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Drug-Induced Pulmonary Fibrosis

by Ian Y. R. Adamson*

The lung is a primary target of cell injury in patients receiving cytotoxic drugs, and in many cases the reaction is severe enough to produce diffuse pulmonary fibrosis. Although many different agents may damage the lung, the patterns of cellular injury and repair are relatively constant. Using bleomycin toxicity as an example, it has been shown that, after IV injection, the selective site of lung injury is the vascular endothelium; this is followed by the accumulation of interstitial edema and later by necrosis of Type I epithelial cells. In normal repair, rapid proliferation of Type II cells, followed by differentiation to Type I, restores the epithelial surface without fibrosis. However, after bleomycin, Type II cell proliferation is frequently followed by abnormal differentiation to a metaplastic form of epithelium. Fibroblast proliferation accompanies this abnormal epithelial response which is related either to selective retention of bleomycin by epithelial cells or to the toxic effects of administering more drug at the time of Type II cell division. It is concluded that diffuse interstitial fibrosis results from prolonged disturbance of the normal epithelial–mesenchymal interrelationships at the alveolar wall. Disruption of the fibroblastic control system by extensive epithelial necrosis or by delayed or inappropriate repair may be the key factor in instigating fibroblast proliferation and subsequent deposition of collagen. This mechanism may account for the development of diffuse fibrosis or fibrosing alveolitis in response to a variety of pulmonary toxins.

The association between the development of pulmonary disease in humans and the administration of various chemicals has been made with increasing frequency in recent years. In particular, the increased incidence of diffuse pulmonary fibrosis in patients receiving various forms of cancer chemotherapy has implicated the lung as a primary target for a number of cytotoxic agents. Drug-induced lung disease is difficult to diagnose with any degree of certainty due to the many variables encountered in studying a human population. Factors such as medical history, variable immune status, possible role of infection and interaction with other drugs or irradiation make it difficult to identify the causal factor(s) in the development of pulmonary disease.

From a series of case reports it is clear that a great variety of chemical agents may induce rather similar clinical signs and symptoms and, on biopsy or at autopsy, the cellular changes seen in the lung, at least in late stages, are also similar. For example, pulmonary fibrosis was reported in 1973 as a side effect of nitrofurantoin treatment (*1,2*). Radiographic evidence of progressive fibrosis and interstitial pneumonitis was confirmed by morphologic studies. Various tests seemed to rule out an immunologic mechanism and, though a direct toxic reaction has been postulated, the precise mechanism of pulmonary injury is unknown. Similarly, diffuse pulmonary fibrosis is associated with busulphan and melphalan therapies for leukemia and multiple myeloma (*3,4*). In one case, where the patient died after receiving only melphalan, interstitial fibrosis was associated with atypical alveolar hyperplasia. This association of epithelial changes and underlying fibrosis was seen in several other melphalan patients and in other studies of different alkylating agents such as cyclophosphamide (*5*) and mitomycin (*6*). In the latter study, histologic examination revealed alveolar septal edema with some interstitial infiltrates and hypertrophy of alveolar lining cells accompanied by thickening of the septal walls with collagen. A similar pattern has been seen in paraquat poisoning where, at biopsy, there was severe damage to the alveolar epithelium with signs of pulmonary fibrosis; subsequently at necropsy, this had progressed to massive intra-alveolar and interstitial fibrosis (*7*).

Many other agents have now been identified as toxic to the lung, and it has been suggested that most cases of idiopathic pulmonary fibrosis are in fact drug-induced. Although the available evidence indicates that there is initial damage to the air–blood barrier with fibrosis developing as a reparative event, no clear pathogenesis can be reconstructed from these scattered clinical reports. In recent years, however, the drug bleomycin has also been shown to induce similar fibrotic changes in the lung (*8*) and it has proved suitable for detailed experimental studies. In dealing with this agent in some depth, it is believed that the cellular mechanisms involved in its toxicity may be valid for drug-induced lung disease in general.

Pulmonary Toxicity of Bleomycin

The antibiotic bleomycin has been used with increasing frequency over the last 10 years in the treatment of several types of tumors, including squamous cell

*Department of Pathology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E OW3.

carcinomas, lymphomas and testicular tumors. The drug was first isolated from *Streptomyces verticillatus* in Japan, where it was shown to be a mixture of glycopeptides with differences in their terminal amine groups (*9,10*). Initially, the drug was found to be effective on squamous cell carcinomas without inducing the renal toxicity that was found with related antibiotics, but with increasing use, it became apparent that bleomycin is toxic to the lung and can produce diffuse pulmonary fibrosis accompanied by epithelial metaplasia.

Bleomycin has some advantages over other cytotoxic drugs principally because it does not have major effects on the bone marrow (*9,11*) or on immunocompetence in animals or humans (*12,13*). In all cells, normal or neoplastic, the drug is initially concentrated in the cytoplasm where it is degraded by an enzyme which cleaves carboxyamide groups (*9*). There is now a clear relationship between responsiveness of a cell type to bleomycin and low levels of the degradative enzyme (*14*). Epidermal cells in general appear to contain low levels of inactivating enzyme, so bleomycin can more readily reach the nucleus where the ultimate damage is fragmentation of DNA with subsequent changes in the synthesis of DNA, RNA and protein (*15*). Some of the DNA strand damage may repair, but usually the cell cycle is affected, often leading to cell death. These effects are therefore more readily seen in rapidly dividing cells such as tumor cells, in particular in tumors of epidermal cell origin with the low level of inactivating enzyme.

The usual limiting factor in prescribing bleomycin is the risk of pulmonary fibrosis. This occurrence appears to be related to total dose administered and the initial guideline of 300 mg total dose to humans has now been reduced to 150 mg (*16*). Previous lung injury may also exclude or limit the use of bleomycin in some patients, and it has also been reported that prior radiotherapy increases the risk of pulmonary toxicity to bleomycin (*17*). Drug administration is usually stopped at the first sign of lung damage as seen by X-rays or pulmonary function tests, but often the injury will progress further to pulmonary fibrosis (*8*).

Biopsies of these injured lungs show diffuse alveolar damage. Figures 1–3 illustrate the pulmonary lesions in a patient who received bleomycin treatment for an epidermoid carcinoma of the esophagus. Although the tumor size decreased, the lungs developed extensive interstitial fibrosis with some areas of intra-alveolar fibrosis (Fig. 1). In some regions of the lung, the alveoli were lined by cuboidal, metaplastic epithelial cells lying on a thick fibrous interstitium (Fig. 2). Electron microscopy showed a variety of epithelial cell forms, including tubules of undifferentiated cells reminiscent of fetal epithelium. More common were areas of proliferated cuboidal Type II cells associated with interstitial fibrosis (Fig. 3).

