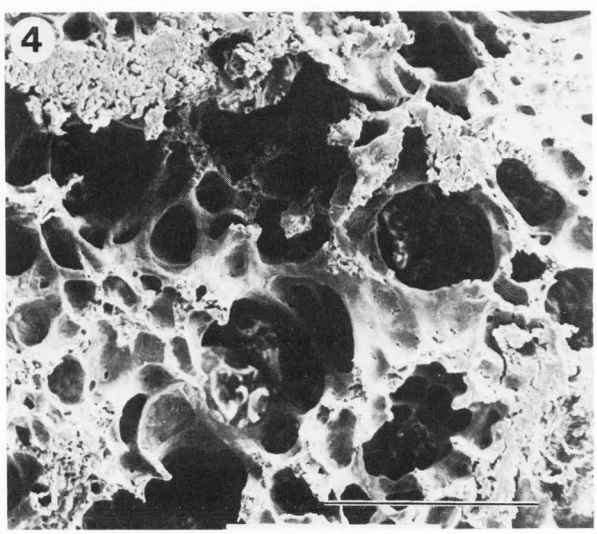
Fig. 3–5: Scanning electron micrographs of lungs recovered 63 weeks PI.

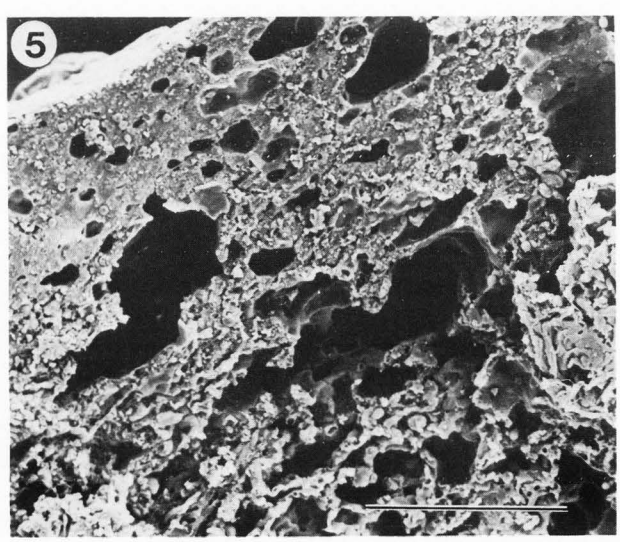
Fig. 3. O Gy (sham-irradiated) exposure. The pleural lining can be noted in the top right. Abundant alveoli and capillaries are present. Bar = 100 μm.

Fig. 4. 5 Gy exposure. Some thickening of the septal walls and coalesced alveoli can be seen. Pleural surface is at upper left. Bar = 100 μm.

Fig. 5. 13 Gy exposure. Considerable septal thickening, loss of alveoli and alveolar coalescence are present. Bar = 100 μm.







Transmission Electron Microscopy: In keeping with the histologic studies, the fine structural morphology of lungs of sham-irradiated animals sacrificed at 63 weeks was essentially normal, confirming morphometric data of available air space. Types I and II pneumocytes, vascular endothelial cells, alveolar macrophages and septal cells all appeared normal (Fig. 6) and almost indistinguishable from those cells in lungs of younger animals.

Radiation-induced fibrosis was present in all irradiated lungs, being more prominent and more compactly organized following the larger exposures (Figs. 7 and 8). From time to time, collagen was present within air spaces (Figs. 8 and 9), and appeared to occur via discontinuities or separations of type I lining pneumocytes and basal laminae.

Type II pneumocytes also exhibited rather dramatic latent modifications. Surfactant-containing lamellar bodies frequently contained atypical small clusters of membranes, quite different from the normal orientation and from multivesicular bodies from which lamellar bodies are thought to arise. These changes are similar to those to be described later following bleomycin administration without or with radiation. In addition, lamellar bodies frequently appeared to coalesce to form huge membrane-containing bodies, sufficiently large to displace the nucleus (Fig. 10). Evidence of degradation, disintegration, and sloughing of type II pneumocytes was frequently encountered, similar to that observed when BLM and radiation are combined as shown later. The alveolar wall never appeared to be denuded when type II cells were sloughed or shed in this manner, but was covered by type I cells.

Occasionally the basal laminae beneath the alveolar epithelial linings were considerably thickened in irradiated lungs (Figs. 7 and 8). To what extent this phenomenon might alter gaseous exchange and/or permeability has not yet been determined, although other studies have demonstrated alterations in the quantity and composition of the basal laminar proteoglycans which could influence permeability (Penney and Rosenkrans, 1984; Rosenkrans and Penney, 1985).

Some endothelial cells exhibited focal pinocytic and coated vesicles and almost all possessed numerous microvillous projections, but lacked any indication of discontinuity or disintegration. Endothelial blebbing was also present.

Bleomycin

Although no deaths occurred in animals given BLM, lungs from animals sacrificed 30 weeks following 10, 30, 60, 100 or 150 mg/kg BLM exhibited dose-related changes, including increased septal collagen, occasional extravasation of erythrocytes into alveoli, disintegration and sloughing of type II pneumocytes and invasion of fibroblasts and macrophages into the septal wall. The basal laminae also appeared to be focally thickened (Fig. 11). By 30 weeks following BLM administration, the populations of intracellular lamellar bodies in type II pneumocytes had been restored following earlier depletion. No significant or atypical damage to endothelial cells or type I pneumocytes was observed. Endothelial projections and blebbing, so prominent at this time in irradiated lungs, were also present, but less common in BLM-treated animals.

Tissues from mice recovered 109 weeks following BLM administration were more fibrotic than those recovered 30 weeks following equal doses of the drug BLM (Figs. 12 and 13). No significant long term damage to type I pneumocytes or endothelial cells was apparent. Coalescence of small alveoli to create larger airspaces was more common in the long term specimens.

Bleomycin and Radiation

Lungs irradiated subsequent to BLM administration evoked no animal mortality by 19 weeks PI, and presented several characteristics which have been associated with a radiation response, such as type II pneumocyte disintegration and sloughing, endothelial blebbing and filipodia, increased septal collagen, thickened basal laminae, extravasation of erythrocytes into alveolar spaces, and increased amounts of intravascular erythrocytes and thrombocytes (Figs. 14–16). Although some type II cells appeared normal, others exhibited characteristics of impaired lamellar body formation (Fig. 14) which has been shown to be radiation-induced (Penney et al., 1982b). Alveolar macrophages are present in alveoli and septal walls. The degree to which fibrosis is developed at 19 weeks PI is considerably greater in BLM-treated animals than in animals exposed to radiation alone (Penney et al., 1981), suggesting potentiation of BLM-induced injury when followed by radiation in the induction of pulmonary fibrosis, a phenomenon also reported for bleomycin therapy following radiation (Catane et al., 1979; Samuels et al., 1976).

Discussion

Radiation-induced damage to normal lung tissue is commonly acknowledged, although ultrastructural support for the development of very late effects of radiation, particularly fibrosis, has been sparse. Prior studies a) established that structural and functional changes of type II pneumocytes were among the earliest detectable effects of radiation exposure to the lung, and b) described fine structural and morphometric changes during the

Fig. 6–10: Transmission electron micrographs of lungs recovered 63 weeks PI. →

Fig. 6. O Gy. Type II pneumocyte (right center) containing prominent lamellar bodies, an alveolar macrophage (left center), type I pneumocytes (arrowheads), and capillary (asterisk) can be observed. Bar = 5 μm.

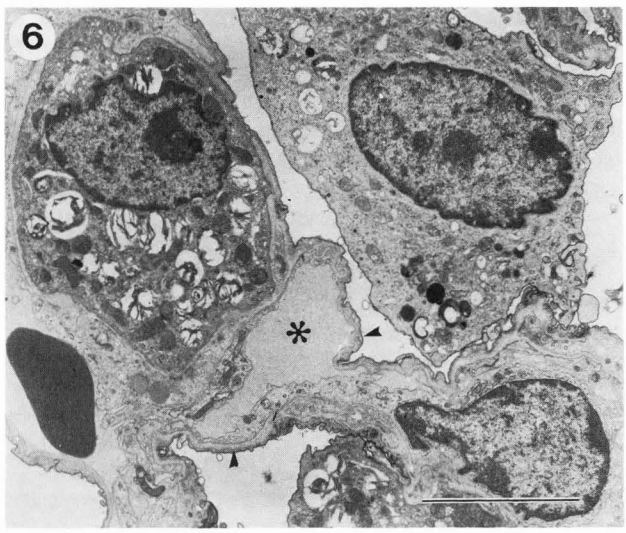
Fig. 7. 5 Gy. Increased thickening and collagen and elastin content of septal walls, with capillaries containing higher numbers of erythrocytes and thrombocytes and focal thickening of the basal laminae (arrows) can be noted. Bar = 5 μm.