Various animal models have now been used to study the pulmonary toxicity of bleomycin. In experiments

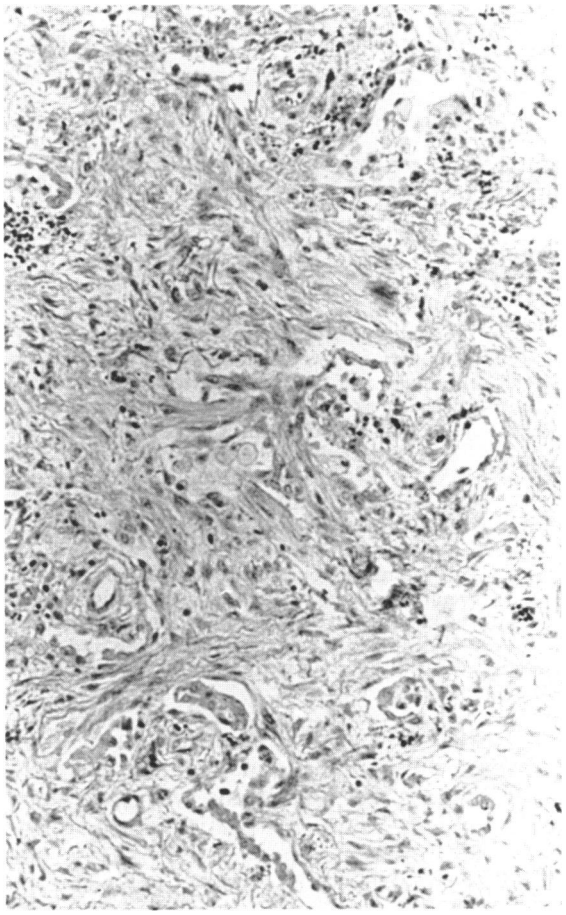

FIGURE 1. Human lung after bleomycin treatment for an esophageal tumor. Lung is consolidated with extensive areas of interstitial and intraalveolar fibrosis. H & E stain, ×250.

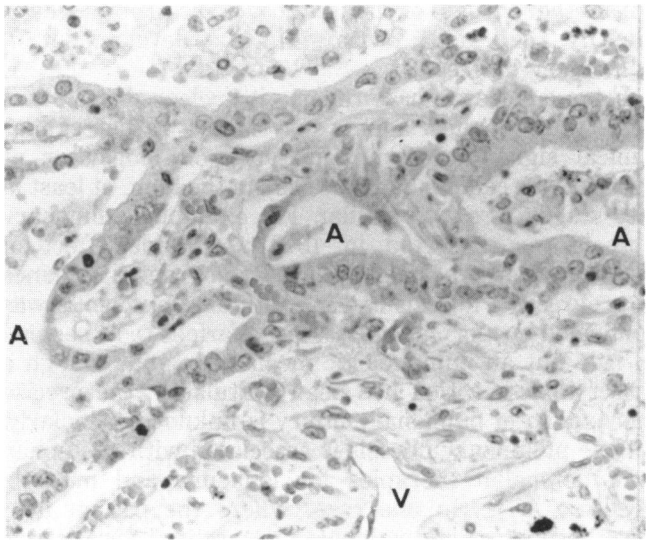

FIGURE 2. Human lung after bleomycin. Metaplastic cuboidal epithelial cells line some alveoli and cover a thick fibrous interstitium: (A) alveolus; (V) vessel. H & E stain, ×665.

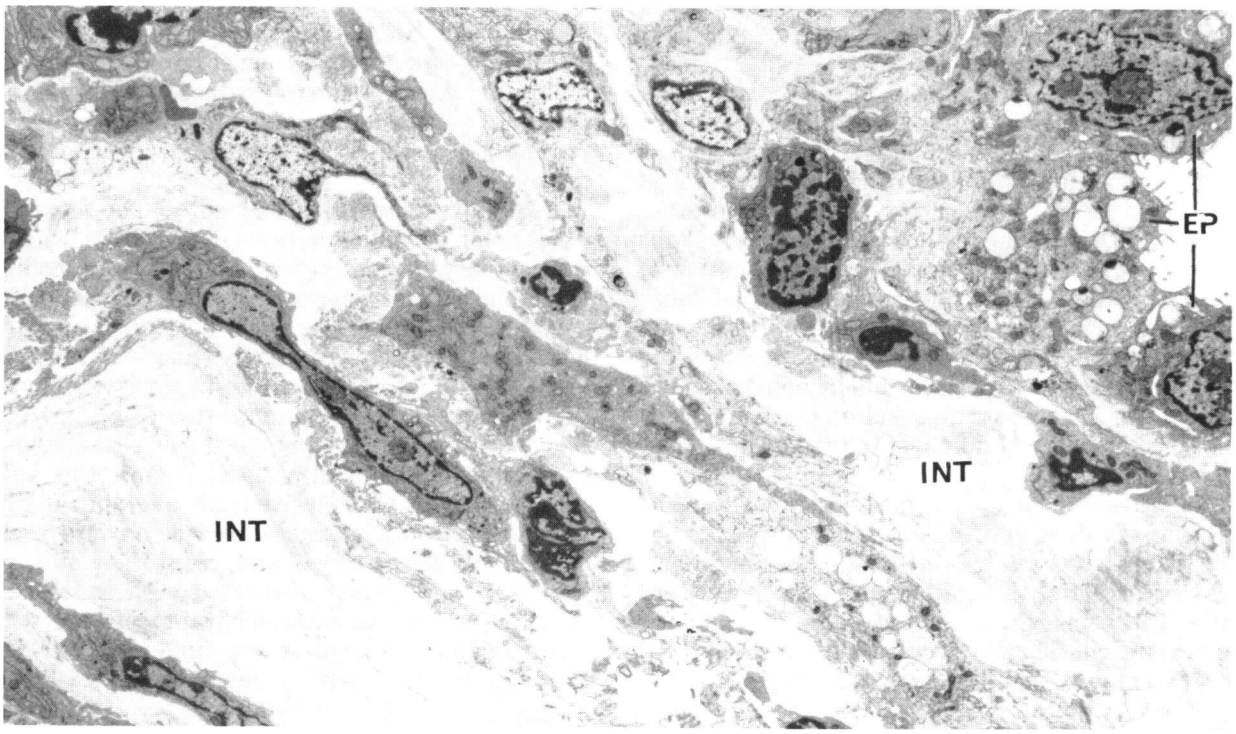

FIGURE 3. Biopsy of human lung after bleomycin. The electron micrograph shows fibrosing alveolitis with a group of Type II cells (EP) overlying a thickened interstitium (INT) containing many fibroblasts and collagen fibers. EM, ×3000.

where dogs received various doses of bleomycin intravenously, Fleischman et al. (*18*) found varying degrees of epithelial reactivity with inflammatory cell infiltrates and fibrosis as post-injection time increased. Later, Adamson and Bowden used multiple intraperitoneal injections of bleomycin to mice, as a model for inducing epithelial metaplasia and pulmonary fibrosis (*19*). From dose/mortality figures, they showed that a higher dose was needed to produce pulmonary changes in mice than in dogs; 0.4 mg/kg produced lesions in dogs (*18*), whereas mice required at least 8–10 times this dose for significant damage to occur (*19*). Following the intraperitoneal administration of 20 mg/kg bleomycin twice per week for 4 to 8 weeks, most animals developed perivascular edema with cellular infiltrates, and, in addition, a proportion of these mice also showed epithelial metaplasia and fibrosis.

Pulmonary fibrosis in mice has also been produced after a single large intravenous dose of bleomycin. The administration of 120 mg/kg bleomycin IV resulted in 50% mortality with most of the surviving animals developing diffuse pulmonary fibrosis (*20*). Autopsies on animals dying with or without lung disease showed that other organs were normal on both gross and histologic examination. It was concluded that the lung is a specific target for bleomycin.

More rapid and severe interstitial pulmonary fibrosis has been produced experimentally by instilling a single dose of bleomycin intratracheally to rats (*21*) and hamsters (*22,23*). In these studies morphologic evidence of fibrosis was correlated with enhanced synthesis of hydroxyproline, collagen and elastin. In hamsters the changes were also correlated with significant deterioration in pulmonary function as measured by lung compliance and capacity. The results of these studies, together with evidence that bleomycin-induced pulmonary fibrosis in baboons (*24*) is similar to that seen clinically, indicate that experimental studies on the pathogenesis and mechanism of lung injury by bleomycin are relevant to the human disease process. The bleomycin model may also have more general application in providing some understanding of cellular mechanisms leading to diffuse fibrosing alveolitis induced by a variety of toxic chemicals.