Fig. 8. 13 Gy. Considerable compact collagen bundles in septal walls, and occasionally (arrows) within alveoli, usually at points of discontinuity of type I pneumocytes. Bar = 10 μm.

Fig. 9. 13 Gy. Extensive collagenous infiltration of an alveolus and two alveolar macrophages can be observed in the region. Bar = 5 μm.

Fig. 10. 13 Gy. Often aberrant lamellar body formation results in huge structures which can displace the nucleus. Normal appearing lamellar bodies appear within the cytoplasm of the same cell. Bar = 5 μm.

Fig. 11. Bleomycin (100 mg/kg), 30 weeks post-administration. Increased collagenous deposition within septal walls, with a widening of the basal laminae (arrows) can be noted. Bar = 5 μm.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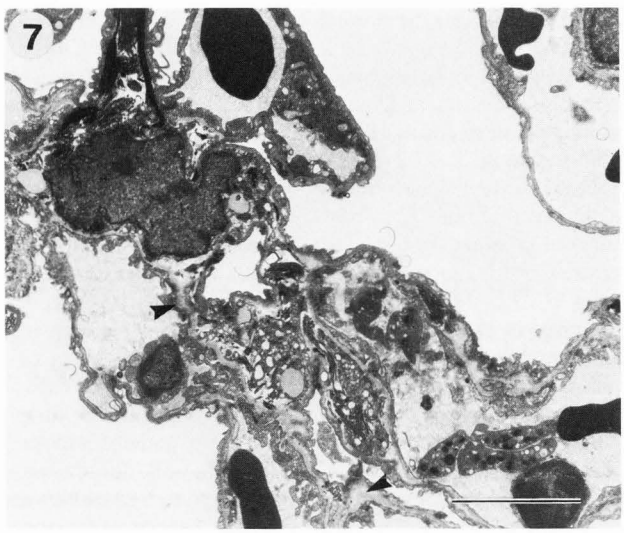
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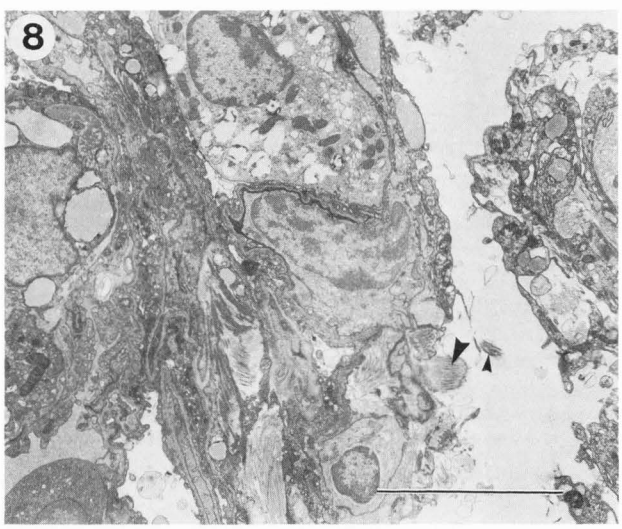
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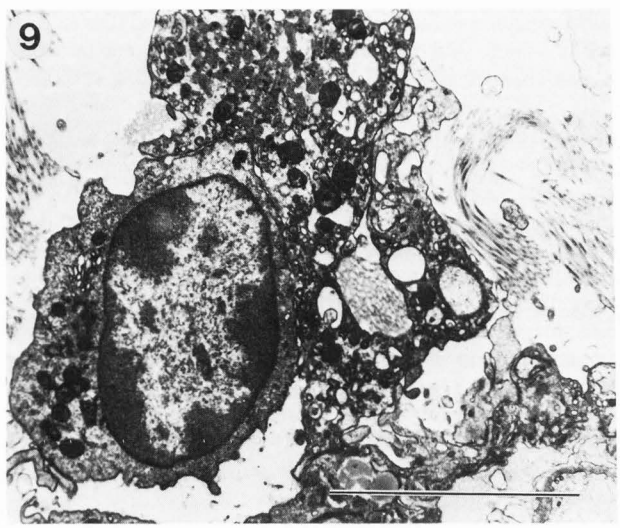
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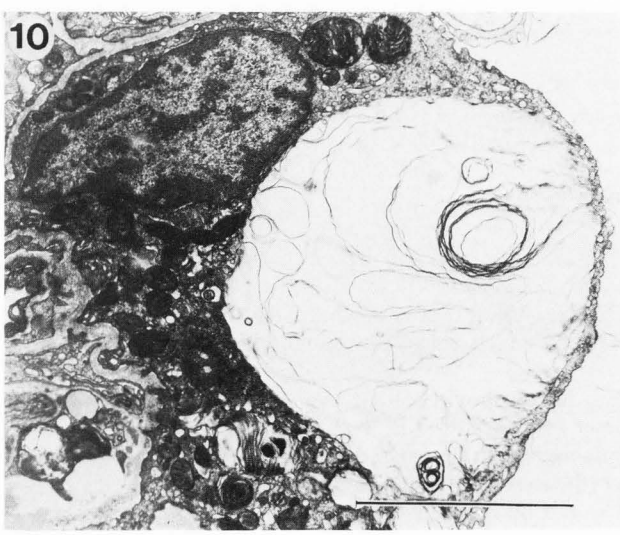
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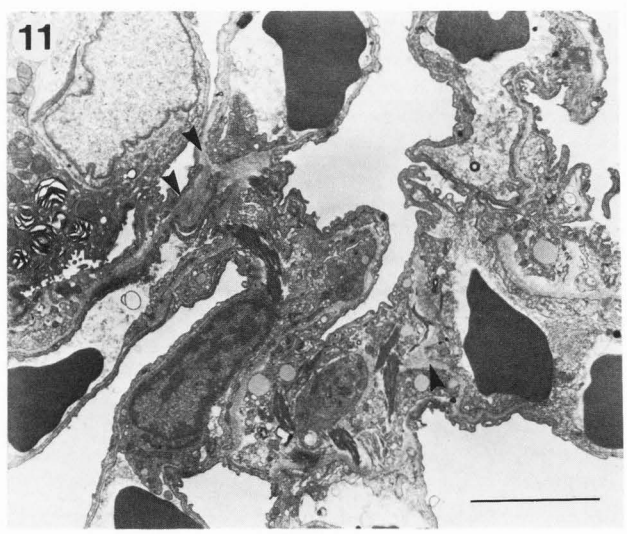
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pneumonitic stage (Penney and Rubin, 1977; Rubin et al., 1980 and 1983; Penney et al., 1981; Shapiro et al., 1982). Current studies extend those investigations to a description of the late effects of radiation, and couple those studies with descriptions of the long-term effects of bleomycin alone and in combination with radiation.

By 63 weeks PI, late effects of radiation are clearly apparent at the doses administered. Based on these and previous studies, survival rates for irradiated mice following 13 Gy exposure decrease sharply, although, in our hands, those deaths do not occur prior to 22 weeks PI. Although animal survival is not compromised at exposures <9 Gy, the loss of available airspace and septal wall thickening suggest latent damage is being expressed, but insufficient to evoke morbidity or mortality. Whether or not the mortality curve for 13 Gy is replicated at times >63 weeks PI for exposures below 13 Gy remains undetermined. Mice also survived bleomycin quite well, despite morphologic changes. Survival of mice exposed to BLM followed by radiation 34 weeks later indicated that despite additive lung injury, mortality was not accelerated, at least by 19 weeks PI.

Clearly these studies confirm our earlier view that the two major radiation-induced sequelae, pneumonitis and fibrosis, can be uncoupled. Although fibrosis always follows pneumonitis, it may also occur in the absence of a pneumonitic response. Gross (1978) has postulated that all radiation exposures evoke radiation damage, the extent of which is dose-dependent. Based on these and other studies (Penney and Rosenkrans, 1984; Rosenkrans and Penney, 1985; and unpublished results), edema, changes in both the alveolar and capillary basal laminae, loss of surfaces for gaseous exchange, and increases in pinocytic and coated vesicles are demonstrable for exposures at and below 5 Gy. These changes, although insufficient to produce pneumonitis, may affect permeability and/or other factors to the extent that latent fibrogenesis is triggered. As would be predicted, the response appears to be dose-related in its severity. Exposures >12 Gy evoke a surfactant release which appears to elicit the histologically-evident pneumonitic response.