Pathogenesis of Pulmonary Fibrosis

Many agents, both airborne and bloodborne, are known to damage the cells of the lung but the patterns of injury and repair are relatively few. The overall determination of lung injury and repair from measurements of protein, DNA, RNA and collagen does not take account of the complex cellular composition of the lung or of the known heterogeneity of cellular responses. Following bleomycin, pulmonary cells demonstrate this differential susceptibility to injury. The cellular events following a large single dose of bleomycin to mice (120 mg/kg) are similar to those observed after multiple intraperitoneal injections (20 mg/kg twice weekly), though the time sequence is accelerated. The earliest

changes observed by electron microscopy involved the endothelium of pulmonary arteries and veins where subendothelial blebs bulged into the vascular lumen, resulting in severe attenuation of the endothelial cytoplasm; in addition, there was intracytoplasmic edema of the endothelial cells (Fig. 4). This was seen as soon as 2 weeks after the start of the intraperitoneal injections and 2 days after the intravenous dose. Subsequently, these changes were detected by light microscopy and were accompanied by accumulation of edema fluid in perivascular spaces. Lesions of large pulmonary vessels occurred before changes were seen in the capillary endothelium where the cells also became swollen and separated from the underlying basement membrane. It is likely that this resulted in permeability changes since evidence of diffuse interstitial edema was found at that time.

The endothelium was therefore identified as the primary site of injury and the anatomic pattern of the lesions is likely related to the mode of drug administration. The preferential susceptibility of endothelial cells of arteries and veins can be related to the fact that the drug is bloodborne. This is in contrast to injury induced by oxygen or other oxidant gases where the cells on the route of maximum gaseous transfer, the capillary endothelium, are preferentially injured (*25,26*). The circulating drug initially must reach and enter the pulmonary endothelium to produce its cytotoxic effects, and the particular sensitivity of this cell type to bleomycin is probably related to a low level of inactivating enzyme.

The endothelium has been traditionally regarded as a waterproof, coagulation-proof lining layer with transport activity. Recently this view has been modified by metabolic studies showing that endothelial cells bear surface enzymes and receptor sites (*27*). Injury to the pulmonary endothelium usually results in the leakage of fluid into the interstitium and, in some animals receiving the large intravenous injection of bleomycin, lung injury did not progress beyond endothelial swelling with the accumulation of interstitial edema; this was also true for animals that received only a few intraperitoneal injections. In both instances, the lesions were reversible; the edema disappeared, and restitution of a normal blood–air barrier was associated with endothelial cell division, as seen in autoradiographs of lung following ^{3}H-thymidine injection at the time of repair (*20*). In instances where there is more severe or prolonged

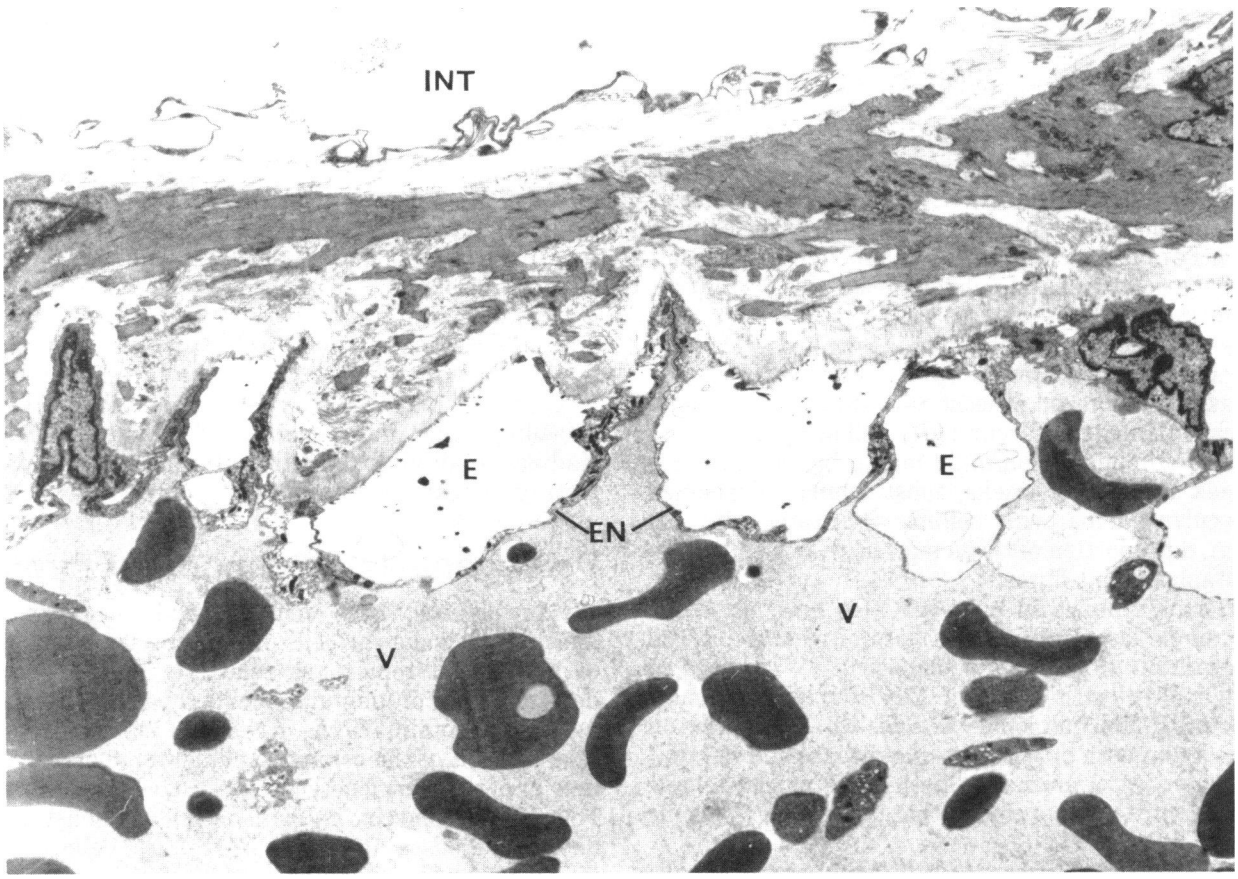

FIGURE 4. Pulmonary vein of a mouse after 2 weeks bleomycin (four injections IP, 20 mg/kg). Severe cytoplasmic edema of endothelial cells is accompanied by subendothelial edema causing swelling of cells into the lumen: (V) vessel lumen; (E) edema; (INT) interstitium; (EN) endothelium. EM, ×12,000. Reproduced from Adamson and Bowden (*19*) with permission.

damage to the endothelium, for example, after irradiation, the disturbance of the endothelial–interstitial cell balance appears to promote fibroblast proliferation. In such a case severe endothelial damage alone, though repaired later, may be associated with interstitial fibrosis.