The fibrogenic capacity of bleomycin in the lung has been widely acknowledged. Contrary to the reports of several investigators (Adamson, 1984, and Bowden, 1984, for reviews), our studies did not reveal damage to type I epithelial or endothelial cells. Inasmuch as our studies were of significantly longer duration than have been reported heretofore, perhaps such damge, if evoked, had been repaired.

The molecular and ionic changes evoked by BLM have been described by other investigators (Wesselius et al., 1984; Raisfeld, 1979). It would appear that a cascade of changes may be evoked, as in radiation (Ts'ao et al., 1983), which may be somewhat general in nature, but sufficient to induce or initiate a fibrotic response. The precise role(s) of macrophage destruction (Penney et al., 1982b), prostacyclin synthesis (Ts'ao et al., 1983) and granulocyte chemotaxis (Wesselius et al., 1984) following radiation and/or BLM remain unclear.

The synergistic evocation of pulmonary damage and tumor reduction in non-pulmonary sites when radiation is coupled with bleomycin, administered either together or within short intervals, is well supported (Collis et al., 1983, Iacovino et al., 1976; Jorgensen, 1972; van Houtte et al., personal communication). The present studies clearly demonstrate that the effects of the two agents can be additive even when their administration is separated by relatively long intervals. These results suggest a remembered "sensitization" of the tissues which is unleashed when a second fibrogenic inducer is administered. The clinical significance of these findings strongly suggests additional caution in the sequencing of therapeutic regimens involving combined modalities.

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References

Adamson IYR. (1984). Drug-induced pulmonary fibrosis. Environ. Health Persp. **55**: 25–36.

Adamson IYR, Bowden DH. (1974). The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. Amer. J. Path. **77**: 185–197.

Adamson IYR, Bowden DH. (1977). Origin of ciliated alveolar epithelial cells in bleomycin-induced lung injury. Amer. J. Path. **87**: 571–579.

Adamson IYR, Bowden DH. (1979). Bleomycin-induced injury and metaplasia of alveolar type II cells. Amer. J. Path. **96**: 531–543.

Fig. 12. Bleomycin (30 mg/kg), 109 weeks post-administration. Increased collagen and elastin in the septal walls are noted, along with endothelial projections. No damage to type I or endothelial cells was noted. Bar = 5 μm.

Fig. 13. Bleomycin (150 mg/kg), 109 weeks post-administration. Intact endothelial and alveolar lining cells were observed. The thickened septal walls exhibit numerous bundles of collagen and infiltrative cells, primarily fibroblasts and septal macrophages. A normal-appearing type II pneumocyte is in the center of the field. Bar = 10 μm.

Fig. 14. Bleomycin (150 mg/kg), irradiated at 10 Gy 34 weeks later, and sacrificed 19 weeks PI. Atypical lamellar body formation consisting of vesicular, rather than lamellar configurations. Septal wall fibrosis with some edematous swelling. Bar = 5 μm.

Fig. 15. Bleomycin (150 mg/kg), irradiated at 10 Gy 34 weeks later, and sacrificed 19 weeks PI. Atypical lamellar body formation in some type II pneumocytes, interstitial edema, and alveolar macrophage can be noted. Bar = 10 μm.

Fig. 16. Bleomycin (150 mg/kg), irradiated at 20 Gy 34 weeks later, and sacrificed 19 weeks PI. Type II pneumocyte desquamation, extensive endothelial microvillous projections, erythrocyte infiltration of alveoli, and a septal macrophage (X) can be noted. Bar = 10 μm.

Aso Y, Yoneda K, Kikkawa Y. (1976). Morphologic and biochemical study of pulmonary changes induced by bleomycin in mice. Lab. Invest. **35**: 558–567.

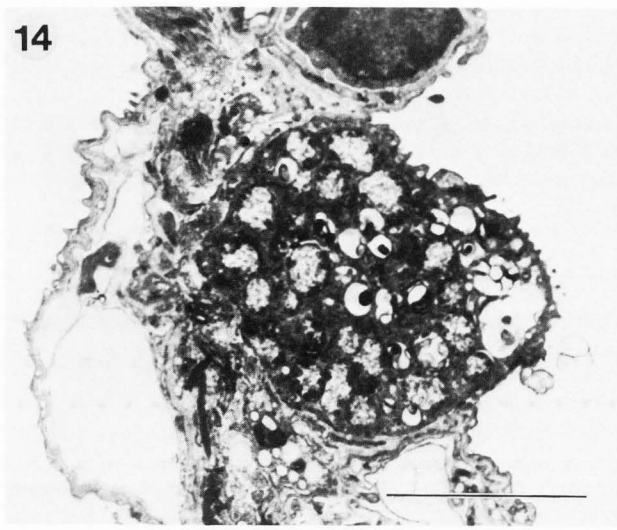
Bedrossian CWM, Luna MA, Mackay B, Lichtiger B. (1973). Ultrastructure of pulmonary bleomycin toxicity. Cancer **32**: 44–51.

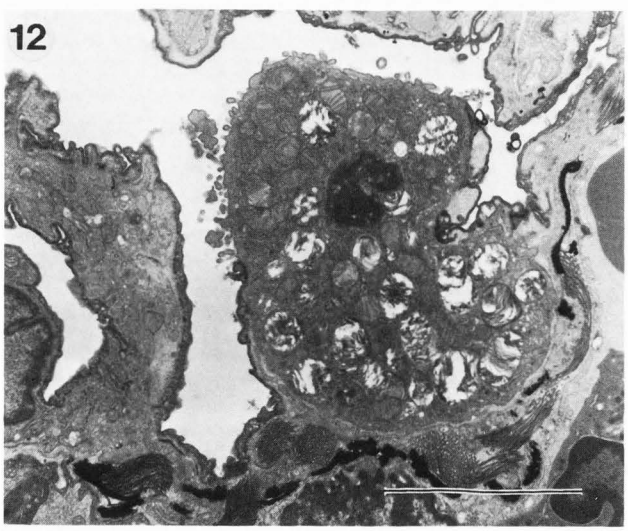
Bowden DH. (1984). Unraveling pulmonary fibrosis: The bleomycin model. Lab. Invest. **50**: 487–488.

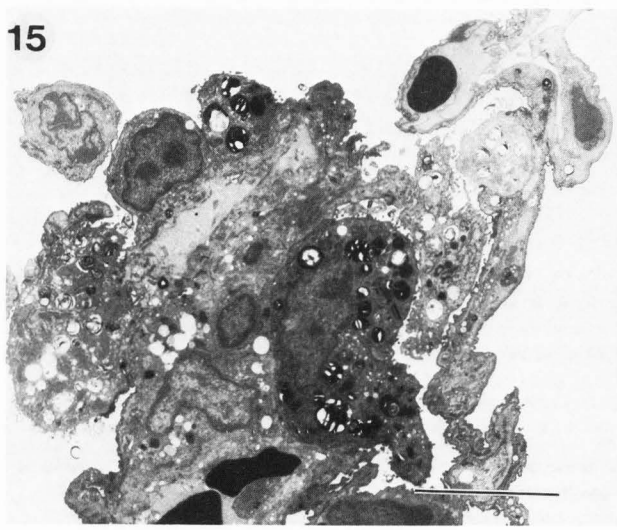
Burger RM, Peisach J, Horwitz SB. (1981). Mechanism of bleomycin action: *In vitro* studies. Life Sci. **28**: 715–727.

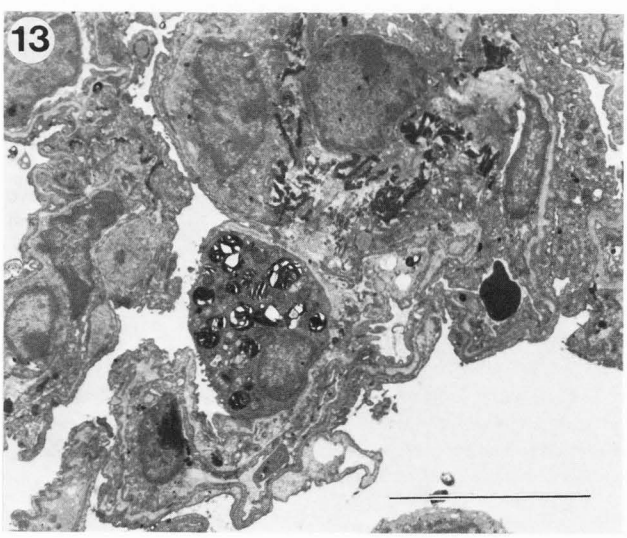
Catane R, Schwade JG, Turrisi AT, Webber BL, Muggia FM. (1979). Pulmonary toxicity after radiation and bleomycin: A review. Intern. J. Radiat. Oncol. Biol. Med. **5**: 1513–1518.