The most critical cellular event following bleomycin administration is destruction of the Type I epithelium. This attenuated cell forms a barrier that initially limits the edema fluid to the interstitium. Since the permeability of the vascular endothelium is altered, circulating bleomycin probably has access to cells of the interstitium and epithelium, and it appears that the drug is toxic to alveolar epithelial cells. As noted in other models of lung injury, there is a differential response of the alveolar epithelium, whereby focal necrosis of the squamous Type I cells was observed (Fig. 5) and the cuboidal Type II cells appeared relatively normal. By destroying this epithelial barrier, the contents of the interstitium and of the blood may leak through damaged endothelium directly to the alveoli, and in fact, necrosis of Type I cells was frequently associated with intra-alveolar aggregates of fibrin (*19*). If this is massive, the animals will die; however, if multifocal, they survive and the lung undergoes a reparative fibrotic reaction.

Following bleomycin, fibrosis was observed in two distinct compartments of the lung. In fibrin-filled alveoli, fibroblasts migrated from interstitium across the denuded epithelium to the alveoli where they surround the fibrin and secrete collagen to produce areas of intra-alveolar fibrosis (*19*). Fibroblastic activity was also prominent in the pulmonary interstitium around both capillaries and larger vessels giving a picture typical of fibrosing alveolitis (Fig. 6). Both the septal and intra-alveolar fibrosis became progressively more extensive and severe with time (Fig. 7). In animals that received a 20 mg/kg dose twice per week for 8 weeks, fibrosis was first observed at 4 weeks, then progressed to become diffuse by 4 to 12 weeks, whereas mice receiving IV bleomcyin developed fibrosis by 2 to 4 weeks.

It can be seen that the morphology of the late stages of the pulmonary reaction is similar in the human (Figs. 1-3) and animal (Figs. 6 and 7) studies. The formation of intra-alveolar and interstitial fibrosis appears to follow the same pattern after bleomycin as that described for experimental paraquat poisoning where the key event is also disintegration of the alveolar epithelium (*28,29*). In the case of paraquat, the alveolar damage is usually much more severe with massive intra-alveolar edema and/or hemorrhage resulting in death (*7*); the few survivors with much less epithelial injury develop

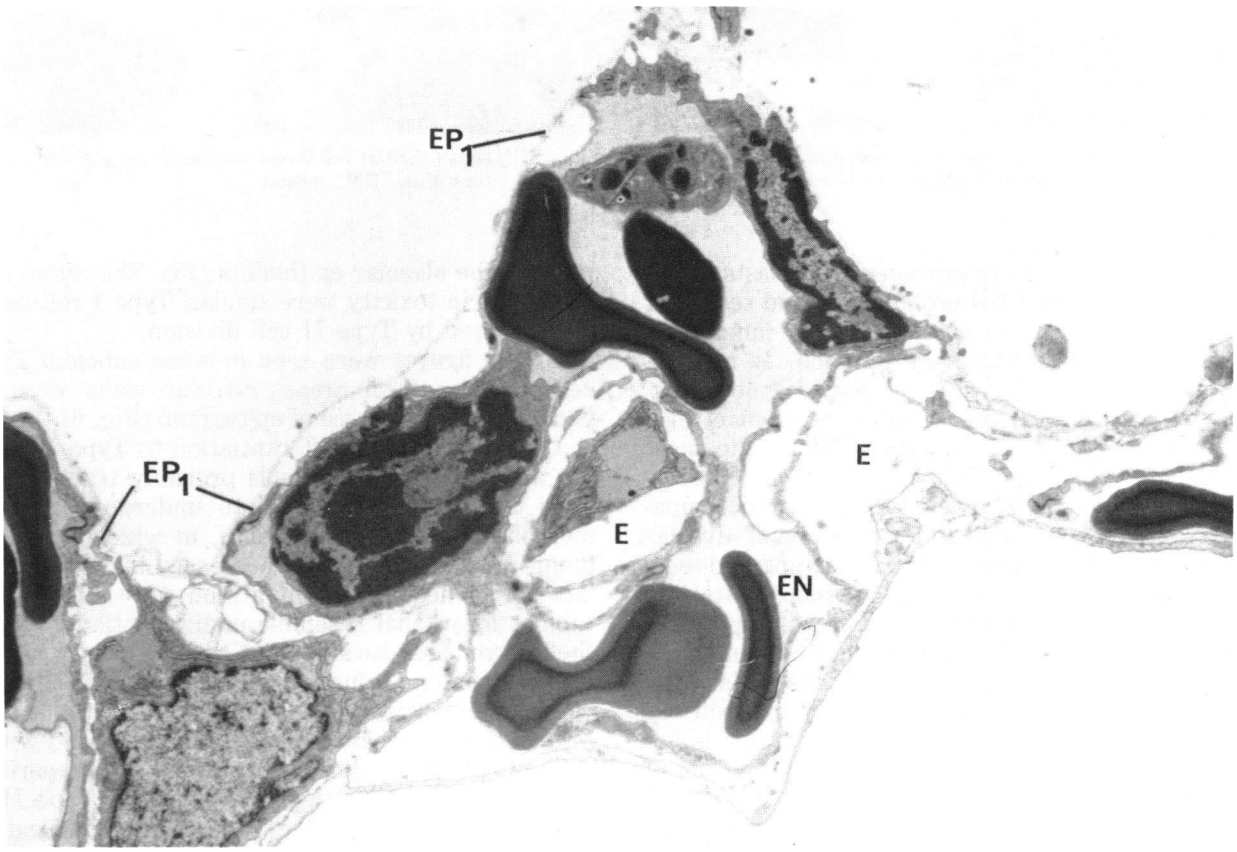

FIGURE 5. Mouse lung after 4 weeks bleomycin IP. Swelling of capillary endothelial cells is accompanied by interstitial edema (E). In several areas there is necrosis of Type I epithelium (EP1). EM, ×4200.