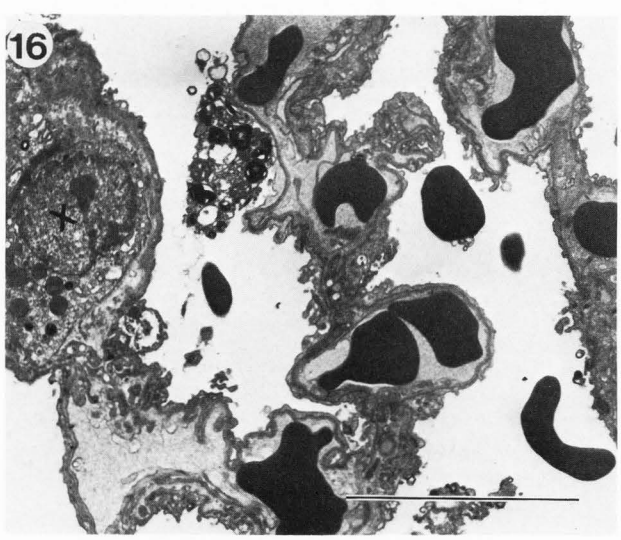
Collis CH, Down JE, Pearson AE, Steel GG. (1983). Bleomycin and radiation-induced lung damage in mice. Brit. J. Radiol. **56**: 21–26.











Comis RL. (1978). Bleomycin pulmonary toxicity. In: Bleomycin: Current Status and New Developments. Carter SK, Crooke ST, Umezawa H. (Eds.): Academic Press, New York, pp. 279–291.

Daskal Y, Gyorkey F. (1978). Morphological manifestations of bleomycin toxicity. In: Bleomycin: Current Status and New Developments. Carter SK, Crooke ST, Umezawa H. (Eds.): Academic Press, New York, pp. 57–59.

Field SB, Hornsey S. (1977). Slow repair after x-rays and fast neutrons. Brit. J. Radiol. **50**: 600–601.

Gross NJ. (1978). Early physiologic and biochemical effects of thoracic x-irradiation on the pulmonary surfactant system. J. Lab. Clin. Med. **91**: 537–544.

Iacovino JR, Leitner J, Abbas AK, Lokich JJ, Snider GL. (1976). Fatal pulmonary reaction from low doses of bleomycin. An idiosyncratic tissue response. J. Amer. Med. Assn. **235**: 1253–1255.

Jones AW, Reeve NL. (1978). Ultrastructural study of bleomycin-induced pulmonary changes in mice. J. Path. **124**: 227–235.

Jorgensen SJ. (1972). Time-dose relationships in combined bleomycin treatment and radiotherapy. Europ. J. Cancer **8**: 531–534.

Law MP, Hornsey S, Field SB. (1976). Collagen content of lungs of mice after x-ray irradiation. Radiat. Res. **65**: 60–70.

Luna MA, Bedrossian CWM, Lichtiger B, Salem PA. (1972). Interstitial pneumonitis associated with bleomycin therapy. Amer. J. Clin. Path. **58**: 501–510.

Moseley PL, Shasby DM, Brady M, Hunninghake GW. (1984). Lung parenchymal injury induced by bleomycin. Amer. Rev. Respir. Dis. **130**: 1082–1086.

Penney DP, Rosenkrans WA Jr. (1984). Cell-cell matrix interactions in induced lung injury. I. The effects of x-irradiation on basal laminar proteoglycans. Radiat. Res. **99**: 410–419.

Penney DP, Rubin P. (1977). Specific early fine structural changes in the lung following irradiation. Inter. J. Radiat. Oncol. Biol. Phys. **2**: 1123–1132.

Penney DP, Shapiro D, Rubin P, Finkelstein J, Averill K. (1980). Effects of irradiation on alveolar type II pneumocytes. Anat. Rec. **198**: 145–146A.

Penney DP, Shapiro DL, Rubin P, Finkelstein J, Siemann DW. (1981). Effects of radiation on the mouse lung and potential induction of radiation pneumonitis. Virch. Arch. B. Cell Path. **37**: 327–336.

Penney DP, Siemann DW, Rubin P, Shapiro DL, Finkelstein, J. (1982a). Responses of the mouse lung to irradiation. Anat. Rec. **202**: 107A.