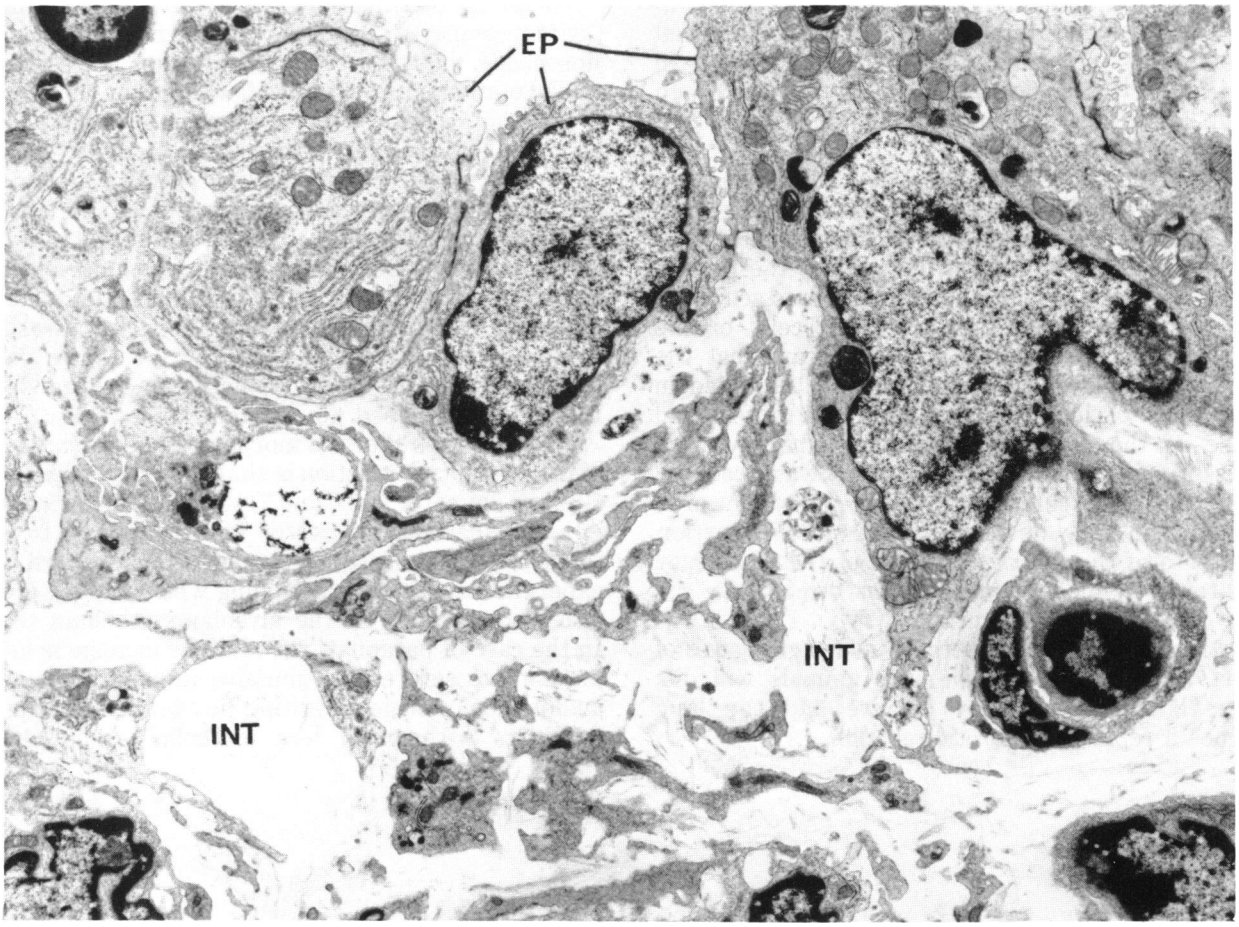

FIGURE 6. Mouse lung after 6 weeks IP bleomycin. Regenerating epithelium (EP) shows various cell forms varying from undifferentiated to typical Type II cell. The air-blood barrier is now thickened by fibrosis: (INT) interstitium. EM, ×8400.

fibrosis. In this case also, migration of fibroblasts to the alveoli as well as interstitial proliferation and secretion of collagen, resulted in consolidation of the lungs with rapidly deteriorating pulmonary function. It may be postulated therefore that the epithelial cell injury destroys the critical epithelial–mesenchymal control relationship and is at least partly responsible for the onset of fibrosis.

In the case of bleomycin toxicity, fibrosis was accompanied by reactive hyperplasia and metaplasia of alveolar epithelial cells and the amount of fibrosis appeared to be related to the severity of the epithelial lesions. After the period of Type I cell necrosis, evidence of reparative activity was found in the alveolar epithelium. It is generally accepted that the Type I epithelial cells of the air–blood barrier are susceptible to injury by a variety of bloodborne or airborne agents. Acute injury is followed by epithelial repair, accomplished by division of Type II cells, some of which transform to the Type I form to restore the normal air–blood barrier. This process, which occurs after oxygen (*30*), nitrogen dioxide (*31*) and intraperitoneal butylated hydroxytoluene (*32*), is a recapitulation of events in the fetal development of the alveolar epithelium (*33*). The initial events in bleomycin toxicity were similar, Type I cell necrosis was followed by Type II cell division.

Mitotic figures were seen in some cuboidal Type II cells (*19*); in focal areas, alveolar walls were lined exclusively by this type of epithelium (Fig. 6). However, the usual process of differentiation to Type I cells did not always occur. Some cells probably transformed to Type I but others seemed to undergo change to a metaplastic form of epithelium, in which the alveolar lining cells were cuboidal, possessed microvilli but not lamellar bodies (*19*). Many alveoli were lined by these tubular forms that resemble undifferentiated fetal epithelium and are also similar to the epithelial forms seen in the bleomycin-injured human lung (Fig. 2).

The frequency of observing metaplastic epithelial cells in fibrotic areas of mouse lung increased with the number of injections following biweekly intraperitoneal administration at 20 mg/kg. Many giant Type II cells were seen at 8 weeks; they were often binucleate and disruption of nuclear structure occurred in some cells (Fig. 8). The development of ciliated alveolar cells was observed from 8 to 12 weeks after the start of bleomycin

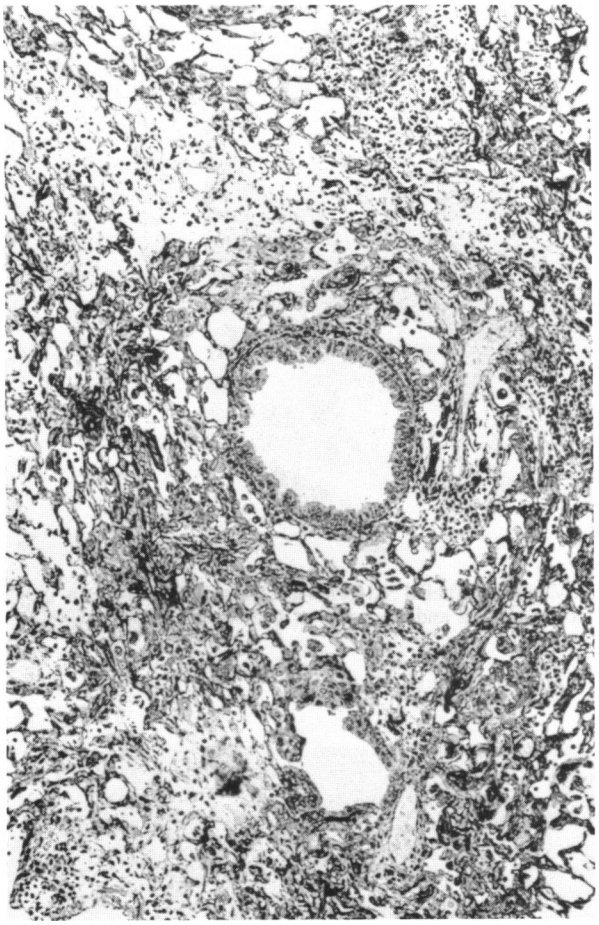

FIGURE 7. Mouse lung after 8 weeks IP bleomycin showing massive interstitial and intra-alveolar fibrosis. Silver methenanine stain, ×130.

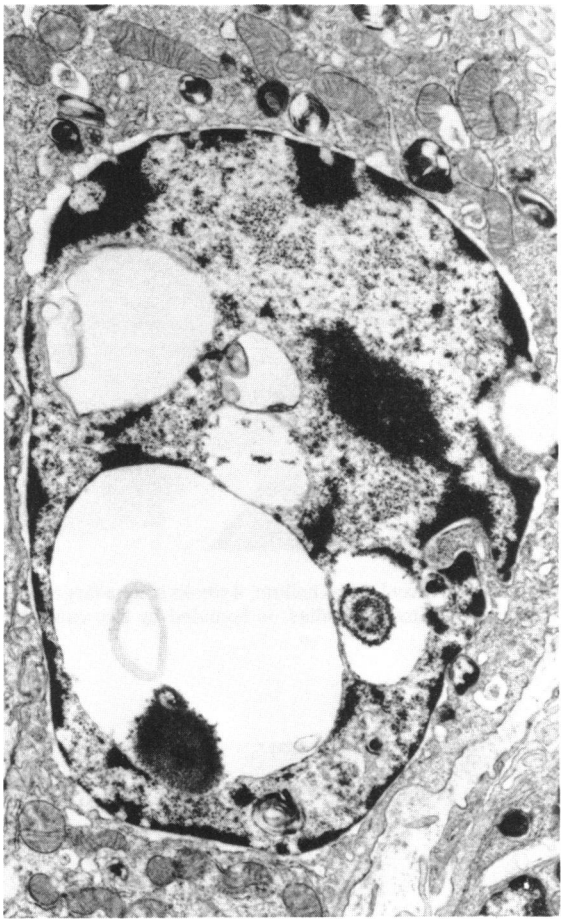

FIGURE 8. After multiple bleomycin injections, nuclear disintegration of some Type II epithelial cells is seen. EM, ×10,000. Reproduced from Adamson and Bowden (*47*) with permission.

injections. This change was focal in nature and, within any one alveolar tubule, a variety of cellular forms was observed including typical Type II cells, ciliated cells without lamellar bodies and intermediate forms with haphazard arrays of microvilli and cilia (Fig. 9). By 12 weeks, occasional foci of squamous metaplasia lined the original alveoli (*19*) and ciliated alveolar epithelium was readily recognized by light microscopy (Fig. 10). These findings suggest that the Type II cells divided to initiate epithelial repair but that the effects of the drug on epithelial DNA subverted the post mitotic processes of differentiation to produce a variety of inappropriate alveolar lining cells.

It could be argued that bronchiolar epithelial cells had grown down into the alveoli to repair the injury but the finding of these metaplastic forms in distal regions of the lung makes it likely that they were derived from alveolar epithelial cells. Additional evidence that the metaplastic cells arose from altered differentiation of the alveolar epithelium comes from a cytodynamic study (*20*). After a large intravenous dose of bleomycin, mice were killed at various intervals, with ^{3}H-thymidine injected 1 hr before sacrifice. From combined morphologic and autoradiographic studies it was shown that, following epithelial injury, regenerative activity occurred promptly. After 12 days many alveoli were lined by large cuboidal cells, a number of which had incorporated tritiated thymidine. The overall labeling of alveolar cells was maximal at 12 days and most of the cells labeled at this time were proliferating Type II cells (Fig. 11). Immediately prior to this peak, endothelial labeling was observed, and in the period 14–28 days, as fibrosis developed, evidence of thymidine incorporation was seen in fibroblasts. Labeling of bronchial and bronchiolar epithelial cells never exceeded control values throughout the study, indicating that the metaplastic epithelial cells were of alveolar origin, the result of inappropriate differentiation after a period of division (Fig. 11).

The standard pattern of repair to the alveolar wall is rapid regeneration of surviving endothelial cells (*25*) and Type II epithelial cell proliferation followed by transformation to Type I (*30*). When lung injury is acute and focal, these events occur rapidly and fibrosis does

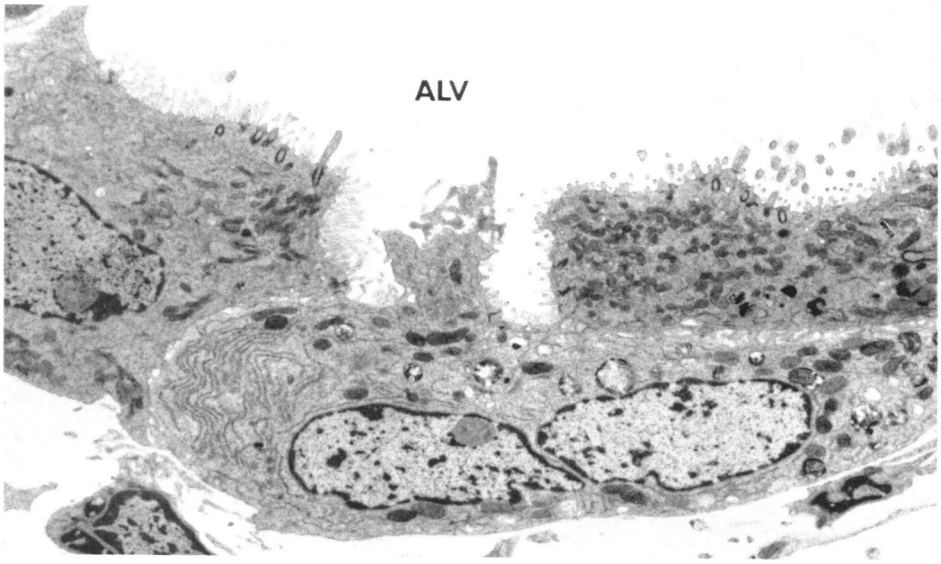

FIGURE 9. Area of alveolar epithelium, 4 weeks after a large IV dose of bleomycin. Various epithelial cell forms line the alveolus (ALV), a Type II cell with typical lamellar bodies, is bounded by two cuboidal cells with a mixture of cilia and microvilli at the surface. EM, ×6000.

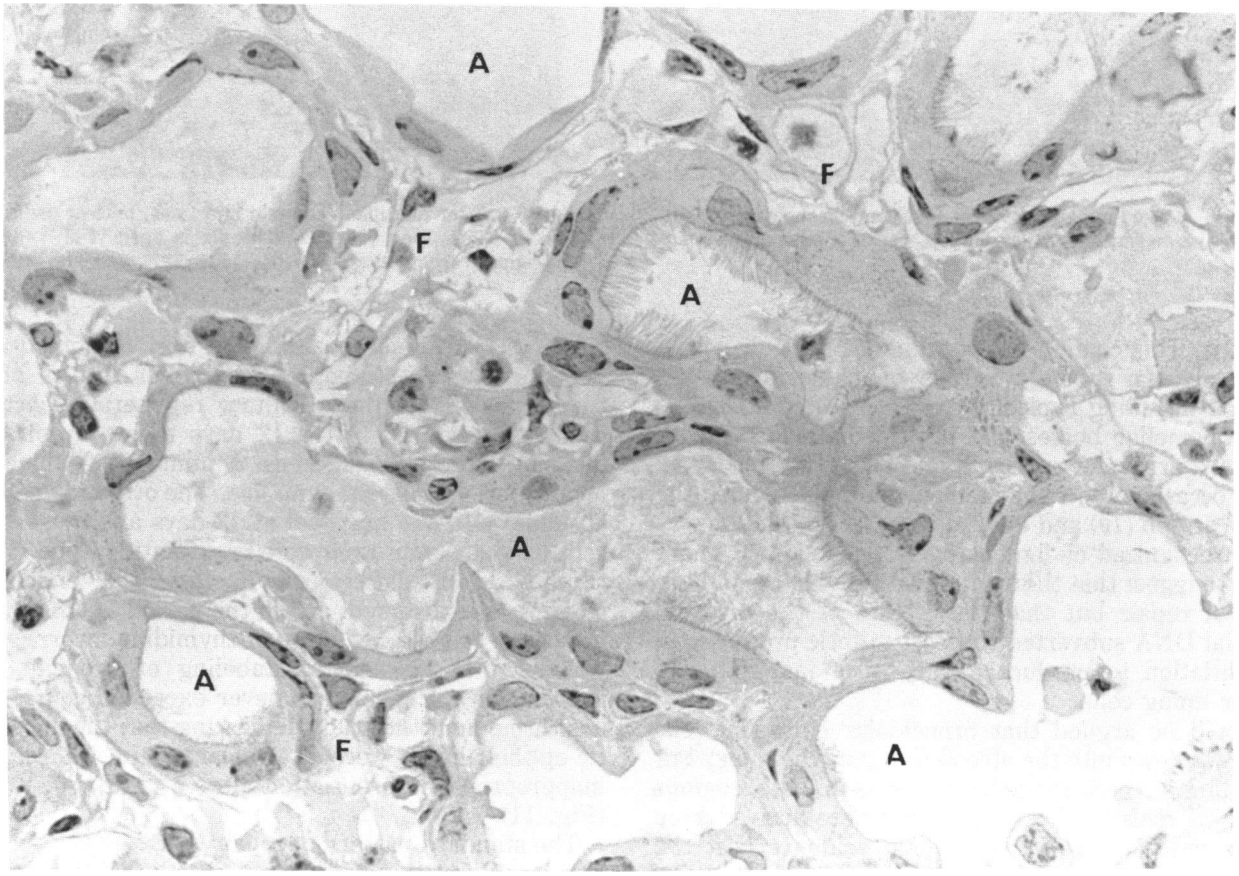

FIGURE 10. Mouse lung 10 weeks after IP bleomycin. In some areas of interstitial fibrosis (F), the alveoli (A) are lined by flattened epithelial cells, some of which are ciliated. Methacrylate section, H & E stain, ×1000. Reproduced from Adamson and Bowden (*20*) with permission.