Penney DP, Siemann DW, Rubin P, Shapiro DL, Finkelstein J, Cooper RA Jr. (1982b). Morphologic changes reflecting early and late effects of irradiation of the distal lung of the mouse. A review. Scanning Electron Microsc. 1982; I: 413–425.

Phillips TL, Margolis L. (1972). Radiation pathology and the clinical response of lung and esophagus. Front. Radiat. Ther. Oncol. **6**: 254–273.

Raisfeld IH. (1979). Bleomycin-induced pulmonary toxicity: A model for the study of pulmonary fibrosis. In: Bleomycin: Chemical, Biochemical and Biological Aspects. Hecht SM. (Ed.), Springer-Verlag, New York, pp. 324–335.

Rosenkrans WA Jr., Penney DP. (1986). Cell-cell matrix interactions in induced lung injury. II. X-irradiation mediated changes in specific basal laminar glycosaminoglycans. Intern. J. Radiat. Onc. Biol. Phys., in press.

Rubin P, Casarett GW. (1968). Respiratory System. In: Clinical Radiation Pathology, Rubin P, Casarett GW. (Eds.), WB Saunders Co., Philadelphia, pp. 423–470.

Rubin P, Shapiro DL, Finkelstein JN, Penney DP. (1980). The early release of surfactant following lung irradiation of alveolar type II cells. Intern. J. Radiat. Oncol. Biol. Phys. **6**: 75–77.

Rubin P, Siemann DW, Shapiro DL, Finkelstein JN, Penney DP. (1983). Surfactant release as a predictor and measure of radiation pneumonitis injury. Intern. J. Radiat. Oncol. Biol. Phys. **9**: 1669–1674.

Samuels ML, Johnson DE, Holoye PY, Lanzotti VJ. (1976). Large-dose bleomycin therapy and pulmonary toxicity: A possible role prior to radiotherapy. J. Amer. Med. Assn. **235**: 1117–1120.

Shapiro D, Finkelstein J, Penney DP, Siemann D, Rubin P. (1982). Sequential effects of irradiation on the pulmonary surfactant system. Intern. J. Radiat. Oncol. Biol. Phys. **8**: 879–882.

Siemann DW, Hill RP, Penney DP. (1982). Early and late pulmonary toxicity in mice evaluated 180 and 420 days following localized lung irradiation. Radiat. Res. **89**: 396–407.

Siemann DW, Kochanski K. (1981). Combinations of radiation and misonidazole in a murine lung tumor model. Radiat. Res. **86**: 387–397.

Sikic BI, Mimnaugh EG, Gram TE. (1978a). Development of quantifiable parameters of bleomycin toxicity in the mouse lung. In: Bleomycin: Current Status and New Developments. Carter SK, Crooke ST, Umezawa H. (Eds.): Academic Press, New York, pp. 293–297.

Sikic BI, Young DM, Mimnaugh EG, Gram TE. (1978b). Quantification of bleomycin pulmonary toxicity in mice by changes in lung hydroxyproline content and morphometric histopathology. Cancer Res. **38**: 787–792.

Thrall RS, McCormick Jr, Jack RM, McReynolds RA, Ward PH. (1979). Bleomycin-induced pulmonary fibrosis in the rat. Inhibition by indomethacin. Amer. J. Path. **95**: 117–130.

Travis EL. (1980a). The sequence of histological changes in mouse lungs after single doses of x-rays. Intern. J. Radiat. Oncol. Biol. Phys. **6**: 345–347.

Travis EL. (1980b). Early indicators of radiation injury on the lung: Are they useful predictors for late changes? Intern. J. Radiat. Oncol. Biol. Phys. **6**: 1267–1269.

Travis EL, Down JD, Holmes SF, Hobson B. (1980). Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. Radiat. Res. **84**: 133–134.

Travis EL, Harley RA, Fenn JO, Klobukowski CJ, Hargrove HB. (1977). Pathologic changes in the lung following single and multifraction irradiation. Intern. J. Radiat. Oncol. Biol. Phys. **2**: 475–490.

Ts'ao C-H, Ward WR, Port CD. (1983). Radiation injury in rat lung. I. Prostacyclin (PGI_2) production, arterial perfusion and ultrastructure. Radiat. Res. **96**: 284–296.

Wesselius LJ, Catanzaro A, Wasserman SI. (1984). Neutrophil chemotactic activity generation by alveolar macrophages after bleomycin injury. Amer. Rev. Respir. Dis. **129**: 485–490.