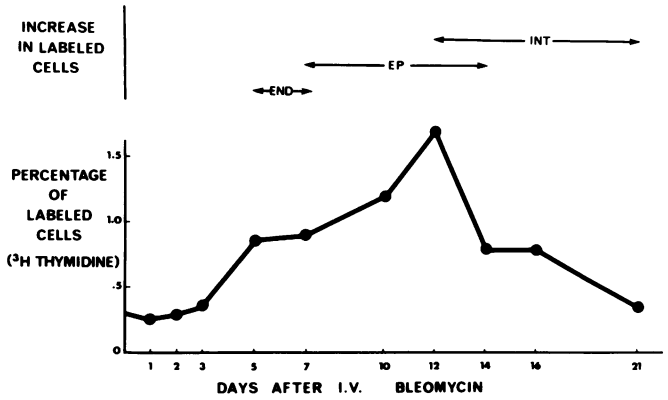

FIGURE 11. Plots of (bottom) percentage of ^{3}H-thymidine labeled cells at various times after a single IV injection of bleomycin to mice (120 mg/kg); (top) pulmonary cell types showing the time span of increased thymidine incorporation as determined from differential counts of labeled cells: (END) endothelium; (EP) epithelial; (INT) interstitial. Reproduced from Adamson and Bowden (*47*) with permission.

not develop. Following bleomycin treatment, endothelial repair occurs quickly but, after a period of epithelial cell division, a variety of inappropriate, poorly differentiated cell forms are seen. The overall sequence of cellular injury and repair reveals that epithelial injury and regeneration started before the fibroblastic response, indicating that metaplasia is not the result of an initial change in the pulmonary interstitium. Rather, the reverse process may be true; altered and/or delayed repair of the epithelium may have promoted interstitial fibrosis. This may be the result of severe, acute epithelial injury or repeated exposure to a toxin which by altering the surface, disturbs the usual epithelial–interstitial cell relationship and promotes fibroblast proliferation. In this way injury to the surface of the lung may resemble a cutaneous wound where epithelial destruction is associated with fibroblast proliferation, which is inhibited subsequently when a normal epithelium is restored. There is evidence to support this concept from the oxygen poisoning model of lung injury. Prolonged exposure to high concentrations of oxygen results in delayed epithelial repair with fibrosis, whereas acute epithelial injury is repaired without fibrosis (*30*, *34*). It is suggested that, in the case of bleomycin, the epithelial injury and the disordered repair that results disturbs the usual interrelationships between epithelial and fibroblastic cells and is a key factor in the development of pulmonary fibrosis. In addition to the concept that surface disruption "switches on" fibroblasts, bleomycin may have a direct effect on these cells since in tissue culture, it has been shown that bleomycin stimulates fibroblasts directly to increase collagen synthesis (*35*).

It has also been proposed that immunologic mechanisms may play a role in the development of pulmonary fibrosis after bleomycin. Although some plasma cells and lymphocytes are occasionally seen in peribronchial areas after many intraperitoneal injections, they are not a prominent or consistent finding. After a large intravenous injection, fibrosis develops in 2 to 4 weeks without such a cellular infiltrate (*20*). Recent studies in both athymic mice (*36*) and baboons (*24*) demonstrate that interstitial lung disease can be produced by bleomycin without the involvement of cellular immune processes.

Mechanisms of Injury and Repair

If, as suggested above, injury to the alveolar epithelium and its subsequent abnormal repair are crucial factors in the pathogenesis of bleomycin induced pulmonary fibrosis, further evidence should be sought for the role of the drug in epithelial reactivity and altered differentiation during repair. The toxicity of bleomycin to cells in division is the basis for its use as an anti-tumor agent and tissue culture studies have shown that the drug acts at various stages of the cell cycle (a) by breaking DNA strands (*37*), (b) by inhibiting the entry of cells into mitosis (*38*) and (c) by retarding the passage of cells through the mitotic cycle (*39*). Two hours after administration of ^{14}C-labeled bleomycin, isotope was detected at the cell membrane and reached the nuclear membrane by 4 hr; necrotic cells had heavy cytoplasmic label (*40*). This is supportive evidence for the theory that cells contain varying amounts of an inactive enzyme in the cytoplasm and that cells with low levels of this enzyme are most susceptible to injury by bleomycin. It has been shown in mice that squamous cell carcinomas, readily controlled by bleomycin, possess less inactivating enzyme than more resistant sarcomas (*14*). In a study by Ohnuma, homogenates of various tissues were incubated with bleomycin and drug inactivation was followed (*41*). Liver, intestine, spleen, plasma and kidney all contained more inactivation enzyme than skin and lung. This is the inverse of drug retention from *in vivo* studies of Umezawa et al. (*10*), where bleomycin content was highest in lung and skin, the organs of lowest inactivation enzyme content. In studies of the reaction of skin to bleomycin, the drug not only inhibited the proliferation of epidermal cells, but it also altered cellular maturation (*42*).

A common factor between lung and skin appears to be the extensive covering by epithelial cells, which contain low levels of detoxifying enzymes to cleave the carboxyamide groups of bleomycin. Once across the cytoplasm, the drug affects the nucleus where it inserts between the double helix, causing strand scission (*37*). This process is reversible; however, if the cell is involved in replication before DNA is repaired, an abnormal cell is produced (*43*), leading to secondary changes in RNA and protein (*44*). *In vitro* experiments have shown that the differential susceptibility of cells to bleomycin depends on the phase of the cell cycle (*15*). Some cells may be inhibited from entering mitosis, others with DNA damage during G_1 and S phases may replicate

abnormally. In mouse skin, bleomycin depresses the number of cells in mitosis, their passage time through the cycle and inhibits cellular maturation (*42*). The effects on epithelial differentiation have been demonstrated in a clinical study using serial biopsies of carcinoma of the tongue. Bleomycin treatment reduced cell division and promoted the accumulation of tonofilaments in tumor cells which later produced keratinic epithelial pearls, indicative of altered epithelial cell differentiation (*45*). These combined results suggest that, whereas bleomycin may partially inhibit cells from division, it has also an effect on differentiating post mitotic epithelial cells.

The present experimental evidence suggests that bleomycin is selectively retained by the lung where it damages endothelial and Type I epithelial cells. It is reasonable to speculate that the drug may also be incorporated into Type II cells at the same time and its effects manifested during the critical period of Type II cell proliferation when, in some instances, severe nuclear damage to Type II cells was observed (Fig. 8). It seems, therefore, that the reactivity of the Type II cell depends upon its phase in the cell cycle. In the resting G_0 phase, the cell appeared resistant to bleomycin whereas in the proliferative and transformation phases, cellular changes were observed. As in other epithelial cell systems described above, the drug may influence the processes of cellular differentiation in the alveolar epithelium. This would also explain the exaggerated metaplastic response observed following multiple injections of bleomycin in which there is continuing insult to the actively dividing or recently divided Type II cell population by newly administered drug.

These theories on the action of bleomycin on differentiating Type II cells and the link between abnormal repair and fibrosis are based on the premise that bleomycin is present at the critical period of repair. It is known that the lung is relatively deficient in an enzyme which detoxifies bleomycin (*9,10*), and this probably accounts for the selective retention of the drug in the lung (*46*). Following 50 mg/kg IP to mice, bleomycin was retained for up to 5 days in skin and lung whereas the drug was rapidly cleared from liver and kidney. To test the hypothesis that bleomycin is present in the lung during the phases of division and differentiation of Type II cells, Adamson and Bowden (*47*) used tritiated bleomycin to correlate cellular injury and repair with drug presence in the lung. After a single intravenous injection, significant levels of bleomycin were present during the periods of Type I cell necrosis and Type II cell proliferation. It was suggested that the subsequent epithelial metaplasia was due to the continuing presence of the drug in Type II cells at the critical phase of mitosis (Fig. 12).

These results were substantiated by experiments in which biweekly intraperitoneal injections of tritiated bleomycin were given to mice. When the drug was administered at the time of Type II cell proliferation, an exaggerated epithelial metaplasia was produced and

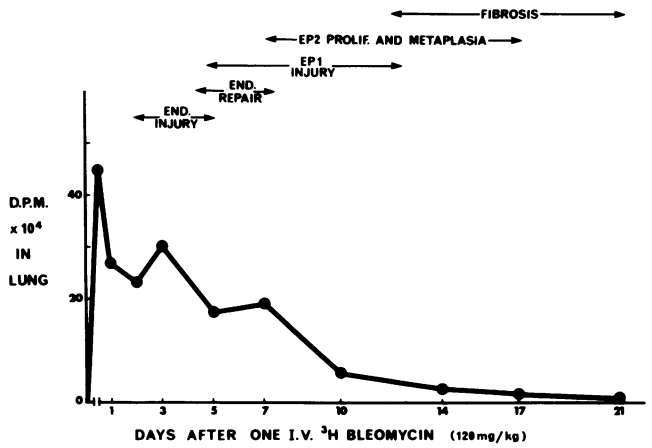

FIGURE 12. Plots of (bottom) mean values for radioactivity (dpm) in 30 mg mouse lung samples at intervals after a single IV injection of ^{3}H-bleomycin (120 mg/kg); (top) time sequence of cellular events after the single IV dose of bleomycin.

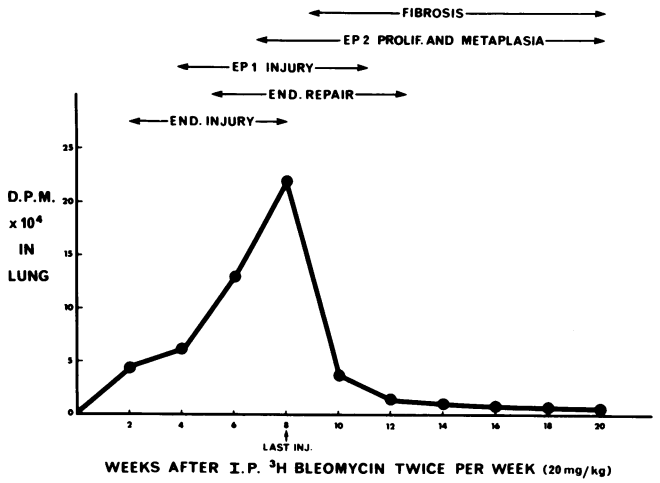

FIGURE 13. Plots of (bottom) mean values for radioactivity (dpm) in 30 mg mouse lung samples at intervals after twice weekly IP injections of ^{3}H-bleomycin (20 mg/kg) for 8 weeks; (top) time sequences of the cellular events after multiple injections of bleomycin.

inappropriate cell types described earlier were found lining alveoli. At this time progressive accumulation of ^{3}H-bleomycin in the lung was observed by scintillation counting (Fig. 13) and the autoradiographs indicated a selective concentration of the drug in Type II cells (*47*). From the sequential data on morphology, DNA synthesis in epithelial cells and drug retention, it was concluded that sufficient bleomycin was present to affect the Type II cell during its proliferative phase. Division of these cells occurred, but the processes of cellular differentiation were disturbed sufficiently to produce cell forms varying from typical bronchial to typical alveolar epithelium in association with areas of fibrosis. Occasionally a few such epithelial cells have been seen in "normal" lung shere they have been attributed to a

developmental abnormality related to the common origin of all pulmonary epithelium (*48*). It appears therefore that the metaplastic cells seen after bleomycin are the result of drug effects on the synthetic mechanisms of the differentiating epithelial cells.

The finding of various epithelial cell types overlying areas of fibrosis suggests that the abnormal differentiation did not restore the normal epithelial–mesenchymal control mechanisms that usually limit fibroblastic activity in the interstitium. The fibrotic reaction with reactive hyperplasia and metaplasia of alveolar epithelium is not specific to bleomycin toxicity, since similar responses have been observed clinically in the various forms of diffuse pulmonary fibrosis or fibrosing alveolitis (*16,49*). It is suggested, however, that a common mechanism may apply in these cases and involve the reaction of Type II cell as the crucial factor. Whereas acute injury to endothelium and epithelium may be rapidly repaired with no fibrosis, continuing injury with delayed or disturbed regeneration is associated with fibroblastic activity (*35,50*). Type I cells are particularly vulnerable to injury and, in the steady state, the Type II cell with its slow turnover is generally considered to be injury resistant. However, in the regenerative phase the total population of these cells may turn over in 3 days (*30*), and Type II cells have now been demonstrated as susceptible to injury during division by persisting or newly administered cytotoxic agents. This is of particular significance in chemotherapy where repeated, frequent administration of drugs such as bleomycin increases the chance of drug interaction with Type II cells dividing in response to previous Type I cell injury. The therapeutic action of bleomycin is dependent on its ability to control the division of rapidly dividing cells, and it is particularly effective on tumors of epithelial origin. These facts are consistent with the present observations of the particular sensitivity of dividing Type II epithelial cells to the drug, with the subsequent development of pulmonary fibrosis during the time of abnormal epithelial repair.

In conclusion, experimental studies indicate that diffuse pulmonary fibrosis may be the result of a general repair mechanism following injury to the alveolar wall. Many different agents may cause the initial toxic insult but factors such as the extent of injury, local concentration and retention of the toxin, and any repetition of insult, determine whether or not the air–blood barrier can be repaired quickly. Focal denuding of the epithelial surface can allow plasma proteins to flow into the alveolus followed by migration of fibroblasts to produce nodules of intra-alveolar fibrosis. Diffuse interstitial fibrosis may result from disturbance of the usual epithelial–mesenchymal cell interrelationships at the alveolar surface. Any disruption to this control mechanism by extensive epithelial necrosis, delayed or inappropriate cellular repair may be crucial to instigating the fibrotic process in the pulmonary interstitium. The picture of diffuse fibrosis with epithelial hyperplasia and metaplasia is common enough to suggest that this proposed mechanism for the action of bleomycin applies to many toxins that damage the alveolar wall.

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